

SECOND EDITION

THE SCIENCE OF HAIR CARE



edited by
CLAUDE BOUILLON • JOHN WILKINSON

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edited by

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L'Oréal Recherche, Clichy, France

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Contents

<i>Foreword</i>	<i>vii</i>
<i>Preface to the Second Edition</i>	<i>ix</i>
<i>Preface to the First Edition</i>	<i>xi</i>
<i>Contributors</i>	<i>xiii</i>
1. Hair Structure, Function, and Physicochemical Properties	1
<i>A.Franbourg and F.Leroy</i>	
2. The Hair Follicle: Structure and Function	75
<i>Bruno A.Bernard</i>	
3. Scalp and Hair Hygiene: Shampoos	92
<i>Bernard Beauquey</i>	
4. Hair Care Products	141
<i>C.Dubief, M.Mellul, G.Loussouarn, and D.Saint-Léger</i>	
5. Temporary Restyling of the Hair	183
<i>R.Beidone, J.M.Sturla, H.Paty, P.Meurice, and H.Samain</i>	
6. Permanent Waving and Hair Straightening	217
<i>Charles Zviak and Anne Sabbagh</i>	
7. Hair Bleaching	246
<i>Charles Zviak and Jean Milléquant</i>	
8. Hair Coloring: Non-Oxidation Coloring	269
<i>Charles Zviak and Jean Milléquant</i>	
9. Oxidation Coloring	296
<i>Charles Zviak and Jean Milléquant</i>	
10. Toxicology of Hair Dyes	328
<i>Gerhard J.Nohynek</i>	
11. Hair Product Safety	370
<i>C.Hourseau, M.Cottin, M.Baverel, P.Meurice, and G.Riboulet-Delmas</i>	
12. Evaluation of Product Efficacy	397
<i>A.Franbourg, F.Leroy, and D.Braïda</i>	
13. The Regulation of Cosmetic Products	487
<i>D.Pierrard-Meillon</i>	
14. Skin Tests	527
<i>J.D.Wilkinson and S.Shaw</i>	
15. Adverse Reactions to Hair Products	547
<i>J.D.Wilkinson and S.Shaw</i>	

16. Occupational Disorders in Hairdressers	570
<i>J.D.Wilkinson and S.Shaw</i>	
17. Hair Shaft Abnormalities	581
<i>Rodney P.R.Dawber</i>	
18. The Hair Pigmentation Unit and Hair Graying	590
<i>Bruno A.Bernard and Stéphane Commo</i>	
19. Alopecia	601
<i>P.Reygagne and O.de Lacharrière</i>	
20. Seborrhea	628
<i>Claudine Piérard-Franchimont and Gérald Piérard</i>	
21. Dandruff (Pityriasis Capitis Simplex): Of Yeasts and Men	656
<i>D.Saint-Leger</i>	
22. Dermatitis and Eczema of the Scalp	684
<i>Pierre G.Agache</i>	
23. Scalp Dermatoses	693
<i>Dominique Blanc</i>	
24. Hair Transplantation	731
<i>Pierre Bouhanna</i>	
25. Hair: A Psychoanalytical Perspective	760
<i>Danièle Pomey-Rey</i>	
Index	778

Foreword

Charles Zviak was a prescient man who, two decades ago, saw the need for a broadbased general text on the scalp and hair that would attract a wide readership across many disciplines. He envisioned that an eclectic volume would promote interactions and collaborations among dermatologists, cosmetologists, pharmacists, chemists, physicists, toxicologists, and even psychologists. These diverse specialists could tap into an authoritative, multi-authored text that would provide practical information on a variety of subjects without plowing through a half-dozen, narrowly focused specialized tomes.

His mission was to cover concisely a wide range of topics relating to every stage of the development of safe and effective products for the care of the scalp and hair in both health and disease. Dermatologists and cosmetologists occupied quite separate domains with little dialogue between the two, the former dealing with disease and the latter with appearance.

The stratum corneum was the boundary between the two with the cosmetologists mainly applying inert substances to the surface to enhance beauty while dermatologists used active drugs to moderate disease processes in the viable tissues.

Zviak was actually the first to recognize that this division was artificial, misleading, and biologically untenable. He had the vision to presage my later definition of “cosmeceuticals,” which referred to a wide spectrum of topical substances lying between the arbitrarily opposite poles of drugs and cosmetics. He clearly recognized that there was a “conversion of interest” and that “the borderline between cosmetology and dermatology is often blurred.” I was unaware of these visionary comments when I wrote my supposedly original concept on “cosmeceuticals.” Moreover, he perceived that the domain of cosmeceuticals between drugs and cosmetics was especially applicable to scalp and hair products whose “intended use verges on the esthetic and the therapeutic.” Thus, instead of claiming credit for what I thought was an original concept, my role in this story was to invent the term “cosmeceutical” to crystallize Zviak’s ideas!

The number and variety of products designed for use on scalp and hair comprise a huge marketplace, whose popularity and usefulness can be quickly calculated in terms of the mega-billions of dollars spent annually, probably exceeding the sums spent on prescription drugs! Accordingly, this newest edition is even more relevant to the modern scene than the original volume. Another development that makes this new edition required reading for specialists in scalp and hair care is globalization. Products made in one country are sold everywhere, a great bolster to world trade. The problem is, however, that each major trading block—notably Europe, Japan, and the United States—has enacted different regulations regarding the sale of foreign products. In many countries, regulations remain set in rigidly outdated views instead of keeping up with strides forward. Rules for establishing safety and efficacy are also different. These thorny matters are also given serious attention in this volume, which leaves no pertinent area outside the scope of product development.

Another recent development is the intensified awareness of the importance of appearance in enhancing the quality of life. Numerous studies by sociologists and psychologists have demonstrated that those who are deemed attractive fare better in all human interactions. The unattractive are at a disadvantage at every stage of the life cycle.

From time immemorial, hair has been celebrated as the “crowning glory” of human beauty. Modern industry has been especially ingenious and innovative in creating products designed for the scalp and hair, not only for beautification but also to provide protection against disease and to maintain a healthy condition. The topic of scalp and hair care warrants even greater emphasis in view of the epidemiological fact that the population is aging rapidly. Mean life expectancy in developed countries has increased from about 65 in 1965 to about 75 in the year 2000, more so for women than for men. People over 85 are now the fastest growing segment of the population. It is a fact of life that no one gets prettier or healthier with aging. This is nowhere more apparent, even to the casual observer, than on the status of hair, which inevitably becomes grayer, sparser, less voluminous and less groomable. These changes are especially disagreeable to women whose self-esteem and social confidence are threatened by these regressive changes. Great credit is due to the manufacturers of hair and scalp care products that these losses and degradations are now largely concealable and ameliorable by a host of novel products that bring satisfaction and pleasure to the elderly.

Zviak anticipated how appearance could adversely affect one's psycho-social well-being by stating, “people seek advice not only for pathologic conditions but for regular hair care. Physicians should have a basic knowledge of scalp and hygiene products, of the composition of shampoos and products to change the appearance, feel, color and shape of hair, the treatment of dandruff and hair loss.” The complementary convergent activities of physicians and cosmetologists have never been greater.

The text serves the all-important function of making knowledge of the scalp and hair accessible to physicians, pharmacists, dermatologists, trichologists, beauticians, and cosmetic chemists. Having this text at hand is an absolute necessity if this motley group of professionals wish to stay au courant.

Finally, it is appropriate to point out that French scientists have played a prominent role in relation to scalp and hair conditions, going back centuries to that incomparable first chief de clinic of the Hospital St. Louis in Paris. In his famous “Tree of Dermatoses,” Jean-Louis Alibert vividly described and graphically depicted various disorders of the scalp and hair. Sabouraud was a great mycologist who described the tinea (ringworms). Few are aware that he also published five books on the scalp and hair, further validating ownership of this domain by French scientists.

It is noteworthy that this edition is the fruit of a French-British collaboration ensuring that the knowledge conveyed by the internationally authoritative contributors to this text will be available to every professional who is practicing the science and art of the care of the scalp and hair.

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Preface to the Second Edition

Fifteen years have elapsed since Charles Zviak passed away. During his time as CEO of L'Oréal Co., as an innovative, incentive scientist and a generous personality, C.Zviak was rewarded by the respect and consideration of all who came into contact with him.

The new edition of this book is intended to pay homage to the man who had the original idea and was the expert who put together the first edition. It also expresses still vibrant recognition of an exceptional man to whom the field of cosmetology owes much. He was indeed an ardent pioneer of cosmetic science and if this has gained respectability it is, for a large part, thanks to his tireless efforts.

But C.Zviak was also an outright militant of the partnership with dermatology: From the moment that he entered the field of applied research and development of cosmetic products, he took pains to forge close links with the field of dermatology, profoundly conscious of the fact that cosmetic science and dermatological science had to go hand in hand and nourish each other. His idea for the original book was founded on his belief in this concept: a special relationship develops quite naturally between those who share the same field of investigation, in the continuum of normal to diseased. The ambition behind this new edition is to continue the mission that C.Zviak entrusted to us—that of producing a reference book, embracing all aspects of scientific and technical knowledge at its most advanced in a perspective resolutely directed toward the future.

Hair develops within the most prolific cellular community of the organism. Its production (12 km of fiber per annum per head of hair, on normal growth) is governed by extremely complex control systems that researchers struggle to decipher, as they do in the case of the mechanisms that regulate the rhythm of its cycle and renewal. Since the first edition, hair and hair-care science have benefited from considerable advances thanks to the accelerated development of various technologies and methods of investigation. C.Zviak held that only progress in understanding could form the basis of and give rise to true innovation in terms of products. It is obvious that he would be fascinated, today, by the almost daily progress of research. He would be amazed by the extraordinary palette of product textures and exploits in all aspects of hair care and beauty, matching the covert expectations of the user. These advances have been accompanied by worldwide internationalization which has enlarged our vision of hair in various ethnic groups, stimulating the search for the most appropriate answers to specific needs.

These changes and steps forward obviously owe much to scientific knowledge and product design as well as consumers' attitudes or habits. Demands are now made by every generation, for the life exhibited by a head of hair is closely linked to the desire for self-expression, and contributes in a pre-eminent fashion to the relationships of the individual with others. With the prolongation of life expectancy, hair care has become an important element in sustaining social relationships and in self-esteem.

The development of new cosmetic products does of course depend upon the conjugation of art and science to arrive at a knowledgeable orchestration that will afford both comfort and pleasure to the user with all the magic of a transformed hairstyle. It

benefits from technological progress but it is also dependent upon the breakthroughs achieved in the assessment of hair and scalp condition and product efficacy, the exact identification of impairment and measurement of performance using instruments capable of detecting and quantifying things that were previously only open to sensory evaluation and not measurable.

Another field that has benefited from marked innovation is that of safety evaluation, with the progressive availability of alternative methods freeing the process ever more from the use of animal testing. In this case, it is clear that innovation has often filled the gap between the desirable and the practicable. The regulations have also seen great changes, with the opening of frontiers between States implying the progressive lifting of obstacles to commercial exchange and the movement toward harmonization of legislation. This new edition is the fruit of the motivation of highly qualified specialists, each of whom, in their domain, has made their expertise available so that this new edition lives up to the first one, which had made its mark due to the richness, novelty and pertinence of the information it contained. Every one of these contributors must be saluted for their efforts to express a clear and up-to-date perspective and awareness of their field. At this time, Professor Pierre Agache, faithful friend of Charles Zviak, is particularly remembered following his tragic death a few months before the completion of this edition. We hope that C.Zviak would have been proud of this new edition and that the reader will find it of great value. This will be the reward that would please us the most, in homage to a scientist and humanist who devoted his life to the cosmetics industry and who gave it its true legitimacy together with his warm human attention.

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Preface to the First Edition

In editing this book on scalp and hair I hope to promote a number of meeting points and closer associations.

The fields of cosmetology and dermatology have different objectives, but are interrelated and in many ways complementary.

The cosmetologist focuses his interest on hygiene, beauty enhancement, and care; whereas the dermatologist is concerned with therapy and has the primary purpose of preventing and curing disease of the skin and its appendages. Yet, in the final analysis, beauty and health spring from the same need, inherent in both women and men who are conscious of the appearance of their own body, skin, and hair.

Cosmetology, and hair cosmetology in particular, has become a full-fledged science based on several disciplines: chemistry, biology, pharmacy, biophysics, medicine and so on. It embraces the study of healthy skin, product development, and the evaluation of product effects on healthy skin, scalp, and hair.

Dermatological research is primarily oriented toward diseases of the skin and scalp, and the development of medication for their prevention and cure. Basic research is often carried out on healthy skin, its normal physiologic mechanisms, and the possible causes of disorder.

A convergence of interest is clearly perceptible. Coexistence and interpenetration are current within the same research units between laboratories studying healthy or diseased skin, normal and abnormal appendages, cosmetics and dermatological medication.

Purely dermatological research may move into the cosmetic field, while in-depth research may result in therapeutic applications.

In the area of legislation the borderline between cosmetology and dermatology is often blurred: there are often no precise definitions. Despite the fact that countries agree on terms describing hygiene and beauty products, wide consensus regarding the definition of a drug, and the distinction between a cosmetic and a medical product, there remains a "no man's land" of products whose intended use verges on the esthetic and therapeutic. This is especially true of many hair products.

In this blurred area the legislator has attempted to define products and set out legal restrictions. In the United States the use of "Over the Counter" (OTC) products is subject to special regulation. In France advertisements for cosmetics "beneficial for health" must be approved by the Ministry of Health. This illustrates that legal ambiguities exist, and that there is a continuum between cosmetics and drugs.

Product safety is an essential consideration. There are important differences between drugs and beauty products, due to their nature, composition, and intended use. A beauty product is aimed at external action, whereas a medical product may cross the cutaneous barrier and act internally. Safety tests may therefore differ fundamentally. Assessing therapeutic risk is accepted for medication, but the rule for a cosmetic in normal use conditions, is to achieve, if not total, at least a maximum level of harmlessness.

Therapeutic risks imply the occurrence of disadvantages and adverse reactions, which cosmetic products should attempt to avoid. Cosmetologists should be aware that new ingredients and formulations may cause unforeseen effects with which the dermatologist may have to deal. Safety is therefore a domain shared between cosmetology and dermatology.

The close relationship between appearance and the psyche has been emphasized by many eminent authors. Dermatology contributes greatly to psychosomatic medicine, the importance of which is constantly increasing. Cosmetology, given its esthetic motivation, has no lesser part.

The cosmetologist should be familiar with and keep abreast of dermatological problems. The dermatologist can no longer ignore cosmetology. I have attempted to describe within the text the divergence and convergence of the two fields, for the benefit of all readers concerned with these matters in relation to the hair and scalp. There is nothing new in this endeavor. Twenty years ago, Doctor Sidi and I edited a book entitled *Problèmes Capillaires*. The need for this was already evident at that time. I should like to take this opportunity to render homage to my friend, the late Edwin Sidi, who passed away too soon. He was an eminent dermatologist who foresaw, almost before anyone else, the benefits to be gained from a symbiotic relationship between the fields of dermatology and hair cosmetology. Today, I feel that I am continuing the work in which he was so deeply engaged.

Charles Zviak
Former L'Oréal Chairman
and Chief Executive

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1

Hair Structure, Function, and Physicochemical Properties

A.Franbourg and F.Leroy

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Hair, the characteristic covering of mammalian skin, probably evolved from the epidermal scales of reptiles. Animals below man on the evolutionary ladder need hair of exact length, density and color to survive. Though human hair would appear to be vestigial, it is in fact still an important organ of touch and a part of normal sexual and physiological development.

Human hair is of the same nature as all epidermal appendages specific to all mammals including different coats or furs, wool, vibrissae and all other types of hair, feathers, nails, horns, hoofs, etc. Its original function is body protection against environmental factors (heat, cold, sunlight, dryness, etc.), injuries and impacts. It contributes to thermal regulation and is endowed with remarkable resistance and outstanding long-term stability and preservation of its structure.

The scalp differs from the skin covering the rest of the body in having an abundance of large hair follicles producing long, coarse hair fibers with large sebaceous glands attached to each follicle. In addition, the network of nerves and blood vessels intimately connected with the follicles is very elaborate.

Irrespective of their origin and body location, all the skin appendages referred to above have a certain number of common features. A major one is that they are essentially composed of a specific class of proteins, the keratins, which are also a major constituent of the horny layer, the outermost part of the integument that protects the internal body from its environment. Keratins are characterized by a high cysteine content, a unique sulfur-containing amino acid able to establish cross-linking between protein chains via disulfide linkages. Moreover, some keratins can take on a spatial arrangement to form crystal-like structures.

The hair shaft is formed of keratinized cells, containing highly organized material whose orientation and biochemical structure are designed to provide the fiber with remarkable resistance to various environmental constraints and attacks, such as friction, tension, flexion, UV radiation, and chemical insult.

The hair has the appearance of an extremely elongated cylinder. Its length varies greatly from one individual to another. Its average maximal length is generally considered to be 1–11/2 m. Some exceptional cases have been reported with hair length of several metres: the longest recorded hair is that of Swami Pandarasannadhi, an Indian monk, reported in 1949 to be 26 feet in length (1). By comparison, its diameter is 20,000 times smaller, but can differ greatly from one hair to another since it can vary from ca. 40 to 120 µm (2).

This great variability is linked to numerous factors among which are age (3), race (4–7), and even the area of the scalp (8). The cross-section of hair helps to explain the great differences: it is in fact far from being circular; it is more of an ellipsis, flattened to a certain extent, but a great variety of forms have been observed (Fig. 1). The shape of the hair is generally related to the section of the fibers from which it is formed: cylindrical hair develops straight fibers (as in the case of Asian hair) while very elliptical or “bean” shaped forms lead to curly or frizzy hair (African hair) (9,10).

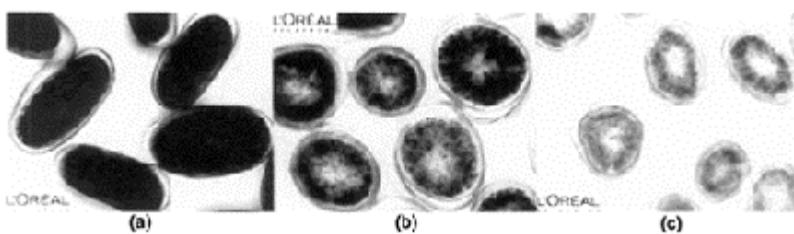


Figure 1 Cross-sectional view of hair from the three major ethnic groups: (a) African hair; (b) Asian hair; (c) Caucasian hair.

1. STRUCTURE AND COMPOSITION

1.1. Morphology

Studies have shown (7,10) that frizzy hair rolls itself around the small axis of the section. In the case of wool, the frizz is explained by the different sub-structures within the fiber (ortho- and paracortical cells). In human hair, the existence of such cells is still under debate (11,12) and the only explanation for the degree of curvature seems to be one of genetic morphology imprinted right from the follicular stage (9,13).

Ethnic origin has the greatest visible influence on the morphology of the hair. African and Asian hair have a cross-section markedly larger than that of European hair (Table 1). Moreover, Asian hair is characterized by a more circular section than the hair of other races, whilst, on the contrary, African hair is the most elliptical (10).

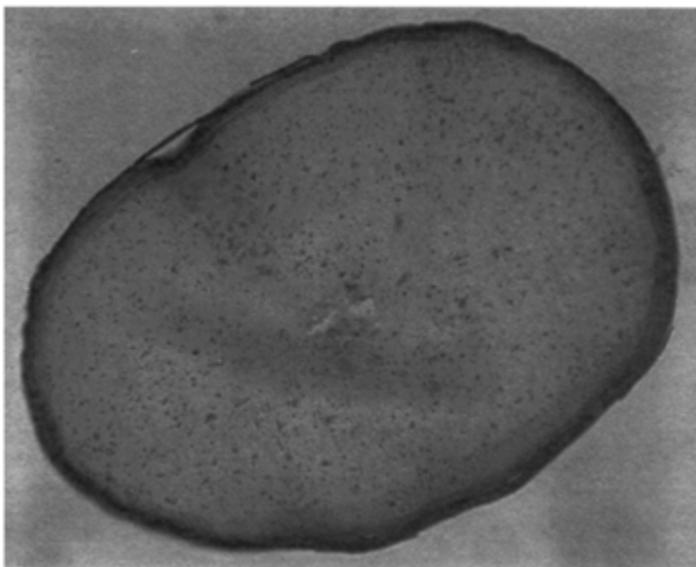
1.2. Hair Structure

The hair shaft is composed of three separate regions (Figs. 2 and 3). The cuticle forms a thick sheath by the superimposition of several cell-like scales. It tightly encircles the cortex that constitutes the most voluminous part and the heart of the fiber. In the cortex are located the fibrous proteins characteristic of hair, the α -keratins. The third zone, the medulla or marrow, is found close to the center of the hair.

Table 1 Influence of Ethnic Origin on Human Hair Shape

	Racial group		
	Caucasian	Asian	African
Diameter (μm)	30 to 100	35 to 125	45 to 120
(Thickness)	(Fine to moderate)	(Moderate to thick)	(Moderate to thick)
Ellipsicity	1.35	1.25	1.75

(From Ref. 10.)

**Figure 2** Cross-section of human hair seen under an optical microscope.

In general, the medulla is discontinuous, and may be entirely absent (14). The natural color of the hair is provided by the melanin pigments distributed in the cortex (Fig. 4).

1.2.1. Cuticle

At the follicular level, a single layer of cells gives rise to the cuticle. Starting out cuboid in shape, the cells flatten as they ascend from the follicle. At the same time they curve towards the top and, when keratinization is complete, they cover each other like

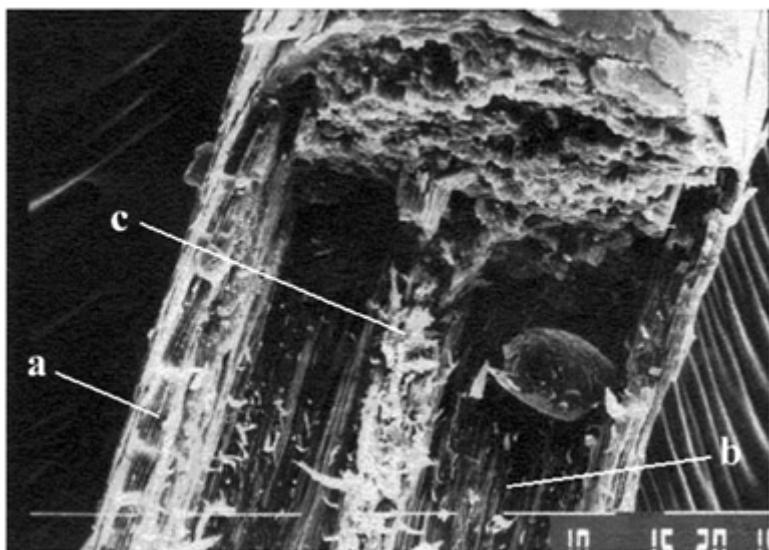


Figure 3 Scanning electron micrograph of broken end of hair showing the internal structure of the fiber: (a) cuticle cells; (b) cortical cells and macrofibrils; (c) medulla.

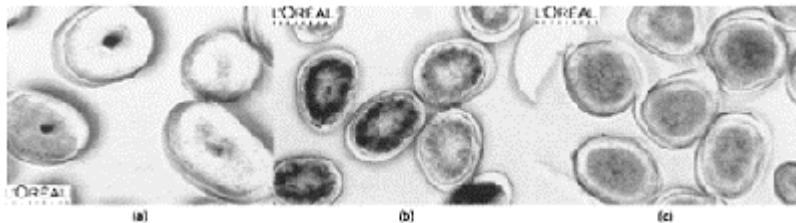


Figure 4 Different hair colors: (a) Scandinavian blonde; (b) European light brown; (c) red hair.

roof tiles (Fig. 5a), the external edge of the cells being directed towards the hair end. Each cell is approximately $0.5\text{ }\mu\text{m}$ thick and $45\text{ }\mu\text{m}$ long. The considerable coverage of these cells means that the cuticle of human hair has, at its root, between 7 and 10 superimposed layers (15).

The cuticle makes an important contribution to many of the physical properties of the hair. As the outermost part of hair, it governs its specific surface properties and it acts as a barrier against external aggressions, i.e., water penetration (16). It is

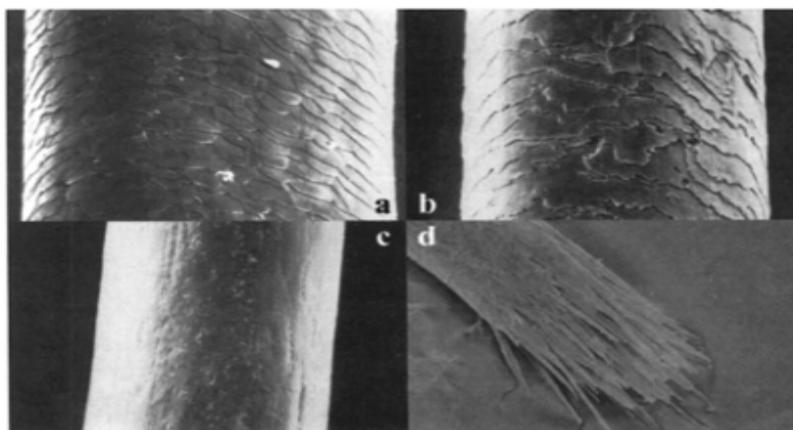


Figure 5 Scanning electron micrographs of one virgin hair fiber from root to tip: (a) root (emergence from the scalp); (b) 10 cm from the scalp; (c) 40 cm from the scalp; (d) tip end showing fibrillation (split end).

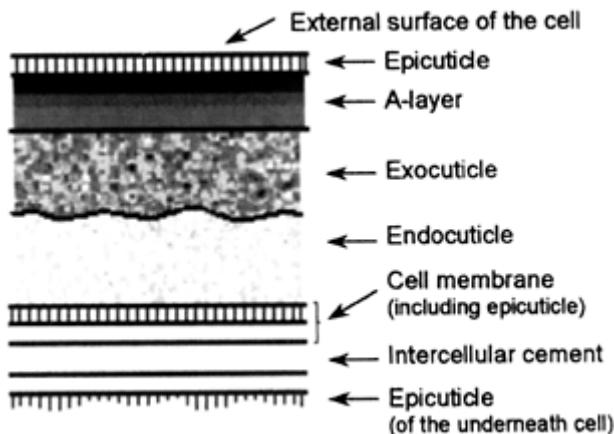


Figure 6 Diagram of cuticle cell structure.

also suspected that it plays an important role in the mechanical properties of the fiber, especially bending properties (17). It is also the sheath which constrains the cortical cells and allows the fiber to keep its mechanical integrity. This is clearly seen when one looks at the evolution of the cuticle along a fiber, and at its consequences. As they emerge from

the scalp, the flat cells of the cuticle have smooth, regular, well-defined edges and they are closely glued to each other (Fig. 5a). Very soon, however, degradation is observed: erosion, breakage of cell edges, and lifting of scales (Fig. 5b), with the appearance becoming jagged, mainly due to regular brushing combined with the action of solar radiation. The tight, enfolding wall formed by the cuticle deteriorates and progressively thins until only two or three superimposed cell layers remain. In the case of long hair, this degradation can culminate in the total disappearance of the cuticle (Fig. 5c). The cells of the cortex whose cohesion is no longer maintained by the cuticle sheath break up, leading to the phenomenon of split ends (Fig. 5d).

The cuticle cell (Fig. 6) is covered with a fine membrane, the epicuticle. Beneath this membrane, the cell is composed of three layers clearly visible under transmission electron microscopy: successively the A-layer, the exocuticle, and the endocuticle (Fig. 7).

The Epicuticle. This is a very thin membrane ca. 50–70 Å in thickness (18). It was first detected by Allworden (19) who observed that bubbles formed on the surface of wool treated with hypochlorite solutions. Covering each cell, the epicuticle consists of a proteinaceous layer covered by a lipid layer covalently bound by thioester linkages (18,20–23) (Fig. 8). These lipids are fatty acids, with methyl eicosanoic acid (18-MEA) being the main constituent. The epicuticle is thought to play an important role in determining the surface properties of hair, both the physical (friction) and the chemical properties (it acts like a semi-permeable membrane) (24).

The A-Layer and the Exocuticle. Underlying and contiguous to the epicuticle, the A-layer is a region composed of proteins particularly rich in sulfur. The cystine content of the A-layer is over 30%, and around 15% in the exocuticle (25,26). This particular composition suggests that the proteins belong to the high sulfur (HS) and ultra high sulfur (UHS) groups of keratin associated proteins (KAP), similar to those composing the matrix in the cortex (see below) (27). The high content of cystine in

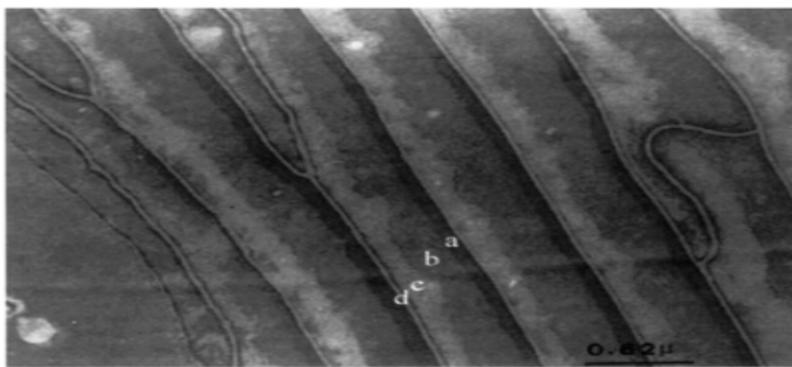


Figure 7 Transmission electron micrograph of the cuticle. (a) A layer; (b) exocuticle; (c) endocuticle; (d) cell membrane complex.

the A-layer and the exocuticle and the presence of iso-peptide bonds in the A-layer (23,28) suggest that the proteins in these regions of the cuticle cells are strongly cross-linked. This part of the cuticle is very poorly soluble and is particularly resistant to chemical attack. It swells little in water, even if some evidence of penetration of water in this area has recently been observed (29). Such a dense structure is predicted to have a very high elasticity modulus (slope in the first linear part of the stress/strain curve), which has been confirmed by nanomechanical experiments using atomic force microscopy (AFM) (30,31); but it breaks at lower strain levels.

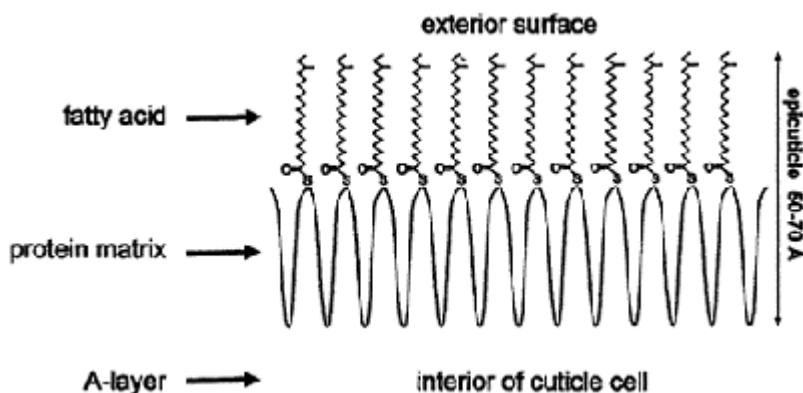


Figure 8 Suggested model for the epicuticle. (From Ref. 16.)

The Endocuticle. This area, at the interface of the internal part of the hair, or cortex, is composed mainly of the remains of cell organelles. It consists of proteins that, unlike those found in the other compartments of the fiber, have a very low sulfur content. Weakly cross-linked, they form a very porous structure readily accessible to water: AFM observations of cuticle scales (32) show a dramatic increase in their thickness in water which is probably accommodated by swelling of the endocuticle. It should also be a very soft and deformable structure.

1.2.2. Cortex

Surrounded by the cuticle, the cortex represents about 80% of the hair and constitutes the core of the fiber on which its fundamental properties are grounded. It is formed of cells themselves made of proteinaceous material with a high level of organization (Fig. 9). It is this particular structure and notably the preferential orientation along the fiber axis of the subunits composing the cortical cells that confer on the hair its unique mechanical properties.

The Cortical Cells. The cortical cells are quite varied in shape and size. They are all spindle shaped, aligned along the main axis of the fibers and, although huge variations in their dimensions are described (33), their length is of the order of 100 μm with a diameter

of 2–5 µm. In the case of wool, several types of cortical cells have been described, ortho-, para- and mesocortical cells (34,35). Each type of cell shows a specific protein

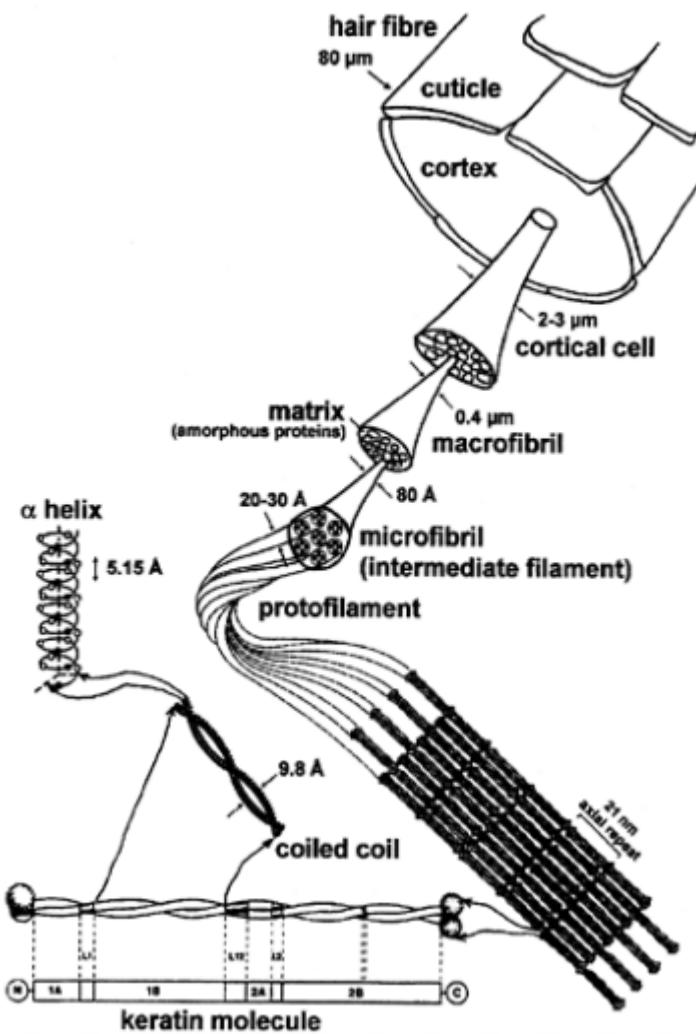


Figure 9 Diagram showing the structure the cortex. (From Ref. 10.)

composition, the orthocortical cells having a lower sulfur content than the paracortical cells (36). Their distribution through the cortex is asymmetrical, and it has been demonstrated that the level of wool curliness is closely related to the amount of paracortical cells found in the fibers (37). In human hair, the existence of different cortical cells is unclear. Mazurkiewicz and Phillips (11) and Mercer (34) considered that

human hair only contained paracortical cells. However, Swift (12), more recently, observed orthocortical cells in curly hair, suggesting that, like in wool, the degree of curliness of human hair might be related to the proportion and distribution of orthocortical cells. Kassenbeck (36) has shown that some cortical cells located on the outermost part of the cortex have a specific shape. He did not consider them as paracortical cells, however, but called them heterotype cortical cells.

The cortical cells are formed mainly of macrofibrils separated by thin membranes probably consisting of the remains of the initial cytoplasmic content of cells. These membranes also contain melanin pigments, variably dispersed, and nucleic residues (Fig. 10).

The Macrofibrils. The macrofibrils are little rods, aligned along the axis of the hair, a few microns in length and between 0.1 and 0.4 μm in diameter (38). They are organized like a perfectly orientated fibrillar composite and made of intermediate filaments (IF) previously called microfibrils, highly structured protein regions, set in amorphous protein matter, the matrix. In human hair, the mass fraction of the matrix is about 40% (39). Electron microscopy images (Fig. 11) do not clearly show any regular distribution of the IF, even if a local hexagonal order can be seen in some regions, and it had been assumed that microfibrils were randomly distributed within the matrix. Fraser et al. (40) suggested that they were hexagonally arranged. Modeling of small-angle X-ray diffraction diagrams has shown that the microfibrils are in fact more or less regularly organized in a pseudo-hexagonal array (38,41). In wool, this organization is more easily seen in mesocortical cells (42,43) than in ortho- or paracortical cells (38).

Intermediate Filaments and the α -Structure of Keratins. A complete description of the structure and formation of IF, and especially keratin microfibrils was published by Parry and Steinert (44).

A common feature of all hard-keratin fibers, whether of human hair, wool, other mammalian coats or even porcupine quills, is their X-ray diffraction diagrams (45) (Fig. 12a, b). The diagrams are complex as a result of the crystallization and association of type I and type II keratin chains (see Chemical composition section) to form the highly structured entities called microfibrils or IF.

The formation of IF can be described as a four-step process:

- a. the formation of α -helices,
- b. the association of one type I and one type II keratin to form a dimer,
- c. the aggregation of two dimers to form a tetramer,
- d. the formation of a pseudo-hexagonal structure (the IF) by the association of seven or eight tetramers.

An X-ray micro-diffraction study (46) has shown that the α -structure of keratins and the formation of dimers is produced at an early stage in the process

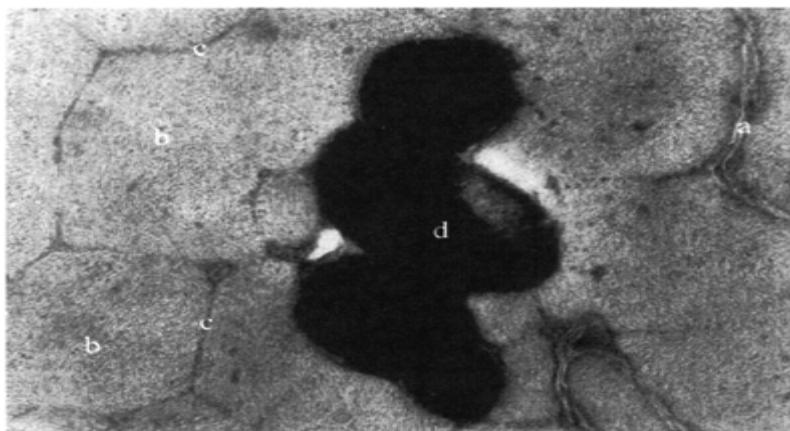


Figure 10 Transmission electron micrograph of a transversal section of a cortical cell: (a) cell membrane complex; (b) macrofibrils; (c) intermacrofibrillar spaces; (d) melanin pigments.

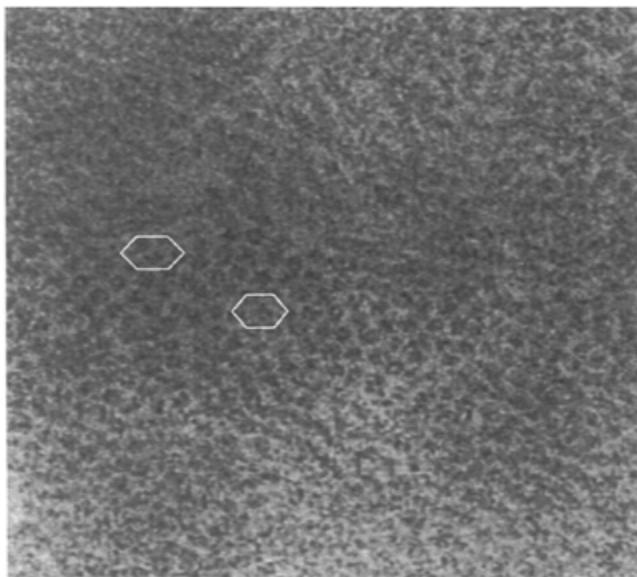
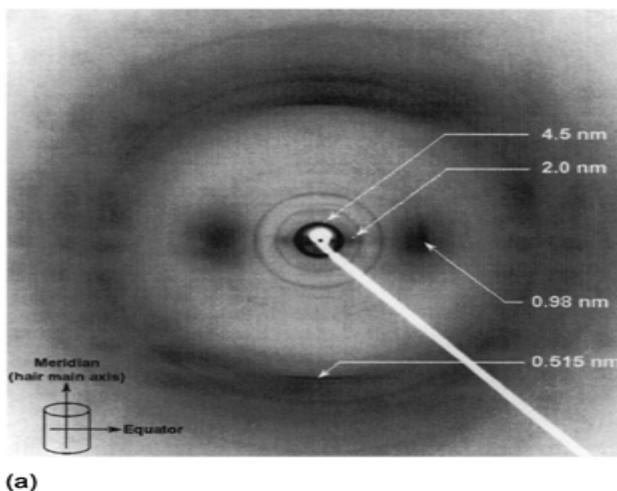
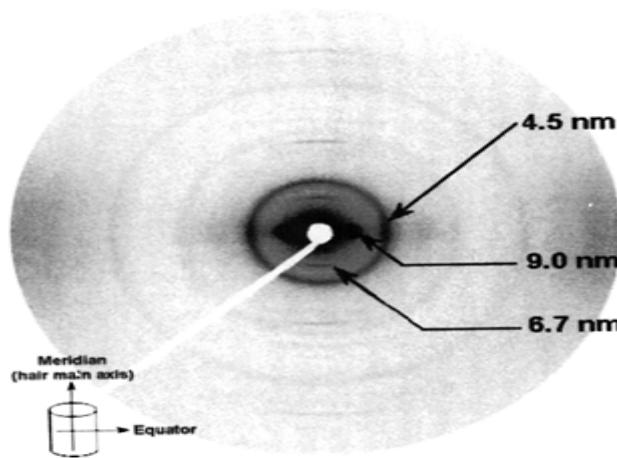


Figure 11 High resolution transmission electron micrograph of a macrofibril showing the local pseudo-

hexagonal array of IF. A ring+core structure of the IF can be seen for some of them.



(a)



(b)

Figure 12 (a) Wide angle X ray scattering (WAXS) diagram of a human hair. The meridional reflection at 0.515 nm is the axial projections along the coiled coil axis of the α -helix. The 0.98 nm equatorial spots

correspond to the distance between the two strands in a coiled coil. The 2.0 nm reflection corresponds to the distance between protofilaments in a microfibril. The 4.5 nm ring is attributed to lipid bilayers. (b) Small angle X ray scattering (SAXS) diagram of a human hair. The 6.7 nm reflection corresponds to the projection of the axial distance between successive lattice points. The 9.0 nm is the center to center distance between microfibrils.

of keratinization, in the lower part of the hair follicle. IF aggregation, the final step of maturation in building hard α -keratin, only takes place at the upper end of the follicle.

The α -helix was first described by Pauling et al. (47) (Fig. 13). This helix contains 3.7 amino-acid residues per turn and is stabilized by the formation of hydrogen bonds between the N–H and C=O groups (from peptidic bonds) of the n and $n+4$ amino acid of the sequence, respectively. The characteristic spots observed on the diffraction diagrams (Fig. 12a) correspond to the distance between the centers of two helices (equatorial repetition, perpendicular to the fiber axis, of 9.8 Å), the distance between two consecutive amino acids of the chain length sequence and the helix pitch (meridian repetition, parallel to the fiber axis, of 1.5 and 5.15 Å, respectively) (48,49).

The theoretical pitch of such a helix is 5.4 Å. To account for the shorter measured distance (5.15 Å), Crick (50) proposed a coiling of two helices. Crewther et al. (51) suggested that each dimer was made by the association of one type I (with acidic side chains) and one type II (with neutral or basic side chains) keratin chains (heterodimer) in parallel configuration (Fig. 14). Each chain contains N- and C-endings with a rod domain in between consisting of four segments (1A, 1B, 2A, 2B) and three linker regions between the segments (L1, L12, L2). In the four segments, the amino acids are arranged in a heptad pattern of the form (a–b–c–d–e– f–g)_n. Amino acids a and d have an apolar side chain, e.g. leucine, isoleucine, valine, whilst e and g bear acidic or basic groups. The stabilization of the coiled-coil structure is ensured by hydrophobic interactions between the apolar parts of a and d, facing each other along the axis of the coiled-coil, and by ionic interactions between polar side groups of e and g (Fig. 15).

A stable intermediate was however isolated by Ahmadi and Speakman (52) from wool microfibrils and found to consist of four coiled keratin chains. On theoretical grounds, Crewther et al. (51) suggested that two dimers aggregated to form a tetramer (or protofilament). These tetramers are stabilized by intermolecular ionic interactions. Crewther described four possible modes of association (Fig. 16) which were later confirmed (53):

a. Dimer.

- b. A₁₁: Antiparallel association with overlap of 1B sections.
- c. A₂₂: Antiparallel association with overlap of 2B sections.
- d. A₁₂: Antiparallel association with almost complete overlap.
- e. A_{CN}: Parallel association with a slight head-to-tail overlap.

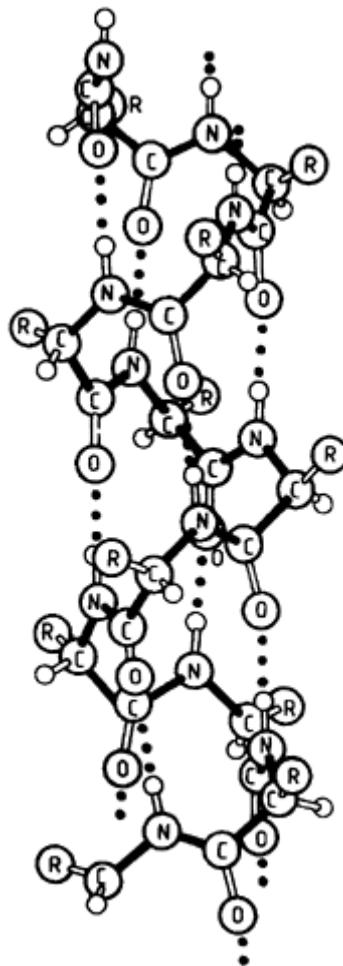


Figure 13 α -Helical arrangement of keratin.

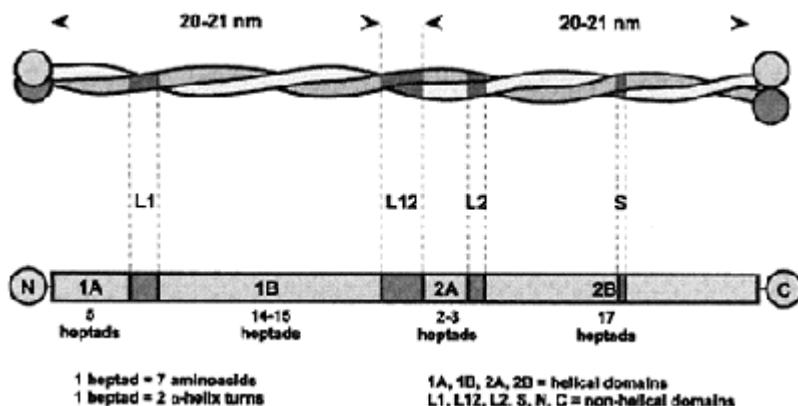


Figure 14 α -keratin heterodimer.
Breaks in the coiled-coil structure
occur in the N- and C-terminal regions,
at the linkers L1, L2 and L12, and at
the stutter S at the center of the
helicoidal 2B domain.

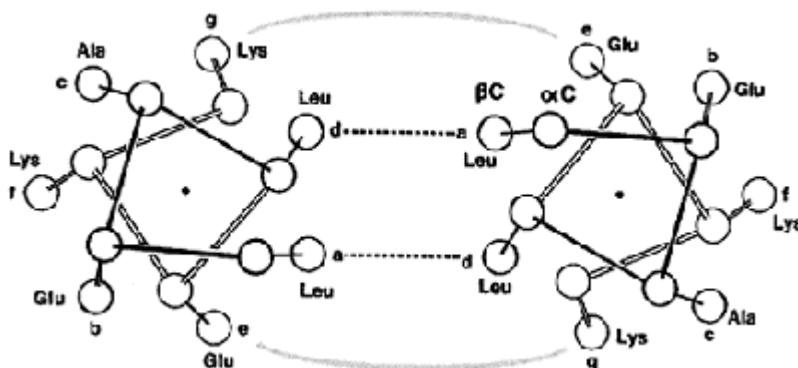


Figure 15 Interactions of α -helices to
form a coiled-coil: apolar interactions
between residues *a* and *d*, electrostatic
interactions between residues *e* and *g*.

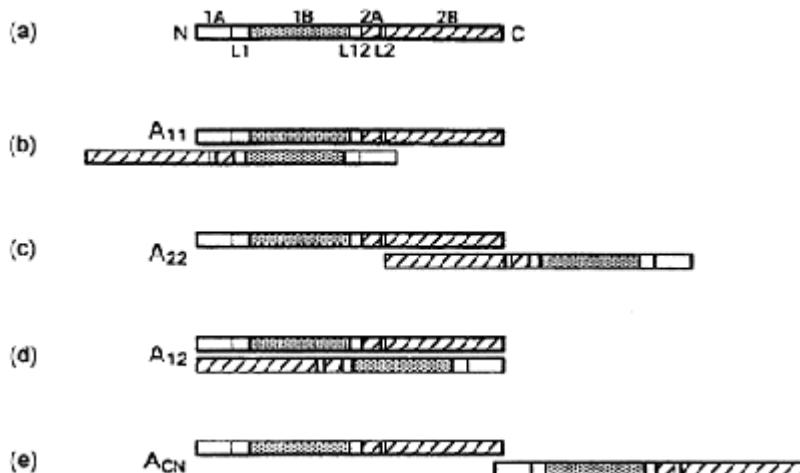


Figure 16 The four different modes of interactions between two IF keratin molecules: (a) the single molecule (heterodimer); (b) the A₁₁ mode; (c) the A₂₂ mode; (d) the A₁₂ mode; (e) the A_{CN} mode. (From Ref. 60.)

To interpret the small-angle X-ray diffraction diagrams, and especially the 6.7 nm meridional and the 2.2 nm equatorial reflections (Fig. 12a, b), Fraser et al. (54–57) built a theoretical model of the IF structure, the surface lattice model, in which seven or eight tetramers are bundled in a helical way with an axial translation of 19.8 nm albeit being affected by a periodic distortion thought to be related to a dislocation along the helical axis (Fig. 17).

Modeling low-angle X-ray diffraction pattern of IF indicates that such a structure could be stabilized by the formation of intra- and inter-protofilament disulfide linkages (58,59). The real 3D structure of the IF would then be obtained by the folding up of this 2D array. There are several other possibilities such as ring or ring+core arrangements, which are still being debated (41,58,60). The exact 3D structure of IF therefore remains unknown.

An important observation is that the structure of the IF keratin is remarkably constant. It is identical in wool, horse hair,...and even porcupine quill. In human hair, there is absolutely no difference between X-ray diffraction diagrams of hair with various degrees of curliness or of different ethnic origins (10). The stability of such a structure is also exceptional: hair collected from pharaoh Ramses II's mummy, more than 3500 years old, exhibits exactly the same X-ray diffraction pattern as current hair (61).

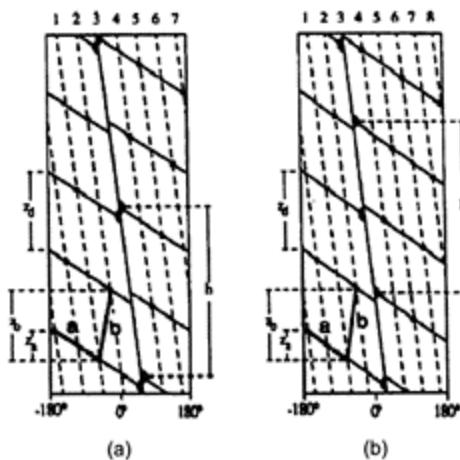


Figure 17 The surface lattice model of hard α -keratin IF based on: (a) seven and (b) eight protofilaments. Each dotted line represents a protofilament, and the full line a discontinuity (dislocation). The axial repeat (h) is 47 nm, and z_a (7.42 nm) and z_b (19.79 nm) are the axial distance between successive lattice points and the axial stagger between adjacent protofilaments, respectively. (from Ref. 57.)

The Matrix. The IF are embedded in the matrix. It is made of different classes of proteins called KAP which, unlike type I and type II keratins of the IF, cannot form crystallized structures, and are therefore often considered as amorphous, even though some specific structural features have been observed (62). The estimates of the relative percentage of matrix proteins in the cortex are very variable, but it is generally considered that about 60% of cortex proteins are KAP while 40% are IF (63).

The KAP are mainly high sulfur and high glycine-tyrosine (HGT) proteins. Despite their high cystine content, their mechanical and physicochemical behavior, and especially water swelling, do not resemble those of a highly cross-linked polymer. In fact, the matrix behaves more like a cross-linked hydrophilic gel (64), and the majority of disulfide bonds are thought to be intramolecular rather than intermolecular (65).

The matrix plays an essential role by binding together keratin IF, thus providing the hair with its structural stability. Matrix/IF interactions are mainly of the disulfide bond type but nothing is actually known about IF-matrix disulfide linkages.

The Intercellular Spaces. The intercellular spaces, also called cell membrane complex (CMC), probably consist of the remains of the original cell membranes. Their role is essential in that they assure cell cohesion in both the cuticle and the cortex, probably thanks to adhesion proteins. Furthermore, several studies have shown that the spaces are preferred routes of penetration and diffusion of substances within

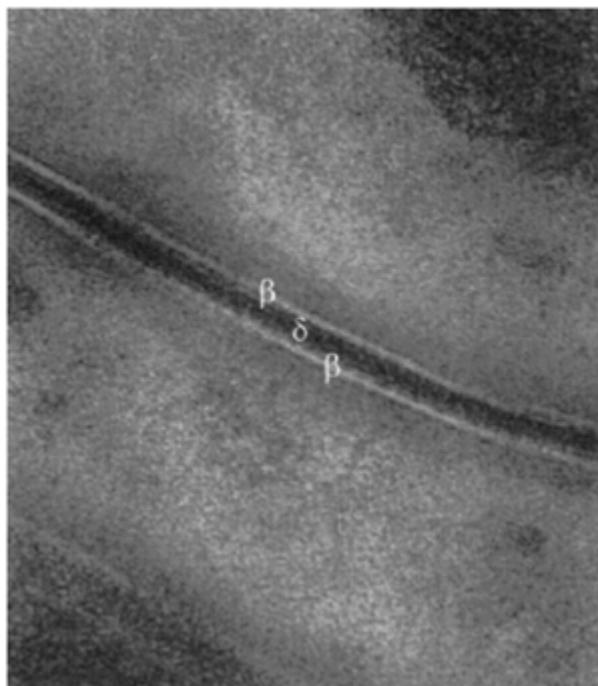


Figure 18 Transmission electron micrograph of the cuticle illustrating the leaflet structure of the intercellular space.

hair (29,66,67). The precise structure of these spaces is yet to be fully elucidated. Numerous electron microscope studies, however, have shown a leaflet structure (Figs. 18 and 19). An intermediate layer, called the intercellular cement or δ layer, is composed mainly of proteins with a low sulfur content but rich in acidic and basic amino acids and hence hydrophilic (68). Its thickness is of the order of 100–150 Å. It is surrounded by two layers called β -layers, circa 50 Å thick, essentially composed of highly cross-linked proteins and lipids (fatty acids and polar lipids) (20) of which some form part of the epicuticle.

Some authors suggest, however, that the composition of these spaces may be different in the cortex and cuticle (20).

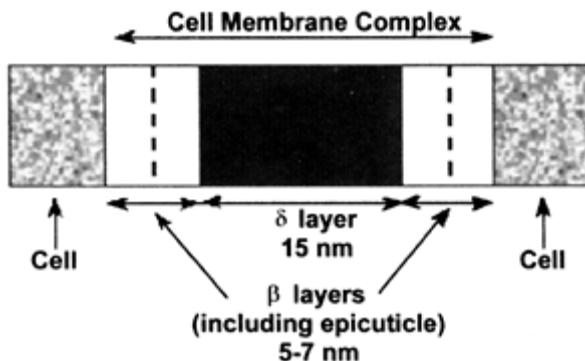


Figure 19 Schemating drawing of the CMC.

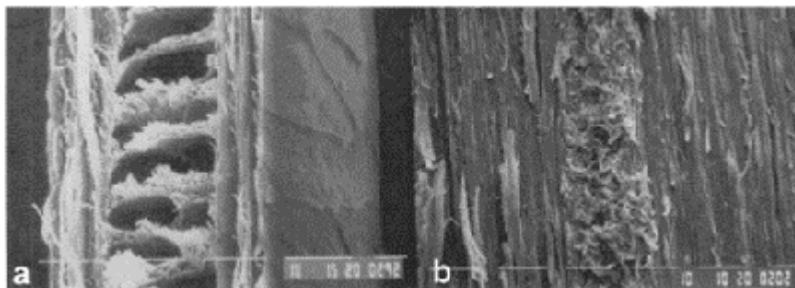


Figure 20 SEM pictures of longitudinal sections of mammals' hair illustrating the huge differences in the relative volume of the medulla: (a) cat hair; (b) human hair.

1.2.3. Medulla

In the fur of many mammals, the medulla forms the major part of the fiber. In the case of human hair, it is often completely absent, and if present, it is intermittent and takes up a modest share of the fiber (Fig. 20).

The medulla is formed of various cells, piled up in a loose fashion and leaving large empty spaces between them. It is likely that in the case of animals, the medulla plays a thermal insulating role. In the case of human hair, it has been little studied because it is assumed that its contribution to the various properties of fibers is negligible (69), although a surge of interest has occurred more recently, mainly in view of its involvement in the optical properties of hair (70).

1.3. Chemical Composition

Like all biological tissues, the hair is mainly made of proteins, and more specifically of keratins, a unique type of proteins characterized by a very high content of sulfur amino acid (around 15% cystine). Lipids are also found, as are various other elements and water. The composition varies in the different morphological compartments of the hair, and this applies especially to the nature of keratins.

1.3.1. The Proteins

Proteins are macromolecules resulting from the polycondensation of various amino acids (Fig. 21).

A large number of studies have been devoted to the analysis of amino acids in human hair (7,71–73). This analysis is generally carried out after acid hydrolysis of

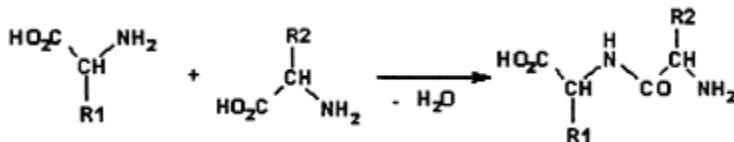


Figure 21 Condensation of aminoacids.

the hair, although this method leads to the destruction of some amino acids namely tryptophan. The mean amino acid content is shown in Table 2.

The amino acid sequence and hence the precise composition of the various proteins are genetically determined. No difference in amino acid composition has yet been detected in relation to the ethnic origin of hair (10).

The main chemical characteristic of keratins is their high cystine level that leads to a high rate of cross-linking through disulfide bonds (Fig. 21). This common feature is associated with a great diversity of types of protein, depending on their location in the hair (cuticle, cortex, medulla), and even in the substructures (endo- or exocuticle, IF, or matrix of the cortical cells). Several methods have been described to extract and isolate the different proteins of keratin fibers (74–76). The most widely used method, particularly for the study of cortex proteins, involves reducing the proteins with dithiothreitol in aqueous urea solutions (74). The reduced proteins are then reacted with iodoacetic acid (Fig. 22), in order to prevent the reoxidation of the thiol groups and to enhance water solubility of the proteins. Isolation is done either by selective precipitation or by gel electrophoresis (1D or 2D).

Medulla. Studying the medulla of the majority of keratin fibers is not simple (it is frequently absent, difficult to isolate and poorly soluble). The only thorough chemical analysis of medulla proteins of a fiber was carried out by Rogers on porcupine quill (77). He found that the medulla contained virtually no cystine, a low amount of hydroxy amino acids (serine, threonine) and relatively large amounts of basic or acidic amino acids (mainly lysine and glutamic acid).

Cuticle. The two main compartments of the cuticle (endo- and exocuticle) have very different chemical compositions. Data were obtained after extraction and separation by enzymatic digestion (26).

The endocuticle is believed to be composed of the nuclear and cytoplasmic remnants of the cells, which is consistent with the very low content of cysteine found (3–6%), and the large amount of hydrophilic amino acids (acidic and basic) (26).

On the contrary, proteins of the exocuticle are particularly rich in cysteine (78). The outermost A-layer contains over 30% cysteine (79), and the internal part of the exocuticle around 15–20% (26). Specific amino acids generally not found in α -helical proteins are also present (80). All these observations suggest that most of the proteins of the exocuticle belong to the class of HS and UHS proteins (81,82). It has been demonstrated that some HS and UHS proteins are expressed in the cuticle of human hair (83). However, other studies showed that some low sulfur keratins (IF keratins) were also expressed in the cuticle (84,85). The proteins of the exocuticle are characterized by the presence of significant amounts of isopeptide bonds [ε -amino-(γ -glutamyl) lysine] (23,28).

Cell Membrane Complex. If the β -layers of the CMC are part of the epicuticle of the cells and are supposed to be composed of lipids (see below), the central δ -layer contains proteins with a low amount of cysteine (12). It also contains polysaccharides, probably bound to proteins (glycoproteins) (86,87). The exact composition of the δ -layer, however, as well as its structure, is far from being fully understood.

Cortex. Fractionation of hair proteins originating essentially from the cortex leads to the identification of several different classes:

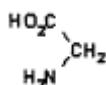
- IF keratins.
- HS KAP.
- UHS KAP.
- HGT KAP.

Table 2 Mean Amino Acid Composition of Human Hair

Amino acids

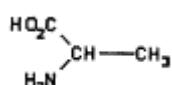
Acyclic

Glycine



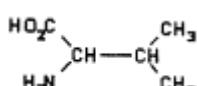
4.5–5.2%

Alanine



2.8–3.5%

Valine



5.0–5.8%

Leucine		6.4–6.9%
Isoleucine		2.3–2.5%
Aromatic		
Phenylalanine		2.2–2.8%
Tyrosine		2.1–2.7%
Heterocyclic		
Tryptophan		0.8–1.2%
Proline		7.0–7.8%
Hydroxylated		
Serine		9.6–10.8%
Threonine		6.5–7.5%
With an acid side chain		
Aspartic acid		5.6–6.5%
Glutamic acid		14.3–15.5%
With a basic side chain		
Lysine		2.6–3.1%

Arginine		8.8–9.6%
Histidine		0.8–1.1%
Sulfur containing		
Cysteine		
Cystine		14.0–16.5%
Cysteic acid		
Methionine		0.5–0.9%
Others		
Citrulline		

IF proteins are the constituents of the microfibrils, while KAP are the major component of the matrix. In human hair as in wool or other keratin fibers, the relative amounts of IF proteins and KAP seem to be very variable, but Dekio and Jidai (88) found that the ratio of IF proteins to KAP was a specific feature of the ethnic origin of the hair (Table 3).

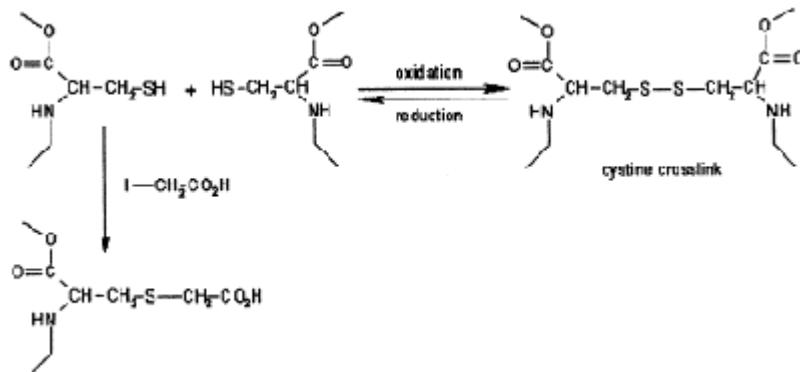


Figure 22 Formation of cystine crosslink, and labeling of sulphydril group of cystein with iodoacetic acid.

Table 3 Amount of IF and KAP Proteins in Human Hair from Different Races

	Caucasian	Asian	African
IF proteins	9.2±0.7	14.3±0.8	8.6±0.8
KAP proteins	31.9±1.3	32.9±2.7	46.0±2.7
IF/KAP	0.29±0.02	0.45±0.03	0.18±0.02

(From Ref. 88.)

The IF keratins. IF keratins, previously called “low sulfur keratins” (their cystine content is about 6%) belong to a class of proteins that can assemble to form filamentous structures (Table 4). They have in common a central domain of about 315 moieties with a very high degree of homology in their amino-acid sequence (89–92). The differentiation of these proteins arises from the size and chemical nature of their N- and C-ending domains.

Fifteen different IF keratins have been identified in human hair (84,85). They are classified into two types depending on their net charge: type Ia (acidic residues) and type IIa (neutral to basic residues). One type Ia and one type IIa are coiled together to form a dimer. This coiling is allowed by the specific heptad substructure of the four domains located in the central part of these proteins (see above). Parry and Steinert (44) gave an extensive description of the primary structure of this central area.

The low sulfur content of these keratins comes from the very low cystine content of the central domain (3%). In contrast, N- and C-endings have a higher cystine content (11–17%) (93). The presence of large amounts of cystine in these regions suggests that disulfide bonds between IF and the matrix are essentially located in the N- and C-ending areas of the IF. The complete sequencing of these areas shows characteristic repetitions of amino acids suggesting that some sections have secondary structures (α -helical in N-endings and β -sheets in C-endings) strongly interacting with the rod domain (94).

Table 4 IF Chain Classification

Type	Name	Approximate number of amino acids	Approximate molecular weight (kDa)
Ia	Hard α -keratin	410	47
Ib	Epidermal keratin	400–550	40–57
IIa	Hard α -keratin	500	54
IIb	Epidermal keratin	480–650	52–70
III	Desmin	380–670	42–53

	Vimentin		
	Glial acidic protein		
	Peripherin		
IV	α -Internexin	500	56
IV-L	Light neurofilament	545	62
IV-M	Medium neurofilament	880	102
IV-H	Heavy neurofilament	1050	115
V-A	Lamin A	570–665	63–73
V-B1	Lamin B1	585	67
V-B2	Lamin B2	600	68

(From Ref. 44.)

Dekio and Jidoi (95) found that the electrophoretic patterns of IF proteins extracted from hair of different ethnic origins were remarkably similar. They concluded that the IF proteins were similar in all types of hair.

The KAP. More recently unraveled, the KAP are less well-known than IF keratins. They are less easily approached as they do not include any well-defined spatial organization. They are classified into three types, depending on the amount of specific amino-acids:

- The High Sulfur (HS) proteins, containing around 20% cystine residues and having a very high molecular weight (50–75 kDa);
- The Ultra High Sulfur (UHS) proteins, with a higher content of cystine (30–40%) and a lower molecular weight (15–50 kDa);
- The High Glycine Tyrosine (HGT) proteins, containing large amounts of these two amino acids and having a low molecular weight (10 kDa).

Twenty-six different families of KAP have been described in human hair, but this catalog is probably not exhaustive (83,96,97).

In wool, two main types of cortical cells are found (ortho- and paracortical cells). Whereas HS proteins are found in each type of cell, the UHS and HGT proteins are each exclusively located in only one type of cell: UHS proteins are found in paracortical cells only (38,98), while all the HGT proteins are to be found only in the orthocortical cells (38,42,98). The nature of KAP surrounding the IF seems to determine the spatial arrangement of IF, the presence of UHS imposing a pseudohexagonal arrangement of the IF (38). The relative amount of HGT also seems to regulate the curliness (crimp) of the fibers (37,99). In human hair, the presence of different types of cortical cell is still under debate and the relationship between the KAP and the crimp of hair is unclear. Shimomura et al. (100) observed that two KAP alleles were specific to Japanese population and were not found in Caucasians.

1.3.2. The Lipids

Hair lipids can originate from two sources. They may arise from cell membrane remains or from the sebum adsorbed by hair in contact with the scalp. Simple extraction of hair with solvents (chloroform, ether, etc.) yields a quantity of lipids amounting to 1–9% of the hair mass and their composition closely resembles that of sebum which implies that the major part of extractable lipids results from sebaceous contamination (101–104). A more thorough extraction using a mixture of solvents at high temperature or following alkaline hydrolysis of the hair yields the constituent lipids of hair (often called internal lipids), part of them being free lipids, and another part enclosing the components of the cell membrane complex or of the epicuticle (105). They represent circa 1–3% of the fiber mass and essentially consist of fatty acids and polar lipids (21,106). Ceramides are also found (107). Some of these lipids are believed to crystallize (108). The exact structure is supposed to be bilayers of soaps of fatty acids (109), based on the interpretation of X-ray diffraction data (Fig. 12a).

It is generally accepted that the internal lipids or bound lipids are located in the intercellular spaces, where they form a part of the β -layers. A recent study using IR microscopy showed that these lipids were essentially found in the cuticle and, more surprisingly, in the medulla (Fig. 23) (110). The same study, confirmed by unpublished analytical results (111), showed that there was a much lower level of bound lipids in hair of African origin than in Caucasian hair.

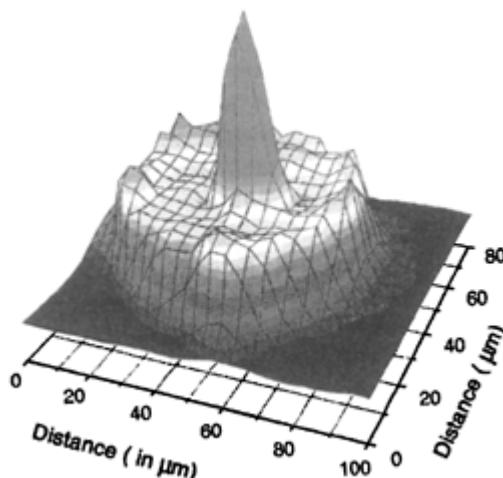


Figure 23 2D Lipid profile across a Caucasian hair section measured by synchrotron IR-microscopy. (From Ref. 110.)

1.3.3. Trace Elements

Hair also contains very variable amounts of inorganic elements, but generally at a low level. The overall content is always lower than 1%. Most frequently encountered are alkaline (K, Na), alkaline earth (Mg, Ca, Sr) and various other metals (Ca, Zn, Fe, Mn, Hg, Cd, Pb, As, Se) as well as metalloids (Si, P). These elements may be of internal origin and incorporated in the hair structure during its synthesis within the follicle. They may also be derived from the environment, taken up by the hair in continual contact with atmospheric dust, ions from water, hygiene products, etc. (112). But before assigning an origin (endogenous or exogenous) to one element or another and using it as a diagnostic tool, the greatest prudence is needed.

The accumulation of certain metals in hair can reflect systemic disorders and the assay can be an excellent tool for diagnosis (113). It is well known, for example, that the level of arsenic in hair is a reliable indication of poisoning with this metal. Similarly, the presence of heavy metals in the air or tap water leads to an increase in their content in the hair of exposed individuals. Hair can thus be used to monitor ambient or atmospheric pollution (114,115).

It should be mentioned that numerous ingested organic compounds can accumulate in the hair. This is the case notably with various drugs (cocaine, opium, amphetamines, etc.). Hair assay is a simple way of detecting the absorption of such substances (116), but the limits of such methods are still controversial (117,118).

1.3.4. Water, An Essential Component

Proteins have a particular affinity for water, an essential factor for the stabilization of their structure. Bound water and water absorbed by hair or wool affect almost all the physical properties, and may also reflect a variety of alterations in hair architecture.

The capacity of hair to absorb large amounts of water is well known (119,120). This capacity was earlier used as a means to continuously measure or control the humidity of ambient air [hair hygrometer (121)]. Absorption isotherms (Fig. 24) show that hair absorbs circa 30% of its dry weight at saturation.

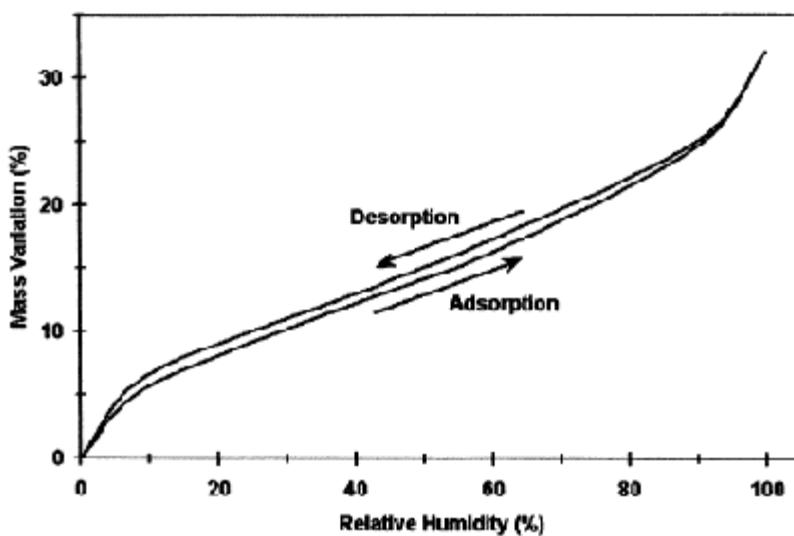


Figure 24 Isotherm of water sorption (absorption and desorption).

Spectroscopic (122–124) thermal (125), and electrical (126,127) studies have shown that water is bound to proteins to a varying extent by hydrogen bonds, either to the acid or basic groups of the side chains, or to the peptide bonds, and that it can condense on itself to form clusters. Many models have been proposed to describe the adsorption curve (Fig. 23) (128–132). The most comprehensive suggest three different mechanisms whereby water molecules bind to hair protein depending on the relative humidity (RH):

- Below 5% RH: Water binds strongly to specific sites of proteins (hydrophilic groups carried by the side chains of acidic and basic amino acids).
- Between 5 and 75% RH: Adsorbed water is involved in weaker interactions with proteins, or with previously bound water molecules. In these two first steps, between 0% and 75% RH, water regain (water uptake absorption) is described by modified BET equations.
- Above 75% RH: Clusters are formed (several molecules of water combined or bunched together).

The mobility of water molecules in the hair, however, is always lower than in liquid water, which demonstrates the existence of a network of hydrogen bonds (122,133). This bonding of water to protein explains the hysteresis observed between the absorption and desorption curves: more energy must be supplied for the water to desorb.

Water absorption varies depending on ethnic origin (10). At a similar relative humidity level, European and Asian hairs absorb the same amount of water while African hair exhibits a much lower rate of water uptake (Fig. 25), even at low relative humidity level (30%) which is reflected by a lesser swelling in diameter. No explanation has been given for this difference.

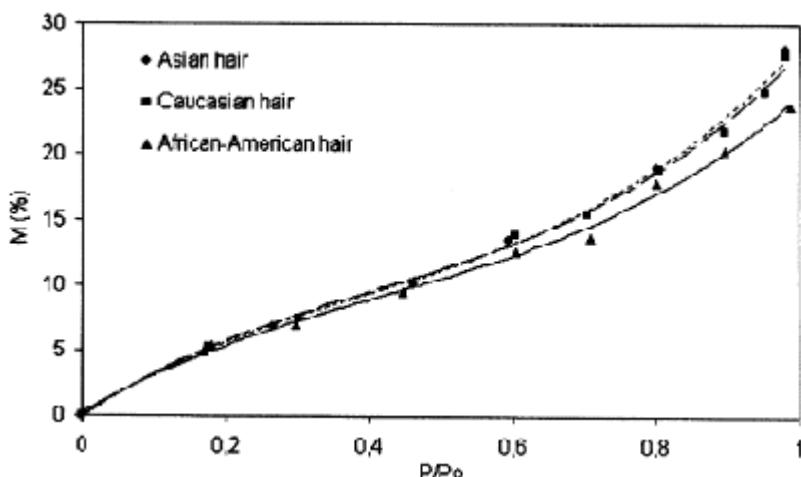


Figure 25 Isotherm of water sorption for the three major ethnic groups (unpublished results).

The most obvious manifestation of water absorption is hair swelling. This swelling is anisotropic: the increase in hair length is only about 2% from 0% to 100% RH, while the diameter increases by over 15% (Fig. 26) (134). The reason is that water is essentially absorbed by the hydrophilic matrix in the cortical cells but does not penetrate the crystalline and dense microfibrils. The incorporation of water in hair has therefore little or no effect on the structure and dimensions of the microfibrils, as evidenced by X-ray diffraction studies (135,136). Adsorption of water molecules however, is assumed to occur at the boundary between microfibrils and the matrix, and water is able to slightly distort the structure of microfibrils as a result (137,138). Hence the microfibrils oppose the longitudinal swelling of the matrix and so its volume is increased by diametric swelling of the hair. Some observations suggest, however, that some non-keratinous areas of hair (notably the CMC, the endocuticle and the intermacrofibrillar spaces) might participate in hair swelling to a certain extent (139,140). Other studies have demonstrated that incorporation of water was also related to the quantity of lipids in hair (141), and that water adsorption modified the structure of the organized lipids (138).

Water absorption and subsequent swelling depends mainly on pH level. Swelling is limited if the pH is highly acid, and greatly aided by an alkaline pH (142).

Water has a very specific behavior. Polar solvents do not produce hair swelling as much as water does. Only ingredients known to break hydrogen bonds have a significant swelling capacity in aqueous solution. Among them are urea, thiourea, formamide, acetamide, and lithium bromide (143,144).

1.4. Origin of Hair Color

It is now generally agreed that hair pigmentation is due to melanin of two types (145,146):

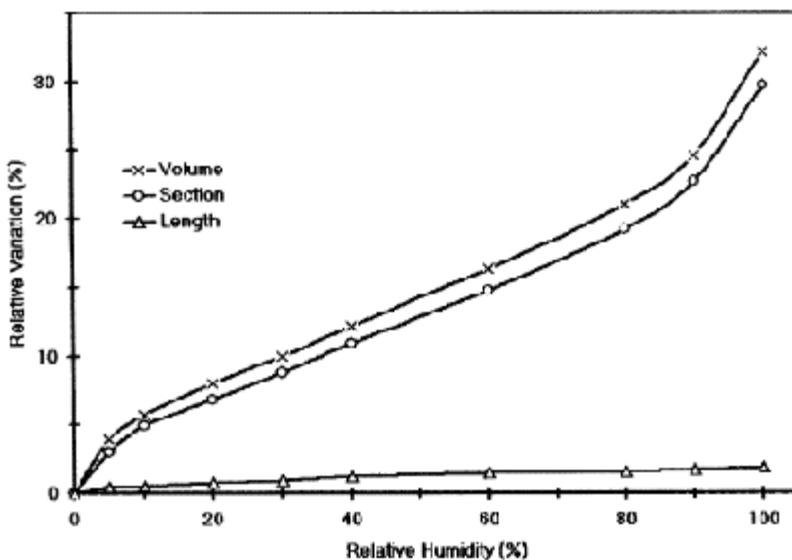


Figure 26 Changes of volume, section, and length of hair as a function of RH.

1. Eumelanin, the commonest, gives the shades from brown to black.
2. Pheomelanin gives the yellow-blond, ginger, and red colors.

The production of these pigments is genetically controlled (147,148). Apart from albinos, all normal humans have melanin hair pigmentation, whatever the color. The actual shade of color in each individual depends not only on which melanin is present but also its quantity and the site, number, and shape of pigment granules in the hair cortex (149–151).

The melanin pigment in hair is the same type as the epidermal pigment that imparts color to the skin (152). The dark eumelanin pigments are generally regarded as the main photoprotective pigments in the epidermis (153). Pheomelanin appears to be less photoprotective than eumelanin and even phototoxic upon irradiation (154). Subjects possessing pheomelanin intend to have little epidermal melanin protection from sun exposure; they become sunburned easily and are more likely to develop chronic sun damage (155) and skin neoplasia (156). Similarly, less-pigmented blond hair has been shown to deteriorate faster than densely pigmented brown hair under sun exposure (157,158).

Melanin is thought to act as an energy sink and free-radical scavenger, preventing the transport of deleterious species into the keratin matrix. Melanin granules are distributed throughout the hair cortex (Fig. 27) but in greater density toward the periphery. When

examined by scanning electron microscopy (SEM), black hair pigments show a rice grain shape (0.8–1.2 µm long; 0.3–0.4 µm thick) with a tight fleecy surface; blond and red hair granules are more sparse, smaller, ellipsoid, or spherical, with a more pitted surface. When extracted (159) from Italian brown hair and Japanese black hair, pigments appear like compact balls—those from Asian being larger—with a cauliflower surface (Fig. 28) as a result of close deposition of

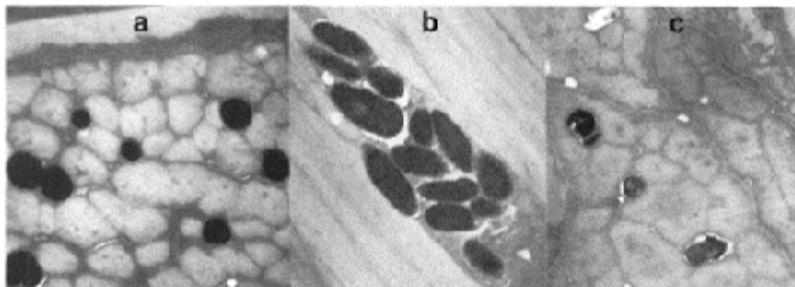


Figure 27 TEM observations of melanin pigments in hair cortex; (a) transversal section of a dark brown hair showing eumelanin pigments in the inter-macrofibrillar spaces; (b) longitudinal section of the same hair; (c) pheomelanin pigments in red hair.

dense melanin grains on a wound protein matrix. Pigments from Irish red hair or Scandinavian blond hair do not keep the shape of organelles after extraction, because of near complete disappearance of protein matrix.

Whatever the type of virgin hair, isolated melanin is brown in color and gives a dark brown solution in aqueous alkaline hydrogen peroxide (159–161). But the quantity of extracted eumelanin is highly variable, depending on the origin of hair (Table 5).

Melanin is a polymer of high molecular weight, insoluble in water and most solvents. It is of very low chemical reactivity, and it cannot be significantly altered except by intense oxidation or by concentrated alkaline solutions.

Its highly complex structure has not yet been established. The prime difficulty when studying melanin lies in isolating pure, unaltered pigment, free from bound proteins as shown by the elemental composition of melanin reported by various authors (Table 6) (159,162–164). Irish red hair pigment shows the highest sulfur content and Scandinavian blond the lowest (Table 7).

All isolated melanins contain approximately 1% of internally associated glycine-rich protein, which clearly differs from hair keratin.

Melanin originates within specialized cells called melanocytes located in the basal layer at the junction of the dermis and epidermis, and in the upper part of

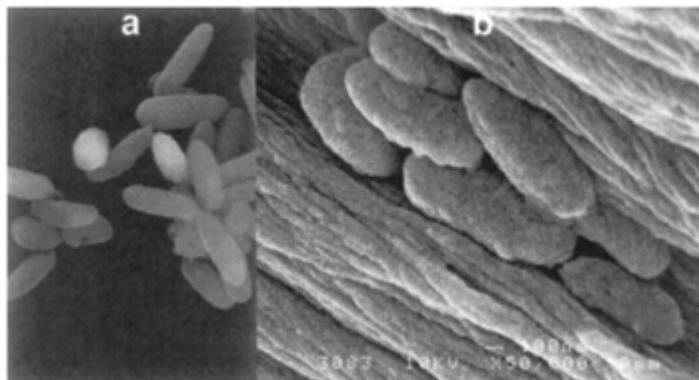


Figure 28 SEM views of eumelanin pigments: (a) isolated pigments extracted from dark brown hair; (b) pigments in a cortical cell.

Table 5 Eumelanin Content of Human Hair Samples

Origin of pigment	Eumelanin content (%)
Italian brown hair	1.1
Japanese black hair	2.0
Irish red hair	0.3
Scandinavian blond hair	0.06
Albino hair	0

(From Ref. 159.)

Table 6 Elemental Analysis of Human Hair Melanins

	C	H	N	S	O
Black ^a	68.0	8.7±0.4	3.7±0.5	2.0±0.3	15.4±0.4
Red ^a	64.9±2.3	9.1±0.3	2.4±0.1	8.5±5.7	11.5±0.6
Eumelanin ^b	611	46	84	37	
Eumelanin ^c	50–53	2.7–3.5	7–8.5	2–2.5	34–38
Synth. melanin ^d	47.2	3.0	7.8	0.06	41.9

^afrom Ref. 160.

^bfrom Ref. 161.

^c from Ref. 159.

^d Synthetic melanin was prepared by in vitro biosynthesis from dopa with mushroom tyrosinase from Ref. 162.

the hair bulb. Melanogenesis involves a complex sequence of chemical reactions corresponding to an oxidative polymerization catalyzed by at least one enzyme.

1.4.1. Eumelanin

The initial propigment molecule is tyrosine (Fig. 29) either as a free amino acid or combined in a polypeptide chain. Tyrosinase, an oxidase with trace amounts of copper in a cuprous state, oxidizes tyrosine to 3,4-dihydroxyphenylalanine (dopa). The enzyme continues to cause oxidation and dopaquinone is formed, a starting point for a series of spontaneous reactions: intramolecular cyclization first converts dopaquinone into leukodopachrome by addition of the amino group on the quinone system; further oxidation generates a red compound, dopachrome, or its tautomeric quinoneimine, which successively undergoes rearrangement and decarboxylation to produce 5,6-dihydroxyindole. Finally, the latter is oxidized to indolequinone (or its quinoneimine form), a highly reactive intermediate from which an oxidative polymerization is initiated, leading to the eumelanin black polymer. This was the classical Raper (165)-Mason (166) scheme, accounting for the regular polymer

Table 7 Sulfur Content of Extracted Melanin Pigments

Origin of pigment	Sulfur content (%)	Carbon/sulfur ratio
Italian brown hair	5.3	13.4
Japanese black hair	4.9	15.7
Irish red hair	8.8	8.8
Scandinavian blond hair	2.3	35

(From Ref. 159.)

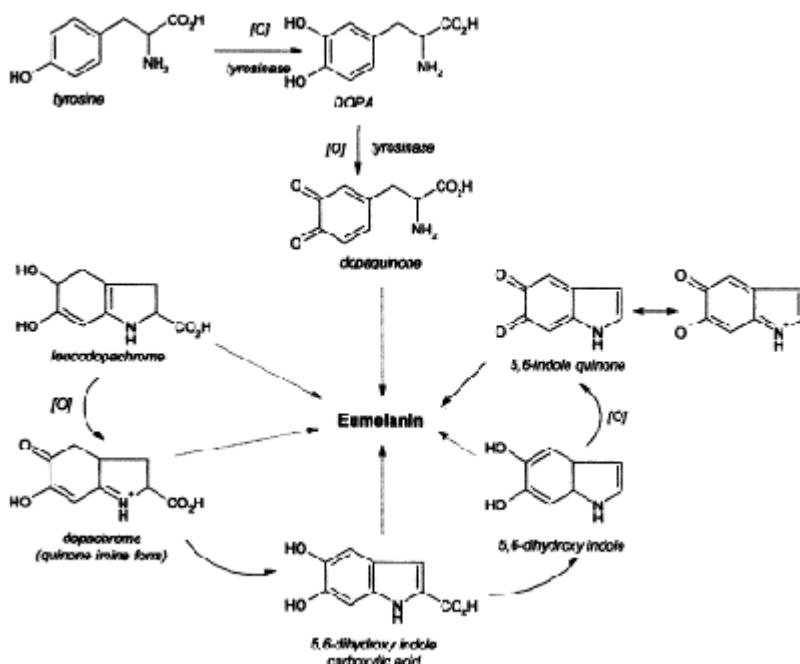


Figure 29 Biosynthesis of eumelanin.

structure. It is now thought that all the intermediate quinones of this pathway can partake in the oxidative coupling process, giving rise to an unpredictable random structure (Fig. 30).

1.4.2. Pheomelanin

The production of this sulfur-rich pigment results from a deviation (Fig. 31) in the previously mentioned biosynthetic pathway. Instead of forming an indole compound by internal cyclization, dopaquinone adds on the sulfur-containing amino acid cysteine to yield cysteinyl dopas (167–169), which may further undergo oxidative cyclization to benzothiazines and higher condensates (Fig. 32) (170), giving pigments whose color ranges from yellow to red-purple.

Eumelanin and pheomelanin are biogenetically related and arise from a common metabolic pathway in which dopaquinone is the key intermediate (171). The cause and control mechanisms for the switch toward pheomelanin still are unknown. Cysteinyl dopas and metabolites are found in the hair of both eumelanic and pheomelanic subjects (172). Thus each individual might be able to produce one or the other melanin type, which is in agreement with the noticeable sulfur level found in eumelanins. But the situation is still more complicated; evidence was reported of the formation of copolymer from both eumelanin and pheomelanin precursors, which strongly suggests an

intermeshing of the pigmentary pathways (171). According to Prota (173), the prevalence of one pathway or its alternate, or mixed oxidative

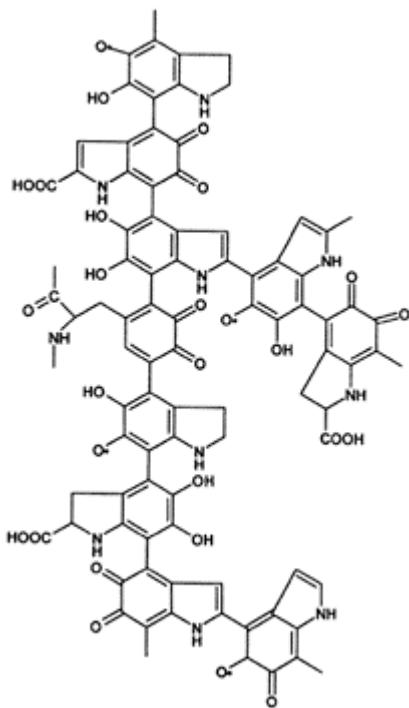


Figure 30 Possible arrangement for eumelanin structure.

copolymerization could be regulated by the cysteine and glutathione levels within the melanocyte, which is under genetic control.

Hair color varies according to age. In general, age produces a color darkening. Then white hairs gradually make their appearance (graying of hair). This color evolution implies that the level of melanin formation is not constant. As the years go by, there is first an intensification and then a slowdown or sometimes even a halt in pigment formation.

It is evident that the formation of tyrosinase in the melanocytes or its enzyme activity can be either activated, reduced, or inhibited. A number of hypotheses have been proposed to explain the graying of hair. Microscopic examinations show a decrease in melanin grain content, but the number of melanocytes does not seem

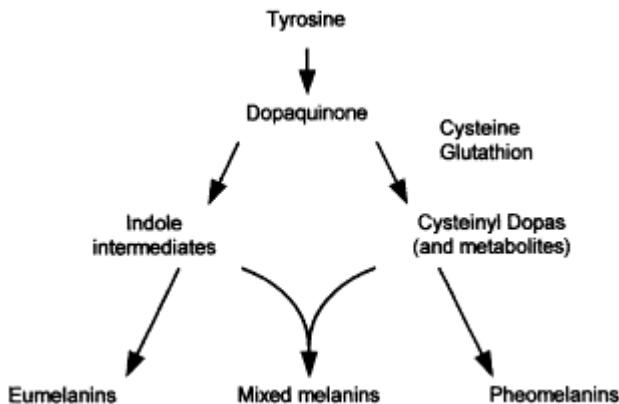


Figure 31 Pathways of melanin production.

to vary. In white hair, only minute amounts of pigment are present, indicating a probable halt in melanogenesis. But this does not mean that the melanocytes have been destroyed. The most plausible explanation is that some unknown substance, inhibiting tyrosinase activity, has been formed. It is well known that the activity of metal-dependent enzymes is relatively easy to block. For example, tyrosinase can be inhibited *in vitro* using a sulfur-bearing substance (e.g., thiourea or its derivatives, cysteine, glutathione, etc.). A possible role of some metabolites accumulating in the hair bulb due to the aging process has been suggested. But it has not yet been possible to identify, *in vivo*, the inhibiting substance or substances, if any exist.

2. PHYSICAL PROPERTIES

The physical properties of hair directly result from the organization of the various structural elements of the fiber: proteins, fibrils, or cells. These properties are to a great extent responsible for hair condition and appearance, and their improvement is the aim of any hair treatment. Some authors (174,175) have even described the behavior of a head of hair as a combination of the various properties of the individual fibers.

The interest in studying the physical properties of hair is thus twofold: first to provide the tools and define criteria for evaluating the integrity of the internal structure of the fibers, its alterations over time or under the effect of an applied treatment or environmental weathering and, secondly, to predict the cosmetic behavior of the head of hair.

The properties affecting a head of hair are essentially mechanical properties. They are a valuable indicator of the quality of the fiber and are closely related to its structure. They also affect certain properties of the hair such as body, styling, etc. The appearance of the hair, on the contrary, is largely dependent upon the surface properties of the hair.

The biological origin of the hair complicates the investigation. The researcher cannot choose the sample on which measurements will be taken (shape, color, etc.) and the properties vary from one hair to another and even along the length of a

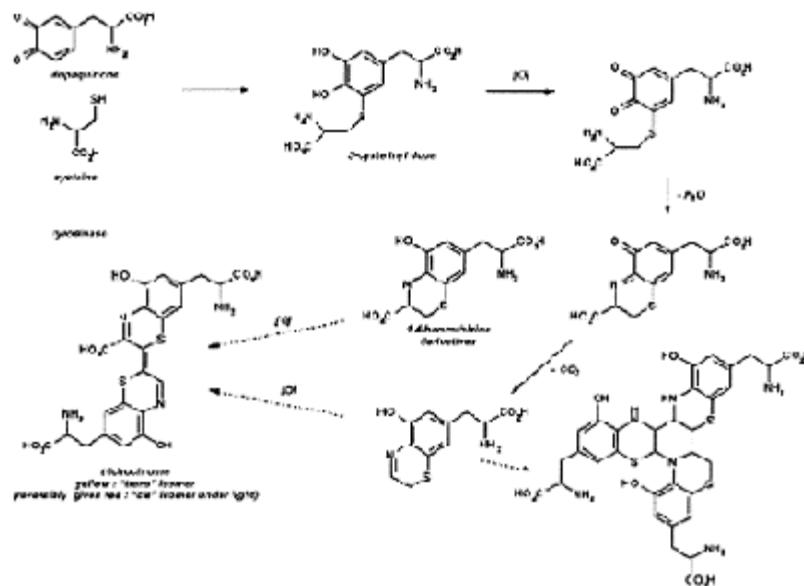


Figure 32 Biosynthesis of pheomelanin.

single hair. All properties of hair are closely related to its structure and are thus subject to variability depending on its biological origin.

2.1. Mechanical Properties

Due to its intricate and unique structure, hair has remarkable mechanical properties. The measurement of these is one of the simplest means of appraising the integrity and attributes of the fiber. It is also one of the oldest, since the behavior of various keratin fibers, particularly that of wool, was described in detail in the 1930s (176–178). Indeed, the slightest modification in the chemical composition or structure of hair may greatly alter its mechanical properties.

Any material (solid or pseudo-solid) subjected to mechanical stress gets its shape altered and resists that stress by opposing forces directed towards a return to its initial state. Four main types of stress are generally considered: the “uniaxial” mode (traction or compression), shearing, torsion, and flexion (Fig. 33).

The stress can be applied in different ways: static (imparted strain or alteration of shape is slow or rapid, but not cyclical, which suggests that the equilibrium is constantly achieved), dynamic (the material is submitted to strain varying cyclically over time), in

relaxation or in creep studies. In this way, the behavior of the material under a strain of varying amplitude until breakage can be investigated.

2.1.1. Tensile Properties

Due to the geometry of hair, the tensile properties are the easiest to evaluate and have long been studied. The usual procedure to appraise the tensile properties of hair is to use a classical device generally called an "extensometer." Figure 34 (179) shows the stress/strain curve of a single hair fiber.

Three areas can be distinguished in which the response of hair to a stress differs:

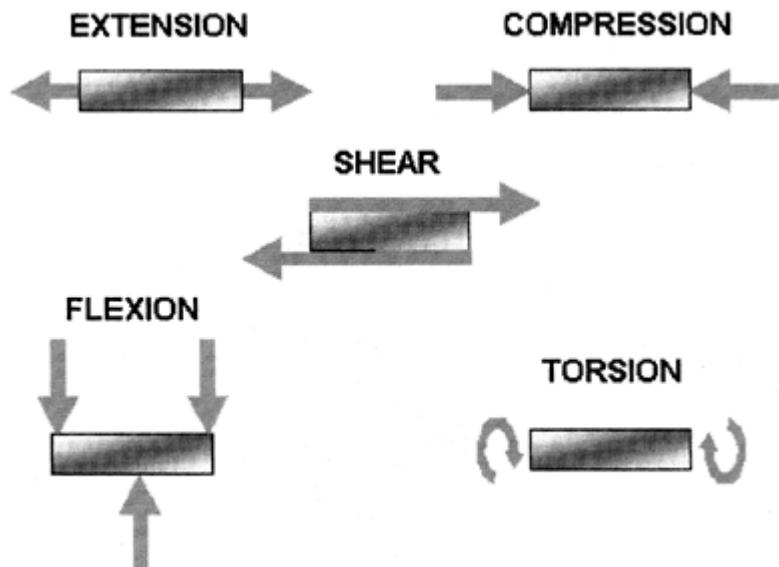


Figure 33 The four main modes to stress a solid.

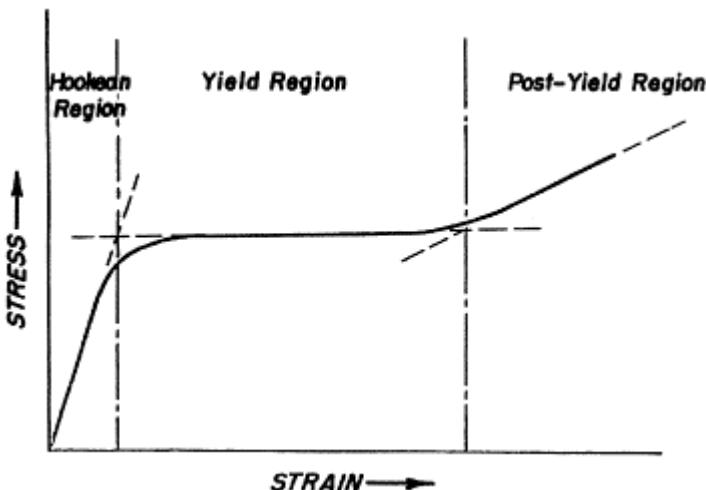


Figure 34 Stress/strain curve of a single human hair. (From Ref. 179.)

1. Between circa 0% and 2–3% elongation, strain is almost proportional to the stress applied. The hair behaves like an elastic material, hence the name commonly given to this area is the Hookean region. This image is now being questioned, and it is believed that the hair in this zone has more of a viscoelastic behavior (180,181).
2. Between 2–3 and 25–30% elongation, strain increases very rapidly without noticeable change in the stress applied. The hair behaves, in this region, like liquid or almost perfectly plastic material. This area is called the yield region. Under specific conditions, however, a recovery may be observed (see below).
3. In excess of 30% elongation, the strain and the stress become proportional again, and the hair behaves again like an elastic solid. It is the post-yield region, in which damage to and finally breakage of the fiber occur.

A number of papers have tried to explain the three regions of this curve as a function of the structural organization of keratin. Some studies on wool identified a transformation of the α -keratin to β -keratin (under specific conditions) just beyond the Hooke region (182,183). In the Hookean part, the macroscopic elongation is mainly attributable to the hydrogen and salt bonds (184) when the hair fiber is stabilized in the dry state. Through the yield region onwards, the amount of β -keratin increases. At the end of this region, approximately one-third of the keratin is in the β -structure (185). Beyond 30% of elongation, the post-yield slope is independent of the hair region. The resistance to strain in this region is controlled by covalent bonds and more specifically disulfide linkages (186).

More recent studies using differential scanning calorimetry (DSC) and X-ray diffraction on wool and hair have been carried out to improve the knowledge of the α – β transition (187–189). From X-ray investigations during extension of fibers, the results show that the α – β transition does not occur simultaneously upon stretching, but occurs during the following steam-setting process (for the stretched fibers). Synchrotron studies

show that the α - β transition is closely related to the “necking” distortion (in the yield region). The “un-necked” segments remain in the α form (and the β form then develops during the following steam-setting process), while the β form is acquired by “necked” segments. This phenomenon, “necking,” commonly seen with other materials, was first described in wool and hair. The distortion process has been described by combining time-resolved small angle X-ray scanning (SAXS) and mechanical stretching of the hair fibers (190), leading to the conclusion that an α helix coil transition of the domains appears instead of the generally admitted α - β transition at 30% RH.

Moreover, it has been shown (135) that mechanical stretching of the microfibril involves a combination of two processes, a stretching of the keratin chains and a sliding of these chains within the microfibril. In water, the sliding process predominates while at 45% RH, the combination of both processes leads to a “melting” of the microfibril supramolecular structure.

The general appearance of these stress/strain curves is similar to that of certain polymers (for example, the “necking” process). However, a surprising phenomenon specific to keratin fibers is their capacity, at least in the wet state, to regain their initial state after relaxation of the stress, even after reaching the “plastic” region corresponding to the yield (up to 30% stretching).

In the case of perfectly elastic materials, releasing stress allows the sample to regain its initial dimensions immediately. In contrast, in the case of perfectly plastic materials, releasing the stress does not cause any shift in elongation.

The hair is in fact never perfectly elastic, even in the Hooke region. The return to the initial state takes place at a certain rate (the hair must thus be considered as a viscoelastic material) and this may be infinitely slow in the case of fibers stretched in water from the start of the post-yield region and relaxed when dry. When the hair is stretched in water, it regains its initial size and properties fairly rapidly (within a few hours). It thus behaves like a visco-elasto-plastic material.

Breakage. The hair is a fiber of great strength. The load required to obtain breakage of a natural, healthy hair varies between 50 and 100 g. The average healthy head of hair (120,000 hairs) may handle 12 metric tons.

African-type hair is fairly fragile (10), due to its highly twisted configuration, flattening as it approaches a collapsed structure in the region of twist and multiple twist reversals along the fiber length (191). Premature failure is increased by tensile and torsional fatigue produced by grooming procedures.

Asian hair is very strong, and the resistance to breakage is similar to that of Caucasian hair.

To visualize the breakage, some studies (191–194) have been carried out using SEM. Three types of fracture are encountered in the breakage of human hair under longitudinal extension: smooth fracture, step fracture, and fibrillated fracture (193). Humidity and conditioning of the hair fiber play an important role in the type of fracture observed.

Hair breakage can also be studied using other types of stress that better reflect real life conditions than the tensile test.

2.1.2. Flexion

When flexion stress is applied to a fiber, the external part is subjected to extension while the internal parts undergo compression (Fig. 35) (195). This type of stress is not “pure” as hair is put through various kinds of strain, but its evaluation is very useful as it affects the external layers of the fiber whose properties are little affected by extension.

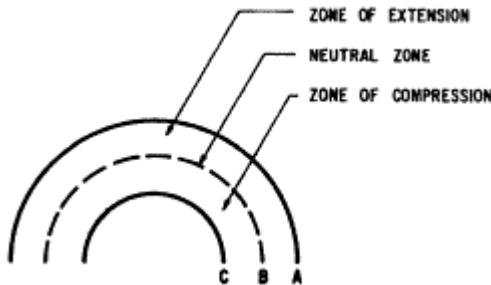


Figure 35 Diagram of a bent fiber.
(From Ref. 195.)

Numerous methods have been proposed for measuring the flexion modulus, including static methods in which the fiber is bent by applying a given force and its strain recorded (196–198), and dynamic methods in which the fibers, attached horizontally by one of their ends, are subjected to vibrations (199).

Using a pendulum, it was observed that the behavior under flexion was directly related to the diameter of the fibers (198) (Fig. 36): the flexion modulus is proportional to the diameter by the power of 4.

2.1.3. Torsion

Due to the geometry of hair and, in particular, its small diameter and elliptical shape, its properties under torsion are hard to evaluate. However, methods based on the torsion pendulum (177,200,201) have succeeded, even in liquid medium (202–204).

Evaluation of the behavior of hair under torsion is very valuable because this type of strain involves different structural zones than those implicated in traction or flexion. Indeed, the axial fibrillar crystalline structure of the proteins means that the microfibrils

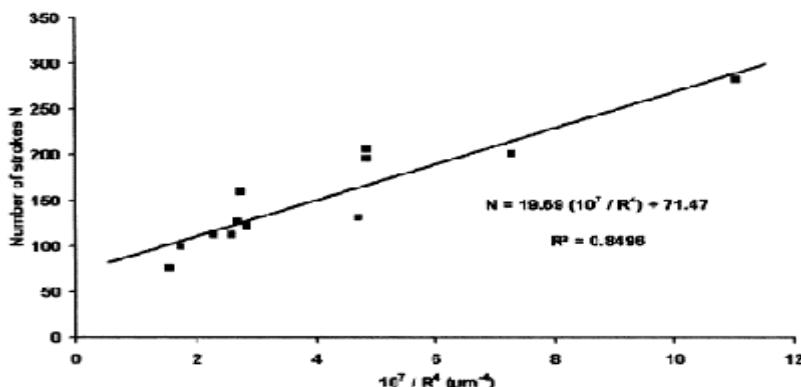


Figure 36 Mean number of strokes N of the pendulum vs. $1/R^4$. (From Ref. 198.)

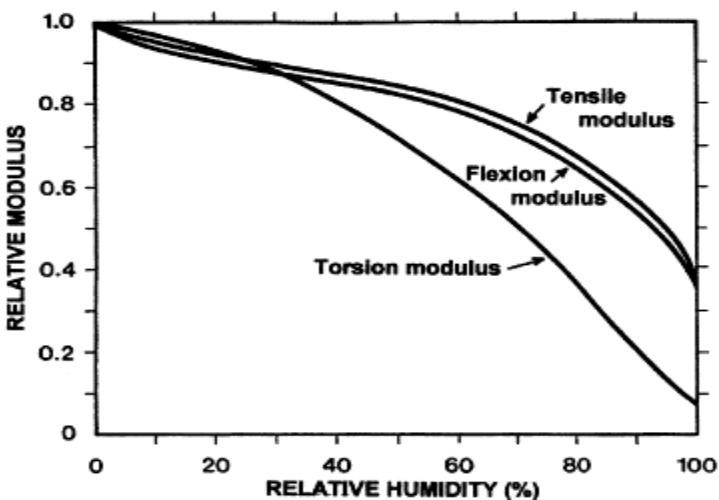


Figure 37 Evolution of the different moduli as a function of RH.

are little affected by torsion. With this type of measurement, an image is obtained of the behavior of the amorphous areas of the cortical matrix (204). This method was therefore used to establish the relationship between the matrix structure and the properties of hair (205,206). It was shown that in the dry state, the torsion and the tensile moduli of hair were not significantly affected by the temperature or disulfide covalent bonds (207–210). In contrast, the sensitivity of the torsion modulus to water regain and pH is far higher than that of the tensile modulus (204,207) (Fig. 37). Thus, ionic bonds and hydrogen bonds are very important for torsion properties.

Different studies have shown that there is not an obvious relationship between the torsion modulus and the fiber diameter (177,211,212). In water the logarithmic decreases vary with diameter. Moreover, irrespective of hair diameter, cuticle thickness is the same, around 3 µm. Wolfram et al. (200) suggested that this observation was due to the plastic nature and low modulus of the cuticle (see also the section on the effects of the cuticle).

Mechanical properties are affected by the water regain, temperature, and cosmetic treatments or damage induced by environmental factors, e.g., ultraviolet (UV) light. The two last points will be dealt with in Chapter 12, and the first ones are discussed below.

2.1.4. The Phenomenon of Relaxation

A number of relaxation studies have been carried out in different media to establish structure/properties relationships and to determine the effects of some components of the structure on macroscopic properties (213,214). A specific study on relaxation of wool fibers after stress (215) showed that two processes occurred when a fiber was stretched in water. One was rapid and played a major part in the course of relaxation: it involved the breakage of non-covalent bonds; the other was slower with relaxation taking place over a longer period of time and resulted from the cleavage of disulfide bonds.

2.1.5. Dynamic Measurements

The viscoelastic properties of materials can be studied by applying a sinusoidal stress. This type of characterization is much used in the field of polymers (216), which do not exhibit a perfectly elastic behavior. Dynamic measurements take into account a parameter that is not involved in static measurements: the time lapse between the stress and the response of the material which reflects an energy dissipation process occurring in the material. Moreover, this type of measurement is ideally suited to monitoring changes in mechanical properties over time as a result of alterations in hair composition.

The first trials on keratin fibers (wool) were carried out by Mason (217). In the 1970s and 1980s, numerous studies were performed by Australian groups on various types of keratin fiber (218–224). These studies were mainly aimed at understanding the interactions between water and wool or hair and succeeded in demonstrating the occurrence of transient phenomena ascribed to reorganization of water within the protein matrix (223) (Fig. 38).

Torsion can also be measured dynamically. Using this method, Mackay and Downes (225) reported that transient stresses occurring during sorption and desorption caused a temporary lowering of the rigidity torsion modulus. This phenomenon could be brought about by the transient sorption-induced stress associated with the front of water penetration. After the passage of the front, the stress would fall and bond reformation could be expected. This observation must be compared with the phenomena described with regard to dynamic tensile properties (223).

Wolfram and Albrecht (200) thus studied the viscous behavior of hair in the dry state and in water. The reported results suggest that hair cuticle, while tough and resilient in the dry state, undergoes water plasticization to a much greater extent than hair cortex.

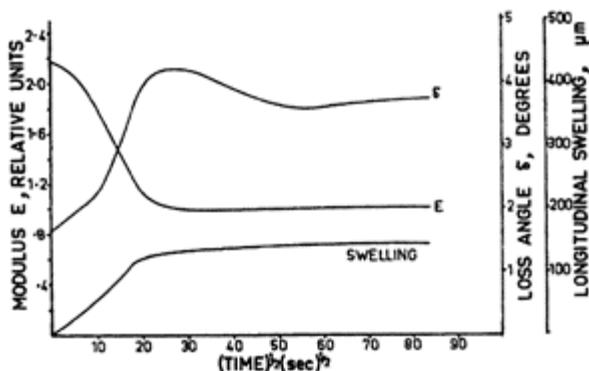


Figure 38 Simultaneous changes of elastic modulus E , loss angle δ , and longitudinal swelling vs. $(time)^{1/2}$ in horse hair at low extensions during water absorption from 52.9% RH to wet condition (1/relative unit=2×10⁹ N/m²). (From Ref. 223.)

2.1.6. Effect of Cuticle on Mechanical Properties

The effects of the cuticle on mechanical properties have been discussed in different papers. It has been shown that no detectable changes in the tensile properties (in wet or dry state) were detectable with or without cuticle (226). It is well known that cuticle cells are essentially inextensible, and that extension mainly causes shear stress between layers of different composition and extensibility within the cuticle cell. Failures appear in the weak endocuticle, and the damage is irreversible (227).

A study was reported on the bending of human hair, and the author suggested that the cuticle might make a contribution to bending resistance (228). With the availability of nano-mechanical techniques and AFM, we have now access to the local modulus of the cuticle and even more precisely, to the moduli of the exocuticle and endocuticle (31). So, it has been pointed out that despite its relative thickness the cuticle had a significant contribution (74%) to the total bending, with 66% from the exocuticle and 8% from the endocuticle.

As for the dynamic torsional properties of wool, it has been shown that a decrease in fiber diameter correlated significantly with the increase in the loss angle at all water regains, i.e., smaller diameter is associated with more viscous properties (229).

2.1.7. Effect of Water

For the most part, the stability of the protein architecture of hair, and particularly in the cortical matrix, is due to the high density of the network of weak bonds that exist between polypeptide chains. These non-covalent, hydrogen or salt bonds are greatly

disturbed by the introduction of water into the structure. As a result, the mechanical properties (see also Fig. 37) of the fibers are considerably changed and, in particular, their response to traction (Fig. 39).

The most marked influence of water occurs at fairly high strain, as reflected by the following observations:

- A reduction in stress in the yield region does not induce any noticeable change in strain at the yield threshold. In water, the stress required to get a given strain is roughly twice less than in dry conditions.
- An increase in strain at the breaking point accompanied by a decrease in stress, without any measurable change of the modulus in the post-yield region.
- To a lesser extent, a decrease in the Young modulus in the Hooke region.

In the same way, relaxation processes are markedly changed (Fig. 40) (230) as mentioned earlier. For example, if hair is elongated until the yield point (115–130% of its initial length) in dry conditions, i.e. low relative humidity, it requires an infinitely long period of time to regain its initial length when the stress is relaxed. In contrast, when the same experiment is carried out in water, the hair only requires a few hours to return to its initial length.

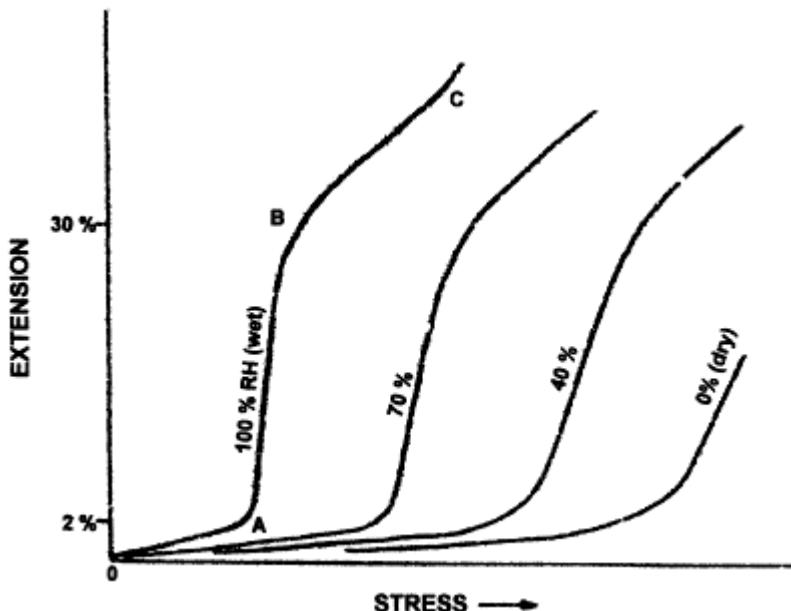


Figure 39 Typical stress/strain curves of keratin fibres equilibrated at four levels of relative humidity (0%, 40%, 70% and 100%). (From Ref. 184.)

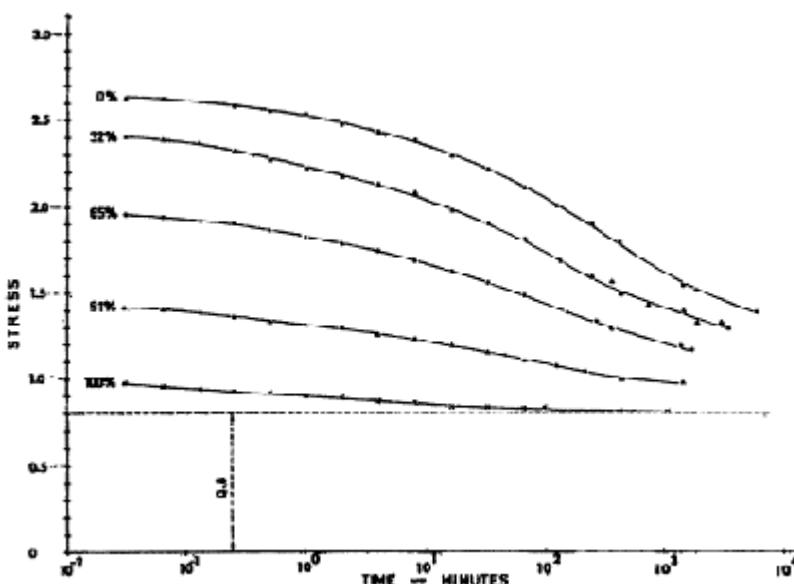


Figure 40 Stress/time curve for wool fiber extended at 0.8% elongation at different RH. (From Ref. 230.)

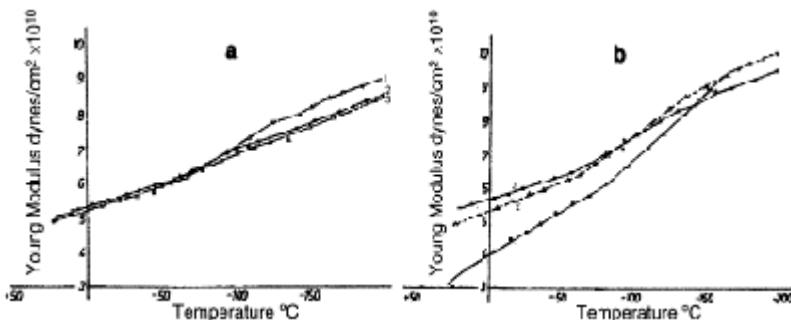


Figure 41 Young modulus of a Corriedale fibre plotted against temperature (from Ref. 231). (a) vacuum dried for different periods: (1) dried for 2 hr; (2) dried for 24 hr; (3) dried for 100 hr; (b) with different water contents: (1) 13% water content;

(2) 3% water content; (3) dried for 17 hr at a vacuum of 10^{-3} torr.

It has also been noted using dynamic measurements that elasticity modulus decreased when water content of hair increased and that hair viscosity increased very markedly (see above).

2.1.8. Effect of Temperature

A number of studies (179,231–233) were carried out on wool to study the mechanical properties as a function of temperature.

Torsional stress was mainly used but other types of stress were also applied. The purpose was both to get a better understanding of structure-property relationships and to describe the thermal transition process (231,234) of wool and hair (see also section on Thermal properties). A decrease in the Young modulus was observed (Fig. 41a) when the temperature increased with an inflection in the modules at -90°C , which may be associated with a transition due to the presence of moisture in the fiber. This decrease is also dependent on the water content of hair (Fig. 41b). At higher temperatures, a sharp decrease is seen (Fig. 42). Thus, for Corriedale wool, at around 130°C or above (in water), the Young modulus becomes very low. At approximately the same temperature, the α -keratin structure, as indicated by X-ray diffraction data is replaced by randomized β -keratin crystallites (233).

Only a few papers have described the alteration of mechanical parameters according to temperature in human hair (179,235). It was found that the elastic modulus, the post-yield modulus and fiber strength decreased with increasing temperature while extensibility increased.

2.1.9. Ethnic Origin

The slope of the stress-strain curve is independent of the ethnic origin of the hair. Account must be taken, however, of differences observed. For example, Asian hair, characterized by a larger cross-section, is considered to be more resistant, i.e., a greater level of traction must be imposed to achieve a given elongation. In African hair, the size of the cross-section greatly varies due to the configuration of curly hair.

To compare mechanical properties, it is necessary to take into account hair diameter. Figure 43 represents the stress/strain curves of hair in water in which the load applied is divided by the hair diameter. Under these conditions, no differences are

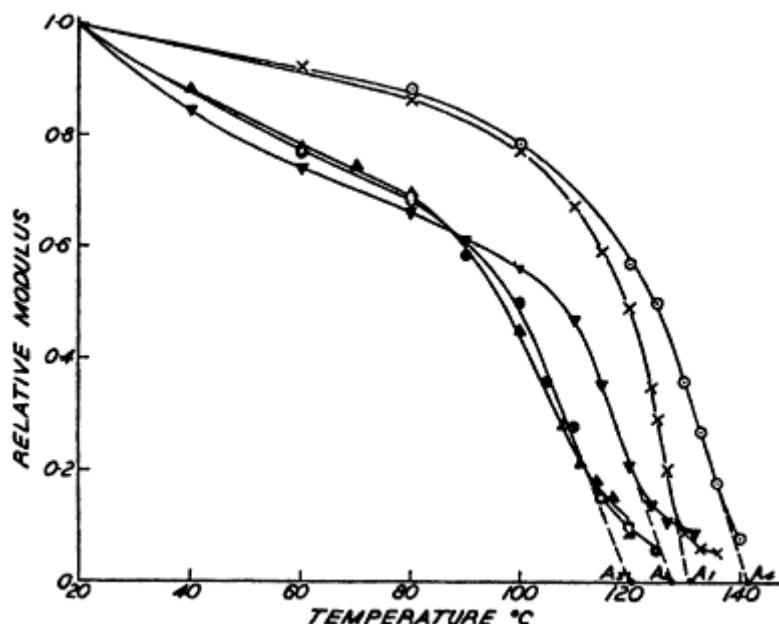


Figure 42 The Hookean modulus in water at various temperatures of wool fibers, expressed as a fraction of the modulus for the same fiber at 20°C for: (X) normal Corriedale wool fibers; (▼) Corriedale wool fibers in which the sulphydryl groups have been blocked by methylation; (●) Corriedale wool fibers in which the disulfide content had been reduced to 42% of normal and the sulphydryl groups methylated; (▲) the same as the latter except with a 30% disulfide content; (○) Corriedale wool treated with formaldehyde. (From Ref. 233.)

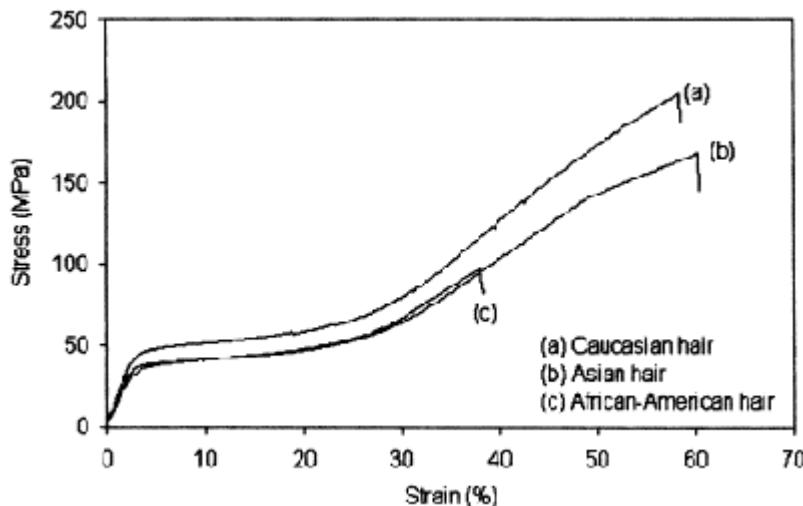


Figure 43 Stress/strain curves of African, Asian, and Caucasian hair (RH=100%). (From Ref. 10.)

observed at different stress levels whatever the ethnic origin of the (natural) hair examined. This is in good agreement with studies carried out on the structure of hair in different ethnic groups. At lower humidities, African hair is differentiated from Caucasian hair by the existence of premature failures at low stresses (10,194).

It seems clear that the above-described types of hair behavior are closely linked to alterations induced in the intimate structure of the hair by mechanical stress. Various models have been proposed to explain stress/strain curves.

2.1.10. Models

From the 1960s, more and more sophisticated techniques have become available for studying the structure of the hair fiber together with ever improving and more potent tools for numerical calculation. As a result, simulation models have been used to describe structure-properties relationships. They were mainly devised originally for wool, whose structure is close to that of hair.

Models Based on Structure. Models propounded to interpret the tensile curves of hair or wool have been the subject of many discussions. A critical review of the structural mechanics of wool and hair has been published by Hearle (236). Currently, three main models coexist: the “series-zone” model developed initially by Feughelman (237) and improved later by Wortmann and Zahn (238), the globular matrix model also proposed by Feughelman (64), and Chapman’s composite model (239). The three models have in common that they consider the elastic resistance to fiber traction, at least under weak stress, to be linked for the most part to the crystalline regions (intermediate filaments) and that the yield is explained by a phase transition in these areas, the keratin chains

changing from the helical shape to a stretched shape, so-called β -leaflets. This $\alpha\text{-}\beta$ transition has been demonstrated by Bendit (240) by studying X-ray diffraction diagrams of wool fibers under stress and different conditions of temperature and humidity.

The so-called “series-zone” model (241) described the hair as an amorphous matrix of weak elastic resistance but high viscosity surrounding more rigid and elastic areas, assimilated to the microfibrils, in which two alternate types of zone, X and Y, existed. X zones underwent $\alpha\text{-}\beta$ transition in the yield region while the more rigid Y zones experienced shifting in the post-yield region only (Fig. 44). This model takes in account the increase in stress in the post yield part of the curve. This model was the basis of several variants which were further proposed by Feughelman and others (242–247).

Significant progress was made based on this model, from studies with the aim of identifying the specific regions of keratin structure of the IF. They were illustrated by Feughelman’s work (248) based on the first descriptions of IF structure (54) and later by Wortmann and Zahn (238) who proposed new definitions of the X and Y zones benefiting from advances in keratin sequencing and knowledge of molecular organization of the IF. All these studies focused on IF structure while the matrix was almost completely ignored.

Chapman (239) published a different model. It was also based on the coexistence of the two areas in hair, but one was the matrix, with an elastomer behavior and the other one was the microfibrils, considered to be both rigid and elastic crystals, capable of undergoing an $\alpha\text{-}\beta$ transition (Fig. 45).

The originality of this model derives from the way the matrix is implicated, being considered as a strongly cross-linked gel with elastomer behavior and, above all, from the fact that stable bonds are introduced between matrix and microfibrils.

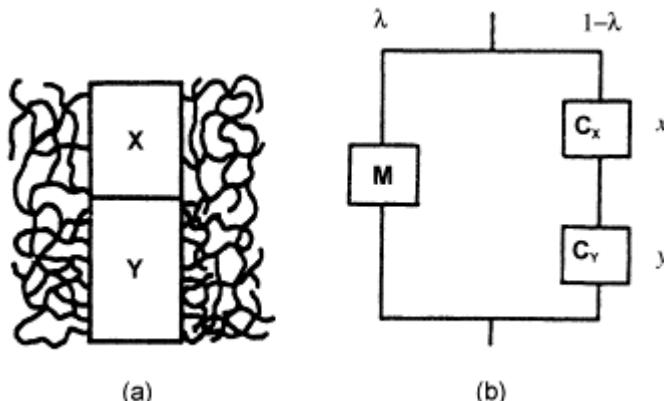


Figure 44 Revised Series-zone model for wool structure: (a) parallel arrangement of a homogeneous M-phase and a composite C-phase; (b) mechanical schematic for the Series-zone model. λ is the area fraction of M-

phase, and x and y are the length fractions of the X- and Y-phase, respectively. (From Ref. 242.)

These bonds allow a transfer of stress between the two areas. Several improvements of this model were further suggested by Chapman et al. (202,249,250). One of the most interesting is the identification of cross-linkages between the matrix and the IF as disulfide bonds linking the end areas of IF fibril proteins and the matrix, itself described as a globular structure, strongly cross-linked by inter- and intra-molecular bonds.

The value of this model is that not only does it fully account for traction curves but, above all, it is also in full agreement with the various relaxation curves of hair,

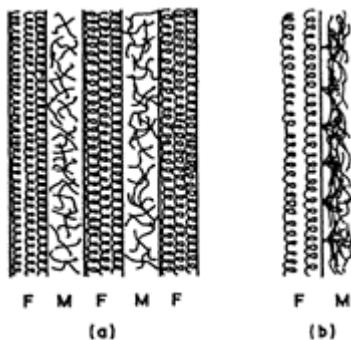


Figure 45 Two-phase model showing parallel crystalline microfibrils F in the less ordered matrix M (a); postulated linkage between microfibrils and matrix (b). (From Ref. 239.)

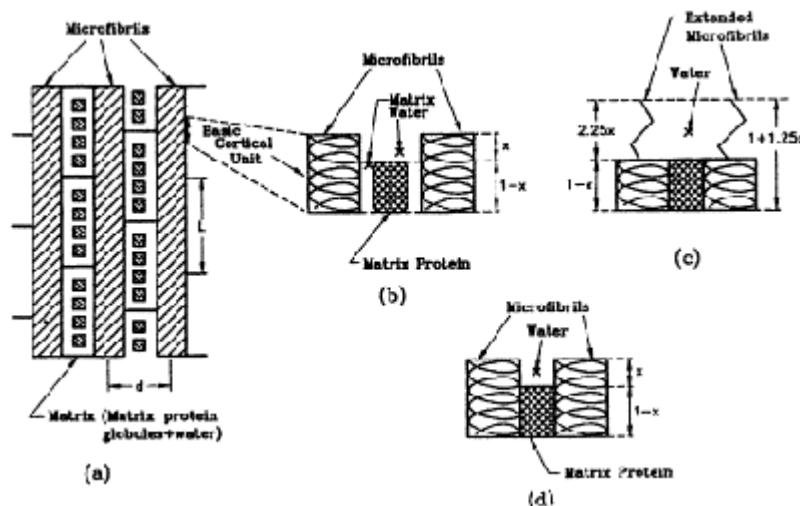


Figure 46 (a) Model for the relationship between the microfibrils, globular matrix protein, and water in the cortex of a wet α -keratin fiber. Also shown are the intermicrofibrillar linkages in the matrix at a longitudinal distance of L apart, where $L \sim 15$ nm. For wet fibers the inter-microfibrillar distance d is ~ 11 nm. (b) The basic cortical unit of the microfibril-matrix relationship. Longitudinal fraction α corresponds to the fraction of the microfibrils in which the α -helical structure is unfolded by the extension of the fiber to the end of the yield region. (c) The state of the basic cortical unit extended to the end of the yield region. The fraction x of the microfibrils is extended to $2.25x$ and the globular matrix protein is jammed against the $(1-x)$ fraction of the microfibrils. (d) The state of the basic cortical unit dried to about 65% RH when the globular matrix protein just

jams into a fraction ($1-x$) of the microfibrils. (From Ref. 64.)

including in water, thanks to the hypothesis of a matrix behaving like a hydrophilic gel and of crystalline zones impermeable to water.

In 1994, Feughelman (64) proposed a new model in which the matrix was likened to a group of proteins surrounded by water, highly mobile. He also postulated the existence of covalent (disulfide) bonds between microfibrils (involving the end regions of proteins) (Fig. 46). The explanation for the yield region of the traction curves was a gel-sol transition of the globular proteins, leading to a new very fluid organization in which water molecules rearranged themselves very rapidly. This model seems very imperfect, notably because it considers very weak cross-linking of the matrix, which is hardly compatible with its high cystine content and with the mechanical resistance of the fibers, as well as the presence of extremely mobile water that should freeze at 0°C, which is never the case.

But the main drawback of these various models is that the relationships between the various structural elements of the hair (notably the proteins) and the mechanical properties are not fully taken in account. Apart from Wortmann and Zahn's model (238), no attempt has been made to precisely identify the role of the various elements. Some authors have offered interpretations of certain specific mechanical properties, taking into consideration the contribution of the different types of disulfide linkages that can exist in keratins. Moreover, the $\alpha-\beta$ transition, essential to the establishment of these models, has been questioned. New suggestions to describe the contribution of structural elements are emerging, which link the mechanical behavior of hair to changes in the organization of matrix-microfibril complex.

Rheological Models. In this type of model, no attempt is made to link mechanical behavior of the material to its structure, but only to describe mathematically the stress/strain curves on the basis of the laws of mechanics. The first model proposed was that of Burte and Halsey (251) but the most useful was propounded by Pichon (252) which takes completely into account the behavior of hair under traction and relaxation, involving various parameters such as the rate of stress or moisture content.

As there is no possibility, however, of linking these models to any structure of the material, their value is limited. Hence the proposal of other models seeking to reconcile the purely mathematical approach with a structural description of hair. It is then considered that hair is composed of two different phases: a continuous viscoelastic phase and discontinuous elastic fibrillar areas. Each of these areas can be described by different mechanical parameters according to the water content of hair. Thus, Tao and Postle (253,254) explained the behavior of hair on the basis of seven viscoelastic parameters linked to these two areas.

Similarly, Curiskis et al. (255,256), in a modeling approach by finished elements, defined a number of important mechanical parameters for each phase. These approaches are very interesting, but difficult to use for understanding the structure/properties relationships of hair.

2.2. Surface Properties

2.2.1. Importance of the Hair Surface

A head of hair represents an important surface area. A calculation for an average head of hair, 20 cm long, leads to a surface area of 6 m² (considering hair to have an average diameter of 80 µm). This explains in part why it is so hard to wet hair and why it is necessary to include wetting agents when formulating most hair products. This also accounts for the preferential adsorption by hair of products that have been applied, rendering the amount likely to reach the scalp negligible. Similarly, hairstyle depends on the numerous (more than 100,000 hairs) fiber-to-fiber interactions and thus, to a great extent, on surface properties.

The surface condition varies greatly from one head to another, between hairs, and according to the location along the shaft. The number of layers of overlapping cuticle scales—usually 7–10 in midshaft—decreases from root to tip, resulting in weakened tightness of the cuticle, reduced resistance—due to abrasive handling—and a decreased barrier function against external forces.

Scanning electron microscopic examinations show dramatic morphological changes in scale architecture, most particularly on long hair (Fig. 5). Smooth, tightly bound with a regular pattern at the root, the scales exhibit increasing scratch marks and eroded areas toward the tip; their edges become jagged, notched, rugged, and saw-toothed, while lifting up from the shaft; complete elimination of the cuticle is frequently seen at the distal end, exposing the cortex unprotected and bringing out “swallow-tailed” fractures known as split ends (257,258).

These changes in the surface condition are mainly due to mechanical abuse (e.g., frequent, harsh brushing, especially wet or dry combing, heat-drying) and sunlight exposure and chemical overprocessing (227,257,259–261). They hamper combing, styling,

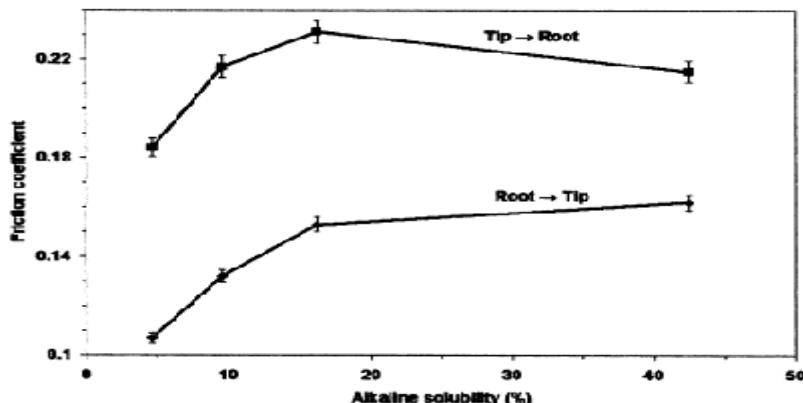


Figure 47 Friction coefficient vs. bleaching level of hair (expressed as alkaline solubility).

and manageability. The cosmetic treatments, notably dyeing, waving, and conditioning, also modify the surface properties of hair. The surface condition is thus a major feature to be taken into account when developing a product for hair care or embellishment.

2.2.2. Friction

The capacity of hairs to slide against one another is of paramount importance. Apart from the feel, this capacity explains a certain number of properties of the head of hair: cohesion, ease of combing or untangling, volume, style retention, etc.

The hair's friction coefficient is greater than that of most natural fibers. Moreover, the coefficient measured from the root to the tip is much lower than that measured in the opposite direction (Fig. 47). This is due to the particular structure of the cuticle formed as a set of overlapping scales. The direction of the free edges of these scales towards the distal ends makes sliding more difficult from the end to the root. Brushing and inappropriate treatments degrade and lift up the scale edges, so increasing the friction coefficients, to an extent depending on the direction of friction (Fig. 47). The more harsh and fierce the handling and its frequency, the more the scale edges are eroded and the friction coefficient decreases and the difference between the two coefficients has a tendency to lessen (Fig. 47).

The friction coefficient also depends upon the physicochemical nature of the surface. The surface of a new hair is composed of lipids and proteins. The particular structure of the epicuticle, and especially the presence of 18-MEA on the outer surface of hair, probably contributes to specific friction properties (15). As the hair ages its surface becomes degraded under the effects of sun exposure and oxidative stress (including UVA), leading to a large number of strongly ionized sites (cysteic acid in particular). The friction coefficient of hair, which is inversely proportional to its surface charge, increases with the oxidation level of the hair surface.

Atomic Force Microscopy is a powerful tool to understand the origin of frictional effects on materials (morphology and physicochemical characteristics). Use of AFM on hair in friction mode (262,263) could give information at a local scale on the causes of modifications of the friction coefficient.

2.2.3. Wettability

Wettability of hair is, as with friction and other surface properties, strongly dependent on the physicochemical structure of its surface. The outer 18-MEA layer makes the hair surface hydrophobic. Consequently hair, though denser than water ($d \approx 1.3$), floats on its surface. The capillary forces exerted on the fiber that are due to its hydrophobicity allow it to float. This explains in part why it is so hard to wet hair and why it is necessary to include wetting agents when formulating most hair products.

Following the various kinds of treatment to which hair is subjected, the surface lipids, especially 18-MEA, are easily impaired. The cuticle proteins, rich in cystine, are readily oxidized, as mentioned earlier, with the formation of strongly hydrophilic sites. The surface of the hair is modified and becomes hydrophilic (264,265).

2.3. Thermal Properties

This aspect refers to the behavior of hair when subjected to a rise in temperature. The first studies on heat-induced alterations of keratin were carried out in wool (266) with two distinct aims:

- To discover the effect of heat, which was analyzed by evaluating the consequences on the properties of traction (267), torsion (266), surface quality (260), and specific volume (268).
- To characterize the thermal transitions of fibers, whether chemically modified or not, in relation to their structure.

In the earliest studies, thermal transitions in wool were approached by measurements of torsion stress (266). More recent studies have made use of thermal analysis techniques such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), etc., applied to both wool and hair. When wool or hair fibers are heated from room temperature to 300°C, different events are successively observed by DSC or Differential Thermal Analysis (DTA) (269–271) (Fig. 48):

- At temperatures lower than or equal to 100°C, free or weakly bound water evaporates.
- The evaporation of water more strongly bound to the structure, i.e., to the protein chains, is indicated by a very large peak culminating at 135°C.
- A vitreous transition has been described and the temperature at which it occurs depends on the moisture content (268,272,273) (Fig. 49). This transition was also ascribed to a toughening transition (271).
- Irreversible endothermal transitions occur at a temperature between 230 and 250°C (274).
- At a temperature higher than 350°C, thermogravimetry shows a rapid loss of mass linked to the breaking and decomposition of keratin chain.

Study of vitreous transition as a function of relative humidity have been carried out on different types of wool and hair by DSC. The results are in good agreement with the calculations based on the Fox equation (275) linking the temperature of vitreous transition to the water content of the fibers. Water does seem to play a part as a plasticizer of the keratin structure as reflected by the fall in vitreous transition temperature with increased water content within the fiber.

DSC has also been used to determine the water content and state of water in wool or hair (276).

Two distinct peaks (Fig. 50) corresponding to endothermal transitions have been observed in hair, one at 240°C, attributed to fusion of the crystalline regions of microfibrils, the other at 250°C, believed to be linked to the degradation of amorphous matrix proteins (277–279). The first peak, was also used by Spei to determine the helix content in wool (280,281). These studies were in general conducted using DSC and small angle X-ray scattering, in order to understand which structural parts of the fibers were involved in the thermal events.

The high pressure DSC (HPDSC) method has led to the conclusion that the two peaks involved two types of cortical cells having a different sulfur content (282). In the case of

wool, these two types were identified as corresponding to the ortho and para cortex, respectively.

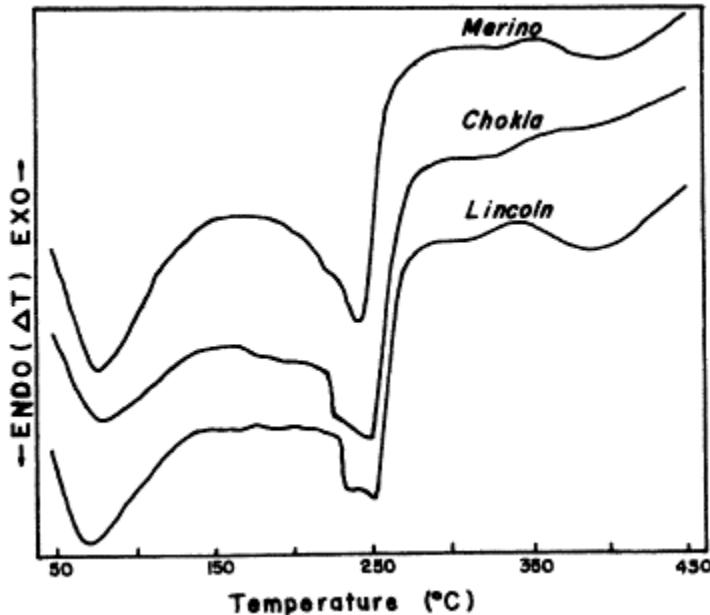


Figure 48 DTA thermograms for control wool fibers. (From Ref. 269.)

In the case of hair, DSC study has shown that there was no difference in the thermal behavior of Caucasian and Asian hair (283).

Effects of heat treatment (271,281) or mechanical stress (280) on the keratin structure have been studied using DSC and X-ray diffraction. The peak area ascribed to α -keratin increased upon annealing from 80° to 150°C and decreased at higher annealing temperatures (271,278).

Using DSC (279,284) or HPDSC (285), the effects of cosmetic treatments on hair have been investigated by measuring:

- the area and temperature of the two peaks (Fig. 50) from the DSC curve,
- the enthalpy and temperature of keratin denaturation from HPDSC.

The authors gave an interpretation of the structural alterations induced by bleaching and permanent waving of human hair.

A cosmetic impact of heat must also be noted. After repeated drying of hair in hot air and under cycles of wetting, major changes have been observed in the surface condition,

with the appearance of fissures depending on the temperature produced by the dryer in the neighborhood of the fiber (260).

2.4. Electric and Dielectric Properties

As for numerous physical properties, wool is the main fiber in which electric properties have been described. Keratin fibers are very hygroscopic and conductivity has generally been studied relative to their water uptake (286–291) and also as a function of temperature: logically, it has been found that resistance drops when atmospheric relative humidity increases (Fig. 51). Apart from the description of conductivity

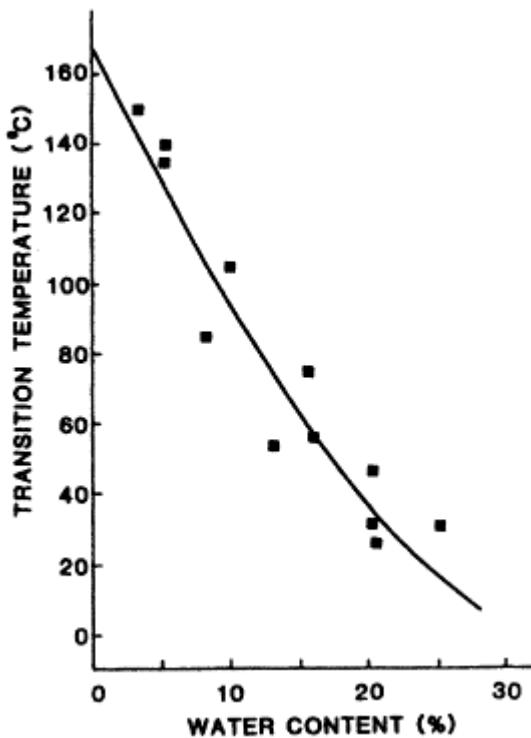


Figure 49 Variations of transition temperature in wool with water content. (From Ref. 273.)

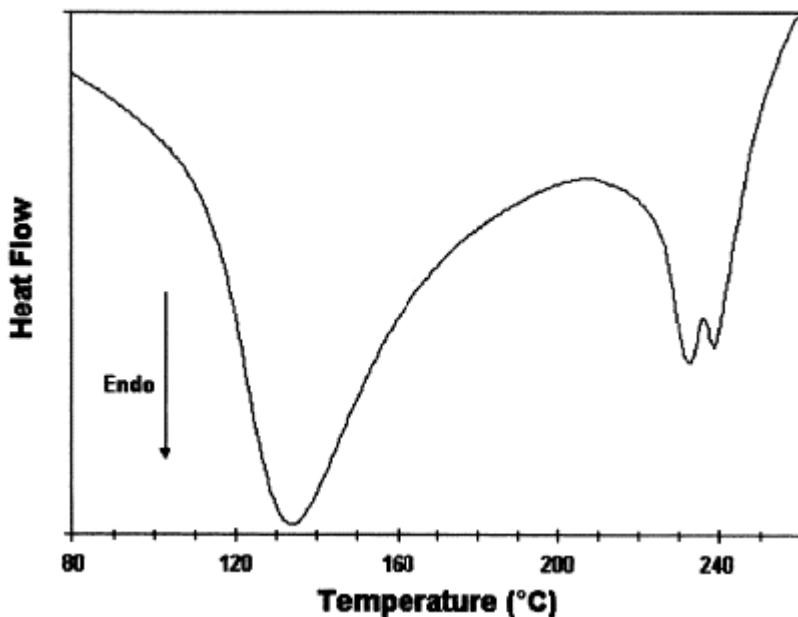


Figure 50 DSC curve of human hair.
(From Ref. 279.)

or resistance in terms of relative humidity, the aim of these studies was to propose models and/or interpretations of water/keratin interactions.

The kinetics of water uptake when the relative humidity varies from 0 to 90% was studied under mechanical stress and without (292,293). The authors reported that, when penetrating into the keratin fibers, water was in a configuration unfavorable to conduction, which led them to formulate the hypothesis that:

1. the water/keratin system behaved like a proton semiconductor.
2. prior to conduction “taking place,” it was necessary for a network of hydrogen bonds to be created and stabilized.

The principle of electric conductivity might thus be based mainly on the diffusion of protons within the network of hydrogen bonds of the keratin fibers.

In the case of hair (291), the results have been similar to those obtained with wool except for low levels of relative humidity where the variation in resistance is smaller than in wool which reflects a lower availability of water molecules to take part in the electric transport (Fig. 52). The term “incorporated water” has been suggested.

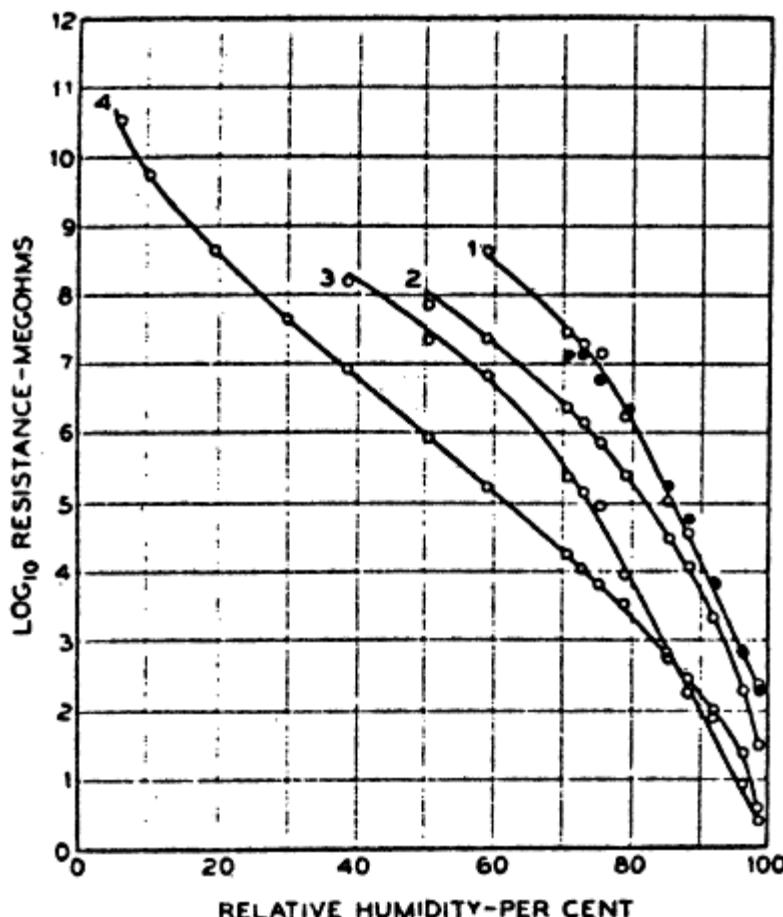


Figure 51 Insulation resistance as a function of relative humidity for Wool, Silk and Cotton. (1) Silk threads II (O sample 1; ● sample 2); (2) wool yarn; (3) silk threads I; (4) cotton threads I. (From Ref. 288.)

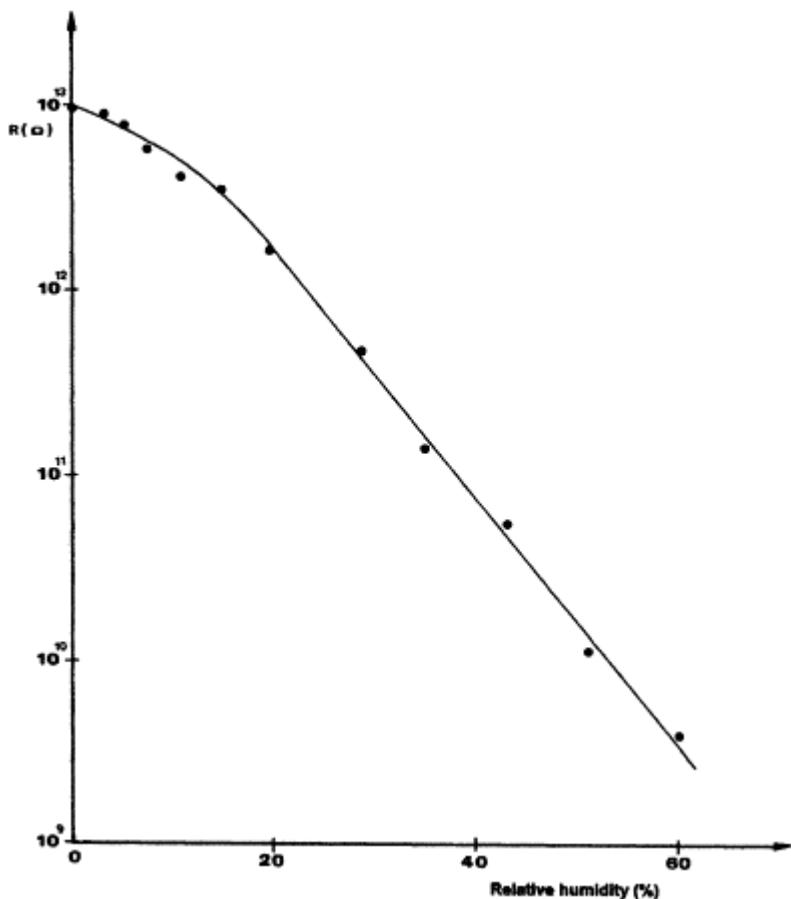


Figure 52 Variation of the electrical resistance of a natural human hair with relative humidity. (From Ref. 291.)

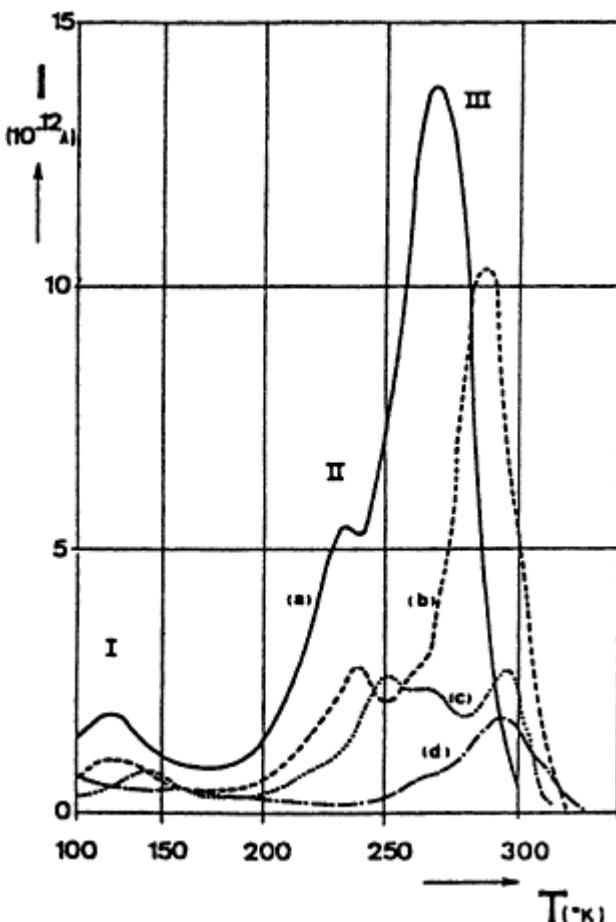


Figure 53 Variation of the curve $i(T)$ in a sample of natural human hair with the change in relative humidity of conditioning. (a) 85% RH; (b) 60% RH; (c) 45% RH; (d) 20% RH. (From Ref. 300.)

The dielectric properties of keratin have been investigated in parallel with studies performed in the field of polymers (294–299). The aim was the determination of the characteristic relaxations determining either “co-operative” or “local” molecular mobility of the keratin chains relative to their water content, the temperature and stress applied. In the case of wool, three absorption bands have been described (298): α , α' , and β bands. The first one is believed to be linked to the mobility of the principal chain, the second to water absorption and the third to the mobility of the side chains. Moreover, water appears

to act like a plasticizer. Further studies carried out in hair (300–302) using the depolarization thermal currents (DTC) method are in agreement with those carried out in wool. They confirm the three relaxations peaks (Fig. 53). The DTC method is particularly appropriate for studying keratin chain mobility and in particular rotation of the polar parts.

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2

The Hair Follicle: Structure and Function

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The hair follicle is a unique structure, characteristic of mammals. It is also a fascinating organ, since it encompasses most of the rules which control body homeostasis, as well as cell and tissue integration into an organized, autonomous and self-renewing biological entity. Indeed, as will be seen later on, hair follicle—like skin—is composed of epithelial and dermal compartments, which interact with each other from the very beginning of its morphogenesis, as early as the third month of human embryogenesis, and throughout life. In the adult scalp, each of the 150,000 (estimated) follicles undergoes, on its own, a cyclical process of degradation and renewal which partly repeats the sequence of events which takes place during its formation during fetal life. Another fascinating feature of the hair follicle is its high degree of autonomy with respect to its dermal environment, since it is apparently able to drive its own androgen metabolism, its own inflammatory response, and maintain both structure and function, namely hair fibre production, when dissected out and kept under *in vitro* culture conditions.

The hair follicle must be looked at as a unique dynamic biological structure as well as a paradigm of tissue homeostasis and integration, harboring most of the clues of normal organogenesis and development. Its self-controlled organization reflects a perfectly tuned internal communication network, the understanding of which could certainly lead to the elucidation of pathological processes or dysregulations affecting organs other than the hair follicle.

1. FUNCTIONAL COMPARTMENTS OF THE HAIR FOLLICLE

Microscopically, the follicle can be separated into four compartments, namely—starting from the scalp surface—the infundibulum, the sebaceous gland, the isthmus and the bulbar region (Fig. 1), which itself should be divided into upper, central, and lower parts. If one considers terminal scalp hair, it penetrates 4 mm deep into the dermis, down to the hypodermis. Histologically, the hair follicle is composed of different cell types of either mesenchymal or epithelial origin, organized into well-defined compartments (Fig. 2). The dermal compartment includes the dermal sheath and the dermal papilla. The epithelial compartment includes the hair matrix and



Figure 1 Whole mount of the hair follicle (HF) with the sebaceous gland (SG) and apocrine sweat gland (ASG).

three concentrical structures, namely the outer root sheath (ORS), the inner root sheath (IRS), and the hair shaft.

1.1. Dermal Compartment: Dermal Sheath and Dermal Papilla

The dermal sheath, which surrounds the entire length of the follicle, is made of two major components: (i) a connective tissue sheath produced by fibroblasts and mainly composed

of type I and III collagen, and (ii) a basement membrane. The basement membrane is made of laminin type 1, collagen type IV (Fig. 3a), fibronectin and proteoglycans, but its composition is not homogenous along the hair follicle, since (i) laminin type 5 is conspicuously absent in the lower part of the follicle (1) and (ii) a substantial swelling, known as the hyaline or vitreous membrane is observed from the central part to the distal part of the follicle. The lower part of the dermal sheath harbors a delicate network of blood microvessels.

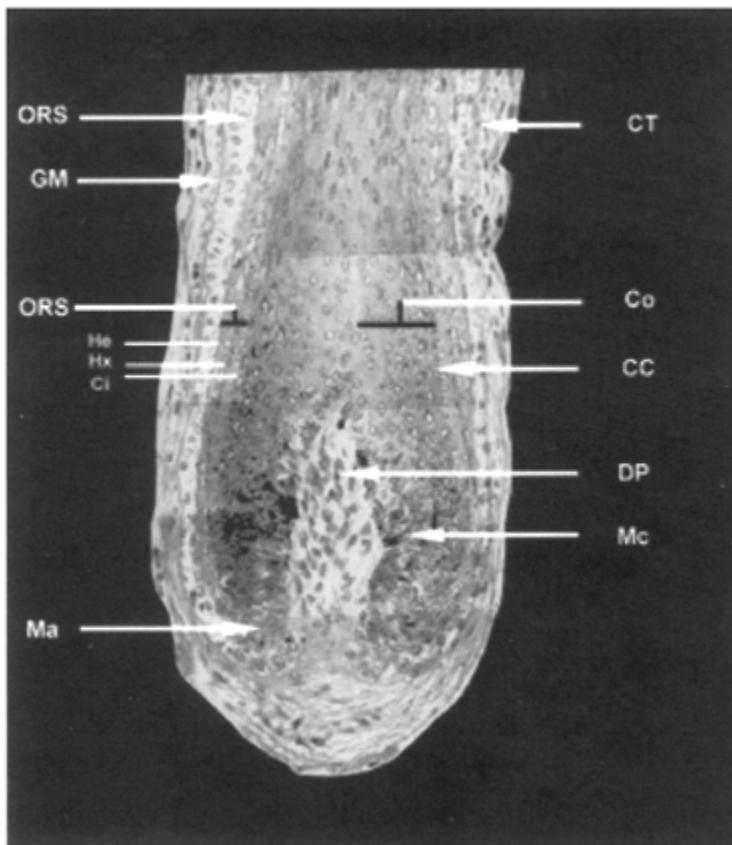


Figure 2 Structure of the anagen hair follicle: lower part of a terminal hair and identification of the different compartments on longitudinal histological section. CT: connective tissue sheath; GM: glassy membrane; ORS: outer root sheath; IRS: inner root sheath (with He: Henle's layer, Hx:

Huxley's, Ci: cuticle); Co: cortex; CC: cuticle cells; Mc: melanocytes; DP: dermal papilla; Ma: matrix cells.

The dermal papilla, almost totally embedded into the matrix epithelium of the lower follicle, is an oval mass of extracellular matrix harboring spindle-shaped fibroblasts. This mass is rich in laminin type 1, fibronectin, glycosaminoglycans, and sparse collagen fibers, while small blood vessels cross it. Due to the large number of low affinity binding sites of these extracellular matrix components, the dermal papilla can be considered as a reservoir for growth factors and the real "heart" of the follicle, since its size apparently controls the size of the follicle and it is capable of inducing follicular development. It is also endowed with specific markers such as the anti-apoptotic protein Bcl2 (2,3), and specific enzymatic activities such as alkaline phosphatase and prostaglandin synthase (Fig. 3b) (4).

1.2. Epithelial Compartment: Matrix, Outer Root Sheath, Inner Root Sheath and Hair Shaft

At the lower end of the follicle, the hair matrix consists of epithelial cells closely surrounded by the dermal sheath (outside) and the dermal papilla (inside). At a critical level corresponding to the widest part of the papilla (Auber line), matrix cells enter various differentiation programs which give rise to the ORS (Fig. 3c), the IRS (Fig. 3d) and the hair shaft (Fig. 3e,f). Under the Auber line of the bulb, matrix cells are poorly differentiated and actively divided (Fig. 3g), probably with the highest mitotic rate in the body. The growth rate of the hair follicle, roughly 0.3 mm/day, directly reflects this mitotic activity. The ORS is composed of two cellular structures, one is the so-called companion (innermost) layer and the other ORS cells. Interestingly enough, even though the ORS lies in continuity with the epidermis, its differentiation pattern is different, particularly with respect to keratin and cadherin expression. Moreover, the ORS expression pattern of $\beta 1$ -integrins, epidermal growth factor (EGF) receptor, desmoglein, K19- and K15-keratins (1,5) clearly shows that this external follicle compartment can be further divided into three parts: lower, central, and upper. The next major epithelial compartment, the IRS, separates the ORS from the hair shaft. The IRS is composed of three cell layers, namely Henle's layer, Huxley's layer and cuticle. A multistep differentiation program characterizes the IRS (6) resulting in a rigid structure which may serve as a guide and/or mold to the growing shaft. The IRS may also serve as a diffusion barrier, involved in the maintenance of hypothetical morphogenic gradients inside the hair follicle. Finally, in the center of the follicle grows the hair shaft, which is composed of three different epithelial cell types, namely cuticle, cortical, and (where present) medulla cells. It is noteworthy that IRS cuticle cells and hair shaft cuticle cells are head-to-tail oriented. The hair shaft is mainly composed of specific "hard" α -keratins, the catalog of which has been recently established (7,8). The maturation of terminal scalp hair is supported by an orderly and finely tuned expression pattern of these keratins. For example, the hHa2/hHb2 keratin pair seems specific to hair cuticle, whilst hHa1/hHb1,

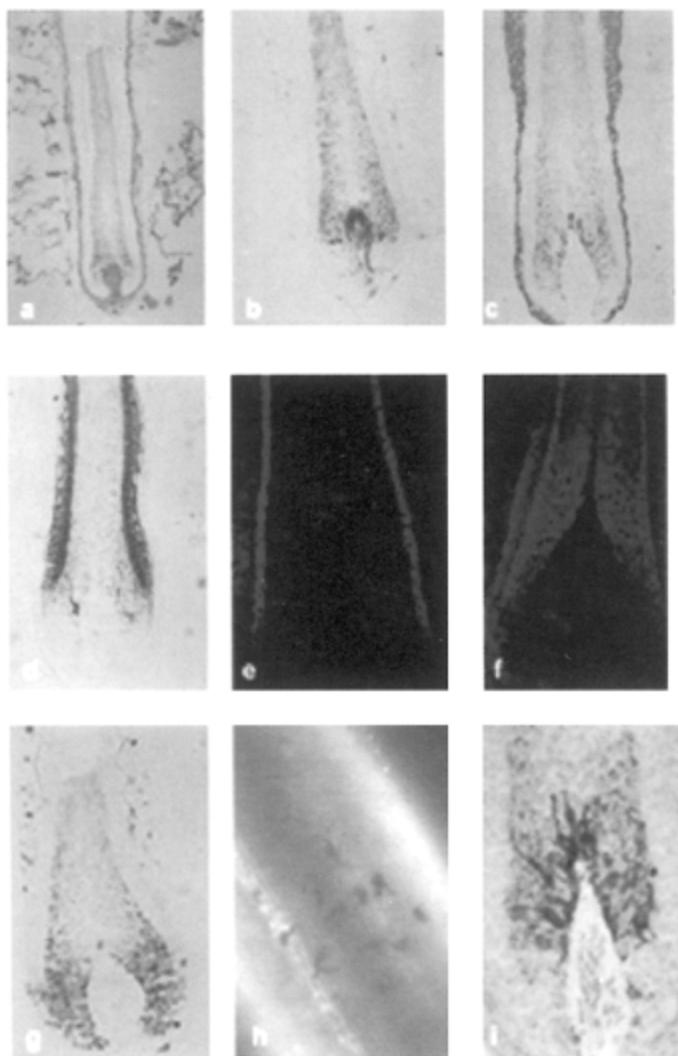


Figure 3 Immunohistological characterization of the different compartments of the hair follicle: (a) collagen IV: connective tissue sheath and dermal papilla, (b) type-1 prostaglandin synthase: dermal papilla, (c) keratin K14: outer root sheath, (d) trichohyalin: inner root sheath, (e) keratin Hb2: hair shaft—cuticle, (f)

keratin Ha5: hair shaft—cortex, medulla, and cuticle, (g) Ki67: proliferative matrix cells, (h) keratin 20: Merkel cells, (i) p-mel 17: pigmentation unit.

hHa3/hHb3, and hHa4/hHb6 keratin pairs are specific to the hair cortex. hHa5 alone might be a medulla marker found in largest hair follicles, while hHa7 might be a specific marker for fine, vellus hair (7). The hair shaft is also characterized by the expression of specific keratin-associated proteins (KAPs) (9–12). Indeed, in the hair cortex, hair keratin intermediate filaments are embedded in an interfilamentous matrix, consisting of hair KAPs, which are essential for the formation of a rigid and resistant hair shaft through their extensive disulfide bond cross-linking with abundant cysteine residues of hair keratins. More than 100 hair KAPs have been found in various species and classified into 17 families (KAP 1–17), exhibiting high sulfur (16–30% cysteine) and ultrahigh sulfur content (>30% cysteine).

1.3. Other Components of the Hair Follicle

Gathered on top of the dermal papilla, numerous large, dendritic melanocytes of neural crest origin form the pigmentation unit of the follicle (Fig. 3h). They lie in the basal layer, closely interacting with the basement membrane and they extend their dendrites into the intercellular spaces of the bulb to reach cortical cells to which they transfer melanosomes, under a very cell-specific and restricted process. One melanocyte thus interacts with five cortical cells. Another set of melanocytes are found, dispersed in the basal layer of the upper third of the ORS. These melanocytes are amelanotic and probably represent a reservoir for the epidermis and cyclically renewed follicles (13). In the upper part of ORS, at the level of the isthmus, and in the sebaceous gland, a few Langerhans cells can also be detected, probably with a role as immune sentinels. A very small number of Merkel cells are also present at the level of the isthmus, disposed as an easily identified ring through specific keratin-20 (K20) expression (Fig. 3i). Their role is unknown, but these Merkel cells may be involved in some neuroregulatory loop, as a network of sensory nerves converges at this level.

2. THE DYNAMICS OF HAIR FOLLICLE MORPHOGENESIS

Hair structure development from primitive embryo epidermis starts at 8–9 weeks. It then follows several ordered stages, namely pre-germ (10–11 weeks), hair germ (11–12 weeks), hair peg (12–15 weeks), bulbous hair peg (15–18 weeks), and lanugo hair follicle (18 weeks to birth). During the bulbous hair peg stage, two swellings appear on the side of the follicle. The uppermost swelling and the lowermost swellings will give rise to the sebaceous gland and the bulge, respectively. The bulge will act as the attachment site of the arrector pili muscle. A third swelling sometimes develops above the sebaceous gland, giving rise to the apocrine sweat gland (14).

The hair follicle is composed of epithelial components (matrix, ORS, IRS, and hair shaft) and dermal components (dermal papilla and dermal sheath). In fact, during the development of the hair structure and from the very beginning of this process, ectodermal and mesodermal elements co-operate through reciprocal interactions and remain in intimate contact throughout the life of the follicular unit (15,16). The scenario of this communication stands as follows. A first signal is given: "make an appendage" (the source, whether epithelium or mesenchyme is still unclear). The epithelium then signals to the mesenchyme to prepare to make an appendage. In return a dermal message induces a local thickening of the epidermis to form a placode which in turn sends a message to the dermis to induce mesenchymal cell aggregation, beneath the placode. A second dermal message signals back to the placode for hair germ development which proceeds further and induces the mesenchyme to make a dermal papilla. Ultimately, the dermal papilla drives subsequent hair follicle morphogenesis. Above the matrix, a cone of cells differentiates to form the hair, while a second concentric cone will form the IRS.

Such a delicately tuned series of events must be tightly controlled, both in time and spatially, as reflected by the ordered stages described above and the patterning of hair growth on the human body. Many factors involved in this communication network have now been identified, and some control pathways deciphered. These factors include homeobox gene products (essential for the patterning and development of many segmental structures in both invertebrates and vertebrates), transcription factors such as lymphoid enhancer factor 1 (Lef-1) and winged-helix-nude (whn), signaling and adhesion molecules such as bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs) and their receptors, transforming growth factor- β s (TGF- β s) and their receptors, Wnt (homologous of *Drosophila* wingless), β -catenin, hedgehog (shh), notch, tenascin, cadherins, epimorphin, nuclear receptors, extracellular matrix, and proteoglycans (17–19). However, most of these factors are also involved in tooth and feather development, and even lung development. This means that even though the actors have been identified, the choreography and the rules that specify hair follicle morphogenesis remain to be established. These rules involve autocrine and paracrine regulatory loops (20), reaction-diffusion mechanisms (21) as well as oscillatory processes which control both spatially and in time the distribution of activating and inhibiting morphogenic fields and/or gradients (22). Moreover, depending on the ethnic background, differences in hair density have been noticed, being twice as high in whites compared to blacks and Koreans (23).

3. THE DYNAMICS OF HAIR FOLLICLE CYCLE

3.1. The Different Phases

From the time it is formed, the human hair follicle grows following a continuous cyclic pattern. A period of growth (anagen) is followed by involution and resting periods, known as catagen and telogen, respectively. After a latency or exogen phase (24,25), during which the hair shaft is shed without regrowth, a new anagen phase is turned on, the early stages of anagen recapitulating fetal folliculogenesis as the lowermost part of the follicle must reform to subsequently produce a new hair shaft. This cycle is driven by deep remodeling events involving both the follicle itself and its immediate dermal

environment. Depending on the body site, the anagen phase can last from 1 to 6 months (arms, fingers, eyelashes) or 5 to 7 months (legs), up to 1 to 10 years (adult scalp). With respect to the 100,000–150,000 scalp hair follicles which grow hair fibers at an average rate of 396 ± 55 and 256 ± 44 $\mu\text{m}/\text{day}$ in Caucasians and Africans, respectively (26), the mean duration of anagen, catagen and telogen is 3 years, 3 weeks, and 3 months, respectively (24). The latency period, which is noted in 80% of hair cycles, lasts from 2 to 5 months on average before a new hair cycle is engaged (24). Interestingly, hair cycles are asynchronous from one follicle to another, and the phase durations of the “*n*th” cycle has no influence on the “*n*th +1” cycle (25). A mathematical model—the follicular automaton—has been developed, which takes into account the stochastic transition of each follicle between successive phases (27). A decrease in the mean duration of the anagen phase as well as a lengthening of the latency period have nevertheless been demonstrated during the course of natural aging (24). A parallel decline in average hair diameter is observed (28). Rarely more than 10% of follicles are in telogen but an overall annual periodicity has been noted, showing a maximal proportion of telogen hairs at the end of summer and the beginning of autumn (29). To possibly control the duration of each phase, and slow down the natural process of hair loss, it is of upmost importance to understand the molecular signals which control the anagen to catagen, as well as telogen to anagen transitions. Unfortunately, little is known with respect to these discrete events.

3.2. Anagen to Catagen Transition

The most obvious and early feature of this transition is the cessation of pigment production and mitosis of bulb matrix cells, as well as the arrest of the IRS differentiation program (6). The lower part of the follicle undergoes apoptotic degeneration and becomes club-shaped, moving upwards to reach the level of the arrector pili muscle insertion. The bulbar region of the follicle collapses, a minimal program of differentiation being observed in the epithelial column, under the club hair (6). Concomitantly, the vitreous membrane becomes thickened and the dermal papilla physically separates from the club hair. However, the dermal papilla ultimately follows the upward movement of the lower follicle in the follicular sheath. Macrophages clean up follicular remnants, at the end of catagen. One-third to one-half of the follicular length is lost during the catagen process. Only the hair papilla and a residue of the matrix remain.

3.3. Telogen to Anagen Transition

Telogen is the resting phase of the hair cycle, characterized by a lack of cell division and the presence of inactive melanocytes in the telogen germ. The control of this phase is still unknown, the dermal papilla appears atrophic, as a tightly packed ball of cells, devoid of extracellular matrix and microvessels. After a few months, the spontaneous onset of a new anagen is observed, the stimulus for initiating this new cycle being also unknown. A possibility is that an inducing signal may arise within the follicular epithelium acting on the resting papilla, thus re-establishing the communication between epithelial and dermal compartments of the follicle, and initiating a new morphogenetic process. The similarity of this process with that observed during fetal folliculogenesis is striking. Anagen onset is marked by a proliferation of matrix cells and down-growth of the follicle, through the

dermis and along a pathway of fibrous streamers. The proliferation of a melanocyte subset is also observed very early after anagen onset, and only those melanocytes which will co-migrate with the newly formed bulb in close apposition with the dermal papilla, will show active melanogenesis (13). The different compartments are progressively put back in place, while the neurocutaneous and vascular networks are remodeled. All these processes clearly require intense tissue remodeling, driven by extracellular matrix synthesis and controlled release of proteases, particularly matrix metalloproteases (MMP) (30), as well as transcription and silencing of growth factors and structural genes. A number of players in this orchestra have been identified, but the full score is still obscure.

3.4. Stem Cells

The succession of involution and renewal phases implies the existence of stem cell reservoirs, from which the follicle and its pigmentation unit will be cyclically regenerated. We have recently shown that the amelanotic melanocytes present in the upper ORS are probably a melanocyte reservoir, with a restricted subset of melanocytes present in the telogen follicle being reactivated both in terms of mitosis and melanogenesis (6). With respect to the epithelial stem cells from which the human follicle is regenerated, their location is not clearly established, at least in the anagen follicle and has been debated for over a decade. Histological data have clearly established that anagen onset is marked by an intense proliferation of keratinocytes located in the lowermost part of the isthmus at the site of attachment of the arrector pili muscle. In the mouse hair follicle, a discrete structure has been identified in the bulge region, which contains slow cycling cells which under activation can be induced to proliferate and give rise to transient amplifying cells (31). In the mouse hair follicle, the bulge is thus considered as the stem cell reservoir, which would be cyclically reactivated by factors thought to be produced by the dermal papilla. This bulge structure cannot be identified in the adult human follicle. In fact, functional (32,33) and immunohistochemical studies (1,5) have recurrently suggested that the epithelial stem cells of the human hair follicle could be located somewhere else in the ORS. A unifying “split-fuse” hypothesis has recently been proposed stating that two stem cell reservoirs identified by $\beta 1$ integrin (1) and keratin 19 expression (5) could exist in the human anagen hair follicle—one in the upper ORS, one in the lower ORS, and that both reservoirs fused in late catagen and telogen and split again at anagen onset (5) (Fig. 4).

4. THE MOLECULAR CONTROL OF HAIR GROWTH AND HAIR CYCLE

Over the past 12 years, the list of factors capable of influencing hair growth and the hair cycle has dramatically increased, emphasizing the complexity of the monitoring network of this integrated organ. The problem today is not so much to identify control factors or groups of factors, but rather the hierarchy of control pathways in which they are active. We shall focus mainly on hormones, growth factors, and cytokines.

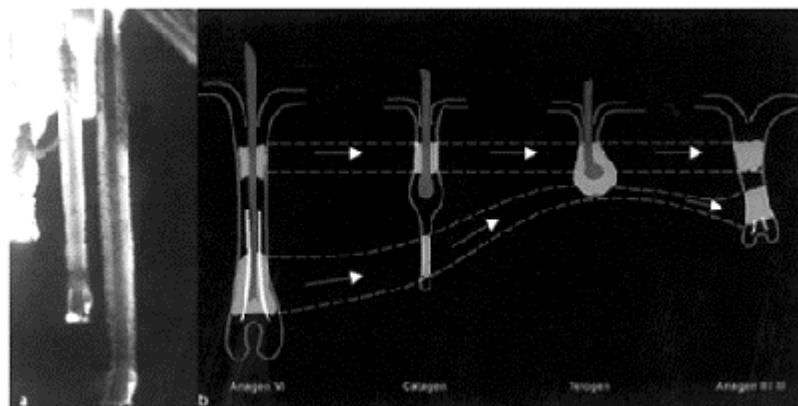


Figure 4 (a) The two stem cell reservoirs identified by keratin 19 expression in the outer root sheath, and (b) the split-fuse hypothesis.

4.1. Hormones

Androgens are among the most important modulators of hair growth, as beard, body hair, and hair loss patterns are key actors of sexual dimorphism in the human species. Strikingly, androgens are necessary for conversion of vellus hair to terminal hair on the body and beard region, but they also play a role in converting terminal hair to vellus hair on certain areas of the scalp. It is generally accepted that testosterone must be activated by the enzyme 5α -reductase into the more potent metabolite dihydrotestosterone (DHT), which then interacts with nuclear androgen receptors to modulate target gene transcription. The human hair follicle, together with the sebaceous gland, have the necessary enzymatic equipment to perform testosterone metabolism in an autonomous way (34). Micrografting of individual follicles has clearly established that androgen responsiveness is at the level of the follicle itself, the primary target of androgens being the dermal papilla (35). The opposing effects of androgens on beard and scalp follicles probably reflect differences in (i) androgen receptor content, higher in beard dermal papillae than in occipital hair dermal papillae (35,36), and/or (ii) 5α -reductase activity, which is higher in beard than in scalp (37). The 5α -reductase isoforms are also differentially expressed depending on body area and even on the scalp, the major form expressed in the scalp hair follicle is the type 1 5α -reductase (34,38). The question of the mode of action of androgens on the hair follicle remains open but the genomics of the dermal papilla will probably give some clues in the very near future.

Other non-steroidal hormones may have a role in hair growth control, as we have recent evidence for the presence of retinoic acid receptors, vitamin D receptors (39), thyroid hormone receptors (40) and peroxisome proliferator-activated receptors (PPARs) (41) in human hair follicles. Most of the natural ligands of these receptors have an effect on *in vitro* hair growth and survival, but depending on the concentration, the effects may

be either beneficial or detrimental. Clearly most of these hormones can favor hair growth, but only within a biological window which may vary from one follicle to another and/or from one body site to another.

4.2. Growth Factors and Cytokines

Beside hormones, numerous growth factors and cytokines have direct effects on the hair follicle, some of them being produced by the follicle itself. Depending on the distribution of their receptors, growth factors could exert their effect on growth and cycling of the follicle either through an autocrine or paracrine route. The list of these factors is already long, and includes members of the EGF-, FGF-, TGF β -family as well as hepatocyte growth factor (HGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), parathyroid hormone-related protein (PTHrP), and interleukin-1 (IL-1). For example, EGF has a defleecing action when injected in sheep (42), FGF5 might be involved in the induction of anagen to catagen transition (43), IGF-1 in the maintenance of the anagen phase (44), and FGF7 (KGF) might be required for normal follicular development (45). However, the effects of growth factors on hair growth and cycle have mostly been observed in animal models such as knockout mice, and the biological weight of these factors in human hair control remains to be established. Nevertheless, by using in vitro grown human anagen hair follicles, we have been able to show that human hair is very sensitive to IL-1 α . It induces growth arrest and disruption of the follicle, together with a quick induction (within 6 hr) of chemokines such as IL-8 and IL-6 as a result of the activation of IL-1 receptors (46). We thus believe that the human hair follicle is able to trigger, in an autonomous way, its own immune response leading to lymphocyte infiltration and localized development of inflammation. This circuit might be involved in the development of alopecia, where infiltrates are often observed at the periphery of infundibulum and sebaceous gland, generally associated with the presence of thickened fibrous tracts (47).

5. THE GENETICS OF HAIR GROWTH AND PIGMENTATION

5.1. Hair Growth and Morphogenesis

Androgenetic alopecia is widespread and its prevalence is a strong argument in favor of a polygenic transmission (48). The inheritance of androgenetic alopecia is now almost taken for granted, but no genes, no loci have definitely been associated with androgenetic alopecia. For example, allele A2 of the CYP17 gene coding for 17 α -hydroxylase has been found to be associated with male pattern baldness (MPB), but was denied as a primary genetic defect (49). Similarly, polymorphism of the androgen receptor gene, resulting in the insertion of polyglutamine stretches at the N-terminal end of the receptor, was found to be associated with MPB, but again not as a primary genetic defect (50). The genes encoding the two 5 α -reductase isoenzymes, although likely physiological candidates, do not appear to be associated with MPB (51). With respect to alopecia areata, a relationship was found between the severity of the disease and the presence of allele 2 of the IL-1

receptor antagonist gene. Moreover, disease proneness was found to be associated with some alleles of HLA-DR, -DQ class II antigens (52). Clearly alopecia areata also appears as a polygenic disease. In the case of alopecia universalis, several mutations have been localized in the *hairless* gene (53,54). This gene encodes for a putative transcription factor, which may function as a co-repressor of the thyroid hormone receptor (55) and be involved in the regulation of keratinocyte apoptosis during the catagen phase. The complete absence of scalp hair, eyebrows and eyelashes in the human *nude* phenotype has been associated with a mutation affecting the human *whn* (*wing-helix-nude*) gene, another transcription factor specifically expressed in the epithelial cells of the skin and thymus and which is developmentally regulated and controls cell-fate decisions (56). Together with the *hairless* gene, this finding suggests a role for cell-type-specific transcription factors in hair-follicle cycling and morphogenesis.

The genetic study of hypohidrotic ectodermal dysplasia, a disease characterized by abnormal morphogenesis of teeth, hair, and eccrine sweat gland, has recently led to evidence of a new control circuit involved in the patterning of hair growth (57) and placode formation. Two partners have been identified, a transmembrane protein called ectodysplasin and its receptor *downless*, a member of the tumor-necrosisfactor receptor superfamily, both of them being possible actors, together with bone morphogenetic proteins, in a reaction-diffusion model. Like the Wnt/ β -catenin/ Lef-1 pathway, such findings confirm the role of specific transmembrane signaling factors in hair follicle patterning and morphogenesis.

The above mutations affect early follicle morphogenesis. Other mutations have been related to hereditary hair dystrophy syndromes such as monilethrix. In this disease, single-point mutations were found in the genes coding for either type II hair keratin hHb6 or hHb1. These point mutations induced non-conservative amino acid substitutions, and thus provided direct evidence for involvement of hair keratins in hair disease.

5.2. Pigmentation

In mammals, at least 80 genetic loci regulate pigmentation and among them 21 genes have been cloned and characterized, in mice and in humans (58). Mutations of these genes are associated with various forms of human pigmentary diseases including ocular and oculocutaneous albinism, piebaldism, Hirschsprung's disease, and Waardenburg's syndrome. Little is known, however, about the genetics of progressive hair graying or hair color. Recently, loss of function mutations of the human melanocortin 1 receptor (MC1R) have been associated with red hair (59), while functionally variant alleles of this gene have been demonstrated to cause a broad range of pigmentation phenotypes.

6. CONCLUSION AND PERSPECTIVES

This journey through the biology of hair aimed at showing the hair follicle as a unique, autonomous, and fully integrated organ, where numerous control networks compete against each other and/or co-operate to finally orchestrate a delicately tuned score of growth, involution, and regeneration processes. The hair follicle is a dynamic organ, oscillating between two equilibrium stages, anagen, and telogen. Although our knowledge of the

molecular cascades that control the hair cycle has dramatically progressed over the past 12 years, we are still like children faced with the pieces of a puzzle. We have to assemble the data and pieces, and try to put in order the key events, signals and scheduling of this tremendous machinery, and to establish some hierarchy in this mass of information. Indeed, the hair follicle is a paradigm of cell-cell interactions aimed at producing one thing, a pigmented hair shaft. The system is so intricate and stable, that even after dissection, it keeps working in a proper way. Clearly, this single organ contains and expresses all the rules of tissue homeostasis, which probably apply to most human tissues.

No doubt the hair follicle is far from a simple organ. It is like a living test tube, containing a myriad of answers to fundamental biological problems. We are just at the very beginning of reaching some sort of understanding, and let's hope genomics, proteomics, and molecular genetics will help to boost research and shed new light to solve the puzzle.

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3

Scalp and Hair Hygiene: Shampoos

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1. INTRODUCTION

“Champo” is a word in Hindi (the main language of India) meaning to massage, to knead. It became “shampoo” in English and, by extension, further designated a product used for cleansing the hair and scalp. Except for a few marginal products, shampoos are available as a liquid, gel or cream formulated using surface-active agents (surfactants) to yield wetting, emulsifying, foaming and detergent properties (detergency being the process of elimination of soils from a surface in the presence of water) capable of cleansing both the hair and the scalp.

Shampoos are the most widely used products for cleansing and care of the hair. They represented about 4 billion units sold worldwide in the year 2000, or approximately 50% of the total hair product market.

While originally confined to a strictly detergent function, when soaps were replaced by synthetic surfactants, they evolved, firstly becoming less detergent and softer on the scalp. Later, they contributed to beautifying the hair to satisfy the growing demands of the consumers, at the same time as other hair treatments were developed, such as hair dyes and permanent waving.

The frequency of their use has also increased in parallel with improvements in living standards and in hygiene. For example, in Europe, in the last 20 years, the frequency of shampoo use has increased from one or two to three times weekly; they are used daily by 20–30% of the population (principally among the under 30s and men in general). In the United States, 80% of people wash their hair every day and even twice a day. This figure rises to 90% in Japan.

The act of hair-washing has become a frequent hygienic measure, on a level with washing one's hands or cleaning one's teeth. Constant progress in the formulation of shampoos has led to their development as beauty products caring for the hair.

Currently, virtually all shampoos can be used frequently and make the hair easier to manage. These two characteristics are not always systematically referred to during market research studies. The main expectations of consumers with “normal” hair, as expounded in a survey carried out in France in 1997, were that it should:

- cleanse the hair well
- not dry the hair out

- not make the hair oily
- be easily rinsed out
- be gentle to the hair
- not weigh it down
- make the hair look beautiful
- make the hair soft to the touch
- make it shiny.

The in-use properties, though not mentioned here, are obviously equally important in the assessment of a shampoo.

The types of dissatisfaction with the product used regularly, generally few in number, concern:

- the feeling that the hair is coated
- a lack of fullness and volume of the hair
- a lack of spring and bounce over time
- insufficiently nourished hair
- failure of the hair-style to last.

The coexistence of the cleansing and hair-care functions represents a true paradox, demanding very delicate adjustment of the balance of a formulation, taking into account a number of factors: in particular:

- Hair type (dry, oily, bleached, permanent-waved, short, long, curly, smooth, European, African American, Asian, etc.) and scalp condition (dandruff, seborrhea, etc.).
- The consumers age, lifestyle and habits (frequency of application and use of other hair products such as conditioners and hair-styling products).
- Hair-styling techniques.

To satisfy the “mosaic” of tastes and needs of consumers, hair cosmetic laboratories must therefore offer a wide choice of shampoos with, of course, varying cosmetic or conditioning properties but also varying in texture, foaming properties and perfume. In addition, all these products must meet the requirements of safety, eye comfort and respect for the environment.

Behind a “simple” shampoo product, often perceived as banal, especially if it is used on an everyday basis, lies in truth a multitude of constraints and complex parameters that are often hard to reconcile.

2. CHEMISTRY OF SURFACTANTS USED IN SHAMPOOS

Surfactants, whose role is considered in Sec. 3, are the basic ingredients of shampoos in that they provide the foaming and detergent properties. These are amphiphilic substances—that is to say molecules within which regions exist that have very different solubility characteristics, i.e., hydrophilic groups having a high affinity for water and hydrophobic groups such as a long hydrocarbon chain having a low affinity for water. They are classified according to their hydrophilic grouping, which may be ionic, i.e., carrying anionic or cationic charges or non-ionic.

Ionic surfactants are electrolytes whose ionic pole is located at the extremity of a long hydrocarbon chain (so-called fatty chain) and are subdivided into categories according to their electrical charge. Anionics bear a negative charge, cationics have a positive charge.

Non-ionic surfactants contain no polar group in their structure and are thus devoid of any charge.

2.1. Anionic Surfactants

2.1.1. Soaps

Soaps were the first cleansing agents used on the hair and skin. They are the salts of fatty acids obtained either by the saponification (from the Latin *sapo*, soap, means the chemical transformation into soap) of triglycerides, i.e., alkali treatment releasing glycerol and fatty acids, or by neutralization of fatty acids.

A great variety of plant oils and fats can be used as raw materials in the production of soaps by saponification. Those most commonly used are coconut (copra), palm, olive, or castor oil. For the transformation of fatty acids into soaps by neutralization, either mineral (soda, potash), or organic bases (usually alkanolamines) are used.

Copra soap lathers readily and the foam is aerated, abundant, and short-lived. Oleic soap yields a more unctuous, oilier foam; castor oil forms a dense, unctuous, and oily foam.

Solubility in water is the primary condition for a soap used as a surfactant in a shampoo. This condition is met in soft water. In hard water, however, alkaline-earth metals are formed which precipitate out and leave a deposit on the hair, causing it to look dull.

Such soaps of alkaline metals (sodium, potassium, etc.) possess, in addition, a negative property: highly susceptible to hydrolysis in solution, they lead to the release of a metal hydroxide according to the following equation (R=fatty chain, M=alkaline metal, MOH=alkali):



The resultant alkalinity is detrimental to both skin and the hair cuticle. The subsequent application of an acidic hair-care product or conditioner (vinegar in the past) partially mitigates these disadvantages but the repeated applications of soap to the scalp is, anyway, undesirable from skin care and esthetic viewpoints.

Hence, it is easy to understand why soaps are no longer used as the detergent basis for a shampoo. They can, however, be used as additives in a formulation composed of synthetic anionic or non-ionic surfactants. The insoluble soaps such as those of aluminum or magnesium are sometimes used as opalescent or thickening agents.

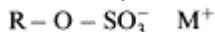
2.1.2. Sulfates

It is clear that the growing popularization of shampoo went hand in hand with the commercial development of sulfated fats. Sulfates share, in general, the following characteristics:

- as monoesters of sulfuric acid, they are potent electrolytes,
- the C–O–SO₃⁻ bond is more readily hydrolyzed than the C–SO₃⁻ bond of the sulfonates.

Long-chain sulfates, preferably where R=C₁₂–C₁₄, have a good cleansing capacity related to their potent emulsifying and solubilizing properties.

Fatty Alcohol Sulfates (or Alkyl Sulfates).



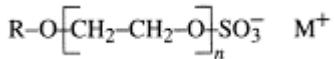
[1]

These are prepared by sulfation of fatty alcohols. Linear primary alcohols obtained by catalytic reduction under high pressure of fatty acids, their esters or triglycerides, are generally used. Petrochemistry is another source of fatty alcohols. A major innovation in this field came from the synthesis of linear fatty alcohols from ethylene: the compounds obtained are globally identical to those obtained from natural fats.

The most appropriate alcohols which yield sulfates with good cleansing power are the mixtures of dodecanol (C₁₂ alcohol) and tetradecanol (C₁₄ alcohol), often referred to as lauryl alcohol. Their sulfates present a range of very valuable properties that can be modulated by the judicious choice of the counter-ion M⁺.

According to Adam and Neumann (1), it is the ammonium salt that presents the best foaming capacity on hair; whereas the triethanolamine salt yields slightly inferior results. It is also well known that ammonium salts are more readily soluble than sodium salts. The authors did not detect any significant difference between these various salts with regard to ocular tolerance.

Polyethoxylated Fatty Alcohol Sulfates (or Alkylether Sulfates) (2,3).



[2]

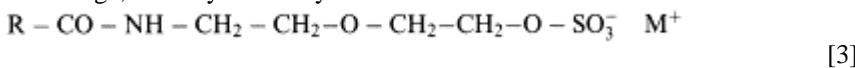
These surfactants correspond to the general formula [2], in which R is a fatty hydrocarbon chain and M⁺ a metallic or organic ion, such as that defined above, where n is the mean number of ethoxy (or ethyloxy) units, generally less than 5. These compounds differ from the preceding ones by the presence of polyethoxy units between the fatty hydrocarbon chain and the sulfate group. As long as n remains of a low value, the ethoxylated fragments contribute toward enhancing the surface activity of this class of compounds and, in turn, its foaming, detergent, and dispersant properties. Additionally, they bring improved solubility in cold water.

Adsorption onto keratin and the cutaneous “response” (potential irritant effects) decrease markedly when the value of n increases. The alkylether sulfates, whose alkyl radical is a C₁₂–C₁₄ chain with an n value of 2 or 3, constitute the best compromise in the search for an ingredient that both lathers and cleanses well while, at the same time, is well tolerated by the skin.

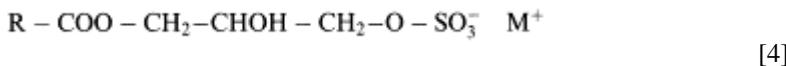
When an extremely mild product is a priority, higher n values are called upon, resulting in inferior foaming properties that decrease significantly when n>3. Magnesium

salts have been used in the past since they are considered as milder than sodium salts, for example.

Alkanolamide Sulfates. The fatty acid ethanolamide sulfates are too unstable for incorporation in an aqueous formulation. Diglycolamide sulfates [3] do not present this disadvantage, but they are rarely used.



Glyceride Sulfates.



This category of anionic surfactants was developed notably by the Colgate-Palmolive-Peet Company (4). Although they are sensitive to hydrolysis, they have been proposed for the formulation of liquid or aerosol shampoos.

Sulfated oils and fats fall within this category but, to date, they remain of little use in shampoos.

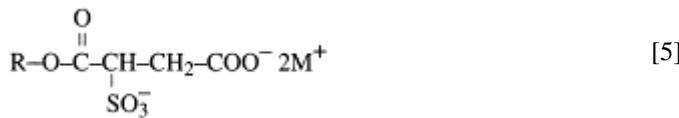
2.1.3. Sulfonates

Sulfonic acids being strong acids, their salts do not hydrolyze in solution, i.e., do not release bases, unlike soaps.

The paraffin sulfonates and alkylbenzene sulfonates developed during World War II are used for household detergents but not in cosmetics with regard to their irritant potencies.

Alpha-Olefin Sulfonates. These are complex mixtures obtained by sulfonation of α -olefins with sulfuric anhydride. They have exceptionally good stability at low pH, little sensitivity to hard water, a high foaming capacity in the presence of sebum, a fairly low cloud point and good solubilizing properties. Moreover, they offer faint color and odor (5). These surfactants are particularly suitable for the formulation of conditioning shampoos, at an acidic pH.

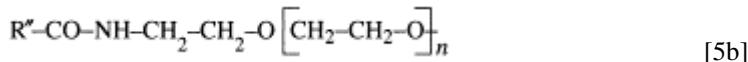
Sulfosuccinates (6). These are the hemiesters of sulfosuccinic acid in which the carboxyl and sulfonic groups are neutralized:



R is a hydrocarbon group that may be polyethoxylated (5a) or a polyoxyethylenated fatty amide (5b):

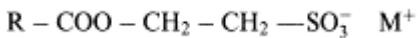


or



The most valuable compounds in formulating shampoos are those in which the group R, R' or R''-CO comprise 12–14 carbon atoms. They present good foaming and cleansing properties. The sulfosuccinates are exceptionally gentle toward the skin and the eye cornea. Their main disadvantage remains the sensitivity of their ester group to hydrolysis. This limits their formulations within a pH range of 6–8, preferably around 6.5

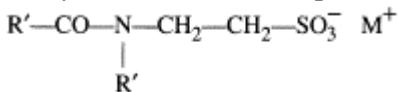
Acylisethionates.



These are among the first synthetic surfactants. Developed by I.G. Farben, they were marketed under the brand name Igepon A®.

Most of the previous comments expressed about mildness and stability of sulfosuccinates also apply to these acylisethionates. Poorly soluble in cold water, they are mostly used in cream shampoos and syndets.

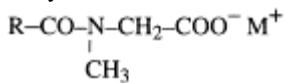
Fatty Acid Sulfoethyl Amides and, more specifically, N-Acyltaurides.



Unlike the isethionates and sulfosuccinates, these surfactants are chemically stable. Their main advantages reside in the quality of the foam developed (rapidity, volume, consistency, and great mildness) and their ability to disperse lime soaps.

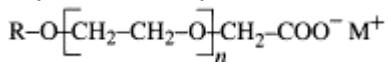
2.1.4. Carboxylates

Alkaline Salts of N-Acy Amino Acids. Of good foaming and cleansing properties, these surfactants are more readily soluble in hard water than soap. The best known are the acylsarcosinates (8). They are skin and hair-friendly, to which they confer a pleasant feel.



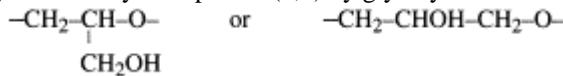
The derivatives of the acylation of protein hydrolysates by fatty acid chlorides also belong to this category. They are known under the commercial names of Maypon® and Lamepon®. These mixtures of “lipo-oligopeptides” and “lipo-amino acids” do not foam as well as the lauryl sulfates but their cleansing properties remain satisfactory. They also afford a good capacity in dispersing lime soaps and an easy rinsing. They are appreciated for their hair-conditioning properties and good skin tolerance.

Salts of Polyethoxylated Carboxylic Acids (7).



Their properties are very similar to those of other carboxylates. With higher values of n , they are still compatible with cationic agents and readily soluble at low pH.

The ethoxy chains may be replaced (8,9) by glyceryl structures such as



Combinations of polyethoxylated carboxylates and alkylether sulfates or sulfonates have also been suggested as the cleansing components of a shampoo.

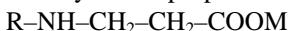
2.2. Amphoteric Surfactants

Formally, amphoteric surfactants behave like cationics (positively charged) at low pH and like anionics (negatively charged) at high pH. At an intermediate pH, known as the isoelectric point, they carry both negative and positive charges (dipolar ion or "zwitterion" structure).

In contrast, other amphoteric surfactants, often called dipolar surfactants, are true zwitterions over a wide range of pH.

Among the numerous amphoteric surfactants commercially available, the simplest, chemically speaking, are the derivatives of carboxyethylated fatty amines such as:

N-Alkylamino propionates:



[10]

or N-alkylamino dipropionates:



[11]

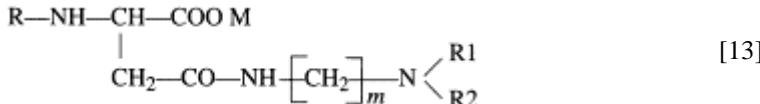
The amphoteric surfactants obtained by carboxymethylation of fatty "imidazolines" constitute another extremely interesting class, of which the chemical structure was long poorly understood.

According to recent studies (10), the main compound of this type of surfactant has a non-cyclic (i.e. non-imidazoline) structure of the following formula:

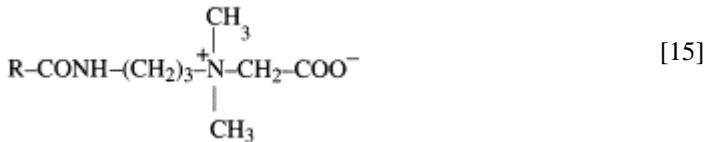


[12]

Other amphoteric surfactants, derived from asparagine, result from the addition of a fatty amine to cationic maleamides. They have been used in the formulation of shampoos dedicated, mostly, to dyed or bleached hair, due to their great affinity for keratin. They have the general formula:



The category of dipolar surfactants is represented, above all, by the long-chain betaines such as the alkyl- and alkylamidobetaines:



Their sulfonated homologs, called sulfobetaines or sultaines, are also commercially available. They display a sulfonate group (SO_3^-) instead of a carboxylate (COO^-).

As a general rule, the amphoteric surfactants are less likely to interact with biological components and are less aggressive than the corresponding cationics. They are generally combined with other surfactants, anionic and non-ionic, in the formulation of very mild shampoos (baby shampoos for example) (11). Added to anionic surfactants, the amphoteric form “complexes” which reduce the tendency of anionics to adsorb onto proteins.

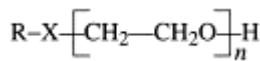
2.3. Non-ionic Surfactants

This category of surfactants comprises a very large number of substances whose molecular structure can be adjusted on demand. It includes good solubilizers and dispersants, which can be “tailored to fit” the detergency requirements. These non-ionic compounds are generally considered to be mild surfactants, being devoid of a protein-denaturing capacity (12,13). This advantage is evident when they are applied in washing simulation experiments (e.g., arm-immersion test), the results of which have been explained by the absence of denaturation of proteins. In addition, non-ionic surfactants absorbed to a low extend onto keratin.

Despite such favorable features, the use of non-ionic surfactants, as cleansing bases of shampoos, occurred only recently, in few products. They are, in fact, more commonly used as additives and formulation aids. This limited usage is generally linked to the lack of appeal of shampoos with a high level of these surfactants (rather low-induced viscosity, mediocre foam volume and softness when compared with anionic surfactant-based shampoos). Several groups of the non-ionic family are, however, very widely used in shampoos, nowadays. The major ones are as the following.

2.3.1. Polyethoxylated Derivatives

These non-ionic surfactants are prepared by reacting ethylene oxide with a fatty chain compound offering one (or more) mobile hydrogen atom(s). The derivatives of the single function are represented by the general formula:



[16]

where R is a hydrophobic group (fatty chain, at least C₁₀), n is the mean number of ethoxy groups per molecule, and X is O, COO or CO-NH.

By varying both R and n, the wetting, cleansing, foaming, dispersant, and emulsifying properties can be considerably modified.

- The polyethoxylated derivatives of fatty alcohols (X=O) are generally incorporated in shampoos for their cleansing properties. The latter are optimal when n is equal to about two-thirds of the number of carbon atoms forming the hydrophobic group R.

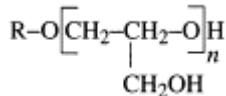
The most detergent and foaming derivatives are the saturated linear C₁₂-C₁₄ fatty alcohols with around 10–15 ethoxy groups. With longer chain-lengths, the foaming properties become impaired. When n decreases, solubility is rapidly undermined, as is the foaming capacity.

As a general rule, the polyethoxylated fatty acids (X=COO) are softer than the corresponding fatty alcohols (X=O). They foam poorly, a defect that is attributed to the presence of polyoxyethylene diesters, generated during the ethoxylation process.

- The polyethoxylated esters of polyols, such as the glycerol or sorbitol derivatives (Tween 20, for example), are basic elements of baby shampoos with regard to their extreme mildness. They also have the virtue of reducing or minimizing the level of irritancy of ionic surfactants, justifying their use in combination with ether sulfates.

2.3.2. Polyhydroxylated Derivatives

For many years, attempts have been made to use polyol derivatives in the detergent bases of shampoos, such as sucrose monolaurate, to gain the benefit of their extreme mildness. However, none of them has yet been introduced onto the market. However, the polyglycerol ethers, corresponding to structure (17) have successfully been incorporated in shampoo formulations (14):



[17]

Both their excellent compatibility with the skin and their exceptional foaming properties, despite a non-ionic structure, have made them valued shampoo bases.

2.3.3. Alkylglycosides (or APGs)

This type of surfactant was first synthesized by E.Fischer in the 1890s (15) but it was only toward the end of the 1970s that commercial products (octyl/decyl polyglycosides) began to become available from several major suppliers. These amphiphilics are designated by the general formula:

RO(G)_n G=carbohydrate or “ose”

[18]

Those used as foam-boosters in hair products are characterized by a degree of polymerization, n , of 1–3 and a group, R, having 8–14 carbon atoms.

These surfactants are generally synthesized via two procedures:

- transacetalization of short-chain (C_3 – C_4) alkyl glycosides with long-chain fatty alcohols;
- or direct glycosidation with fatty alcohols.

These reactions are complex and require the elimination of residual fatty alcohols by distillation, followed by discoloration of the end products.

The amphiphilic compounds obtained have excellent foaming properties but appear as rather average detergents. They are well tolerated by the skin and easily biodegradable. Furthermore, their synthesis strictly require renewable resources of natural raw materials.

More than 200 patents currently cover the synthesis and formulation of these surfactants. Their use in shampoos remains, however, very limited.

2.3.4. Acyl Glucamides

These are polyhydroxylated derivatives with properties similar to those of APGs.

They are obtained by reductive amination of glucose in the presence of ammonia or short-chain primary amines. The resulting glucamines are further acylated by methyl esters of fatty acids (16,17), of the general formula:



The behavior of *N*-methyl *N*-lauroyl glucamide, with regard to eco-toxicity for example, is considered to be very satisfactory (18) but the development of this family of amphiphilics is hindered by, among other things, uncertainty as to the possible formation of nitrosamines from *N*-methyl glucamine (17,19).

Despite the existence of numerous patents, this class of surfactants has yet to find acceptance for use in hair products.

2.3.5. Alkanolamides

The copra monoalkanolamides and, more precisely, the monoethanolamides



[20]

or mono-isopropanolamides



[21]

are not true detergents or foaming agents, but they help to promote and stabilize foam in formulations based on fatty alcohol sulfates, whether ethoxylated or not. The longer-chain compounds are used as thickeners or pearlescents.

2.4. Cationic Surfactants

Owing to their positive charge and their affinity for keratin fibers, these surfactants are mediocre detergents and tend to encourage the re-deposition of soil onto the hair.

They are sometimes combined with non-ionic surfactants in specific applications, for example, shampoos dedicated to dyed hair or in post-coloring rinses. They are used, above all, at low concentrations (max. 1%), as additives in anionic shampoos to modify the rheology or cosmetic effects of the products.

2.4.1. Amine Oxides

Amine oxides are semi-polar compounds whose value in shampoos derives from their multifunctional character: foam stabilizers, viscosity builders, and efficient hair conditioners (20).

Given their weak cationic nature, notably in an acidic medium, they are classified by some authors as non-ionic surfactants. They are designated by the general formula:

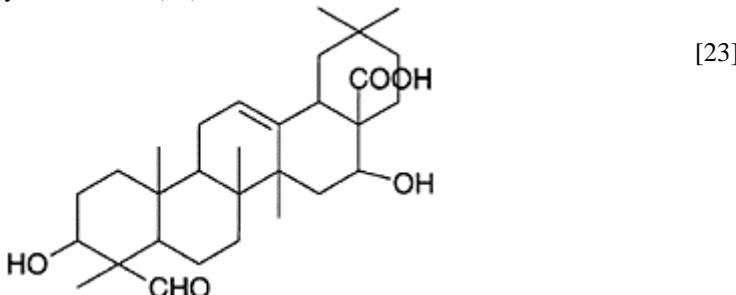


2.5. Saponins

For a long time saponins have been acknowledged as natural surfactants, abundant in nature. They appear in very diverse vegetable species and very different climates: soap bark, soap-wort, sarsaparilla, ivy, agave, etc. In the past, they were believed to have cleansing properties, but have not been used for years. Modern fashion for natural products has brought them back into favor.

In view of the chemical structure of their hydrophilic group, which is a sugar residue, it might be stated that saponins fall roughly into the category of non-ionic surfactants, although some of them are acidic or basic.

The hydrophobic part, aglycon sapogenin, the non-sugar moiety, is either steroidic or triterpenic. Thus, the triterpenic aglycone of the soap bark saponin is quillaic acid, represented by the formula (23) shown below.



Saponins are used as emulsifiers, solubilizers, and foaming agents. Discussion of their properties must allow for uncertainty in the composition of commercial extracts.

Laboratory tests on preparations of soap bark (*quillaia*), soap-wort, and sarsaparilla have revealed the poor detergent qualities of these products.

3. BASIC MECHANISMS OF SHAMPOO ACTION

The adult human scalp has an area of some 650–700 cm², bearing some 100,000–150,000 hairs whose total area is several square meters (5–20, depending on its length). These figures alone indicate that the act of shampooing, taking a few minutes, is equivalent to the rapid cleaning of a whole room.

But this approximation fails to take into account the nature of the hair surface, consisting of scales overlapping and partially covering each other to form, on average, four to five superimposed layers. Additionally, the porosity of the hair fiber surface can vary with sun exposures, intemperate weather or with some chemicals such as alkaline, oxidative, or reducing agents. Another factor to consider is the various squamous conditions of the scalp.

First of all, the primary function of a shampoo is to cleanse a considerable area that is rough, porous to a degree and strongly retentive of contaminants that, again, vary in both type and origin, affecting the global efficacy of the shampoo to a varying extent. These may be summarized as follows:

- sebum, produced continually by the sebaceous gland and the products of its bacterial or oxidative breakdown constitute the dominant fraction;
- keratin debris from scalp desquamation, proteins, and organic or mineral components transported by sweat;
- particles deposited by the atmosphere, including pollutants (minerals contained in water, hydrocarbons, dust, airborne microorganisms, mites, tobacco, etc.);
- residues of hairdressing products (gels, sprays, etc.) especially since sebum behaves like a physical "trap" for all these elements.

In brief, the surface of the scalp constitutes an ecosystem having two undesirable aspects:

- the unattractive appearance of the hair which becomes, over time, greasy, dull, sticky, and hard to manage, which the consumer globally qualifies as "dirty";
- the presence, on the scalp, of numerous compounds formed by the biological or chemical transformations of sebum, the "toxic" aspects of which are now being further documented.

In fact, external hair lipids (sebum, mostly) constitute a highly complex mixture whose composition varies with age, sex, diet, and time of year (see Chapter 20). These lipids are susceptible to chemical transformation such as hydrolysis and oxidation, peroxidization, etc., which induce a marked effect on their physical properties. Their consistency and polarity are determinants in resistance to their removal.

Cosmetic ingredients may interfere during the process of cleansing. When brilliantine was in fashion, its liquid hydrocarbons hampered the action and efficacy of shampooing. Nowadays, the selection of ingredients in formulating shampoos is based on their capacity to eliminate such poorly soluble materials (e.g., film-forming resins used in hairstyling sprays, gels, mousses, etc.).

3.1. The Physical Chemistry of Hair Cleansing

3.1.1. *The Role of Surfactants*

Because of their consistency and adhesive properties, all these contaminants cannot be removed from the hair by simple mechanical efforts. It is also important, as well, to avoid damaging the epicuticle during the cleansing process, since it is highly sensitive to frictions parallel to the axis of the hair shaft. The direction of the scales must be respected. Any action in another direction may result in tearing the epicuticle, the ultimate protector of the hair, also damaging the scales and leading, sometimes, to the phenomenon of felting comparable to that seen in woolen fabric.

The main role of a detergent is to weaken the physicochemical bonds between the soil and its substrate and to transfer it in the aqueous medium. Surfactants are compounds endowed with dual affinity: they are both lipophilic and hydrophilic. Such duality derives from their intrinsic molecular structure: they are composed of a long hydrocarbon chain with at least 10-12 carbon atoms, a so-called fatty chain, which accounts for their affinity with fats and a polar, or hydrophilic head, conferring affinity with water.

Soap is a typical example of this so-called amphiphilic class of compounds, whose behavior in an aqueous medium varies according to its concentration:

- at a very low concentration, it dissolves at the molecular level, i.e., as individual molecules (molecular solution);
- beyond a certain concentration, the critical micellar concentration (CMC), the molecules assemble in small aggregates called micelles (Fig. 1).

Within the micelles, the hydrocarbon chains clump together, forming, within these aggregates, a hydrocarbon core while the polar heads are arranged around the periphery. The molecules and the micelles of surface-active agents carry an electrical charge and are escorted by ions of the opposite charge called counter-ions. With non-ionic surfactants, the pattern is slightly different: the hydrophilic groups are deployed in the aqueous phase (Fig. 2).

From a thermodynamical point of view, the micellar solution represents a stable state. The micelles behave like tiny reservoirs: they release surface-active agents that are adsorbed onto a wide variety of surfaces or interfaces and may incorporate substances of very different kinds. This capacity defines the phenomenon of micellar solubilization. Transparent liquid shampoos are a typical example of such micellar solutions.

At higher concentrations, surfactants take the shape of liquid crystals, so-called because their properties resemble those of both crystals and liquids. These liquid crystals also form when hydrosoluble surfactants combine with lipids and can lead, for example, to hexagonal, cubic, or lamellar structures (cf. Fig. 3).

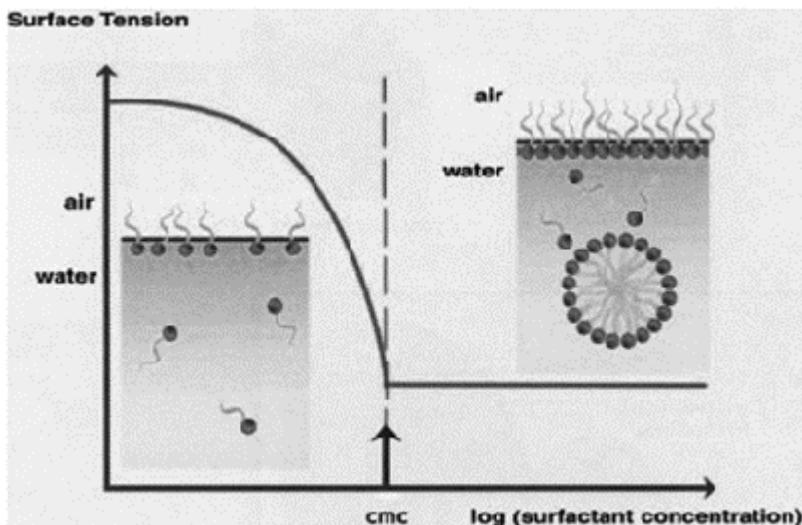


Figure 1 Surface tension plotted against surfactant concentration.

A direct consequence of the amphiphilic character of surfactants is their adsorption onto water-air and water-solid interfaces either in the form of a monolayer or multilayers. The monolayers that form at the water-air interface have been particularly investigated: the surfactant molecules assemble in a configuration by which they are anchored to the water by their polar head while the hydrocarbon chains emerge in the gas phase (Fig. 4).

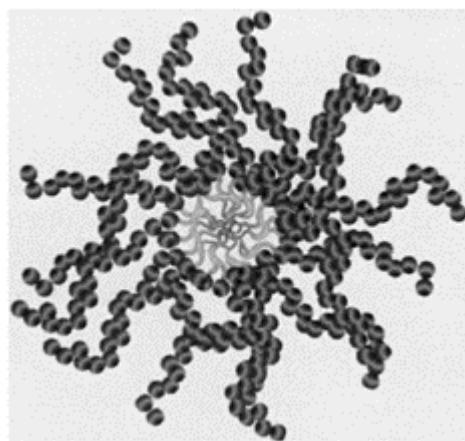


Figure 2 Model of non-ionic surfactant micelle.

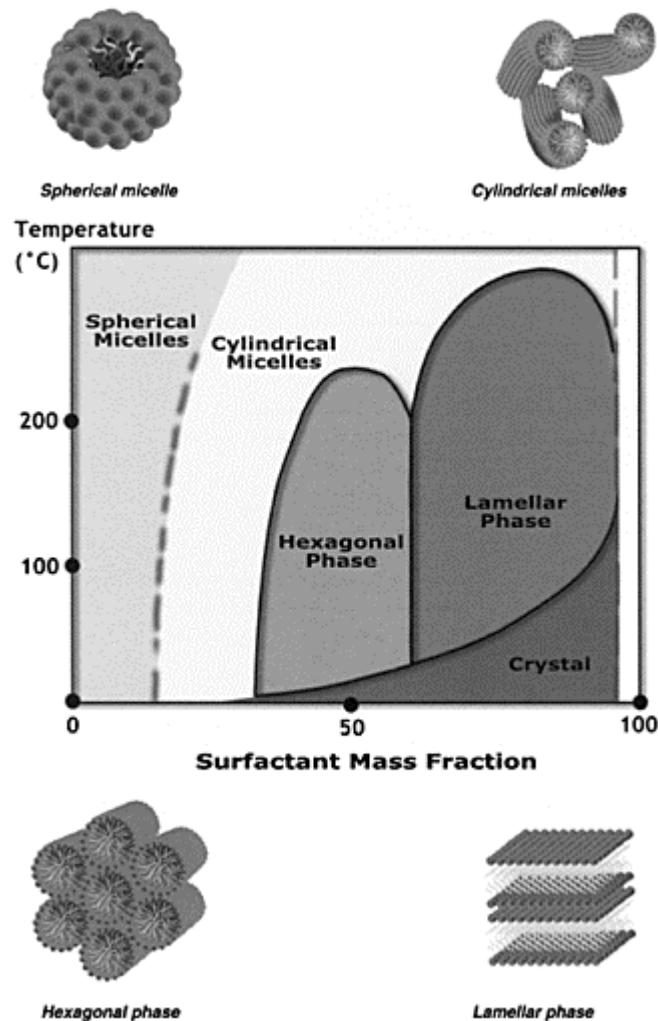


Figure 3 Diagram of surfactant phases.

The pressure exerted by the monolayer as it spreads over the water surface causes a reduction in the surface tension of the water. This property is often considered to be of primary importance but is, in fact, of only relative significance since the reduction in surface tension is not a primary factor in surfactant properties: it cannot be used to predict or evaluate the cleansing efficacy.

3.1.2. *The Lather*

The lather is a characteristic of shampoos. It results from the insertion of air bubbles in water. It is stabilized by surfactants that adsorb onto the newly formed interfaces.

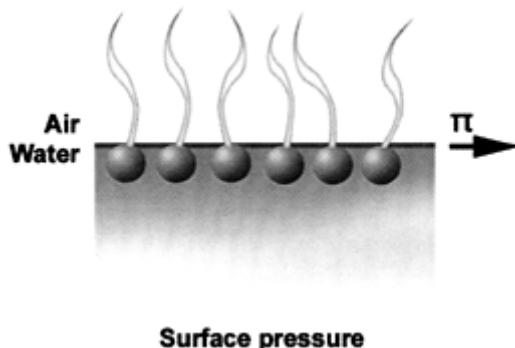


Figure 4 Surfactant interface adsorption.

Foam is not a simple dispersion of air within a liquid. In a solution containing a foaming surfactant, the latter is adsorbed on to the air bubbles introduced into the solution, forming an initial monomolecular layer. Because of their low density, the bubbles rise, lift the adsorption layer on the surface of the liquid and become covered by a second layer on their exterior (Fig. 5). Water containing the foaming agent is trapped between these two layers. It runs between the two monomolecular layers without the bubble breaking; implying that the layers must be neither too solid nor too fluid, i.e., there must be a certain degree of both cohesion and elasticity. This elasticity of the liquid film stretched out between the bubbles is allied to the Gibbs-Marangoni effect which, by inducing migration of the surfactant molecules from the low tension zones toward the high surface tension zones, takes the liquid with it and therefore compensates for any fluctuations in thickness.

The principal characteristics of the foam are:

- rate of foam formation, for example, “flash foaming”,
- foam abundance,
- expansion, i.e., the ratio between the volume of foam and volume of liquid leading to foam abundance,
- density,
- viscosity,
- bubble size or airiness,
- stability (rate of drainage and breaking).

The foam is subjected to four major forces that determine its evolution over time (21):

- the force of gravity which causes segregation of the bubbles according to their size (upthrust buoyancy is greater with large bubbles) and the drainage of liquid responsible for thinning the film separating the bubbles;
- the capillary forces which appear when the bubbles become polyhedral and which accentuate the drainage of liquid from the center of the films toward the edges (called Plateau borders, cf. Fig. 6);

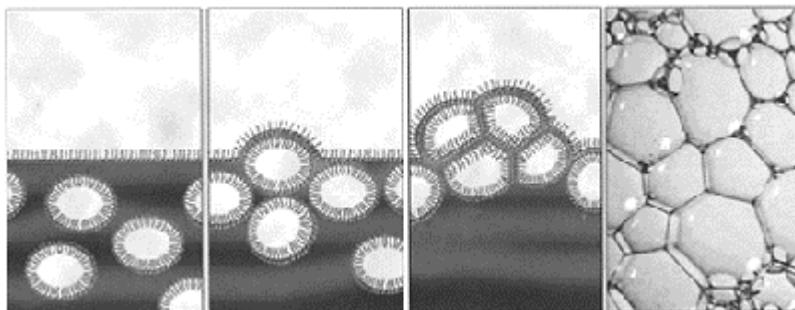


Figure 5 Foam formation.

- the internal pressure according to Laplace law ($\Delta P=2\gamma/R$) is greater in small-sized bubbles and induces inter-bubble air diffusion;
- electric or steric repulsion, which limits the thinning of the liquid film.

A number of additional factors may sometimes modify foam behavior in an antagonistic fashion (22). These are, for example:

- an increase in viscosity of the liquid (presence of hydrosoluble polymers for instance) will reduce the rate of drainage. It can also, if too important, hamper the generation of lather by agitation;
- the formation of liquid crystals can, by increasing the interfacial viscosity, improve the stability of the inter-bubble film;
- fine hydrophilic particles in suspension, or fine emulsified droplets may, by accumulating at the Plateau borders, limit drainage;
- the adsorption of amphiphilic particles (proteins or polymers, for example) onto the liquid/air interfaces leads particularly, when the angle of contact is close to $\pi/2$, to an improvement in the interface visco-elasticity;
- the presence of electrolytes, by reducing electrostatic repulsion between the two surfaces of the liquid film, tend to accelerate drainage. But it also tends to reduce the repulsion between polar heads and thus improves the adsorption and adhesion of surfactants to the interfaces;

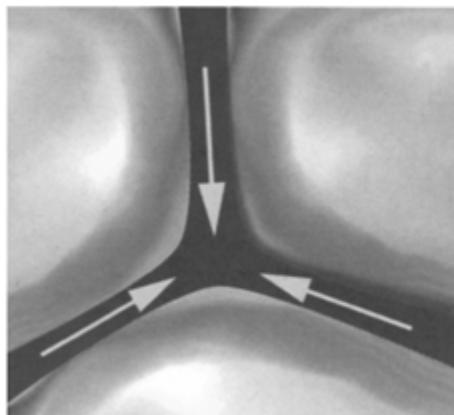


Figure 6 Plateau borders.

- environmental factors, such as temperature and pH, may alter the hydrophilic nature of surfactants and consequently induce changes in their properties of adsorption and adhesion to the interfaces. In the case of shampoos, foaming properties are optimal at a temperature in the region of 30–35°C and a pH close to neutral (5–7).

Given that the mode of use of shampoo hardly varies, the main factor that can be modifiable is its composition. The foaming properties are greatly influenced by the structure of the foaming agents and by the presence of specific additives called foam-booster and stabilizers.

The surfactants may be characterized in a geometric fashion by the critical packing parameter (23): $\text{CPP} = v/a_0 l_c$ where v is the volume of hydrophobic fraction, a_0 is the area of the polar head group, and l_c is the maximum length of the fatty chain (cf. Fig. 7). The foaming properties and CPP, which are closely linked, are optimal at $\text{CPP}=0.5$.

The destabilization of foams by fats result from two main causes:

- in the case of fluids: spreading → film-thinning → rupture,
- in the case of solids: bridging → de-wetting → rupture.

Sebum has a strong anti-foam capacity. Foam is consequently a very useful indicator of the progress of cleansing.

3.1.3. *The Detergency*

Several elementary and very distinct mechanisms have an effect on detergency (24,25).

Wetting. This is the first stage in the cleansing process, in the course of which the liquid spreads over the individual fibers and penetrates deep into the hair. Among the numerous parameters that regulate these phenomena, the surface properties of hair play an important role. In fact, the hair surface remains remarkably hydrophobic, even after prolonged extraction with solvents. This has been shown by measurements of the critical surface tension (26,27). According to these results, hair appears more similar, on this criterion, to polyethylene than to nylon, explaining why it tends to be more easily wetted

by oily substances. One of the functions of the surfactants used in shampoos is to reverse this situation.

The process has been described as "rolling up." It takes place in systems in which three phases come into contact: oil, water, and solid. The solid surface, wetted initially by oil, is then wetted in the course of the process by the aqueous phase. The

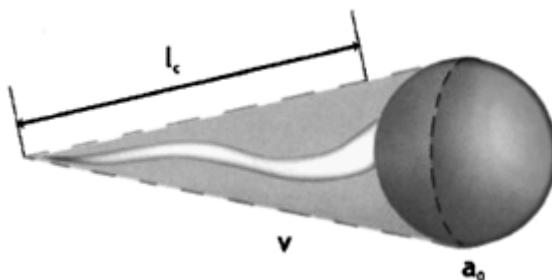


Figure 7 Critical compact parameter.

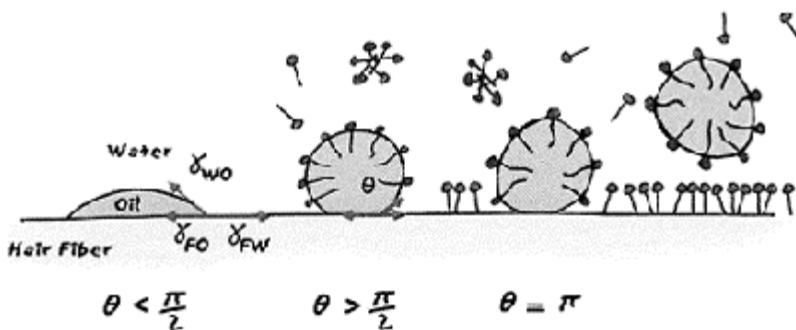


Figure 8 "Rolling up" of oil or sebum on hair fiber in the presence of surfactant.

droplets of oil are detached little by little and float due to their own buoyancy or due to mechanical action (cf. Fig. 8).

Some authors have suggested that sebum is not detached in such a way, but that its different constituents are extracted in varying proportions, according to the chemical structure of the surfactants used (28).

Micellar Solubilization. Liquid soil can also be solubilized in the micelles. This solubilization remains limited as long as the surfactant/soil ratio is low. In this respect, non-ionic surfactants are more effective than ionic surfactants since they form micelles at a lower concentration.

Nonetheless, this phenomenon is of a modest yield. A micelle with an aggregation number (number of surfactant molecules composing the micelle) ranging 50–100 will only solubilize a few lipophilic molecules.

Dispersion and Peptization. In a dispersion, the insoluble material, broken up into the state of fine particles, is coated with a surface layer of surfactants presenting a strong affinity for the surrounding medium. This surface layer also has the function of preventing aggregation of the particles, a function assured by the forces of repulsion that prevent their assembling.

In an emulsion, the dispersed material is in a liquid state. The amphiphilic substances, due to their dual affinity, have a natural tendency to settle in the interface separating the non-miscible phases—between oil and water, for example. Sebum and its by-products contain a certain number of amphiphilic substances that promote their dispersion in an aqueous medium. Such is the case, among others, of fatty acids, which are very effective in an alkaline medium, due to the formation of soaps. This process forms the basis of the spontaneous so-called peptization of certain oils or oily materials. Some polymers, in particular proteins, are also capable of facilitating the suspension of fatty soils in water. However, such spontaneous dispersions do not suffice to achieve satisfactory cleansing of the hair fibers.

The amphiphilic constituents of sebum play another positive role in detergency. They can combine with the shampoo surfactants to form liquid crystals in the presence of water (Fig. 9). These appear under the microscope in the form of droplets or cylindrical structures called myelinic figures.

The “penetration” by surfactants of oily solid soil takes place at temperatures lower than their melting point. It is achieved with a low surfactant concentration in the solution (29,30), whose chemical nature then plays a crucial role in such a process.

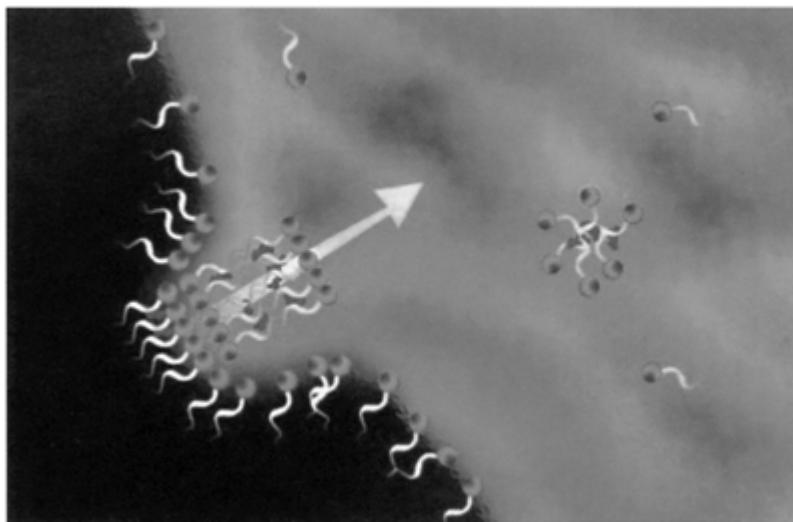


Figure 9 Dispersion of sebum components by surfactant structures.

The non-ionic surfactants, having appreciable solubility in both lipids and water, are particularly suited to penetration of an oil phase and dispersing it in water (24).

Dispersions are generally stabilized, however, by the forces of electrical repulsion, afforded by surface ion groups. Most of the colloids, and particularly those found in soil, carry a negative charge. They are thus flocculated by positively charged compounds, particularly quaternary ammonium salts. This explains why the latter cannot be used as detergents: they must be combined with other substances to prevent the precipitation they induce.

The multivalent ions, mostly calcium present in hard water, produce similar effects. Dispersions formed by detergents sensitive to these ions will have a strong tendency to destabilize and be re-deposited. This phenomenon is amplified when rinsing with hard water as the dispersing agent finds itself in the presence of an excess number of antagonistic ions.

In the stabilization process of these dispersions, another mechanism can intervene, known as "steric stabilization." The stabilizing effect is provided by the presence, at the interfaces of substances having a high molecular weight, of a part of the molecule being anchored in the dispersed phase and another balancing in the dispersant medium. The effect of certain additives called "protective colloids," aiming to prevent the re-deposition of soil, belongs to this phenomenon to a large extent.

As in solvent extraction, the efficacy of detergents can be assessed qualitatively or quantitatively. Sebum adhering to the hair is never 100% removed. According to Koch et al. (31), a commercial shampoo only removes some 40–60% of the lipids that can be extracted by water saturated ether and even repeated shampoos lead to a proportion that does not exceed 70–90%. The composition of the residual lipids is also significant. As mentioned above, the latter can be classified into two groups depending upon their accessibility. Other factors such as the physical state and polarity, can affect, too, the selectivity and efficacy of the process. There is strong evidence that the liquid components of sebum are extracted more easily than the solid ones.

The situation with fatty acids is more complex as they can be bound to keratin by calcium and magnesium "bridges."

3.2. The Effects of Surfactants on the Skin and Hair

The use of surfactants in the cosmetic field is essentially based on their cleansing action—in shampoos, shower gels, toothpastes, etc. The aim is the removal, by solubilization or emulsification, of undesirable materials, both endogenous (sebum, keratin material, etc.) and exogenous (soil, pollutants, etc.).

The secondary question of their effects, other than cleansing, is obviously raised. Are their various structures and classes accompanied by different effects on the skin and hair? If so, are they characteristic of the various classes of surfactant?

This theme has been the subject, in the past two decades, of numerous studies that benefited from the availability of new non-invasive techniques to assess the condition of skin and hair.

3.2.1. Interaction of Surfactants and Proteins—Theoretical Aspects

While the effect of surfactants on proteins has been recognized from the very beginning, the study of its mechanisms is fairly recent. It was initiated and conducted by biochemists

investigating the structure of proteins (using electrophoresis, etc.). They found some valuable “tools” in surfactants for the elucidation of these structures. An excellent review has been published (32).

The effect of surfactants on a proteinaceous structure in solution can be generally described as coupling/binding and denaturation, which are mostly related.

Coupling/Binding. The protein/surfactant interaction depends upon their respective ionic states and, hence, upon the pH and ionic strength of the medium. It results from attraction between the polar head group of the surfactant and the ionic residues of protein and leads to denaturation.

The anionic surfactants bind with the cationic side chains of protein (lysyl, arginyl, histidyl). Conversely, cationic surfactants fix onto anionic polar head groups (glutamyl, aspartyl).

In addition to this type of electrostatic bonding, there is, for example, the interaction of fatty chains with hydrophobic protein side chains. This interaction is of the so-called “co-operative” type, the bonding of one group influencing the next. What is more, the protein structure often being globular, this phenomenon only impacts on the surface groups initially, i.e., those most accessible to the surfactant. From there, the co-operative effect leads to an increasing accessibility of the deepest parts of the protein structure.

These phenomena are quantifiable by the study of sorption isotherms, based on Scatchard modeling. The various stages in the formation of the protein/surfactant complex can then be described. On surfactant saturation, the stoichiometry indicates that the level of bonding is high. Globular proteins can, for example, bind some 1.4 g of sodium dodecyl sulfate (SDS) per gram of protein, i.e., circa one molecule of SDS per two amino acids.

Denaturation. The protein/surfactant interaction, depending on the polarity of both components, can lead to the deployment of the spatial arrangement (called tertiary and quaternary) of the protein structure. As a general rule, ionic surfactants denature to an extent depending on their structure while non-ionic surfactants denature very little or not at all.

The highly theoretical assumption according to which the denaturing capacity of a surfactant reflects ipso facto an aggressiveness toward structures and functions has led to the development of methods of testing in vitro (33–37). These methods enable surfactants to be classified according to their capacity for denaturing albumin or keratins. The predictive aspect and pertinence of these simplified tests have been reviewed (38). While they obviously cannot reflect the complexity of the situation in practice, their value in the screening of defined molecular substances is certainly not negligible.

3.2.2. Practical Aspects: Approaches to the Complex Reality

In practice, in real-life situations, these tests and principles are the first “victims” of their (excessively) reductive character.

Major factors that models in vitro do not (or cannot) take into account should be considered:

- Skin and hair protein substrates, insoluble, are remarkably organized (stratum corneum, desmosomes, cuticle, epidermal lipids, etc.) and naturally protected by what is termed the cutaneous barrier. The intimate contact between surfactant and protein, such as

that modeled in a test tube with soluble proteins, in no way corresponds to the contact between surfactant and skin. This is the basic reason why, in most of the available tests *in vivo*, the investigator is forced to maximize the conditions (occlusive patch, high concentration, unrealistically repeated applications, etc.) in order to observe and quantify any effect. Under normal usage conditions, with the exception of rare allergic reactions, the manifestation of a reaction to a given surfactant is hardly ever observed.

- A formulation product never, or very rarely, contains only one surfactant. The presence of other surfactants, polymers, thickeners, etc., can modify the intrinsic characteristics of a surfactant, sometimes in a radical manner. This explains why, very sensibly, formulators tend to test the complete formula using the procedures at their disposal.
- The time for which the surfactant or the product is in contact with the skin and hair is, in the case of shampoos, extremely brief (in the minute range, or less) followed by copious rinsing with large volumes of water.
- Skin reactivity also depends on numerous and potent endogenous (sex, age, genetics, etc.) and exogenous (e.g., seasonal) factors. Winter is in fact a period in which responses are amplified in predisposed terrains, such as a sensitive scalp, for example.

3.2.3. Effect of Surfactants on the Skin

This aspect is, with no doubt, the domain of research that has received the greatest attention over the last 20 years, in cosmetology. References in the literature are numbered in their hundreds and there are some excellent reviews. The predictive aspect and relevance of these simplified tests have recently been summarized (39–41).

They will not be analyzed in this chapter because the great majority pertain to products for the face and body. Furthermore, the safety and monitoring procedures for hair products are the subject of a special chapter (cf. Chapter 11).

This field has benefited greatly from increasing innovations in non-invasive techniques yielding detailed information on any repercussions of surfactants on the skin surface, desquamation process, induced dryness, erythema, hydration, intimate organization of the stratum corneum, cytokine production, and various cellular signals depending upon the array of methods used. The most recent and complete reviews are found in references (40,41).

The increasing availability of reliable and relevant test procedures *in vivo*, applicable to the human, closely illustrates the remarkable progress made by the manufacturers of raw materials in the quality of the surfactants they supply.

In brief, the surfactant industry is now technically expected to develop and supply cleansing agents or compositions free from the potential to harm cutaneous tissue, given the amplitude and thoroughness of the arsenal of tests, from the most simple to the most complicated, at its disposal.

3.2.4. Effect of Surfactants on the Scalp

The effect of surfactants on the scalp, like that of finished products, has not been the subject of a great number of publications. This may seem paradoxical in view of the immense shampoo market and usage. Several reasons likely explain this apparent contradiction:

- The scalp is closely related to the rest of the skin, in its structures and functions. Although epidermal renewal seems greater than elsewhere (see Chapter 20 on Dandruff), the keratinization process and its regulation do not much differ from those of other skin sites. Hence, knowledge of the effect of surfactants on skin in general is logically transferable to their effect on the scalp.
- This transfer of information is all the more justifiable given that, per se, the act of shampooing, as mentioned earlier, significantly limits possible aggressiveness (brief contact time, substantial hair surface area, abundant rinsing, etc.).
- On the quantitative level, the amount of surfactant transiently present on the scalp is minimal. It can be estimated by considering some 10 g of product (containing, say, 20% surfactant), spread over the two areas of the scalp and the hair. From the ratio of the respective areas (ca. 600 cm² versus several square meters) it can be assumed that the quantity of surfactant briefly in contact with the scalp would range 20–50 µg/cm². Such a trivial amount of material, soluble by definition, would easily be eliminated by rinsing.
- The exfoliative, or keratolytic, capacity of some surfactants, described in certain procedures applied to the skin, has no bearing on the scalp. Indeed, the release of isolated corneocytes, when shampooing, results more from the friction exerted by the hair rubbed on the scalp (hard keratin on soft keratin) which represents 20–30 times (42) the effect that could be attributed to the surfactant. In other words, the very act of shampooing predominates, as far as changes to the skin are concerned, to such a great extent that the action of the surfactant on the scalp remains a mere “blip on the horizon.”
- Maintenance, or even improvement, of skin structures becomes meaningful, however, in the event that it has been impaired. This is the case particularly with the sensitive scalp (see Chapter 4) and above all that affected by dandruff, where the detergent has to be as neutral as possible. In this respect, ingenious techniques suggested by Piérard and coworkers (43,44) are a valuable guide to formulation.
- For these reasons, shampoo formulation is more focused, as regards safety, on the more subtle ancillary problems. These may concern, for example, absence of eye irritancy, the ease with which the product is rinsed away, the absence of accelerated re-greasing and, of course, its qualities in use and the end-result of the hair style.

3.2.5. Effect of Surfactants on Hair

Excellent papers (45–47) have been dedicated to the study of the hair fiber. The hair, its properties, the action of cosmetics, etc., have been the subject of a vast amount of documentation (see Chapter 1). But here too, and to an even greater extent, the intrinsic effect of surfactants on the fiber is poorly documented. The reasons are even more obvious than in the case of the skin:

- The fiber is remarkably structured, protected by a cuticle that, by comparison with the stratum corneum, appears even more impermeable.
- The huge majority of injuries caused to it are of external origin and represent natural erosion of the fiber (so-called “weathering”), i.e., combing, brushing, styling, and UV, whose effects are all mostly amplified by water. By comparison, the effect of surfactants, whatever their nature, can only be minimal if perceptible at all.

- The logical consequence has been to endow the shampoo with, cleansing action apart, the function of caring, repairing, “conditioning.” The provision of conditioning vectors, for example, the cationic polymers, and any interaction they may have with surfactants mean that the latter are not longer considered as isolated substances with all their intrinsic potential. This combination of functions decrees that priority is given by the formulator to the in-depth study of the complete formulation.

It is in this spirit that certain elegant investigations have assessed the effects of the intimate interaction of shampoo formulations with hair fiber. They are based, for example, on determination of the protein/peptide loss (48), the surface liberation of SH sites (49) and, above all, the quantification of soluble and insoluble material (cuticle debris) on combing (50).

These ingenious and helpful studies, dedicated to complete (or quasi-finished) formulae, illustrate again the massive contribution of the friction induced by combing. They also enable the reparative effects of conditioning agents to be measured.

4. SHAMPOO FORMULATION

4.1. Principle

The development of a shampoo results from the complex mixture of some 10–20 components in general, which fall into three categories:

The *cleansing base*, constituting the heart of the formulation,
 The “*active*” ingredients vis-à-vis the hair and scalp,
 And *other ingredients* which confer the texture, appearance, feel, perfume, and stability on the product.

Some optional ingredients, such as vitamins, vegetal extracts, protein derivatives, etc., can also be added for specific purposes.

4.2. Cleansing Base

Surfactants are the principal constituent of a shampoo, representing about 10–20% of the formula.

The various surfactants have been mentioned earlier in Sec. 2. The cleansing base is developed by the judicious combination of these surfactants in the search for the following characteristics:

- wetting, emulsifying and dispersing properties,
- the generation of a good-quality foam in terms of rapidity of foam development, abundance, smoothness, stability, and ease of elimination,
- sensitivity to the action of thickeners,
- compatibility with “active” ingredients, i.e., sustaining or, better, potentiating, their contribution.

The surfactants most often used are the alkyl sulfates and alkyl ether sulfates, due to their excellent foaming and cleansing capacities, of their reasonable behavior in terms of irritant potential toward the skin and eyes and their competitive cost.

They may be combined with other anionic surfactants presenting enhanced tolerability by sensitive skin, but developing less foam, such as alkyl ether carboxylates or alkyl sulfosuccinates.

In Asia, and particularly in Japan, they are currently combined with acyl amino acids (acyl glutamates or acyl methyl taurates) and with acyl isethionates.

More commonly, the cleansing bases combine one or two anionic surfactants with an amphoteric surfactant. This mixture offers many advantages such as a tighter, softer, more stable foam that is more resistant to hard water. Water hardness, i.e., its divalent Ca^{2+} and Mg^{2+} ion content, does limit the development of foam.

Additionally, this type of surfactant combination facilitates thickening and improves compatibility with the skin and eyes.

4.3. “Active” Ingredients

Grouped under this heading are the “conditioning agents” whose roles are to provide care for and beautification of the hair, and the “specific active ingredients” to mitigate and correct an imbalance affecting the scalp.

4.3.1. Conditioning Agents

Among the various ingredients described in the chapter on Hair Care Products (Chapter 4), the favored substances for a shampoo formulation are the cationic polymers and silicone oils, under many different (chemically) modified forms (51).

Introduced in the early 1970s, cationic polymers began to be used, with the added benefit of their compatibility with anionic surfactants, to provide ease of untangling, suppleness, softness, and discipline on hair that needs this, such as dry hair.

The cationic derivatives of cellulose and guar gum are the agents most commonly used.

The effect of their properties is most evident on damaged hair: this type of hair, acting like a resin with a pronounced anionic character, is very ready to take up substances of a cationic nature.

Their efficacy is however limited on undamaged or only slightly damaged hair: less active due to lower affinity, they have to be used at a higher concentration and then risk weighing the hair down (a “coated” feel) and are difficult to rinse off.

The incorporation of silicones in shampoos has been the subject of numerous patents (52–55). The first “2 in 1” shampoos containing silicones appeared in the United States in 1985. Nowadays, silicones are frequently used. Essentially, these are non-ionic polydimethylsiloxanes, insoluble in an aqueous medium, dispersed in the form of fine droplets maintained in suspension. Hence they are prone to being deposited on hair, with a tendency to adhere preferentially to non-sensitized parts of the hair fiber. They also enable swifter drying of the hair.

They yield a smooth feel, i.e., hair with no rough surfaces from root to tip (for evaluation of this criterion, a lock of hair is taken between two fingers which run from

root to tip). Moreover, the hair is lightweight and untangled, and the final result is particularly good on undamaged hair.

Given these properties, the combination of the two conditioning agents became self-evident. New generations of shampoos were thus born, combining the cationic polymer(s) and silicone derivative(s) in varying proportions. They achieve an homogenous conditioning effect from root to tip of the hair and meet the needs of the whole range of hair-types, from the healthiest to the most damaged.

To achieve these hairdressing properties, other categories of ingredients are used: anionic polymers, amphoteric polymers, cationic silicones (with properties intermediate to those of the cationic polymers and non-ionic silicones), and mineral or plant oils.

Additionally, other conditioning agents of first choice should be mentioned for use in hair that has been damaged, e.g., sun-exposed, lightening or bleaching, oxidation coloring, etc.

- Ceramide (56,57), a sphingolipid obtained by the condensation of a fatty acid with sphinganine, very close (double bond excepted) to that naturally found as structural element of the hair cuticle (58). A cohesive factor of the hair cuticle which reinforces it and contributes to its smoothness.
- Alpha-hydroxy acids, such as citric acid, enable reconstitution of the saline bonds between the ammonium and carboxylate functions of the protein chains of the hair fiber, particularly in damaged hair, via an ion exchange mechanism. This phenomenon induces an increase in the cross-linkage of proteins and hence reinforcement of the hair (59).

4.3.2. Specific Active Ingredients

In chapters 20–21, are listed agents likely to re-establish an equilibrium in the skin of the scalp. Some of these are commonly used in shampoos designed specifically for action on the scalp.

Anti-dandruff. Two main categories are in use in order:

- To limit overgrowth of “*Malassezia ovalis*” a yeast commonly resident on the scalp that may lead to excessive desquamation or scaling and chronic irritation of the scalp, accompanied by itching. Piroctone olamine (the monoethanolamine salt of 1-hydroxy-4methyl-6-trimethyl pentyl-2-pyridone) and zinc pyridinethione are the agents most commonly used, but selenium sulfide and ketoconazole are also used at a rate of up to 1% in shampoos.
- To facilitate the elimination of squames, keratolytic agents such as salicylic acid are often included.

All the ingredients in the product, particularly the surfactants and additives, are selected and used in such a way as to optimize the contact between the antidandruff agents and the scalp and so enhance their efficacy.

Greasy Hair Treatment. Certain derivatives of chitosan are used to impede the migration of excess sebum on to the hair fiber (60). Agents promoting a healthy scalp such as essential oils may also be incorporated.

Scalp Comfort. Moisturizing agents (glycerol, sorbitol) or soothing agents (α -bisabolol, calophyllum oil, etc.) are often included in shampoos designed for sensitive or irritated scalps.

Miscellaneous Agents. Antiparasite treatment, etc.

4.4. Other Ingredients

The consumer generally measures the amount of shampoo considered adequate in the palm of the hand, and spreads it over the hair and scalp. To facilitate these operations, shampoos are presented in the form of a liquid whose viscosity is adjusted with thickeners.

While transparent formulations are appreciated by those with oily hair, users of a “conditioning” shampoo will favor textures that appear creamy and “rich” to the feel, which contributes toward reinforcing the “care” image. Pearlescents and opacifying agents are used to modify the appearance of shampoos.

To guarantee the user a range of uniform characteristics on application (consistency, smoothness of the foam, etc.) and in terms of the cosmetics result, stabilizing agents are added.

The final touches given to the shampoo are an appropriate perfume and coloration, if required.

4.4.1. Thickeners for Surfactant Preparation

Chosen for their compatibility with the surfactant mix and sometimes for their pH, they fall principally in the following categories:

- Electrolytes, in particular sodium chloride and/or ammonium chloride and the salts of citric acid (61). The incorporation of these ingredients is delicate because the thickening effect reaches a maximum and then drops rapidly. The fall in viscosity can be accompanied by cloudiness of clear formulations or destabilization of pearlescent formulations. A thorough study of the complete formula is therefore imperative.
- Fatty acid amides and more specifically the alkanolamides such as ethanolamides and isopropanol amides. These are additives valued for their multiple roles since they also contribute to enhance foam softness, smoothness and stability. As with electrolytes, the viscosity can decrease, however, beyond a certain concentration.
- Polyethoxylated fatty alcohols, such as the lauryl alcohols with two to five oxyethylene groups.
- Modified polyethylene glycols of hydrophobic character such as polyethylene glycol distearates or polyurethane block polymers.
- Polyethoxylated and esterified sugars such as the highly oxyethylenated dioleates of methyl glucose.
- Polymers of natural origin, whether chemically modified or not: xanthane gum (obtained by bio-fermentation of sugars), alginates and carrageenates (algae extracts), pectins (fruit extracts), carob, hydroxyalkylated or carboxymethylated derivatives of cellulose, or guar gum. These substances often have stabilizing and softening effects on foam. The cellulose derivatives, particularly the hydroxyethyl-, hydroxymethyl-

and hydroxypropyl-derivatives, also provide softness and ease of untangling of the hair and play a role in preventing re-deposition of soil.

- The synthetic polymers such as the cross-linked homopolymers of acrylic acid.

4.4.2. Pearlescents and Opacifiers

- The derivatives of fatty alcohols or acids, the latter derivatives having one or two fatty chains, are greatly used at concentrations of 1–3%. In addition to providing an opaque or pearlescent appearance, they also have a softening role. Among them are included:
 - a. the ethylene glycol stearates or behenates,
 - b. the amides of C18 (or greater) saturated fatty acids (stearic acid or behenic acid) that are poorly soluble,
 - c. the fatty alcohols (cetyl, stearyl, behenic), and their ether derivatives.
- The copolymers of acrylic acid and styrene in the form of milky dispersions giving an equally milky appearance to the finished product. They are used at low concentrations (of about 0.1%).
- Dispersions of mineral particles: oxides of titanium or silica, nacre and mica, are used at varying concentrations and often combined with the ingredients described in the first group to obtain a shinier texture. Some of these pearlescents contribute toward foam stabilization (62).

4.4.3. Perfumes

Used at concentrations around 0.5%, their role is twofold: technical and hedonistic.

Their primary mission is to mask the odors of the shampoo's raw materials, particularly the surfactants, but also some active ingredients whose smell might be considered unpleasant (for example, selenium disulphide, used as an antidandruff agent).

Care must be taken to evaluate any possible interactions between the perfume and the other ingredients such as, for example, the preservatives. These interactions may take the form of changes in color that may be gradual. Prudence is required particularly when the perfumes contain certain perfumery ingredients such as citral, citrus essential oils, vanilla compounds, etc.

Perfume plays a more fundamental role in the hedonistic perception of the shampoo. Among the major olfactory families: floral, fruity, oriental, aromatic notes, etc., the choice will depend upon the aim of the product. Sometimes it is simply a question of evoking cleanliness, at other times softness or haircare aspect or even the efficacy of an anti-dandruff shampoo for example.

Finally, perfume is a potent vector of brand personalization. What is more, products in the same range (shampoos, conditioners, styling products, etc.) must exude an homogenous perfume because the user often combines the products.

4.4.4. Colorants

Local legislation governing their use has to be taken into account. Attention must also be paid to any interactions with the packaging material and the stability of product

coloration, especially when the packaging is transparent or translucent. The stability of the color can often be improved by adding a UV filter to the shampoo composition or by incorporating it in the container material together with chelating agents such as the salts of ethylene diaminotetraacetic acid to prevent catalytic degradation induced by traces of metal salts. The chelating agents are endowed with the property of entrapping polyvalent metal ions with a complex, stable structure in which the metal is bound in such a way that it loses all the characteristics of a metal ion.

4.4.5. Stabilizing Agents

The most usual are pH buffers, chelating agents, UV filters eventually, and preservatives. Special attention should be given to preservatives. Their aim is to inhibit the growth and proliferation of microorganisms (bacteria, yeasts, and molds) in the product during its shelf life so that the benefits of the product remain fully unimpaired and available.

The preservatives used and their concentration must comply with the regulatory requirements in any given country. They must be carefully selected for various reasons:

- the numerous ingredients that make up a shampoo formulation may interfere with and impede their ability to prevent contamination;
- they can be unstable in certain mixtures, leading to a rapid drop in efficacy;
- they may alter the color of the product. As an example, phenol derivatives frequently make the shampoo turn brown.

Among the preservatives currently used are the following: esters of *p*-hydroxybenzoic acid, namely methyl, ethyl, propyl, butyl and isopropyl parabens, 1,3-dimethylol-5,5-dimethyl hydantoin (DMDM hydantoin), phenoxyethanol, benzyl alcohol, benzoic acid, salicylic acid, sorbic acid, imidazolidinyl and diazolidinyl ureas and, lastly, a methylchloroisothiazoline/methylisothiazolinone mixture.

5. SHAMPOO CATEGORIES

Although it might appear simplistic and risky to try to classify shampoos, given the diversity of the products and their different targets, missions to be accomplished and multiplicity of presentations, certain major categories are definable.

Shampoos can be prepared in the form of:

- clear liquids
- gels
- opaque liquids, pearlescent or not
- creams of varying fluidity
- aerosol foams or powders (more rarely)

All these products are formulated according to the principles defined in Sec. 4, but it is obvious that their physical form and texture affect their ease of use as well as the complexity of the formulation (Fig. 10).

Clear, liquid shampoos, once the most common, are the “simplest” and, hence, the cheapest. The need for transparency often prohibits the introduction of some active

ingredients but certain cationic polymers with a weak charge can be incorporated without difficulty. On rinsing the hair, these polymers bond with the hair, making it very soft and easy to comb out.

Shampoos in gel-form have the same basic characteristics as clear liquid shampoos but with added thickeners. Their advantage is that they do not run but they must remain easily spreadable and rinsable.

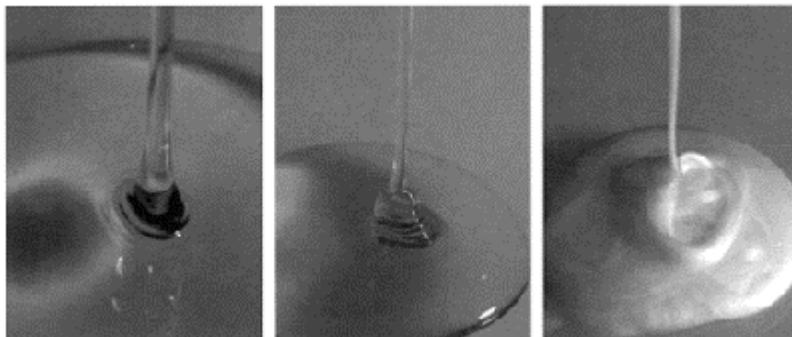


Figure 10 Textures of shampoo.

Opaque liquid shampoos are the most numerous on the market. They are easy to measure out, spread, and rinse. Their more complex formulation in the form of a semi-liquid emulsion enables the incorporation of a large variety of substances that contribute to the care and appearance of the hair. Their development is a delicate matter and it is necessary to monitor the stability of the product's properties under different storage conditions as these can vary greatly from country to country.

Cream shampoos are used, more or less, like gel shampoos, but the cream form allows the use, if desired, of a greater range of conditioning agents. They are for use particularly on damaged or dry hair.

Shampoos in the form of an aerosol foam are not a great favorite with consumers. Furthermore, this type of packaging is expensive, leading to high prices, and raises difficult storage problems in metallic packaging. They are only used for certain very specific shampoos.

Powder shampoos, whose use and elimination are rather impractical, have virtually disappeared from the market due to the increase in frequency of use of other shampoo forms.

To sum up, there is a great variety of products in different physical forms, corresponding, above all, to different objectives and needs which can vary in the case of a single user depending on the time of year and the condition of the hair. To simplify, it is possible however to define eight main classes of product:

5.1. The “Classic” Shampoos with Hygiene as the Priority

The aim is to obtain a generous mousse, to cleanse the hair well without excessive detergency, without leaving the hair too soft while ensuring that the hair may be readily untangled and shiny.

These shampoos are generally formulated using anionic surfactants (alkyl sulfates or alkyl ether sulfates) alone or combined with other surfactants such as the amphoteric derivatives of betaines. They are very well suited to natural hair but can also be adapted to suit different types of hair (dry, greasy, etc.) by playing on the nature, concentration, surfactant ratio, and certain additives.

5.2. Beautifying, Conditioning Shampoos

There are a great many of these on the market and they must develop a rich, creamy, and abundant foam. Their cleansing properties are regulated and adjusted by the choice of surfactants used and modulating the concentration of these to promote bonding of the conditioning agents in the course of the shampooing process. They are divided into two major classes:

5.2.1. “Conditioning” Shampoos

In this category of products, also called “2 in 1”, the targeted performances are those which will achieve the dual aims of a shampoo+conditioner, even if these are slightly inferior to the results with two separate products.

They must confer special conditioning properties on the hair, meeting the particular needs of the hair-type: on dry hair, they provide ease of untangling, suppleness, softness, smoothness, and shine. On dyed hair, they must also enhance the vividness of the coloring.

These properties are mainly provided by cationic polymers and silicone derivatives. Opacifiers, pearlescents, and fatty extracts (plant and mineral oils) also have a substantial effect on the cosmetic result.

The presence of a silicone oil, insoluble in the commonly used surfactant media in shampoos, is immediately detectable in the form of fine droplets under a microscope, as are the pearly crystals (Fig. 11).

5.2.2. Styling Shampoos

The aim is to obtain a finished hairstyle that is maintained over time. All hair-types are concerned, but the products must take into account what type of hair is involved: fine, thick, curly, etc. These hair types do not have the same needs and do not react in the same manner. Thus, individuals with fine hair expect an increase in volume,

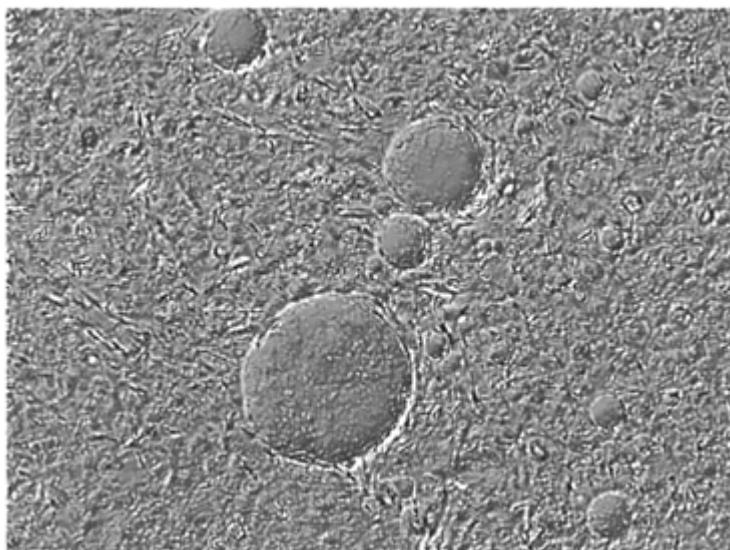


Figure 11 Microscopic view of silicone droplets dispersed in a shampoo.

texture and hold over time whereas those with permanent-waved hair seek an advantage in terms of curls (being well defined) with spring and vitality.

The development of these types of formulation is particularly delicate, for it demands the subtle modulation of hair conditioning and hair-dressing agents to avoid excessive bonding to the hair that would cause the hair to be weighed down, built-up, or the loss of untangling properties after repeated shampoos.

The styling properties are provided by anionic, amphoteric and non-ionic polymers and sometimes by certain high-molecular-weight cationic polymers with a weak electrical charge. A large formulation program and study of the molecular interaction with hair fiber has been carried out on cationic/anionic or amphoteric polymer combinations in this regard (63,64).

5.3. Treatment Shampoos

Their basic function is to help correct an imbalance in the skin of the scalp (dandruff, greasy condition) or to cleanse with the greatest gentleness (in the case of sensitive scalp).

5.3.1. *Antidandruff Shampoos*

These contain active ingredients capable of re-establishing the natural microbial flora on the skin surface. Their cleansing action must be gentle enough to avoid affecting an

already irritated scalp. Leaving the product on the scalp for 2–3 min often leads to increased efficacy, although the consumer rarely follows such instructions in practice.

These products take effect after a few applications and, when used regularly, prevent the re-appearance of dandruff.

5.3.2. Shampoos for Greasy Hair

Their cleansing power must be strong to eliminate excess sebum, but mild so as not to irritate the skin, often adversely affected by the greasy hair condition.

Conditioning additives such as cationic polymers and silicone derivatives often have a negative impact on the rate of re-greasing of the hair fiber.

5.3.3. Shampoos for the Sensitive Scalp

These often include active ingredients promoting comfort (cf. Sec. 4) and must be particularly gentle on the scalp. The cleansing base therefore combines anionics selected for their extreme mildness with amphoteric and/or non-ionics.

5.4. Shampoos for Babies and Children

The keyword is obviously optimal harmlessness on the scalp and hair and above all the eyes. Any risk of eye irritancy, even benign, any sensation of discomfort, of an effect on the eyes, even in the absence of clinical signs, must be excluded.

The first step, in developing these shampoos, consists of using the smallest possible number of ingredients. The selection of basic surfactants and their combination are based on the results of physicochemical testing and studies of irritation and discomfort. The cleansing base usually consists of large quantities (sometimes more than 10%) of high-molecular-weight non-ionic surfactants such as the oxyethylenated (30 OE) copra monoglycerides, mixtures of oxyethylenated (200 OE) palm glycerides and oxyethylenated copra (7 OE), or also the oxyethylenated sorbitan monoalkylates.

The mode of action of these oxyethylenated non-ionic surfactants seems to result from molecular rearrangements causing the formation of more voluminous micelles and altered kinetics of adsorption on to interfaces, and in particular, on to the surface of the lacrymal film.

5.5. Shampoos for Professional Use

The requirements for products for professional use are different. There are three types:

- the “current,” universal shampoo that will be followed by another process and must, in no way, interfere with that operation, e.g., permanent waving, treatment product, etc. It has no other purpose other than cleansing the hair and must ideally be reasonably priced, present good usage properties (rapid and easy foam building, problem-free rinsing) and not weigh down the hair. This type of shampoo is offered to hairdressers in concentrated form, to be diluted eight to 10 times prior to use, or in ready-to-use form in individual doses for “all hair types.”

- the “specific” shampoo, compatible with the technical operation that has preceded it or that which will immediately follow it. In this category, come “post-bleaching” shampoos with anionic surfactants, but acid for neutralizing the alkalinity of the bleaching process, without interfering with the coloration that will generally follow it. Another example is the “post-coloring” shampoo that constitutes an excellent neutralizing rinse, and enhances the color of the hair.
- the “treatment” shampoo that is an end in itself for it will not be followed by the application of any other product. It is expected to provide beneficial effects, often more evident than in products sold exclusively to the public, in the case of dry hair, dandruff, greasy hair, or sensitive scalp. Sometimes, a special application may be called for (by serial partings of the hair, for example) to achieve optimum results.

5.6. Dry Shampoos

These belong in an entirely separate category since their formulation is not based on surfactants. They are powdery products that are shaken on to the hair, left to act for a few minutes and then eliminated with vigorous brushing.

The principle behind their formulation is a simple one. They generally contain three types of substance:

- a fat absorber: starch, maize starch,
- an abrasive to dislodge soil: infusorial earth (kieselguhr and various other earths),
- an alkalizer: borax, sodium carbonate, etc.

Dry shampoos can be of value to individuals wishing to avoid wetting their hair or for those (the elderly or the handicapped) who experience problems with the usual washing process (the shower, bath, etc.).

6. SHAMPOO EVALUATION

6.1. In-use Qualities and Cosmetic Performance

6.1.1. *In Vitro*

The appraisal tests described in Chapter 12 are for the most part applicable to the shampoo, but evaluation of the foam and of detergency are specific to this type of product:

Foam. According to the ISO and AFNOR standards, foam is defined as a group of gaseous cells separated by thin layers of liquid, and formed by the juxtaposition of bubbles that yield a gas dispersed in a liquid.

The determination of foaming capacity illustrates perfectly the problems encountered in the evaluation of shampoos in the laboratory. One only has to consider the number and complexity of techniques proposed to have an idea of the problems.

The aim of all these techniques is to measure the aptitude of hair products to form a foam. In use, the foam is produced by friction from an aqueous solution with a high

concentration of cleansing agents and in the presence of soil. However, few techniques reproduce this aspect. The methods described in the literature can be classified in two categories:

- Methods “without substrate”
- Methods “with substrate” (including a brush or a mixer to simulate the shampooing process).

Among the methods “without substrate”, an ISO recommendation (65) and an AFNOR standard (66), inspired by the technique of Ross and Miles (67), are based on the production of foam from the pouring of a diluted hair product solution onto a liquid surface of the same solution.

Another AFNOR standard (68), as well as the methods of Bikerman (76) and Ross (70) recommend the diffusion of a gas through the foaming solution.

The DIN 53902 standard (69,77) is based on the method of Barnett and Powers (75). A perforated disk fixed to the end of a shaft, is displaced vertically in a tube.

Some very simple techniques recommend the stirring of solutions in tubes or graduated flasks (69,75,77).

Some mechanical methods simulate closely the process of shampooing, such as the method of Schlachter-Diercke (71), using a polyamide (Perlon) brush rotating at constant speed. A mixer has also been used in some techniques such as those of Bromley (72), Neu (73), and Hart & Degeorge (69,74,79). However, all the mechanical methods, although more realistic than methods “without substrate,” fail to reproduce exactly all aspects of the phenomenon and it is worth complementing the tests with some *in vivo* (on-head) validations.

Detergency. The phenomenon of detergency is the action of soil removal from a material. While, initially, detergency was of particular interest to laundries, techniques have been created specifically for the hair. The soil used is often an artificial sebum that resembles natural sebum in composition. The sebum is generally applied by dipping the material to be soiled in a sebum-enriched solvent. The sebum concentration and the amount of solvent can vary depending upon the author (78,79). The material can be hair (78,79) or wool fiber (80). Initial experiments used wool yarn spun in wool fat (lanolin). The detergent concentration was 0.25% and the temperature 38°C. The highest rate of grease removal was obtained with alkyl sulfates, soaps were less efficient, and the cationic surfactants gave negative results.

The shampooing required to remove sebum is carried out according to various methods ranging from a simple soak in a shampoo solution (a method often used) (78,79), to manual action in order to simulate on-head application (78).

The assay methods measuring residual sebum after shampooing are chemical, physical, or sensory methods.

The chemical methods use extraction. The choice of extractant, the temperature and extraction time are very important. Chemical analyses then carried out on the extraction liquid use gas-phase chromatography (78,79), spectrophotometry (81), gravimetry (82,83), refractometry (82,84), viscosity measurement (82,84), or infrared spectroscopy (82,84).

There are few physical methods. The friction test (83) can be cited which recognizes that the presence of sebum reduces the forces of friction, and the glass slide method (85) by which sebum can be gathered directly from the hair.

Finally, sensory analysis (86) can also be used to evaluate the detergent capacity of products.

6.1.2. In Vivo

An original product is often the result of a complex and lengthy development process. The ingredients must be carefully selected and their respective concentrations minutely adjusted to obtain the best performance.

The relevance of this work has to be verified in multiple studies: “on-head” evaluation of the product is an indispensable tool in guiding the work of the formulator, in positioning the new formula by comparison with others already present on the market and to validate its capacity to fulfill its designated tasks.

The principle of the tests is described in Chapter 12. The operation of washing the hair involves a number of stages, each supplying specific information. Specific aspects concerning shampoos include the following:

- foam building, development, quality (appearance, smoothness), hold and ease of elimination by rinsing,
- cosmetic properties of the wet hair (untangling, softness, suppleness, smoothness, etc.),
- ease of combing, hairdressing, and setting,
- rate of drying of the hair,
- cosmetic properties of the dried hair (untangling, softness, shine, appearance of the tips, discipline, spring, volume, body, etc.)

Depending upon the hair care and conditioning needs or improvement in appearance sought, assessment of the performance may be repeated in the hours or days that follow application, so as to monitor such parameters as volume and the hold of a hairstyle over time.

Superimposed applications at close intervals may also be carried out to detect any build-up effects that might lead to the hair becoming “weighed down.”

Finally, when the shampoos are offered as part of a range including conditioners, hair-styling aids, etc., application of the twinned products (shampoo followed by conditioner or styling product), or even of the group of products, must be simulated and the characteristics of each element in the range adjusted if necessary to achieve the optimum end-result.

The results so obtained after many dozens of on-head trials by professionals in close collaboration with development laboratories can be consolidated with evaluation by a panel of experts in “shampoo sensory analysis,” testing the products on their own hair under normal usage conditions (cf. Chapter 12).

The appraisal of products destined for international markets entails taking into account cultural, behavioral, environmental, and climatic data. A product that is used as universally as shampoo can be used in many different ways: mode of application, quantity applied, frequency of use, length of time it is left in contact, how long the rinsing process lasts, combination with other products that might affect the ultimate performance

of the developed product. The behavior of the user is the dominant feature, here. This behavior may be influenced by disparate elements such as:

- the level of maturity of the market,
- domestic appliances,
- social pressure,
- seasonal climatic variations, etc.

These data add their influence to that of the individual's own physical data (hair-type, length, levels of sensitization by the sun or other hair treatment, etc.) Some examples will illustrate the disparity of the situations:

- The curly hair of some Brazilian women, exposed all year long to sun and sea, calls for the regular use of dual (shampoo+conditioner) or triple (shampoo+rinsed-off conditioner+leave-on conditioning cream) as the only way to provide sufficient levels of shine, softness, suppleness, and untangling. For them, the shampoo represents just a first stage in the sequence of treatments applied to the hair.
- Studies carried out in the region of Beijing, which has an extreme continental climate, show that there are great differences between the frequency of shampoo use in summer and winter.
- The nature of water, and principally its hardness, can have a negative influence on foam building and on the performance of a shampoo or a conditioner. And water hardness, i.e., the level of calcium and magnesium salts it contains, can vary greatly within a single territory. In France, for example, it varies from 6 to 55 degrees of hardness (French scale) (87).

These few examples show the extent to which local conditions can strongly modulate the performance of a single product. To evaluate these conditions, all the local parameters have to be taken into account if the consumers are to be offered a product most ideally suited to their specific needs and habits.

6.2. Treatment and Care Efficacy

Shampoo, via a natural act of hygiene, performed on a regular basis, can offer the real benefits of treatment and care of the scalp and such states or conditions of imbalance as dandruff, excess sebum, hypersensitivity, etc.

These shampoo formulae include one or more active ingredients whose efficacy has previously been demonstrated in tests *in vitro*. Obviously, the activity of the finished product then has to be confirmed, *in vivo*, under normal usage conditions.

Accordingly, such studies are carried out by volunteers and the results evaluated using non-invasive and objective methods based on strict protocols in which all the conditions are rigorously defined. The protocol is drawn up to meet a precise objective, which is usually to verify the efficacy of the shampoo in treatment of a specific condition, notably in terms of its rapidity and intensity of action. Secondary objectives may be, for example, to monitor the persistence of the effect after termination of treatment.

Complementary studies may be undertaken to elucidate the mechanism of action of the active ingredient, to verify how accurately it hits the target and measure its availability on the scalp. It may also be necessary to determine the optimal usage

conditions of a new type of technology to reduce associated constraints (duration of contact time, frequency of application, etc.).

Apart from these objectives, the protocol defines the techniques employed, minutely details the timetable of operations, the analysis, and the data processing. In particular, the following points demand vigilance:

- Selection of the panel: volunteers are recruited according to inclusion criteria concerning either particular characteristics (age, scalp condition, hair length, etc.), or the result of a pre-selection test (mg sebum per cm² of scalp, etc.). For example, in the context of the evaluation of shampoos destined for the care of sensitive scalp, the study populations will be limited to those aged under 50 years, since scalp reactivity diminishes with age. The sensitivity of the scalp of these volunteers will be pre-tested using a detection test in which an inducer such as lactic acid, for example, is employed.
- Usage conditions: frequency, mode of use, amount applied, absence of interference with other products, etc.
- Test period: any seasonal influence on dysfunction of the scalp must be taken into account. For the evaluation of an antidandruff shampoo, the summer season is avoided given that dandruff problems tend to regress naturally then.
- Techniques applied: these combine instrumental and clinical evaluations. They must also include self-evaluation by the panel not only to verify the actual perception of efficacy by the volunteers themselves, but also to evaluate phenomena otherwise non-measurable objectively, such as itching. This multi-parametric approach is designed to shed light on the full complexity of the individual response.

By way of example, the range of techniques used to explore the antidandruff efficacy of a shampoo is described in Chapter 4.

7. SAFETY OF SHAMPOOS

7.1. Consumer Safety

Safety testing must take into account all the conditions under which shampoos are used: level of dilution, duration of contact time, etc., in order to provide realistic data on the possible undesirable effects run by the user, without forgetting the conditions imposed on professionals in the case of products destined for hairdressing salons.

Shampoos are safe products in normal use, but may generate safety concern in case of misuse such as ingestion (children) or exaggerated ocular contact.

7.1.1. Identification of the Risk

Evaluation of the risk related to the use of shampoos takes into account both systemic and local effects.

Systemic Risk. The ingestion of shampoo is a serious risk in young children in particular. Indeed, between the ages of 18 months and 3 years, the sense of taste is not mature yet and contact with new objects is often made orally. The accidental ingestion of a small

amount of product does not pose a serious toxicological problem in view of the low toxicity of the ingredients at the concentrations used. Particular attention is paid to the acute oral toxicity of the constituents of shampoos.

The main risk is, in fact, allied to the foaming characteristics of these products that can lead to respiratory problems and asphyxia. In the event, the subject should not be made to drink or vomit; a physician should be contacted, who will call the appropriate health care services.

Local Risks. The risk of introduction of the shampoo, generally in dilute form, into the eyes in the course of use and particularly when rinsing, must also be considered. The accidental projection of the undiluted product into the eyes is also a possibility. In such cases, the eyes must be immediately rinsed abundantly with water.

The excessive or inappropriate use of shampoo, e.g., daily use with overgenerous massage, inappropriate contact time and/or insufficient rinsing can lead to irritation of a particularly sensitive scalp. Such event is not widespread, taking into consideration the countries in which usage is daily or even twice-daily.

Irritation of the hands may occur among professional hairdressers performing a number of shampoos which involves frequent rubbing of hair with hands soaked in water—the use of gloves is not yet sufficiently widespread.

Furthermore, the risk of sensitization related to ingredients such as preservatives and perfumes cannot be completely eliminated but it is, again, a very low risk given the dilution, short contact time and the rinsing process.

7.1.2. Shampoo Safety Evaluation Procedures

The safety of shampoos, as for other cosmetic products, is based on the knowledge of constitutive ingredients toxicology and the experience acquired during their use in other cosmetics (cosmetovigilance data).

Indeed, except in the case of new ingredients, the data available on shampoos of similar composition and the monitoring of products on the market constitute an essential element in the evaluation of product safety and subsequent selection of tests (in vitro and *in vivo*) required to confirm this safety:

- If the test product is very close to a well-tolerated product already marketed, and if the differences are considered not toxicologically relevant, it will not be necessary to carry out new testing.
- Otherwise, the safety assessor will define the test(s) required. The studies are carried out in a comparative fashion so that the test product may be classified relative to the level of tolerability of the shampoos representative of the state of the art (benchmarking).

7.1.3. Evaluation of Eye Irritant Potential

Particular attention must be paid to the evaluation of eye compatibility. This is no longer monitored using the Draize eye test (88), animal tests on cosmetics having been banned many years ago by most manufacturers of cosmetic products (89,90).

Currently, eye irritant potential is evaluated on the basis of knowledge of the ingredients, of the formulations in which they are included and of the results of alternative methods in current use.

A final verification of tolerability and eye comfort can, if necessary, be carried out in man under controlled technical conditions and in compliance with strict ethical rules.

The eye irritancy data on the various ingredients are communicated by the raw material supplier. They constitute one of the elements required by the legislation relative to chemical products. This type of information concerns the undiluted ingredient. Evaluation of the eye irritant potential of a substance in a finished product is based on its concentration, its interaction with the other product ingredients, its bioavailability to the ocular tissues, the pH of the product...etc. The safety assessment of shampoos shows that their eye irritant potential is usually due to a very small number of ingredients, particularly the surfactants because of their intrinsic irritant potential and their concentration in the finished product. The irritant potential can be limited by the judicious combination of surfactants.

Alternative Methods. The alternative methods developed for the prediction of eye irritant potential aim at detecting, *in vitro*, the phenomena arising on accidental contact of cosmetic products with the eye.

From the clinical and anatomo-pathological data in ocular irritation, a range of tests have been developed to detect:

- the principal corneal damage i.e. epithelial damage, using cytotoxicity tests or the integrity of the epithelial barrier function,
- the main vascular damage i.e. using a test mimicking the vascular organization of the conjunctiva.

It is only the simultaneous use of various *in vitro* tests within a battery appropriate to the test substance that can supply information on its eye irritant potential.

The main tests used are the following:

Test on Isolated Bovine Cornea (91–94). The test substance is applied to the surface of the isolated corneal epithelium derived from bovine eye. After various contact times, modifications in the transparency and permeability caused by the test substance are evaluated.

The main advantage of this test is the use of the target tissue for the test substances and ability to quantify the results. It is simple and rapid but difficult to apply to substances with low irritancy or those not readily rinsed off. Recently this test has been transferred to the porcine cornea.

The Agarose Overlay Method (95–99). This test assesses the cytotoxicity of the test substance. Evaluation of cosmetic products that are not soluble in water is enabled by the inclusion of cells in a semi-solid medium (agarose gel). The test substance is applied to the surface of this gel, within which it must diffuse to reach the cells. Cytotoxicity is evaluated by measurement of the diameter of cell lysis around the treated zone. This test is simple and rapid. However, non diffusion of some cosmetic ingredients is a limitation to its use for certain types of cosmetic.

The HET-CAM Test (100,101). In this test, the test substance is deposited on the chorioallantoic membrane of a hen's egg. The vascular system of this membrane mimicks the vascular system of the conjunctiva.

It is thus possible to evaluate, after various contact times, the level of hyperemia, hemorrhage or thrombosis as well as coagulation induced by cosmetics. The intensity of the lesions, evaluated on a defined scale, is proportional to the irritant potential of the test substance.

This is a reliable, simple and rapid method of testing formulations, especially water-soluble formulations such as surfactant-based cosmetics. However, substances that are not readily rinsed away, or that are colored, may lead to false positives.

This method is now recognized by the French regulatory authorities for use with finished cosmetic products containing surfactants.

Other Tests (102–111). Other methods have been proposed for evaluating eye irritant potential: estimation of the denaturation of macromolecules such as proteins, cytotoxicity on isolated corneal cells, measurement of the product effect on the cohesion of the intercellular junctions or on cell metabolism.

The evaluation of cytotoxicity on three-dimensional fibroblast cultures has also been proposed. Methods of 3D reconstruction of the human cornea are under development. This model would, perhaps, permit the evaluation of all kinds of substance with the help of relevant parameters of the damage induced.

It should be underlined that, currently, none of the existing tests is capable of evaluating the cumulative effect of repeated contact or the phenomena of reversibility of the effects. There is no alternative test that totally replaces *in vivo* determination of eye irritant potential (112–114).

Clinical Evaluation. Data on the constitutive ingredients, close shampoos and alternative methods can be complemented by clinical trials to confirm that the product is harmless and compatible, particularly as regards eye comfort. These studies are carefully monitored and conducted in strict compliance with ethical rules (115–120).

Use Tests Under Ophthalmological Supervision. The use test consists of evaluating the product under normal usage conditions. The panel is recruited on the basis of pertinent criteria relative to the shampoo type.

An ophthalmological examination will be carried out, for example, before and after the first shampoo, then after 2 or 3 weeks of use at home.

In addition, the completion of a questionnaire yields information relative to eye comfort: stinging, discomfort, palpebral itching, etc.

7.2. Environmental Safety

During the shampoo formulation process, the selection of ingredients must also take into account their environmental impact.

The potential impact depends upon the level of ecotoxicity of the ingredient, its aptitude for bioaccumulation and its biodegradability.

Ecotoxicity is estimated using standardized tests on algae (OECD 201), on daphniae (OECD 202), and sometimes on fish (OECD 203).

The aptitude for bioaccumulation correlates well with the coefficient of octanol/water distribution. Where $\log k_{oE}$ is >3 the substance is considered to have a bioaccumulation potential in the lipophilic phases of aquatic species.

The third parameter relative to biodegradability is of particular relevance to shampoo formulations.

Indeed, shampoo inevitably enters wastewater and thence water treatment plants. The ingredients, and principally the surfactants, undergo biodegradation (in the activated slurry of the stations) that eliminates the majority of these elements. This aptitude for biodegradation is determined by standardized test procedures.

The Scientific Committee for Toxicity, Ecotoxicity and the Environment (SCTEE), of the European Union, has proposed the following definitions with regard to biodegradability:

- *Primary biodegradability.* Alteration of the chemical structure of a substance, provoked by biological action, having as its consequence the loss of the specific properties of that substance.
- *Ultimate biodegradability.* Level of biodegradability attained when the test compound has been entirely used by micro-organisms leading to the production of carbon dioxide (under aerobic conditions), water, mineral salts, and new microbial constituents (biomass).
- *Ready biodegradability.* Arbitrary classification of chemical substances that have passed certain tests specific to ready biodegradability. These tests are so rigorous that it is predictable that such compounds biodegrade rapidly and completely in the aquatic environment under aerobic conditions.
- *Inherent biodegradability.* Classification of chemical substances for which there is unequivocal proof of biodegradability (primary or ultimate) in any biodegradability test whatsoever.

The assessment of biodegradability is carried out using tests developed by the Organization of Economic Cooperation and Development (OECD).

- *The first level* corresponds to ready biodegradability (OECD 301 A–F, principally methods B and D). The conditions of these tests are severe in terms of the high concentration of test substance and the low concentration of microorganisms (obtained from water treatment plant sludge). In the event of success, this test gives an indication as to the aptitude of the material to degrade completely.
- *The second level* corresponds to inherent biodegradability (OECD 302 A–C). This test is used in the event of failure in the preceding test. It corresponds to the most optimal conditions: higher concentrations of microorganisms and a longer test period.
- *The third level* simulates the actual purification station conditions (OECD 303 A currently being revised). This test is only used as a last recourse.

The aim is to use only surfactants that have passed the ready biodegradability test. The residual amounts in the environment will then be extremely small. The development of shampoo formulae should therefore favor materials that present the lowest levels of ecotoxicity and aptitude for bioaccumulation as well as the most advantageous results in the biodegradability tests.

It is the combination of all these criteria that will provide the greatest guarantees of safeguarding the environment.

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4

Hair Care Products

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1. SCALP AND HAIR

Hair condition may be adversely affected by the state of the underlying, fostering, nourishing, and hair-bearing scalp, which means that maintaining the scalp in a good condition is a prime requisite to prevent hair from potentially unpleasant, unattractive, or disfiguring defects. Hence a paramount need for providing the most appropriate care to those scalps which are prone to be greasy, dry, itchy, scaly, all conditions that are commonly exacerbated by stress, upsets, disturbances or irregularities in life habits, etc. Scalp is therefore part of hair care and must be dealt with by the cosmetic scientist with particular focus on the following conditions: greasy scalp, dandruff, and hair loss.

1.1. Scalp Care

1.1.1. *The Normal Human Scalp*

The normal scalp (i.e., not involved by alopecia or skin disease) is a peculiar skin site. It represents an invisible surface of about 650–700 cm² (in adults), covered by 100,000 to 150,000 terminal hairs of various natural colors, leading to an average follicular density of 200–250 per cm². The latter being of about 1100–1300 per cm² in infants (1) and assuming a definitely programmed (genetic) constant total number of follicles, these figures indicate that normal growth, from infancy to adulthood, makes scalp surface extend by about five times. Although not easily accessible to the eyes, the scalp is a skin site where sensory manifestations (itching, reactions to external stresses, etc.) are daily experienced by about 25–30% of the subjects, irrespective of gender and ethnic group (2).

Although of a comparable structure than any other skin site, scalp differs by very distinct and specific features (which are reviewed in more details in Chapter 21) (2):

- Both keratinization and epidermal renewal show a quicker turnover, up to about twice as that of other sites. In most cases, scalp has a thicker horny layer, albeit of a loose structure and an unpronounced (flat) microrelief (Fig. 1a) at least at the vertex area, when compared with the nape (Fig. 1b).

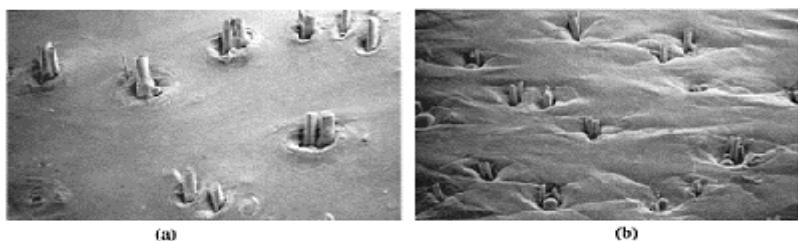


Figure 1 (a) The appearance of a normal scalp (SEM): Vertex area. (b) The appearance of a normal scalp (SEM): Nape area.

- Every scalp shows in fact some foci of abnormal desquamation. The presence of flakes (aggregates of hundreds of corneocytes) is a hallmark of any normal scalp, a phenomenon greatly amplified in dandruff (Fig. 2) or seborrheic dermatitis.
- Histological examinations of the normal human scalp reveal the permanent presence of foci of inflammation, i.e. dilated capillaries under the dermalepidermal junction and dermal infiltrates of inflammatory blood cells [see Chapter 21 (2) for more details].
- It is probably the only skin site that combines, at the same location the expression of fully active skin appendages (sebaceous glands, sweat glands, terminal follicles). These obviously participate in the daily presence of a rich hydro-lipidic medium (sebum, water, salts, amino acids, peptides, etc.) in the gram-range (3) which, in turn, permanently fuels a dense (10^4 – 10^8 per cm^2) commensal and lipophilic flora.
- In contradistinction to other skin sites the scalp is certainly the skin region most adversely affected by various elements: heat from hair-dryers, brushing carried out with sharp metal combs, friction from towels. It is noteworthy that hair/scalp friction invariably leads to a strong increase in the

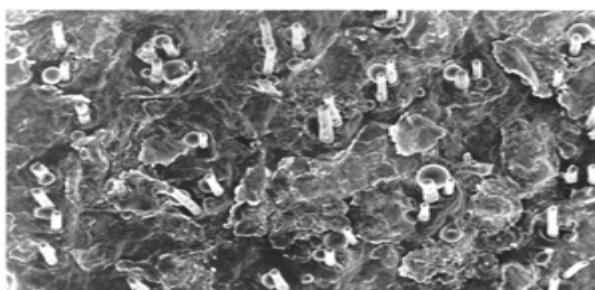


Figure 2 The appearance of a scalp with dandruff (SEM). Note the presence of flakes.

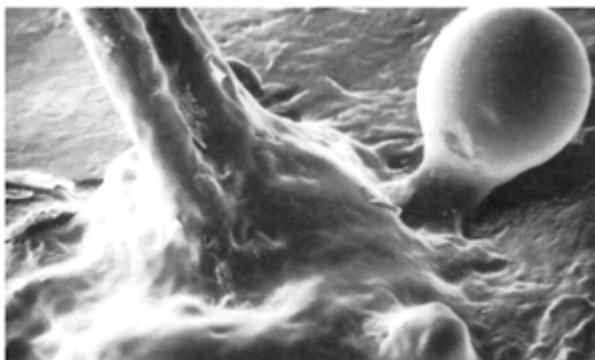


Figure 3 Scalp and hair surface at 24 hr post-shampoo. SEM picture showing sebum excretion and spreading from follicle ostium.

extraction/shedding of scalp corneocytes (4) as a result of the physical erosion by hard keratin (hair) onto soft keratin (skin).

Together, these principal scalp-specific criteria define three large domains of products dedicated to scalp care. These include:

- seborrhea of the scalp and any consequent hair regreasing process,
- dandruff or abnormal desquamation of the scalp (see Chapter 21),
- excessive hair loss (alopecia).

1.1.2. Scalp Seborrhea

In this section, the word seborrhea refers to the normal function of sebaceous glands, not to the hyper-seborrheic state, i.e., excess of sebaceous production, most often resulting from hormonal or inflammatory disorders (5), of clear medical relevance.

In its normal state, the scalp is a naturally greasy skin site, with highly active sebaceous glands [see Chapter 20 (5)], constantly delivering large amounts of sebum (Figs. 3 and 4) that spread onto the scalp surface, which then comes into contact with hair (see below), making it progressively greasy. The perception of seborrhea, mostly subjective, results from a series of alterations that can greatly vary inter-individually. These are the following:

- The hair rapidly becomes greasy and sticks in clumps.
- Hairstyles do not last long after being set, for the excess sebum weighs the hair down.
- Dust and pollutants accumulate on greasy hair, which gets dirty very quickly.
- The sebum can undergo peroxidative transformations, leading to disagreeable odors (6,7).

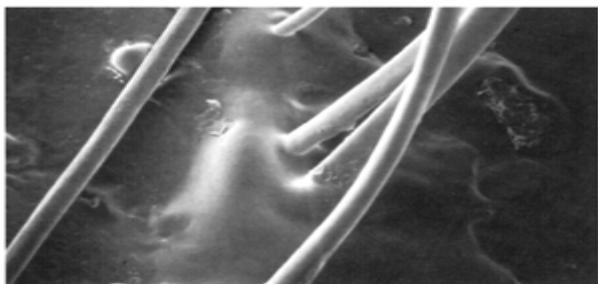


Figure 4 Scalp regreasing at 48 hr post-shampoo (SEM picture).

The regreasing kinetics of the scalp is a well-described process (Fig. 5), where the amount of sebum reaches comparable casual levels to those found on the forehead in a 2–3-day period, whereas these are reached in some 3–4 hr on the forehead area. The scalp sebaceous excretion appears therefore to be slower not only because of an intrinsic lower rate of production (Fig. 6) but also because a significant part of sebum (and derived products) is transferred onto the hair shafts. Nowadays, such kinetics no longer correspond to real-life conditions with regard to the considerable increase in the frequency of shampooing, i.e., up to 14 times a week at the most extreme range, three to seven times a week on average (at least in the Western world and Asia) according to culture, gender, climatic conditions, etc. (see Chapter 3) (6,7).

With high frequencies of shampooing, sebum is eliminated before it generates its unsightly effects. For a long time, at least in European countries, a strong belief was held in what was called “reactive seborrhea,” implying that too much (or too frequent) shampooing would, in turn, exacerbates the sebaceous gland, i.e., stimulating its production and delivery. Our studies and those of others have shown that the sebaceous gland is in fact insensitive (Fig. 7) to these external frequent washing procedures, sebaceous secretion being unchanged by increased frequency of shampooing (3,8,9).

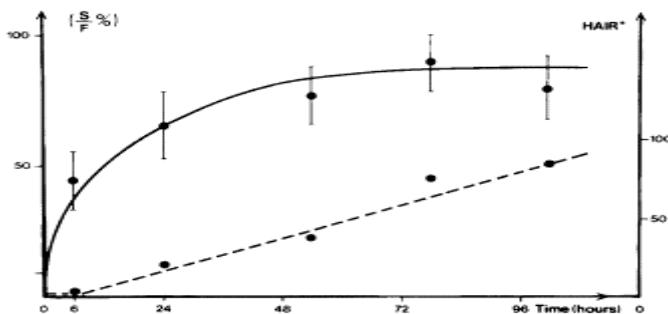


Figure 5 Regreasing kinetics of the scalp surface (*f*) and hair root (—) of a male population (*n*=15) after

shampooing. The amounts of lipids (S) found on the scalp of a given subject, are expressed as a percentage of the casual level of lipids (F) found on the forehead of the subject. Vertical bars represents M \pm SE of the whole population.

Subject Number and Age	Forehead SER ($\mu\text{g}/\text{cm}^2/\text{min}$)	Scalp SER ($\mu\text{g}/\text{cm}^2/\text{min}$)
1 (39)	1.56	0.07
3 (23)	0.74	0
4 (45)	1.12	0.02
5 (43)	0.81	0.07
6 (46)	0.90	0.24
9 (34)	0.82	0.3

Figure 6 Sebum excretion rate (SER) of scalp and forehead.

Scalp Antigrease Agents. Briefly stated, there is as yet, no efficient *topical* agent for controlling or down-regulating sebaceous production at its source. Interestingly, even most potent oral drugs, generally used as anti-acne treatments, are quite disappointing, when applied topically (5). These include cyproterone acetate and 13-cis retinoic acid, which, when taken orally, lead to a strong reduction in sebum output in every sebaceous rich region, scalp included. Male acneic subjects receiving 13-cis retinoic acid by the oral route wash their hair once a fortnight in most cases (10,11).

In the cosmetic domain, it is however possible to formulate good products to mitigate milder cases of seborrhea. “Antiseborrheic” agents and formulas recommended should possess the following characteristics:

- Be non-toxic.
- Eliminate excess sebum without excessive detergent action or drying. This excludes shampoos with high detergent power and solvents that are too efficient in removing grease/fat.
- Stop itching when necessary.
- Bring adequate bactericidal and fungicidal activities.
- Contain elements that can induce a return to normal keratinization or reinforce the stratum corneum cohesiveness.

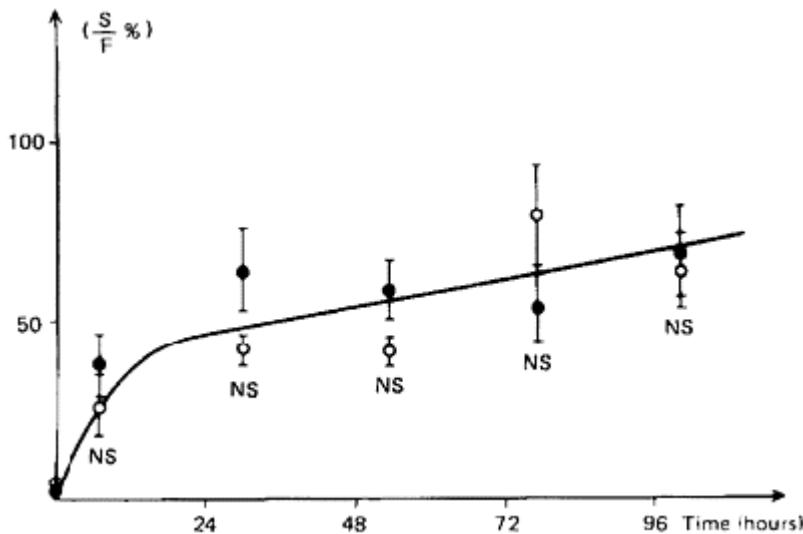


Figure 7 Regreasing kinetics of the scalp surface following a single shampoo (●) or eight consecutive shampoos (○ S/F=ratio of the amounts of lipids found on scalp (S) and forehead (F), respectively; NS=no significant difference between (●) and (○).

With regard to scalp seborrhea, the considerable increase in frequency of shampooing has lessened the need for topical anti-regreasing agents. These, albeit of only limited activity, are still found in a large variety of products such as:

- Sulfur and sulfur derivatives, used for a long time, in the form of colloidal dispersion, but now abandoned in many countries because of their weak efficacy associated with questionable tolerance (irritation, induced skin dryness, etc.) and unpleasant odor. Selenium disulfide, which is largely used as a highly efficient ingredient for controlling dandruff, does not show any anti-seborrheic properties. Conversely, as with the other anti-dandruff active compounds, the success of treatment (elimination of scales) always leads to increased hair regreasing through a physical effect (see Chapter 21 on Dandruff), i.e., the lessening and/or disappearance of lipid absorbing structures, stratum corneum and flakes, respectively.
- Sulfur-containing amino acids and thio-ethers have been extensively used in the control of scalp regreasing. They were shown, a long time ago (12), to be useful in slightly reducing the level of sebaceous excretion. These compounds included, for example, S-carboxymethyl cysteine, thiolanediol, 2-benzylthioethylamine, etc. Again, the presence of such compounds is, now, less and less justified in view of the high

frequency of shampooing which leads to the removal of sebum before it produces its undesirable unesthetic effects.

- Tars (Cade oil, Juniper oil, etc.) which were widely used as ingredients for scalp hygiene and very helpful in treating various chronic disorders (psoriasis, eczema, etc.), are no longer authorized, at least in Japan and E.U. for safety reasons (potential carcinogenic risks, mostly). They are still used in the United States. However, their anti-seborrheic action has not been well-documented.
- Nowadays, the control of sebaceous activity by the enzyme 5- α reductase [mainly type I, see Chapters 2 and 20 (12)] has led to a large variety of ingredients or vegetable extracts claimed as inhibitors of the enzyme. See, for example, Patents FR 2643375A, WO 9422900A1, WO 9640180A1, JP 2000044439A. In most cases, the resulting effects have not been clearly supported yet, *in vivo*. Ultimately, the effect on the sebaceous function by the potent drug Finasteride (13), an inhibitor of 5- α reductase type II, taken orally for hair-regrowth, has not been reported, although this compound exerts some inhibitory action, *in vitro*, on 5- α reductase type I activity.

1.1.3. Hair Regreasing

Sebum does not move spontaneously over the hair (14) nor migrates along the hair shaft by some capillary process. In fact, transfer of sebum from scalp to hair surface is driven by a passive phenomena, by direct contacts, in addition to external events such as combing, contacts with pillow, touching by the hands, etc. The root of the hair, being the closest to the greasy scalp surface, is logically the more rapidly affected part of the fiber (8).

Apart from quantitative aspects (a greasier scalp leads to greasier hair), some other factors control the hair regreasing process. Sebum rheology is obviously influenced by physicochemical properties such as viscosity which, in turn, is controlled by the ratio of unsaturated fatty acids or triglycerides, the more unsaturated the more fluid. The fusion curves obtained by differential analysis show that rheological properties highly control both the rates of flow and migration onto the hair (15). With time, sebum transforms rapidly, as a result of lipase activity, leading to higher content of released unsaturated free fatty acids, or oxidation generated byproducts which may lead to short fatty chains (highly fluid) by intra-molecular rearrangements.

In addition to the unesthetic aspects brought about by the coating of hair by sebum, the latter makes hair more prone to trap external, airborne contaminants or pollutants (smoke, particles, etc.). In other words, from greasy, hair rapidly becomes dirty, as perceived and stated by the consumer.

Evaluating the Greasy State of the Hair and Scalp. Various methods for measuring scalp and hair seborrhea can be used. They are reviewed in-depth in Chapter 20 (15). The purpose is to evaluate the condition of the hair and scalp, qualitatively or quantitatively, to measure or follow the effects of a treatment and to identify the factors and parameters determining the greasy appearance of hair. In addition to the always necessary clinical scoring by trained technicians (through analogical scales), several objective methods may be routinely used. These range from the most tedious and time-consuming to the simplest and commonly used. For research purposes, sebum may be extracted by various ways: solvents or detergent solutions although the latter implies, in most cases, a difficult (and

low yield) further extraction of lipids by solvent. In all cases, however, the extraction carried out *in vivo* appears global, i.e., it does not allow one to differentiate lipids according to scalp or hair origin. Irrespective of the method of extraction used, the lipids are further estimated quantitatively, using either gravimetry or spectrophotometry and qualitatively, using gas liquid chromatography (GLC), high-pressure liquid chromatography (HPLC), or high-performance thin layer chromatography (HPTLC) (16).

In applied research, simpler and quicker methods can be used which, in many aspects, closely differentiate sebum in relation to its origin. In routine, these methods are preferred. For example, absorption methods involve the application of a strip of absorbent paper (or any adequate physical support) to a swatch of hair from root to tip allowing further extraction and quantification of lipids. The regreasing process after shampooing and the spread of sebum along the hair shaft can therefore be followed. The same holds true with regard to the photometric method (8,17), using either a Lipometer® or Sebumeter® which allows the recording of the amount of sebum on hair or scalp surface. Again, the sebum replacement rate on the scalp and/or hair refatting can be both determined rapidly on a routine basis. Another interesting and clever approach has been more recently proposed by Pr Piérard, in Liège, through the use of sebum absorbent tapes which can be combined with photometric measurement or further estimated by image analysis (see Chapter 20) (48,17).

Agents Used for Controlling Hair Regreasing. *Substances retarding sebum recovery.* Another approach to slowing down sebum uptake by hair consists of depositing an oleophobic film on the surface of the hair. Illustrative of such a physicochemical treatment, perfluorinated fatty acids or acrylic resins have been proposed for use (18–22). Being both hydrophobic and lipophobic, these compounds, used in low concentrations, can retard sebum transfer from scalp to hair.

Different hydrophilic polymers are also very efficient. For example, poly(β -alanine) (23), and monosuccinylamide chitosane (24) have been used in marketed products. Their film deposits all over the follicle and limits the propagation of the sebum from hair to hair. Styling properties are enhanced as a result.

Grease Absorbers. Rheology modifiers. Proteins such as gelatin or casein, in addition to finely powdered silica, have been used to absorb sebum and give it a more waxy consistency in order to make the seborrheic condition less obvious. They are reasonably effective and satisfactory, but they leave hair looking dull.

Products for Greasy Hair. Different types of formulation have been developed for treating greasy hair. Lotions would seem to be the most effective in modifying the regreasing process. They are applied preferably at the root; active ingredients are deposited on the scalp or along the hair shaft and, being left there, can be active over lengths of time. Liquid or as more or less fluid gels, these lotions are formulated for daily use or as an after-shampoo lotion.

1. Lotions for daily use have a higher alcohol content (40–50%) for quicker drying and to dissolve a portion of the sebum to be desorbed onto the towel during the drying process. In general, they contain a small amount of an anionic polymer to impart greater volume to the hair.
2. After-shampoo lotions have a lower alcohol content. They usually contain gums or hydrocolloids to act as absorbers and help in setting the hairstyle. Lotions for reinforcing hairstyle hold and duration also include specific polymers.

3. Hydroalcoholic gels are an interesting variant. Their consistency makes localized application on the greasy roots and scalp possible and makes dosage by an appropriate device easy. The gels liquefy when lightly rubbed in after application; this creates a sensation of freshness and lightness. Good results have also been achieved with gels in which a gelling polymer is associated with non-ionic polymers, acting as sebum absorbers and simultaneously “unsticking” the hair from the scalp. The swelling imparted to the root retards sebum uptake, renders styling easier, and favors more long-lasting hairstyle retention and volume.
4. Antigrease compounds can also be incorporated into hair rinses, but it is difficult to obtain a significant effect during the brief time before rinsing or ensure adequate affinity so as to avoid removing active agents when rinsing. To overcome this difficulty while still rinsing with water, gelling emulsions have been developed. Their viscosity is favorable for distribution throughout the hair and they have good penetration. The product is left to act for a period of time, then rinsed out. These emulsions contain clays, plant extracts, protein extracts, and/or polymers to strengthen the hair. A small amount of surfactant is often included to ease emulsification and ensure proper rinsing.
5. Another type of product is the dry shampoo. It is based on combinations of sebum-absorbing substances (starches, clays, vegetable powders). They help reduce greasy deposits without wetting the hair and therefore do not require setting and drying. Their main failing is that they tend to leave hair dull.
6. More recently, a new approach has been developed which involves formulating shampoos for regular use based on surfactants with the property to greatly influence the scalp condition and the kinetics of hair regreasing (25).

The surface condition of a scalp with seborrhea is often highly disturbed, heterogeneous, weakened, and highly sensitive to physical (massage, etc.) or physicochemical demands made upon it. It must be treated with great caution and the utmost gentleness. However, hygiene products for greasy hair must have sufficient detergent strength to eliminate greasy deposits on hair and the abundant variety of dirt they trap. Shampoos are therefore crucial, and their formulation comes under very close scrutiny.

It has been found that the regular use of certain surfactants, in particular the nonionic derivatives of polyglycerol (26), which have good foaming and detergent properties, leads to progressive and marked lengthening of the hair regreasing process without requiring any additional treatment or antigrease ingredient. This improvement probably arises from a gradual return to a normal scalp condition, as the disappearance of itching in affected subjects would seem to indicate. They probably act upon the hair surface as well.

1.1.4. Dandruff Condition

This is reviewed in Chapter 21 (26). Dandruff, which concerns a large part of the human population, is a benign disorder, unsightly and a source of discomfort (itching). Chapter 21 attempts to illustrate how dandruff condition fits within these previous and following paragraphs, i.e., how it can be influenced by sebum, its effect upon the hair cycles, etc. Dandruff is a fascinating field of research and a clear example of a complex ecosystem where large domains of biology admix (physiology, microbiology, immunology, etc.).

1.1.5. Evaluating Hair Loss

The mechanisms and main features of hair growth, hair loss, alopecia, etc., are reviewed in Chapter 2 (26). Anti-hair loss products refer, here, to compositions mainly aiming at preventing excessive hair loss, being seasonally induced or resulting from inflammatory processes, rather than stimulating a significant hair regrowth, as induced by drugs such as Minoxidil® or Finasteride® (13,27).

From a legal point of view, anti-hair loss products are diversely regulated (see Chapter 13). Despite hair loss, e.g., masculine and/or feminine baldness, is not regarded as a disease, as shown by a recent poll to MD's organized by the *British Medical Journal* through the web (28), anti-hair loss products are still considered as drugs by certain authorities.

In fact, the threshold between cosmetics and drugs is clearly defined by safety. Cosmetic products are not normally associated with benefit/risk ratios nor with potential side effects or contraindications as drugs are. That makes the difference as reflected by Minoxidil® or Finasteride®.

Minoxidil has been, historically, a pioneering product as a potent inducer of hair regrowth. Its development resulted from a side effect, when taken orally as an antihypertensive agent (29). The first report of such a side effect goes back as early as 1979 (30), but remained rather neglected for years. Despite numerous hypotheses, its mechanism of action is still largely unknown and since the drug patent has expired, many Minoxidil-based preparations have been put on the market. Minoxidil had the valuable merit, in the 1980s, to "awake" a scientific community who believed that alopecia could not be efficiently reversed, i.e., that the decline in hair follicle biology was an irreversible natural process. Subsequently, hundreds of patents have been issued with claims about products with hair regrowth properties, ranging from the most rational to the most poorly grounded, herbal traditional medicine included.

Finasteride has been marketed as an oral drug (Propecia®) with substantiated evidence of hair regrowth potential. As a potent inhibitor of 5- α reductase type II, it was initially developed and marketed for the treatment of prostate neoplasm. Its use to correct alopecia, at a 30 times lower dosage, was secondary and based on the same rationale, although the hair follicle expresses both types of 5- α reductase (see Chapter 19) (30). Both its efficacy on androgenetic alopecia and side effects are well-documented (13,31–33).

With regard to cosmetic products with anti-hair loss properties (at least in E.U.), 2,4-diamino-pyrimidine-3-oxide (2,4-DPO), has been marketed (Aminexil®) and used to slow down the typical increased hair loss observed in spring and autumn (seasonal hair loss) on the basis of the results of multi-center studies (34). Other marketed preparations contain procyanidins, flavanone, immunostimulating peptides, etc. which aim at targeting different growth factors (VEGF, TGF- β , FGF, TNF- α , etc.) (35,36), or a simple combination, i.e., a lotion of a bactericide and an anti-fungal agents, exhibiting a substantial anti-inflammatory effect that leads to an improvement in the hair cycles ratio (37). In addition, repeated applications of anti-fungal agents through anti-dandruff shampoos have been showed to slightly increase the diameter of the hair shaft (38).

Irrespective of the type of products and targeted mechanism, the anti-hair loss or hair regrowth approaches share common features:

- Basically, none can be considered to date as a true, complete and definitive solution to stop and/or fully reverse the balding process. They all, at best, mitigate the evolution of the disorder by maintaining hair growth phase duration and thus retarding the miniaturization process of terminal hair.
- Controlled studies, as compared to the vehicle, clearly show that the so-called placebo effect is far from being nil, suggesting that other ingredients (propylene glycol, ethanol, etc.) used as vehicle for topical applications (together with massage) might possess intrinsic action on the follicle. Even by the oral route, it is quite surprising to observe that the “innocent” pill used as placebo/control in the Finasteride studies can induce some slight but significant side effects in the hormonal sphere, and libido included (39). Brain is a fascinating organ of command.
- The efficacy of the final product should be determined *in vivo* in human volunteers, in controlled studies for the reasons expressed above and to allow for variations, spontaneous or seasonally driven (40–42). In addition, the vehicle effect (so-called placebo) has to be assessed when studying hair growth. Rigorous control of these studies appears as the best way of avoiding biased claims.
- Irrespective of the mechanism assumed to be involved, mostly based on *in vitro* findings, they should lead to objective and significant modifications of the hair cycles *in vivo*, increasing either hair diameter or its rate of growth. These parameters can be strictly followed by the use of techniques such as those described as follows.

Trichogram (43–47). This technique was initially described by Van Scott et al. in 1957 (48). The hair-collecting procedure is based on the brutal and rapid pulling up of about 50 hairs, when firmly held between the rubbed jaws of a homeostatic pincer. The collected hairs are subsequently mounted between a glass slide and a cover slip for microscopic observation. Visual assessment of each proximal part allows one to establish their individual situation with regard to the various phases of the hair cycle, i.e., anagen, catagen, or telogen. In normal conditions, 10–15% hair are in the telogen phase, whereas 85–90% are in anagen phase. An anagen/telogen ratio below 4 will suggest a telogen effluvium status. It is a simple technique, easy to carry out and needs only one visit. It has the advantage of being independent of hair color and allows detection, when present, of “abnormal” states such as dystrophic anagen (49) or the teloptosis phenomenon (50). The distal part of the hair can, in addition, be used for quantifying hair diameters and their statistical distribution. This technique cannot, however, determine hair density. This is one of the reason that led Rushton et al. (51) to propose the Unit area trichogram which consists in clipping all the hairs present within a standardized area of the scalp which are further classified by their growth stage, i.e., telogen, resting anagen, growing anagen, etc.

Phototrichogram (52–58). In this technique, photographs are taken from pre-shaved patches of the scalp, of about 1 cm² each. Two or 3 days later, new photographs are taken of the same precisely delineated patches (see Figs. 8 and 9). By comparing the pictures, the growing hairs in anagen phase and those of unchanged length, in telogen, can be differentiated. Being non-invasive and non-traumatic, this method can be performed as frequently as necessary, allowing the study of the entire cycle of individual hairs.

Like the trichogram technique, it shows whether a hair is in growing or shedding phase, but has the added advantage of providing a measurement of the number of hairs per surface unit, the hair growth rate and the duration of the growth phases. Other parameters can also be determined, such as hair pattern, hair thickness, lapse of time

between the shedding of one hair and the appearance of the new hair (latency or exogen phase), etc.

A variant of the phototrichogram, the videotrichogram, uses a video camera in an optic fiber, thus replacing the classic photograph camera (59–61).

When combined with image analysis techniques, these photo or video visualization methods offer the precious advantage to allow automated recordings of some parameters (59,60,62).

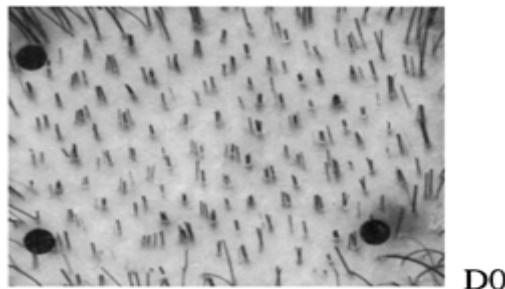


Figure 8 Typical images obtained by the phototrichogram technique at D0 (just after shaving).

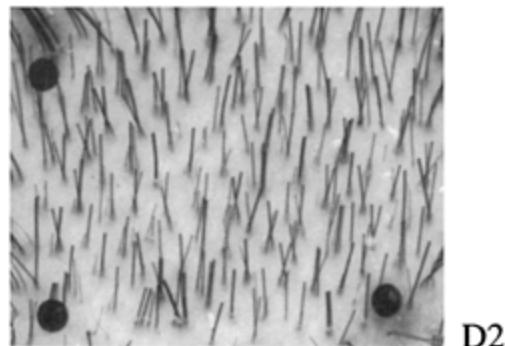


Figure 9 Typical images obtained by the phototrichogram technique two days after shaving (D2).

The continual development of this non-traumatic technique, combined with statistical analysis, has resulted in a body of important information on the growth cycle of the human hair (41,63). The duration of the growth cycles of hairs on an individual head, even those of neighboring hairs, vary greatly, ranging from several months to several years. Variations also occur in hair diameter. The androgenetic alopecia process has the effect of greatly increasing the proportion of hairs of a short cycle (Fig.10). Frequently,

the percentage of hairs in the telogen phase is taken into account in diagnosing alopecia. It is only, however, an average figure that does not reflect hair diversity and evolution of the whole head of hair.

Tractiophototrichogram. This method was originally described by Bouhanna (64). After a scalp area of 0.25 cm^2 has been delineated, soft and repeated tractions are performed to pull out hairs in the telogen phase. Other hairs, supposedly in the anagen phase, are then cut trim with the scalp surface and a photograph of the whole area is taken, allowing their counting. Adding both numbers (telogen and anagen hair counts) provides the total density of hairs in this area. Although this method avoids the need for two consecutive visits unlike phototrichogram, its limitations lie in the non-standardization of the pulling force, a likely source of variable results from one investigator to another.

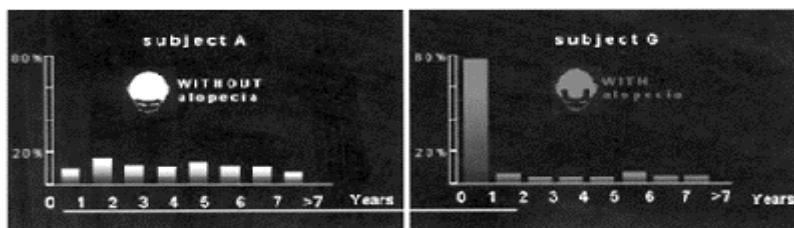


Figure 10 Examples of the range and distribution of durations of anagen hair phase with and without alopecia. (Data from Ref. 41.)

Target Area Hair Counts (65–67). This method, involving one single visit, aims at counting hairs present within a 1-inch diameter circle target area, previously clipped. Counting the hairs can be performed either *in vivo* using low magnification or on the photographic snap. This approach appears less informative than the phototrichogram, since it is limited only to the measurement of hair density.

Hair Weight (68). In this technique, a scalp area of about 1 cm^2 is previously delineated and shaved. Six weeks later, the hairs present in this area are successively clipped, washed, dried, and weighed. This method possesses the major advantage of being global, i.e., integrating many factors such as hair growth rate (length), diameter, and hair counts. It requires, however, much meticulousness and a high level of training of the technicians involved in the investigation.

Biopsy. A biopsy, analyzed in horizontal section (69), can be performed, allowing the assessment of follicles according to their respective hair cycle phase, to differentiate terminal hair from lanugo and to measure hair diameter. With regard to its invasive nature, it should be mostly dedicated to study factors that may negatively affect hair growth (fibrosis, inflammatory reaction, etc.).

Hair Pull Test (70) and Clinical Scales (71). Hair pull test consists in exerting a manual traction on about 50 hairs and counting those which have been pulled up to get an estimate as percentage. A ratio over 10% would suggest a telogen effluvium process. It is

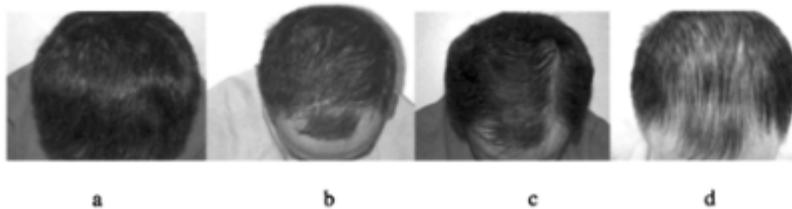
more a method for helping in diagnosis rather than a true quantitative approach. Furthermore, it shows strong variations from one investigator to another.

Clinical scales, based upon different photographic aspects of both hair density and diameter, are useful and provide standardized charts/guides for the global assessment of the various grades of baldness. With regard to its simplicity, the technique of clinical scales can be easily and routinely applied to studies on large cohorts.

In short, phototrichogram, target area hair counts and hair weight, are non-invasive methods that can be used on clearly defined scalp areas, allowing a rigorous follow-up of the observed follicles over time. These methods offer the major advantage over the invasive ones (trichogram, biopsy) of being suitable for recording the variability of hair growth parameters on various sites of the scalp.

In addition to these methods which measure specific parameters, some global techniques may be used.

Global Photographs. Modifications in hair counts, diameter, and rate of growth will obviously affect, in the long term, the overall aspect of the whole head of hair. It then becomes logical to follow such changes through global pictures of the whole head of hair. Such an approach, however, requires a high level of standardization (72) of technical conditions for correct interpretation and validity such as distance, lighting, film characteristics, and development—all steps that are not always easily controlled. Special emphasis should be placed on the photographed head of hair. Great care should be taken with its standardization (length of hair/ haircuts, mode of combing, color, etc.). The latter aspect is of paramount importance since it may lead to strong bias and false feelings of hair regrowth or hair scarcity. To illustrate this point, the same head of hair has been photographed by us on two consecutive days in different situations such as before/after hair cut, wet hair, different hair styles, etc. (see pictures a,b,c,d below). One can realize, from such photographs, that the effects of a particular hair regrowth treatment could be easily made to look positive, based on misleading photographic evidence if the evaluation did not follow and stick to stringently defined procedures ensuring relevance of the comparison.



(a) Before a hair cut, day 1; (b) After a hair cut, day 2; (c) Wet hair before a hair cut, day 1; (d) Wet hair after a hair cut, day 2

To be properly and reliably interpreted, global pictures should be evaluated by several investigators, who should preferentially be trained for such assessment. However, some automated techniques, using image analysis, allow a direct assessment without human intervention (73). Nevertheless, global photographs do help in the classification process, allowing reference to published topographies proposed for male alopecia (74–76) or for female alopecia (77,78).

Hair loss can be globally assessed too. Derived from the method used to quantify dandruff (79), the method called “Trichanalectia” (42) allows one to collect and count hairs that are removed and trapped by a $100\text{ }\mu\times 100\text{ }\mu$ filter, during a standardized shampooing.

Additionally it is possible to ask volunteers to collect, daily, their lost hair from various sources (pillow, comb, brush, etc.). Counting can be performed either by the volunteer or by the investigator who can further observe the morphological appearances at the distal part (70,80). Although a useful addition in the quantification of hair loss, the technique should nevertheless be cautiously interpreted: since all lost hairs in a full day are not necessarily counted, these techniques naturally underestimate the actual amount of daily hair loss.

Such a large arsenal of available techniques allow investigators to adapt these to their specific needs in the design of protocol, taking into account reliability, easiness, practicability, and accuracy. Not only restricted to evaluation of hairloss, some of these techniques can be used in the assessment of the efficacy of hair care products.

1.2. Hair Care

1.2.1. Hair Type and Condition

The feel of natural, healthy hair is both firm and soft. It is easy to disentangle when wet or dry. When kept clean, it has a glossy, non-greasy look given by the sebum taken up by the hair.

Dry Hair. We have looked at the consequences of excessive sebaceous secretion. If, on the other hand, secretion is insufficient, the scalp appears taut and dry, and the hair becomes dull looking and brittle to the touch. This is known as “dry” hair and it has physiological causes. When hair is traumatized by over-vigorous mechanical or chemical treatments, it also ends up looking this way. It happens to hair that is often wound too tightly onto curlers, to hair that is highly bleached, and to hair treated with too alkaline permanent wave lotions or with shampoos with excessive detergency power. Or, it can occur when brushing is too frequent or excessive; hot blow-drying intensifies the drying effect to an extent determined by its frequency.

Dry hair is prone to tangles. Combing enhances shaft damage (81) and split ends when unsnarling; cuticle cells are uplifted and alignment of loops formed along stationary Fibers mostly entail longitudinal splitting, if not breaking.

A further factor in the frailty of hair is weathering. This term covers the cumulative effects of climatic exposure, namely sunlight, air pollutants, wind, seawater and spindrift, or chlorinated water of swimming pools (82). The prevailing inducer of damage is sunlight which, apart from bleaching brown hair and yellowing blond hair, causes photooxidative splitting of cystine linkages, initiates free radicals detrimental to the protein matrix, and increases porosity of the cuticle. Degradation processes are enhanced by ambient humidity. The physiochemical changes are more pronounced on bleached, waved, or dyed hair, but intact hair itself is rendered more sensitive to cosmetic handling. The alterations are not restricted to surface properties—tactile, frictional, glossy, etc.; tensile strength has been shown to decrease progressively with increasing exposure (83,84). It is not a matter of inadequate sebum level, but an alteration in fiber texture.

Fine, Limp Hair. Fiber texture also is a concern associated with another hair type very commonly found in northern Europe, the United States and in Anglo-Saxon countries in general. It relates to fine hair which often creates problems for the women and men having such hair. This is neither a deficiency nor a natural or artificially induced anomaly. Some hair is healthy whilst lacking firmness, rigidity, and volume. It is subject to fly-away, is hard to style and tangles easily. With a diameter below average, it is weaker and more sensitive to external traumatic agents than other hair types. It splits and breaks easily and the ends are often spliced, especially if the hair is long, making it look droopy and unattractive.

These defects can be attributed for the most part to the mechanical characteristics of the hair, which are closely related to the cross-section. For two fibers, having diameters in a ratio of 1:2, their respective rigidity toward torsional stress ratio is 1:16. Moreover, as shown by electron microscopy, the number of cuticle layers and their thickness are about the same in fine and coarse hair. Therefore, the only region of hair affected by reduced section is cortex, which is the dominant contributor to the overall mechanical properties.

The total surface area of hair is also important. The specific surface (developed surface of a given mass) of finer hair, i.e., the part of the hair directly exposed, will be greater, as shown in the following table. The problems of fly-away and lack of control will also be greater, and the entire head of hair will suffer more from the consequences of surface alteration, or rapid regreasing (the more so as sebum spreads much more quickly through fine hair). Fine hair is sensitive to aggressive or too frequent treatments; the diffusion and penetration of the reagents inside the hair affects a greater proportion of the hair fiber structure.

Hair type	Hair diameter
Fine	<60 μm
Average	60–80 μm
Thick	>80 μm
Hair diameter	Specific surface (cm^2/g)
40 μm	760
80 μm	380
110 μm	250

In addition, although this is not necessary the case, fine hair can be “limp” hair, lacking in firmness. As mentioned above, fine, droopy hair must be given firmness, body, spring, elasticity, and fullness (volume) without loss of suppleness, luster or silkiness.

All physicochemical and chemical alterations of hair can be defined and measured (see Chapter 12) (83,84):

- Loss of sheen.
- More brittleness to the touch, with a loosening of cuticle scales and increase in the friction coefficient.
- Increased porosity with subsequent increase in drying time.

- Lower disruption point. The hair breaks more easily. This is due to the disruption of certain linkages providing fiber cohesion, e.g., cystine, hydrogen bonds, etc.
- Decrease in sulfur content.
- Degradation in polypeptide chains leading to the elimination of oligo-proteins, etc.

1.2.2. Basic Ingredients for Hair Care

The main active ingredients used in formulating hair care products specifically designed to treat and improve hair condition are the following.

Organic Acids. Acids are classical agents used in hair care products. Everyone knows the virtues of rinsing with lemon juice or vinegar! They are useful for several reasons. After washing hair with soap, hair looks dull. This is due to insoluble lime soap deposited on hair as a result of soap interaction with hard water. Alkali produced by soap hydrolysis can also impair hair surface and appearance. The acid rinse brings the epidermal pH and the "hair pH" back to a normal level. It also dissolves the soap deposits, and through the calcium salts of fatty acids released, gives back its normal feel and sheen to the hair. After bleaching, an acid rinse precipitates proteins and thus prevents the elimination of amino acids or oligoproteins resulting from the fiber degradation. It is obvious that aging of hair due to the repetition of external stresses from various origins (photochemical oxidation, overprocessing, inappropriate or damaging treatments, vigorous mechanical handling with brush, e.g. 200–300 times over 3 months, etc.) causes a decrease in the resistance of hair to repeated combing. Flexabrasion (85) is a convenient test method for this evaluation. Treatment of bleached hair with solutions of carboxylic acids restores the initial fatigue resistance to hair. Aromatic sulfonic acids such as toluenesulfonic or naphthalenesulfonic acids also have some effect, but much less significant than that of carboxylic acids. These findings led to the design of specific shampoos for restoring resilience to cosmetically altered hair as illustrated by distinct effects obtained with shampoos formulated with a substantial amount of an α -hydroxyacid, e.g., citric acid, or fruit acid, their pH being adjusted at 5 (86).

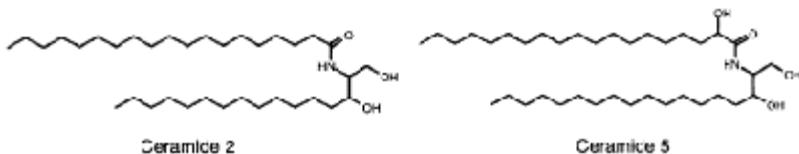
Fatty Compounds and Their Derivatives. Fatty compounds are naturally present in hair not only as sebum but also as constitutive elements of the fiber.

Several authors have published the results of human sebum analysis. The composition generally accepted includes free unsaturated fatty acids (20%); free saturated fatty acids (10%); triglycerides (30%); fatty esters other than triglycerides (20%); cholesterol, pure or combined (5%); and squalene (15%).

Other studies have demonstrated that significant amounts of lipids are strongly associated with hair in such a way as to be resistant to solvent extraction (87,88). These internal hair lipids include free fatty acids, wax esters, ceramides, hydrocarbons, free cholesterol and cholesterol esters, and triglycerides (I).

The prevalent fatty acid was identified as 18-methyl-eicosanoic acid (88) presumably covalently linked to the proteins of the cuticle (89).

Ceramides present in a lipidic extract from human hair are related to ceramides classes 2 and 5 in which the basic long-chain is predominantly sphinganine. These differ from ceramides found within the stratum corneum which are sphingosinederived (90):



Representative structures of main human hair ceramides

It has been shown that permanent waving (91) and irradiation with sunlight (92), degrade the internal lipids of hair.

Providing hair with suitable fatty material to compensate for the lack of fatty elements seems to be a logical step. It was traditionally used in all civilizations as essential care products: vegetable oils have been prized for their ability to beautify, protect, lubricate the hair and lend it softness and sheen. In Egypt and Greece, olive oil was used; in Africa, karite oil; in India, ratanjot, shorea, or neem oil; in Polynesia, monoi oil; and among the American Indians, jojoba oil. Nowadays, the most frequently used products belong to a number of categories:

1. Fatty acids: oleic, stearic, behenic, ricinoleic, linoleic, and linolenic (vitamin F) acids.
 2. Fatty alcohols: lauryl, myristyl, oleyl, cetyl, and stearyl alcohols.
 3. Natural triglycerides: almond, castor, peanut, avocado, corn, olive, monoi, and karite oils.
 4. Natural waxes such as beeswax, spermaceti, and jojoba oil.
 5. Fatty esters such as glycol stearates and synthetic short alcohol fatty esters.
 6. Oxyethylenated or oxypropylenated waxes, alcohols, and fatty acids.
 7. Partially sulfated fatty alcohols, lanolin and its derivatives, other animal waxes, phospholipids, fatty acyl lactylate salts used years ago as conditioning agents are less and less included in hair care products.
 8. Ceramides, which are fatty acylated sphinganine, sphingosine, or phytosphingosine. Synthetic ceramides have also been shown to restore the integrity of damaged hair cuticle and re-establish its protective role. Among them, *N*-oleoylsphinganine (93) has been developed and extensively studied in hair care applications. In the late 1980, other ceramides or closely related substances (pseudoceramides) were patented (94,95), but few of them have been used in marketed products. Furthermore, vegetable-derived ceramides have been proposed by ingredient suppliers.

Refined techniques have been used to demonstrate ceramide affinity for the hair (Fig. 11). Present in the fiber between the overlapping scales of the cuticle (96), it imparts a tighter junction and enhances smoothness as a result.

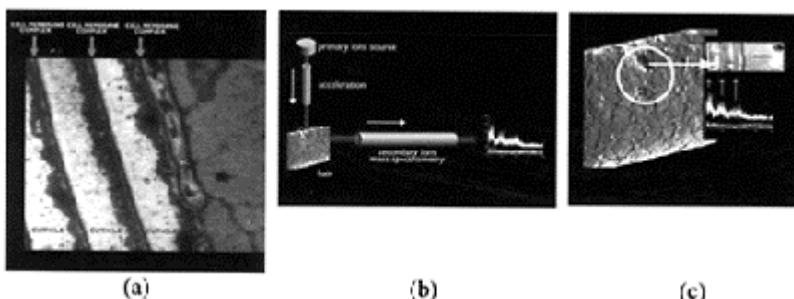


Figure 11 (a) Transmission electron microscopy (TEM) of a cross-section of brown natural hair ($\times 50,000$) stained with uranyl acetate and lead citrate to reveal non-keratinous components. (b) Secondary ion mass spectroscopy (SIMS) representation. (c) SIMS image of hair treated with radiolabeled ceramide. Erosion crater caused by ions beam is visible which makes it possible to localize the radioactive species in the voids of the cell membrane complex.

Ceramide protects hair from chemical and physical damages such as that caused by excessive exposure to solar radiation in wet conditions. It maintains the cohesion of the cuticle when submitted to physical insults. In particular, African-American hair weakened by relaxing treatments are protected by ceramide during brushing (97). A number of hair care products have been developed based on these properties (93–98).

Vitamins. Vitamins are mostly chosen from those of groups A and B and can be provided by miscellaneous extracts. Wheat germ, separated out during the milling process, has gained favor: it contains liposoluble or hydrosoluble vitamins, auxin, agents promoting the development of yeasts, diastases, microelements, fatty acids, phosphatides, amino acids, and sugars, their relevance to hair remaining to be substantiated. It is especially rich in group E vitamins, in particular α -tocopherol, useful because it is involved in the regulation of oxidoreductive phenomena.

D-panthenol, the precursor of D-pantothenic acid (vitamin B5), a natural constituent of healthy hair, is also extensively used. In addition to its affinity for hair, it can penetrate the hair shaft. It has a humectant properties and makes hair more manageable.

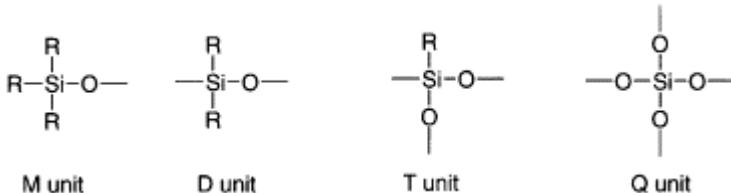
Protein Derivatives. Protein molecules are too large to penetrate the hair and fix onto hair keratin. They can be used either in the form of partial hydrolysates, a peptide mix, or, even in the form of amino acids resulting from total hydrolysis. Extracts or hydrolysates of keratins (cow horn, hoof, horsehair, wool, hair), silk proteins, collagen, gelatin, casein,

isinglass, protamines from fish milt, vegetable proteins (soybean, oat, wheat) are used. Sometimes use is made of their condensation products with fatty acids.

The protein retention by hair, especially when collagens obtained by enzyme hydrolysis are used, has been studied. The greater the degree of hair damage, the higher the sorption level will be, and sorption is controlled by ionic equilibrium phenomena; sorption is highest at pH 9–11 for natural hair, at pH 6 for strongly bleached hair, and has been proved to be optimal for the polypeptide fraction of average molecular weight 1000 (99,100).

Silicones. The use of silicones in hair cosmetics dates back to the 1950s. Silicones denotes the following polymer structure: $(R_nSiO_{[4-n]2})_x$, where n is between 0 and 3, x is 2 or higher. R is generally a methyl group but it may be substituted by long-chain aliphatic moieties, phenyl groups, or by functional groups, e.g., amine, mercapto, fluoro, or hydroxyalkenyleneoxide.

A classical nomenclature applied to define silicones consists, when R is methyl, in referring to M, D, T, Q as the four major siloxane chain units. M refers to the end unit ($R_3SiO_{1/2}$); D refers to dialkylsiloxane unit (R_2SiO); T refers to the branch group silsesquioxane ($RSiO_{3/2}$); Q represents silicate (SiO_2):

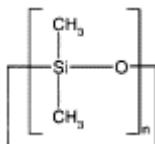


Silicones cover a wide range of compositions and architectures including silicone homopolymers, silicone random copolymers and silicone-organic block copolymers.

Polydimethylsiloxanes. Polydimethylsiloxanes are the largest volume of silicone homopolymers produced to date. They exhibit the characteristic properties of the silicone family: chemically inert, low surface tension, low glass transition temperature, weak cohesive forces, and water insolubility. This causes them to spread easily on most surfaces to give a quite uniform, smooth, hydrophobic deposit. Several silicone homopolymers find use in hair care products:

1. Cyclic siloxanes (Cyclomethicone)

Basic structure is as follows:



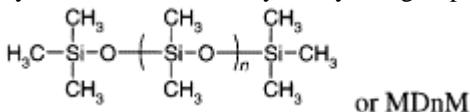
or D_n , where n can vary from 3 to 6.

The most common cyclic siloxane used in cosmetics is decamethylcyclopentasiloxane (D5). It is a colorless, odorless, volatile liquid which is compatible with numerous hair care ingredients and give a very soft, non-greasy touch.

It is used as solvent for other silicones or polymers, in conditioning and styling products, as an additive in rinse conditioners or as a volatile plasticizer in hair spray polymers.

Cyclomethicones are considered as non-volatile organic components (VOCs) by the U.S. Federal and State Environmental Protection Agencies.

2. Linear dimethylsiloxane with trimethylsiloxy end groups (Dimethicone).

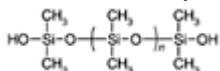


A large variety of polymers belongs to this family, with viscosity ranging from 0.65 to 1,000,000 mm²/s at 25°C. The silicones with lower viscosity (approximately 4 mm²/s) are volatile.

Hexamethyldisiloxane (MM) is more volatile than decamethylcyclopentasiloxane (D5). It is used as solvent for other silicones in leave-on products.

Silicones having higher viscosity appear like oils or gums insoluble in water but soluble in aliphatic hydrocarbons. Dimethicones with medium or high viscosity (50,000–1,000,000 mm²/s and more) are extensively used as conditioning agents in shampoos.

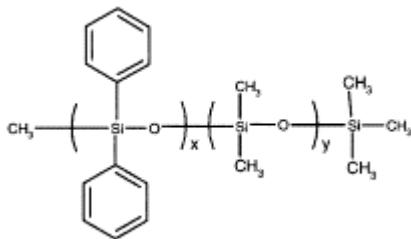
3. Linear polydimethylsiloxane with dimethylsilanol end groups (Dimethiconol)



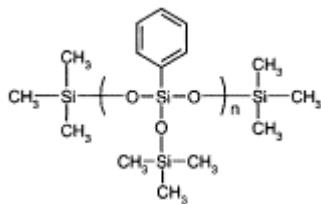
This type of silicones are generally produced by kinetically controlled hydrolysis of chlorosilanes. Dimethiconols are available with a viscosity ranging from about 20 to 1,000,000 mm²/s. Their uses are quite similar to those of dimethicones, they are employed as conditioning agents.

4. Phenyl-containing siloxanes.

The most commonly used phenyl siloxanes in hair care products are the linear dimethyl siloxane/diphenyl siloxane copolymer, so-called diphenyl dimethicone (Fig. a) and the “T” structure polymer phenyltrimethylsiloxane (phenyl trimethicone) (Fig. b).



(a)

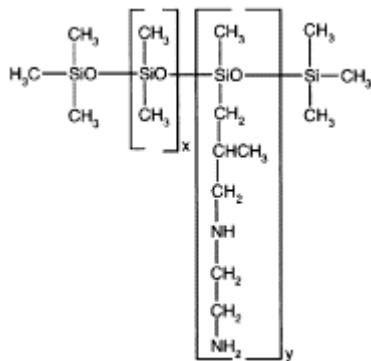


(b)

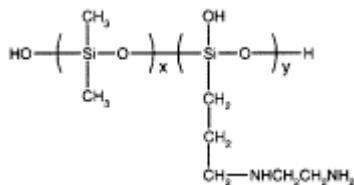
When methyl groups are replaced by phenyl groups in a polysiloxane structure, the compatibility with organic materials is improved and refractive index increased. These polymers are used to improve hair condition, and to enhance the shine and gloss of hair mainly in leave-on products.

Dimethylsiloxane Copolymers with Functional Groups. They include a great variety of ingredient categories. The most used are:

1. *Copolymers of dimethylsiloxane and (di) amino alkyl methylsiloxane.* Trimethylsilylamodimethicone (Fig. c) and Amodimethicone (Fig. d) derivatives are preferred.



(c)

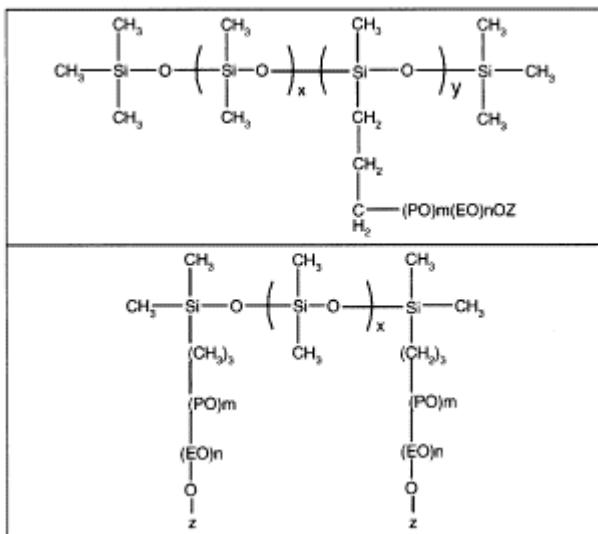


(d)

Amodimethicone differs from trimethylsilylamodimethicone by its reactive silanol groups. A wide range of polymers with various molecular weights and amine contents are

available. The presence of amine groups in silicone structure increases hydrophilicity of the polymer and makes it easier to formulate. When the silicone derivative has a high content of amine groups, neutralizing with an organic or inorganic acid can make it soluble in aqueous medium. Furthermore, silicones with amine groups are cationic polymers with good affinity to hair. They have therefore become important components of conditioners, mousses, perms, and hair coloring products, taking advantage of their dual properties of silicones, and cationic polymers.

2. Siloxane-polyether copolymers. Various structural types of copolymer have been synthesized. They most frequently contain a polyether or a linear block polymer consisting of ethylene oxide and propylene oxide units (silicone glycol). Typical silicone glycols are random or block copolymers. Examples are represented by the following formulas:



where EO, ethyleneoxy; PO, propyleneoxy; and Z, hydrogen or a lower alkylradical.

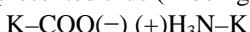
The addition of some polyoxyalkylated substituents increases the solubility of the polymer in polar solvents.

Depending on their structure, molecular weight, type and size of side chain, silicone glycols may be soluble in water or alcohol and used as conditioners, wetting or foaming agents, plasticizers or modifiers of the properties of film-forming polymers in styling products.

Silicone glycols have been recognized as additives that reduce eye and skin irritation associated with some anionic surfactants.

The CTFA designation of silicone glycols is Dimethicone Copolyol.

Cation-Active Surfactants. A natural hair can be regarded as an amphoteric gel possessing basic and acidic groups with nearly equal and inverse strengths. It can be represented thus (K being the polypeptide chain):



A slight predominance of acid groups suffices to give hair the nature of an anionic polymer. It happens in the case of damaged hair, especially bleached hair, which becomes very rich in free acid groups as a result of cystine linkage disruption and oxidation. These groups are sulfonic acids, strong acids that give the hair surface a strongly anionic valence. Intense exposure to sun can bring about this kind of transformation through ultraviolet light action on the cystine linkage, increasing hair damage.

The cation-active derivatives are surfactants with a hydrophilic cationic group carrying one or two lipophilic hydrocarbon fatty chains. When a cation-active compound comes into contact with a damaged hair possessing numerous anionic sites, an electrochemical bonding between the negatively charged fiber and the positively charged cationic site of the cation-active substance can take place. Cation-active compounds have a high affinity for the keratin fiber. Due to their polarity, they can neutralize and eliminate the discrepancy in electrical charge between the different hairs.

Basically, the action of a cation-active, i.e., cationic surfactant entails fixing a monomolecular film to the hair through electrochemical bonding. Immediately, even very damaged hair is given a pleasant, soft feel and excellent combability, because the film is composed of a fatty chain, which provides lubrication, reduces fiber friction, and minimizes abrasive effects of combing and brushing. These qualities are most dramatic on wet hair. The lubricant contribution, however, is less noticeable on dried hair, where weighing down should be avoided.

There are many cation-active substances. Depending on the type of anion or cation, their properties (detergent, emulsifying, conditioning, and adsorption properties) can be varied. The longer fatty chain compounds, such as the stearyls or the behenyls are far more effective conditioners than those with shorter fatty chain, such as the lauryls (101). This category includes the following:

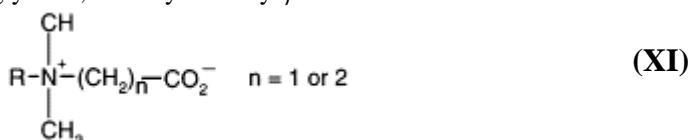
1. Quaternary ammonium salts with one or two fatty chains such as the behenyl or stearyl or oleyl dimethylbenzylammonium, lauryl pyridinium, and distearyldimethylammonium salts.
2. Fatty amines, such as stearyldimethylamine.
3. Ethoxylated fatty amines, quaternized, or non-quaternized (structure X) (102), e.g., Quaternium 52*:



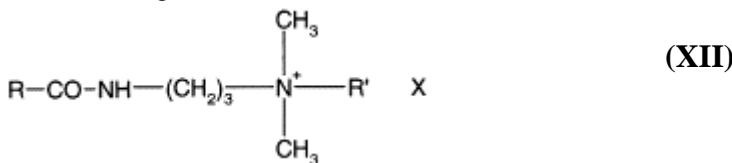
R=hydrocarbon fatty chain

*CTFA (US Cosmetic Toiletry and Fragrance Association) adopted name

4. The quaternized α or β fatty amino acids (structure XI), such as the alkyldimethylglycines, the alkyldimethyl β -alanines:



5. Sugars or derivatives having a quaternary ammonium group, such as the quaternized gluconic amino amides, e.g., Quaternium 22*.
6. The quaternized (structure XII) or non-quaternized amino amides of fatty acids, such as stearoylaminopropyldimethylamine, e.g., Quaterniums 61, 62, 63, and 70*, or the amides of lanolin acid, e.g., Quaternium 33*(103):

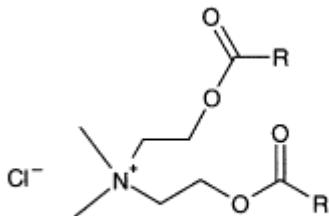


7. Esterquats: The term “esterquat” is commonly used to describe a class of surface-active quaternary ammonium derivatives having the general formula $\text{R}_4\text{N}^+\text{X}^-$, where the hydrophobic part of the radical R, which contain more than four carbon atoms, is linked to the positively charged head group via an ester bond. Depending on the starting materials and the process used, different mixtures of mono, di- and triesterquats may be obtained.

While traditional alkylquats are stable under the conditions of use, esterquats are stable in aqueous medium only within a narrow pH window otherwise they decompose releasing fatty acid and non-toxic amino alcohol derivative devoid of surfactant activity.

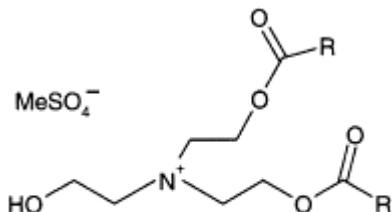
Esterquats were first used in the 1990s as textile auxiliaries. Nowadays, they are extensively patented and developed commercially for hair care applications. The most important classes of esterquats are those derived from alkanolamines and fatty acids. Some examples are given below.

Diestequat of dimethyldiethanolammonium chloride



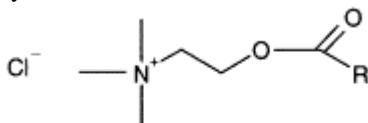
R = alkyl or hydroxyalkyl (C_{12-22})

Diestequat of triethanolammonium methosulfate



$\text{R} = \text{alkyl or hydroxyalkyl (C}_{12\text{-}22}\text{)}$

Monoester of dimethylethanolamine



$\text{R} = \text{alkyl or hydroxyalkyl (C}_{12\text{-}22}\text{)}$

The major interest of cationic surfactants lies in their conditioning properties on moist hair after washing. Optimal effects are obtained in non-ionic vehicles.

These are also useful in the form of anion-cation complexes produced by mixing a cationic compound with an anionic compound. For example, if ammonium oleate is mixed with a fatty amine, in stoichiometric proportions, the result is the fatty amine/oleate complex. When a hydroalcoholic solution of the amine oleate is applied to a bleached hair, this is what is thought to occur:

1. The free acid groups in the hair keratin are stronger than, and displace, the oleic acid and fix the fatty cationic compound, i.e., the fatty amine by electrovalent bonding.
2. The fatty acid released can fix itself to the hair by adsorption.
3. Thus, the hair is conditioned both by anion-cation exchange (as it occurs with a cation active product) and by adsorption (as with fatty compounds).

When strongly anionic surfactants are present, such as an alkylsulfate or an alkylethersulfate, the effects are significantly lowered.

Cationic Polymers. Cationic surfactants are the ideal compounds to normalize the hair surface, protect damaged areas, smooth out the cuticle scales, impart softness, and facilitate disentangling, combing, and brushing. But they do not improve hair texture. Moreover, the use of these compounds has certain limitations. Some are not well tolerated by the eye, but the main drawback is that most of them are incompatible with the major anionic surfactants used in formulating shampoos. Hence, developed the idea of a new category of cation-active compounds, having a similar affinity for hair and able to sheathe the surface with a continuous film and thus impart body, texture, and firmness while protecting it.

These are the cationic polymers, in which the hair-binding cationic groups are no longer attached to a fatty chain, as they are in cationic surfactants, but grafted or integrated into a polymeric structure. Cationic polymers have made a breakthrough in

conditioners, given their specific properties and the compatibility shown by many with the most commonly used anionic surfactants. They were also revolutionary in the way they protect weakened or fragile hair against external attack. The first polymer used was a cellulose resin called "Polymer JR" (Polyquaternium 10*), introduced in an anionic shampoo in 1972 (104). The average molecular weight of this type of polymer lies between 250,000 and 2,000,000.

Interactions of quaternized cellulose polymers with human hair were extensively studied (105–108) and the effects of molecular weight, concentration and charge of the polymers, pH (106) and composition of the solutions [salts (107), surfactants (106,108)] were pointed out. Since then, many cationic polymers have been patented, marketed, and used in formulating dry hair treatment products (109,110). The main categories of interest to cosmetic chemists are summarized as follows.

Cationic Polysaccharides. Examples of these polymers are:

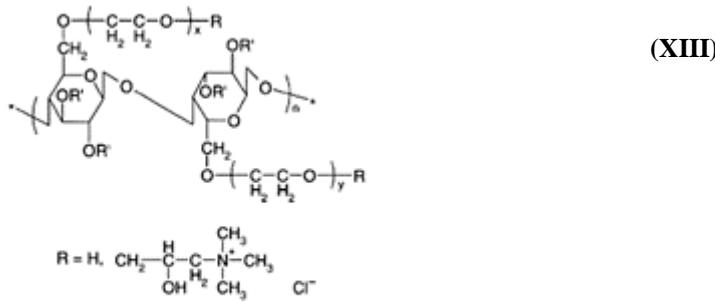
Ucare Polymer JR 30 M, 125, 400 from Amerchol (structure XIII);

Quatrisoft Polymer LM 200 (Amerchol), a hydroxyethyl cellulose reacted with lauryl dimethyl epoxypropyl ammonium chloride;

Celquat L 200 (National Starch), e. g., Polyquaternium 4* a graft copolymer of hydroxyethylcellulose with diallyl dimethylammonium chloride (structure XIV);

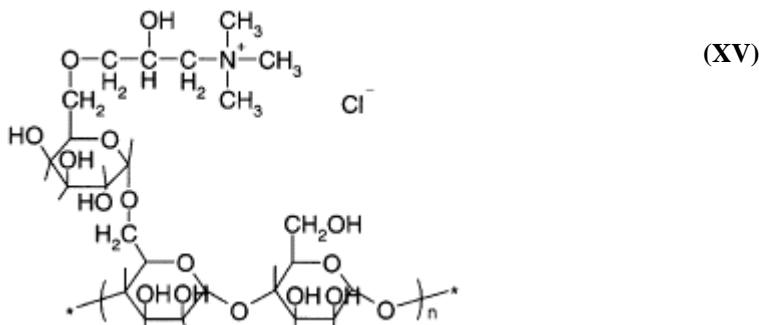
Guar derivatives, e.g., Jaguar C 13 S, C17, C 14S (Rhodia) and Cosmedia Guar C 261 (Henkel)(structure XV);

Kytamer KC (Amerchol), e.g., Polyquaternium 29*, reaction product of chitosan (deacetylation product of chitin) with propylene oxide then quaternized with epichlorhydrin.



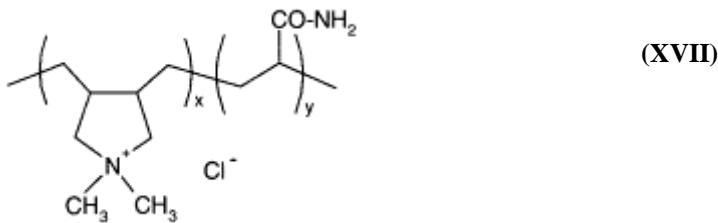
R=H,



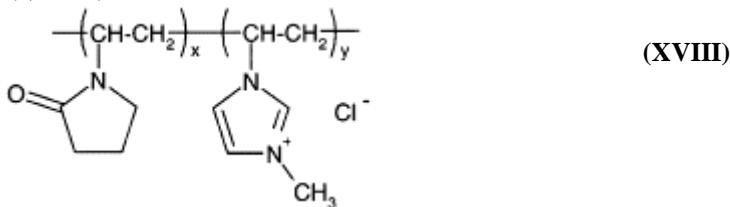


Dimethylsulfate Quaternized Copolymers of Vinylpyrrolidone and Dimethylaminoethyl Methacrylate (Polyquaternium 11*). An example is Gafquat 734 and 755 (from ISP), with average molecular weight of 100,000 and 1,000,000, respectively.

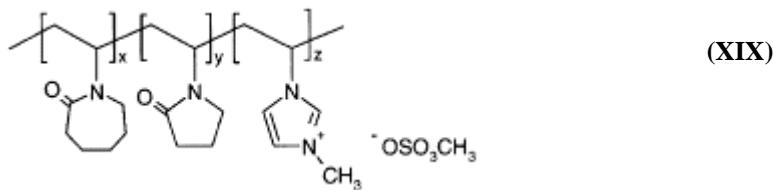
Diallyldimethylammonium Homopolymer (Polyquaternium 6*) and Copolymer with Acrylamide (Polyquaternium 7*). Examples are Merquat 100 (structure XVI) and 550 (structure XVII) from Nalco.



Vinylpyrrolidone/Methylvinylimidazolium Chloride Copolymer. An example is Luviquat FC 905 (BASF)(XVIII).

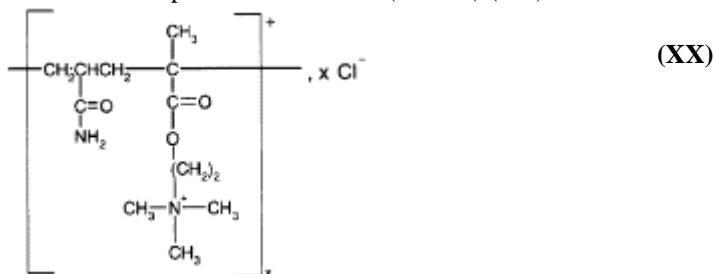


Vinylcaprolactame/Vinylpyrrolidone/Methylimidazolium terpolymer (XIX) (Polyquaternium 46*) developed by BASF (Luviquat Hold) especially for styling products:

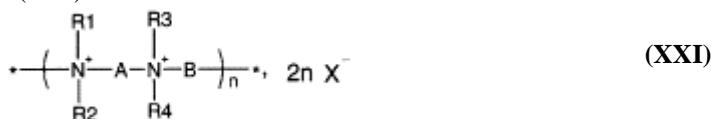


Vinylcaprolactame/Vinylpyrrolidone/Dimethylaminoethyl methacrylate terpolymer such as Gaffix VC-713 (ISP) which combines both conditioning and holding properties.

Acrylamide/Quaternized Dimethylaminoethylmethacrylate or Methacrylate Copolymers, e.g., Polyquaternium 32*. Example is Salcare SC63 (Rhodia) (XX).

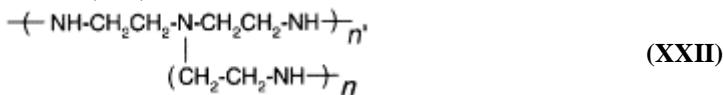


Ionene polymers (XXI).

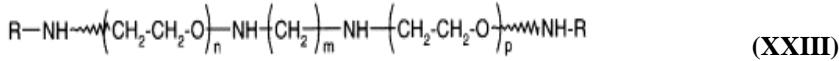


These polymers generally have much lower molecular weight, between 1000 and 20,000 (111). Examples are Mirapol A-15 (Rhodia) (Polyquaternium 2*), A=(CH₂)₃-NHCONH-(CH₂)₃, B=CH₂-CH₂-O-CH₂-CH₂, and Mexomere PAK (Chimex)(Polyquaternium 34*), A=B=(CH₂)₃, R₁=R₂=C₂H₅, R₃=R₄=CH₃X=Br⁻.

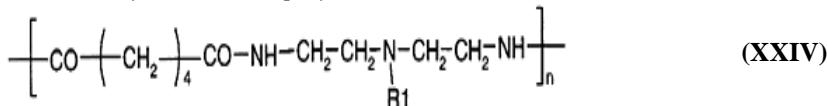
Polyethyleneimines (112).



Polyoxyethyl polyamines. An example is Polyquart (Henkel) (XXIII)



Adipic acid/diethylenetriamine polyamides (XXIV).



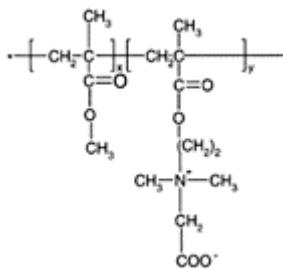
Among these cationic polymers are Cartaretine F4 and F23 (Clariant) with $R_1=CH_2-CHOHCH_2-N(CH_3)_2$ or Delsette 101 (Hercules) or Mexomere PK (Chimex) which is the reaction product of adipic diethylenetriamine with varying amounts of epichlorohydrine, i.e., from partial to total quaternization.

Piperazine cationic polymers (113,114) with general structure (XXV)



Amphoteric and Betainic Polymers. More recently introduced on the market (115) these polymers provide a good mix between conditioning and styling properties.

Examples of amphoteric polymers are Polyquaternium 22* (dimethyldiallyl ammonium chloride and acrylic acid copolymers), e.g., Merquat 280 and Merquat 295 (Nalco), Polyquaternium 39 (acrylic acid, diallyl dimethyl ammonium and acrylamide terpolymer), e.g. Merquat Plus 3330 and 3331 (Nalco) or Polyquaternium 47 (acrylic acid, methyl acrylate and methacrylamidopropyl-trimonium chloride terpolymer), e.g., Merquat 2001 N (Nalco). Polyquaternium 30, e.g., Mexomere PX (Chimex) illustrate betainic polymers



1.1.3. Formulation of Hair Care Products

The aim of hair care products is above all to restore the hair's natural beauty, i.e., to give it lightness, volume, spring and control while also providing suppleness, softness, and sheen (116,117).

The demands vary depending on the nature, abundance, and condition of the hair. For hair that is dry or very dry, creams are required that are highly conditioning, that nourish, soften, and make for ease of untangling and styling. Natural or hardly damaged hair will need cosmetic creams basically providing manageability, lightness and suppleness and enhancing the natural qualities of healthy hair. For fine, limp hair without volume, the need is above all to give it texture, consistency, "body," spring, resilience and fullness while keeping it supple and silky. Coarse, thick, straight hair needs emollience, suppleness, and manageability. In the case of severely damaged hair, rendered porous by hard processing or cumulative weathering, the fiber must be strengthened, restructured, protected and its softness, shine and bounce restored. Hence, the necessity for varying the product formulations and compositions but also the presentation and texture in order to properly fulfill the very different, specific requirements and needs of each type of hair and consumer habits as well.

The great majority of formulations available, known as "rinses," combing aids or conditioners are cationic emulsions of waxes, mainly fatty alcohols. The relative amounts of cationic conditioning agents [surfactant(s) and/or polymer(s) and wax(es)] will determine the appearance and the rheology of the beautifying product but also the level of care (suppleness, untangling, softness, etc.) provided to the fiber.

* CTFA (US Cosmetic Toiletry and Fragrance Association) adopted name.

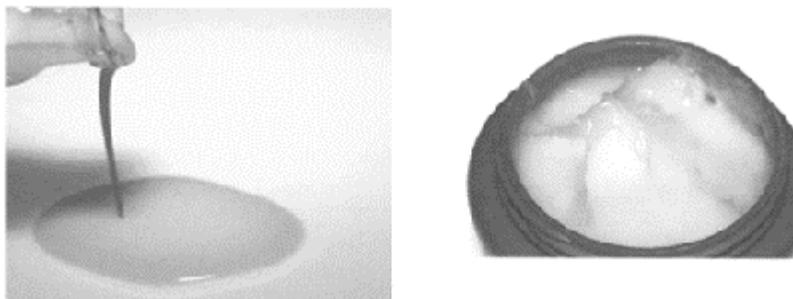


Figure 12 Examples of different consistencies of conditioning emulsions or creams.

The choice of cationic surfactants is made not only on the basis of the intensity of care required, but also more and more by taking into account the characteristics of ecotoxicity and safety of the substances (118–121). The viscosity of these preparations can range from 1000 cps to 50,000 cps; the pH is acid from 3.5 to 5.5 to maximize the affinity of the cationic agents for the hair fiber (Fig. 12).

Conditioning Creams and Emulsions. These are the most widespread products. They are applied after shampooing the hair and are rinsed off almost immediately afterwards; leaving the product on the hair for 1 or 2 min results in a better conditioning effect. Their main aims are to make combing out easier and provide softness, suppleness and shine to the hair when it has dried. They are available in the form of lotions, creams varying in consistency as in the case of balms or conditioners, or in the form of gel emulsions (using PEG or cellulose derivatives). These products are easy to apply and they penetrate the whole head of hair rapidly. They can be used each time the hair is shampooed, whatever the hair type, to beautify the hair, but they are particularly suitable for hair that is dry, damaged, or normal. The regular use of a conditioning cream after the shampoo corresponds to a hair care ritual characteristic of some countries.

Deep Conditioners. Also known as masques or packs, these are stick creams packaged in jars or tubes. They are very rich in conditioning (cationic) agents. Their consistency allows the application of a thick layer (cataplasma) to the particular area in which the hair is most damaged. Applied and left on the hair for a prolonged time, sometimes with added heat (under a hair-dryer or application of moist, hot towels), these creams are used specially for the treatment of very dry hair in which the hair shaft is impaired, fragile, rough, and porous. They are able to flatten the scales, to make them tightly overlap each

other, leveling out the surface and making it smooth, protecting the fiber thanks to the preferential fixation of the conditioning agents to all the damaged sites. Whether the creams are highly untangling care or deep conditioner, modern formulations often combine cationic surfactants and silicone derivatives that act synergistically not only to ease combing-out of wet hair but also supply lightness and individualization to the fiber (122–127).

Lotions or Liquid Gels. Clear or opacified “emulsified fluids,” liquid or with the texture of a slight gel and non-oily, these formulations are meant particularly for normal hair or hair with an oily tendency and for fine, limp hair. The conditioning agents, generally solubilized in water, are mainly formulated in a simplified environment (absence of fatty alcohol, for example) so as to avoid any weighing down or coating that could spoil the appearance of the hair. These lotions provide lightness, shine, an anti-static effect and, depending on the hair’s needs, body and volume. The hot oils, aqueous solutions of cetrimonium chloride and polyquaternium 80 are good illustrations of this type of formulation.

Besides these lotions presenting a slight conditioning effect, it is possible to formulate very liquid treatments, also called serums or essences that have a marked conditioning, untangling and repair effects e.g., by preparing very fine emulsions of waxes and fatty substances, micro-emulsified with a cationic surfactant such as Quaternium 87 (128–131). These formulations combine ease of use (application, rinsing) of a serum and the benefits of a conditioning cream.

Conditioners Free from Cationic Surfactants. This type of hair care presents a much lower level of conditioning (suppleness, untangling). It is of particular value on hair that has suffered little damage or fine hair that does not tolerate being weighing down. Many formulation attempts have been described (132–134) but few have been marketed due to their failure to untangle the hair adequately. More recently, the family of associative polymers has opened the way to significant improvements in the conditioning properties of hair care products without cationic surfactants. Thus the combination of two polymers, e.g., Polyquaternium 37 and saturated methylenediphenyl diisocyanate (SMDI)/PEG 150 stearyl alcohol yields a conditioning cream gel characterized by a melting effect on the hair (Quick Break effect) linked to the associative polymer (135).

Hair Treatments with a Vector Effect: The Nano-Emulsions. To treat, beautify, and repair the hair usually means depositing on the surface of the hair the conditioning agents that will alter and improve the feel and appearance of the fiber. For a greater, more lasting effect, it is sometimes desirable to transport those agents into the deepest layers of the cuticle. Formulations with a vector effect must therefore meet specific criteria and in particular be suited not only to the dimensions of the hair fiber but also to the physical and physicochemical structure of the lipid layers of the inter-scale cement, for example.

Hair Dimensions

Element	Characteristic	Value
Shaft	Diameter	≈70 μm
Scale	Diameter	5–10 μm
Scale	Thickness	0.5–1 μm
Inter-scale cement	Thickness	0.2–0.3 μm

Several vector systems are described even though the difficulties faced in their formulation, stability, and mode of preparation continues to pose problems in their implementation. For example, lamellar systems (136) and liposomes (137–142) carry lipophilic agents such as sunscreens, vitamins, ceramides, etc., effectively to the surface of hair (143–150). Oil nano-emulsions especially with vegetable oils are representative of these hair care products with a vector effect. They are characterized by oil globules less than 100 nm in size, making them transparent. Depending on the volumetric fraction of the oil, the nano-emulsified preparation is presented in the form of a liquid or a translucent gel (Fig. 13).

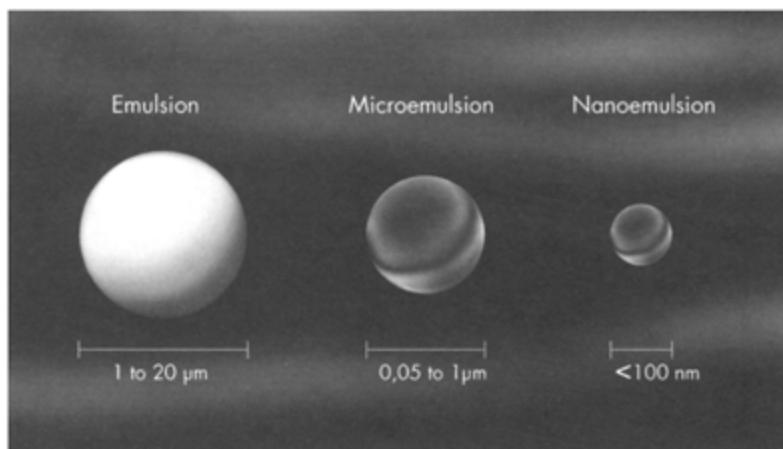


Figure 13 Typical ranges in the sizes of emulsion, microemulsion, and nanoemulsion.

These nanoemulsions are produced by high-pressure refinement (800–1500 bars) of a primary emulsion (Fig. 14) using the technology called “HPH” (high-pressure homogenization).

With this type of formulation, the hair fiber can be provided with a greater amount of lipids than would be the case with classical emulsions of the same composition thanks to the extremely small size of the globules that are diffused. The presence of cationic surfactants within the emulsifying system of the nano-emulsion increases its vector capacity yet further (150).

The value of such a formulation for hair care is its ability to nourish and protect the hair fiber thanks to the lipids contained in plant oils without leaving an oily, heavy, sticky feel often associated with oils. The nano-dimension of the oil globules affords a greater, more effective impregnation of the outer layers of the cuticle which results in an effect on the fiber that remains perceptible after several shampoos, as shown in Fig. 15.

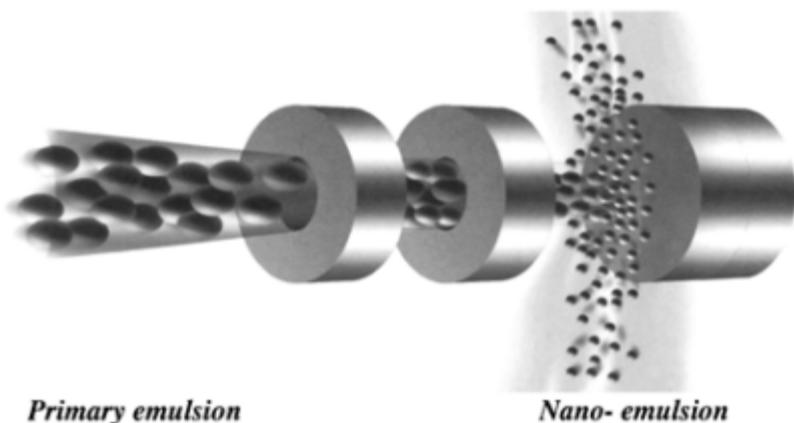


Figure 14 Scheme of the physical process leading to nano-emulsification.

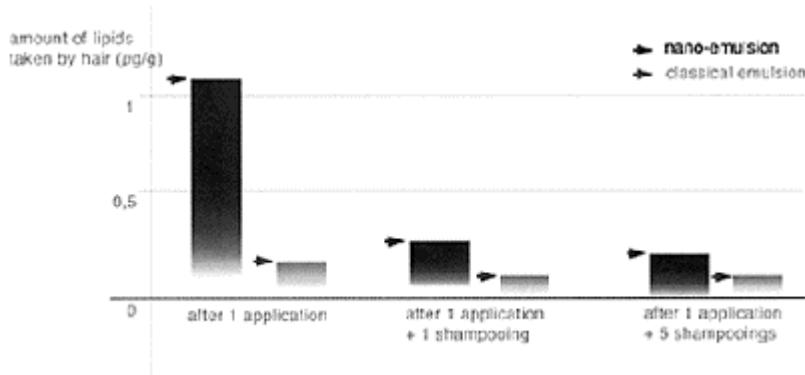


Figure 15 Comparative differences in the amount of lipids bound to the cuticle layers using a nanoemulsion refers a classical emulsion.

Leave-on Conditioners. This class of products, applied after shampooing, aim at improving combing out, providing a soft feel, smoothness, shine to hair, and ease in hair styling. Since not rinsed off, their composition must be fine-tuned to avoid build-up.

Like conditioning products followed by rinsing, they are mostly based on the affinity of cationic derivatives for the hair fiber. Similar cationic surfactants as those present in rinsed conditioners are mainly used but in a lower amount since the whole product is left on hair. Quaternary ammonium salts having a long fatty chain such as behentrimonium chloride (i.e. C₂₁) are generally preferred. However, when the product need to be entirely soluble in aqueous medium, shorter chains (e.g., C₁₇) are used such as cetrimonium

chloride. Some cationic polymers are also found in a variety of products, taking advantage of their efficient conditioning effects, eg., polyquaterniums. Their film-forming properties further contribute to hair styling and dressing which is not commonly provided by cationic surfactants characterized by deep conditioning effects.

In addition to cationic derivatives, silicones are widely used agents for hair care as they both provide soft feel and lightness. Early silicone derivatives of polydimethylsiloxane type have given way to functional silicones with targeted effects: thus cationic aminosilicones enhance affinity and thus impart cosmetic/conditioning benefits; phenylsilicones add luster; ethoxylated silicones help making water-soluble, etc. Molecular weight also influence viscosity and the appearance of the product.

Finally, emollients such as glycerol or various polyols are introduced in some products taking advantage of their high compatibility with aqueous or aqueousethanolic medium.

Fluid Products. This type of product is supplied as a clear aqueous or aqueous-alcoholic lotion mainly single dose- or pump-spray. They were the first leave-on products to be introduced on the market. Although they may contain a limited amount of ethanol, the formula is most generally aqueous and is based on the following combination:

- silicone derivatives, either water soluble or pre-emulsified in order to be easily blended during the preparation,
- cationic surfactants, preferably water soluble,
- emollients such as glycerol, propyleneglycol, etc.

These fluid products are still marketed nowadays but with a much larger diversity of presentations.

Aerosol Foams. Also available as hairstyling products which appeared on the market in early 1990s, they gain favor because of their fine, soft, smooth consistency, spreading and melting evenly and nicely into the whole head of hair. In addition, they offer the advantage of allowing to apply a more easily controlled amount, thus avoiding possible build-up. The hair care components are overall similar as in previous types of products: silicones, cationics, and emollients. Added ingredients are:

- propellants, mostly mixtures of hydrocarbons,
- one or several foaming agents selected among cationic or non-ionic surfactants.

Leave-on foams met with some success and are still widely used in the field of hairstyling. They have been, however, now superseded by emulsions.

Creams and Emulsions. With the growing availability of gelling agents saving both emulsifying and conditioning properties, a variety of products from light emulsion to thickened cream could be obtained by proper formulation. Due to their innovative textures, they gained a tremendous success that has never failed. Their appeal comes from the great diversity of consistencies which can be achieved by creative cosmetic scientists and formulators, making use of a palette of gelling agents, used alone or combined.

Oils and Serums. They are generally water-free products, based on silicones or oils. These oils can be applied before or after shampooing. Before shampooing, oils treat the hair or protect it from the combined action of sun and salt water, from chlorinated swimming pool water, wind, and low atmospheric humidity. The time required for the massage and the waiting interval can be a few minutes or up to an entire night. Spanish

women put olive oil on their hair in the morning and covered their heads with towels to give the oil a chance to really penetrate and do not shampoo until the evening. After shampooing, oil is used as a regular conditioner.

Oils are intended for thick or very dry hair. They are often used in sun care hair products: they aim at nourishing hair damaged by repeated bathing associated with sun exposure. Much less oily, silicone derivatives enable us to formulate more versatile care products varying in texture, from fluid to thickened serum depending on the viscosity of the components. Thus, available products include sprays that provide a silky effect, fluid serums that smooth the hair, thicker serums for targeted applications on split or damaged ends, etc.

To give an example of product formulation, a silicone gum or a mixture of gums (e.g. dimethiconol and/or dimethicone) is made soluble in a volatile silicone (e.g. cyclopentasiloxane)

It should be noted that the very designation “serum” also includes aqueous products which are emulsions of cationics and/or silicones. These products are actually leave-on hair care formulations presented as fluids or gelling creams as described above.

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5

Temporary Restyling of the Hair

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1. INTRODUCTION

To persuade the hair to adopt temporary restyling, the primary agent required was water, to plasticize the hair and stretch it slightly, then drying was used to maintain the elongation and shape achieved.

Setting was initially used as the styling aid. It required the use of curlers, a hair dryer and sometimes setting lotions. Fixing the hair with hair spray was then necessary to prolong the hold of the hairstyle for several days.

Blow-drying, i.e., styling the hair using a brush and hair dryer, was introduced in the 1970s and changed hairdressing habits while creating a demand for new hairstyling products. This phenomenon was accompanied by the abandonment of curlers and a move toward structured hairstyles.

During the 1980s, hairdressing practice changed: hair became shorter, with more frequent hairwashing, and hair styling with the fingers became a widely used technique.

In the 1990s, new effects were sought: on the one hand, a natural, supple, flexible hairstyle achieved using hairdressing products with a clean feel, leaving no residues; on the other hand, very marked, sophisticated effects, obtained by dressing the hair using an accessory or the provision of heat.

2. PRINCIPLES OF HAIR SETTING

It is known that the structure of hair keratin makes it elastic. Slight elongations are completely reversible, which is a characteristic of all elastic materials. However, the rate at which the return to the initial state occurs can be a function of the condition under which the strain is carried out.

The levels of stress corresponding to various extensions can be measured with a specially adapted tensile meter. This type of instrument gives the necessary information to draw a load-elongation (stress-strain) diagram (Fig. 1); examining this diagram, one can try to interpret what occurs during the hair set. The following observations can be made from the diagram.

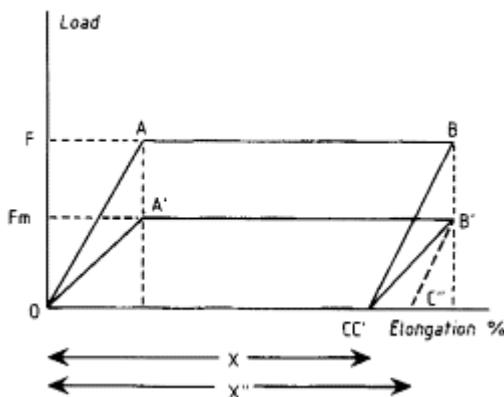


Figure 1 Load elongation diagram for human hair.

2.1. Cycle of Dry Hair

For low extension values (up to about 2%), the elongation is proportional to the load (curve OA). Then, starting at a critical load F , it is clear that the hair can be easily stretched to high extension levels without increasing the load (curve AB). If, at this point, the hair is rapidly released, it retains a residual elongation ($OC=X$) even though the load level has become zero (curve BC). The residual elongation slowly decreases until the hair recovers its initial length. The total cycle is represented by the parallelogram OABC.

2.2. Cycle of Wet Hair

If the entire operation described above takes place in water, the diagram OA'B'C' (Fig. 1) is obtained. It has the same shape as in the dry state, but with the following characteristics:

1. The angle of the curve OA' with the abscissa is less than the angle of the curve OA (lower slope).
2. The load value F_m giving the same extension increase is lower than the value F (approximately half).
3. The points C and C' for the same hair are identical.
4. The displacement rates for the curves A'B' and C'O are much higher than for dry hair.

The same phenomena can be observed for wet and dry hair, but in the case of wet hair, lower load values and higher rates prevail. Schematically, this means that wet hair, for low extension values, is more elastic than dry hair. The return to the original position is effected more rapidly.

2.3. Extension of Wet Hair, Drying Under Stress, Releasing After Drying

Let us take the preceding cycle and stop at B', allow the hair to dry maintaining the same load, and release it suddenly once it is dry. Relieving the load is accompanied by a residual elongation X" greater than X, and the curve B'C" is parallel to BC, therefore to OA.

This observation is of the utmost importance, because it provides an explanation for the setting process. It demonstrates that wet hair with a low extension value of about 2%, dried under a load, and then released retains more elongation than dry hair or wet hair that has undergone the complete cycle described above. The return from the position C" to the position 0 will occur, but it will be slower than the return from C or C' to 0.

Thus, temporary strain or reshaping will have been given to each hair. Sets carried out in hair salons are done in similar fashion, with wet strands of hair being rolled either in curls around the finger or on curlers. The rolling must be done tightly. Then the hair is dried. Unrolling the curlers after drying corresponds to the release from a load. The shape obtained will last for several days, but will not be retained if the hair is wetted. For setting operations, hair is not stretched to the 2% mark referred to previously, but it may be so at the molecular level.

The mechanical observations above are probably related to physicochemical changes at the protein structure level (1). Above a certain value, stretching causes partial transformation of α -keratin into β -keratin. This is accompanied by a shift in relative position of the polypeptide chains, facilitated by water moistening the hair. This shift brings about disruption of the ionic and hydrogen bonds. During the drying procedure, new ionic and hydrogen bonds are formed, which block the return to α -keratin. Gradually, the new linkages give way under the natural forces that cause the hair to return to the original state and length. If the hair is moistened the return to the α -form is virtually immediate.

These explanations are confirmed by the following experimental observations:

1. Curling hair does not cause any reshaping.
2. Rolling and immediate unrolling of wet hair yields no reshaping, even if rolling is done tightly.
3. Only rolling wet hair, drying under stress, and releasing after drying produces temporary restyling. But it is well-known that the tighter the winding, the longer lasting will be the new shape. This is often not compatible with the dictates of fashion: when loose waves are in style, rolling is done loosely (given that tight rolling yields tight curls), although all these produce a short-lived set.

It was noted that points C, C', and C" on the diagram (Fig. 1) are not fixed. They tend to be drawn toward the initial position. The problem lies in slowing down their return to the original position, in order to obtain hairstyles that last as long as possible.

It is logical to seek a delayed action in the moistening of dried hair with various solutions containing film-forming polymers either natural or produced by synthesis. In aqueous or aqueous-alcoholic solutions, these polymers will leave a film on the hair when it dries. This film ensures cohesion and increased stability. This is why setting lotions have been devised to maintain the hold of the hair set.

It is also logical that hairstyle "stability" be an objective, through the fixation of the new shape after drying and the subsequent finishing touches made with the comb. The idea is to cover the styled hair with an invisible net that will give it a degree of rigidity and protect it against wind and humidity. This is the rationale of hair sprays.

3. HAIR STYLING AND SETTING PRODUCTS

3.1. Shaping the Hairstyle

The shape given to a hairstyle is not only a very important esthetic element, it aims to be in harmony with the shape of the face, but it is also an expression of the personality of the wearer.

In hairstyling, the shape of the head of hair is modified by positioning the hair with the hands or with the help of tools such as the comb, brush and perhaps hair dryers or curling tongs. There are numerous variables in the hairstyling technique. They are chosen according to the length, shape and nature of the hair (curly, smooth, straight, fine, etc.) and the desired hairstyle. Among them are included blow-drying, rollers and natural drying.

- Blow-drying combines the simultaneous working of the hair with a brush and application of hot air from the hairdryer. It is particularly suited to giving volume to fine, straight hair or to giving curly hair a smooth hairstyle.
- Setting the hair, which involves winding the hair on rollers and drying it under a hooded drier, is an effective way of endowing fine hair with body or curls.
- Natural drying consists of giving the hair its desired shape with the help, in general, of a comb or brush and then leaving it to dry naturally in the shape given to it.

Hairstyling techniques demand different kinds of work and dexterity: the set and blow-drying require time and technical expertise, whilst natural drying has the advantage of being extremely simple. They differ also in the extent to which they hold the imparted shape over time. Techniques involving traction and heat have a longer-lasting effect. Thus a hairstyle achieved by blow-drying and setting can maintain its shape for several days.

All these hairdressing techniques have in common the fact that, since they only affect the ionic and hydrogen bonds in the fibers, they do not resist shampooing. A single shampoo suffices to annul the shape of a hairstyle.

3.2. Products to Prolong the Hold of a Hairstyle

It is appreciated if the shape given to a hairstyle lasts over time, if possible until it is next washed—particularly if the hairdressing session has been a lengthy one.

But after completion of the hairstyle, this is naturally subject to many factors that can impair its shape—hair weight, the wind, everyday activities, humidity, etc.—and completely cancel out all the hairstyling efforts. Such factors can, for example, lead in straight hair to a loss of volume, drooping of the hair as it emerges from the scalp, the appearance of kinks, loss of symmetry. Curly hair that has been straightened regains its

natural curl. To avoid constant retouching, products have been developed to prolong the hold of a hairstyle.

However, it must be stressed that these products can make a hairstyle last longer but do not allow it to resist the effects of shampooing.

3.3. Finishing Products and Style-Controlling Products

There are two main categories of product: “finishing or setting products” and “style-controlling products” and their ways of working and efficacy are very different:

- The first (which will be tackled in Sec. 4) act by binding the hairs together. As a result the hairstyle is secured in place and very resistant, but has, on the contrary, a rather stiff appearance.
- The second produce the styling effect without binding the hair together. The resistance of the hairstyle conferred by controlling products is not as great as that conferred by hold products. Some of these products are able to leave the head of hair looking very natural with hairs freely mobile and nimble.

The shape desired, the natural strength of the hair, the length of time it must retain its shape all play a role in the choice of products. To keep a natural appearance, styling products are preferred but if the hairstyle is sophisticated or if the aim is for a long-lasting effect, then holding products have to be used. The two types of product can be combined—a styling product to give the hairstyle its shape and a hold product to prolong its durability.

Finishing products also differ from style-controlling products by their usage after completion of hairstyling and several times daily instead of once a day at the most. Once the hairstyle is complete, they are used to hold it in place. Then they are called “finishing products.” Style-controlling products are applied before or during hairstyling and are thus also called “modeling products” or “shaping aids.” However, the adaptability of products is such that the order in which they are used can be inverted. Both types of product can be applied before, during or after hairstyling.

3.4. The Evolution of Hairstyling and Setting Products

A few decades ago, when the fashion was for very elaborate hairstyles and the hair was washed less frequently, the hairstyle needed to last for several days or even a week or more. To achieve sufficiently long-lasting hold, products with very strong effects and the most effective hairstyling techniques (setting and then, later, blow-drying) were used.

Nowadays, habits have changed and hairstyling follows virtually daily shampoos. New products have therefore emerged for the needs of rapid and frequent hairstyling. The characteristics of the products have been following this evolution:

- On the one hand, it is no longer necessary for the hairstyle to keep its shape for almost a week. Maintenance for 2–3 days is adequate.
- And, on the other hand, simple and rapid hairstyling techniques are preferred (using the fingers, natural air drying or rapid blow-drying). Shorter hairstyles abound. The hairdressing techniques being lightweight, the styling products must, in compensation, be more effective.

The changes in frequency of application are not the only ones to have had an impact on product development. The shape of the hairstyle also evolves. Thus hairstyles with a very natural appearance are now in favor.

The demands for new shapes appear (or reappear). For example, strands of hair are particularly suited to short hairstyles. It is a matter of sticking the hair together forming strands between 1 and 2 cm wide. Treated in this way, the hair takes on a very unusual appearance reminiscent of wet hair, or forms prickly spikes defying gravity. Products change rapidly with fashion and are now available in many forms to yield a variety of results.

Finally, products are designed to bring additional benefits such as greater shine or conditioning to ease the process of blow-drying and improve the appearance of the hair.

3.5. Mode of Use of Setting and Styling Products

As mentioned earlier, style-controlling products are often applied prior to the process of hairstyling. In this event, they are applied to wet hair (generally after a shampoo) or on dry hair before restyling, especially in the morning. Style controlling products can also be applied after hairstyling when the hair has dried. In this event, the products may re-wet the hair, grouping it into strands to create stranded effects or help it to take on a new shape (curl definition, hair straightening). To avoid locally overloading the hair, the product must be applied with caution: one technique currently used consists of spreading the product on the palm of the hand to create a fine film before applying it to the hair. The product can also be applied in little dabs. Another technique consists of separating hair that would otherwise have stuck together using the fingers, a comb or a brush.

As explained in the section on “principles of formulation” the spreading properties of the product play an essential role in its efficacy.

3.6. The Principles of Product Function in Hair Styling and Setting

The efficacy of the products is derived from the fact that, after application and drying, they leave a film on the surface of the hair. The properties of the material constituting this film determine the end-result. This material must benefit from a certain number of “intrinsic” or “physical” properties. The most important characteristics are:

- “hardness”: hard, soft or liquid
- “resistance”: solid or fragile
- “surface properties”: sticky or non-sticky.

In parallel with these intrinsic properties of the material, the “hair-coating morphology” on a microscopic and macroscopic scale also plays an important role.

- On a microscopic scale, the coating may be smooth and completely cover the hair fibers, or take the form of little plates.
- On the macroscopic scale, the coating may be homogenous (along the whole length of the hair) or heterogeneous (for example, a greater amount on the tips than at the roots). The material can also “weld” the hair together to yield a stranded effect.

The end-result of a finishing product, i.e., keeping not only the shape over time but also providing the sensory achievements of feel and shine, depends upon all these factors. The following three examples describe products with very different effects:

1. A product leaving a very homogenous film on each hair fiber, both microscopically and macroscopically. The coating being very evenly spread, its thickness will be slight. The setting effect is thus limited. However, if the properties of the material are well chosen, this holding effect can last for some time. The result can be especially worthwhile as it will keep natural, resistant over time and the tactile properties will be perfect thanks to the smooth, fine coating. The product will hold the hairstyle in place in an inconspicuous way.
2. A product producing a badly distributed film will look as if it is covered with bumps when viewed through a microscope. The friction that this causes between individual hairs can provide cohesion to the hairstyle and thus a substantial and lasting hold. The limits of this approach lie in the fact that the bumps generated on the surface of each hair can impair the feel and appearance of the hair, leaving it dull and dry to the touch. This must be taken into consideration during formulation.

There are other products that provide a thinly spread and fluid coating. This coating can act as a barrier to humidity and help the hair to stick together. These products help the hairstyle to keep its shape in humid climates. They have the disadvantage of giving the hair a somewhat waxy appearance.

3.7. Principles of Styling and Style Controlling Product Formulation

As mentioned earlier, products must be simple to apply and they must spread easily over the hair fiber if they are to retain the desired cosmetic properties. The properties of the material deposited after the product has dried are critical and the formulator must be well aware of these in order to develop a new product with novel effects.

Thus the ease of application, the distribution of the product over the hair fiber, the capacity for maintaining the hairstyle and the cosmetic performance are decisive when considering possible formulation routes.

The texture and presentation of the product play a primary role in the properties of spreadability and ease of application of the product. The first presentations were in the form of a “lotion” (destined particularly for hair setting) or a “wax.” Other more suitable forms were invented: the “gel”, the “foam” and, more recently, the “spray-gel” form.

The “gel” is often used by men as it is particularly suited to the needs of short haircuts.

“Foams” are products designed to hold the hairstyle in place and give the hair volume while leaving the hair fiber clean and regular. These are more feminine products.

“Waxes” and “pastes” are also enjoying renewed popularity, notably because they can be applied to specific areas of the hair.

The following guidelines are useful:

- Gels must not become soapy when spread on the palm of the hand.
- The rheology of gels is one of the key elements in their spreadability over the hair. The “rheofluidifying” effect (firm at rest and fluid in movement) gives the best results, with a melting texture that spreads very readily.

- The foams must be firm so that they do not break down in the hand but must be able to melt into the hair. The proportions of foaming agents used relative to the propellant mean that the texture can be adjusted as required.
- The products must neither impede nor facilitate the hairstyling. For example, the ease with which the brush passes through the hair must not be hampered. This point is particularly important in the case of products with a high holding capacity. Indeed, a high film-forming polymer concentration of these makes the surface of the hair temporarily sticky when the product has been applied during the drying process. The use of lubricants such as silicones can improve the ease of application and spread of products over the hair.

Long-lasting capacity and cosmetic performance define the properties required of the material and thus the ingredients of which it is composed. The product must, after having dried on the hair, provide the coating desired through its intrinsic properties and its spreadability.

- In choosing the ingredients that will form the intrinsic properties of the coating material, the rules of polymer physics have to be followed. In brief, there is a whole variety of polymers with differing intrinsic physical properties, ranging from the most flexible (called low glass transition temperature T_g materials to the most rigid high T_g materials). Materials having a low T_g can become sticky and, on that basis, lead to undesirable tactile properties. The choice of polymers and of additives that might modify the T_g yield the standard of material required.
- As to the choice of ingredients leading to the required spreadability, no simple guideline has been drawn up, mainly because the texturing agents (thickeners, foam boosters), but also other ingredients (silicones, etc.) can have drastic effects on spreadability.
- For ease of elimination of the coating when shampooing the hair, polymers readily soluble in water are favored.
- One of the great difficulties encountered by the formulator consists of the fact that the user chooses which hairdressing technique to use and this sometimes without regard for the recommended mode of use. Hence it is necessary to check that each product can be used and remain effective for all known hairdressing techniques. The performance and the type of product usage must therefore be evaluated in a large number of tests calling for participation by both consumers and hairdressing professionals. To allow the formulator to progress more rapidly in the choice of formulation numerous laboratory tests have been developed (see Sec. 5).

In choosing the components, the formulator must reason in the following way. The formulator knows that each ingredient has a major role to play and can be selected in accordance with the end-result sought:

- The main task of the polymers is to provide hold.
- Solvents serve as vehicles for the polymers.
- A thickener gives the product its texture.
- Lubricants or conditioning agents may be added to aid the passage of the brush.

However, it is also necessary to bear in mind that each ingredient can have an effect on the overall properties of the product. For example, the thickener will modify the

properties of the material. The addition of a silicone to soften the surface properties of the material will often change its structural properties and so modify its spreadability on a microscopic scale.

After the product has dried, each component, with the exception of the solvents and gases, will form part of the composition of the material deposited. Consequently, all these ingredients will play a role in the intrinsic properties and the spreadability of the material. This interactivity of the ingredients complicates the formulation of style controlling products and implies a step-by-step approach to the construction of a product. That said, the ingredients can still be classified relative to their principal role in the formulation.

3.8. Structural Ingredients of the Material

The main agent of a hairdressing product is the polymer (2,3). The polymers are selected for their film-forming properties, i.e., their ability to produce after drying a material presenting stiffness, hardness and the surface properties desired and depending on their aptitude for inclusion in a formulation/vehicle (generally aqueous or hydroethanolic). Hydrosoluble polymers are preferred to those in latex form. They may be nonionic, anionic, cationic and even sometimes amphoteric (cf. Sec. 4 for the chemical structures).

3.8.1. Non-ionic Polymers

These polymers, historically the earliest ones, have the advantage of being compatible with numerous ingredients. In contrast, they can sometimes offer a limited holding capacity and form a slightly sticky film in a humid atmosphere, whence the weak resistance to humidity. For example, the polyvinylpyrrolidones (PVP) and the vinylpyrrolidone/vinyl acetate copolymers (copolymerization with vinyl acetate lowering the water uptake potential).

3.8.2. Anionic Polymers

These are more widely used because of their greater holding capacity due to the quality of the fairly rigid film they are often able to form. For example, acrylic polymers (acrylic acid/acrylate copolymers), methylvinylether/hemi-esters of maleic acid copolymers, etc. These copolymers must be neutralized using an alkali or alkanolamine to adjust film hardness, to achieve total solubilization in the solvent medium and ensure thorough removal on shampooing (2). Neutralizing agents of the alkanolamine type are often used, such as 2-amino-2-methyl propanol (AMP), mono- or triethanolamine, etc.

3.8.3. Cationic Polymers

Their introduction into styling and style-controlling products came later. Their positive charge lends good affinity with the hair. Their excellent conditioning properties make them the ingredients of first choice for styling foams. The quaternary ammonium derivatives of cellulose or guar provide, in addition, the benefit of giving gels their texture.

3.8.4. Amphoteric Polymers

These polymers, of more marginal use, are characterized by a high holding capacity and they must be partially neutralized to yield adequate solubility.

The selection of polymers is made on the basis of their physical and physicochemical properties. The ideal polymer must create, with the other ingredients, a film having high affinity for the hair and must be readily washed out. It must not form white particles on brushing or become sticky under high humidity conditions. Co-polymers are often used. These polymers are obtained from various types of monomer by co-polymerizing, i.e. polymerizing together two or more monomers. The choice of monomers decides the properties of the copolymer: its solubility in water, its mechanical properties, and its interaction with other ingredients in the formulation. Hence polymers formed from acrylic monomers are often called upon because they are readily soluble in water. Polymers formed from vinylcaprolactame monomers increase rigidity.

The structure of the polymer can affect its properties. Thus sequential polymers (so-called block-polymers) are used to yield specific properties.

The properties of product can be modulated by combining several polymers in a single formulation. In particular, the combination of anionic or amphoteric polymers with cationic polymers can be synergistic. For example, an anionic polymer for style control and a cationic polymer for conditioning provide the hairstyle with long-lasting hold and the hair fiber with a soft feel (4).

In actual fact, no type of polymer is recommended for a specific intrinsic property. All kinds of material can be prepared from a single chemical class of polymer. The polymers, even if restricted to soluble polymers, can yield a palette of intrinsic properties of the film formed on the hair: from the most flexible to the most rigid, from fragile to resistant, from plastic to elastic. The choice of concentrations of the various additives can again vary the properties of the film and improve the conditioning effects.

3.9. Cosmetic Additives

To compensate for certain undesirable effects due to polymers (dry feel, difficulty in untangling, surface irregularities), certain additives are incorporated (2).

The most effective are the silicone derivatives: these ingredients modify the surface condition of the hair fiber, making it smoother. Hence the hair is softer and silkier to the touch (5). By facilitating the passage of the brush, they protect the hair in blow-drying. By altering the surface properties and limiting the friction between individual hairs, they contribute towards reducing the static electricity arising under dry conditions, during blow-drying.

Fatty acids, like fatty alcohols or ceramides, have lubricating properties that can nourish and smooth the hair to protect it from the mechanical effects of repeated blow-drying. Moreover, ceramides are used in setting lotions to give the hair more body (6-8).

Polyols such as glycerol can be used for their emollient properties. They can be used to change the appearance of the hair by providing a “wet” effect or giving curl definition. In the case of gels (9), they can be used to limit the formation of white residues on hair-types that do not require very strong hold (straightened curly hair) but require a less dry feel.

3.10. Other Ingredients

These include:

- surfactants (10): amphiphilic compounds are used as emulsifiers, foam boosters, and perfume-solubilizers;
- preservatives: added to an aqueous or aqueous-ethanolic medium where the level of ethanol is insufficient to guarantee adequate protection from contamination;
- coloring agents;
- perfumes, etc.

3.11. Ingredients Providing Texture

Two types of ingredient are essential contributors to the spreadability of a product and to determine its form and texture. The solvents solubilize the active ingredients, transport them on to the hair and deposit them there while they evaporate. The thickeners, determine the ease of distribution into the hair and spreading properties over the hair fiber. The solvents most used are water, ethanol, fatty alcohols, and oils. Volatile silicones are good solvents for silicone gums. Depending upon the solvent chosen, this may facilitate styling by providing a wetting effect and help to regulate the rate at which a product dries. The thickeners used are either water soluble or not, depending on the solvent used (11).

Here are a few examples of various presentations of styling and style-controlling products.

3.11.1. Hair Lotions

These are aqueous or aqueous-ethanolic solutions to which are added suitable film-forming polymers and additives and which are applied to the hair using a spray.

3.11.2. Hair Gels

The gels are aqueous or aqueous-ethanolic formulations thickened with a gelifier. Several thickening systems are currently used: anionic gelifiers (Carbomer[®]), natural gelifiers (guar gum derivatives such as hydroxypropyl guar), combining polymers, etc.

They are chosen on the basis of their rheological properties on the one hand and for the impact that they have on the microscopic spreadability and properties of the material on the other. For example, Carbomer[®] and the modified guar gums do not have the same rheology. In the former case, the gel is fairly firm. In the latter, the gel is more transient and easier to distribute over the hair, with a more melting texture. The impact of Carbomer[®] on the material is not negligible: it tends to soften the material and give it a sticky appearance. In contrast, guar gums reinforce the hardness and solidity of the material. They contribute to maintenance of the hairstyle.

3.11.3. Hairstyling Foams (12)

They consist of:

- a liquid containing the active ingredients, including one or more polymers, the additives and possibly surfactants acting as foam-boosters;
- a propellant gas, generally a mixture of hydrocarbons (butane/isobutane/ propane).

The proportion of liquid to propellant controls the expansion and the texture of the foam. Generally, the propellant represents 4–10% of the mixture.

3.11.4. Waxes

At the boundary between conditioning and style-controlling products, waxes are finishing products that structure the hairstyle and make the hair shine by coating the hair strands with an oily film.

They are anhydrous bases composed either exclusively of fats, or emulsions. In both cases, the fatty phase includes waxes behaving as thickeners and hardeners (hydrocarbon waxes, beeswax, polyethylene waxes, etc.). These waxes are transported in solvents such as mineral oil, hydrocarbons (isododecane), volatile silicones (cyclomethicone). Silicones yield a more melting texture and thus a product that is easier to use. However, given the problems with removing fats when washing the hair, waxes must retain a solid texture that limits the amount used and the application to small dabs (to the tips, for example) in finishing. The use of non-ionic surfactants aids removal by the shampoo.

3.12. Perspectives

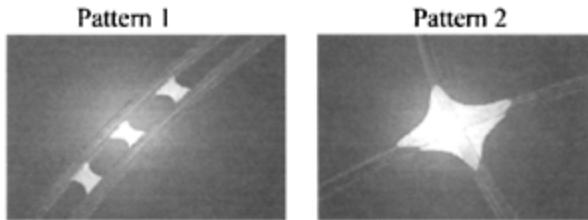
One of the greatest challenges is the development of new products combining extremely long-lasting hold with a natural visual and tactile effect, vibrant and clean, suggesting that the hair has not undergone any treatment. The ease of application remains a primary factor as there is no effectiveness without good distribution throughout the head of hair. The block-polymers or organo-mineral polymers form the basis of materials endowed with original properties, opening up interesting avenues for the development of new products.

4. HAIR SPRAYS

Hair sprays are used on their own or as a finishing touch after styling. Applied to the whole head of hair, they hold and prolong the shape of the hairstyle. The use as localized touches to pre-dried hair allows modeling the hairstyle prior to fixing. In some markets, a distinction between sprays is made based on wetting character and degree of hold imparted.

4.1. Hold Principle

Unlike hair gels, lotions and mousses that essentially work by coating the hair, this type of product deposits fine droplets of polymers in solution in a volatile solvent. On drying, transparent welds are created among the hairs (patterns 1 and 2).



This invisible network of junction points gives the hair a degree of hold and protects it from going out of shape under the influence of the movements of everyday life.

4.2. Properties Sought

To meet the needs of users, a hair spray must have many qualities (see Table 1). Depending on the expectations of the user, specific technical characteristics are called upon, which determine the choice of formulation. One major difficulty is to combine strong hold with acceptable cosmetic properties.

Table 1 Relationship Between Consumer Needs, Technical Characteristics, and Formulation

Properties required by the user	Technical characteristics	Choice of formulation
Ease of use, precision, ease of activation	Good balance between the equipment and the type of spray	Dispensing system: valve, actuator compatible with the propellant and solvents used
Rapidity and ease of hairdressing	Manageable, controlled drying	Solvent/propellant ratio and aerosol output
Hold with movements of head or hair	Welds that can move without breaking during movement of head of hair	Manage the weld characteristics by selection of polymers and additives
Spray fineness	Good vaporization in fine droplets	Nature and quantity of propellant and of dispensing system
No visible residues/powdering	Formation of a transparent or translucent, colorless weld	Property of the polymers and their combination
Clean and natural feel	Absence of sticky feel, absence of synthetic feel	Quantity and quality of the polymers and additives
Hairstyle maintenance suited to the nature of the hair, with the desired result	Different levels of hold and setting	Quantity and quality of the polymers and additives
Maintenance or provision of shine	Maintenance of shine/good shine	Addition of brilliance additives if necessary
Reversibility/restyling possible	Ease of untangling and	Management of weld

	elimination with a brush	characteristics by selection of polymers and additives
Suppleness of the head of hair	Natural appearance, suppleness, light-weight, not "stiff as a board"	Management of weld characteristics by selection of polymers and additives
Ease of removal	Removed readily when hair-washing	Neutralization of acidic and amphoteric polymers

4.3. Need for the Aerosol or Pump-Spray

Originally, these products were applied using a manual spray and they contained ethanolic solutions of natural gum lac that is difficult to remove. These hair sprays using shellac, sprayed coarsely from hand vaporizer, gave minimal satisfaction and they have practically disappeared. The development of aerosol technology allowing very fine spraying and the availability to cosmetic chemists of a varied range of film-forming polymers meant that highly effective hair sprays came on to the market. Their use in aerosol form is nowadays extremely widespread.

Two systems are used: one under pressure (self-propelled) and the other a mechanical system, the atomizer. They yield the qualities expected of this type of product.

4.3.1. Principles of Aerosol Vaporization

Its main technology is defined in European Directive 75/324/EEC dated May 20, 1975 (13) of a unit consisting of a disposable container in metal, glass or plastic, containing a compressed gas, liquefied or dissolved, under pressure, with or without liquid, paste or powder and equipped with an insert allowing the discharge of the contents in the form of a foam, of paste or powder, or in the liquid state. Current usage has extended the meaning of the word "aerosol" to cover a mode of packaging under pressure.

In the particular case of hair sprays, it is a two-phase aerosol consisting of a metal or plastic container equipped with a valve activated by an actuator (Fig. 2). The valve is equipped with a dip tube reaching the bottom of the container. The contents comprise two phases.

In the case of liquefied gas:

1. A liquid phase consisting of a mixture of the active agent concentrate and of liquefied propellants.
2. A gas phase surmounting the liquid phase and consisting of gas under pressure, in equilibrium with the liquid phase.

Operating Principle. The gas phase exerts pressure both on the walls and on the liquid phase, forcing it to rise through the dip tube up to the valve. By pressing the actuator, the orifice is exposed. The liquid phase is then expelled. As the liquefied propellant gasses it contains in solution have a boiling point very far below room temperature, these are instantly reduced to the state of vapor. Each drop expelled then explodes into an infinity of very fine droplets consisting of the liquid active agent. At

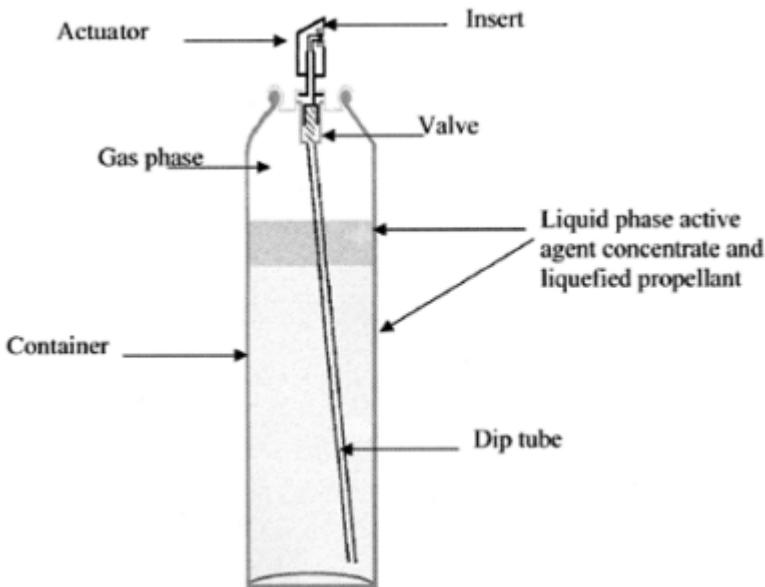


Figure 2 Aerosol can equipment.

the same time that this very fine vaporization takes place, equilibrium reactions come into play within the system, keeping the pressure constant: when part of the liquid is expelled, the volume of the gas phase increases. In this way, part of the liquefied gas vaporizes and re-establishes the pressure equilibrium. This is therefore always the same and the quality of the vaporization remains constant through the life of the aerosol product.

In the case of compressed gas:

1. A liquid phase consisting of a mixture of active agent concentrate.
2. A gas phase surmounting or surrounding the liquid phase and made up of gases under pressure.

Operating Principle. The gas phase exerts pressure both on the walls and on the liquid phase, compelling the latter to rise through the dip tube up to the valve. By applying on the actuator, the orifice of the valve is exposed. Vaporization is only created by the ejection rate and depends upon the viscosity of the liquid and the pressure applied by the gas. The latter plays the role of a gaseous piston. The spray is therefore coarser than in the case of liquefied gas. The characteristics of spray thus change during the period of use of the aerosol (14).

4.3.2. Principle of the Pump-spray

This vaporization system consists of a container and a pump (Fig. 3) with which it is possible, without propellant, to dispense a measured volume of product. Unlike the aerosol, the container is not pressurized. It contains the product to be vaporized. Vaporization occurs on mechanical activation of the pump. A dosage of the liquid is

drawn up through the dip tube then compressed in the compression chamber by activation of the actuator. It is then expelled through the insert, so forming a coarser spray than that obtained with an aerosol.

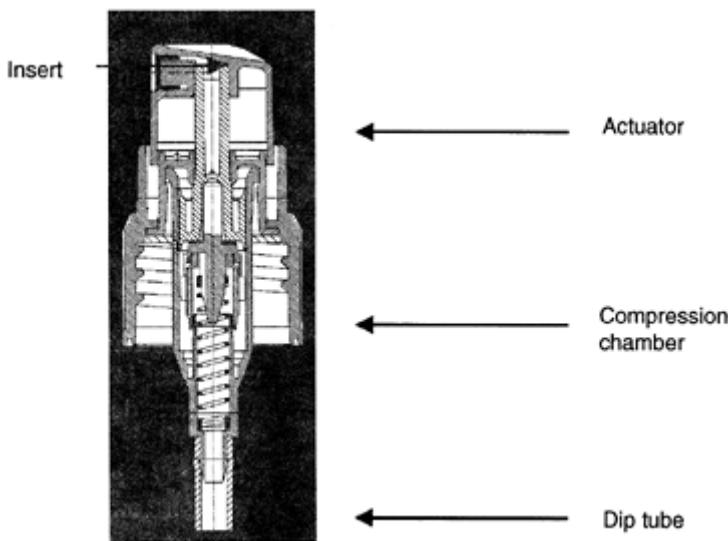


Figure 3 Diagram of a pump.

There are several types of pump, with or without air uptake, dispensing various dosages (from 0.08 to 0.7 mL in the case of hair sprays).

4.4. Formulation

The formulation chemist is subject to three constraints:

- to attain the properties sought for the product (see Table 1),
- to take into account economic limitations,
- to remain within regulatory requirements, which can differ, in the countries in which the product will be marketed.

4.5. Principle of Formulation

The development of a hair spray in an aerosol container requires a study of packaging and propulsion technology and a chemical and technical study of the formulation itself. In this chapter, we will mainly approach the development of the formulation with the main ingredients used and will only touch upon the general criteria of the choice of container. Specialized works (15,16) deal with aerosol containers in greater detail.

The characteristics of spray fineness (size of droplets, angle, force and nature) and the flow rate can be studied in the laboratory. The same is true of the physicochemical characteristics of the films obtained by polymer crystallization after evaporation of the

solvents. But laboratory tests only yield a very imperfect mimicking of what happens in reality. It is evident that hair keratin and its degree of affinity for film-forming polymers play a decisive role. Hygrometric conditions and the plasticizing action of sebum must also be taken into account. Development can only be finalized under real usage conditions requiring a very large number of on-head tests together with a sensory evaluation. The study of a high-quality hair spray, aside from safety, storage and compatibility testing, demands a considerable length of time.

The characteristics of a hair spray are defined as much by the aerosol or vaporizing containers as by the ingredients of the formulation which are:

- the propellant gases in the case of aerosols,
- the solvents,
- the polymer(s), with neutralizer if necessary,
- the plasticizers, if necessary,
- the additives: softness and shine providers,
- the perfume.

4.5.1. Packaging Items

The Aerosols. These consist of a container and a dispensing device.

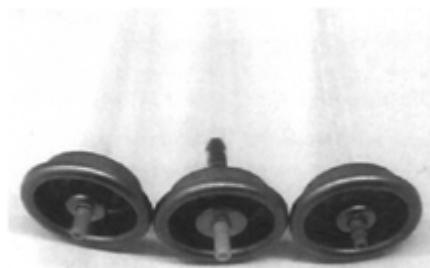
Metal (tin-plate or aluminum) or plastic containers are used. For safety reasons, they must meet the criterion of resistance to pressure in accordance with legal requirements.

In the case of compressed gas use, an aluminum or plastic pouch can be used as a reservoir for the active ingredient concentrate, to guarantee the presence of gas around the pouch throughout the period of use (17).

A valve, superimposed on the container, enables the product to be dispensed on demand. This valve is characterized by three orifices: the housing orifice, the stern orifice and, if necessary, the vapor-tap providing additional supply of gas phase to dry the spray that has been formed. The combination and careful choice of these three orifices dictates the flow rate and quantity of product dispensed (18).

The valves are equipped with a dip tube and are of two types:

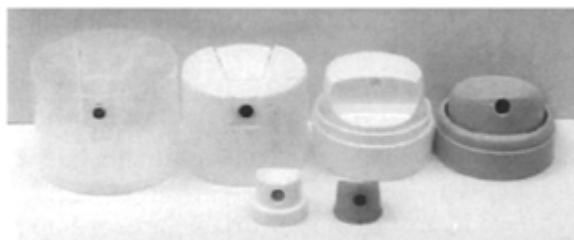
- male or female vertical action valves,



Male vertical action valves

- toggle or tilt action valves, which are more readily actuated.

The actuator opens the valve—Downstream of the dispensing mechanism, it consists of a channel with an insert at its end. Definition of the geometry of this insert enables the quality and shape of the spray to be controlled. The external shape of the actuator can take various forms.



Spray caps and actuators

For safety purposes, these items of equipment are subject to regulations that vary from country to country. In the European Union, Directive 75/324/EC dated May 20, 1975 and its technical amendment 94/1/EC dated January 6, 1991 (19) together define all the rules to be respected when placing such packaging on the market.

The Pump Sprays. A container and an extracting device make up the vaporizer. Containers of recyclable plastic are the ones currently used most often. Metal or glass containers also exist. The extracting device consists of a pump that defines the metered volume of product delivered. It is surmounted by an actuator that forms the spray.

The choice of pump is guided by the usage dose, and the dispenser by the shape required for the spray depending on the specific rheology of the formulation.

4.5.2. Propellant Gases

Liquefied Propellant Gases. Propellant gases are used to create the ejection force but they can also play the role of solvent for the diverse ingredients in the formulation. The chemical nature of each gas imposes its own characteristics of pressure, solubility, and compatibility. These characteristics, as well as the relative quantity of propellant, can influence drying time and spray fineness. In general, a low boiling point favors a short drying time and finer droplets.

In the 1970s, the ozone layer controversy led to a search for propellants free from interaction with this layer of the stratosphere. This research led to the development of sprays of a fluorocarbon propellant containing no chlorine: difluoro-1, 1-ethane (F_2HC-CH_3) or HFC 152 a (Dupont de Nemours) (20,21). Other propellants have also found greater use, such as dimethyl ether and C_3 and C_4 hydrocarbons.

Dimethyl Ether (DME or Dimethyl Oxide). Unlike the hydrocarbons, its physicochemical properties (22,23) endow it with a good solvent capacity as regards both polar and apolar polymers. It also offers the advantage of excellent miscibility with water (up to 34% by weight at 20°C), and with the majority of the usual alcohols and solvents. It allows the use of hydroalcoholic media (24,25). These properties enable a marked reduction in the quantity of alcohol required in the formulation of hair sprays. The use of water as a solvent helps to reduce the level of volatile organic compounds (VOCs) in the propellant

formula (see Sec. 7). In countries regulating VOC emissions, it becomes a propellant of choice. The fact that it is highly inflammable is a problem, however. Its good solvent capacity requires special industrial measures.

Hydrocarbons. Prior to the implication of chlorofluoro-propellants in impairment of the ozone layer, propane, butane and isobutane found little use because of their mediocre solvent capacity and inflammable nature. They have now become the basic propellants in many aerosol formulations (26,27). They are characterized by their low density, their non-miscibility with water, good toxicological properties and a relatively low price.

A major defect of hydrocarbons when compared with DME is their mediocre solvent power toward polymers, so that the level of propellant has to be lowered and co-solvents introduced. The availability of new polymers has allowed them to be used in a sufficient quantity to obtain good vaporization.

The mixture of these hydrocarbons in variable proportions yields a wide range of pressures from 1.2 bars (*n*-butane) to more than 7 bars (propane).

Hydrofluorocarbons. In this family of non-VOC products, only HFC 152a is used in cosmetic aerosols in countries where it is permitted, e.g., the United States. However, poor solubility in water and a high cost are obstacles to its development. Its inflammable nature is less pronounced than that of DME and the hydrocarbons. Its solvent capacity toward polymers is intermediate (see Table 2).

Compressed Propellant Gases. Air, nitrogen, nitrous oxide (N₂O) and carbon dioxide (CO₂) can be used. They are present in the aerosol solely in gas form and create pressure destined to eject the active agent concentrate at great speed to vaporize it. The initial usage pressure is around 10 bars and falls to around 4 bars at the end of usage.

They have the advantage of a low cost-of-goods and they are not inflammable. Conversely, an incorrect usage position makes a classic aerosol lose its pressure very rapidly with this type of gas and it then becomes unusable. Pressurization of products with these propellants demands special industrial tooling.

4.5.3. The Solvents

Ethyl alcohol is the best solvent from every point of view (safety, odor, solvent properties, evaporation rate, stability, corrosion). For cost reasons, in countries where

Table 2 Characteristics of Liquified Propellants

Propellant	Molecular weight	Boiling point (°C)	Density at 20°C	Vapor pressure in bars at 20°C	Vapor pressure in bars at 50°C	Formula
Dimethyl Ether	46	-24.8	0.66	4.2	11.3	CH ₃ -O-CH ₃
Butane	58	-0.5	0.58	1.1	4.1	CH ₃ -CH ₂ -CH ₂ -CH ₃
Isobutane	58	-11.7	0.56	2.2	6.1	(CH ₃) ₂ -CH-CH ₃
Propane	44	-42.1	0.50	7.2	16.3	CH ₃ -CH ₂ -

Difluoro ethane (HFC152a)	66	-24	0.91	4.2	10.9	$\text{CF}_2\text{H}-\text{CH}_3$	CH_3
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(From Refs. 28,29.)

ethyl alcohol is relatively expensive, attempts are made to reduce the proportion or even to replace it completely. Sometimes isopropyl alcohol whose boiling point is similar (81°C vs. 78°C) is used, but it evaporates much more slowly. Isopropyl alcohol has a rather disagreeable odor, difficult to mask with perfume.

Other solvents such as acetone, ethyl formate, and methyl acetate have sometimes been tried. Chlorinated solvents have been abandoned due to problems with safety and compatibility with the environment.

Water, due to its physicochemical characteristics has been of limited use (incompatibility with the chlorofluoro-propellants used previously, poor solvent capacity toward polymers, corrosion and slow evaporation). The appearance of new classes of polymers soluble in water and the ecologic constraints on the use of VOCs (see Sec. 7) have made it an undeniably economical solvent. It has the advantage of reducing the inflammable nature of products. The use of anti-corrosion additives [sodium benzoate, monoethanolamine (MEA) or monoisopropanolamine (MIPA) borates, etc.] impedes the corrosion of containers made from tin-plate.

The emergence of volatile silicones should also be mentioned; considered not to belong to the category of VOCs, they present not only the advantage of a very high evaporation rate, but also the twofold drawbacks of a relatively high price and inflammability.

The “active agent concentrate/propellant” equilibrium of the formulation is influenced by considerations of solubility and cost of goods. In simple terms, it can be said that this equilibrium may depend upon the ratio of the prices of the propellants and alcohol. When alcohol is markedly more expensive than the propellants, it is worth reducing the proportion of active agent concentrate and thus increase the polymer concentration. When alcohol is very cheap, it is of great advantage to reduce the gas phase drastically. Of course, these cost-of-goods considerations cannot be determining factors where priority is given to the properties of the product. With low levels of propellant, it is difficult to obtain a product with a low wetting capacity.

4.5.4. *The Polymers*

The basic ingredients for the formulation of hair sprays are the polymers called film-forming polymers. After the rapid evaporation of the propellants and solvents included in the product, they leave fixation points between the hairs in the form of a film whose characteristics are outlined below:

- small size,
- transparency or slight opalescence,
- good affinity for keratin,
- sufficient flexibility to withstand rupture when the hair moves,

- non-hygroscopic,
- non-sticky to the touch,
- easy removal of the holding effect by brushing,
- complete removal on washing.

The physical and chemical characteristics of the polymers and their formulation influence the properties of the welds formed when they have dried. The material must be neither too soft nor too friable. The weld must accept the minor deformations occurring when the hair moves around in everyday life, but it must be sufficiently fragile to fracture when brushed or combed. However, the ease of brushing must not destroy the structure of the hairdo, which must be easy to restore.

Practically all the polymers used are soluble in alcohol and liquefied propellant gases. Some are carboxylic polymers that must be neutralized if the fixation points are to be correctly removed with shampoo. The main neutralizers used are ammonia, strong bases like soda or potash (rarely used because of corrosion problems), monoethanolamine, triethanolamine (yielding hygroscopic films), aminomethylpropanediol, aminomethylpropanol, triisopropanolamine, stearylamine, etc. Some alkanolamines mentioned play a plasticizing role to provide the polymer film with greater flexibility.

The polymers or copolymers used in hair sprays and lacquers are distinguished by their ionic nature. The presence and nature of the charge borne by the film-forming polymer determines the quality of the film making up the bonds and its affinity with the hair. The least-used polymers are the cationic polymers, given their excessive affinity for the hair fiber and hence the difficulty of removing them.

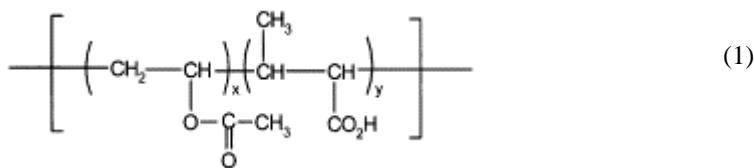
Anionic Polymers (Carboxylic Acid Derivatives). The structure of these polymers comprises carboxylic groups that give them valuable properties.

Anionic Polymers of Natural Origin. One of the first polymers of natural origin to be used in atomizers or aerosols was lac gum, also called shellac. It is practically the only natural polymer that has found a use as the basis of a hair lacquer. It is extracted from the secretions of an insect called *Laccifer laccata*. It consists essentially of polyhydroxylated acids and esters, among which has been isolated, in particular, aleuritic acid with the chemical formula:



The lac gum yields very hard, very bonding films (30). It has also been used for the provision of excellent shine and for resistance to humidity. Conversely, its limited solubility in solvents and propellants used previously required neutralization by alkali or ammonia. Moreover, it is very difficult to remove by brushing or washing, as the film that it creates readily breaks up into powder, leaving white residues. Lac gum is now only rarely used alone in aerosols. It does sometimes constitute an additive to other polymers. Shellac and other natural polymers (*Canarium luzonicum*, *Commiphora myrrha*) are still used in combination with synthetic polymers.

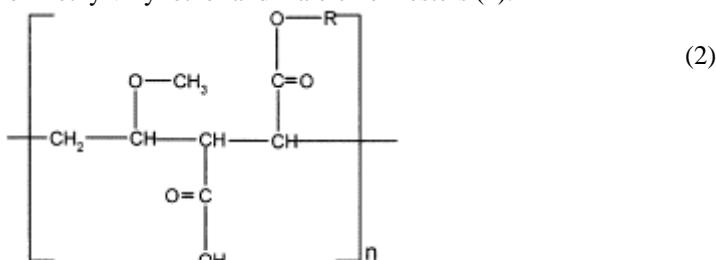
Anionic Polymers of Synthetic Origin. Copolymers of vinyl acetate and crotonic acid (1): in general, they contain something of the order of 10% crotonic acid. The properties of the film (hardness, hygroscopicity) and solubility can be modulated by the level and nature of the neutralizing agent of carboxylic acid groups. The preferred neutralizer is AMP (2-amino-2-methyl-1-propanol).



This type of polymer yields clear, fairly hard, fairly shiny films and so provides spray products with holding and style-controlling properties. The films obtained are poorly hygroscopic. Moreover, careful plasticizing can overcome their friability and lack of suppleness.

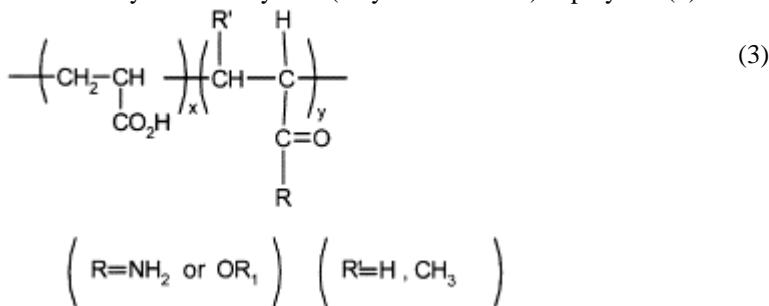
Terpolymers, e.g., vinyl acetate/crotonates/vinyl neodecanoate copolymer, vinyl acetate/vinyl butylbenzoate/crotonates copolymer—are used particularly in aerosol formulations containing hydrocarbons. Neutralization is made as for the preceding polymers.

Copolymers of methylvinyl ether and maleic hemiesters (2):

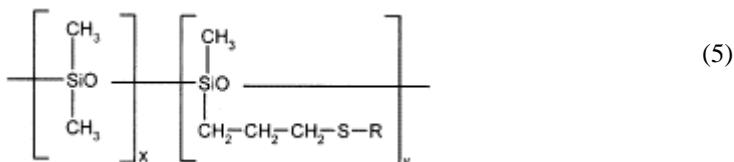
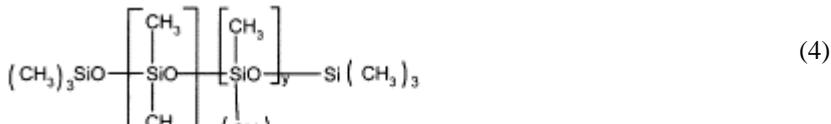


Less soluble in water, these polymers yield very hard, clear and shiny films leading to reinforced hold, but a little friable. Plasticization of the films can be adjusted by the nature of the neutralizer (triisopropanolamine or amine with a fatty chain). Solubilization is obtained for a fairly high alcoholic content.

Polyacrylic Polymers. These compounds form fairly hard, very transparent, flexible, fairly shiny films. But they are rather hygroscopic and can become sticky. The polymers most used for their good compatibility with hydrocarbon propellants are: acrylic acid/acrylamide or acrylic acid/acrylates (acrylic acid esters) copolymer (3).



The last generation of polyacrylic polymers was developed by grafting or combining silicone polymers to improve cosmetic properties such as softness to the touch and flexibility of the bonding (31). They are of three types (32–34): acrylates/dimethicone copolymer, polysilicone 9 (4), and polysilicone 8 (5).

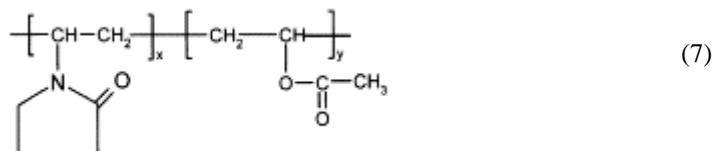


The final category of anionic polymers is derived from polyurethanes. Similar developments have been achieved by grafting silicones on to polyurethanes (35,36). These polymers have the advantage of being compatible with water and are thus most suitable to develop products with limited VOC content (see Section 7).

Non-ionic Polymers. These are used in formulation for their compatibility with the other ingredients. They can be used in all media. They are easy to handle and do not require neutralization. The first synthetic polymer used in hair sprays was polyvinyl pyrrolidone (PVP) (6).



This polymer was originally used as a substitute or diluent for blood plasma. Numerous grades exist, corresponding to various degrees of polymerization. The formulation of hair sprays using this polymer yields transparent and flexible films, somewhat limp, with some affinity for keratin (thanks to its slightly cationic character). But its main drawback is its hygroscopy: the films become sticky in the presence of humidity. It is essential to lower its affinity for water and to harden the films by the use of additives or by copolymerization with less hygroscopic monomers such as the copolymers of vinylpyrrolidone and vinyl acetate (PVP/VA) (7).

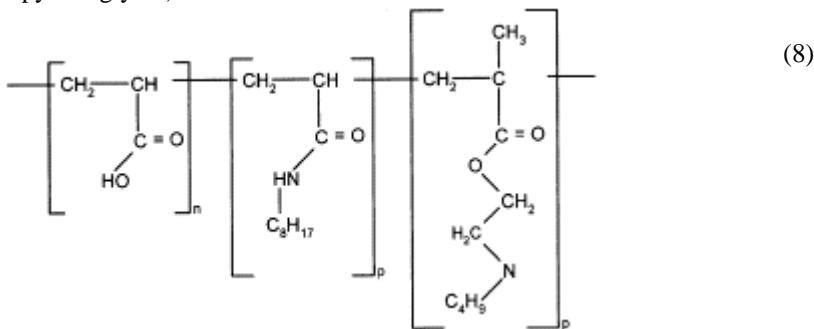


These copolymers represent improvements over PVP. They yield clear, limpid films with reduced powdery effect (formation of white particles) when the hair is brushed or combed. The greater the proportion of vinyl acetate, the harder is the film and the greater its resistance to humidity but the disadvantage is that it is harder to remove with shampoo. It should be noted that despite PVP films properties being significantly improved by copolymerization with vinyl acetate, the hygroscopic character of these copolymers is never completely eradicated, even with a high level of vinyl acetate. The usual PVP/VA copolymers contain between 30% and 70% PVP.

Terpolymers. it has been suggested, in particular, that these offer better compatibility with hydrocarbon propellants. Examples are: PVP/VA/vinyl propionate copolymer, vinyl caprolactam/PVP/dimethylaminoethyl methacrylate (DMAEMA) copolymer and acrylates/PVP copolymer.

Amphoteric Polymers. These are acrylic polymers having both cationic (amine) and anionic (acid) groups, e.g., octylacrylamide/acrylates/butylaminoethyl methacrylate terpolymer (8). They are readily soluble in water and have good affinity for hair fiber. The films obtained are hard but not always very cosmetic. These polymers are used to achieve high levels of hold and a reduced VOC content.

The polymers or copolymers mentioned above are often combined together to yield very high-quality hair sprays. They must either be mixed together or their characteristics must be modified by adding agents capable of altering the plastic properties of the film they produce. The plasticizers may be esters (isopropyl, ethyl or methyl esters of adipic, phthalic, sebacic, myristic acids, etc.), or they may be polyols (glycerol, diethylene glycol or dipropylene glycol) or silicone oils.



The ingredients that produce shine or softness are often the derivatives of fatty alcohols or esters and silicone oils, particularly phenyl silicones (cf. Chapter 4). These additives must be compatible with the aerosol (or vaporized) mixture and form a homogenous film with the polymers. Their use enables modulation of the initial properties of the polymer film formed, in particular to reconcile the cosmetic qualities and its holding capacity.

Numerous other additives are used such as pro-vitamins or vitamins (e.g., panthenol, niacinamide, tocopherol), UV filters (e.g., benzophenone, octyl methoxycinnamate), protein derivatives (e.g., ethyl ester of hydrolyzed silk, hydrolyzed wheat gluten), or lipids (e.g., 2-oleamido-1,3-octadecanediol).

Finally, the perfumes used in hair sprays must be subjected to extremely demanding stability testing in the final formulation and packaging.

5. EVALUATION OF TEST METHODS

A series of laboratory tests can be used to assess, qualitatively and quantitatively, the properties of hair styling and setting products. Although of value and highly useful for the development of products, these techniques can only give a sketchy evaluation of the basic qualities of the product tested. They cannot replace the assessment that will ultimately come from the users. These techniques are described in Chapter 12. An important trial in the early stages of product development is the investigation of polymer films. The evaluation is carried out on films of standard thickness and

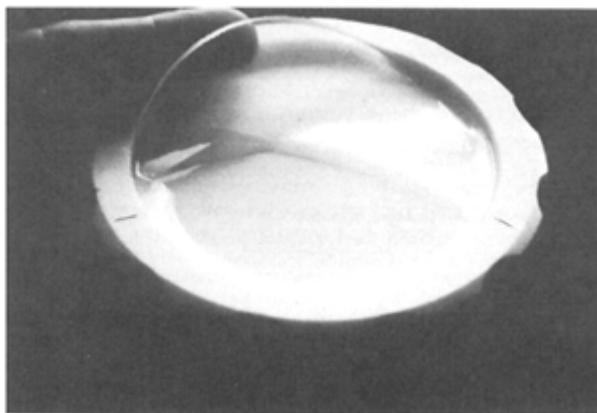


Figure 4

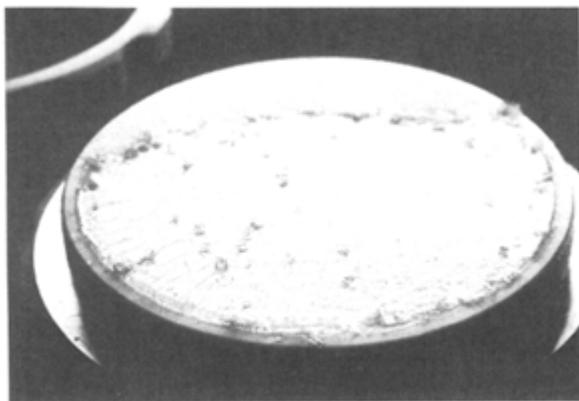
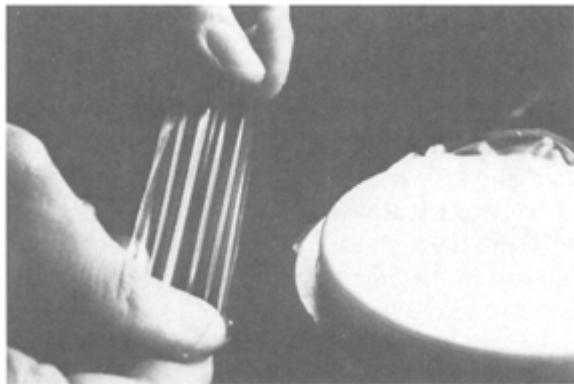


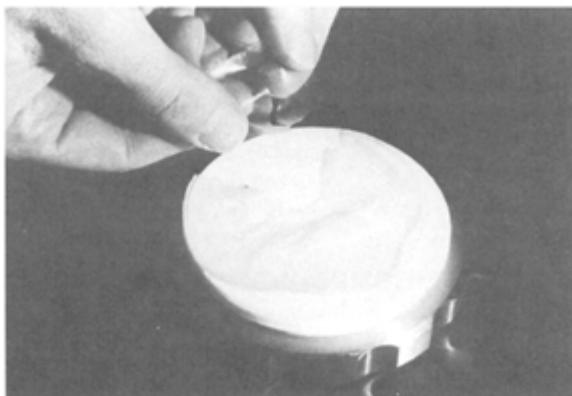
Figure 5

surface area in various controlled (temperature, humidity) conditions (Figs. 4–7). Assessment involves a variety of criteria such as:

1. Visual assessment—clarity, gloss,
2. Tensile properties of the film—elasticity, plasticity (e.g., resistance to bending),
3. Film hardness, flaking,
4. Feel properties—tackiness, stickiness,
5. Hygrometric properties—weight gain measurement from the moisture uptake as a function of time, in controlled atmospheres.



Figures 6



Figures 7

Figures 4–7 Studies on polymer films for hair sprays. The polymer solution is poured onto a perfectly even teflon matrix. Figures 4 and 5: Two types of film obtained after evaporating in standard atmosphere [22±2°C; 50% relative humidity (RH)]. Studies on polymer films for hair sprays. The polymer solution is poured onto a perfectly even teflon matrix. Figures 4 and 5: Two types of film obtained after evaporating in standard atmosphere [22±2°C; 50% relative humidity (RH)]. Rheologic features: the assessment is carried out, before and after conditioning for several hours at various RH, on physicomechanical (hardness, resistance to extension, water uptake, etc.) and cosmetic (feel, softness, etc.) criteria.

6. SAFETY OF HAIR SPRAYS

The safety of sprays has been the topic of numerous discussions concerning the possible risks of inhalation in poorly ventilated confined spaces or in places where a considerable amount of spraying is carried out.

Doubts first emerged in the late 1950s, when an article was published that reported two cases of abnormal deposition of foreign material (thesaurosis) of the lungs and suggested that the inhalation of aerosol products containing PVP could have been the cause (37). More than 70 studies published between 1962 and 1966 were done on the potential correlation between the various cases of thesaurosis and the use of hair sprays. Despite the fact that several patients examined used hair spray to the point of abuse, almost of obsession, biological analyses did not lead to relevant presumptive evidence (38).

Many experiments have been carried out on animals (rabbits, hamsters, guinea-pigs, monkeys) in an attempt to produce granulomatous inflammation of the thesaurosis type (39–42). After long-term exposure to repeated sprays of high-concentration aerosols containing the polymers employed in hair sprays formulation (PVP-VA, copolymers of vinyl acetate and crotonic acid, or methylvinylether and maleic hemiesters), no lesions could be detected by histological examination (43), even where conditions were extreme, where the high aerosol content in the inspired air would cause polymer material deposits in the lung tissue; it was shown that the lungs rapidly eliminated these deposits (44,45).

The risk of thesaurosis seems to be very limited. This is confirmed by epidemiological studies on high-risk, exposed populations (hairdressers, perfumers, cosmeticians) (46,47). But it is nonetheless necessary to try to foresee and prevent, insofar as it is possible, any increase or aggravation of preexisting disorders, for example, in allergic subjects (48,49). This is why an entire methodology has been established to check spray safety and assess the risk associated with repeated inhalation of substances intended for use in an aerosol (50,51).

For the most part, this risk is related to the probability of aerosol-dispensed particle penetration as far as the pulmonary tract. The multitude of studies in this field demonstrate that this hazard is very low for particles above 10 µm in size (51,52). A number of devices are capable of analyzing the distribution of particles size in the aerosol. It is therefore possible to decide on the formulation without having to carry out inhalation experiments.

7. LEGISLATION ON VOLATILE ORGANIC COMPOUNDS AND THE ENVIRONMENT

Hair setting, styling and dressing products must comply with VOC regulations.

7.1. What Is a Volatile Organic Compound?

In very general terms, the VOCs are organic substances that can readily evaporate into the atmosphere.

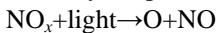
They can be of natural origin (biogenic) or they can be manufactured by man (anthropogenic). The legislator therefore differentiated between the biogenic methane VOC and the anthropogenic non-methane VOC.

The VOCs used in cosmetics are non-methane VOCs. In the case of hair sprays, they are propellants or solvents such as the hydrocarbons, dimethyl ether, ethyl alcohol, etc.

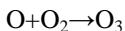
7.2. The Consequence of the Presence of VOCs in the Atmosphere

Generally, VOCs react, in the presence of sunlight, with nitrogen oxides (NO_x) to form ozone, O_3 (53).

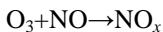
In a very simplified form, the following photochemical reactions take place:



(a)



(b)



(c)

When VOCs are present, these react more rapidly than O_3 with NO (reaction c) so contributing to an increase in the O_3 concentration in the low atmosphere (troposphere). For an increase in the ozone concentration to be observed, the following conditions are required:

- sunny weather,
- a good ratio of NO_x to VOCs.

The VOCs do not all make an equal contribution toward ozone formation. This depends upon their photochemical reactivity that is often referred to as the photochemical ozone creation potential (POCP). POCP values are first estimated and refined later as progress is made in this field.

The solvents and propellants used in cosmetics are considered to have low POCP values.

The increases in ozone concentration can have consequences on people's health (the elderly or those suffering from respiratory disorders, asthma, for example) as well on the vegetation. However, in 2000, in the European Union, VOC aerosols (all products together) only represented 1.9% of the total VOC emissions (biogenic and anthropogenic) (source CEFIC: Conseil Européen des Fédérations de l'Industrie Chimique). The small proportion, added to the low POCP values, means that their true contribution is very low (European Aerosol Federation, Aerosols and VOCs, 2000; data not published).

7.3. Current Regulations

7.3.1. Europe

The VOC emissions due to transportation and to industrial activities (54) are covered by EU regulation. According to the latter regulation, every organic compound with a vapor pressure of 0.01 kPa or more at 293.15°K is considered to be a VOC.

Switzerland has implemented a VOC taxation system covering consumer products that came into force in January 2000 and an increase in the level of taxation was foreseen for 2003.

The Netherlands already have, based on agreements between industry and the authorities, a VOC reduction program (KWS 2000) concerning certain consumer products and they

are now considering a new program (National Environment Strategy Plan) aiming at lowering VOC quantities by 30% between 2000 and 2010—that will include cosmetic products among other things.

Other regulations aim to set “National Emission Ceilings” (55) for some pollutants, including VOCs, and ozone target values (56).

Another pertinent document is the UN Economic Commission for Europe—Multi-pollutant protocol) dated December 1999, implicating more than 30 countries. The aim of this protocol was, among other issues, to reduce VOC emissions in the signatory countries.

The restrictions on VOCs entail a willingness to reduce VOC use in professional and consumer products. Discussions began in the EU Commission in November 2001; the paint and lacquer domain is the first to be examined but the cosmetic field is in second place.

7.3.2. Canada

Environment Canada, in the context of the Environmental Protection Act of 1999, published in November 2002 its guidelines on VOCs in consumer products (57).

The limitations are as follows:

Products	Limits (VOC% w/w)
Hair sprays	80
Hair mousses	16
Hair gels	6
Shaving foams	5
Nail polish removers	85

As for deodorants and antiperspirants, only highly volatile organic compounds (HVOC) with a vapor pressure in excess of 80 mmHg at 20°C were taken into consideration:

Aerosol deodorants	20% (HVOC)
Aerosol antiperspirants	60% (HVOC)

VOC content must be declared to Environment Canada on an annual basis.

The guidelines will become mandatory when the VOCs are published in the list of toxic substances. The date of publication is not yet known.

7.3.3. United States

In the United States, the Federal Clean Air Act (CAA) defines the objectives as regards air quality with the help of the National Ambient Air Quality Standards (NAAQS). For the requirements of the NAAQS to be met, it was necessary to introduce regulations aiming to reduce VOC emissions in:

- industry,

- transportation,
- consumer products.

California was the leader in this field with the California Air Resource Board (CARB) then a regulation was drawn up covering the whole of the USA under the control of the Environmental Protection Agency (EPA).

The general definition is common to both the EPA and the CARB (58):

A VOC is any compound containing a carbon atom with the exception of carbon monoxide, carbon dioxide, carbonic acid, and metal or ammonium carbonates that participate in chemical reactions in the atmosphere and hence contribute to photochemical pollution.

Compounds that are weakly reactive are excluded, e.g.:

- Methane, ethane, acetone, methylene chloride, 1,1,1-trichloroethane, CFC, HCFC, HFC, volatile silicones, and methyl acetate.
- The case of *t*-butyl acetate is currently being examined.

However, the classes of substances restricted by the EPA and the CARB are not identical.

- At the federal level, the EPA only defines:

High VOC: Pv (vapor pressure) >80 mmHg at 20°C

- In California, three classes are defined:

High VOC: Pv (vapor pressure) >80 mmHg at 20°C

Medium VOC: Pv >2 mmHg at 20°C

Low vapor pressure VOC (currently exempt): Pv <0.1 mmHg at 20°C

or Pv <2 mmHg at 20°C in deodorants and antiperspirants

or substances with more than 12 carbon atoms if the Pv is unknown.

For example, volatile hydrocarbons and dimethyl ether are high VOC while ethyl alcohol is medium VOC.

7.4. Existing Limits in Cosmetics

- At the federal level in the USA (EPA):

Products	VOC limits (% w/w)
Hair sprays (aerosols and pump vaporizers)	80
Hair styling gels	6
Hair styling mousses	16
Aerosol antiperspirants	60 (high VOC)
Aerosol deodorants	20 (high VOC)
Shaving foams	5
Nail polish remover	85

- In California (CARB):

Products	VOC limits (% w/w)
Hair sprays (aerosols and pump vaporizers)	55
Hair styling gels	6
Hair styling mousses	6
Hair shine sprays	55 on January 1, 2005
Shaving foams	5
Non-aerosol deodorants	0 HVOC/0 MVOC (ethanol exempted)
Aerosol deodorants	0 HVOC/10 MVOC (ethanol exempted)
Nonaerosol antiperspirants	0 HVOC/0 MVOC (ethanol exempted)
Aerosol antiperspirants	40 HVOC/10 MVOC (ethanol exempted)
Perfumes <20%	75
Perfumes >20%	65
Nail polish remover	75, then 0 on December 31, 2004

7.5. Alternative Options

The EPA and CARB have identified two alternative options for products that cannot meet the VOC limits:

- The first corresponds to the term “innovative product” aimed at authorizing products with a VOC rate exceeding the limit but with a technique for its application (often linked to innovative packaging) that limits the VOC released each time it is used to a level that complies with the standard.
- The second corresponds to the “alternative compliance plan” that allows a company to sell a product that exceeds the limit if it can be proved that, based on total sales, the VOC quantity does not exceed the level at which it would be if all its products complied with the standards.

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6

Permanent Waving and Hair Straightening

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1. HISTORICAL REVIEW

One of the earliest permanent waving methods was that used at the time of Louis XIV of France, known as the *frisure infernale* ("hell's own curls"); it was used on their wigs. The tresses were rolled on cylinders made of baked clay, maintained in boiling water for 3 hr, "cooked," and dried in an oven (1,2).

It was not until 1906 that the first waves were produced on a human head by a London hairdresser of German descent called Nestlé (his real name was Nessler). These waves "resisted water, washing, and all atmospheric influences." He soaked strands of hair in borax, rolled them on metal curlers, and enclosed them in a heater, a veritable electric oven brought to 145–150°C. A quotation from Hillier's (3) humorous description of the whole operation is illuminating:

It took a whole day to do the permanent wave.... Women bragged about their burns to other women.... The heaters were so heavy that only a few curls could be baked at a time.... It was necessary to tap the heaters with a hammer to release them.... The hairdresser would use a nutcracker.... Sometimes the hair would come off with the pad....

There is no doubt it was complicated, troublesome, and often painful. But it was also the decisive step, the discovery that hair could be given a permanent deformation using certain chemical products and heat.

Nessler's system was perfected in the course of the next 30 years (4,5). Equipment was simplified, and direct heating (by electric wire devices) was replaced by indirect heating (special devices using curlers preheated on rods). At the same time, formulations were improved; the initial alkaline solutions were made more and more active by the addition of reducing agents (sulfites) (6).

Hot waving systems were also devised at this time (7,8). They required heating sachets, but no equipment. They used the same liquids. Heat was generated by the sachets when soaked in substances producing an exothermic reaction by contact with water.

[†]Deceased.

The first heatless permanent waving products based on the use of sulfides appeared around 1935–1940 (9,10).

A new era was ushered in for hairdressers and specialized researchers about 1940, when the first patents were taken out for the use of thiols (mercaptans) in cold waving (11,11a). The advent of the cold wave coincided with the instigation of largescale research programs in cosmetic laboratories.

The procedure was still more improved around the 1960s by using a buffering system that results in lowering pH value while maintaining alkaline level high enough during the reduction process. This technique is among the most used nowadays since the occurrence of hair damage is significantly reduced.

Permanent wave products and methods are now developed taking into consideration the most precise chemical, physical, and biological data available.

2. HISTORICAL TECHNIQUES

Permanent hair (re)shaping or curling can be described in simple terms. Hair is elastic. Therefore, when (re)shaped, it tends to return to its previous shape. It is not plastic but must be plasticized in order to retain a permanent new shape. All permanent waving systems aim at plasticizing (softening) hair while the wave is given, and at wave retention thereafter.

The frisure infernale used on wigs in Louis XIV's time has been mentioned. In this instance, during the 3 hr of immersion, the wig hair was plasticized by chemical means, by hydrolysis.

2.1. Hot Waving

In hot waving, hair is plasticized by chemical and thermal means. Hair is moistened with an alkaline solution or, more generally, an alkaline reducing solution. Then it is rolled on curlers and maintained at a temperature of 100–200°C for several minutes by a heating mass. Basically, the time of reduction under heat is that necessary for plasticizing the fiber. When cool, hair recovers its normal properties of elasticity, but it will have taken on the new, permanent shape given by the curlers.

How can this action be explained? Nessler's theory, which he considered to be the basis for his invention (12), was:

A naturally straight hair is a tube, perfectly enveloped, which is only able to absorb through its root or its extremity, if it has been cut. On the contrary after waving, a hair resembles a tube, the entire surface of which has been perforated and allows air, water, and any foreign substance to penetrate. Natural curls are the result of atmospheric humidity, the archenemy of all the different kinds of artificial wave. The wave produced by my method separates and opens up the tiny scales overlapping hermetically in sleek hair, letting in the beneficial humidity. In the end, the hair resembles naturally curly hair which, under the influence of humidity, undergoes a tightening of the curls. Particles of moisture penetrate the hair tube by these tiny pores and cause the curling effect.

We have come a long way since Nessler. There are much more precise physicochemical explanations for the permanent shaping of the keratin fiber by steam, alkaline products, and reducing agents. These products are efficient not only because they affect the electrostatic and hydrogen bonding but also because they bring about the cleavage and transformation of covalent disulfide linkages that confer on keratin its structural rigidity.

2.1.1. Action of Steam and Alkaline Agents

There has been a great deal of studies devoted to the mechanism of action of steam and alkaline agents and to the nature of the new linkages created and their role in the mechanical and chemical properties of hair keratin (13,14).

The formation of a thioether derivative of cystine was suggested as early as 1933 (15) and confirmed in the 1940s when lanthionine was isolated in hydrolysates of wool treated with a boiling solution of sodium carbonate (16,17). These transformations are initiated by the hydroxide ion, and their extent increases with increasing alkalinity. Among the reaction sequences proposed to explain lanthionine formation, two seem to prevail.

The first (18) assumes a direct attack by the hydroxide ion on the disulfide linkage, according to a bimolecular nucleophilic substitution mechanism (Fig. 1).

The second (19) postulates a β -elimination initiated by hydroxide ion attack on the hydrogen atom located on the carbon atom in the β -position to the disulfide bond (Fig. 2). This leads to the formation of the dehydroalanyl residue, (II), a highly reactive structure, which in turn can react with thiol or free amine groups. Thus, the addition of cysteine groups (III), resulting from disruption of the disulfide bonds or of the free-amino groups carried on the lysine side chains, leads to the formation of lanthionine and lysinoalanine, respectively (20,21). This intermolecular rearrangement seems the most likely when the hair is treated by alkali, as shown by the fact that lanthionine formation in an alkaline medium increases rapidly with the ionic strength of medium (22,23).

Unlike disulfide ones, sulfide cross-linkages (thioethers) with one sulfur atom, are not sensitive to the action of reducing agents. Thus, the degree of lanthionization shows up in the solubility of the hair in concentrated urea-bisulfite or phenolthioglycollic acid reducing media: when the lanthionine ratio increases, solubility decreases.

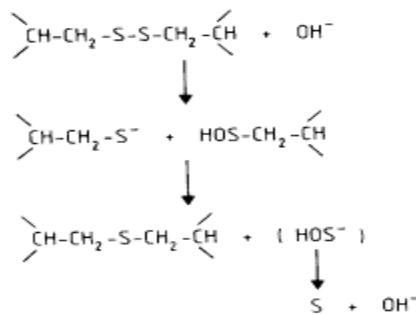


Figure 1 Nucleophilic pathway of lanthionine formation.

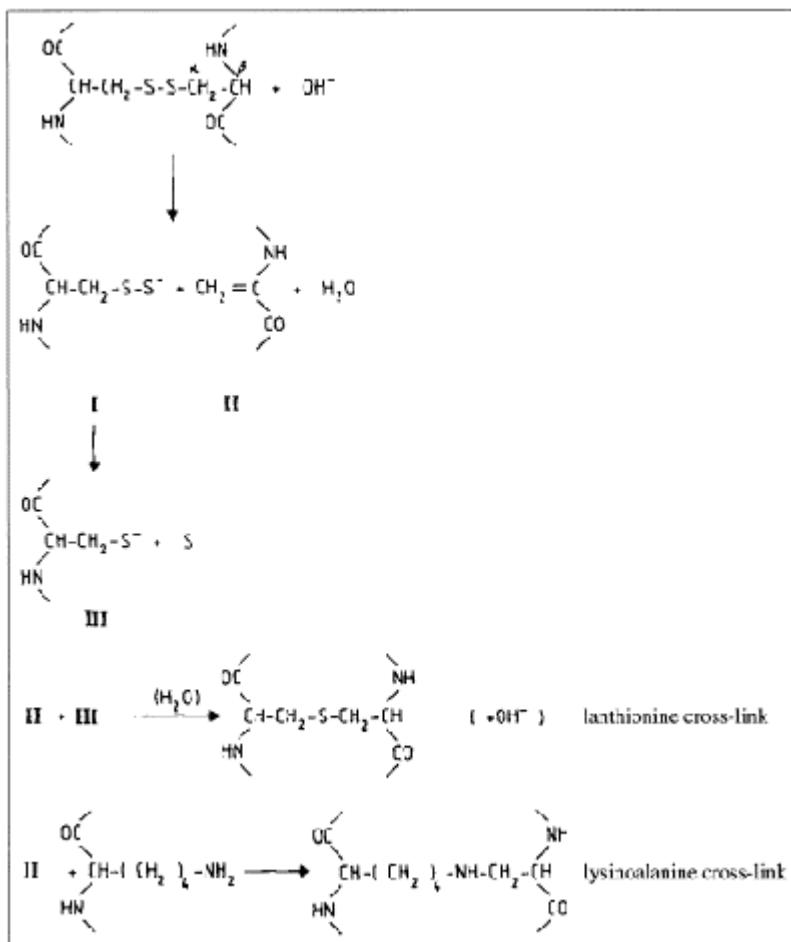


Figure 2 Alkali-induced transformation of cystine into lanthionine and lysinoalanine cross-linkages.

2.1.2. Action of Reducing Agents

The reducing agents used in hot waving methods were generally sulfites in highly alkaline media (see Sec 3.9). The reaction of sulfites with disulfide bonds is not simple because the basic processes involved are both reversible and highly sensitive to pH. It seems to follow a typically nucleophilic mechanism (24) (Fig. 3).

The optimum reducing action of sulfite is in the range pH 3–6, but there may well be two maxima, one at pH 3.2–3.5 and the other about pH 5 (25,26). In practice, an acidic medium is however avoided because of the instability of sulfites under these conditions;

pH 6 is preferred. At pH 7 (30 min, 30°C) about 15% of the disulfide bonds can be reduced. To increase the number of disulfide bonds reduced and thus

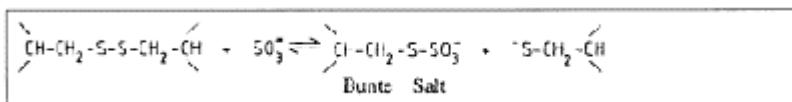


Figure 3 Reduction of disulfide bond by sulfite.

increase the degree of waving, the reaction equilibrium (Fig. 3) has to be shifted towards the right; this is done by heating.

The major shortcoming of the hot processes becomes apparent from the description of these reactions. From a chemical standpoint there can only be disadvantages. The hair fiber is weakened because most of the reactions involved are irreversible. Thus, linkages are disrupted that cannot reform. In addition, there is an inevitable decrease in the sulfur level.

Hot waving has virtually disappeared, so only the products and techniques will be discussed.

The solutions used were either alkaline solutions based on aqueous ammonia, sodium, potassium or ammonium carbonate, borax, monoethanolamine or triethanolamine, or neutral or alkaline solutions of various sulfites (sodium, ammonium, mono- or triethanolamine, morpholine sulfites, etc.). The use of swelling agents, derivatives of urea or amides (formamide, for example), reinforced the action of the solutions; this allowed a reduction in their concentration or in their pH. Using these basic principles, the specialized chemist sought to make these formulations distinctive by adding wetting and softening agents.

The technique consisted of rolling 30 strands, moistened with the selected lotion, on metal curlers 7–8 cm in length and 4–10 mm in diameter. Before rolling, each section of hair was isolated from the scalp by a rubber protective clip. Then a mass of metal heating material, or heater, was placed on the wound curl. In the direct or progressive system, where heat was supplied by electric wires, the heater was connected to a heating device containing a resistance that was heated “progressively” to the desired temperature. In the indirect system, the heater was preheated to the appropriate temperature on the heating rods of a special device.

This hot waving process has virtually disappeared, not only because it is too complicated and excessively damaging to the hair, but also because hairdressers could not be certain that they would consistently obtain the desired results.

2.2. Heating Sachets

The principle remains the same: to get permanent hair shaping through the action of alkaline reducing solutions under heat (100–120°C in hair sections). In practice, almost exactly the same solutions are used as those mentioned above. However, the heat is no longer supplied by a “direct” or “indirect” electric heating device. A special sachet

soaked with water is wound over the rolled strands. It contains a substance or a mixture of substances, which in contact with water produces an exothermic reaction. The heat generated in this way is sufficient to obtain the necessary temperature.

The desired exothermic reactions can be simply neutralization, hydration or, most often, redox reactions.

These imperfect techniques, difficult to use and sometimes dangerous, are no longer in favor.

2.3. Tepid Wave

This technique resembles hot waving and requires the same kind of "indirect" apparatus, but different solutions and a lower temperature are needed. There are two types of tepid wave: the "heated cold wave" or the "cooled hot wave." The names refer to two ways of formulating solutions, but the techniques and the results obtained are comparable.

The "heated cold wave" lotions are in fact cold waving lotions at approximately the same concentration. Given the temperature level, diluting the cold waving lotions based on thioglycollic acid by about 30% suffices. Sometimes, these lotions contain sodium sulfite in order to lower the mercaptan level slightly.

The "cooled hot wave" lotions are based on ammonium sulfites or sulfites of organic bases like monoethanolamine.

Inside the swatch-tress the temperature employed is 70–80°C instead of the 100–120°C needed for hot waving.

The tepid wave in the forms described has gradually been replaced by the cold wave. And it is now an outdated technique.

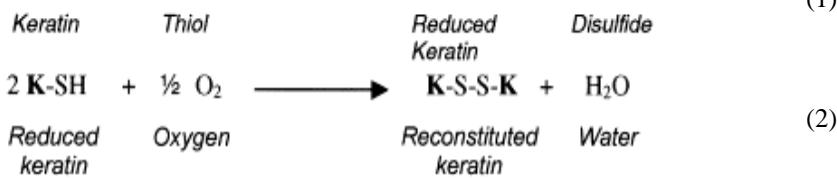
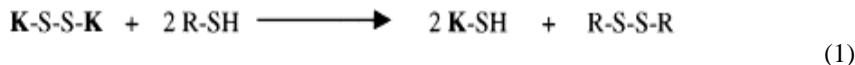
3. COLD WAVING

As its name implies, cold waving is effected at room temperature without supplying heat. The hair strands are rolled on curlers after being moistened with waving lotion. Then, after a specified process time, the curly shape is rendered "permanent" by the application of a "fixing" lotion.

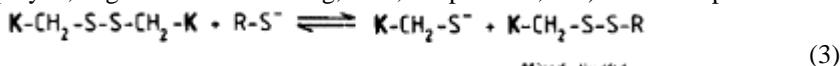
There are two different phases: 1) the reducing action of the waving lotion and 2) the action of the fixing product (or neutralizer), which is generally oxidizing. The first phase is intended to act chemically on the bonds determining the cohesion of the protein structure: covalent disulfide cross-linkages, ionic bonds, or hydrogen bonds. The object is to plasticize the fiber momentarily, so as to be convertible into a new shape without elasticity. The hair is wetted with a reducing solution and rolled on curlers so that the imposed deformation is in the shape of curls. In the second phase, the curls are set by recreating disulfide cross-linkages to restore hair architecture.

Rupture of ionic linkages or hydrogen bonds only leads to temporary hair shaping, namely the hair set. Cleavage of cystine links and their subsequent reforming in a new position is the process that affords permanent shaping. This is now described in more detail.

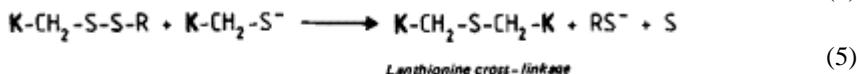
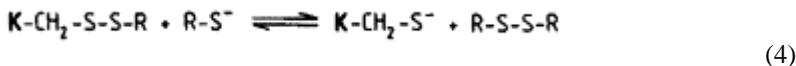
Reducing solutions are primarily based on thiols and fixers are mostly oxidizing solutions; so the probable generalized reactions on the cystine link may be set down as follows:



The reducing action is governed by a number of complex equilibria that depend on various parameters (pH, concentration, stress during rolling, nature of the thiols employed, degree of fiber swelling, time, temperature, etc.). For example:



Mixed disulfide



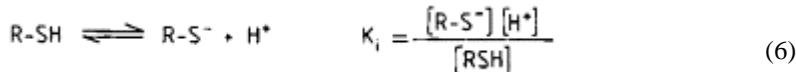
Lanthionine cross-linkage

The first stage of the reaction gives rise to the formation of a mixed disulfide (27), which may then undergo further reaction in two ways, as shown by the experiments of Boré and Arnaud (28): a second thiol molecule acts on the mixed disulfide, with the formation of reduced keratin (Sch. 4) or an internal reaction between the reduced keratin and the mixed disulfide produces a lanthionine cross-linkage (Sch. 5).

It has been shown (29) that, whatever the reducing conditions, the number of disrupted disulfide bonds does not exceed 65–70%, corresponding to the estimated quantity of keratin matrix embedding α -keratin fibrils in the hair. In practice, the reduction level falls somewhere between 19 and 43%.

3.1. Thiols

The reducing agents most commonly used in producing cold waving lotions are thiols or mercaptans. The reducing action of thiols on keratin is due mostly to the thiolate anions RS^- resulting from the dissociation of the RSH thiol groups (30,31);



The higher the RS^- ion rate in the waving lotion, the faster the reaction will occur. This rate depends in turn on the ionization constant K_i of the given thiol (Sch. 6).

The nature of the thiol compound, expressed by its pK , determines the equilibrium level of the preceding reactions and the nature of the competitive reactions that could occur according to pH.

Table 1 gives the pK of a number of thiols. Examination of the pK value reveals that a number of thiols can only be of acceptable efficiency at high pH: others, with a lower pK and a high ionization constant, can be used at lower pH levels. Experience has confirmed this. It is well known, for example, that thioglycollic acid (as ammonium salt) has acceptable waving efficiency only if the pH of the solution exceeds 9. But it has been observed that amides or esters such as thioglycolamide or the glycol thioglycollate can be used at virtually neutral pH and even at slightly acid pH.

These considerations served as justification for many experiments on thiols, especially those that are efficient at neutral or acid pH (32,33). The latter have been found to be very promising because of their many advantages: sometimes a fainter

Table1 pK of Thiols

Thiol	Formula	pK
Cysteine	HS-CH ₂ -CH-COOH NH ₂	10.8
Dimercapto-adipic acid	-(CH ₂ -CH-CO ₂ H) ₂ SH	10.8
Cysteamine	HS-CH ₂ CH ₂ -NH ₂	10.8
Thioglycollic acid	HS-CH ₂ -COOH	10.4
Thiolactic acid	HS-CH(CH ₃)-COOH	10.4
β -Mercapto-propionic acid	H S-CH ₂ CH ₂ -COOH	10.4
Monothioglycerol	HS-CH ₂ -CHOH-CH ₂ OH	9.3
Thioglycolamide	HS-CH ₂ -CONH ₂	8.4
Thioglycolic hydrazide	HS-CH ₂ -CO-NH-NH ₂	8.0
Glycol thioglycollate	HS-CH ₂ -COO-CH ₂ -CH ₂ -OH	7.8
Glycerol thioglycollate	HS-CH ₂ -COO-CH ₂ -CHOH-CH ₂ OH	7.8

odor, less swelling of the hair during the reducing stage, etc. But Voss's data (34) and our own experience have shown that it could be associated with lower skin compatibility.

The following list (Table 1) includes major thiols that have been used in practice or have been the subject of considerable investigation:

1. *Thioglycollic acid* (or mercaptoacetic acid or 2-mercaptopethanoic acid) HSCH₂-COOH is by far the most commonly used thiol in the formulation of cold waving lotions. It is a colorless liquid, soluble in water and in the usual solvents, which has a characteristic and unpleasant odor. Many reducing agents have been tried as substitutes for thioglycollic acid, but none has all of its advantages:

- Adequate efficiency
- Higher scalp compatibility and low irritant potential in the conditions of use (8–11%).
- Faint odor of the solution, if the substance is pure enough at the start.

2. *Thiolactic acid (α -mercapto-propionic acid)*. It is less efficient than thioglycollic acid and has a slightly stronger odor. It is sometimes added to thioglycollic acid in countries where legislators have restricted the permitted use concentrations of the latter.
3. *Cysteine* is specifically used in Japan. Superficially, it seems the ideal thiol, being related to the bonds on which it is intended to act in hair keratin. In fact, it is a weak reducing agent, difficult to stabilize in solution (risk of cystine precipitate and leaving hair in a moderate condition).
4. *Thioglycerol (α -monothioglycerol)*. It has been used commercially in the United States. The resulting waves are of very good quality, and its odor is less disagreeable than that of thioglycollic acid. However, it has been associated with problems of intolerance and it is no more used.
5. *Glycerol monothioglycollate*. It is used in formulations of what are called “acid” waving solutions (mostly professional market).
6. *Cysteamine (β -mercaptoethylamine)* gives results similar to thioglycollic acid on a wider range of pH values. It is particularly more efficient than the latter at a pH close to neutral. Hardly smelling in solution, it however develops a strong residual smell in hair with time. A number of studies have been carried out to try to alleviate this drawback (35).

3.2. Oxidizers or Neutralizers

The oxidation stage results in the fixation of the shape given to the hair during the reducing step and the restoration of the hair's initial physicomechanical properties by reforming the disulfide cross-links between keratin chains.

1. For the most part, fixation or “neutralization” is done after thorough water rinsing using hydrogen peroxide solutions at acid pH. The hydrogen peroxide acts quickly and fairly completely. Sometimes, hydrogen peroxide is supplied in the form of urea peroxide capsules.
2. Sodium perborate in its tetrahydrate or monohydrate form is often used as a powder to be dissolved in water immediately before use (because of its lack of stability in water). In an aqueous solution it gives off hydrogen peroxide. Sodium perborate is highly alkaline, and if desired, the pH of its solutions can be regulated by adding organic acids. It is very efficient, and in fact the best fixation results are obtained at alkaline pH.
3. *Bromate*. The sodium salt is the only bromate used, either in neutral or slightly acid solutions (at acid pH there is a risk of bromine formation). It is an effective fixer, although less so than hydrogen peroxide or the perborates. The risk of bleaching natural shades of hair is very low.
4. Other fixers include the following:

Tetrathionates (36), especially potassium tetrathionate, have given interesting results. Fixation occurs without involving oxygen, in a reaction that takes place as follows:



(7)

The reaction is accompanied by a fall in pH. Fixation is effective. It leaves the hair in good condition, without risk of bleaching or secondary reactions characteristic of most other oxidizers. However, their use have been short-lived due to unpleasant odors that may develop in hair with time. Tests have also been carried out with aldehydes. Fixation can be good, but it leads to the formation of new linkages such as K-S-CH₂-S-K in the case of formaldehyde. Obviously, it is out of question to use a sensitizer like formaldehyde as a reagent. Interesting laboratory results have been obtained with other aldehydes such as glyoxal and glutaraldehyde, but they have the disadvantage of coloring the hair.

Other substances have been investigated, but they are not yet marketed. Among them are: divalent metal salts (37,38), α -alkylene dihalides (39), activated double-bond compounds such as dimethacrylates (40), maleates (41,42), and polymerization into hair of vinyl monomers (43).

3.3. Self-Neutralizing Systems

Attempts have been made to effect the reduction and fixation steps in a single operation. Success in this would achieve a great simplification in technique and a considerable saving in time. Unfortunately none of the techniques investigated has led to a satisfactory self-neutralizing system, and virtually no progress has been made beyond the laboratory stage. Several approaches are outlined in this section.

3.3.1. Oxidation by Atmospheric Oxygen

If hair were left on rollers for at least 6 hr after being moistened with the reducing liquid, rolling, waiting, and rinsing, atmospheric oxygen fixation would suffice to obtain permanent hair deformation. This kind of process has been applied in some home permanent wave systems. But the fact is that the fiber is not integrally restored to its initial state (44). Gradually, the hair is damaged and dried out. Despite the appeal of this simple technique, users prefer the regular two-step system.

Attempts have also been made to speed up oxidation by atmospheric oxygen by adding catalysts to the waving lotion, either heavy metal salts (manganese or cobalt sulfate) (45,46) or terpene derivatives (47). None of these techniques constitutes a satisfactory self-neutralizing system.

Since then, it has been shown (48) that atmospheric oxygen in normal conditions is not very effective for disulfide bond reconstitution. A swatch of hair was reduced under standard conditions using thioglycollate and left exposed to the outside atmosphere for a year. At the end of this period, analysis showed that the reduced keratin had not yet undergone significant reoxidation.

3.3.2. The Thio-Dithio System

The idea of combining a thiol and its own disulfide (e.g. thioglycollic acid and dithiodiglycollic acid) or the disulfide of another thiol (e.g. thioglycollic acid and dithiodiglycerol) is exploited in some new products in the market (49). The principle is as

follows: the thiol reduces the cystine linkages of the hair keratin. This frees-SH residues, which can be oxidized by the disulfide, thus instantly reforming the cystine links.

The redox equilibrium (4) is therefore shifted toward the left by the excess of disulfide, which acts as an oxidizing agent for the reduced keratin.

This attractive technique was considered with a view to avoid the fixation step. However, its limits come from the excessive concentrations of disulfide needed to achieve total neutralization. It is not yet known how to complete fixation without having recourse to a normal oxidizer (hydrogen peroxide or a bromate). Nevertheless, more flexible hair waving may be obtained without any fixation step or fixing without process time (50). On the contrary, the thio-dithio approach is currently used, followed by applying a conventional oxidizer. It has been shown that thioldithio system enhances mechanical properties of permed hair.

3.4 Thioglycollic Acid Formulations

The vast majority of waving lotions are based on thioglycollic acid.

3.4.1. Regular Formulation at Alkaline pH (9–9.3)

The simplest cold waving lotions are ammonia and ammonium thioglycollate solutions at a pH between 9.0 and 9.3. The curve on Fig. 4 represents all the pH/ thioglycollic acid combinations having the same waving potential, that of a cold waving lotion for natural hair. For example, it can be stated that 6% thioglycollic acid at pH 9.8 has the same effectiveness as 10% thioglycollic acid at pH 9.35.

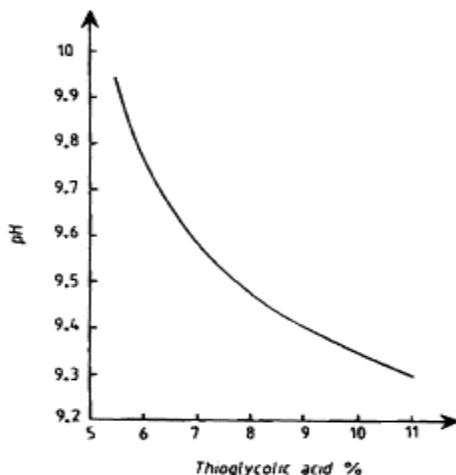


Figure 4 Curve showing ammonium thioglycollate solutions possessing the same waving potency as an average

lotion used by hairdresser for normal hair.

The first solution is much less costly than the second, but is potentially more irritant and, because of its high concentration of ammonia, has a disagreeable odor.

In the majority of cases, waving lotions for natural hair have a pH between 9.0 and 9.3, and therefore a thioglycollic acid concentration between 7.5 and 11% (lotions for hairdresser use).

It is evident that the strength of the lotion employed must be appropriate for the quality of the hair to be waved. For example, bleached hair, which is very porous, brittle, and lacking in S–S links, must be treated with low-concentration waving lotions. The following list indicates the different concentrations of thioglycollic acid employed in professional waving lotions at pH 9.4–9.5 as a function of hair quality:

“Difficult” natural hair, 8 or 9%

“Average” or “easy” natural hair, 7%

Slightly bleached hair, 5%

Heavily bleached hair, 1%

Lotions intended for home use are formulated in comparable fashion, but their strength is reduced by about one-third.

Bases other than ammonia have been tried. Ammonia is useful because, at the level of concentrations used, it is not intrinsically damaging. This is due to the fact that its volatility causes a drop in pH during use and thus provides a guarantee against the risk of excess waving. But its volatility enhances the odor of the products. To reduce unpleasant smell, attempts have been made to use stable bases such as sodium or potassium carbonate, ethylenediamine, triethanolamine, monoethanolamine, diethanolamine, ethylamine, etc.

It is not enough to compare the strength of the bases, their dissociation constants and their effectiveness in this kind of medium. Their penetration rate into hair structure and molecular size should also be considered. Thus, the salts of thioglycollic acid with strong bases mentioned here are less active than ammonia, except monoethanolamine, the only one still considered useful. But it is not desirable to neutralize completely the thioglycollic acid with monoethanolamine alone. It softens and damages the hair too much. However, to diminish the odor of lotions, mixed formulations using ammonia and monoethanolamine can be considered. Limiting or covering the smell of a perming lotion remains a difficult challenge. But the residual odor of reduced hair is also another challenge to overcome.

3.4.2. Buffered Formulations at Lower pH (7.8–8.5)

If an ammonium thioglycollate solution containing 8% thioglycollic acid is buffered at pH 7.5 or 8 with ammonium bicarbonate or carbonate, the result is a permanent wave lotion that is much more active than a regular lotion at the same pH 7.5 or 8 (which results from the neutralization and alkalinization by ammonia alone). It is true that this

lotion is somewhat less active than the regular lotion at pH 9–9.3 having the same thioglycollic acid concentration and the same alkali “reserve” (determined by titration); but its strength is perfectly adequate. This kind of buffered, carbonated lotion possesses a number of important advantages:

- Diminished odor
- Softer hair
- Less hair swelling during the reducing stage
- More regular curling, less stiffness
- More progressive development of the curl

The disadvantages are:

- More difficulty in controlling wave development because there is a lesser degree of curl.
- Need for high concentrations of thioglycollic acid if high-strength lotions are desired.
- The hair and curls are slightly less springy.

This kind of formulation has now widespread usage as it leaves hair in a better condition and is comfortable on the scalp.

There is no advantage in replacing ammonia in the regular liquid formulations. After running the same tests on buffered lotions, the results are not necessarily comparable. Certain bases such as ethylamine whose thioglycollate salt is less efficient than ammonium thioglycollate, can give better waving and hair softening results than with ammonia when used in carbonated lotions.

3.4.3. Additional Ingredients

Cold waving lotions are not simply solutions made up of thioglycollic acid salts with ammonia or other bases, carbonated or non-carbonated, and adjusted to the desired concentration and pH. Frequently other useful ingredients are included.

Boosters. These are intended to accelerate the reducing activity, and in particular, to lower the amount of thioglycollic acid or reduce pH. Such boosters may be other reducing agents (sodium sulfite, cysteine, cysteamine), swelling agents (urea), or solvents (ethanol, isopropyl alcohol or propylene glycol).

Wetting and foaming agents. These agents are intended to facilitate localized application of the lotion as well as its penetration inside the hair swatches, especially during the so-called “saturation” operation, when the moistening of the hair swatches with the waving lotion is renewed after rolling. All the non-ionic or anionic surfactant wetting and foaming agents can be used in very low concentrations. The hypothesis was advanced that these surfactants could enhance thiol diffusion into hair and migration to cystine linkages by interacting with the hydrophobic bonds of the keratin structure. Reduction in the presence of anionic surfactants did not show any potentiation of thioglycollate except at pH 3, where it is ineffective for waving (51). Cationic (quaternary ammonium)

substances are sometimes preferred to nonionics and anionics because of their hair conditioning effect.

Thickening agents. They are mainly used in products to be applied on parts of hair or very specific areas (e.g., products for roots). Carboxymethyl cellulose and guar derivatives are mostly used (52).

Opacifiers. These are intended to produce a more attractive, milky appearance. They are often available as emulsions of acrylic, vinyl, or styrene polymers and copolymers.

Conditioning agents. One may postulate that the hair is particularly "receptive" during the reducing stage and will "absorb" conditioning agents more easily. Fatty alcohols modified to render them more or less hydrophilic, cationics (53), mineral and vegetable oils (54,55), hydrolyzed proteins (56), and silicones (57,58) are used for conditioning and embellishing hair, e.g., impart softness, smoothness of hair surface, and gloss.

The reactive sites created provisionally during the reducing stage offer propitious conditions to strengthen hair by providing tailored care products with high affinity to keratin, such as certain polymers with quaternary ammonium groups (59,60). Hair swelling may indeed reach 300% which helps large molecules such as polymers to diffuse into hair. Moreover, reducing conditions involve a pH level far higher than the hairs isoelectric point (pH 3.6) which suggests that the whole charge of hair is clearly negative and as a result is likely to promote the building of strong ionic bonds with cationic derivatives. In this way the most significant improvements in hair condition during the waving operation have been gained (61).

Chelating agents for heavy metals, most particularly iron. It is well known that thioglycollic acid solutions develop a violet coloration in the presence of traces of iron salts, which catalyze the formation of hydrogen sulfide. Therefore, complexing agents for trace metals should be added, e.g., derivatives of ethylenediaminetetra acetic acid, which have already been mentioned.

Perfumes (62,63) and coloring agents. These must be chosen with the utmost care, as there is a risk that the highly reducing medium might alter them during storage.

3.4.4. Non-liquid Formulation

The formulation of permanent waving creams is attractive in theory, because conditioners can be relatively easily introduced when dealing with an emulsion. But this kind of product, which may have very useful features, has not been successful for several reasons: creams have a slippery feel, which makes rolling difficult; creams are harder to rinse out than liquids, and elimination of the waving product after the process time is sometimes inadequate. Thus, fixation is difficult under these conditions. This type of product is no longer used since it involves direct application of reducing cream to hair before rolling.

Waving lotions have been marketed in aerosol dispensers (64,65). Under pressure, the product comes out as a foam, and is applied to the hair, strand by strand, after it has been rolled. The advantage is that rolling is done using water, and the hands do not come into contact with the actual waving lotion. In addition, the application of the foam, curler by curler, helps avoid running. This seems attractive, but it is a rather delicate process due to the difficulty of obtaining even application of the waving foam onto the hair swatches.

3.5. Neutralizer Formulation

3.5.1. Liquid Hydrogen Peroxide Neutralizers

Hydrogen peroxide neutralizers (or fixers) are supplied either as concentrates, to be diluted immediately before use, or as ready-to-use solutions. The preference of Hairdressers for one or the other depends on their work habits, whether they find it more practical to use a large amount of liquid with a low volume of hydrogen peroxide, or a small quantity of a more concentrated lotion. In general, the concentration for use is between 6 and 9 volumes of hydrogen peroxide. In all cases, the difficulties encountered in formulation are the same:

1. The fixer should be acid to ensure the stability of the peroxide solution. Acetic, citric, and tartaric acids are employed for this purpose.
2. The wetting potential of the solution should be as great as possible, so that it penetrates the rolled hair. It should also be foamy so as to minimize running. Fatty alcohol sulfates or nonionic compounds such as polyoxyethylene fatty alcohols can do this, but wetting and foaming cationic compounds are preferable in that they leave hair soft and easy to comb after rinsing. It is difficult to select the products, given the problem of hydrogen peroxide stability in the presence of organic derivatives.
3. Hydrogen peroxide solutions must be stabilized. Therefore, the usual stabilizers should be used, such as those described in the next chapter entitled "Hair Bleaching".
4. The fixing stage can be regarded as an opportunity to condition the hair, as it is the final operation of the permanent waving process. Thus, it can compensate for any possible damaging effects of the reducing step, and leave softening, disentangling, and glossing agents on the hair.

Formulations are sometimes designed to provide a synergistic effect between the conditioning agents in the reducing lotion and those in the fixer. For instance, a cationic compound in the reducing lotion can form a high affinity treatment product in conjunction with an anionic agent in the fixer (66,67).

Self-emulsifying waxes and fatty alcohols, etc. are often used in addition to cationic surfactants or quaternary polymers (68–70) and cationic silicones (57).

3.5.2. Liquid Neutralizers Containing Bromates

These are always based on sodium bromate. The way they are formulated is similar to hydrogen peroxide products with the proviso they should never be formulated at acid pH, as this would lead to a release of free bromine, or at high (alkaline) pH, for their excessive stability would slow down the oxidation/neutralization process. Only pH levels close to neutral should be employed.

Most frequently, 200 mL of an 8 or 9% sodium bromate solution, or 100 mL of a 12 or 13% sodium bromate solution are used.

3.5.3. Neutralizers in Powder Form

These have been looked on favorably because of their low-bulk packaging possibilities. They are based on oxidants such as sodium perborate, or sodium bromate.

Naturally, powdered neutralizers must be dissolved in water prior to use, and the resulting solutions must possess the same technical characteristics as the liquid fixers previously described which includes wetting and foaming potential and softening effect.

In general, additives are included to regulate pH after dilution (sodium carbonate, sodium bicarbonate, monosodium phosphate, tartaric acid, etc.), as well as foaming agents such as fatty alcohol sulfates or cetyl trimethylammonium bromide.

3.6. Cold Waving Techniques

It is not difficult to carry out a cold waving operation, but success depends on scrupulous attention to detail, especially if it is not performed by a skilled professional hairdresser. Every move has significance and physicochemical repercussions. The following are the crucial points to keep in mind:

1. The choice of lotion is the first essential, and should be made in accordance with the hair texture. Choosing a high-strength liquid to get a tight curl, or a low-strength liquid to get a loose curl is erroneous, because the degree of curl desired has nothing to do with the choice of the lotion to be used. The hair quality is the only thing that counts. In the case of bleached hair in particular, it is often necessary to treat hair ends and roots differently, using low-strength lotions for distal ends.
2. The choice of curlers and the width of the hair strands are the factors that determine the degree of curl. Curler diameter usually falls somewhere between 6 and 20 mm. Thirty-five to 50 curlers are placed on the head, depending on the degree of curl desired. Rolling procedure should be performed carefully with a perfect smoothing down of hair to avoid the occurrence of acute angles which might result in untoward shape or breaks.
3. The hair should be moistened liberally with the waving lotion, saturating strand by strand with a sponge, or squeeze bottle after rolling. Great care should be taken to avoid running onto the scalp in order not to provoke irritation.
4. The process time should be short. Most lotions used on natural hair are intended to have a process time of between 5 and 20 min. If this is extended further, the hair will be excessively softened without the result being improved.
5. The neutralization (fixing) stage is a crucial operation and on it hinges the hold of the wave. It is also responsible for the integral rebuilding of hair keratin. But all too often, it is done too quickly; neutralization requires very careful previous rinsing to eliminate the maximum amount of reducing lotion. Then two steps are performed: (a) Application, using a sponge or squeeze bottle, of two-thirds of the neutralization solution to the rolled curls, strand by strand, followed by a 5-min process time. (b) Unrolling, without pulling on the strands. Application of the remaining one-third of the neutralizing solution on the ends, followed by a few minute process time, then careful rinsing.

It is strongly recommended that hairdressers wear protective gloves during the whole operation.

3.7. Other Techniques Derived from Cold Waving

Various adaptations of the cold waving process are available to users. They are briefly described below.

3.7.1. Hair Protective Pre-Treatment

Hair ends and roots are not always of similar texture. The ends have often been damaged by previous permanent waving, or bleaching, and by air moisture and sunlight. It can be advantageous, therefore, to try to treat hair ends in a different way from the roots and shafts.

Several approaches have been proposed. Among them are the following:

- A pre-treatment of damaged cuticle parts (bleached distal ends or streaked locks) using a protective care product, possibly soaked in a special paper intended to cover or wrap weakened areas before applying reducing treatment. Rolling is then done followed by saturation with reducing lotion.
- Pre-rolling lotions with a low reducing strength (71), containing disulfides (72), cationic polymers (73,74), fatty additives or silicones.

All these indirect methods are aimed at ensuring regular curling along the entire hair length, avoiding frizzing the ends and preventing excess damage. The results can be excellent if they are applied when the hair condition warrants it, and if saturation is carried out very carefully. If it is done carelessly, then the resulting wave can be uneven or inadequate.

3.7.2. Permanent Setting

This subject was discussed in the previous chapter on setting hair. It was seen that moistening was carried out using normal reducing lotions, using normal-sized setting rollers, which are cylindrical and have diameters ranging from about 12 to 30 mm. Two techniques are then possible: drying the hair, in the hope that drying alone will suffice to rebuild the cystine linkages and fix the wave, or neutralization followed by rinsing and drying-all of which are performed while the curlers remain in place on the head.

Thus, the permanent waving and setting techniques are combined together in a single operation.

With the modern technique, and using lotions based on ammonium thioglycollate, it is difficult to ensure sufficient reformation of the S–S linkages. Moreover, the permanent shape obtained using setting rollers can only be in the form of very loose waves. Therefore, the waving process must be repeated every 4–6 weeks, and excessive damage to the hair can result.

3.7.3. “Self-Regulated” Permanent Waves

Sometimes cold waving lotions based on thioglycollic acid and containing a proportion of dithioglycollic acid are advised. They are called "stop action" perm lotions (61). The idea is to "regulate" the process time and afford a certain margin of safety, even if the process time is inadvertently prolonged.

The presence of large amounts of thioglycollic acid disulfide slows down the reducing action of the thioglycollic acid by shifting the equilibrium of the reversible redox scheme (4) (cf. sec. 3.3.2). It was shown that mechanical properties of hair were improved by this "self-regulating" procedure (75).

3.7.4. "Self-Heating" Permanent Waves

Sometimes the following are used to augment the client's comfort during the waving process:

"Exothermic" reducing lotions

"Exothermic" fixing lotions

Waving systems including both "exothermic" reducing and fixing lotions.

To produce an exothermic reducing lotion, a certain amount of an oxidant, such as hydrogen peroxide, is added to the reducing lotion immediately before use. The resulting oxidation reaction is then exothermic, and the temperature rises. Applying a warm liquid increases comfort, and the *in situ* formation of dithiodiglycollic acid possibly causes a kind of self-regulation, as was previously described (76).

To obtain an exothermic neutralizing lotion, a reducing lotion or powder such as sodium sulfite or monoethanolamine sulfite is added immediately before use (77). An exothermic redox reaction will then occur.

These partially or totally self-heating permanent waves are very popular in hairdressing salons in the United States. When the waving lotion is self-heating, it may no longer be appropriate to refer to "cold" waving, but the principles are basically the same.

Some hairdressers combine cold waving and the use of a heating source to speed up the curling uptake and obtain more pronounced results. It is advised to restrict this type of procedure to natural hair.

3.8. Acid Permanent Waving

These are the permanent waves generally carried out at pH between 6 and 7 with thioglycollic acid esters or amides. Valued for the properties of hair after waving, they raised great expectations. Unfortunately, most of the thiols producing an adequate curling at neutral pH are associated with problems of scalp discomfort and intolerance. Glyceryl monothioglycolate remains the only one used in the so-called "acid waves."

The instability of the thioglycolate used necessitates two-part packaging: it generally contains 30% glycerol. The thiol should be packaged in an anhydrous medium, separately from the aqueous solution containing the other ingredients. The two parts are mixed together immediately before use.

With a view to get significant improvement of comfort and compatibility in sensitive scalp, perming products have been developed based on thioglycollic acid but at a pH

close to neutral. As with the previous products based on thioglycollate esters, in order to keep pH stable with time the reducing lotion also needs two separate products to be mixed just before use, one containing the reducing thiol only, the other one including base for pH adjustment, conditioning agents, perfume, etc. pH of the mixture is usually around 7.5 (78–80).

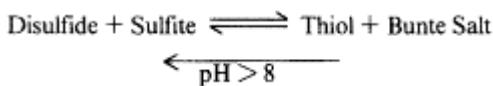
3.9. Cold Waving Without Thiol Compounds

Lotions based on ammonium sulfites and bisulfites at a pH around 7 have been marketed as neutral permanent waves. Schematically, they act as shown in Figure 3.

After the waving process, oxidizing agents produce fixation and thus the reformation of disulfide bonds out of the $-S^-$ residues. The $-S-SO_3^-$ (thiosulfates, so-called Bunte salts) moieties do not lend themselves readily to bonding, and so the restoration of the hair keratin structure remains incomplete. Furthermore, the action of sulfites can only result in loose waves unless rolling is carried out on many, fine hair strands which means a large number of curlers using a fairly long process time.

This is why neutral permanent waves based on sulfites are hardly ever used in salons.

Carrying out the process at a pH around 8 gives good fixation, the increase in pH leading to a reverse in the dynamic equilibrium (8), as a result of an increased amount of thiol in the more oxidizable thiolate form:



However, the waving potential is very low, because of the shift of the reaction equilibrium towards the left, and also because of the low diffusion of the sulfite in the hair fibre at this pH conditions.

In addition to thiols and bisulfites, attempts have been made to use thiourea, formamidine sulfonic acid (81), hydrosulfite (82), sodium hydroxymethyl sulfinate (sodium formaldehyde sulfoxylate) (83), tetrakis (hydroxymethyl) phosphonium chloride or tris (hydroxymethyl) phosphine (84,85), potassium borohydride (86,87), and synergistic mixtures of potassium borohydride and sodium sulfite (88), etc., but their reducing activity was found to be inadequate to alter the shape of hair.

3.10. Assessment

The evaluation of the permanent wave, like that of the other hair treatment operations, can only be made by *in vivo* testing. But the complex reactions caused by the reduction and oxidation produce measurable modifications of the physicochemical properties of the hair. It is most important that these modifications be studied in the laboratory. Naturally, *in vitro* testing will not provide enough information to draw conclusions about the ultimate performance of a permanent waving process in every respect, but the chances are very good that products of little or no promise will be recognized and eliminated.

The following is a list of laboratory techniques used in the selection of permanent waving products.

3.10.1. Yield Assessment

Perming yield is the ratio of the diameter of the curler used to that of the curl obtained. Measuring the perming yield of a given product is easily done in the laboratory, with the proper equipment capable of creating experimental conditions that are reproducible: calibrated cylindrical curlers, constant temperature and degree of moistening, a given rolling stress, etc. The hairs themselves are calibrated, and measurements can be carried out on a single hair. Curl diameters are measured after releasing the hair and immersion in water for about 10 hr. The operation is repeated on 30 hairs so as to increase accuracy and make the results statistically significant.

These *in vitro* studies of perming efficacy lead to the following observations:

1. At room temperature, for a lotion of constant composition, perming yield increases as a function of time up to a limit (about 65% for average lotions and natural hair).
2. For a given lotion, and constant contact time with the reagents, perming yield increases with increasing temperature.
3. For a given lotion, and constant contact time and temperature, perming yield is independent of the diameter of the curler.
4. Perming yield increases abruptly at an applied stress of between 2 and $3 \times 10^5 \text{ g/cm}^2$. It can then reach 100%. Moreover, this increase is independent of both curler and curl diameters.

These observations are only valid under the experimental conditions employed. They have two important features that differentiate them from practical permanent waving in the salon:

1. A single hair is rolled, so the problems encountered in real conditions due to the size of hair strands are not taken into account.
2. The wound curls are totally immersed. This is the only way to provide constant humidification, but there is a great excess of reducing or fixing liquid in the process as a result.

Nevertheless, this method can be used for comparing various raw materials in formulating permanent waving products and in the evaluation of the influence of formulation changes on the performance of the product.

3.10.2. Assessment of Hair Swelling

It has been noted previously that during the reducing stage the various forces ensuring keratin fiber cohesion could be partially disrupted (89–91). Water and liquid penetration is then facilitated and fibre swelling can be seen as a result.

The swelling can be observed and measured by microscopic examination. However, the results are more precise and more easily reproducible using gravimetric measurement of the liquid absorbed by the hair after reduction or after a complete permanent wave cycle. Measurement is carried out after centrifugation; strictly speaking, this is not a measurement of swelling, but of the degree of retention of absorbed liquids (92). This method is extremely useful because various thiols or reducers can be compared using it.

Microscopic examination reveals rapid fibre swelling due to the breaking of cohesion linkages (93,94). There may be further swelling after rinsing the hair with water. The hair

behaves like a semi-permeable membrane. After rinsing, the saline concentration is greater inside the fiber than outside, so an inward flow of water across this semi-permeable membrane can occur. Swelling then decreases during fixation.

These studies of hair swelling revealed that thiols lacking ionizable groups other than -SH groups are very different in their action from those possessing other ionizable groups. At alkaline pH all the thiols containing other polar groups, such as thioglycollic acid, cause much greater swelling than those that do not, such as the glycollic ester of thioglycolic acid or thioglycolamide. This may be related to the repelling effect of the charge on thioglycolic acid vis-à-vis the anionic sites of hair fibre.

3.10.3. Other Methods

Other chemical or physical techniques can measure the different steps of permanent waving. All can be useful. Some of them are:

1. Evaluation of tensile properties
 - a. Determination of the 15, 20, or 30% index, which is the ratio of the work required to stretch the fiber to 15, 20 or 30% elongation after a treatment to that before the treatment (95–97).
 - b. Measurement of load at break, elongation at break, and disruption energy (98).
 - c. Measurement of stress relaxation, the fibre being maintained at a constant stretched length (99).
 - d. Determination of elongation curves at constant load, with the hair immersed in the various reducing solutions to be compared (creep).
 - e. Post yield slope evaluation (43).
 - f. Mechanical behavior of hair immersed in a perming lotion through repeated load-elongation cycles (100,101).
2. Analytical methods for the determination of free sulfhydryl groups and lanthionine (28,29).
3. Colorimetric methods (see Chapter 3) (102).

4. HAIR STRAIGHTENING

Hair straightening, like permanent waving, is an operation in which a permanent deformation of hair is the objective. Permanent waving effects a lasting transformation of straight hair into waved hair. Hair straightening does just the opposite, taking naturally curly or kinky hair and making it more or less straight.

The art of straightening hair has been practiced since the beginning of the twentieth century when C.-J. Walker had the idea of using a heated metal comb on hair imbibed with oil. This temporary styling with the help of heat is still used, in the form of a straightening iron and an oil or lotion applied to the hair to protect it. Around 1959, more elaborate chemical methods based on sodium hydroxide entered hairdressing salons. Then other methods based on sulfites or thioglycollate were used, even at home. These

products achieved permanent straightening that resisted shampooing and are still in use today (103,104).

To impose a permanent transformation on hair, making it change from a curly type to a smooth type after treatment, the principle is as follows:

- Cleavage of disulfide bonds.
- Mechanical straightening during the rupture phase.
- Fixation of the new shape.

There are two main types of technology, with different characteristics: mechanism of action, performance, reversibility, durability, quality of hair fiber, tolerability, and comfort of the scalp.

- Reducer/oxidant technology, based on the use of thiols (reducing process).
- Alkaline technology.

4.1. Reducer/Oxidant Technology

Its principle is the same as that of cold perms (see Sec. 3): reducing keratin's disulfide linkages with mechanical smoothing of hair followed by re-forming disulfide cross-links by oxidation (fixation of the new shape). Breakage of the cystine bonds and the reformation of these bonds in a new position lead to permanent straightening. It is a two-step procedure.

Reducing process is achieved using thiol compounds (principally thioglycollic acid). The reducer concentrations are of the same order as those of the classic cold perms (see Sect 3).

One of the main characteristics of smoothing lies in the use of reducing compositions with a thickened texture that contributes to the process of straightening by keeping the hair straight during the processing period. Usually, these straightening products are in the form of a gel or emulsion (principally oil in water). The alkaline reducing agents (thiols) are incorporated in cream vehicles containing glyceryl stearate, glycol stearate, self-emulsifying waxes, various fatty alcohols, etc. The composition of the cream is developed by optimizing of its flattening capacity (keeping the hair straight during the processing period), its ease of application and distribution, and its rinsability.

The oxidizing compositions (or neutralizers) used in the second stage are based on hydrogen peroxide or bromates and are of the same type as those used in cold perms.

4.1.1. Effectiveness

This technology achieves straightening or smoothing of curly or wavy hair. But for tightly curled hair, in particularly frizzy hair, it is not effective enough. Indeed, as indicated in Chapter I "Hair structure and properties", this type of hair is essentially distinguished from other hair types by its form and very great variation in its section right along the hair shaft. For this type of hair, reducing technology can nonetheless be used with rollers so that the hair is partially uncurled, yielding regular, well-defined curls. It is therefore called the "curl" technique.

4.1.2. Techniques

The diagnosis of hair type and the choice of appropriate product must be made with the greatest care. Hair can react in a very different way to straightening treatment depending on its physicochemical and mechanical characteristics, in particular: its fineness, abundance, resistance to mechanical and chemical attack, and its porosity. The choice of product, the quantity applied and the processing time must be adjusted to take account of these different factors.

The usage technique is simple: the hair is impregnated with the straightening product (reducer) that is generally creamy and thick. Application is made strand-by-strand. During the processing time (from 5 to 20 min depending on product type and hair type), smoothing is achieved mechanically with, for example, the back of a non-metallic comb. This smoothing promotes the slippage of the polypeptide chains relative to each other, so determining the final degree of straightness of the fibre.

This stage is extremely delicate; the hair having been chemically reduced is temporarily very fragile. From the moment that it comes into contact with the reducing product, it must be manipulated with great care. This is why, in cold perms, it is recommended to roll it softly, without pulling. This is not the case here. From the start of impregnation and again after the processing time, smoothing of the hair is recommended. But it is very difficult to smooth without pulling. This is contradictory and explains why the procedure is so difficult. Great care must be taken and the products and hair quality well understood if straightening without damage is to be successful.

As in the case of a perm, fixation or neutralization is an important stage. It must be performed extremely carefully after meticulous rinsing of the straightening cream to limit the risk of breakage that can occur a few days after the straightening procedure. After rinsing the cream away, the neutralizer is applied with a sponge or from a hot water bottle, often in two stages, with a few minutes' rest in between.

It is strongly recommended for the hairdresser to wear protective gloves throughout the hair straightening operation.

Straightening can lead to perfect results but new growth is curly. So the "roots" pose a real problem and the process must be repeated every 4–6 weeks. If reapplication is limited to the new hair growth, damage will not be a problem. But the superimposition of applications to areas already straightened can lead to dry and fragile "sensitized" hair.

4.2. Alkaline Technology

Although technology involving reducer is also a type of alkaline technology (cf. Sec. 2), this name is usually reserved for those that use a strong base for straightening, such as sodium, potassium, lithium, or guanidine hydroxide (105,106).

The chemical action of strong bases has been described earlier in the Perm section (cf. Sec. 3). The hydroxyl ion transforms the disulfide linkages into sulfide bonds with the loss of a sulfur atom transforming the cystine into lanthionine. The properties of these sulfide bonds are very different from disulfides. In particular, they are particularly insensitive to the action of reducing agents. Thus, the action of a concentrated reducing agent on the hair decreases when the proportion of lanthionine increases.

Highly effective, the products of alkaline technology must be handled with care. They can be aggressive to the scalp and over-brutal usage can damage the hair. These are emulsions with a relatively high oil-phase content. The oil phase has two objectives:

- as in the reducing step of reducer/oxidant technology, the use of a thickened texture keeps the hair as straight as possible during the processing time;
- it tempers the aggressivity of the alkaline agent while assuring close contact that promotes the action of the hydroxyl ions on the keratin.

The application of these products usually called relaxers is sometimes preceded by the application of an oily product, to protect the scalp.

4.2.1. Formulation

To respond to the various hair types, there are ready-to-use products containing variable proportions of strong bases (2–4%) that are mineral bases such as sodium, potassium and lithium hydroxide. The lithium hydroxide bases are particularly aggressive, they require prior application of a scalp protective cream formulated using oils. As mentioned earlier, the composition of the emulsion used will play a determining role in the efficacy of the product and protection of the scalp. Studies have been carried out (107,108) on the rate of release of active ingredients from emulsions prepared with mineral oil and emulsifiers of a different nature. The kinetics of release show that an active ingredient, hydrophilic in character, introduced into an emulsion, is more readily released in the case of an aqueous continuous-phase emulsion. The alkaline straighteners are therefore aqueous continuous-phase emulsions, the oil phase serving to protect the scalp and allowing adjustment of the texture.

Other products are presented in two parts for mixture at the time of use. Guanidine hydroxide, an unstable compound, is formed *in situ*, from guanidine carbonate and calcium hydroxide. Precipitation of the calcium salt in this reaction can influence the final cosmetic result due to a deposit on the hair. But these products are particularly appreciated for their efficacy and scalp compatibility (109). The need to prepare a mixture at the time of use and the cost of this type of formulation mean that this technique has not entirely replaced the other alkaline technologies offering less comfort on the scalp.

4.2.2. Usage Technique

As in the case of reducing technology, the choice of product depending on hair type is a deciding factor. A strand test is often recommended, although it cannot guarantee a representative result. After application of the protective cream to the skin around the hair and to the scalp (if necessary), the straightening cream is applied with a spatula or brush. Mechanical smoothing is carried out with the spatula or the back of a comb, strand by strand, without pulling. The hair is carefully rinsed, the head is massaged lightly with the lukewarm rinsing water to dislodge the particularly adhesive cream from the hair. A neutralizing shampoo is then applied to complete the elimination of the cream from the hair and neutralization. This shampoo must have highly effective detergent properties and it is often used in several successive applications. Some of these shampoos contain a

colored indicator to verify that excess alkali has been completely neutralized and that the hair has returned to its initial pH.

4.3. Comparison of the Modes of Action of the Two Techniques

As mentioned previously, the chemical processes used in the two types of technology are different and therefore each induces specific chemical modifications to the hair. The mechanical properties of hair straightened by one or the other method are different:

- Hair straightened by the reducer/oxidant technique, while having a plastic behavior after the reduction phase, regains its elasticity after fixation.
- Conversely, hair straightened using the alkaline technique presents mechanical properties modified in an irreversible fashion. In particular, its behavior becomes plastic to the extent of elongation.

The alkaline technology is the most effective on hair that is difficult to straighten, yielding a durable modification of its shape. Nonetheless, this effectiveness is accompanied by a transformation of the hair fibre. Given the differences, it is important to stress that the two techniques are wholly incompatible with each other. Total regrowth of the hair is necessary before changing techniques.

At the current state of the art, the methods of straightening hair remain difficult to master. The use of rinsed and unrinsed treatments after straightening is systematic, particularly in the case of the alkaline technology. In the case of reducer/oxidant technology, hair care products to control the volume by smoothing and impart discipline and manageability to the hairstyle are often applied after straightening.

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7

Hair Bleaching

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1. HISTORICAL REVIEW

When Caesar came back to Rome bringing with him captive blond-haired female Gauls, it was a revelation to the brunette women of Rome: here, indeed, was a new source of physical attraction (1,2). The captives were disdained as *pictae* (painted women), but according to Ovid, there was much enthusiasm for their blond hair.

First of all, aristocratic Roman ladies fashioned blond wigs from the hair of the captured Gauls. Then they took to bleaching their hair with pomades brought specially from Gaul. Pliny noted the composition of these pomades: "tallow and ashes." The most effective were made of beech ashes and the tallow from natural goat fat. They were used in paste or liquid form. He added that the men of the Germanic peoples "used it more than the women did."

Much later, toward the end of the sixteenth century, the taste for blond shades once again became the rage in Venice. The *arte biondeggiante* became the major preoccupation of elegant ladies of the era. The Venetian beauties, immobilized on their terraces, stayed seated in the sun during the hottest 3 or 4 hr of the day; their hair was drenched in a caustic soda solution, and stretched out and over the "solana," a sort of hat with no crown. In this manner, they obtained the Venetian blond shade immortalized by Titian. In France, the Venetian method was introduced by Marguerite de Valois.

During the nineteenth century, the flamboyant blond color preferred by the Parisian ladies was obtained by using a solution of potassium lye. Thus, for centuries, the only chemical way to lighten hair was to use alkaline solutions or very alkaline soaps. Accounts show that occasionally alum, borax, and decoctions of various plants (birch bark, saffron, rhubarb, etc.) were added.

Hydrogen peroxide did not come into use until about 1860. The hair of Cora Pearl, mistress of Napoleon III, was bleached using hydrogen peroxide. In 1867, at the International Exhibition in Paris, the advantages of hydrogen peroxide were

[†]Deceased.

demonstrated by a London chemist, E.H.Thiellay, and Leon Hugot, a Parisian hairdresser. At the time, their lotion was called *Eau de fontaine de Jouvence dorée* (Golden water from the fountain of youth) or *teinture blonde anglaise* (English blond dye). It was a 3% solution (10 vol.). Their method rapidly became popular.

To date, oxidizing products are still the basis of bleaching preparations. Hydrogen peroxide is used alone, or together with ammonia, or mixed with peroxy salts or peroxides.

2. DEFINITION

Apprentice hair stylists are taught that bleaching is “the lightening of the natural hair shade.”

Bleaching has two objectives: to give hair a lighter look or, more often, to prepare it for the application of a dye preparation, generally yielding a shade lighter or more vivid than the natural one. The natural hair color depends on the quantity of pigment contained and on the size and distribution of the pigment. Bleaching, or lightening, modifies these characteristics of the melanin pigments.

Microscopic examination of the hair sections shows:

1. pigment grains or granules, the color varying from light brown to deep black;
2. diffuse pigmentation, ranging in color from very pale yellow to brownish red.

In a given hair, there will always be a relationship between the tones in the two pigment forms. The cross-section of a brown hair with reddish highlights, for example, will reveal diffuse yellow-red pigmentation, against which are brownish-red grains. It is interesting to note that red hair shows much diffuse pigmentation and very few pigment grains, whereas hair with ash highlights has little of the first and a varying number of grains, depending on the background color accompanying the ash highlight.

If one follows the bleaching process step by step by hair sections under the microscope, one can see that the number of pigment granules decreases regularly, while the diffuse pigmentation becomes more and more apparent. It is therefore likely that the grains react more strongly to oxidants. Perhaps they are destroyed, which would render the diffuse part more apparent by contrast. Perhaps they are “solubilized,” transformed into diffuse pigment.

Gradually, the grains disappear, and the diffuse pigmentation itself is lightened. The total disappearance of the latter can also be obtained; this is what is known as the platinum shade. Whatever the case may be, the result of controlled bleaching is to make the color of the diffuse pigmentation emerge in stages. This phenomenon illustrates a well-known experimental fact: some hair will have red highlights when bleached, others yellow, but all the intermediary colors are possible.

The dominant highlight reveals what is, in fact, the diffuse pigmentation of the natural hair. In the past, this led to the distinction between “ferruginous” and “sulfurous” hair types. This convenient distinction has no scientific basis (Chapter 1): the iron and sulfur contents are not such that a correlation can be drawn with the phenomenon; the true significance of the latter remains to be determined.

Therefore, hair bleaching or lightening means going to a lower tone level (the hair gradually becomes less dark, grows lighter and lighter) and a change in highlight

Table 1 Lightening Levels and Bleaching Power

Lightening Level and color	Bleaching power [‡]		
	Medium	Strong	Powerful
1. Black			
2. Brown			
3. Dark chestnut			
4. Chestnut			
5. Light chestnut			
6. Dark blond			
7. Blond			
8. Light blond			
9. Very light blond			
10. Light, light blond			

The diagram illustrates the relationship between lightening levels and bleaching power. It consists of a vertical line with horizontal branches extending to the right at each lightening level. Three downward-pointing arrows are positioned along this line, indicating the degree of lightening. The first arrow is labeled '2 tones' and is located between the horizontal lines for 'Light chestnut' (level 5) and 'Dark blond' (level 6). The second arrow is labeled '4 tones' and is located between the horizontal lines for 'Very light blond' (level 9) and 'Light, light blond' (level 10). The third arrow is labeled '6 tones' and is located at the bottom of the line, indicating the maximum lightening level shown.

[‡]Starting from level no. 4.

(appearance of more or less "warm" highlights varying in reddishness, which become less and less deep). This is the basis for establishing scales indicating the different degrees of bleaching.

Lightening degrees can be defined in the same way as the natural shade levels, leaving out to some extent the question of highlights, except in the case of very light shades. Table 1 gives the present terminology used by European hairdressers. It shows the different lightening degree levels and the bleaching power obtained.

The value of lightening "tones" is expressed somewhat differently in the United States. The grading system is more complicated, but it can be said that tone 5 in Europe corresponds to 4 in the United States. When the degree of lightening is referred to in this chapter it will be to the European practice.

The notion of highlights is useful when lightening is done to prepare the hair for a dye application. When the bleaching produces red or orange highlights, it will be easy to obtain a result with a golden, red, or dark-auburn highlight. But it will be virtually impossible to get an ash tint. To obtain beautiful ash-blond colors, it is first necessary to obtain a background of pale yellow or platinum. The lightening degrees can also be expressed in terms of highlights, a single tone being the difference between the following highlights: black, brown, red, red orange, orange, yellow, pale yellow, and platinum.

3. THE CHEMISTRY OF BLEACHING

In practice, hair lightening is done by oxidizing melanin pigments. This oxidation can lead, if carried to extremes, to total solubilization and elimination (Figs. 1-3).

It is difficult to attempt a description of melanin transformation by oxidants. It can be said, however, that a depolymerization occurs, giving rise to carboxylated derivatives, which are soluble in an alkaline environment and can therefore be eliminated by rinsing (3-7). The nature and relative content of derivatives generated by alkaline oxidative medium clearly depend on the lightening degree and highlight of the hair (8,9).

For the most part, melanin takes the form of grains, clusters. It is very likely that these grains are linked to keratin by polypeptide residues. To get to the melanin

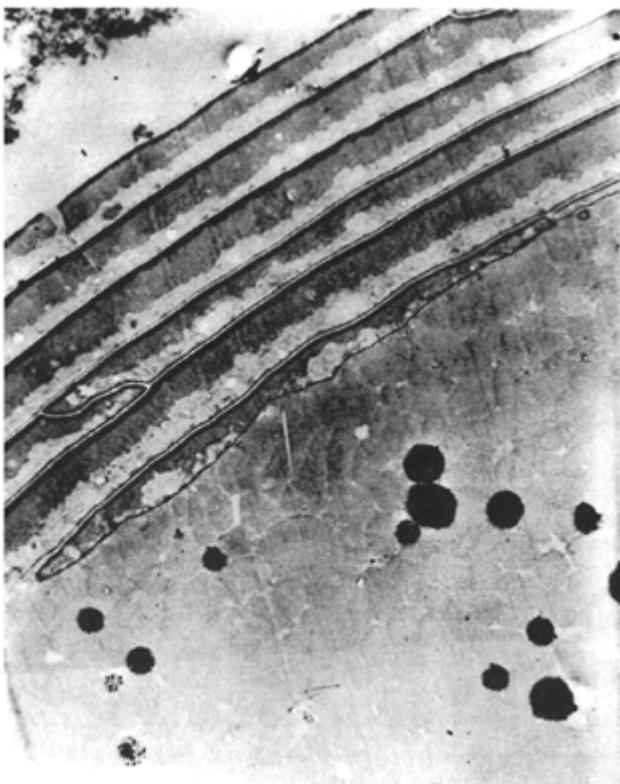


Figure 1 Cross-section of natural brown hair. Melanin grains are visible in the cortex.

grains, the keratin itself must be dealt with and then the peptide residues linking the melanin and keratin.

It was shown that the very gradual process of pigment bleaching on its own should involve the solubilization of melanin granules as a first step (6a,6b). This solubilization would result from oxidative breakage of disulfide cross-links of protein weft (10). Studies in vitro have shown that melanin, which is highly resistant to reducing agents is, on the contrary, fairly easily broken down by oxidation when it has been dissolved in hydrogen peroxide solutions at high pH value, with a specific role of ammonia (6b). The situation is infinitely more complicated *in vivo* because melanin is dispersed throughout an intricate structure.

In practice, all bleaching treatments are oxidative alkaline treatments. All oxidizing bleaching systems are capable of acting on the keratin itself. It is important to examine

the reactions in detail, because they will cause a whole series of modifications in hair properties in addition to bleaching.

Hair keratin has a great number of accessible reaction sites with which alkaline oxidative compositions may interact:

- Hydrogen bonds
- Salt linkages
- Cystine linkages (disulfide cross-links)
- Side-chain amido or free amino groups, e.g., lysine, arginine
- Hydroxyl groups of certain amino acids, such as serine, threonine, and tyrosine
- Amide groups in the polypeptide chain, etc.

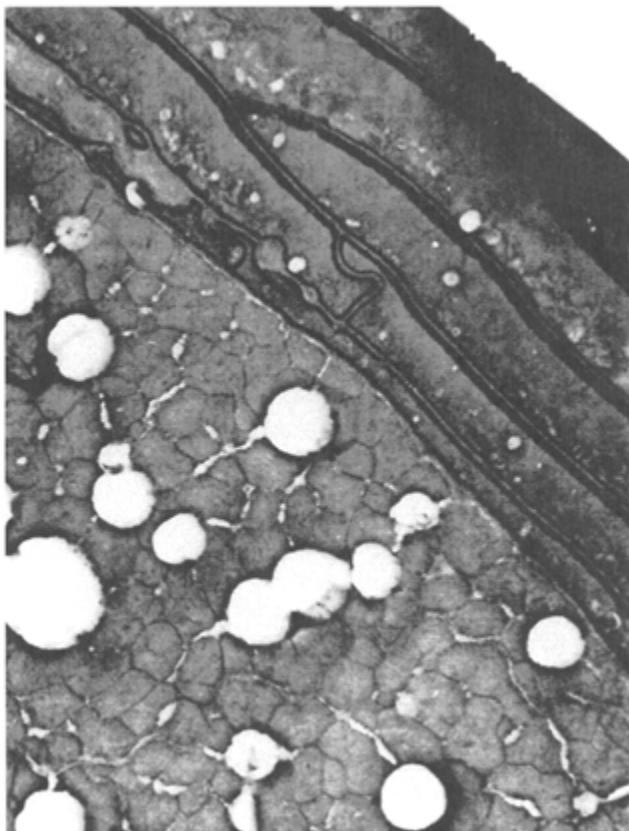


Figure 2 Cross-section of natural brown hair after 1.5 hr bleaching by hydrogen peroxide, 30 vol. Melanin grains have totally disappeared.

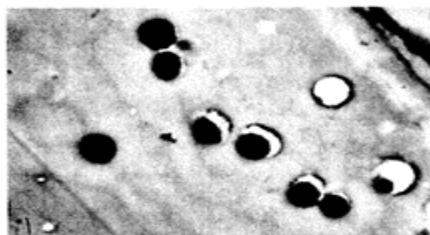
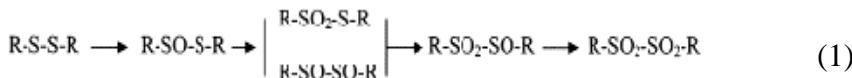


Figure 3 Sun bleaching. Natural brown hair exposed to light and weathering (terrace in the open air) during two summer months. Melanin grains have totally disappeared at the cuticle vicinity, where sunlight struck. Melanin degradation decreases with increasing depth.

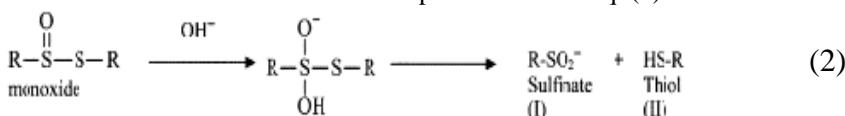
The most important effects to be emphasized are the following:

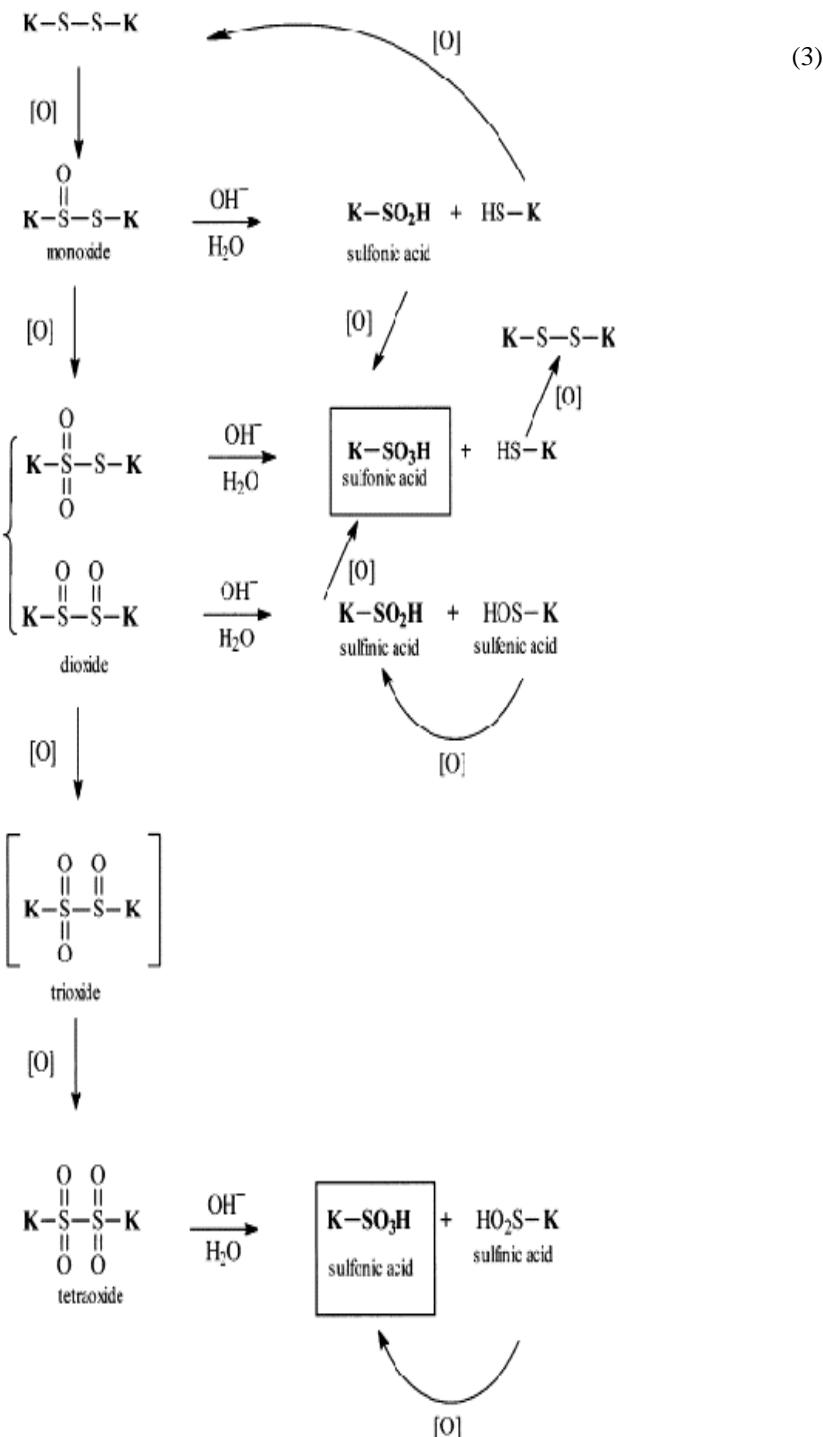
1. The cystine linkages can fix oxygen and yield a number of compounds. Several mechanisms have been put forward to account for this transformation leading to the cleavage of the disulfide bonds (7,11,12). It is currently assumed that it mainly results from the combination of two kinds of reaction: an oxidative process on the sulfur atoms and an alkaline-induced disproportionation of the oxidized species.

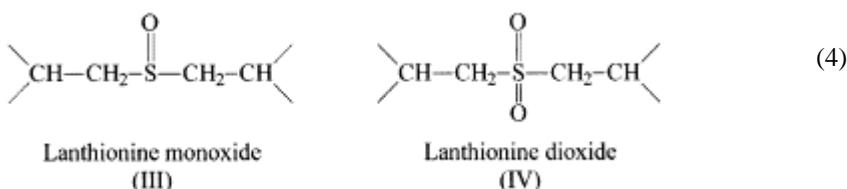
a. *Oxygen fixation:* Studies carried out on the amino acid cystine demonstrated that four oxygen atoms could be successively added to give the various possible oxides (13). Intermediate oxides were isolated, except trioxide. However, these oxides could not be found in bleached hair because of analytical conditions that involve acid hydrolysis. Cysteic acid was the only oxidized species detected which is also the main component evidenced by spectral analysis (14,15):



b. *Alkaline scission*: The oxidized derivatives of cystine show increasing sensitivity to alkali with increasing levels of oxidation. The polar nature of the S-O bond enhances the attack by the hydroxide ion, entailing a scission between the two sulfur atoms, which produces an oxysulfur acid and either a homolog with a lower level of oxidation or a thiol. An example is shown in Eq. (2):







From monoxide, sulfinic acid salt (I) is formed together with a thiol (II). In the oxidizing medium, sulfinic acid salt is further oxidized into sulfonate $\text{R}-\text{SO}_3^-$, and the thiol into disulfide, which may in turn enter the preceding oxidative process.

The whole complex oxidative chain is shown in Eq. (3).

It is currently thought that $\text{R}-\text{SO}_3\text{H}$ is the final step in oxidation process, but one cannot exclude the possibility that oxidation, if taken to extremes, can reach the point where sulfur is released and inorganic sulfates are generated.

2. The action of an alkaline medium per se can lead to the disruption of some disulfide cross-links with the loss of a sulfur atom and the formation of lanthionine, as described in Chapter 6. The latter undergoes oxidative transformation into the corresponding sulfoxide (III) and sulfone (IV):

3. Alkaline oxidizing agents can act on peptide bonds. The hydrolytic attack on the amide bonds can give rise to oligopeptides, which are soluble in water and can therefore be removed by rinsing.

4. Various amino acids can react or be altered: under severe bleaching conditions, 20% of tyrosine can be degraded, 15% of histidine, and 10% of lysine (16).

5. Salt and hydrogen links can be disrupted. The formation of SO_3H groups on side chains increases the ratio between free acids and free bases, and generates strongly acidic groups. The isoelectric point of hair is lowered, and the interchain cohesive forces are greatly modified and redistributed as well as available interaction sites.

6. Hydrogen peroxide can be adsorbed on free NH_2 groups and be fixed as relatively stable complexes. If vigorous final rinsing does not remove it completely, it will continue to react.

The various reactions, taken as a whole, result not simply in the bleaching effect, but also in a modification of specific characteristics of the hair fiber:

- Mechanical properties;
- Increased alkaline solubility through the creation of free acid groups;
- Increase in porosity and water-intake capacity;
- Modification of surface properties;
- Different behavior vis-à-vis various hair treatment operations.

To refer to these hair alterations, the words "sensitization" or "sensitized hair" are often used. These aspects are discussed later (Sec. 8).

4. THE OXIDIZING PRODUCTS

All the bleaching methods used in current techniques are oxidation processes. Hydrogen peroxide can be used alone to bleach hair, particularly for home use. But it reacts very

slowly. If lightening by two tones is desired, for example, a hydrogen peroxide (20 vol.) solution would have to be left to act for one night. In general to obtain hair lightening within a reasonable time (1 hr maximum), it is necessary to apply after mixing with an alkaline solution. Neither molecular oxygen nor stable oxidizing product solutions (acid hydrogen peroxide, bromate, alkaline perborate, etc.) give lightening effects. Even when using reactive oxidants such as persulfates, it is always essential that the product applied onto hair contains hydrogen peroxide.

One must now consider the different ingredients in the formulation of bleaching products:

1. Hydrogen peroxide is delivered to the user as a 6 or 9% solution, or rarely as a 12% solution. The titer is generally expressed in volumes. The volume of a hydrogen peroxide solution is the number of liters of oxygen in its gaseous form released by the decomposition taking place in a liter of that particular hydrogen peroxide solution. The correspondence between volume and weight concentration is as follows:

Volume	Weight Concentration (%)
10	3
20	6
30	9
40	12

Usually, 20 vol. hydrogen peroxide is the product made available for home use, rarely 30 vol. Hairdressers make use of products at 10, 20, or 30 vol, or exceptionally, 40 vol. It is hazardous to go any higher.

Hydrogen peroxide solutions are unstable. The presence of traces of metallic salts or certain organic products suffice to catalyze its decomposition. They cannot be kept stable except at acid pH, in the presence of stabilizers and or chelating agents. These include phosphoric acid, etidronic acid, quinine sulfate, pyrophosphates, acetanilide, phenacetin, oxyquinoline sulfate, ethylenediaminetetraacetic acid (EDTA), pentasodium diethylene triamine pentacetate or certain stannates, etc. It should be noted that certain stabilizers are strictly regulated depending on the country.

Hydrogen peroxide solutions may be replaced by crystals or tablets. In this case, the hydrogen peroxide is in a stable addition complex, such as urea peroxide at 30 or 33% H₂O₂, melamine peroxide powder, etc. In order to facilitate dissolution of the tablets, dispersing agents like agar-agar are sometimes added.

As stated above, hydrogen peroxide cannot react rapidly unless it is mixed just before use with an alkaline solution. Proportions in general use are: H₂O₂ (20 vol.), 40 mL; 20% aqueous ammonia, 7 mL.

2. Persulfates are often used in the formulation of bleaching powders that are watered down just before use, in particular in the form of their salts: sodium, potassium, and ammonium. The ammonium salt is the most active, simply because diluting it at alkaline pH leads to the formation of ammonia; this facilitates product penetration. However, it is now used less and less so as to get optimal tolerance of the products. Sodium and potassium salts are less hygroscopic. Persulfates are rarely used alone, simply diluted in water. They are used with additives to adjust pH and mixed with hydrogen peroxide (20,

30, or 40 vol.) just before use. Persulfates and hydrogen peroxide are clearly complementary in their bleaching effect on hair melanin. Such complementarity is still unexplained. Martin et al. (17) have studied the effects of potassium persulfate on melanins but at high temperature, using acid conditions and melanins from fungi which are quite different from human melanins.

3. Sodium percarbonate can be used, diluted in water or in hydrogen peroxide just before use. But it has slowly fallen out of favor because it is not stable, not even in powder form.

4. Perborates, i.e., sodium perborate and magnesium perborate, are rarely used in the formulation of bleaching products. They have little effect on melanin at alkaline pH because they are too stable.

5. Besides hydrogen peroxide and the peracid salts, bleaching powders may sometimes contain *peroxides* like magnesium dioxide, or strontium dioxide which replaced barium bioxide for safety reasons.

5. THE FORMULATION OF LIGHTENING AND BLEACHING PRODUCTS

There are a variety of products available. To distinguish their technical or esthetic uses, they are classified as follows:

- Hydrogen peroxide solutions and emulsions;
- Lightening and bleaching creams;
- Lightening and bleaching shampoos;
- Bleaching powders or pastes;
- Bleaching oils or gels.

5.1. Hydrogen Peroxide Solutions, Oxidizing Solutions, Oxidizing Emulsions, Lightening Setting Lotions

Simple aqueous solutions (20–30 vol.) are available. They are used by hairdressers after mixing with varying proportions of aqueous ammonia, or other products that are mentioned in the following sections. The simpler formulation only requires choice of the proper pH and stabilizers, to ensure effective solution preservation. The pH should be very acid, preferably under 4, and this is done by using mineral acids, usually phosphoric, or organic acids like citric and lactic. The stabilizers commonly used and the danger of even traces of metal salts have already been mentioned (Sec. 4).

The hydrogen peroxide and ammonia mixture is very liquid. It is hard to localize its application and to avoid it running onto an already-bleached portion of the head. This is why hydrogen peroxide is often available as a thickened oxidizing emulsion or a fluid oxidizing cream. These emulsions are generally obtained by using self-emulsifying waxes containing oxyethylenated fatty alcohols partly sulfated or not or polyglycerolated fatty alcohols or fatty amides. Certain polymers stable in acidic medium can also be used. Mixing with ammonia or any other alkaline product must be done just before use, under the same conditions (Sec. 4) as those for a simple hydrogen peroxide solution.

The thicker composition makes it possible to ensure localized application with greater success. Moreover softeners or conditioning agents may be added, whereby the mixture will afford hair smoother feel, combability, and sheen. Softening agents may be either alcohol derivatives or fatty amides such as those just mentioned, or cationic-surfactant derivatives, or cationic polymers, among others. The cosmetologist knows that great care must be taken to obtain good stability in this kind of formulation, which contains a high proportion of organic ingredients. To date, these oxidizing emulsions are replacing the simple hydrogen peroxide solution in all its uses.

Oxidizing emulsions containing hydrogen peroxide are mixed just before use with an alkaline agent, usually ammonia. According to the hydrogen peroxide volume, these mixtures enable one to obtain 1–3 tones lightening within 20 min. It is useless to try to extend the reaction time, because after 20 min the partially decomposed hydrogen peroxide has virtually no further effect. Increasing the proportion of ammonia increases the likelihood of obtaining rather unesthetic red highlights, without adding to the lightening power while increasing the probability of skin itching or irritation.

When hydrogen peroxide is made available to home users, it may be without ammonia, which simplifies everything. As mentioned previously, acid hydrogen peroxide does not act on melanin effectively in relatively short time periods (under 1 hr). This is why the product should be left to act for a number of hours, in general for 12 hr, to let the hair go about 2 tones lighter.

A practical formulation for these hydrogen peroxide solutions, which do not require a final rinse, is the setting lotion or the conditioner providing a smoothing effect and giving the hair better combability. These lotions can lighten if they contain the necessary proportion of hydrogen peroxide (usually 6–10 vol), in addition to the polymers and conditioning agents already contained in this kind of product. They may be available as sprays. All these types of lotion give appreciable, progressive lightening results when applied regularly, for example, once a week.

5.2. Lightening and Bleaching Creams

These creams generally contain ammonia and sometimes other alkaline agents such as monoethanolamine. They are made up of self-emulsifying waxes, fatty alcohols, fatty acids, lanolin derivatives, cationic surfactants, quaternary polymers, etc.

The creams contain 10–15% of 20% aqueous ammonia. Just before use, they should be mixed with a quantity of hydrogen peroxide or oxidizing emulsion (20–30 vol.). At the time of use, the composition of the mixture resembles that obtained with oxidizing emulsions mixed with ammonia. But the cream form can be applied locally with more success. Moreover, it is easier to add conditioning agents to a cream. Last, given the same concentration of hydrogen peroxide and ammonia, the lightening is slightly more effective because evaporation of the ammonia is slowed down. The hair can be lightened up to 3 tones and half.

Even more than 4-tone lightening may be reached thus converting to a bleaching product by adding a strengthening persulfate-based powder to the hydrogen peroxide and ammoniated cream mix.

5.3. Lightening and Bleaching Shampoos

It is possible to produce a ready-to-use shampoo containing a certain proportion of hydrogen peroxide, if great care has been taken in stabilizing it. But the time needed for a hair wash is adequate only for slight hair lightening. As a result, this type of product is not often used.

Lightening shampoos are actually ammonia-containing shampoos to which hydrogen peroxide (10, 20, or 30 vol.) is added just before use. The composition obtained will possess both detergent and foaming characteristics. After the normal waiting period (20 min to 1 hr), simple rinsing is all that is needed. Obviously, a final shampoo is not required as it is for the preceding products.

Bleaching shampoos are sometimes made up of bleaching powders, like those referred to in the following paragraph, to which detergent or foaming powders (fatty alcohol sulfate, etc.) have been added. Just before use, the powder is diluted in water or in a hydrogen peroxide solution. This is the easiest way to provide a strong bleaching shampoo; the hair can lighten up to 4 tones.

5.4. Bleaching Powders and Pastes

These products are the most frequently used when strong bleaching is to be obtained within a reasonable time (40–60 min), especially if the hair is dark to begin with (from chestnut to black).

These powders contain peroxygenated salts such as sodium, potassium, or ammonium persulfates, and sometimes percarbonates or peroxides like strontium dioxide. They are alkaline; their high pH is obtained using either metasilicates or phosphates or carbonates. These powders have a very finely divided mineral content: such as magnesium carbonate or magnesia, the role of which is to modulate oxidizing process after dilution. A swelling agent such as carboxymethyl cellulose or a vegetable gum is often added to bleaching powders; this gives the desired emollient quality to the resulting paste and slows down drying when applied to the head. Occasionally, small quantities of blue or violet colorants are added in order to give the hair light shading: this avoids an excessively yellow shade.

The powders are mixed just before use with the amount of hydrogen peroxide (20 vol.) needed to obtain a paste fluid enough to be easily applied but thick enough to avoid running.

Application of pastes is easy, and the results are usually highly satisfactory. However, their disadvantage is that they dry on the scalp during the waiting period (about 1 hr), and sometimes create a sensation that the skin is tightening. These compositions are very effective, as they are capable of lightening by 4 or 5 tones. They are therefore ranked as bleaching products.

In order to streak a tress or swatch of hair, a hairdresser mixes these powders with more concentrated solutions of hydrogen peroxide (30–40 vol.). Higher concentrations of hydrogen peroxide, even used with utmost care, would be harmful for scalp and very damaging for hair.

5.5. Bleaching Oils or Gels

Bleaching oils or gels are products with an oily appearance and texture. They usually contain ammonia, but sometimes organic bases (monoethanolamine for instance). Mixed with a quantity of hydrogen peroxide (20–30 vol.), used as such or as oxidizing emulsion they yield a transparent or translucent gel.

Gel formation is obtained by several methods:

- A careful choice of fatty oxyethylenated or polyglycerolated derivatives.
- The use of ammonium or alkanolamine soap solutions such as ammonium oleate.
- Solution of stoichiometric anion-cation complexes, etc.

There are many advantages to using a gel formulation:

- Their texture is such that application is easy and easily localized.
- They are transparent; it is possible to follow the progress of the color lightening without difficulty.
- They are much more “comfortable” than pastes, they do not draw the scalp tight because they do not dry.

When the gel is obtained by mixing soluble oil and hydrogen peroxide (20 vol.), the hair can only be lightened 2–4 tones as with creams. To obtain high-strength bleaching powder, three products are mixed:

- Soluble oil;
- Hydrogen peroxide;
- Two or three sachets of a reinforcing powder.

The main ingredients of this powder are the persulfates. But the powder must be soluble so that the gel remains clear; therefore it does not contain as much mineral content, as there is in the case of bleaching pastes. It must be alkaline; so sodium metasilicate or phosphate is usually added. This composition has all the advantages described above and bleaches as effectively as pastes.

6. THE BLEACHING TECHNIQUE

Many techniques are used. We will therefore restrict discussion to emphasize some points. Before bleaching, the hair must not have been washed. It is undesirable to remove the lipids on the hair surface, because the preparations have a tendency to run and it is hard to localize their application. Another reason is that the scalp will then retain its natural surface lipid layer to protect it against the risk of irritation.

However, it is important to use a shampoo or a rinse after application, except in the case of bleaching shampoos. The choice of shampoo and the way it is applied is also important. It is preferable to select an acid pH product with a low level of detergent action and to apply it without energetic scalp massage.

It is crucial that bleaching be localized to the portions where it is needed. Extension onto areas that have been previously bleached can only increase the damage to the hair. The application must be made keeping in mind the well-known fact that the part of the

hair near the root bleaches more easily than the shaft and ends. This difference is hard to explain. It is sufficient to say that there is enough heat emanating from the scalp to cause an acceleration of the process. This is why, in first-time bleaching, the application must be done in two steps: the ends first and then the roots. In this way, the waiting interval will be longer for the ends, thus compensating for the difference, and the final result will be homogeneous. Bleaching compositions should be applied with brush applicator.

It must be remembered that all bleaching preparations are highly active. It is not advantageous to accelerate their reaction by increasing the quantity of ammonia, the volume of hydrogen peroxide, or the proportion of reinforcing powders, especially in the case of bleaching gels. The idea of speeding up the process seems attractive in itself, but the goal is to limit modification in hair keratin and to avoid producing any dermatitis of the scalp.

7. REMOVING HAIR DYES

Bleaching is the lightening of a natural hair shade. Sometimes, however, one needs to carry out partial or complete removal of semipermanent or permanent dyes. It is always more difficult to remove artificial pigments than natural ones, and their removal is a delicate operation demanding all the skill of an experienced hairdresser.

Two methods and two products are employed:

(1) Reducing agents: Sodium hydrosulfite or sodium or zinc formaldehyde sulfoxylate are dissolved just before use in water rendered acid by an organic acid (often tartaric acid, oxalic acid being more effective but impossible to use because of its toxicity). When applied, this solution will produce good dye removal in the case of oxidation dyes, but the resulting shade is unstable. In the days following, it will darken; this is due to reoxidation. One can ensure fixation of the hair color after dye removal, on the same day as the application, if an alkaline rinse or oxidizing alkaline rinse is carried out. The shade will deepen a little, but fixation will occur. Recently some products based on ascorbic and citric acids came to the market for home use. In principle, such products are less damaging for hair but their efficiency is variable depending on the shade to be removed. In particular, they perform moderately on natural shades and it may be harder to yield a color after such a treatment.

(2) Oxidants: This is a stronger solution, and more reliable, as no color changes occur after application. High-strength bleaching such as those mentioned are called for in this case.

In both instances, dye removal damages and “sensitizes” the hair, especially if the shades to be lightened are dark or very dark. Furthermore, the shades obtained are frequently quite unattractive, and a fresh dyeing operation should be done to correct them.

8. EFFECTS OF BLEACHING ON HAIR

Bleached hair will no longer behave like natural hair:

1. It is drier, more brittle to the touch, and tangles more easily.

2. It is porous and more likely to be affected by humidity. It takes up water more readily and therefore takes longer to dry.
3. It is weaker, with a lower disruption point. However, its elongation to the disruption point is greater, especially when wet.
4. It absorbs, and adsorbs more readily. If bleaching is not uniform all along the hair shaft, then dye absorption will be heterogeneous (selectivity).
5. It lends itself better to permanent waving solutions. Weaker solutions dosed and formulated especially in consideration of the degree of damage apparent in the bleached hair are required.
6. It sorbs and retains to a greater extent cationics due to enhanced density of anionic sites and increased diffusion (18–20).

In order to understand these phenomena, we must refer to the chemical effect of the oxidants. The primary effects are on the disulfide bonds, a number of which are disrupted. As a result, the mechanical properties of the fiber are no longer the same. It also follows that the physicochemical processes such as dyeing and permanent waving treatment have greater impact on hair keratin.

During oxidation by peracid salts or peroxides, the polypeptide chains themselves may well be modified. In any case, degraded, water-soluble proteins are formed and can later be recovered in the rinse water and analyzed qualitatively and quantitatively. It is even thought that this kind of degradation might continue after the conclusion of the bleaching operation: each shampoo can result in the elimination of oligoproteins. That is why it is important to terminate the bleaching process with a shampoo or an acid treatment that will more or less precipitate the proteins into the keratin fiber, render them insoluble, and fix them there.

Slight or average lightening will cause only minor modifications which can be virtually prevented by protective polymers. But high-strength bleaching, especially if superimposed frequently on the hair, will alter the hair fiber to a considerable extent.

Bleaching requires great care and skill, and the products and techniques employed should be adapted to each specific case. Specialists in the field of cosmetology are familiar with the physicochemical and cosmetic characteristics of bleached hair; they have made great efforts to prevent, limit, or compensate for the undesired damaging effects of bleaching. To this end they have tried to accurately measure the consequences that bleaching agents could have on hair. The main techniques used are briefly discussed in Sec. 8.1.

8.1. Physical Methods

8.1.1. Tensile Properties

Measurements include wet and dry tensile strength, determination of breaking load, stress-strain curves, and 15 or 20% index (7,21–23). Contrary to what one might expect, modifications in tensile strength following a single bleaching operation are not marked. The values for stress at the breaking point of hair immersed in water are lowered, while elongation at the breaking point shows an increase. Measurements of the 20% index

reveal a drop of only 10–15% even after strong bleaching. But repeated bleaching may induce a much greater decrease.

8.1.2. Surface Properties: Changes in Friction

The feel of bleached hair indicates a change in surface condition. As shown microscopically, the scales become more apparent, delineated, clear-cut, uneven, less tight. Hair is drier, more brittle, and rougher to the touch which suggests that frictional characteristics are likely to be modified.

Like an animal fiber, hair presents two coefficients of friction: one from end to root, the other from root to end. These mechanical parameters may be assessed using a very accurate tensile meter to measure the force (F) necessary to move smoothly a parallelepiped load (L) along the surface of two parallel hairs laid horizontally. Repeating the process in both directions on several pairs of hair allows the determination of the mean frictional coefficient (24): $\operatorname{tg} \psi = F/L$.

The more bleached the fiber, the higher will be the coefficient. Feel is usually well correlated with the data obtained.

In other rubbing tests a loaded hair is curled around a mandrel or a cylinder, and the frictional forces developed as it rotates are recorded (25,26).

Another approach to these properties is to measure the strength needed to comb a tress or swatch of hair from the root down the end (sliding test) (27,28). Swatches 15–20cm long are suspended from an Instrom-type tensile tester (see Chapter 12); a comb is run through the entire length of the strand at a constant speed, and the force necessary is recorded. This force will increase with tangling of hair and the alteration of the hair shaft (raised scales, etc.). It is usually markedly higher near the tip of the swatch.

Breaking induced by brushing is another test method proposed to reflect hair surface condition providing that brushing conditions applied do not produce any breakage on hair having a perfect even surface (Chapter 12).

To interpret the data obtained in these various tests, following spectral techniques may be useful: ESCA (14), FTIR (15), SMIS (29) or confocal microscopy (30), and AFM (31).

8.1.3. Determination of Weight Loss After Bleaching

The vigorous action of alkaline pH oxidants can lead to a decrease in the sulfur content, sulfur being released in the native form or as oxidized derivatives including, in extreme cases, sulfates. In addition, a decomposition of the polypeptide chains and the elimination of soluble oligoproteins can occur, present in the rinse water.

Weight loss was estimated as a means of comparison between different bleaching products of varying type, all possessing the same bleaching strength. The treated hair is weighted under controlled atmospheric conditions to avoid errors due to atmospheric moisture uptake. After strong bleaching, the weight loss may reach 2 or 3%.

8.1.4. Water Uptake Curves

Using gravimetry, under controlled atmospheric conditions, one can measure the amount of water absorbed by a given weight of hair in a given time. Thus, one can demonstrate that bleached hair is more hygroscopic than normal hair, which indicates the freeing of hydrophilic groups.

More recently, near infrared spectroscopy (NIR) has been evaluated as a tool to determine the moisture content of hair (32). It is noteworthy that this technique is useful to study bleached hair and can be used to measure the lifting power of hair colors even in the presence of synthetic dyes.

8.2. Chemical or Physicochemical Methods

8.2.1. Alkali Solubility Assay

This method was first studied in the case of wool (33). It is primarily based on the fact that the oxidation of disulfide linkages leads to the formation of strongly acid groups. Fiber solubility in alkaline solution is thereby increased. Determination of the level of solubility in a given alkaline solution, for a given time and temperature, can be used to measure the degree of oxidation and the level of alteration (34,35) (Fig. 4).

8.2.2. Quantitative Analysis of Cysteic Acid

Oxidation of cystine linkages can lead to the formation of SO_3H groups. Acid hydrolysis of the oxidized keratin and the separation of cysteic acid by electrophoresis on paper or ion-exchange chromatography give interesting results (36).



Figure 4 Bleaching level and alkali solubility (AS). Left to right: natural brown hair, slightly bleached (AS)

10%), medium bleached (AS 20%),
highly bleached (AS 50%).

Knowing that the average cystine content of hair keratin is 16.5%, the total amount of potentially generated cysteic acid may reach 23.2%. Quantitative analysis after bleaching shows a measurable degree of degradation: 4–6% for slight or average bleaching and 10–15% for strong bleaching.

8.2.3. Analysis of Solubilized Protein Material

The possible degradation of polypeptide chains in keratin can be assessed by the protein material solubilized during a bleaching process. Peptide bonds are not significantly affected under use conditions in the salon. The values obtained ranged from 0.4 to 0.8%, which is about the level found after shampooing hair merely exposed to normal weathering.

These findings emphasize that the only perceptible action of bleaching on keratin is the oxidation of cystine linkages. Similar results have been observed on hair bleached by excessive exposure to sun. This oxidation entails most of the changes noted in hair properties.

8.2.4. Colorimetric Methods

Alterations of the superficial shaft, i.e., cuticle structure, and an increase in the available anionic sites raise the porosity and the polyelectrolyte nature of hair. The capability to be dyed is therefore greatly modified (kinetics of adsorption, absorption, diffusion, fixation).

The uptake of cationic dyes is markedly enhanced on bleached hair. Methylene blue is readily fixed on the sulfonic and carboxylic groups of keratin in neutral and acid media. The more acidic groups generated by bleaching, the more methylene blue will be fixed on the hair. In practice, the decrease in the dyeing agent content in the solution before and after immersing a swatch of hair for about 24 hr is recorded by a colorimeter or spectrophotometer.

The uptake of acidic dyes, which are anionic, is also increased with the porosity (37). However, acidic dyes are mostly used for assessing the uptake of cationic derivatives by damaged hair. The more bleached the hair, the greater will be the number of sulfonic groups and the amount of fixed cationics. The affinity of acidic dyes will increase with the number of cationic groups retained by hair. Various colorimetric methods have been developed that help to depict the ionic and physicochemical conditions of the fiber and thus measure the alterations elicited by a treatment. They are most often based on Orange II (38) and Red 80 (39) dyes.

In summary, the chemist has a whole battery of tests from which to select. This helps in the determination of the optimal conditions for bleaching. He chooses the method and the product which, possessing equal bleaching strength when compared with others, will minimize hair damage. In addition, these measurements help him to develop and evaluate techniques and products that can be used to prevent or compensate for the disadvantages of bleaching.

Hence, one can see why the study of oxidized hair constitutes a major area in the assessment of hair condition and properties.

9. THE IMPORTANCE OF HAIR BLEACHING

The bleaching of hair or even mere lightening is often thought of as an operation of minor importance. Some publications cover the subject in a few pages, without giving more than general indications on formulation of individual products. This might make one conclude that bleaching is a simple process, or of little significance. But the contrary is true; bleaching is one of the most intricate and crucial areas in the field of hair treatment, a fact well-known to specialists.

Bleaching is complex, because it calls on a range of chemical reactions involving not only the pigment it is intended to lighten, but also the keratin fiber itself. It is not yet possible to act on melanin without using reagents that modify polypeptide (side) chains and the network of linkages binding them together.

We are as yet unable to prevent or compensate for the effects of strong oxidizing products in an entirely satisfactory manner. Interesting results have been obtained using techniques to create cross-links between loose keratin chains.

Restructuring or strengthening of the keratin fiber has been achieved using certain cationic polymers (40–43), or their mixture with divalent metal salts (44), or in situ polymerized products (45–48).

Bleaching is important because it concerns more than just the subject of the lightening of hair: it is involved in the majority of all coloring operations. Fine coloring, which is transparent and subtle, cannot exist without simultaneous lightening, and as mentioned previously, all coloring systems based on oxidation dyes use it to some extent. All hair dyed with oxidation dyes is, in fact, lightened hair. Bleaching and lightening should therefore be considered as hair treatment operations of major and maybe of utmost importance.

A method of lightening hair that acts only on the pigment, and not on hair keratin, would be a great step forward in the fields of dyeing and bleaching. So far only methods and products have been designed to prevent and compensate for untoward effects of bleaching, helping bleached hair retain or recover the physicochemical characteristics of natural hair.

10. CHEMICAL BLEACHING AND INTOLERANCE

Some reactions of intolerance by the skin have been observed following the application of bleaching agents: these usually consist of transient urticarial reactions, more rarely forms of eczema and a few cases of respiratory problems in hairdressers. This question is dealt with later on, in Chapter 15.

The mechanism of these manifestations has not been elucidated. It appears that they occur essentially in the presence of ammonium persulfate (49). Few reactions have been reported with other persulfates. No explanation has been found for this specificity. Formulated correctly, persulfates remain acceptable compounds however (50).

To minimize the respiratory problems noted principally in professionals, several “non-volatile” powders have arrived on the market. These compositions, derived from the powders mentioned previously, are “coated” with the help of anhydrous compounds, such as mineral oils (51) or hydrosoluble anhydrous surfactants (52). Indeed, these compounds agglomerate with the finer particles among them while still presenting a powdery appearance. These coating agents mainly reduce the volatility of the powders but can also play a role in the quality of the bleaching paste. In the event that a sufficient amount of the anhydrous coating agent is added, it is even possible to arrive at a rather worthwhile cream form (53).

To retain the powdery appearance, coating has also been proposed by granulation with film-forming polymers (54,55). However, in this case, the formation of the paste when mixed with the hydrogen peroxide is very time-consuming. In addition, following repeated jolting due to transportation, these “granules” are capable of crumbling into particles of a smaller size again, during storage.

11. BLEACHING BY THE SUN AND ARTIFICIAL LIGHT

It is evident from simple observation that hair can be lightened by sun exposure. This is particularly noticeable on light-colored hair in the summer, and that of sailors, exposed to sun and sea-spray. Unfortunately this action by the sun also leads to “sensitization” of the fiber, which can become very fragile (56–62), particularly if the hair has already undergone other hair treatment (63).

Nonetheless, this phenomenon suggests a procedure whereby hair might be treated with an optical photosensitizing agent, then irradiated with light capable of activating the photosensitiser (64). Under the effect of the light, the photosensitizer could trigger a succession of chemical reactions culminating in a lightened or bleached effect. Indeed, this bears a resemblance to the classic lightening system with an unrinsed hydrogen peroxide solution.

More precise studies show that the keratin in hair does not absorb light in a wide range of wavelengths and in particular in the visible domain. Only melanin, responsible for the color of the hair, absorbs light in this domain. Consequently, if the hair is subjected to intense radiation with visible light, the energy absorbed by the melanin can:

- Be converted into heat;
- Destroy chemical bonds.

This second type of conversion only occurs if the energy received is greater than that of the chemical bonds. In the case of light, the energy is given by Planck's law: $E=hv=hc/\lambda$, where E is the energy, h the Planck constant, v the frequency, c the speed of light, and λ the wavelength. With a visible wavelength at 532 nm, the energy supplied exceeds 50 kcal/mol, which is greater than the bonding energy of melanin (ca. 40 kcal/mol).

The hope is therefore reasonable that we may be able to lighten hair with radiation in the visible region without damaging it, under the following three conditions:

- Emission strictly limited to the visible spectrum as keratin also absorbs in the infrared and ultraviolet ranges;

- Sufficiently high energy density to destroy the chemical bonds of melanin;
- The shortest possible duration of emission so as to avoid its conversion into heat (in fact 1 μ sec; at most, the thermal relaxation time of melanin).

This working hypothesis suggests the use of *pulsed light* sources, delivering a large amount of energy over a very short space of time, i.e. lasers. This has led to the application for various patents (65–68).

Nonetheless, at this time, non-concrete application of these novel procedures has yet emerged.

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8

Hair Coloring: Non-Oxidation Coloring

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1. INTRODUCTION

For centuries the use of hair coloring was restricted to the fashion needs of a privileged few. For a long time, necessity alone dictated its use in hiding white hair to give a more youthful appearance. Today, people in ever-greater numbers—women and men alike—change their hair color to make themselves more attractive. There are various reasons for this: to hide white hair, to lighten hair color or add an additional highlight, to remove the yellow look from gray hair, or to enhance the color of the natural gray, and so on.

From the technical standpoint, extensive laboratory and research work is essential because of the diverse problems to be solved and the large number of products already developed.

The indiscriminate use of certain colorants may result in technical drawbacks and raise dermatological or toxicological concern. It is well known that “hair dyes” may be associated with allergic reactions.

There are a number of factors that, taken as a whole, afford ample justification for in-depth specialized study. Among them are the market size; the diversity of requirements and products; the complexity of laboratory research; and the need to take in account the technical, dermatological, and toxicological aspects.

For the non-specialist, the complicated terminology employed by manufacturers, scientists, and hairdressers referring to various products can be bewildering. Even specialists themselves hesitate sometimes in selecting the right term. It is often difficult to perceive the difference between the “rinse,” “highlighter,” “color shampoo,” “dye,” “color intensifier,” “progressive coloring,” and so on. In this book a simplified nomenclature is adopted, taking into account both user convenience, and the chemical and technical characteristics of the products.

1.1. Temporary Coloring

The object is to attain a slight change in natural or modified hair color. This should last for several hours or days, or even longer, from one shampoo to the next. In this man-

[†]Deceased.

ner, the products improve or correct an existing dye shade, add a slight tint, or brighten up a natural shade. They can be really considered as *make up products for hair*.

1.2 Semipermanent Coloring

This is “direct” hair coloring, i.e., ready for use without prior mixing. It yields “semipermanent” results, in that it slowly and evenly fades away with cumulative shampoos. The products are useful in covering up the first white hairs, in heightening natural shades with highlights or in rendering gray hair more attractive.

1.3. Permanent Coloring

The object here is to produce a real change in the natural hair color, either by covering white hair, by imparting a lighter or darker color, or by first bleaching and then coloring hair. The color obtained is “permanent” (Chapter 9), but about every month the problem of 1 cm of new growth of hair is raised. The products used for permanent coloring are hair dyes and color shampoos, which contain oxidation dyes. (They require mixing with an oxidizing agent prior to use).

To make it clear, “tone-on-tone oxidation coloring” is dealt with in Chapter 9 since the same dye materials are used as for oxidation coloring but with a softer change of shade which lessens the issue of one monthly centimeter growth of hair.

2. HISTORICAL REVIEW

The most ancient documents and objects attesting to the use of coloring products were found in Egypt in the tombs of the Pharaohs. Alongside the perfumes and makeup products of the age, were found powders made from henna leaves. Women were already using it to paint their fingernails and dye their hair. We also know that the Egyptians used other vegetable extracts and metallic compounds to change their hair color. There is a story that one Egyptian dignitary, in order to restore his graying hair, had the blood of a black cow mixed with oil and then had the whole concoction boiled before application to his head!

It is now known that many other peoples who were contemporaries of the ancient Egyptians used the same dyeing methods. The use of henna to color finger-nails, hair, and the soles of the feet was widespread in India and Persia, and the Hebrews too were known to have made frequent use of it. Not only did women and children use henna but men as well, especially to dye their beards. For beards, the red hue produced by henna was deepened to a brown-black color by applying an indigo preparation.

In Greek tombs tinting products made from white lead and vermillion were found.

The Romans commonly used lead combs and dyes made from various lead compounds. It is said that they also made use of red and yellow colorants in the form of pastes made of talc and vegetable saps. “They blackened their eyebrows with soot or charred ant eggs; to dye their hair, they used a walnut strain and lead or copper acetate” (Torok). It is a known fact that the ladies of the Roman aristocracy had a penchant for blond hair; they bleached their hair or wore blond wigs (Chapter 7). According to Servius, one of the female slaves, called a *ciniflo*, assigned to the lady of the Roman household, was given the task of procuring and applying a powder (*cinis*), which gave the hair a light, ash-blond color. Virtually every Roman writer mentioned the fashionable use of hair coloring. Pliny alone cited more than 100 recipes, including the following: green

walnut hulls, black elderberries, cypros (henna), the charred dregs of vinegar, etc., and also “a crow’s egg beaten in a copper vase and a decoction of putrefied leeches, steeped for 60 days in a lead vase with black wine and vinegar.”

During the French Renaissance powder was held in high esteem, “powdered violets for brunettes and powdered iris for blondes.” Powder remained stuck to the hair only after the application of a mucilage. Consequently, many hair washes were necessary before a comb could be used on a sticky head of hair (Quicherat). The stylish Marguerite de Valois had her hair color changed constantly. She had it either bleached and then dyed or used a powder made from oak moss.

In the seventeenth century, many English women dyed their hair red in honor of Queen Elizabeth’s red-blond royal head. They soaked their hair in an alum solution followed by a rhubarb decoction.

Louis XV preferred white or very light ash-blond hair. During his reign and that of Louis XVI, powders once again became the fashion. In 1789, Boileau reported that 24 million pounds of starch were used to make hair powder every year. Poor girls could not afford starch and resorted to using the dust from decomposed wood.

Only at the end of the nineteenth (1883) century was it discovered that a number of synthetic substances—like paraphenylenediamine, discovered by Hofmann in 1863—already employed in dyeing animal fibers could also be used for coloring human hair.

From then on hair dyes progressed, but only very slowly, due to the fact that they were exclusively used to hide white hair. Things began to change after the mid-1920s. Oxidation dyes were greatly improved, and the barriers of preconceived ideas began to fall: the fashionable use of hair coloring boomed.

Since the end of World War II in 1945, many new colorants have been developed and added to the extensive range of existing hair dyes. It is not an exaggeration to say that hair coloring has become a necessity in today’s world.

3. VEGETABLE DYES

The use of vegetable dyes is a very ancient practice. The way they produce effects on hair vary and the lastness of result obtained depends on the product.

3.1. Henna

As already mentioned, the use of henna dates back thousands of years. In Arab countries it is still prevalent and is used to dye hair, nails, and the palms and soles. Henna—its Egyptian name is Khenna, Arab name is Al Khanna, and Indian name is Mendee—grows as a shrub, and only one species exists, *Lawsonia inermis*. It is a small and attractive tree with tapering branches, whitish bark, and bifid leaves of a pale-green color. The white flowers give off a sharp odor. It is cultivated mostly in India, Tunisia, Arabia, and Iran.

The leaves are dried and crushed into a greenish-yellow powder and then used as a hair colorant. Just before use the powdered leaves are mixed with hot water to produce a “henna pack,” a paste applied to the head and left on for 30–40 min before

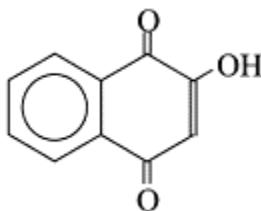


Figure 1 Lawsone.

rinsing. In Arab countries a towel is quite often wrapped around the head and the paste left to act all night in order to obtain very intense coloring effects.

When used on dark hair, henna produces an auburn color or, to be more precise, a brown-orange color highly valued in Moslem countries (it is said that Mohammed's beard was henna dyed). On white and light-colored hair, an unattractive "carrot" shade is produced.

Henna's active ingredient is 2-hydroxy-1,4-naphtoquinone, called lawsone (Fig. 1). It is thought that henna leaves contain about 1% lawsone as glucoside. They also contain tannin, which leaves the hair with a certain degree of stiffness.

Henna is occasionally used in Western countries, both by hairdressers and by women at home, but its popularity fluctuates mainly following the trends of the "back-to-the-earth" type. It is true that henna is a natural product and that generally it does not have any side effects, but it does have a number of major drawbacks: complicated preparation and application, unbecoming shades, the contrary effect of showing white hair off, hindrance to waving uptake, lack of compatibility with further hair treatments, etc. Henna has been virtually completely replaced by semi-permanent dye products.

Various studies have been devoted to henna and henna coloring (1–6).

3.2. Chamomile

The Roman Chamomile (*Anthemis nobilis*) cultivated in Western Europe and the United States, and the German chamomile (*Matricaria chamomilla*) grown in Germany and Hungary, yield flowers that can serve to tint hair. These flowers contain a yellow coloring substance, apigenin or 4',5,7-trihydroxyflavone (Fig. 2).

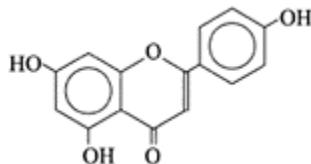


Figure 2 4',5,7-Trihydroxy flavone.

Chamomile can be used either as a hot-water decoction of the flowers, or as a paste like henna. In the latter case, the flower heads are crushed with kaolin into a fine powder and mixed with hot water just before use.

The yellow tint given to the keratin by repeated applications of the chamomile extract or paste leads to a faded brown color of the hair, thus producing a lightening effect.

3.3. Indigo

Indigo powder comes from plants of the indigofera family found in the West Indies and in South America; it yields a blue-green hue. Sometimes it is used in conjunction with henna to obtain shades ranging from chestnut to black.

3.4. Miscellaneous Extracts

Other extracts made from wood, bark, leaves, or flowers have been used in the past. Examples are walnut hulls and nutgall. Their use, like that of henna is disappearing. They cannot compete with modern direct dyes, which are much easier to apply, provide a much more varied shade potential and are more flexible to fit fashion changes.

4. METALLIZED DYES

Dyes containing metallic salts are as ancient as vegetable dyes, but they tend to disappear from the market. They are rarely used today except for what is known as “progressive” dyeing because daily applications result in some coloration of white hair.

These dyes are still rather popular with men for home use. Hairdressers no longer use them, because they are incompatible with perming and bleaching operations. There is still some doubt as to how they work. In general, it is thought that the hair shaft is colored by a deposit of metallic sulfide formed at the expense of keratin sulfur. There have also been exponents of the theory that metallic salts are a true “restorative” for natural hair shades, but those impressive-sounding arguments do not have any scientific basis. In addition, the progressive development of shades leads to the supposition that there is, simultaneously, a formation of insoluble sulfides at the expense of the keratin sulfur and of insoluble oxides from contact with oxygen in the air.

Metallized dyes contain, for the most part, lead and silver salts. Formerly, copper, nickel, bismuth, cobalt, and manganese salts were occasionally added to solutions to “vary” shades but it is no more the case for toxicological reasons. Lead salts are employed in the acetate or nitrate form. Sometimes, finely divided sulfur is added to solutions. Application should be made daily: slowly, white hair develops a grayish tint that darkens progressively until it reaches a final, rather dull black (leaden).

Silver nitrate has been used since the beginning of the nineteenth century to dye hair. It has been called: *eau d'Egypte*, *eau de Grèce*, *eau de Chine* (Egyptian, Greek, Chinese water). Its mode of action is dual, in the sense that the silver salts darken when exposed to light, and silver combines with protein yielding a dark-colored proteinate. The two forms available have been the progressive dye and the instant dye.

Progressive coloring is done by daily application of a silver nitrate solution. Instant coloring is done by applying two lotions successively. The first lotion is a

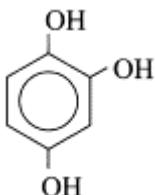


Figure 3 1,2,4-Trihydroxy benzene.

solution of 1,2,4-trihydroxybenzene (Fig 3.) and the second contains ammoniated silver nitrate. The instant coloring is probably obtained in a dual-effect system: the silver salt acts as the mordant for the trihydroxybenzene development, while the latter serves as the reducing agent for the silver salt. By varying the proportions of silver nitrate and trihydroxybenzene, it is possible to get darker or lighter shades. These types of product are still used to dye eyelashes and eyebrows.

Compounded hennas, now virtually abandoned, were available as early as 1907 when Schueller first proposed their use. They were based on the formulation of hair lacquers using phenol compounds such as pyrogallol, and nickel, iron, copper, cobalt, or lead salts in the presence of a reducing salt and a medium containing henna. These compounds produced a full range of colors and were quite popular in hair salons.

The disadvantages of hair dyes containing metallic salts are numerous. Most of them are toxic to some extent, and their use is strictly supervised. They nearly always create a dull, leaden color with a flat, metallic appearance. Moreover, the metal fixed on the hair shaft acts as a catalyst, causing an abrupt breakdown of the hydrogen peroxide in bleaching products or permanent wave fixers. This breakdown can result in rupture of the hair shaft and burns on the scalp.

5. TEMPORARY COLORING

These products are intended to effect a change, rapidly and simply, in natural or modified hair color. The change must be temporary. It should be easily brushed or washed off. At most, it should last until the next shampoo. The most accurate description of temporary hair dyes can be found in a slogan used from time to time, "a rinse brings it, and a rinse takes it away." A number of specialty products with multiple purposes answer this description. They are capable of the following:

1. Beautifying, "personalizing" or, sometimes, correcting a semipermanent or permanent hair dye color just produced. In hair salons, this is very common practice.
2. Restoring a slightly dull natural shade.
3. Bringing back the natural shade to hair lightened after exposure to air and light.
4. Eliminating the yellowish tint in white hair or adding a shade of gray.
5. Restoring color between two applications of semipermanent or permanent dyes.

6. Giving hair a new tone, a new color “just to see what it looks like.” In the latter case, light shaders might be used, or products with a pronounced coloring capacity that are able to go as far as to shade off white hair.
7. Correcting yellow or red off-shades after bleaching.
8. Creating “special effects” such as streaks of colored hair just for the evening.

5.1. Formulation

At first glance, the idea of formulating temporary hair dyes seems simple. The requirements appear to be few in number. It is not necessary to get the rich tones of semipermanent dyes, or the strong dyeing capacity of oxidation dyes. Nor is it necessary to obtain highlights or a cover-up for white hair lasting 4 or 5 weeks. The only real requirement is that the color fastness under sunlight is satisfactory between two shampoos and quite a few dyes seem to meet it.

In practice there are many obstacles. The dyes should possess a number of often contradictory characteristics:

1. Easy removal by shampooing.
2. Reduced affinity for altered keratin, so as to avoid patches on damaged hair shafts.
3. High affinity if strong tones are desired, a good cover-up of white hair. In this instance, the “keratin-dye complex” should be unstable enough to break apart during an ordinary shampoo, or even by strong brushing as for hair mascaras.
4. Sufficient resistance to friction to avoid loss into clothing or pillowcases. This is hard to accomplish with a particular type of transient hair dye product, namely, colored hair sprays or setting lotions or hair mascaras.
5. Color-fastness in rain or in contact with perspiration.
6. Non-selectivity in the case of dye mixes (almost always the case). As for semipermanent dyes, it is essential to strive to avoid the following:
 - a. Selectivity in affinity,
 - b. Selectivity in resistance to sunlight,
 - c. Selectivity when shampooing off.

Apart from these problems in choosing appropriate dyes, there are also difficulties in the formulation process itself. Temporary dye products often have a dual objective, and the dye function is not the main one. Colored setting lotions, for instance, contain primarily film-forming anionic polymers. It is therefore important to select dyes compatible with this type of coloring process and formulation, which is often not easy.

The predominant temporary hair dyestuffs are the following:

1. “Azo” derivatives (7);
2. Basic dyes of the triphenylmethane class such as methyl violet, sometimes in the form of their leuko-derivatives (8,9);
3. “Azine” derivatives like safranine;
4. Indoamines or indophenols (10–17).

Semipermanent dyes are sometimes used: nitro derivatives, anthraquinone derivatives, etc. (Sec. 6.2) at concentrations weak enough to avoid excessive duration and overintense shades.

Hair mascaras also contain pigments, pearlescent ingredients and cosmetic colorants. The use of colored polymers has also been reported (18,19). Melanin produced by chemical synthesis has been further patented (20–22).

5.2. Types of Product

There are a number of technical forms for this kind of product. The list below, which is not complete, includes products that have proved their worth at various times.

- Capsules or sachets of powder to be diluted;
- Aqueous solutions, several drops of which are diluted at use;
- Foaming or non-foaming lotions for after-shampoo application;
- Shampoos containing temporary dyes;
- Shading strengtheners (setting and dressing);
- Colored hair sprays;
- Mascaras.

1. *The powders to be diluted* are simply dye mixes in the presence of an organic acid. It is a good product, easy to prepare and apply. Preservation of the dyes is satisfactory, provided the package is airtight. However, today's user no longer turns to this product, preferring ready-to-use products that have other advantages in that they leave the hair soft and combable. These effects are difficult to obtain from powders.

2. Professional hairdressers show a marked preference for the *concentrated aqueous solution to be diluted in warm water*. With experience and the right selection and dosage of appropriate dye mixtures, they can vary the shades obtained with permanent dyes without limitation. The formulation is relatively simple once the choice of dyestuffs has been made, as it is an aqueous acid solution. The use of basic dyes in their leuko-derivative form, which are oxidizable by atmospheric oxygen at the time of application, have sometimes lead to very good results regarding evenness of color.

3. *Coloring conditioners*, applied after shampooing without subsequent rinsing, are generally dispersions of cationic surfactants, or cationic polymers containing basic dyes. Or they may be “acid dye-cationic surfactant” complexes redissolved by a solvent or a cationic surfactant.

4. *Shampoos* contain temporary colorants in an anionic, amphoteric, or cationic medium, the dyes being selected as a function of polar compatibility with the main surfactant. The formulation is rather difficult, as the shampoo medium is generally an excellent dye peptizer, thereby causing a considerable reduction in dye affinity for the hair fiber.

5. *Shading strengtheners* are an interesting application of temporary hair coloring. These lotions, containing plasticizers, are hydroalcoholic solutions of polymers or copolymers, such as polyvinylpyrrolidone, or the copolymer of vinyl acetate and crotonic acid. They are applied on wet hair, after the shampoo and immediately before hairstyling. After drying, each hair is surrounded by a sheath of a transparent or translucent film; this film is considered very important in giving good hair keratin affinity. If the lotions contain dyes of low affinity, but soluble in the polymer film, the result will be coloring as durable

as the film; it will last, at best, until the next shampoo. Hair porosity is not a factor, and these products can be applied even to damaged hair without the risk of increasing selectivity in the most bleached areas. These shading strengtheners containing plasticizers to a greater or lesser extent, can be formulated—like conditioning lotions—as an “aerosol foam,” easy to apply because foam does not drip. The formulation of this kind of coloring product is more difficult when anionic and cationic polymer combinations, cationic and non-ionic polymer combinations, cationics or cationic polymers alone, are used instead of only anionic polymers.

6. *Colored hair sprays.* To formulate a colored hair spray, a dye and a filmforming polymer are juxtaposed in an aerosol spray unit identical to that of a hair spray. As in the case of above setting and dressing lotions or strengtheners, the film obtained is colored. But application on dry hair with a spray unit gives much less uniform results. It is hard to prevent color stains and leaking onto clothes, pillow-cases, etc. In fact, colored hair sprays have never been considered satisfactory from a technical standpoint. They are, however, one way of giving a quick highlight, an instant touch of color to hair that has been styled.

7. *Hair mascaras* are formulated in a similar way as mascaras for eyelashes. They are built with the same pigments, coloring and pearlescent agents, and should comply with similar constraints. As presented, they make it easy to create streaks of various shades in the moment. Such special effects are designed to last for the time of the evening and are swept off by brushing hair.

6. SEMIPERMANENT OR DIRECT COLORING

The category of semipermanent dyes includes any product capable of effecting to some extent a change in the natural hair color that fades progressively with cumulative shampoos.

This kind of product must be ready-to-use and easy to apply. They are usually intended for application on wet, freshly washed hair, and subsequent rinsing. They are sometimes called direct coloring products: the dyestuffs are “direct,” technically speaking; they directly impart to hair their coloring potency as opposed to oxidation dyes that “develop” when mixed with hydrogen peroxide just before use.

Semipermanent dyes are intended generally to fulfill three expectations:

1. *Add various tones* to natural hair color: golden, red, auburn, purple-blue, ash, the so-called “modish” highlights (*reflets fantaisie*).
2. *Dye white hair a natural shade* by restoring the original, natural hair shade. They can be applied to hair containing up to 30% white hair. In this manner, natural shade can be restored without difficulty to the entire head of hair. This is a “direct tone-on-tone” coloring.
3. *Rid gray or white hair of unseemly yellowish tones* and give them the desired level of gray: either a neutral shade, or with a slight slate-colored, lilac, or bluish tone. These are “gray-tone” products.

Semipermanent dyes are normally formulated for application on natural, non-bleached hair. Some products, however, have been developed to:

- brighten up dye shades between applications;
- shade warm highlights produced by bleaching;
- bring a pastel shade to previously bleached hair.

6.1. Formulation Requirements

As already mentioned, semipermanent dyes, or toning products, are simple and easy to use. Even the amateur home user should experience no technical difficulties in using these products.

Developing an easy-to-apply product always raises a number of obstacles. It is not surprising, therefore, that the list of formulation requirements is lengthy. The major points are included in this section.

6.1.1. Simplicity and Safety

Semipermanent dyes must be simple and easy to use. As well they must present optimal safety in use which could well lead to the exclusion from product formulation of dyes subject to doubt or insufficient skin tolerance.

6.1.2. Affinity for Hair Keratin

The semipermanent products should normally be used at room temperature. However in order to increase dyestuffs diffusion into hair, pausing under a cap or even hairdryer in hairdresser's salon may be used. The pause is rather short: usually, 5–15min will suffice. The implication of so short time to perform the objective is to select dye molecules of small size, with a molecular structure ensuring adequate hair affinity (8,9). It is easy to see why the smallest dye molecules are useful. This is the case with the "nitro" dyes (23,24). If the molecules are larger (naphthalene derivatives, anthraquinone derivatives, methinic derivatives, azo derivatives, etc.) steps must be taken to ensure adequate penetration using solvents, swelling agents, anion-cation complexes, etc.

A number of reports have been published which deal with diffusion and distribution of direct dyes into hair (1,3–6,8,9,23–28).

6.1.3. Physical and Chemical Fastness

What we are referring to here is the fastness of the color of the dyed hair. It should be extremely fast to sunlight, atmospheric oxygen, friction, and hair washing. However, while retaining the same tone, the dye must gradually weaken to make way for successive applications without risk of excess coloring on the shafts and ends and without taking any particular technical precautions.

A semipermanent dye is generally ranked as satisfactory if the shade completely fades after six shampoos, but some products may delay fading until 12 shampoos.

6.1.4. Compatibility with Other Treatments

The application of a shampoo, setting lotion, or permanent wave lotion should not result in unforeseen color alterations. This is why it is wise to avoid dyes that act like acid-base pH indicators. It is preferable to avoid certain azo dyes, which due to the chemical reduction brought about by permanent wave lotions, can yield new compounds producing undesirable hair tints. In the past certain azo derivatives produced a lovely gray tone in white hair. Unfortunately, during chemical reduction by a permanent wave product, they turned the hair a very bright and, alas, exceeding stable pinkish color.

6.1.5. *Absence of Selectivity*

This is a considerable hurdle that must be taken when working with dye mixes, in other words, most of the time. Application is made onto a substrate reacting to dyes in very heterogeneous fashion. In particular, hair ends damaged by previous treatments, e.g., perming or by air, sun, and so on, will be much more receptive than the roots or shafts. The hair ends can be "choosy" and selective, absorbing some dyes preferentially and rejecting others, while the shafts behave differently.

This phenomenon of selectivity has several aspects to watch out for:

1. *Selectivity in affinity*, which might be responsible for different colorings on different portions of the same, individual hair, and between hairs.
2. *Selectivity in the way the colorants hold*, leaving, after several days, unforeseen shades.
3. *Selectivity in chemical behavior* vis-à-vis permanent wave lotions: certain dyes can thus be destroyed or changed by the reducing action of thioglycollates, whereas others are able to resist.

A simple case illustrating possible disadvantages is the following: a chestnut shade may be obtained by mixing a small-sized nitro dye like 1,4-diamino-2-nitrobenzene (2-nitro-*p*-phenylenediamine), red-orange, with a basic dye of large size, such as malachite green. If the hair ends have been damaged, they may selectively absorb the green dye, while the roots will absorb the chestnut color produced by the mixture. But if the entire head of hair takes on a homogeneous tint, the unstable basic dye shade will inevitably fade at a rapid rate under the action of light. The highlight will then deepen, and the process will probably produce uneven results along the hair shaft. Unfamiliarity with this crucial information has led to serious failures.

In order to avoid selectivity in its various manifestations, efforts should be directed toward using dyes of the same chemical class (29). Even this is not enough however: their size and basic qualities should also be very nearly identical, so that their affinity for hair and chemical behavior will be of the same order.

Unfortunately, it is often necessary to use dyes belonging to different chemical classes. Consequently, one can expect to meet the shortcomings described previously. Laboratory tests cannot predict all eventualities, and only application to a large number of heads in all possible technical conditions can give the information required in estimating possible mishaps.

6.1.6. Stability in Solution

This is included as a reminder; but the chemist concerned with product development will regard this as of primary importance.

6.2. Possible Chemical and Physicochemical Systems

The development of a simple product is a complicated process. In-depth study of systems has been carried out in response to the great variety of products and the multiplicity of their possible applications. Based on our own experience, existing products, literature and patents, the main ways of formulating that have been considered and explored are described in the following. Even though the chemical classes mentioned may offer a sufficient palette to build a homogeneous product, there are often several families of dyes used within a single formula (30).

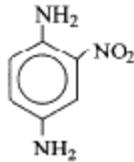
6.2.1. "Nitro" Dyes

These include aromatic amines, aminophenols, and nitrophenols. Adding one or more nitro groups to the benzene ring imparts a dyeing potential even in the absence of oxidant. These dyes have a remarkable affinity for hair keratin. Simultaneously, these added substituents on the ring generally eliminate the possibility of oxidative condensation. The "nitro" derivatives are not, as a general rule, oxidation dyes but true powerful, direct dyes.

This class features a great number of yellow, orange, and red dyes (31–40). Multiple substitutions, performed in particular on the amino groups attached to the ring, yielded a variety of pink, purple-violet, violet, blue colors. Cosmetic chemists have a wealth of shades at their fingertips, providing an impressive formulation potential.

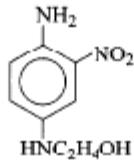
Substituting the hydrogen atoms of the amino groups by groups such as the following (Fig. 4) results in a deepening of the shades (bathochromic effect, i.e., shift toward shorter wavelengths) (41,42).

This is how the orange-red, for example, of the 1,4 diamino-2-nitrobenzene (a) becomes purple-red (b) and even violet-blue (c):



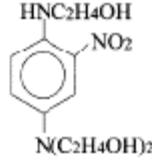
1,4-Diamino-2-nitro benzene

Orange-Red
(a)



1-Amino-2-nitro-4-β-hydroxyethylamino benzene

Purple-Red
(b)



1-β-Hydroxyethylamino-2-nitro-4-bis(β-hydroxyethylamino)benzene

Violet-blue
(c)

This deepening of colour is obtained at the expense of increased molecular weight which is often accompanied by a decrease in keratin affinity. As a result, significant differences may be observed between root and sensitized or damaged tip regarding dyeing strength.

But certain groups increase solubility (43). The decrease in keratin affinity can therefore be compensated for by using a higher concentration. The basic or acid character of the groups introduced also modifies the tinting characteristics and fibre affinity according to the pH at use.

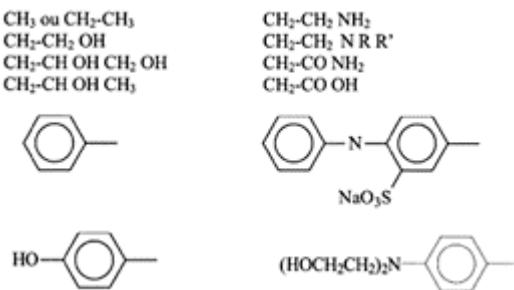


Figure 4 Potential linkages to amino groups.

The following classification gives a proposed nomenclature for some of the main dyes in use:

Nitro derivatives of 1,4-diaminobenzene (Fig. 5; Table 1,5a-k)

1,4-Diamino-2-nitrobenzene	Orange-red
1-Amino-2-nitro-4- β -hydroxyethylamino-benzene (HC Red 7)	Purple-red
1-Amino-2-nitro-4-bis-(β -hydroxyethyl)-amino-benzene (HC Red 13)	Red-violet
1,4-bis-(β -Hydroxyethyl)amino-2-nitro-benzene	Violet
1- β -Hydroxyethylamino-2-nitro-4-bis-(β -hydroxyethyl) amino-benzene (HC Blue 2)	Violet-blue
1- β -Hydroxyethylamino-2-nitro-4-amino-benzene (HC Red 3)	Purple-red
1-Amino-3-methyl-4- β -hydroxyethylamino-6-nitro-benzene (HC Violet 1)	Purple-red
1-Amino-2-nitro-4- β -hydroxyethylamino-5-chloro-benzene	Purple-red
1-Amino-3-methyl-4- β -aminoethylamino-6-nitro-benzene	Red
1- β -Hydroxyethylamino-2-nitro-4-(ethyl, β -hydroxyethyl) amino benzene (HC Blue 12)	Blue-violet
1- γ -Hydroxypropylamino-2-nitro-4-bis (β -hydroxyethyl) aminobenzène (HC Violet 2)	Violet-blue

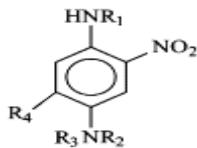


Figure 5 Nitro derivatives of 1,4-Diaminobenzene.

Table 1 (5a-k)

R ₁	R ₂	R ₃	R ₄	
a. H	H	H	H	Orange-red
b. H	H	CH ₂ -CH ₂ OH	H	Purple-red
c. H	CH ₂ -CH ₂ OH	CH ₂ -CH ₂ OH	H	Red-violet
d. CH ₂ -CH ₂ OH	H	CH ₂ -CH ₂ OH	H	Red-violet
e. CH ₂ -CH ₂ OH	H	H	H	Purple-red
f. H	H	CH ₂ -CH ₂ OH	CH ₃	Purple-red
g. H	H	CH ₂ -CH ₂ OH	Cl	Orange-red
h. H	H	CH ₂ -CH ₂ NH ₂	CH ₃	Red
i. CH ₂ -CH ₂ OH	CH ₂ -CH ₂ OH	CH ₂ -CH ₂ OH	H	Violet-blue
j. CH ₂ -CH ₂ OH	CH ₂ -CH ₃	CH ₂ -CH ₂ OH	H	Blue-violet
k. CH ₂ -CH ₂ -CH ₂ OH	CH ₂ -CH ₂ OH	CH ₂ -CH ₂ OH	H	Violet-blue

Nitro derivatives of 1,2-diaminobenzene (Fig. 6; Table 2, 6a–b and Fig. 6 bis; Table 2 bis, 6c)

1,2-Diamino 4-nitro benzene Yellow-Orange

1-Amino 2-β-hydroxyethylamino 5-nitro benzene (HC Yellow 5) Orange-Yellow

1,2,3,4-Tetrahydro-6-nitrochinoxaline (44) Orange-Yellow

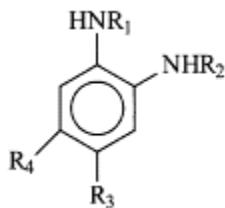
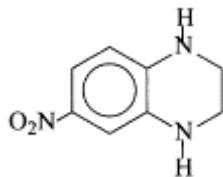


Figure 6 Nitro derivatives of 1,2-Diaminobenzene.

Table 2 (6a–b)

	R ₁	R ₂	R ₃	R ₄	
a.	H	H	NO ₂	H	Yellow-orange
b.	H	CH ₂ -CH ₂ OH	H	NO ₂	Orange-yellow

A link between the nitrogen in position 1 and the nitrogen in position 2 by a saturated bridge may be of interest.

**Figure 6 bis** 1,2,3,4-Tetrahydro-6-nitro quinoxaline.**Table 2 bis (6c)**

	R _{1,2}	R ₃	R ₄	
c.	CH ₂ -CH ₂	NO ₂	H	Yellow-orange

Nitro derivatives of aminophenols (Fig. 7; Table 3, 7a–f)

1-Hydroxy 2-amino 4,6-dinitrobenzene	Orange
1-Hydroxy 3-nitro 4-aminobenzene	Orange
1-Hydroxy 3-nitro 4-β-hydroxyethylaminobenzene	Red
1-Hydroxy 2-amino 3-nitrobenzene	Yellow-orange
1-Hydroxy 2-β-hydroxyethylamino 5-nitrobenzene (HC Yellow 11)	Yellow
1-Hydroxy 2-amino 4-nitro 6-chlorobenzene	Red-orange

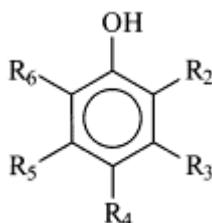
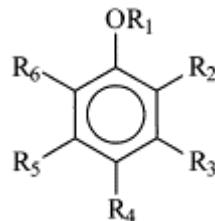
**Figure 7** Nitro amino phenols.

Table 3 (7a-f)

R ₂	R ₃	R ₄	R ₅	R ₆	
a. NH ₂	H	NO ₂	H	NO ₂	Orange
b. H	NO ₂	NH ₂	H	H	Orange
c. H	NO ₂	NH-CH ₂ -CH ₂ OH	H	H	Red
d. NH ₂	NO ₂	H	H	H	Yellow-orange
e. NH-CH ₂ -CH ₂ OH	H	H	NO ₂	H	Yellow
f. NH ₂	H	NO ₂	H	Cl	Red-orange

Nitro derivatives of aminophenyl ethers Fig. 8; Table 4, 8a-c)

1-β-Hydroxyethoxy-2-β-Hydroxyethylamino-5-nitrobenzene (HC Yellow 4)	Yellow-green
1-Methoxy-2-β-hydroxyethylamino-5-nitrobenzene	Yellow-green
1-β-Hydroxyethoxy-3-methylamino-4-nitrobenzene	Yellow-green
1-β-Aminoethylamino-4-β-hydroxyethoxy-2-nitrobenzene	Orange
1-Methoxy-3-β-aminoethylamino-4-nitrobenzene	Yellow-green

**Figure 8** Nitro aminophenylethers.**Table 4** (8a-e)

R ₁	R ₂	R ₃	R ₄	R ₅	
a. CH ₂ -CH ₂ OH	NH-CH ₂ -CH ₂ OH	H	H	NO ₂	Yellow-green
b. CH ₃	NH-CH ₂ -CH ₂ OH	H	H	NO ₂	Yellow-green
c. CH ₂ -CH ₂ OH	H	NH-CH ₃	NO ₂	H	Yellow-green
d. CH ₂ -CH ₂ OH	H	NO ₂	NH-CH ₂ -CH ₂ -NH ₂	H	Orange
e. CH ₃	H	NH-CH ₂ -CH ₂ OH	NO ₂	H	Yellow-green

Similar types of dyestuff are conceivable with pyridine ring instead of benzene ring. Various examples have been reported but very few applications.

6.2.2. Solvent-Assisted Dyeing Systems

The use of suitable organic solvents is the adaptation to semipermanent hair coloring of techniques studied for textile-fiber dyeing. Solvents such as glycol ethers, cyclohexanol, furfuryl alcohol, benzyl alcohol, and phenethyl alcohol increase the affinity for hair keratin of dyes with low uptake under normal conditions of use (45–47). These solvents can be miscible in water in any proportion, like butyl ether of ethylene glycol (butylcellosolve) or have a relatively low solubility, like benzyl alcohol or the hexyl ether of ethylene glycol. These solvents work at optimum concentrations and when they are poorly miscible with water, the optimal level can be the limit of solubility.

It is not well understood how these solvents work, but it is generally accepted that they facilitate the absorption of dyes onto the surface of the hair fiber. The accelerating effect of the solvents seems to be closely related to their dielectric constant. Although it is unwise to assume that the efficiency of the solvent increases as the constant decreases, observation shows that promising results are only obtained through the use of solvents with a dielectric constant lower than 15 (at 25°C). When semipermanent coloring is carried out under acid conditions, the use of polar solvents which acts strongly on proteins but not on lipids, such as propylene carbonate, allows to reduce the possible occurrence of stains on scalp while keeping with an optimal coloring potency (48).

The use of this kind of solvents made it possible to effect direct dyeing with a number of dyes whose solubility characteristics and chemical structure at first glance, might seem inadequate. Such are the disperse dyes and metallic dyes.

The *disperse dyes* are not readily soluble in water. They are reduced to extremely fine particles and maintained in suspension by a dispersing agent until absorption by the fiber takes place. In this class are a large number of azo and anthraquinone dyes; for example CI 11220 Disperse Red 17 (Fig. 9) and CI61100 Disperse Violet 1 (Fig. 10).

The *metallic dyes* are of interest as well. These are metallic complexes in which all the coordination numbers of the metal are saturated. The characteristic properties of the metallic ion then disappear. The compound (Fig. 11) is representative of such metal complexes.

These dyes are of some interest for hair dyeing only if they are used with solvents selected with the utmost care, which will ensure good affinity at room temperature.

They are derived from azo dyes, which means that their color possesses the richness of the azo dye range. But introducing the metal atom “flattens” the shades,

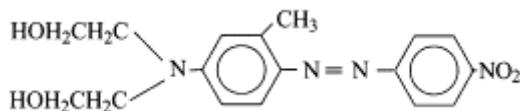
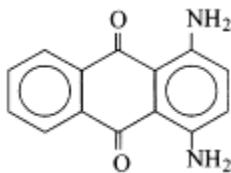
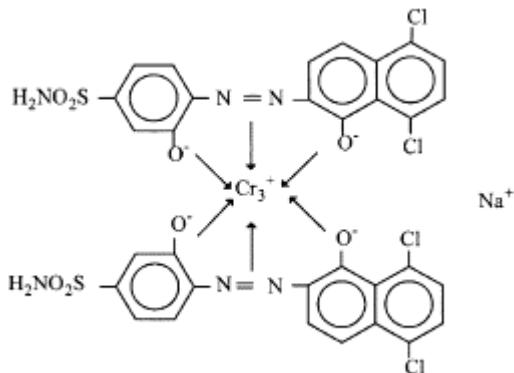


Figure 9 CI 11210 Disperse Red 17.

**Figure 10** CI 61100 Disperse Violet 1.**Figure 11** CI Acid Blue 170.

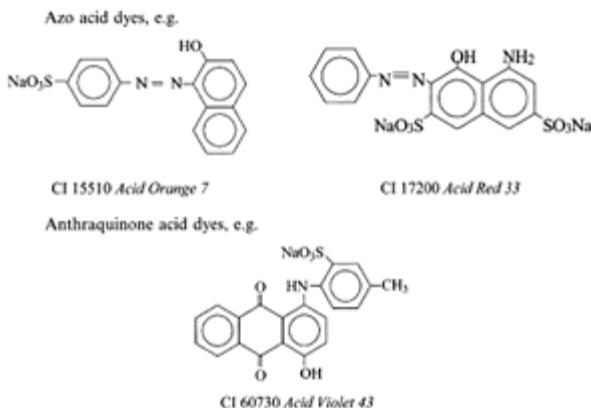
makes them more stable. This last feature is of special importance in the case of hair coloring. Moreover the N=N (azo) group is being in a way blocked, these dyes are very stable in solution and highly stable to sunlight. In such conditions, metallic dyes are particularly suitable for the development of gray shades.

6.2.3. Anion-Cation Complexes

The advantages of cationic agents have always been attractive in relation to affinity, as a possible means of facilitating dye adsorption onto the keratin fiber. A two-step operation has actually been proposed: first, treatment of the hair by a cationic surfactant, followed by application of an acid dye solution. But this kind of operation is too complicated and as a result was unsuccessful.

However, the properties of a great many acid dyes can be considerably modified by forming a complex with a cationic surfactant. If the proportions are stoichiometric, an anion-cation complex is obtained, which acts as a new dyestuff, but is generally insoluble. It is therefore essential to disperse it or solubilize it using a surfactant most often a non-ionic one. The anion-cation complex put in solution can entail much better affinity for hair keratin than the initial acid dye.

The dyestuffs especially well suited to this sort of formulation are as mentioned, the acid dyes, that is to say, those having essentially the function SO₃H or COOH in their molecular structure. The following dyes are most used.



Some azinic dyes, such as Indulines, e.g. CI Acid Blue 20 or Nigrosines, e.g. CI Acid Black 2 have met with some success. However, they are hardly used nowadays.

The cationic surfactants used are of the quaternary (ammonium, pyridinium, quinolinium) or tertiary amine-type. The non-ionic surfactants used in redissolving the stoichiometric anion-cation complex include fatty ethoxylated or polyglycerolated alcohols. They can be associated with or replaced by suitable solvents (butylcarbitol, etc.). If the cationic surfactant is itself sufficiently ethoxylated, it can fulfil the double function of cationic complexing agent and solvent for the anioncation complex.

The formulation of anion-cation dye complexes is always difficult, the complex must be *precisely*:

- equimolecular for maximum affinity,
- redissolved by the non-polar solvent system.

If the amount of the latter is insufficient, the anion-cation complex “sticks” to the fiber, thus yielding opaque, very irregular patches that are not resistant to friction. If the amount is excessive, affinity decreases considerably; the partition coefficient of the anion-cation dye favors solubility at the expense of the hair shaft.

However the anion-cation complexes are of especial note, because they give acid colorants with low affinity a significantly increased uptake by hair keratin. This is particularly useful for the development of semipermanent gray rinses to be applied on white hair. It is a fact that the different forms of selectivity are very evident in white hair, and the best way to avoid them is to avoid dye mixes when formulating a suitable product. But homogenous grays are rare. There are some among the metallic dyes; their affinity can be increased using the solvent system. Others can be found in the series of azinic dyes like the Induline or Nigrosine dyes but as said before they have gradually fallen in use.

Anion-cation complexes offer a second advantage in that they may produce even coloring of the fiber, which is relatively rarely achieved. Yet, inherent in their use lies a disadvantage: they work by adsorption, i.e., by a surface phenomenon. This casts doubt on their fastness. It relies on the strength of the initial acid dye, and it is clear that the anion-cation system also helps in the formulation of temporary dye products (see Sec. 5).

Promising results have also been obtained using cationic dye/anionic surfactant, or anionic dye/cationic dye combinations. In practice, however, the former has proved the most useful, if only because it retains the properties contributed by the cationic surfactant: easy disentangling, hair softness, etc.

6.2.4. Self-Oxidizing Dyes

Numerous aromatic amine or aminophenol derivatives, are capable of self-oxidation in contact with atmospheric oxygen, and thus yield colored polymers and dyes. But in most cases, oxidation by air contact leads to unstable, intermediate compounds: the oxidizing polymerization continues and, for that reason, the hair shade will continue to evolve, sometimes with dramatic end-results. Triaminobenzene does this, rapidly producing a nice black-blue color on a head of hair at room temperature. It is so unstable, however, that after several days a red shade emerges. A similar though less eye-shocking result occurs with *m*-diaminophenol: in contact with atmospheric oxygen the original light-chestnut shade will turn to chestnut-auburn after several days. Stable shades can only develop after intense oxidation, which explains why its correct place is in the list of oxidation dyes.

There are, however, a number of polyphenols or aminophenols derivatives that yield, after rapid oxidation, interesting and stable shades that develop on the hair at room temperature. The major ones are derivatives of trihydroxy-benzene (49), amino-hydroquinone, or 2,4-diaminophenol.

The products are of interest because it is possible by mixing to obtain rather natural tones with fair cover-up intensity. On the whole, they behave as intermediates between true (nitro) direct dyes and oxidation coloring. They provide products simple and easy to apply, of adequate intensity to conceal white hair in a satisfactory manner. These products have been successful in the third quarter of the twentieth century and even in the early 1980s. However, they have since given way to tone on tone oxidation hair dyes which are dealt with in the following chapter.

But the goal coveted for a long time by chemists was to be able to reproduce the natural process of hair pigmentation which means re-create natural pigment within gray hair. A number of attempts have been made to induce biosynthesis from the primary aminoacids (tyrosine, dopa) involved in melanin pathway using biological oxidation catalysts (tyrosinase, peroxidase, phenolase). One of the key steps in melanin biosynthesis is 5,6-dihydroxy indole (DHI) (Fig. 12).

This highly oxidizable intermediate ultimately generates a brown pigment of eumelanin type. Many studies were devoted to control DHI production. Many

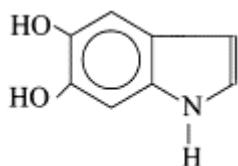
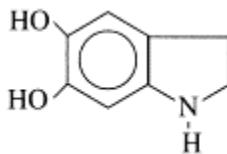


Figure 12 5,6-Dihydroxy Indole.

**Figure 13** 5,6-Dihydroxy Indoline.

chemical processes were patented as well as appropriate ways to keep it stable and operating procedures to ensure and enhance its uptake by hair, in order to get *in situ* oxidative polymerization toward brown pigment production. Both acid (50) and basic (51) conditions were suggested with or without adding oxidant. Two-step operations were also patented whereby either DHI application was followed by pigment development using an oxidation catalyst such as cupric chloride (51), or prior “sensitization” of hair using a metal salt before applying DHI (52).

Studies (53) further focused on a close derivative of DHI, i.e. dihydroxyindoline (Fig. 13) which also leads to the production of a brown melanin-like pigment.

Both DHI- and dihydroxyindoline-based products were marketed. In both cases, the oxidant was oxygen from air and the natural shade was obtained by cumulative close together applications. It should be noted, however, that these products can only give natural shades, they are unable to generate a variety of highlights.

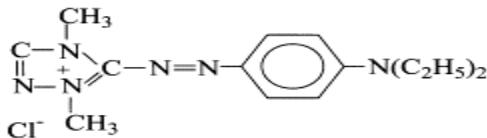
Another point to be mentioned is that self-oxidizing dyes are by nature highly sensitive to oxidation and as a result difficult to stabilize in aqueous medium which makes it difficult to develop a product.

6.2.5. Cationic or “Basic” Dyes

Cationic derivatives have a special affinity to hair all the more when it has been previously “sensitized” by oxygen from air or sunlight together with moisture, or oxidizing cosmetic treatments which gives its surface a more or less strong anionic character.

The same goes for dyestuffs. For a long time, cosmetic chemists have used such ingredients as a support with a view to correct highlights and also as major component of dyeing products.

Ideally the cationic group is part of dyestuff. It provides vivid, strong dyes with a high affinity to hair. Albeit the utmost beautiful cationic derivatives of triphenylmethane or phenylphenazinium have almost been given up due to high weakness under daylight, other dyes remain of interest (e.g. Fig., 14).

**Figure 14** CI 11055 Basic Red 22.

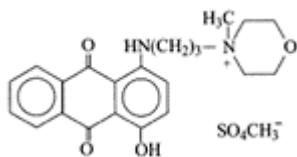


Figure 15 Cationic anthraquinonoid dye.

Cationic groups may also be coupled with the structure of a dyestuff. Anthraquinone derivative is given as an example (Fig. 15) (54).

Azo dyes or quinone-imine derivatives having a quaternary ammonium group may also be used. Among them, the best known dyestuffs belong to the Arianors family. A fairly diverse color range allows for homogeneous formulation in all shades. (for examples see Fig. 16).

For certain uses, however, their brightness and fastness may be inadequate.

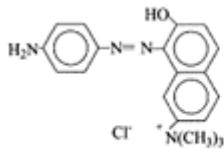
6.2.6. “Reactive Dyes” and “Dyeing Reactions”

Developed for cellulose or wool fiber dyeing, “reactive” dyes contain a group capable of reacting with the alcohol groups of the fiber to yield covalent bonds between dye and fiber (55–57). The possible reactive groups are numerous. Among the more frequently mentioned in patents are the following:

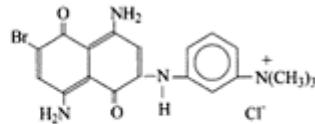
- Dichloro-triazinyl group (the most reactive, because only traces of water are sufficient to initiate hydrolysis);
- The chloro-amino-triazinyl group;
- Chloropyrimidine derivatives;
- Vinylsulfones, $-\text{SO}_2\text{-CH=CH}_2$;
- Acrylamides, $-\text{NHCO-CH=CH}_2$ and others;
- Isothiouroniums (58).

These reactive groups may be linked to various nitro, anthraquinone, metallic, phthalocyanine, acid “azo” dyes, etc.

Despite a real affinity to keratin and a very good fastness, these “reactive dyes” have still not found any application in the dyeing of hair. The only “reactive dye”



CI 12250 Basic Brown 16



CI 56059 Basic Blue 99

Figure 16 Arianor dyes.

actually used is lawsone from henna which is thought by several authors to covalently bind to hair keratin (1,3,5).

From the mid-1980s, many patents have described reactions whereby dyes were obtained without using an oxidant (59–62). They involved a carbonyl group reacting with one of the following:

Amino-acid or oligopeptide;

Primary or secondary amine;

Phenol derivative;

Anilin derivative;

Amino sugar, etc.

The key step for dye generation can be summed up by the following reaction:



As a hair contains a number of NH_2 groups, the latter can be involved in the above reaction or otherwise induce a similar reaction in the presence of carbonyl group of an aldehyde ($\text{R}_1\text{HC}=\text{O}$), as published in a patent which describes the combination of indoles with aldehydes (63). Other patents mention the use of imine derivatives to react with amino groups (64):



It cannot be excluded that such “coloring reactions” eventually find applications in hair coloring but provided their safety is adequately supported and scalp staining properly mastered.

6.3. Practical Developments of Direct Dye Products

As mentioned previously, direct dye products must help in producing three types of result:

1. “Fancy” highlights to blend with the natural color of the hair: golden, ashblond, red, light auburn, dark auburn, purple-violet, etc.
2. “Tone-on-tone” coloring to cover up white hair by tinting it to the natural hair color: blond, light chestnut, chestnut, brown, black, etc.
3. Gray highlights designed to rid white hair of yellowish hues and give a gray shade.

Besides these three major types of result, it is possible to brighten up permanently (oxidation coloring) dyed hair shades and to give semipermanent pastel shades to previously bleached hair (65).

“Fancy” highlights are generally obtained by using “nitro” dyes or cationic direct dyes, especially for the warm shades. The use of “nitro” pyridine dyes was also put forward and quinone derivatives related to natural dyes as well (66,67).

The “tone-on-tone” coloring can also be done with “nitro” dyes, as the available dye range goes from yellow to blue. Yet, it is often necessary to deepen the shades by combining with additional dyes belonging to other chemical classes, such as the

anthraquinone or azo dyes. To improve shade evenness or fastness under repeated shampoos applied to hair previously "sensitized" by bleaching or perming, more or less significant amounts of basic dyes, possibly with nitro group (68) or cationic dyes such as of "Arianor" type may be added. Self-oxidizing dyes can also be considered.

Grays can be obtained with anthraquinone, metallic, and acid "azo" dyes. To formulate them, it is often appropriate to use anion-cation complexes.

The semipermanent dyes available to professional hairdressers or directly to the consumer for home use are often products to be applied to the wet hair after shampooing and rinsed out carefully after waiting 10–30 min. They are available in all kinds of presentation: disentangling or conditioning lotions, gels, creams or mousses. Users find the pressurized unit very convenient; it produces the product as a creamy foam (aerosol foam). This means that it is non-drip and easy to apply all through the hair. Semipermanent dyes are sometimes available in shampoo form. The whole process including shampoo, waiting period, and dyeing is thus combined into a single operation. In all cases, the formulation presents numerous difficulties (69–71).

Formulation requirements imply very strict criteria for dye selection. The remaining problem is achieving perfect compatibility in all respects between the dyes and the various elements of product composition.

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9

Oxidation Coloring

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The so-called “oxidation” dyes are the only dyes capable of giving permanent hair color in an infinite variety of shades and a perfect coverage of white hair. The formulation of almost all permanent hair dye products uses oxidation dyes. In this class are all the dyes and color shampoos used in hair salons or for home use, offering the following possibilities:

- Cover-up of white hair in a variety of shades, in particular, the natural shades,
- Simultaneous bleaching and dyeing to obtain intense highlights,
- Shading after lightening in all possible tones,
- Sufficient durability so that the user only requires one application a month.

Nowadays the permanent dyes are by far the most frequently used hair coloring products and hold the dominant share of the market.

1. OXIDATION DYES

The term “oxidation dyes” is not entirely accurate. In chemistry, what are known as colorants are colored compounds capable of producing, under determined conditions, visible coloring in certain materials. The substances used as permanent hair dyes do not answer this description. In fact, most of the time these compounds are colorless or faintly colored. It would be more precise to regard them as intermediates rather than colorants. But, when mixed with oxidizing products just before use, these intermediates can produce colored compounds or colorants through a process of oxidative condensation.

In general, they are aromatic ring derivatives belonging to three major chemical families: the diamines, aminophenols, and phenols (or naphthols).

The oxidation dyes most currently used are in the following classes:

Diamines

B₁ 1,4-Diamino-benzene (*p*-PHENYLENEDIAMINE)

B₂ 2-Methyl-1,4-diamino-benzene (*p*-TOLUYLENEDIAMINE or TOLUENE-2,5-DIAMINE)

[†]Deceased.

-
- B₃ 2-Chloro-1,4-diamino-benzene (2-CHLORO-*p*-PHENYLENEDIAMINE)
- B₄ 4-Amino-diphenylamine (*N*-PHENYL-*p*-PHENYLENEDIAMINE)
- B₅ 1- β -Hydroxyethyl-2,5-diamino-benzene (HYDROXYETHYL-*p*-PHENYLENEDIAMINE)
(1)
- B₆ 1-Amino-4-bis-(β -hydroxyethyl)amino-benzene (*N,N*-Bis-(2-HYDROXYETHYL)-*p*-PHENYLENEDIAMINE) (2)
- C₁ 1,3-Diamino-benzene (*m*-PHENYLENEDIAMINE)
- C₂ 2,6-Diamino-pyridine (2,6-DIAMINOPYRIDINE)
- C₃ 1-Methyl-2,6-di-(β -hydroxyethylamino)-benzene (2,6-DIHYDROXYETHYLAMINOTOLUENE) (3)
- C₉ 2,6-Dimethoxy-3,5-diamino-pyridine (2,6-DIMETHOXY-3,5-PYRIDINEDIAMINE) (4)

Aminophenols

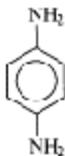
- B₇ 1-Hydroxy-2-amino-benzene (*o*-AMINOPHENOL)
- C₄ 1-Hydroxy-3-amino-benzene (*m*-AMINOPHENOL)
- B₈ 1-Hydroxy-5-methyl-2-amino-benzene (6-AMINO-*m*-CRESOL) (5)
- C₅ 2-Hydroxy-1 -methyl-4-(β -hydroxyethylamino)-benzene (2-METHYL-5-HYDROXYETHYLAMINO-PHENOL) (6)
- B₉ 1-Hydroxy-4-amino-benzene (*p*-AMINOPHENOL)
- B₁₀ 1-Hydroxy-3-methyl-4-amino-benzene (4-AMINO-*m*-CRESOL) (7)
- B₁₁ 1-Hydroxy-4-methylamino-benzene (*p*-METHYLAMINOPHENOL)
- C₆ 1- β -Hydroxyethoxy-2,4-diamino-benzene (2,4-DIAMINOPHOXYETHANOL) (8)
- C₇ 2-Hydroxy-1-methyl-4-amino-benzene (4-AMINO-2-HYDROXYTOLUENE)
- C₈ 1-Methoxy-2-amino-4-(β -hydroxyethylamino)-benzene (2-AMINO-4-HYDROXYETHYLAMINO-ANISOLE)
- C₁₀ 1-Hydroxy-2,4-dichloro-3-amino-benzene (3-AMINO-2,4-DICHLOROPHENOL) (9)
- C₁₁ 1-Hydroxy-6-chloro-2-methyl-5-amino-benzene (5-AMINO-6-CHLORO-*O*-CRESOL) (10)

Phenols and naphthols

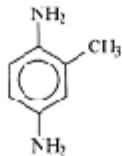
- C₁₂ 1,3-Dihydroxybenzene (RESORCINOL)
- C₁₃ 1,3-Dihydroxy-2-methyl-benzene (2-METHYLRESORCINOL)
- C₁₄ 1,3-Dihydroxy-4-chloro-benzene (4-CHLORORESORCINOL)
- C₁₅ 1,2,4-Trihydroxybenzene (1,2,4-TRIHYDROXYBENZENE)
- C₁₆ 1,2,4-Trihydroxy-5-methyl-benzene
- C₁₇ 1,5-Dihydroxynaphthalene (1,5-NAPHTHALENEDIOL)
- C₁₈ 1,4-Dihydroxybenzene (HYDROQUINONE)
-

C₁₉ 1-Hydroxynaphthalene (1-NAPHTHOL)

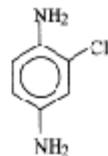
C₂₀ 1-Acetoxy-2-methyl-naphthalene (11)



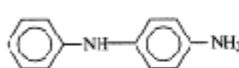
B₁
1,4-Diamino-benzene
(*p*-PHENYLENEDIAMINE)



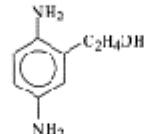
B₂
2-Methyl-1,4-diamino-benzene
(TOLUENEN-2,5-DIAMINE)



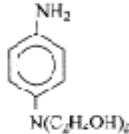
B₃
2-Chloro-1,4-diamino-benzene
(2-CHLORO-*p*-PHENYLENEDIAMINE)



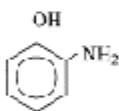
B₄
4-Amino diphenylamine
(N,N'-BENZYL-*p*-PHENYLENEDIAMINE)



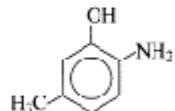
B₅
1,β-Hydroxyethyl-2,5-diaminobenzene
(HYDROXYETHYL-*p*-PHENYLNEDIAMINE)



B₆
1-Amino-4-bis(β-hydroxyethyl)amino-benzene
(N,N-BIS(2-HYDROXYETHYL)-*p*-PHENYLENEDIAMINE)



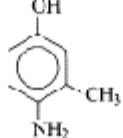
B₇
1-Hydroxy-2-amino-benzene
(*o*-AMINOPHENOL)



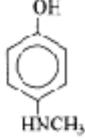
B₈
1-Hydroxy-2-methyl-2-amino-benzene
(6-AMINO-*m*-CRESOL)



B₉
1-Hydroxy-4-amino-benzene
(*p*-AMINOPHENOL)



B₁₀
1-Hydroxy-3-methyl-4-amino-benzene
(4-AMINO-*m*-CRESOL)



B₁₁
1-Hydroxy-4-methylamino-benzene
(*p*-METHYLAMINOPHENOL)

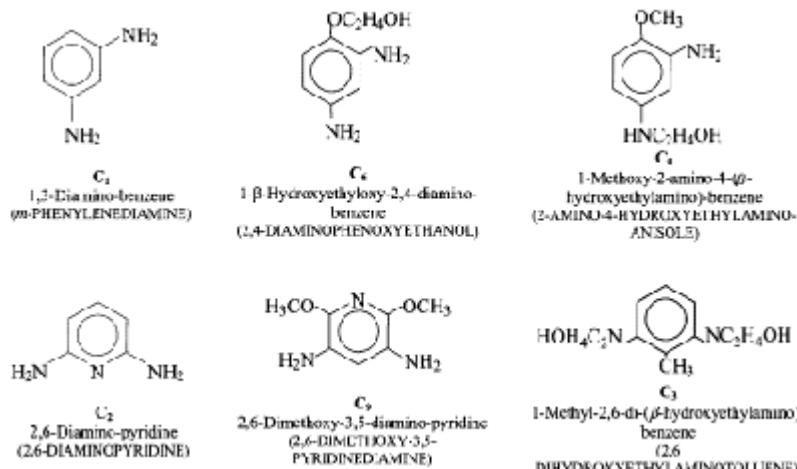
However, from these various intermediates, two major structural classes are to be distinguished. Actually only those with two amine functions or one amine and one phenol functions in *ortho* or *para* to each other are able to give deep shades on white hair. Thus only intermediates capable of producing the quinone monoimine or quinone diimine

forms yields highly colored pigments through oxidation. Similarly, it has been observed that copolymerization does not result in deep shades unless quinone monoimines or quinone diimines participate in the condensation process. This observation leads to a classification of oxidation products that is not a simple differentiation by chemical class.

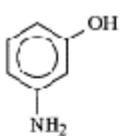
The so-called *bases* or primary intermediates are the aromatic diamines, the diaminophenols, and the aminophenols with amino or hydroxy group *ortho* or *para* to an amino group. These intermediates are essential to create the basic shade and to dye the white hairs. The main oxidation *bases* which meet these requirements are the following:

The “modifiers” or *couplers* are the *m*-diamines, the *m*-aminophenols, and the polyphenols. Taken separately, all these modifiers yield only feeble coloring through oxidation; cooxidation of modifier mixes, too, yield only slight coloring (yellow, blond-beige). But when they are combined with *bases* (or primary intermediates) they contribute developing highlights. The main oxidation couplers are the following:

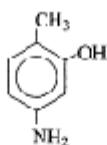
1.1. The *m*-Diamines



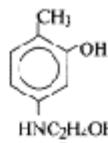
1.2 The *m*-Aminophenols



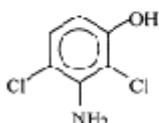
C₄
1-Hydroxy-3-amino-benzene
(m-ANINOPHENOL)



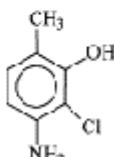
C₅
2-Hydroxy-1-methyl-4-amino-benzene
(4-AMINO-2-HYDROXYTOLUENE)



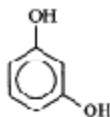
C₆
2-Hydroxy-1-methyl-4-(β-hydroxyethylamino)-benzene
(2-METHYL-5-HYDROXYETHYLAMINO-PHENOL)



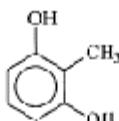
C₁₀
1-Hydroxy-2,4-dichloro-3-amino-benzene
(3-AMINO-2,4-DICHLOROPHENOL).



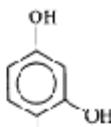
C₁₁
1-Hydroxy-6-chloro-2-methyl-3-amino-benzene
(3-AMINO-6-CHLORO-2-METHYLPHENOL)



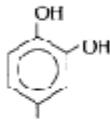
C₁₂
1,3-Dihydroxy-benzene
(RESORCINOL)



C₁₃
1,3-Dihydroxy-2-methyl-benzene
(2-METHYLRESORCINOL)



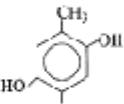
C₁₄
1,2-Dihydroxy-4-chloro-benzene
(4-CHLORORESORCINOL)



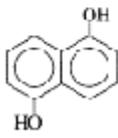
C₁₅
1,2,4-Trihydroxybenzene
(1,2,4-TRIHYDROXYBENZENE)



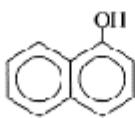
C₁₆
1,4-Dihydroxybenzene
(1,4-DIHYDROXYBENZENE)



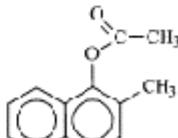
C₁₇
1,2,4-Trihydroxy-3-methylbenzene



C₁₈
1,3-Dihydroxynaphthalene
(1,3-NAPHTHALENEDIOL)



C₁₉
1-Hydroxynaphthalene
(1-NAPHTHOL)

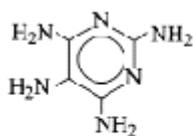


C₂₀
1-Acetoxy-2-methyl-naphthalene
(1-ACETOXY-2-METHYLNAPHTHALENE)

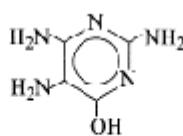
In addition to these considerations on the relative positions of the auxochrome groups, one can attempt to define the influence on the nature and intensity of color of certain substitutions in the benzene ring or in the auxochrome groups themselves.

1. The introduction of nitro groups in the benzene ring yields yellow, red, or purple-violet shades. Actually, it produces the semipermanent dyes described in the preceding chapter.
2. Increasing the number of amino groups facilitates and accelerates the dyeing process. However, this does not necessarily mean a deepening in color. The triamine compounds produce a red or brown color, whereas the corresponding diamines yield black or dark brown.
3. Increasing the number of hydroxy groups in relation to the amino groups diminishes the intensity of the shade.
4. Only the polyhydroxylated compounds with OH groups *ortho* or *para* to each other possess useful dyeing properties. *m*-Diphenol (resorcinol) only colors very slightly. But all of these can participate in copolymerization through oxidation.
5. Substituting methoxy or ethoxy groups or β -hydroxyethoxy for hydroxyl groups weakens color but renders shades more durable.
6. Methyl or ethyl or β -hydroxyethyl group in the benzene ring results in a reduction of dyeing power.
7. A chlorine atom *para* to the NH₂ or OH group will deepen the shade, but if it is *ortho* or *meta*, the shade will be attenuated.

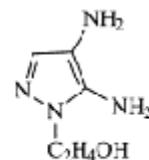
Besides primary intermediates having a benzene ring, pyrimidine (12) and pyrazole (13) derivatives were introduced in the last quarter of the 20th century. They behave like oxidation bases and have been more particularly used to develop shades with red highlights:



2,4,5,6-TETRAAMINO-PYRIMIDINE

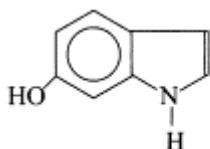


2,5,6-TRIAMINO-4-PYRIMIDINOL

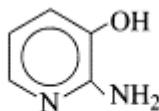


1-HYDROXYETHYL-4,5-DIAMINOPYRAZOLE

Heterocyclic derivatives are also used as couplers or modifiers to produce highlighted shades with increased durability through repeated shampooing. Most illustrative is the use of 6-hydroxy indole the structure of which is close to the basic structural element of the natural pigment of hair (14). The development of oxidation coloring products with pyridine derivatives was restrained due to the sunlight sensitivity of the dyes they generate. However, it should be emphasized that 2-amino-3-hydroxy-pyridine is not associated with such drawbacks and therefore is found in a variety of products. Although it looks structurally related to a primary intermediate, it behaves like a *coupler* (15).



6-HYDROXYINDOLE



2-AMINO-3-HYDROXYPYRIDINE

Sometimes, and in particular to obtain shades with reddish highlights (coppery, purple-violet, dark auburn), semipermanent dyes are added to the oxidation dyes. More generally, nitro derivatives are used (16) which although belonging either to the amine or diamine class or the phenol or aminophenol class, cannot be considered as oxidation dyes. They do not participate either in the oxidation itself or in the oxidative condensations. Their unique role as an additive is to provide highlight. However they often exhibit a lower fastness than oxidation dyes after cumulative shampooing; as a result, they are less and less found as a component of oxidation coloring products.

"Azo" dyes or triphenylmethane derivatives may also be added as far as they are stable enough under sunlight and in solution.

2. THE MECHANISMS OF OXIDATION

This is a complicated process. Each of the precursors defined above (*base* or *coupler*) can, through oxidation and polymerization, produce a pigment that fixes onto hair. Where oxidation of a *base* on its own or of binary *base-coupler* mixes is concerned, the series of chemical reactions can be described without much difficulty. However, what looks rather well understood in the jar may be far from the reality when hair is involved. Hair indeed takes a large part in the process because of selective diffusion of the precursors, *bases* and *couplers*, into and within the fiber and because hair acts as a catalyst promoting the selective formation of certain dyestuffs. In the presence of three, four, or five precursors such as commonly found in hair coloring formulations, the precise definition of the copolymerization mechanism becomes extremely complicated and rather unpredictable.

Several papers on this subject have been published over the past 100 years or so. Without forgetting the founding papers (17–19), more recent studies are of particular interest such as those (20–29). To these studies, it is essential to add those by Corbett, Brown and Pohl between 1968 and 1999 (30–39).

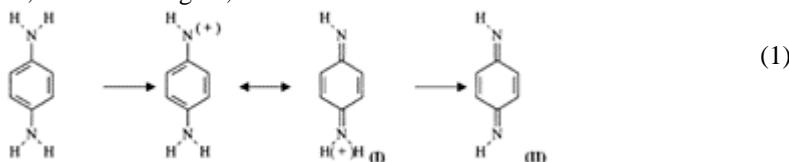
This wealth of information shows that it is worth considering, in succession:

- the case of *bases* on their own,
- these same *bases* in the presence of a *coupler*,
- and, finally the general case.

2.1. Oxidation and Polycondensation of *p*-Phenylenediamine

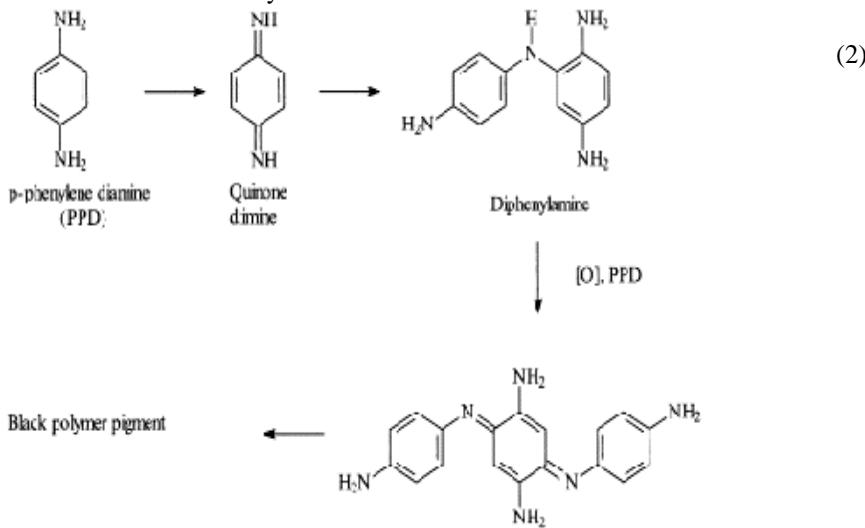
Many authors have begun by considering the condensation of *p*-phenylenediamine (PPD) on itself (17–19,21–25,29–32,37). In the presence of an oxidizing agent, PPD is converted (Eq. 1) into quinone diimine (II), an oxidation product that readily reacts with

nucleophilic agents, i.e., substances rich in electrons such as the *couplers* mentioned earlier and, to a lesser degree, with PPD itself or other *bases*.



The formation of oxidation colorants thus implies prior oxidation of the PPD *base* to give rise to a reactive intermediate derivative. Strictly speaking, it is not entirely certain that this pivotal precursor of oxidative coupling reactions is quinone diimine (II); the true reagent could well be quinone diiminium (I), the first stage in the PPD oxidation process (Eq. 1). These mechanisms have been the subject of many studies and developments; to simplify, the main chemical reactions (Eqs. 2–4) are shown, taking quinone diimine (II) as the key reactive intermediate.

Quinone diimine (II) can react with PPD to form a diphenylenamine that, in its turn, is oxidized and reacts with PPD to yield a deep-blue derivative with three benzene rings. The chain reaction continues and leads to the formation of a black polymer pigment whose structure is not fully established.

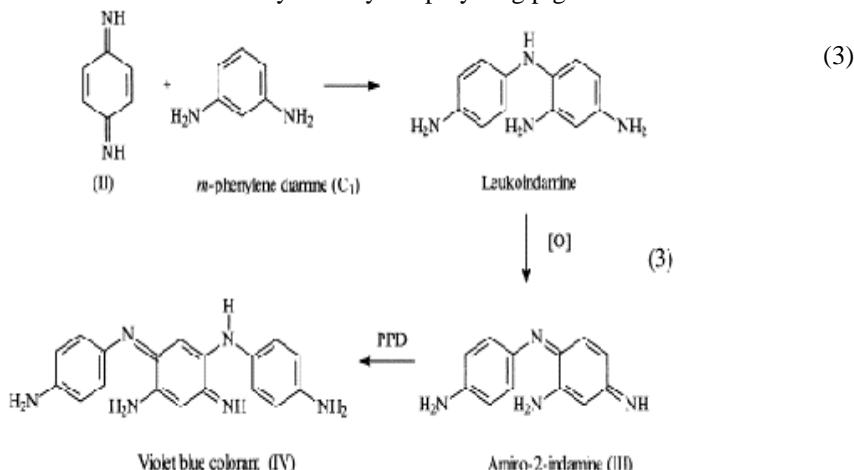


The coupling of PPD with itself does not yield a great variety of colors. The reaction of PPD with *couplers* is thus preferred.

2.2. Co-oxidation of *p*-Phenylenediamine and Condensation with *m*-Phenylenediamine

The oxidation of *p*-phenylenediamines hence leads to the corresponding quinone diimines. In the presence of a *m*-phenylenediamine, there are four stages in the process (20,25,27,30–32,34,35,37):

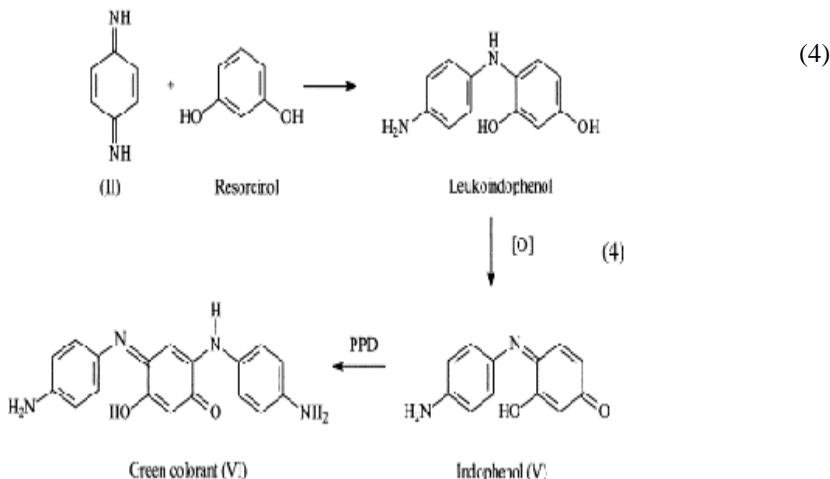
- Formation of the quinone diimine as above by oxidation of the *p*-phenylenediamine.
- Formation of leukoindamine from the reaction of *m*-phenylenediamine with quinone diimine (Eq. 3).
- Oxidation of the leukoindamine yields a blue amino-indamine (III)
- Amino-indamine reacts with *p*-phenylenediamine to form a three-ring violet-blue colorant (IV) that can, in its turn, react with quinone imine (III) or enter into new oxidation/condensation cycles to yield poly-ring pigments.



2.3. Oxidation and Condensation of *p*-Phenylenediamine with Diphenols and Aminophenols

In the presence of *meta*-diphenols or *meta*-aminophenols possibly substituted on the benzene ring by halogen atoms or alkyl or alkoxy groups, and provided that the *para* position on the phenol remains free, the quinone diimines condense with these *couplers* to form indoanilines or indophenols (10,20,23,26,29,30,32,34,35,37).

- The reaction (Eq. 4) of quinone diimine (II) with resorcinol gives rise to a leuko-indophenol readily oxidized to indophenol (V).
- Through condensation with PPD, the indophenol yields a three-ring green colorant (VI) which, in its turn, can react with indophenol (V) to form a five-ring colorant, or undergo further oxidation followed by coupling.



Strictly speaking, given the high pH used in oxidation coloring, the structural diagrams of reactions with ionized forms (phenolates) should also be shown, but the final forms obtained are identical (30,32). A similar reaction scheme can describe the oxidative coupling of PPD with *m*-aminophenol.

2.4. Special Cases

In addition to the general rule, it is worth considering a few special cases frequently encountered in hair coloring, notably with products including:

- PPD or derivative and *para*-aminophenol (PAP) or derivative simultaneously,
- a *coupler* substituted on the benzene ring,
- a tertiary base.

2.4.1. PPD-PAP Combination

In many coloring compositions, both PPD and PAP are present simultaneously. In solution, the oxidation of PAP occurs much more rapidly than that of PPD (33). Also considering the oxidation-reduction potentials (39) shows that the equilibrium:

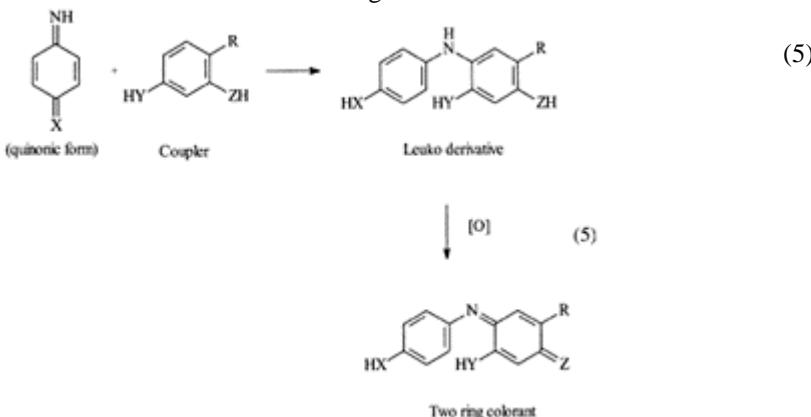


is rapidly shifted toward the right whatever the pH. This means that under the usual conditions of hair coloring, PAP is consumed in the coupling reactions before PPD.

This has been confirmed experimentally by bubbling oxygen through aqueous solutions containing equimolar amounts of *p*-aminophenol, *p*-phenylenediamine and of the *coupler* *m*-phenylenediamine or α -naphthol or *m*-aminophenol (33,34). In every case, the PAP derivative was the first to be formed.

2.4.2. Couplers Substituted on the Ring

In the general case described earlier, when the *base/coupler* ratio is large, the reaction between PPD and resorcinol, *meta*-aminophenol, or *meta*-diaminobenzene can lead to the simultaneous formation of not only a two-ring, but also a three-ring colorant. Moreover, with *meta*-aminophenol, substitution can occur either at the *para* position with the OH group or at the *para* position with the NH₂ group. This leads to a mixture of colorants and thus to less vivid shades than when a single

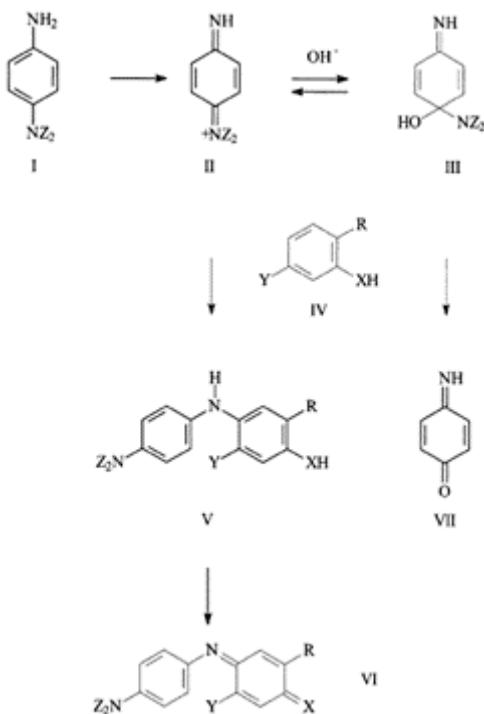


compound is formed. To avoid the formation of mixtures and achieve vivid highlights, one has recourse to couplers substituted at the *para* position of one of the two functions (OH or NH₂) carried by the coupler. If we consider the general reaction (Eq. 5) deduced from the preceding structures (38), where X, Y and Z are oxygen or nitrogen atoms, it appears that a two-ring colorant is formed preferentially if the *base* used can only lead to a monoimine form (the case with PAP), if R is a substituent other than hydrogen and if the *base/coupler* ratio is close to 1. The coupling of PAP with 2,4-diaminophenoxyethanol, 2-methyl-5-hydroxyethylamino-phenol and 4-amino-2-hydroxytoluene are examples of this. However, the gain in shade intensity is achieved at the expense of fastness, as the smaller size of the two-ring structure promotes diffusion toward the outer layer of hair during shampooing.

2.4.3. The Case of Tertiary PPD Derivatives

The bases of the PPD family doubly substituted on one of the nitrogen atoms should lead, a priori, to better-defined coupling with couplers having substituent on the ring but this is not always the case. Indeed, with these tertiary *bases*, the diimine generated can form, with the hydroxyl ion (alkaline pH), a relatively stable compound (III) that does not react

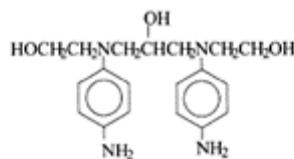
with the *couplers* (36). Since, at above pH 8.1, more than half of the diimine is transformed to (III) which then evolves toward a quinone monoimine form (VII), this has marked consequences for the coloring (36,40).



At a given pH, the kinetics of the coupling reaction are controlled by the ion (II) concentration, itself affected by the formation of complex (III). Nonetheless, taking respective *pK* values into account, there is a greater concentration of the cationic form (II) of the doubly substituted diimine (*pK*=8.1) relative to the diimine derived from the non-substituted PPD (*pK* 5.75). Thus, even if the compound (III) concentration is high, the total rate constant is greater by two orders of magnitude in the case of a doubly substituted PPD.

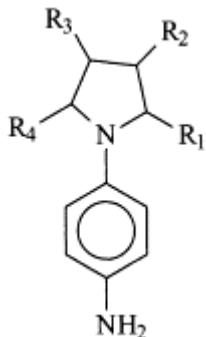
The mechanism indicated above also shows that part of the doubly substituted diimine can be hydrolyzed into *p*-benzoquinone monoimine (the case of PAP). This compound is capable of reacting with the *couplers* present. Hydrolysis is reduced to a minimum at low pH and a relatively high *coupler* concentration. As with the oxidation colorants, the pH is set at between 9 and 10 to promote melanin lightening, therefore the formation of colorant (VI) is prevalent if the *coupler* content is adequate. Finally, in the simultaneous presence of PPD and its *bis*-hydroxyethyl derivative, the doubly substituted (i.e., Z=hydroxyethyl) compound (VI) is the first to be formed (instead of Z=H).

Some coloring compositions contain, a “double” tertiary base which leads to coupling products of greater size. This improves the fastness, after shampooing of the colors obtained on the most fragile parts of the fiber:



(HYDROXYPROPYL-BIS-(N-HYDROXYETHYL-p-PHENYLENE DIAMINE))

It should also be mentioned that the strength of the tertiary *bases* is reinforced when nitrogen forms part of certain five-atom rings.

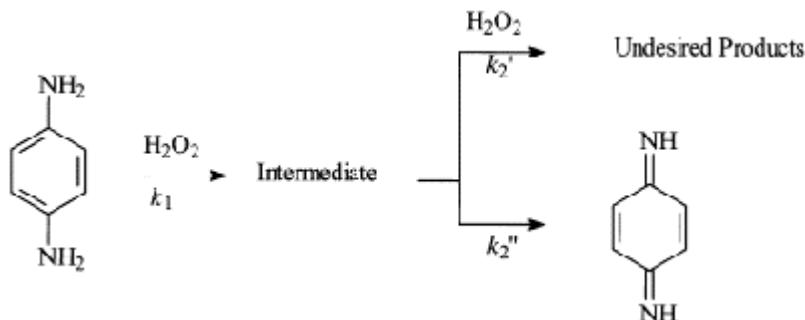


So, despite the size of the precursor, coloring strengths are obtained, even on natural hair that has been little damaged by repeated colorings, which are comparable to that observed with PPD under the same conditions (41,42). Nonetheless, these *bases* have yet to find commercial applications.

2.5. Parameters Affecting Color Uptake by Hair

2.5.1. Influence of Oxidizing Agent

Most of the processes have been studied using ferricyanide as the oxidizing agent. The formation of indo-aniline is instantaneous in this case while it is much slower in the presence of hydrogen peroxide. The quantitative determination of *p*-benzoquinone diimine or any products derived from PPD is practically impossible due to the interference between PPD and hydrogen peroxide but kinetic modeling can lead to valuable information on the probable mechanisms. For the reaction between PPD and the 2,6-dimethylphenol *coupler* model, in the presence of hydrogen peroxide, Feng and Chan (29) have thus been able to propose the following pathway for the initial step:



According to this pathway, the conversion of PPD into an efficient intermediate is governed by the ratio k_2''/k_2' [H_2O_2]. The authors were able to show that, under their experimental conditions, when hydrogen peroxide was at a low concentration, the reaction 2' was negligible relative to reaction 2'' while, when the hydrogen peroxide content was increased, it was reaction 2' that predominated.

2.5.2. Influence of pH and pH Adjusting Agent

The nature of the pH adjusting agent (soda, potash, ammonia, or ethanolamine) has a particularly weak effect, quite the opposite of the case when bleaching. The effect of the pH, however, is very important. It has been given careful consideration (30,32,33,35–37). From these studies, it has emerged that red and coppery highlights are practically impossible to obtain using PAP and the conventional *couplers* at low pH.

2.5.3. Influence of Hair

The study of reactions in solution constitutes an initial approach to the processes of permanent oxidation coloring. Nonetheless, it is the shade produced in the fiber that constitutes the main aim of the operation. It is therefore worth adding, to the items mentioned earlier, the constraints related to the diffusion of the precursors of coloring in the hair. The diffusion is governed by their ionization level or that of intermediates present on the one hand and on the swelling and condition of the fiber on the other. Ammonia promotes swelling of the fiber. In alkaline medium, phenols are partially ionized and their negative charge prevents or slows down their progression into hair: this is particularly the case with resorcinol and methyl resorcinol. Similarly, the size of the precursors can hinder their penetration of the hair. A single methyl substituent on an aromatic ring considerably reduces the coloring strength.

2.5.4. Influence of Surfactants and Other Constituents of Oxidation Coloring Products

As well as the precursors, hydrogen peroxide and pH adjusting agents, hair coloring products include surfactants that:

- solubilize the precursors,

- promote wetting of the hair,
- increase the viscosity of the medium,
- eliminate in the final rinse those colorants that have not been taken up by the hair.

Furthermore, with anionic surfactants and in particular the soaps of fatty acids such as oleic acid or lauric acid, via an electrostatic effect:

- the penetration of diamines is slowed down,
- the penetration of polyphenols is accelerated.

Finally, it should be noted that the kinetics of coloring can be affected by the reducing agents introduced to protect the precursors from oxidation (*bases* are highly sensitive to oxidants) until the moment of use and by chelating agents whose role is to restrain the influence of any metals that may be present and act as oxidation catalyst.

3. FORMULATION OF OXIDATION DYES

The complex chemical processes that come into play in oxidation coloring lead to quinones, quinoneimines, indamines, indophenols and phenazines, and finally to complex pigments. This knowledge is obviously useful in the formulation of hair dyes but considerable judgment is required in the application of this knowledge.

Laboratory studies demonstrate the final terms of the oxidation process but, in hairdressing practice, it seems that the chemical reactions must be complete in order to ensure shade stability (notwithstanding resistance to wear-and-tear or light-fastness).

There is also the time factor. In practice, given that new hair emerges and that applications are repeated every 4–6 weeks, stability lasting 6 weeks should be perfectly acceptable. Some dyes or dyes mixes that might be rejected in laboratory studies may well function satisfactorily in practical hairdressing.

In the final analysis, the formulation of oxidation colorants is the result of theoretical studies, a wealth of empirical data and a multitude of practical tests carried out on actual heads of hair.

The variations in shade intensity—from very light blond to black, the whole range of ash blond, golden, red, auburn, mahogany, iridescent, deep purple, also the pastel tones can be achieved with the dye precursors that specialized chemists have at their disposal (43–54). It is not unusual to find more than 70 different shades in a manufacturer's formulary. And this very large number of shades is yet further extended to every imaginable hue by mixtures of them. Professional hairdressers can produce all the natural shades of human hair, and many others besides, without placing undue strain on their technical prowess and experience.

A first step in developing a hair dye formulary or range of shades consists of carrying out a series of equimolar solutions of various precursors (*bases and couplers*) at a certain pH 9.5, for example, using ammonia. Then a series of binary mixtures (*base+coupler*) is prepared and applied to tresses of white hair, after mixing with an equal quantity of hydrogen peroxide (20 vol.). After leaving it on for 30–40 min, followed by washing and rinsing, the resultant colored tresses are examined. The table in the Appendix gives a general idea of the shades obtained on white hair with the main binary oxidative coupling reactions used in the formulation of hair dyes.

Of course, this is only a preliminary approach, because it only involves equimolar, binary mixtures, in aqueous solution, at a given pH. But it is the only approximation possible. From this starting point, progress can be made in a stepwise fashion, each change being capable of grossly affecting the end result. In fact, it is the start for an infinitely complex process.

The clue to the research is the “vehicle” selected, as the binary or ternary mixtures can yield different results depending on the cream or liquid used. In fact, each coupling is subject to its own specific kinetics that depends on various factors such as the temperature and pH of the reaction—as do diffusion phenomena that can influence the development of the color (55–57).

It is also essential to decide what degree of lightening is desired even before carrying out tests on the formulary, i.e., the quantity of ammonia and the required pH must be decided upon first of all.

For acceptable stability of oxidative dyes in bottles or tubes, the preparations must contain an antioxidant or a reducing agent. It is usually a thiol such as thioglycollic or thiolactic acid or a sulfite, possibly supplemented by hydroquinone (or its derivatives). Ascorbic acid (58) or its isomer erythorbic acid is also used.

Finally, in gel (or gelling) dye products, an oxidation curb such as phenylmethylpyrazolone (59) is often added in order to avoid overly rapid oxidation once the mixture with the oxidizing agent has taken place.

Thus it is only after having determined the precise formula of the vehicle, its pH and its ammonia concentration that the study of the coloring properties can proceed by the binary mixture method. Subsequently, the proportions of the precursors will be modified by introducing new *couplers* or new *bases*, bearing in mind that each additional compound brings its own reactivity into the system of *bases/couplers*.

In summary, the various elements involved in the formulation of an oxidative dye are as follows:

1. The “vehicle” itself: solution, liquid or creamy emulsion, oil convertible to gel, gel, shampoo, powder, etc.
2. The primary intermediates (*bases*).
3. The modifiers (*couplers*).
4. The direct colorants, intended to determine the highlights.
5. The *alkali* (generally ammonia).
6. The *antioxidant* (generally a sulfite or a thiol)
7. In some products, a *curb agent* to control oxidation process.

The development of a high-quality oxidation dye demands a huge amount of meticulous care, huge experience and a huge number of practical tests on actual heads of hair of every kind including every degree of graying hair.

3.1. Coloring Mixture

Oxidation dyes require the simultaneous use of *bases* and *couplers*. Following a cascade of reactions, colorants and pigments form in the hair to achieve the desired shade. The nature and mode of action of the various precursors used have been amply described above. The application of coloring mixture to the hair obeys certain complementary rules:

3.1.1. Quantity

The actual coloring mixture is prepared extemporaneously, prior to application to the hair, by mixing, generally weight for weight, a solution containing the precursors and the other components of the formula with a solution containing hydrogen peroxide (10, 20, or 30 vol. etc.) called developer.

In general, each of these two solutions amounts to 50 g but it is not uncommon to use 40 g of a colorant formula mixed with 60 g of developer. With non-lightening oxidative coloring the amount of developer may be twice that of colorant formula.

The final mixture applied to the hair thus amounts to some 100 g but it can vary slightly according to the amount of hair to be treated.

3.1.2. Concentration

Given that it is diluted by the developer, if the original concentration of a precursor (*base* or *coupler*) is x , its concentration in the final mixture coming into contact with the hair is, at most, $x/2$.

In practice, the initial concentration x used lies between 0.006% and 7%. This range of concentrations corresponds to a spectrum of shades from very pale blond to black, via the most vivid highlights. In this way, the 20–70 shades of an oxidation dye formulary are built up. It is worth noting that:

- Shades containing no more than 0.5% precursors or colorants as a whole represent about 50% of the market.
- Shades containing no more than 0.75% overall precursors or colorants increase the percentage market share to about 75%.
- Only a few shades have a total precursor or colorant concentration reaching or exceeding 3.5% before mixing with developer.

The concentrations above represent the total content of precursors or dyestuffs (sometimes as many as 10) that are necessary to achieve the desired shade. More precisely, oxidation dye products consist of a mixture of:

- two or three *bases*,
- four or five *couplers*,
- sometimes one or two direct colorants (see Chapter 8).

The concentrations of each of the components is thus always much smaller than the overall concentration.

3.1.3. Waiting Time and Temperature

The time that the colorant mixture is left on the hair varies from 10 to 45 min for the initial application. For subsequent applications, such a time-lapse is only recommended for the undyed roots. In this case, the mix is only applied to hair dyed the previous month at the end of the process and left in contact with the hair for only a few minutes. Coloring takes place practically at normal room temperature.

3.2. Coloration Models

Very early on, the designers of dye products sought to link the color observed to the concentration of colorants in the coloring bath or at least in the fibers treated. Kubelka and Munk's theory developed in 1931 was thus widely utilized in coloring practice (textiles, stationery, printing, etc.) (60). At each wavelength of the visible spectrum (400–700 nm), it is possible to relate reflected light (reflectance: R) to the concentration C of the colorants. Various equations and systems of equations are based on Kubelka and Munk's study, yielding two particularly useful ratios:

The first links the ratio of the coefficient of absorption K over the diffusion coefficient S to the reflectance R:

$$K/S = (1-R)^2/2R$$

The second links the same ratio K/S to the concentration of colorants C_i in the matrix via an additive property:

$$K/S = \sum [a_i * C_i + b_i] \quad (i=1, \dots, n)$$

where a_i and b_i are constants linked to the colorant and the medium to be colored.

The reflectance values in the visible domain are obtained experimentally using a spectrophotometer. These data can be input in a calculator to attempt to relate the concentrations of a mixture to the color observed.

In 1985, Brown et al. (61), in an investigation of this type, showed that for two particular couplings, the intensity of the color absorbed by a fiber depends not only on the colorant concentration formed in the fiber but also on the surfactant concentration in the vehicle, the pH of the solution, and the reaction time.

This approach takes account of the spectra of colorants actually formed and of their concentration in the fiber. It differs therefore from that proposed by Nerenz et al. (62), which links the colorimetric values observed on the hair to the composition of the coloring mixture.

3.3. Lightening Coloring

Oxidation coloring develops normally in the presence of an excess of hydrogen peroxide at a pH between 9 and 10 achieved with ammonia or monoethanolamine. And the hydrogen peroxide-ammonia mixture is precisely the combination used for hair bleaching. Oxidation coloring thus always makes the hair a lighter color. In one step, two apparently contradictory operations are achieved: lightening and coloring.

Given this principle, the number of variables to be mastered in the achievement of *one-step* lightening coloring shades is rather high:

1. The natural hair to which a lightening shade is applied should have a certain degree of aptitude to being lightened.
2. The percentage of gray hairs should be considered, as these do not respond to coloring in the same way as naturally pigmented hair.
3. The need for the natural hair color to be not overly dark relative to the desired color, otherwise the limited lightening capacity of the dye will not yield the desired shade.

Without needing to enter into greater detail, it is clear that the formulation of lightening dyes consists of the search for a balance between the concentration and nature of the dye precursors on the one hand and the alkali content on the other. This search can unfortunately only be carried out *in vivo*, i.e., on the head of hair. Laboratory tests and application to swatches of hair do not correspond entirely to actual in-use results.

With regard to the lightening effect, in simple terms it responds to the following general principle: the precursors blend being mixed at the time of use with its weight or volume of hydrogen peroxide (20 vol.) will lighten:

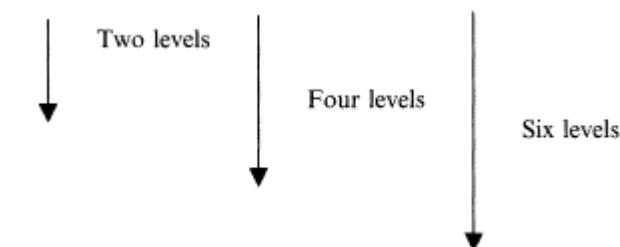
1. by 1 tone if the coloring product contains 5% of 20% aqueous ammonia,
2. by 2 tones approximately if it contains 10% of 20% aqueous ammonia,
3. by 2.5 to 3 tones approximately if it contains 15% of 20% aqueous ammonia.

The use of hydrogen peroxide 30 vol. will make the tone a little lighter but, at the current state of the art, it is difficult to go further than this. The increase in alkalinity and/or the hydrogen peroxide concentration would lead to overly aggressive and irritant products. Furthermore, beyond 4 tones, the pigments resulting from the oxidation are themselves destroyed by the excess oxidant in the dyeing mixture and the coloring becomes inadequate. In this case, it is necessary to bleach the hair first and apply the dye product afterwards. To avoid this two-step process, the highlight can be enriched by using specific cationic colorants in addition to the precursors *bases* and *couplers*. The shades obtained are particularly vivid and fairly long-lasting (63).

To gain a clear idea of the above points, it is necessary to become familiar with the notion of "tones" or "levels." It is based on the classification of natural shades as shown below, one tone separating each shade from the one that precedes or follows it.

Tones or Levels (Color)

1. Black
2. Brown
3. Dark chestnut
4. Chestnut
5. Light chestnut
6. Dark blond
7. Blond
8. Light blond
9. Very light blond
10. Light light blond



Any product that changes hair color from natural chestnut hair to dark blond is capable of lightening by 2 tones. An oxidation dye product lightening by two tones is thus capable

of changing natural chestnut hair comprising 60% gray hair to a uniform dark blond with coverage of the gray hair.

3.4. Non-lightening Oxidation Coloring

This coloring called “tone-on-tone” in Europe and “demi-permanent” in the United States, changes hair color in various ways. It can be used to improve hair color in the same tone, by darkening the natural color or by adding highlights. The highlights can be made more visible by a slight lightening effect.

These results can be achieved by the use of oxidation dyes with a low alkaline component. To reach a satisfactory pH, ammonia can even be dispensed with and a small amount of an alkanolamine used, allowing the product to be pleasantly perfumed. The alkanolamines most frequently encountered are methylaminopropanol and especially monoethanolamine. However the levels used must be carefully controlled as, when used at too high a concentration, they may pose problems of scalp incompatibility in some individuals. Sometimes, these products are at a lower pH than that of lightening dyes, but lowering the pH modifies the coupling reactions and the diffusion of precursors within the fiber. This has to be taken into account when formulating.

This non-lightening coloring gives satisfaction as long as the gray hairs are well distributed and represent no more than 50% of the head of hair. It respects variations in the natural pigmentation, providing a non-uniform coloration with a blurred effect. With no, or very little lightening effect, the roots problem and impairment of the surface of the hair are avoided. With these products, the coloration provided is permanent. Only regrowth causes the reappearance of heterogeneous pigmentation of the hair. The color obtained fades much more slowly than with semipermanent coloring with “nitro” dyes. These attributes explain its success and, in the course of the last two decades, consumers have often shown a preference for oxidative tone-on-tone coloring over non-oxidation semipermanent coloring.

Non-lightening oxidation coloring systems using atmospheric oxygen with the help of appropriate enzymes have also been described (64,65) but these procedures have not yet led to practical developments.

4. TEXTURE OF OXIDATION COLORING

The products on offer to consumers take many forms. As they contain oxidizable precursors, they are never ready for use. Only the powder form allows amalgamation, in a single formula, of the precursors and oxidant: only water needs to be added at the time of use. However, in the great majority of cases, oxidation dyes products are presented in the form of a solution or cream.

The oxidants are then added at the time of application. These oxidative products may consist of:

- solutions or emulsions of hydrogen peroxide 20 or 30 vol.;
- crystals or tablets of urea peroxide or melamine peroxide to be dissolved in water prior to mixing, or to be dispersed directly into the dye;
- crystals of sodium perborate.

The physical properties of oxidative hair coloring products represent a compromise between the characteristics required at the various stages of the process. During application, the coloring mixture must be fluid enough to spread and cover each hair. However, if too fluid, it would drip in an unacceptable way and if too viscous, it would not be sufficiently spreadable.

The ideal mixture has to be markedly thixotropic. These general constraints have been summarized by Iscowitz et al. (66). For a suitable product, the viscosity must exhibit a large decrease relative to the shearing rate. It is therefore imperfectly defined by a single viscosity value at a mean shear rate, it will be preferably characterized by at least two values defining its behavior under extreme conditions. The high shear rate corresponds to the passage of the mixture into the nozzle of the applicator bottle, while the low shear rate corresponds to the waiting period.

Permanent dye products are produced in the form of liquids, creams, gels, shampoos, or powders. Each type has its own characteristics.

4.1. Liquid Dyes

The first oxidation dyes were simple aqueous solutions. They were succeeded by aqueous ethanolic solutions (15–20% ethanol), the ethanol easing the penetration of the oxidation colorants into the hair structure. The coloring properties of these liquids were highly satisfactory. However, they suffered from a number of defects:

1. Liquid dyes run. It is difficult to limit their application to the centimeter of hair regrowth each month.
2. With a liquid base, it is difficult to add various softening additives such as fats, waxes, lanolin, etc.
3. The overly rapid evaporation of ammonia has a detrimental effect on the even quality of the dyeing. The lightening capacity decreases too rapidly and the dye affinity for hair is thus less pronounced.

This is why these dyes have gradually disappeared, ceding their place to more sophisticated products.

4.2. Cream Dyes

Coloring creams have always enjoyed great popularity. Their advantages are numerous: as they do not run, it is easy to limit their application to the desired section. As the vehicle is creamy, it is relatively easy to add softeners and conditioners. In addition, packaging in tubes makes for good stability of the precursors even after they have been opened.

One objection often raised is the difficulty in dosing when mixtures are desirable. In addition, the cream is opaque, which prevents the precise following of the color development on the hair.

For ease of elimination by rinsing, these creams must be readily dispersible in water. This is why they are not generally true emulsions, neither water-in-oil nor oil-in-water. They are, in reality, pseudo-emulsions formulated using ingredients that are self-emulsifying: oxyethylenated fatty alcohols, which may or may not be sulfated, fatty

amides, oxyethylenated vegetable oils, etc. Conditioners such as lanolin derivatives, fatty alcohols, cationic surfactants or polymers (67,68) and various thickening polymers may be added to them.

4.3. Gel Dyes and Coloring Shampoos

Color shampoos constitute the simplest form of permanent coloring products. They have the following characteristics:

1. The vehicle in which they are formulated must exhibit the detergency and foaming capacity of a shampoo.
2. Application must be as simple as that of a shampoo: on hair that has not been washed but is damp, carry out an initial application then rinse. Make a second application, developing the foam, leave on the hair and then rinse. Or application should be possible in a single step, detergent, and foaming. In reality, this characteristic is somewhat theoretical and things are never quite so simple for two reasons:
 - a. The hair often has to be shampooed again to ensure that none of the colorant mixture is left on the hair or scalp. If this further shampoo is not carried out, a "neutralizing rinse" should be applied.
 - b. Even if the product is of good quality, if it has provided adequate coloration and coverage and if the coloration is sufficiently stable, it is not possible to avoid the problem of visible regrowth at each further application. Applying to the entire head of hair in the form of a shampoo is no longer possible. It would have a satisfactory effect on the 1-month grown roots, but with an excess of colorants along the length and tips of the hair still-colored and sensitized from previous applications. The localized application to the roots is thus indispensable from the second or third application on.
3. For application to remain a simple task and to avoid excessive technical problems, coloring shampoos are often weaker dyes and lighteners than the other oxidation coloring products.

The imperatives of simplicity obviously do not yield the perfect success for every type of coloring. Under these conditions "gel dyes" are often preferred to the shampoos. It should be more proper to call these gelling dyes. They are also products with an oily appearance, packed in bottles. After mixture with hydrogen peroxide, generally weight for weight, a clear jelly is obtained.

Gelifiable oxidation products have been hugely popular because they present the cumulative advantages of liquid and cream dyes. Like liquids, the correct dose is easy to measure. Like creams, they are easy to apply. Moreover, their transparency allows the development of color to be monitored without difficulty.

Numerous formulation systems can be envisaged. The three cited below only represent an overview, all combinations being possible:

1. Solutions of soap, generally ammonium or potassium oleate.
2. Solutions of oxyethylenated non-ionic surfactants, generally polyalkoxylated or polyglycerolated fatty alcohols.

3. Solutions of anion-cation complexes.

As with the creams, various thickening polymers can be added to them. These help in the achievement of "tone-on-tone" dark colorations (e.g., the application of a light chestnut on natural light chestnut hair comprising a moderate percentage of gray hairs), and light or moderate colorations that are slightly paler than the natural tones.

For various reasons, the gel and shampoo dyes constitute a large section of the oxidation coloring products for use at home. They are also used in hairdressing salons as rapid, permanent, and easily applied coloring products.

4.4. Powder Dyes

The provision of precursors and oxidant combined in a single homogenous powder is a tempting proposition. The powder only has to be dispersed or dissolved in water at the time of use. The system appears simple and attractive.

In reality, this type of product is inconceivable unless very good stability can be assured. This imperative condition demands the use of very stable oxidative agents in powder form, such as sodium perborate for example. It is therefore only possible to produce non-lightening shades.

The procedure finds its application in the formulation of dark shades. As the main requirement is good coverage of gray hairs in dark shades, these products are only offered on the retail market and in countries where the natural shades are very dark: Africa, India, China, Japan, etc.

5. EFFECTS OF OXIDATION COLORING

It is accepted that colorants, at various stages in their oxidation, are fixed within hair fiber; it is also accepted that the effects of this fixation on the properties of the hair are negligible. What is of the greatest importance is the effect of alkaline lightening that accompanies coloring process.

Hair colored by tone-on-tone oxidation dyes undergoes very little change from its natural condition. This is not quite the case in hair that regularly received lightening oxidation coloring. After several applications, the hair no longer has the same condition and physicochemical characteristics. It tends to resemble bleached hair.

It is therefore natural to seek to re-design the products themselves and to benefit from the successive steps of application with a view to limiting, or canceling, if possible, the negative effects of lightening: tangling, rough feel, greater porosity, etc.

As mentioned earlier, it is relatively easy to add conditioners such as fatty alcohols, lanolin derivatives, cationic surfactants and polymers to dyeing emulsions (creams, coloring shampoos etc.) to protect the hair fiber during the coloring process. It is also necessary to ensure that these conditioning agents maintain their protective effect in the mixture that will be made with the oxidant, a mixture that is both strongly oxidative and strongly alkaline. Some cationic surfactants and polymers possess these properties (67). The effect of polymers having affinity for hair is intimately related to their structure:

- A *low density* and *high molecular weight* lead to an improved surface condition, i.e., improved untangling (68).
- A *high density* and *low molecular weight* promote penetration into the fiber, i.e., hair strengthening (67).

The simultaneous presence of polymers falling in these two categories can be beneficial but it requires vehicles capable of incorporating high concentrations of cationic polymers (69).

The incorporation of such additives in developer solutions or emulsions can also be considered, but the conditioning agents used must not cause a lack of stability of hydrogen peroxide, which constitutes the basis of these oxidants.

6. HAIR CONDITIONING AFTER DYEING

The process of hair dyeing today is almost always followed by a shampoo or conditioner. As fixation of the conditioning agents to the hair is needed and they must also resist the detergency of a shampoo, cationic polymers with a varying degree of affinity are often used nowadays as they may hold for several shampoos.

With the aim of avoiding any allergic reactions, Schueller (70) had recommended “neutralization of the dye” with what he called “the chloride peroxide”:

Sodium chloride	150 g
Hydrogen peroxide, 20 vol.	50 mL
Water	1000 mL

In fact, this type of solution perfects oxidation and facilitates elimination of excess dyes that were not rinsed out. Without going as far as to impose such a final rinse, legislators in a few countries do recommend a final shampoo. To the cosmetologist, it was evident that this final step afforded the opportunity to improve the appearance of hair and provide it with maximum softness, combability, volume, promote sheen, magnify color, etc.

The formulations proposed for what is sometimes called the “neutralizing rinse” are acidic or neutral shampoos, generally cationic or amphoteric that contain, in addition to cationic or amphoteric surfactants, cationic softeners, cationic polymers with a varying degree of affinity for hair, etc.

Hair conditioning emulsions are sometimes used instead of a shampoo and these should be left on the hair for a few minutes before rinsing. They can contain the same ingredients as the shampoos above, and also some protective surfactants or polymers.

Nowadays, with or without regulatory action, virtually all modern oxidation dyeing is followed by a most appropriate shampoo or conditioner. This is a considerable technical advance.

APPENDIX

Dyeing Conditions

An equimolar mixture (2×10^{-2} M) of *base* (B) and *coupler* (C) in a vehicle containing 10% of 20% aqueous ammonia is diluted weight for weight, just before use, with a 20 vol. hydrogen peroxide solution. The dyeing mixture obtained is applied on standardized swatches of 90% white natural hair (N) and 90% white permed natural hair (P). After 30 min, the swatches are rinsed, shampooed and dried prior to evaluating the shade obtained.

Evaluation

Scale of shade tone: very pale, pale, very light, light, medium, deep, dark, very deep, very dark.

Highlight Scale

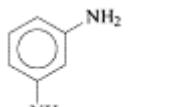
For example, bluish-gray, blue gray, blue.

We have considered the most representative *bases* and *couplers* involving successively the following *couplers*:

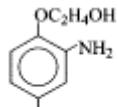
- metadiamines
- metaaminophenols
- diphenols
- naphthols

Appendix Table Shades Achieved with Simple Binary Mixtures (*Base*, *Coupler*) on Natural White Hair (N) and Permed White Hair (P)

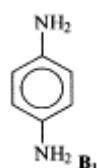
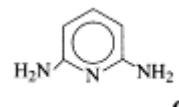
Metadiamine coupling



C₁



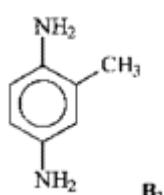
C₆



N Deep blue

Very dark bluish purple

Dark grayish blue



N Dark blue

Purplish dark grayish blue

Deep greenish blue

P Very deep blue

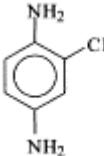
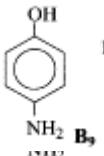
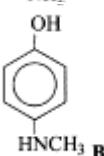
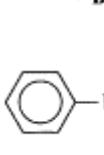
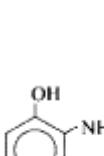
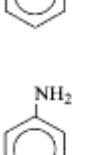
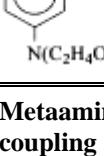
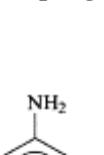
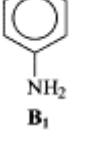
Very dark grayish purplishblue

Dark greenish blue

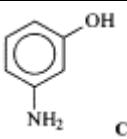
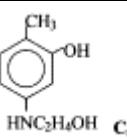
N Pale purplish blue gray

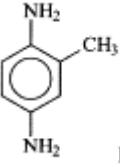
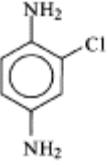
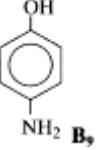
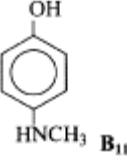
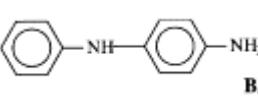
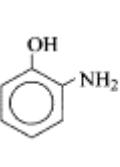
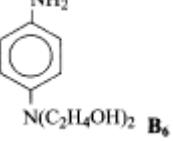
Light purplish gray

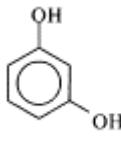
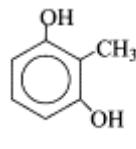
Very light bluish green gray

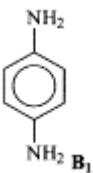
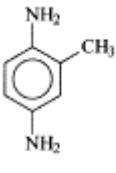
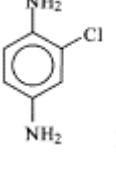
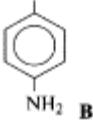
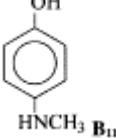
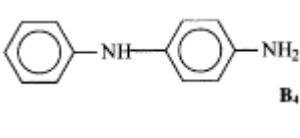
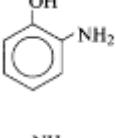
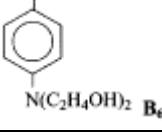
	N	Pale purplish blue gray	Light purplish gray	Very light bluish green gray
	N	<i>Pale purplish blue beige</i>	<i>Slight purplish light red</i>	<i>Very bluish green</i>
	P	Light purplish blue gray	Dark red	Grayish blue green
	N	Very pale, slightly grayish beige	Very pale, slightly orangey beige	Very pale, grayishgreenish yellow
	P	Light, slightly grayish beige	Light grayishpinkish beige	Pale grayish greenish yellow
	N	Very pale, grayish greenish yellow	Pale bluish gray	Very pale, grayish greenish yellow
	P	Slightly pale grayish yellow green	Moderately bluish gray	Pale grayish greenishyellow
	N	Slightly grayish light yellow	Slightly greenishpale grayish yellow	Slightly grayish pale yellow
	P	Moderately grayish yellow	Moderately greenishgrayish yellow	Moderately grayish yellow
	N	Deep greenish blue	Deep greenish blue	Bluish green
	P	Very dark greenish blue	Very dark blue	Dark bluish green

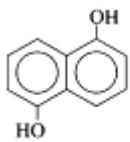
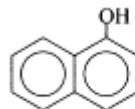
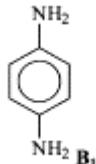
Metaaminophenol coupling

	N	Deep grayish purple	Slightly purplish light grayish red	Deep purplish red
	P	Dark grayish purple	Deep slightlypurplish red	Dark purplish red

	N P	<i>Dark purplish gray</i> <i>Dark mauve gray</i>	<i>Light purplish red</i> <i>Dark purplish red</i>	<i>Deep red purple</i> <i>Dark red purple</i>
	N P	<i>Pale pinkish beige</i> <i>Slightly purplish deep grayish pink</i>	<i>Very pale orangey beige</i> <i>Pale orangey pink</i>	<i>Slightly orangey pale pink</i> <i>Light purplish red</i>
	N P	<i>Pale slightly pinkish beige</i> <i>Slightly pinkish light orangey beige</i>	<i>Pale grayish orange yellow</i> <i>Slightly reddish mid orange</i>	<i>Light grayish yellow orange</i> <i>Mid-orange</i>
	N P	<i>Slightly pinkish pale beige</i> <i>Slightly pinkish pale beige</i>	<i>Slightly pinkish pale beige</i> <i>Light orangey beige</i>	<i>Slightly orangey pale beige</i> <i>Light orangey beige</i>
	N P	<i>Slightly yellowish pale beige</i> <i>Slightly grayish pale beige</i>	<i>Slightly pinkish pale beige</i> <i>Slightly violaceous pinkish mid-beige</i>	<i>Slightly pinkish pale beige</i> <i>Slightly violaceous pinkish light beige</i>
	N P	<i>Greenish mid-yellow</i> <i>Grayish green</i>	<i>Slightly grayish green very pale yellow</i> <i>Slightly pinkish light grayish yellow</i>	<i>Slightly orangey pale grayish yellow</i> <i>Slightly grayish light yellow</i>
	N P	<i>Light bluish gray</i> <i>Dark bluish gray</i>	<i>Light grayish violet</i> <i>Dark violet</i>	<i>Light grayish bluish violet</i> <i>Dark bluish violet</i>

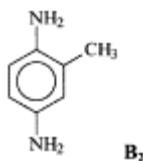
Diphenol coupling**C7****C9****C14**

 B₁	N <i>Slightly greenish mid-brown</i>	<i>Yellowish mid-brown</i>	<i>Grayish pinkish beige</i>
	P <i>Dark greenish brown</i>	<i>Deep yellow brown</i>	<i>Light grayish beige</i>
 B₂	N <i>Slightly grayish light yellowish beige</i>	<i>Slightly pinkish mid-beige</i>	<i>Slightly grayish light beige</i>
	P <i>Slightly greenish mid-brown</i>	<i>Light yellowish brown</i>	<i>Grayish mid-beige</i>
 B₃	N <i>Pale yellowish beige</i>	<i>Light grayish beige</i>	<i>Slightly yellowish light beige</i>
	P <i>Slightly greenish pale yellowish light beige</i>	<i>Light grayish yellowish beige</i>	<i>Light beige</i>
 B₉	N <i>Slightly grayish very pale light beige yellow</i>	<i>Pale yellowish beige</i>	<i>Light pinkish pale beige</i>
	P <i>Violaceous pinkish mid-beige</i>	<i>Light beige yellow</i>	<i>Light pinkish beige</i>
 B₁₁	N <i>Pale yellowish beige</i>	<i>Slightly yellowish pale beige</i>	<i>Pale yellowish beige</i>
	P <i>Light yellowish beige</i>	<i>Slightly yellowish light beige</i>	<i>Slightly grayish light yellowish beige</i>
 B₄	N <i>Slightly grayish pale beige</i>	<i>Slightly yellowish pale beige</i>	<i>Slightly grayish pale beige</i>
	P <i>Light beige</i>	<i>Light beige</i>	<i>Slightly grayish pale beige</i>
 B₇	N <i>Slightly greenish light yellowish gray</i>	<i>Pale greenish yellow</i>	<i>Slightly grayish pale yellow</i>
	P <i>Greenish yellowish mid-yellow</i>	<i>Light greenish yellowish gray</i>	<i>Slightly greenish pale grayish yellow</i>
 B₆	N <i>Slightly mauve pale gray</i>	<i>Slightly beige very pale gray</i>	<i>Slightly greenish light grayish beige</i>
	P <i>Deep grayish purple</i>	<i>Mid-beige gray</i>	<i>Slightly greenish light beige gray</i>

Naphthol coupling**C₁₇****C₁₉**

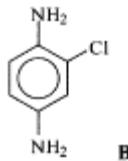
N *Slightly grayish mauve light beige*
P *Mid grayish mauve beige*

Mid grayish mauve beige
Deep grayish mauve



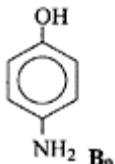
N *Slightly beige pale gray*
P *Mid violaceous gray*

Pale violaceous gray
Deep violaceous gray



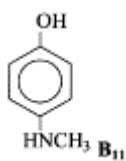
N *Very pale beige gray*
P *Palemauve beige gray*

Very pale grayish beige
Mid grayish mauve



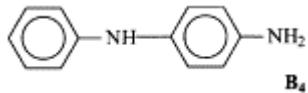
N *Pale pinkish beige*
P *Grayish mid-orangey beige*

Pale orangey beige
Light pinkish orangey



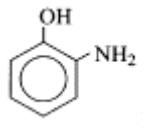
N *Very pale pinkish beige*
P *Mid-grayish pinkish purple*

Slightly pinkish pale beige
Mid pinkish beige



N *Pale grayish beige*
P *Mid bluish gray*

Slightly grayish pale beige
Light grayish beige

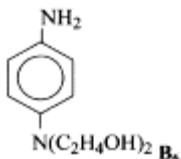


N *Slightly pinkish light yellow*
P *Deep orangey beige*

Slightly grayish light yellow
Slightly grayish mid-yellow

N *Bluish gray*

Light bluish gray

N *Bluish gray**Light bluish gray*P *Deep grayish greenish blue* *Deep grayish blue*

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10

Toxicology of Hair Dyes

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Men are disturbed, not by facts, but by their notions which they form about facts. (Epictetus, A.D. 50–138, Eucheiridiou)

1. INTRODUCTION

For thousands of years, cosmetics have been a part of all human cultures. The use of hair dyes can be traced back at least 4000 years; evidence from Egyptian tombs indicates the use of henna for dyeing hair. Today, millions of consumers use cosmetic dyes and pigments to improve the appearance of their skin, lips, nails, or hair. Given the intrinsic human need to use cosmetics, these products play an important role in our quality of life.

Application of dyes to the human body surface does not necessarily result in absorption or systemic exposure. However, taking into account the extent and frequency of human contact with colouring products, their ingredients must be safe. Toxicology is the science of investigating the interaction of chemical or natural substances with living organisms. Over the past 50 years a new field of toxicology has emerged with the aim of evaluating the safety of cosmetic ingredients. Initially, safety evaluation of cosmetic ingredients included mainly local tolerance, such as skin, eye and mucous membrane irritation, and allergenic potential. The recognition that human skin is not an impermeable barrier to some topically applied substances initiated the investigation of percutaneous absorption of chemicals used in cosmetic products. Occurrence of systemic absorption requires investigation of potential systemic toxicity, such as acute, subchronic, reproductive toxicity and, if appropriate, carcinogenic potential. Finally, during the last decade of the 20th century, innovative in vitro test methods were introduced and validated; today these alternative tests are increasingly replacing in vivo safety tests.

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Risk assessment of cosmetics and their ingredients, including that of hair dyes, must take into account a highly restrictive aspect of their safety: in contrast to drugs, which are evaluated in consideration of a risk-benefit relationship, cosmetics must not be harmful to human health under normal or foreseeable conditions of use. Thus their regulation is based on a virtual, zero-risk situation, which rarely exists for any human activity or human exposure to any natural or synthetic substance. This dilemma is illustrated by a

recent EU proposal concerning cosmetic use of substances that are considered to be carcinogenic, mutagenic or toxic to reproduction (CMR substances) according to the EU Chemical Directive which, in practice, would preclude their use as cosmetic ingredients (1). Although there is general agreement that cosmetic products or their ingredients should not pose such health risks to the consumer, the relevance of this recommendation may be questioned since it is a restriction based on the potential *hazard*, and not the potential *health risk* associated with the use of such ingredients. For example, although ethanol is recognized to be a human reproductive toxicant and carcinogen, its risk to human health is negligible when it is used as, e.g., a perfume ingredient. The potential health risk of ethanol can be put into perspective when one considers the ubiquitous human exposure to natural residues of ethanol in food such as bread and fruit juices, endogenous ethanol levels in mammalian organisms or human intake of alcoholic beverages. Given that some CMR substances have valuable cosmetic properties without posing any risk to human health, there is no rationale to prohibit their use. The reconciliation of the desirable, but unachievable ideal of zero-risk in a real world, which is never free from minute risks remains the major challenge for safety regulation of cosmetic ingredients.

In the following chapter an attempt is made to propose a realistic approach to the contentious field of cosmetic toxicology, the safety of hair dyes. Hair dyes use a wide range of ingredients, and the exposed population is large, i.e., more than 60% of the female and an increasing part of the male population of industrialized countries. Numerous toxicologic and epidemiologic studies have been conducted on the safety of hair dyes and their ingredients. Although most studies indicated no or negligible risk to human health, some results appear less favorable. To illustrate the complexity of this field, examples of important hair dye ingredients that were subject to regulatory scrutiny are included, i.e., *para*-aminophenol, Lawsone, *p*-phenylenediamine, resorcinol, and others. The task of toxicologists is not only to identify possible hazards of substances, but also to assess their potential health risk under conditions of use. Thus the following attempts to shed some light on the pivotal question: does individual or professional exposure to hair dyes pose a risk to human health?

2. INTERNATIONAL REGULATORY SAFETY SCHEMES ON HAIR DYES

2.1. European Union

The key EU document for the regulation of cosmetics is the Cosmetic Directive (2), which is regularly updated and contains positive lists of approved ingredient categories (e.g., ultraviolet filters, pigments, preservatives), a negative list of substances prohibited for use in cosmetic products (Annex II of the Cosmetic Directive) and a restricted-use list (Annex III of the Directive). A list of approved coloring agents that include some direct hair dyes is in the Annex IV, Parts 1 and 2 (3). In the EU, the safety of cosmetics is reviewed by the Scientific Committee of Cosmetics and Non-Food Products (SCCNFP) of the European Commission, an independent group of expert toxicologists and dermatologists. The SCCNFP advises the EU Commission and EU member states on the

safety issues of cosmetics and has issued guidelines for the safety evaluation of cosmetic end products and their ingredients.

In the EU, cosmetic producers are responsible for the safety of their products and required to use only safe ingredients. Cosmetic manufacturers in the EU are obliged to compile a dossier in support of the safety of marketed cosmetic products; the safety dossier must be accessible upon request of the competent authorities. Safety assessment of cosmetics and their ingredients is the responsibility of a qualified safety assessor who approves use of cosmetic products and ingredients.

A safety evaluation scheme for hair dye ingredients has been proposed in the “Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation” issued by SCCNFP (4). The SCCNFP issued a discussion paper proposing additional requirements for the safety assessment of hair dyes, notably data on purity/impurities and tests on oxidative hair dye precursors alone and/or in combination with other substances under conditions of use (5). The SCCNFP Notes of Guidance emphasize the preference of alternative (in vitro) toxicity studies when validated in vitro assays are available. General safety requirements for hair dye ingredients are listed in Table 1. Overall, their safety is determined by a “Margin of Exposure” or “Margin of Safety” (MOE/MOS) approach: a nominal human systemic exposure is calculated on the basis of the amount of the hair dye ingredient applied, its in vitro percutaneous absorption rate, a standard surface area of the human scalp (600 cm^2), and a human body weight of 60 kg. The resulting “systemic exposure dose” (SED), representing a nominal human bioavailability in mg/kg body weight per topical exposure is then compared with the No-observable adverse effect-level (NOAEL, mg/kg/day) of a pivotal toxicological study. This is generally a repeated-dose study of 13 weeks’ duration performed in the species that is most sensitive to adverse effects of the compound.

The result of the calculation (NOAEL/SED) yields the MOS, an uncertainty factor, which should be at least 100-fold the NOAEL. The figure of 100 is empirical

Table 1 Safety Requirements for a New Hair Dye Ingredient in the EU

Endpoint/study type	Comment
Acute oral and dermal toxicity	—
Percutaneous absorption	In vitro study, SCCNFP Guidelines
Skin and eye irritation	—
Skin sensitisation	Maximization test (Magnuson/Kligman)
Subchronic toxicity	Generally 90-day oral toxicity in rodents
Mutagenicity/Genotoxicity	<i>Salmonella</i> assay and in vitro chromosome aberration test. Additional tests, if appropriate
Human data	HRIFT ^a or other human safety tests
Reproductive toxicity	Embryofetal toxicity, fertility, peri-/postnatal toxicity, if appropriate
Toxicokinetic studies	Optional (depending on the toxicological profile)

Metabolism studies	Optional (depending on the toxicological profile)
Long-term toxicity, carcinogenicity	Optional (depending on the toxicological profile)

^a Human repeated insult patch test. (From Ref. 4.)

and takes into account potential 10-fold variability of inter-species (human vs. animal) and intra-species (human vs. human) susceptibilities. A minimal MOS of 100-fold is applied to non-specific toxicological findings, such as a reduction in body weight or an increase in organ weights whereas, for more severe toxicological effects, higher MOS values may be applied.

2.2. United States

Although the safety of cosmetics in the United States, including that of hair dyes, is the responsibility of the U.S. Food and Drug Administration, the safety of their ingredients is largely self-regulated. To this end, in 1976 the Cosmetics, Toiletry and Fragrance Association (CTFA) established the Cosmetic Ingredient Review (CIR), an independent expert panel for the safety assessment of ingredients (6). During the past 25 years, the CIR Expert Panel has reviewed and published safety assessments on more than 650 ingredients, most of which were found to be "safe as used" or "safe under specific use restrictions."

The U.S. State of California has issued additional, more restrictive safety regulations for consumer products, the so-called "Proposition 65," which defines acceptable limits of consumer exposure ("no significant risk level" or "maximum allowable daily level") for substances known to be reproductive toxicants or carcinogens. These levels are generally very low, i.e., in the range of nanograms to micrograms/person/day. All products containing substances at concentrations that may result in consumer exposure exceeding the maximum acceptable limit must bear respective reproductive or cancer warning labels (7).

2.3. Japan and East Asia

In Japan, the safety of cosmetics products and their ingredients is regulated by the Ministry of Health and Welfare. Certain products categories, including hair dyes, skin bleaching agents and many others, are considered to be "quasi-drugs" and are subject to drug approval. This requires evidence of their safety and, if appropriate, their efficacy (8). Other Asian countries, namely Korea, Taiwan, and China are presently developing regulatory schemes for cosmetics and their ingredients.

3. HAIR DYE CATEGORIES AND CHEMISTRY

Hair dyes are classified into the following categories:

- Oxidation (permanent) dyes

- Direct (temporary or semi-permanent) dyes
- Metal salts
- Natural dyes.

Oxidation hair dyes are by far the most important group and have a market share in the EU or the United States of approximately 80%. They differ from the other categories since they consist of two components that are mixed before use. Modern oxidative dyes contain several classes of ingredients with different functions:

- *Primary intermediates*: *para*-phenylenediamine(PPD), *para*-toluylenediamine (PTD), substituted *para*-diamines, *ortho*- or *para*-aminophenols (Fig. 1).

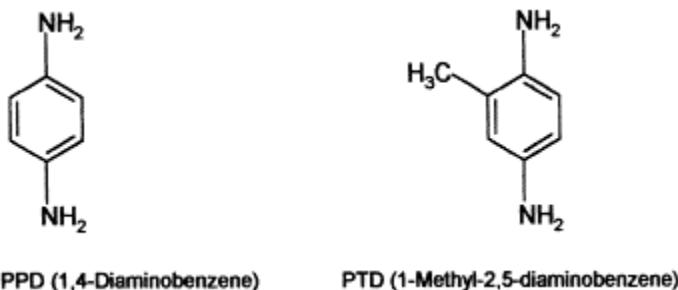


Figure 1 Structures of primary oxidative hair dye intermediates: *para*-phenylenediamine (PPD) and *para*-toluylenediamine (PTD).

Oxidation of these substances and coupling with modifiers result in colored reaction products.

- *Couplers or modifiers*: *meta*-substituted aromatic derivatives such as *m*-phenylenediamines, *m*-aminophenols and resorcinol (Fig. 7). They determine the final shade by reaction with the oxidized form of primary intermediates, followed by further oxidative coupling reactions.
- *Oxidants*: hydrogen peroxide, urea peroxide, sodium percarbonate, and perborate.
- *Alkalinating agents*: ammonia, monoethanolamine, or aminomethylpropanol.

The second category of economic importance are direct dyes, which include semi-permanent (resist six to 12 shampoos) and temporary dyes (removed by one shampoo). Temporary coloring agents include azo-, triphenylmethane-, anthraquinone- or indamine dyes, whereas semi-permanent coloring agents contain *nitro*-phenylenediamines, *nitro*-aminophenols and azo dyes. Metal salts are mainly used for coverage of graying hair and are generally based on lead acetate, which is restricted to a maximum of 0.6% lead content in the EU.

Natural dyes extracted from plants are of a relatively small but growing economic importance. The majority of natural dyes use henna [produced by extraction of the leaves of a North African shrub (*Lawsonia inermis*)] or its pure dye ingredient (Lawsone, i.e., 2-hydroxy-1,4-naphthoquinone).

4. TOXICOLOGICAL ASPECTS OF HAIR DYES

4.1. Acute Toxicity/Human Poisoning

The results of acute toxicity tests suggest that hair dye ingredients have moderate to low toxicity. Cases of accidental human poisoning by hair dye ingredients are extremely rare and have only been reported following oral ingestion. Although sporadic cases have been reported for *p*-phenylenediamine (PPD), these were generally a consequence of attempted suicide or homicide. The principal adverse effect of high acute doses of PPD in man and higher mammals was angioneurotic edema leading to acute respiratory distress and acute renal failure secondary to rhabdomyolysis, i.e., necrosis of skeletal muscle (9). In most of these cases, the form of ingested PPD consisted of concoctions based on solid or powdered PPD. Although such forms permit the oral intake of high doses, the low concentrations in standard hair dye formulations used today in industrialized countries (maximum 2% PPD in a 100 mL dye solution) make the possibility of accidental poisoning negligible.

The results of acute dermal toxicity studies suggest that hair dye ingredients are non-toxic via the topical route. With the exception of allergy or rare cases of irritation due to the presence of alkaline additives, hair dyes have a good local tolerance under standard conditions of use.

4.2. Contact Allergy

Para-phenylenediamine, one of the most common primary intermediates of oxidative hair dyes, and derivatives such as *para*-toluylenediamine (PTD), have a potential to elicit contact allergy. This has been confirmed by numerous animal and human studies (5). Consequently, labels of commercial oxidative hair dyes in the EU bear warning phrases such as "Can cause allergic reactions. Contains phenylenediamines. Do not use to dye eyelashes or eye brows." Consumers are advised to perform an allergy test prior to the use of the product, whereas hairdressers are strongly advised to prevent skin exposure by wearing gloves during the hair dyeing process (Table 9).

In the United States, hair dye labels bear similar warning phrases, such as "This product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should first be made. This product must not be used for dyeing of the eyelashes or eyebrows: to do so may cause blindness." The warning for blindness is due to the presence of alkaline additives (e.g., ammonia) in hair dye formulations. However, there is no evidence that the use of hair dyes around the eye may cause blindness in humans. This is confirmed by the results of tests in animal models, which showed that hair dye formulations cause no permanent damage to the eye (10).

The allergenic potential of PPD and its derivatives remains an important cause of cosmetic allergy, particularly in cosmeticians and hairdressers. Standard patch tests in Germany reported an incidence of contact allergy against PPD in 4.8% of 36,000 eczema patients, PPD being the fifth most frequent contact allergen after nickel, fragrance mix, balsam of Peru, and thimerosal (11). However, the incidence in eczema patients is unlikely to be representative for the entire population.

The incidence of PPD-induced allergy appears to have decreased in recent years, despite the growing use of hair dyes in the industrialized world. For example, the North American Contact Dermatitis Group reported a decreasing "Significance Prevalence Index Number" (the relative clinical importance of contact allergens in the population) for allergenic reactions to PPD, i.e., from rank 3 (1984) to rank 10 (1996) (12). This decrease has been attributed to the successful introduction of risk management measures, i.e., precautionary labeling, increasing compliance with occupational safety measures, such as wearing protective gloves, and the increased risk awareness of the consumer (13). Although the majority of hair dye allergy cases are due to PPD and/or its derivatives, some direct dyes (nitro- and anthraquinone class dyes) are also known to have sensitizing potential; however, the incidence of human allergy to these dyes appears to be low.

In summary, some ingredients of oxidative and direct hair dyes possess allergenic potential. Taking into account their widespread use and the huge number of consumers exposed, they cause relatively few adverse reactions in the order of one per 1 million applications. Today, occupational allergy has been successfully managed by simple precautionary measures and strict control of the purity of dye ingredients.

4.3. Genetic Toxicity

Genetic toxicity tests were developed as short-term screening studies for the prediction of the carcinogenic potential of substances. One of the first genetic toxicity tests was the *Salmonella* assay (Ames test), which measures the incidence of reverse mutations in histidine synthesis-deficient mutants of *Salmonella typhimurium*. In 1975, the results of a *Salmonella* test started the controversy about the genotoxic and/or carcinogenic potential of hair dyes, when Ames et al. suggested that nearly 90% of oxidative hair dye ingredients were mutagenic in *Salmonella typhimurium* and might therefore pose a carcinogenic risk to consumers (14).

In the meantime, a number of publications have reported positive results of hair dye ingredients in *Salmonella* and other *in vitro* genotoxicity assays. Although a recent review of the *Salmonella* assay suggested a correlation of 77% for a series of 368 rodent carcinogens and non-carcinogens (15), the experience of the past 30 years suggests that positive results in *Salmonellae* are poorly predictive for the carcinogenic potential of aromatic amines, the chemical class of the primary intermediates of oxidative hair dyes. Aromatic amines, whether carcinogenic in rodents or not, tend to be positive in the *Salmonella* assay. For example, 33 *Salmonella*-positive aromatic amines were found to be non-carcinogenic when tested by the U.S. National Toxicology Program in mice and rats (16,17). Conversely, a pure sample of the direct hair dye, HC Blue 1 (3,3'-(3,3'-dimethoxy-4,4'-biphenylene)bis(azo)bis(5-amino-4-hydroxy-6,8-naphthalenedisulfonic acid, tetrasodium salt) was negative in the *Salmonella* assay, although the substance was carcinogenic in mice and, possibly, in rats (18,19). Similarly, the results of *in vitro* mutagenicity tests in mammalian cells such as the thioguanine resistance (HPRT) test in V 79 cells, the Mouse Lymphoma TK +/- assay and traditional cell transformation assays were shown to lack predictivity for rodent carcinogenicity of aromatic amines (18,20) (Table 2).

Overall, it is nearly impossible to perform *in vitro* genotoxicity tests under conditions that are identical to the complex oxidative hair coloring process. For example, the

standard protocol of *Salmonella* or other in vitro assays requires incubation of the test organism with the test substance for many hours, whereas the oxidative hair dye development on human hair is completed within 30 min. In addition, most in vitro genotoxicity investigations on hair dyes attempted to eliminate hydrogen peroxide, a key ingredient that is cytotoxic and potentially mutagenic (21), thereby increasing the variability of the test conditions and their results.

Given the wide variety of test conditions, it is not surprising that the results of in vitro genotoxicity studies on oxidative hair dyes were inconsistent. For example, PPD tested alone was negative in *Salmonella*, but positive when left standing at room temperature for 4 hr (22). Even though PPD was found positive in *Salmonella* in combination with hydrogen peroxide only (23), commercial hair dyes always include couplers such as resorcinol. Another study reported that the topical application of a “green reaction product” in rats, from the reaction of a mixture of PPD, hydrogen peroxide and resorcinol, resulted in a *Salmonella*-positive metabolite in their urine (24). However, the test substance of the study was prepared by a 1 hr chemical reaction of an untypical dye mixture (PPD and resorcinol in a 1:3 ratio) that is not representative of actual hair dye reaction products (they typically contain PPD and

Table 2 Predictive Power of In Vitro Genotoxicity Tests for the Rodent Carcinogenic Potential of Hair Dye Ingredients

Chemical	Carcinogenicity (NTP)	Salmonella assay	Mouse lymphoma assay	Cell transformation test ^a
2,4-diaminoanisole	+	+	+	+
4-amino-2-nitrophenol	+	+	+	NT
2-nitro-p-phenylenediamine	–	+	+	+
4-nitro-p-phenylenediamine	–	+	+	+
<i>m</i> -phenylenediamine	–	+	+	+
<i>p</i> -phenylenediamine	–	+	+	+
<i>p</i> -toluylenediamine	–	+	NT ^b	NT
HC Blue No. 1	+	+	NT	NT
HC Blue No. 2	–	+	NT	NT
HC Red No. 3	–	+	NT	NT
Disperse Blue No. 1	+	+	NT	NT
2-amino-4-nitrophenol	–	+	NT	NT

2-amino-5-nitrophenol	-	+	NT	NT
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(+)=Presence, (-)=absence of genotoxic/carcinogenic activity.

^a (Non-modified) Syrian hamster embryo cell transformation test.

^b Not tested (mouse lymphoma and cell transformation tests were discontinued by the NTP on more recently assayed hair dyes due to excessive levels of false-positive results).

(From Ref. 18.)

resorcinol in an approximate 1:1 ratio). In addition, the duration of exposure was not representative of the oxidative hair dyeing process. Moreover, the conditions of the chemical reaction described in the study are likely to result in polymeric dye products of high molecular weight, which are unlikely to penetrate the skin to an appreciable extent. Therefore, significant systemic exposure or excretion of mutagenic substances in the urine after topical administration appears implausible.

An in-depth study revealed that fresh solutions of hair dyes containing PPD, hydrogen peroxide and resorcinol at realistic proportions were non-mutagenic in *Salmonella* TA98 up to 1 hr; however, when stored for 5 or 7 hr at room temperature, they became weakly positive. The reaction of PPD and hydrogen peroxide alone yielded mutagenic activity at 1, 3 and 7 hr of storage (25) (Table 3). These results confirm that mutagenic reaction products may be generated by oxidative hair dye mixtures in the absence of couplers, or when allowed to react beyond 1 hr (26). Additional investigations confirmed that the reaction products of PPD and hydrogen peroxide alone were genotoxic in *Salmonella*, mouse lymphoma cells and human lymphocytes; however, genotoxicity could be prevented by adding the correct proportion of resorcinol and using realistic exposure duration. The genotoxic activity was believed to be due to the formation of Bandrowski's base, a known mutagenic and sensitizing reaction product of PPD and hydrogen peroxide (Fig. 2) (25). These results were confirmed by an earlier study, which detected a reduced mutagenic activity of hair dye formulations after adding couplers such as resorcinol (27).

Overall, the presence or absence of in vitro genotoxic activity of oxidative hair dye mixtures appears to depend on the proportion of the primary intermediate to the coupler(s), their reaction time and the presence and concentration of hydrogen peroxide and other chemicals. Although PPD and hydrogen peroxide alone or an excess of PPD may result in the formation of reactive, potentially sensitizing and genotoxic by-products such as Bandrowski's base, the excess of couplers in commercial hair dyes prevents the formation of genotoxic substances. Overall, the results of all studies suggest that a correct ratio of PPD to couplers and hydrogen peroxide and a realistic exposure time (<1 hr) prevents genotoxicity.

Table 3 Number of Revertants per Plate^a of *Salmonella* TA98 (+S 9) Exposed to *p*-Phenylenediamine+3% H₂O₂ Alone and *p*-

Phenylenediamine+3% H₂O₂+Resorcinol in Fresh and Aged Oxidative Hair Dye Solutions

Test article	Concentration (μmol/plate)	0	0.5	1	3	7 hr
Vehicle control	0	26	30	23	33	28
PPD+3% H ₂ O ₂	1.38	71	120	123	180	237
PPD+3% H ₂ O ₂	2.75	82	126	97	143	302
PPD+3% H ₂ O ₂	5.50	96	55	52	90	250
PPD+resorcinol+3% H ₂ O ₂	0.69+0.83	43	38	46	81	169
PPD+resorcinol+3% H ₂ O ₂	1.38+1.65	52	52	53	95	201
PPD+resorcinol+3% H ₂ O ₂	2.75+3.30	57	45	62	100	250
PPD+resorcinol+3% H ₂ O ₂	5.50+6.60	55	44	42	82	110
Positive control (1 μg 2-aminofluorene)						168±14

^a Mean of two plates; standard deviations not shown.

(From Ref. 25.)

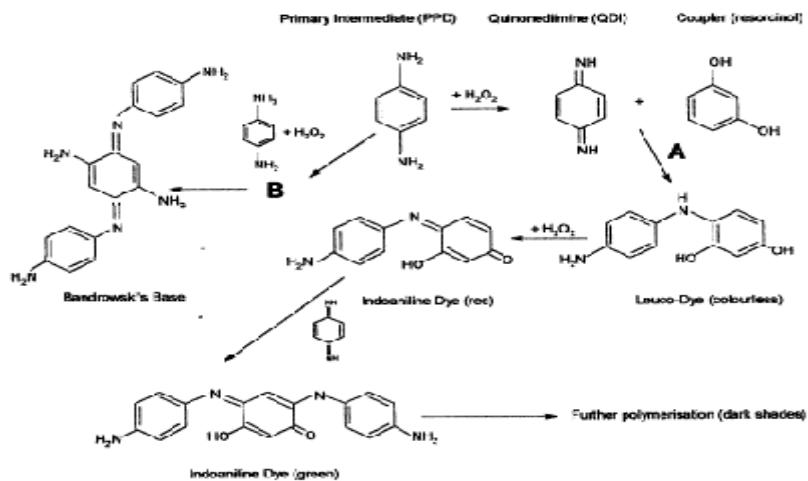


Figure 2 Theoretical chemical pathways of oxidative dye formation. Reaction pathway (A) in the presence of a coupler (resorcinol) results in the desired dye. Reaction pathway (B) may occur in the absence of couplers,

resulting in formation of Bandrowski's base, a genotoxic and sensitizing by-product (From Ref. 26.).

A recent in vitro genotoxicity/carcinogenicity test, the modified (pH 6.7) Syrian hamster embryo (SHE) cell transformation assay appears to have a superior predictive power over the *Salmonella* test for assessing rodent carcinogenicity of aromatic amines: for example, 10 single-ring aromatic amines or nitro-amines (five rodent carcinogens and five non-carcinogens) were all positive in *Salmonella*, whereas the results of the SHE assay yielded a 100% correlation with rodent carcinogenicity or non-carcinogenicity. PPD was negative in this test (28) (Table 4). In an extensive validation study on 30 rodent carcinogens, 18 rodent non-carcinogens and eight inconclusive substances, the SHE assay revealed an exceptional degree of sensitivity (87%) and specificity (83%), including a high sensitivity for *Salmonella*-negative rodent carcinogens (78%). Interestingly, the SHE assay was able to distinguish the *Salmonella*-positive rodent carcinogen 2,4-diaminotoluene from its equally *Salmonella*-positive, but non-carcinogenic isomer 2,6-diaminotoluene (29).

In general, reports of positive in vivo genotoxic activity of hair dyes are rare. A number of studies in humans were published in the scientific literature. Two studies in humans exposed to up to 13 cumulative hair dye applications found no increased incidence in sister chromatid exchanges (SCEs) or chromosome breaks/aberrations in peripheral lymphocytes (30,31). No increase in SCEs over control values was found in lymphocytes of 13 female volunteers at 6 hr or 7 days after application of a commercial hair dye (32), and no increased incidence of chromosome breaks or aberrations were detected in 60 professional hairdressers, when compared with an unexposed control group (33). Moreover, a group of 15 heavily exposed hairdressers who had an extensive history of work without protective measures showed no increased incidence of DNA strand breaks or SCEs in peripheral lymphocytes or *Salmonella*-positive mutagenic activity in their urine (34). No increase in mutagenic activity was found in urine concentrates of a group of female users prior to and after hair dyeing with dark shade dyes that have a high content of aromatic amines (35). Thus all human studies consistently yielded negative results for genotoxicity.

It is important to note that, even when in vivo genotoxic activity of a hair dye ingredient has been identified, such findings do not necessarily indicate a human health risk. For example *para*-aminophenol (PAP), a primary ingredient of hair dyes, was reported to be positive in in vivo genotoxicity assays, causing an increased incidence of micronuclei in the bone marrow and splenocytes of mice (37). In addition, PAP was positive in several in vitro assays, such as chromosome aberration, mouse lymphoma, and sister chromatid exchange tests. On the contrary, *Salmonella* assays, bone marrow chromosome aberration and dominant lethal tests in rats, SCEs in peripheral lymphocytes of human volunteers or the sex-linked recessive assay in *Drosophila* had negative results. The absence of a carcinogenic potential was confirmed by negative results of an oral carcinogenicity study in rats (38). Overall, these data suggest that, although PAP is non-carcinogenic in rodents, it may pose a potential in vivo genotoxic hazard according to the EU chemical classification of "CMR Class 3"—provided that the use of PAP in hair dyes results in human systemic exposure. However, there is evidence that this is not the case.

Table 4 Single-Ring Aromatic/Nitro-Aromatic Amine Compounds: Correlation of Test Results in the *Salmonella* and Modified (pH 6.7) Syrian Hamster Embryo (SHE) Cell Transformation Assays with Rodent Carcinogenicity

Substance	Salmonella assay	SHE assay	Rodent carcinogenicity rating (U.S. NTP)
2-Amino-4-nitrotoluene	+	+	+
2,4-Diaminotoluene	+	+	+
2,4-Dinitrotoluene	+	+	+
<i>o</i> -Anisidine hydrochloride	+	+	+
<i>o</i> -Toluidine hydrochloride	+	+	+
2,6-Diaminotoluene	+	-	-
2,4-Dimethoxyaniline hydrochloride	+	-	-
4-Nitro- <i>o</i> -phenylenediamine	+	-	-
HC Blue No. 2	+	-	-
<i>p</i> -Phenylenediamine	+	-	-

(+)=Presence, (-)=Absence of genotoxic/carcinogenic activity.

(From Ref. 28.)

The results of in vitro metabolism studies suggested that human skin quantitatively transformed PAP into an acetylated metabolite, *N*-acetyl-*p*-aminophenol, i.e., paracetamol (APAP, acetaminophen), a common analgesic and antipyretic drug (Fig. 3). Further in vivo metabolism/toxicokinetic studies in rats and non-rodents revealed that topical application of ^{14}C -PAP resulted in minimal blood concentrations (nanogram range) of ^{14}C -equivalents (Fig. 4), that were shown to consist of APAP and/or its conjugates. ^{14}C -PAP could not be detected in blood, although the studies were performed under maximized exposure conditions (high topical doses, large application surface area, 24-hr exposure under occlusion). Thus the evidence suggests that topical application of PAP results in systemic exposure to APAP, and not to PAP.

The comparison of the quantitative systemic exposure dose in the two animal models after topical application of PAP ($\text{AUC}_{0-12\text{hr}}$ values) with human data, after the intake of a single oral standard therapeutic dose of 500 mg APAP yielded toxicokinetic safety margins of 22- to >700-fold (Fig. 4). Taking into account that

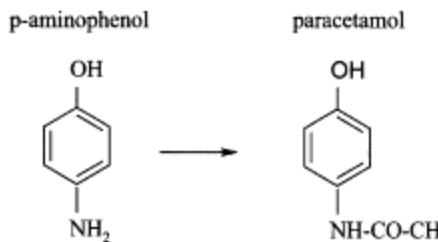


Figure 3 In vitro metabolism of *p*-aminophenol (PAP) in re-constructed human epidermis (Episkin®, Epishin SNC, Lyon, France).

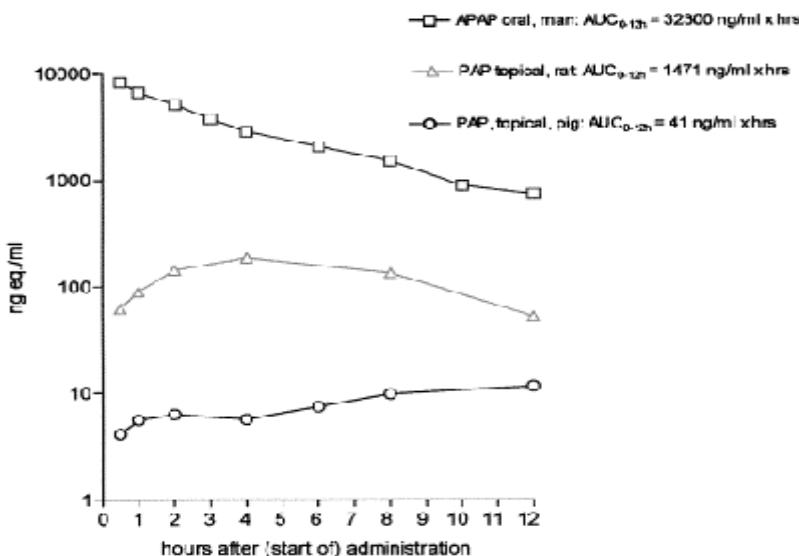


Figure 4 Plasma concentrations after a single therapeutic oral dose of 500 mg paracetamol (APAP) in human volunteers, compared with plasma concentrations (^{14}C -ng Eq/mL) in rats or a pig after topical treatment (10% of body surface, 24-hr exposure under occlusion) with 12.5 or 4.7 mg/kg ^{14}C -*para*-aminophenol (PAP), respectively. (From Refs. 38,106.)

APAP (paracetamol) is (a) a widely used human drug, (b) a pediatric drug, (c) recommended for daily use at an *oral* dose of up to 2 g (4×500 mg), and (d) toxicokinetic-based safety margins of >25-fold are considered to reflect a high degree of safety (39,109), the intermittent and minimal human systemic exposure to traces of APAP following a hair dyeing process with PAP clearly poses no or negligible risk to human health.

This example underlines the importance of metabolism and toxicokinetic data and illustrates that *oral* toxicity data resulting in a high exposure to the parent compound may be irrelevant for the hazard assessment of a topically applied substance that is quantitatively metabolized in the skin.

Another example of a hair dye substance with positive *in vivo* genotoxic activity is Lawsone, the natural dye ingredient contained in Henna (Fig. 5). Topical use of Lawsone as a hair dye has a highly favorable toxicokinetic-based margin of safety, when compared with the systemic exposure after oral administration (Fig. 6). Lawsone has an interesting toxicity and genetic toxicity profile: it was hematotoxic in oral subchronic rodent and positive/borderline-positive in *in vitro* genotoxicity studies (mouse lymphoma assay, chromosome aberration test in CHO cells), but negative in *Salmonella* and CHO-HPRT tests. Although negative in a series of *in vivo* assays (bone marrow chromosome aberrations in hamsters, UDS test in rats,

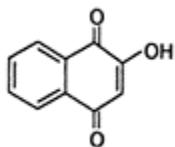


Figure 5 Structure of Lawsone (2-hydroxy-1,4-naphthoquinone), the natural dye of henna, contained in the leaves of the North African brush *Lawsonia inermis*.

sex-linked recessive lethal assay in *Drosophila*, chromosome aberrations in peripheral lymphocytes of subchronically treated rats), Lawsone produced a weak, but statistically significant increase in micronuclei in the bone marrow of mice at 72 hr after oral administration, whereas no response was observed at 24 or 48 hr. On the basis of these data, Lawsone was considered to have an *in vivo* genotoxic potential, corresponding to a “CMR 3 substance,” according to present EU chemical regulations.

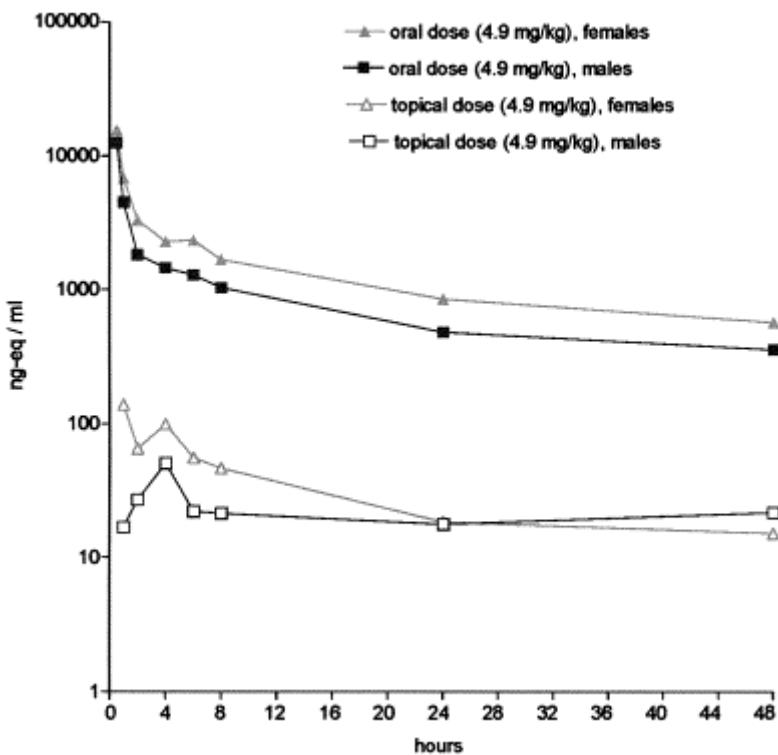


Figure 6 Plasma concentrations (ng^{14}C Eq/ml) in Sprague-Dawley rats after single oral or dermal (10% of body surface area) administrations of 4.9 mg/kg ^{14}C -2-hydroxy-naphthoquinone (Lawson). Calculated $\text{AUC}_{0-48\text{hr}}$ values were 74 and 117(oral), or 1.1 and $1.3 \mu\text{g} \times \text{hr} \times \text{mL}^{-1}$ (topical) for males and females, respectively. (From Ref. 99.)

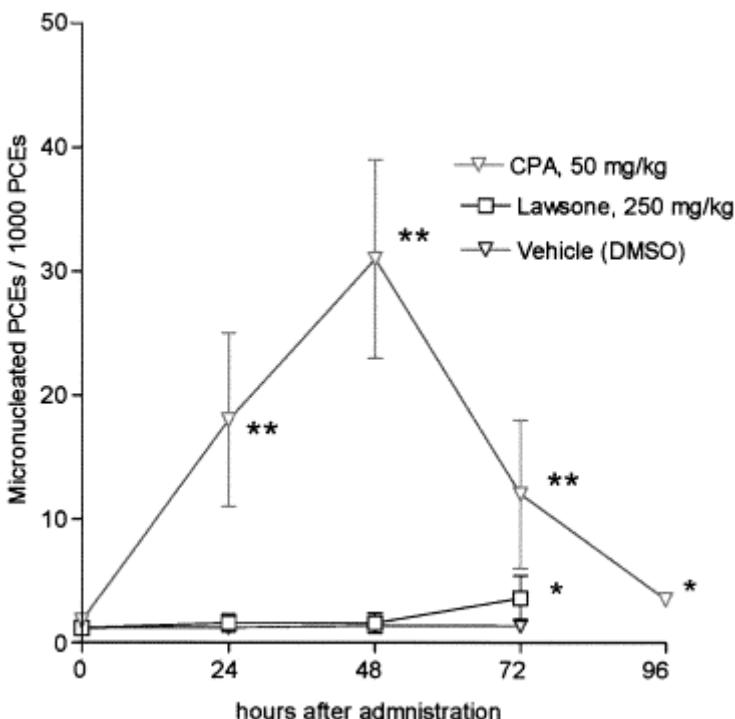


Figure 7 Kinetics of micronuclei in the bone marrow of mice after oral administration of the in vivo clastogenic agent cyclophosphamide (CPA) or Lawsone (* $P<0.05$; ** $P<0.01$). (From Refs. 40,42.)

However, even slow-acting in vivo clastogens cause a maximal increase in micronuclei in mice as soon as 48 hr after administration (Fig. 7). Since all known in vivo clastogenic (chromosome-damaging) agents produce significant increases in bone marrow micronuclei as early as 24–48 hr after administration (therefore, the 72-hr sampling point has been abandoned in modern test guidelines), the delayed and weak activity of Lawsone is inconsistent with a known in vivo clastogenic activity. Therefore, a non-genotoxic mechanism is likely to be responsible for the weak and delayed response.

A similar, slight and delayed increase in the incidence of micronuclei also occurs at 72–120 hr in mice following bleeding, administration of hematotoxic agents such as phenylhydrazine (40) (Fig. 8), or administration of erythropoietin (41), all of which stimulate erythropoiesis. Therefore, the mechanism of the slight and delayed increase in the incidence of bone marrow micronuclei is likely to be consistent with the known hematotoxicity of Lawsone, rather than an intrinsic in vivo genotoxic activity of the substance. A non-genotoxic mechanism was supported by additional micronucleus tests

and bone marrow chromosome aberration studies in mice, all of which had negative results (42) (Table 5).

The lack of specificity of in vitro genotoxicity assays for aromatic amines together with the confounding role of hydrogen peroxide, the presence or absence of couplers and different test conditions have resulted in 30 years of continuous and continuing discussion about the relevance for human risk of in vitro genotoxicity results on hair dyes. For example, a recent EU opinion concluded that PPD in

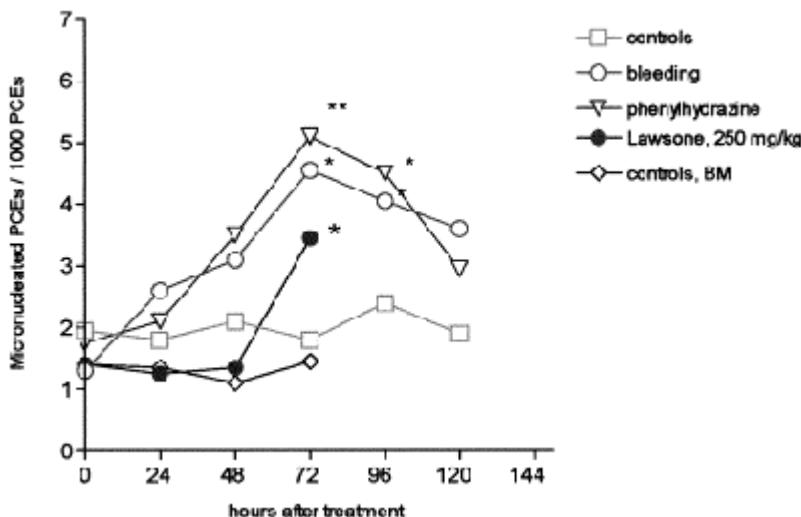


Figure 8 Appearance of micronucleated PCEs in mice after bleeding, 50 mg/kg of the hemolytic agent phenylhydrazine (peripheral blood) or a single dose of Lawsoe at 250 mg/kg (bone marrow) (* $P<0.05$; ** $P<0.01$). (From Refs. 40,42.)

combination with hydrogen peroxide may be potentially mutagenic. Typically, the conclusion mainly relied on *Salmonella* results, although more specific genotoxicity tests, such as the in vivo Comet test and a series of in vivo investigations (bone marrow micronucleus, *Drosophila* or UDS tests), as well as multiple carcinogenicity studies on PPD and PPD-containing hair dyes were negative.

In summary, the complexity of the genotoxicity data on PPD, Lawsoe and *p*-aminophenol underlines the difficulty of a definitive assessment of the relevance of in vitro or in vivo genotoxicity tests for the assessment of a human health risk of hair dyes, keeping in mind that genotoxicity data on their own are only a *surrogate*

Table 5 Lawsone—Bone Marrow Chromosome Aberrations in Mice at 24 or 72 hr After Oral Administration of Lawsone and Cyclophosphamide

Test	Vehicle control	Lawsone (250 mg/kg)	Cyclophosphamide (50 mg/kg)
Total of structural chromosome aberrations per 1000 cells, 24 hr	14 (4) ^a	15 (4)	174 (143)
Percentage of cells with structural chromosome aberrations, 24 hr	1.3 (3)	1.4 (0.7)	33.2 (31.2)
Total of structural chromosome aberrations per 1000 cells, 72 hr	4 (2)	5 (3)	—
Percentage of cells with structural chromosome aberrations, 72 hr	0.4 (0.2)	0.6 (0.4)	—

^a Figure in brackets represent aberrations excluding gaps.

(From Ref. 42.)

endpoint for rodent carcinogenicity, and their positive results do not necessarily suggest a carcinogenic potential in humans. Given the poor specificity of the *Salmonella* assay for aromatic amines, the question may be raised whether this test should be performed on hair dye ingredients at all. Without consideration of the preceding chemical reactions of the test article, class effects, the nature of human systemic exposure (metabolism) and its magnitude (toxicokinetics), an uncritical application of genotoxicity data for human risk assessment of hair dye use appears unlikely to yield meaningful information. Most importantly, one should take into consideration the weight of evidence: not a single study performed in humans has yielded a hint of evidence for a possible human genotoxic risk of hair dyes.

4.4. Carcinogenicity

The potential carcinogenicity of hair dyes has attracted the attention of toxicologists for more than 100 years, mainly due to the fact that some hair dye ingredients belong to the large family of aromatic amines. This class includes known human carcinogens, such as benzidine, 4-chloro-2-methylaniline, 4-aminobiphenyl and 2-naphthylamine, some of which were recognized to cause an increased incidence of bladder cancer in workers of the dye industry as early as 1898. The potential carcinogenicity of aromatic amines and hair dyes has been reviewed by the International Agency for Research of Cancer of the World Health Organization (43) and comprehensive reviews of the toxicology/carcinogenicity of aromatic amines were published in recent toxicology textbooks (44).

Although some aromatic amines are known to be human or rodent carcinogens, many substances of this large chemical class have no carcinogenic activity. Given the potential class hazard of aromatic amines, the carcinogenic potential of most major hair dye

ingredients has been investigated by the dye and hair dye industries, the U.S. National Toxicology Program and independent investigators.

4.4.1. Epidemiology

Numerous epidemiological studies have been published on cancer incidence in hair dye users and professionally exposed populations. Although a recent case-control study reported an increased incidence of cancer of the urinary bladder in hair dye users (45), the majority of studies, including a series of cohort studies suggested no association of hair dye exposure with increased human cancer risk, including bladder cancer (43). Taking into account the greater reliability of cohort over case-control studies, recent reviews of the association of hair dyes and bladder cancer concluded "that the available evidence, despite some inconsistency, excludes any appreciable risk of bladder cancer from personal use of hair dyes" (46,47).

A review of Japanese data on the association of hair dye use and exposure with cancer of the urinary bladder, breast, lung and other sites came to a similar conclusion, i.e., no or little evidence of a relation of hair dyes and human cancer (48). These findings are particularly significant given that Japan predominantly uses dark shade hair colors that have a high content of aromatic amine ingredients.

A large prospective study on the cause of death of more than 547,000 women over a 7–12-year period found that hair dye use was unlikely to be a contributing factor for non-Hodgkin's lymphoma and multiple myeloma in the United States (49). The absence of a cancer risk was confirmed by the U.S. Nurse's Health Study, a prospective epidemiological investigation on a population of 121,700 nurses that started in 1976 and monitored hair dye use and incidence of a range of cancers in this population (50). Moreover, a recent review of the results of 20 different epidemiological studies on the association of hair dye use and breast cancer in hairdressers concluded that (a) the association of hair dye use and breast cancer was well-researched and (b) there is no convincing evidence for an increased risk of breast cancer in hair dye users (51).

Although the incidence of mainly smoking-related bladder cancer appears to be increasing in developed countries, a stabilization of the bladder cancer incidence in women and a decrease in men was noted in the United States during the decade of 1990 to 1999 (presumably due to cessation of smoking), although the use of hair dyes increased in both men and women during the preceding period (52).

In summary, none of the criteria for causality recognized by modern epidemiology, i.e., (a) strength of association, (b) consistency of association in different studies, (c) specificity of a cause resulting in a single effect, (d) magnitude of the association, (e) presence of a biological gradient (exposure-response), and (f) biological plausibility (53,54) applies to the various studies on a potential association of hair dye use and human cancer risk.

4.4.2. Results of In Vivo Carcinogenicity Testing on Hair Dyes

A large number of studies on the carcinogenicity of hair dyes and their ingredients are available in the literature; their results were reviewed in a recent textbook (10). An early 2-year skin painting study in rats using twice-weekly application of the dye ingredients *p-*

toluylenediamine (PTD), resorcinol and *m*-diaminoanisole found no evidence of systemic toxicity or carcinogenicity (55,110). Numerous oxidative hair dye mixtures were tested in carcinogenicity studies under conditions corresponding to human exposure, i.e., topical application of commercial formulations that included coupler substances and hydrogen peroxide. A topical carcinogenicity study on the hair dye ingredients *p*-aminonitrophenol, sodium thioglycollate and *p*-phenylenediamine in mice and rabbits for up to 130 weeks found no evidence of systemic toxicity or carcinogenicity (56). Lifetime topical application in mice of three different commercial hair dye formulations containing *p*-phenylenediamine, 2,5-toluylenediamine, resorcinol, *m*-phenylenediamine, 2,4-diaminoanisole and 2,4-toluylenediamine found no evidence of systemic toxicity or carcinogenicity (57). In another study, 23-month once-weekly topical application of 12 different hair dye formulations to mice resulted in no evidence of systemic toxicity or carcinogenicity (58).

An in-depth study investigated the reproductive toxicity and carcinogenicity of six different hair dye formulations by twice weekly topical application in rats for two generations, followed by a complete 2-year topical carcinogenicity study; three of the commercial formulations tested contained PPD (concentration range: 2.0–4.0%). No evidence of adverse reproductive effects, systemic toxicity or carcinogenicity was found (Table 6) (59). Interestingly, two carcinogenicity studies in mice topically treated with 2,4-toluylenediamine alone or hair dye formulations containing 2,4-toluylenediamine, PPD and resorcinol revealed no evidence of systemic toxicity or carcinogenicity (57,60), although 2,4-toluylenediamine alone was found to be carcinogenic in subsequent oral carcinogenicity tests performed by the U.S. National Toxicology Program (NTP) (61). Overall, with the exception of occasional local irritation, no evidence of adverse effects including systemic toxicity or carcinogenic activity was observed when commercial formulations were tested in rodent carcinogenicity studies via the route of human exposure.

Table 6 Topical Combined Multi-generation Reproduction/Carcinogenicity Study in Rats on Six Commercial Hair Dyes (No. 7401–7406) Containing Various Ingredients. No Evidence of Reproductive Toxicity, Systemic Toxicity, or Carcinogenicity Was Observed

Ingredient (%)	No. 7401	No. 7402	No. 7403	No. 7404	No. 7405	No. 7406
<i>p</i> -Phenylenediamine	2.0	1.0	—	—	—	4.0
<i>p</i> -Toluylenediamine	—	3.0	6.0	—	—	—
2,4-Diaminoanisole	2.0	4.0	—	—	—	—
2,5-Diaminoanisole	—	—	—	—	6.0	—
Resorcinol	1.7	1.7	—	1.0	2.0	—
<i>m</i> -Aminophenol	—	—	0.7	—	—	0.7

<i>p</i> -Aminophenol	—	—	1.0	—	—	—
2-Nitro- <i>p</i> -phenylenediamine	1.1	—	—	—	—	—
4-Nitro- <i>o</i> -phenylenediamine	—	—	0.25	—	—	—
1-Naphthol	—	—	0.50	—	—	—
<i>N</i> -Methyl- <i>p</i> -aminophenol	—	—	—	1.0	—	—
<i>p</i> -Aminodiphenylamine	—	—	—	2.0	—	—
<i>m</i> -Phenylenediamine	—	—	—	1.5	—	—
<i>o</i> -Phenylenediamine	—	—	—	1.0	—	—
<i>o</i> -Aminophenol	—	—	—	—	0.3	—
2-Amino-4-nitrophenol	—	—	—	—	0.4	—
2-Amino-5-nitrophenol	—	—	—	—	—	0.5
4-Chlororesorcinol	—	—	—	—	—	2.0

(From Ref. 59.)

A series of dye ingredients was tested for their carcinogenic potential within the framework of the U.S. NTP. Given that NTP studies are designed to investigate the potential *hazard* and not the *risk* of substances to human health, the studies used daily *oral* administration of doses up to the maximum tolerated dose (MTD) via dietary exposure or daily oral gavage of mice and rats. Although the majority of hair dye ingredients were found to be non-carcinogenic, some ingredients such as 2,4-diaminoanisole, 2,4-toluylenediamine and HC Blue 1 were carcinogenic in rodents and were assessed as having “clear evidence for carcinogenicity,” i.e., carcinogenic in both species, multiple organs and/or both sexes. As a result, these substances are no longer used by the hair dye industry. Other hair dyes were rated by the NTP as having “some evidence for carcinogenicity” or “equivocal evidence for carcinogenicity,” i.e., effects were limited to one species and/or sex (Table 7). In response to the NTP findings, a series of hair dye ingredients were banned or restricted in the EU (Tables 8 and 9).

It may be argued that some of the results of the NTP studies have only limited relevance for an assessment of the human health risk when mechanistic aspects and/or potential human exposure is taken into account. The following examples show the difficulty of assessing the meaning of some NTP data for human health risk.

The direct dye ingredient Disperse Blue 1 (9,10-anthracendion) caused bladder tumors in rats at the highest dose tested, i.e. 217–240 mg/kg/day, resulting in the classification of “clear evidence of carcinogenicity in rats” (19). However, the animals at the highest dose had a high incidence of urinary bladder calculi consisting of crystalline dye, suggesting that tumors resulted from a well-known secondary mechanism, i.e., tumor formation subsequent to chronic, mechanical irritation of bladder tissue by dye calculi. This mechanism was supported by the presence or absence of calculi and bladder tumors in individual, tumor-bearing or non-tumor-bearing animals,

Table 7 Assessment of the Results of Oral Carcinogenicity Studies on Oxidative or Direct Hair Dye Ingredients Performed by the U.S. National Toxicology Program

Dye ingredient	Rat (Fischer 344)	Mouse (B6C3F1)	Overall carcinogenicity rating
HC Blue 2	NE	NE	Negative
HC Yellow 4	EE(M); NE(F)	NE	Negative
HC Red 3	NE	EE(M); NE(F)	Negative
Acid Orange3	NE(M); CE(F)	NE	Positive
Disperse Blue 1	CE(M); CE(F)	EE(M); NE(F)	Positive
HC Blue 1	EE(M); CE(F)	EE(M); NE(F)	Positive
2,5-Diaminotoluene	NE	NE	Negative
<i>p</i> -Phenylenediamine	NE	NE	Negative
4-Nitro- <i>o</i> -phenylenediamine	NE	NE	Negative
<i>N</i> -Phenyl- <i>p</i> -Phenylenediamine	NE	NE	Negative
2-Chloro- <i>p</i> -phenylenediamine	NE	NE	Negative
Resorcinol	NE	NE	Negative
2,4-Diaminophenol	NE	NE	Negative
2-Amino-4-nitrophenol	SE(M); NE(F)	NE	Negative
2-Amino-5-nitrophenol	SE(M); NE(F)	NE	Negative
Hydroquinone	SE(M); SE(F)	NE(M); CE(F)	Negative
2-Nitro- <i>p</i> -phenylenediamine	NE(M); CE(F)	NE(M); CE(F)	Positive
4-Amino-2-nitrophenol	CE	NE	Positive
2,4-Diaminotoluene	CE	NE(M); CE(W)	Positive
2,4-Diaminoanisole	CE	CE	Positive

(M)=male; (F)=Female animals. Rating: NE, no evidence; EE, equivocal evidence; SE, some evidence; CE, clear evidence.

(From Ref. 107.)

respectively. Therefore, the carcinogenic activity of Disperse Blue was most likely due to a secondary, non-carcinogenic mechanism that is irrelevant to the potential human exposure to the substance—humans are not reasonably expected to develop urinary bladder calculi following the use of commercial hair dyes.

Table 8 Hair Dye Ingredients Banned in the EU
(Annex II of 76/768, EC)

Substance	Rationale
<i>o</i> -Phenylenediamine	Positive carcinogenicity rating (NTP)
2,4-Diaminotolulene	Positive carcinogenicity rating (NTP)
2,4-Diaminoanisole	Positive carcinogenicity rating (NTP)
2,4-Diaminophenol	Structural similarity with 2,4-diaminoanisole
2,4-Diaminophenylethanol	Structural similarity with 2,4-diaminoanisole
2,5-Diaminoanisole	Structural similarity with 2,4-diaminoanisole
2-Amino-4-nitrophenol	Positive carcinogenicity rating (NTP)
2-Amino-5-nitrophenol	Positive carcinogenicity rating (NTP)
4-Amino-2-nitrophenol	Positive carcinogenicity rating (NTP)
Catechol	Possible metabolite of benzene
Pyrogallol	Possible metabolite of benzene

(From Ref. 3.)

Table 9 Hair Dye Ingredients Restricted in the EU
(Annex III of 76/768, EC)

Substance	Restriction (max. content in %)	Comments
Oxalic acid, its esters and salts	5%	Professional use only. Warning label required
Ammonia	6%	Above 2%: warning label required
<i>p</i> -Phenylenediamines, <i>N</i> -substituted derivatives and their salts	6% (as free base)	Warning label required
Methylenephenylenediamines, <i>N</i> -substituted derivatives and salts	10% (as free base)	Warning label required
Diaminophenols	10% (free base)	Warning label required
Hydrogen, urea or zinc peroxides	12% H ₂ O ₂	Warning label required
Hydroquinone	0.3%	Warning label required
Alpha-naphtol	0.5%	Warning label required
Resorcinol	5%	Warning label required
Lead acetate	0.6% (as Pb)	Warning label required
Dialkanolamine derivatives of fatty acids	0.5%	Not to be used in presence of

		nitrosating agents
Monoalkanolamines	Max. 0.5%	Not to be used in presence of nitrosating agents
Thioglycolic acid esters	Max. 11%	Professional use only
Trialkanolamines	Max. 2.5%	Not to be used in presence of nitrosating agents
Strontium peroxide	Max. 4.5% (as Sr)	Limited to professional products. Warning label required

(From Ref. 3.)

The hair dye ingredient HC Red No. 3 was evaluated as having “equivocal evidence for carcinogenicity” on the basis of the increased liver tumor incidence in 35/50 mice at the highest dose (250 mg/kg/day), as compared with that in controls, i.e., 25/50 (62). However, mice of the mid-dose group (125 mg/kg/day) had an incidence of liver tumors of only 15/50, i.e., lower than the control group. This example illustrates the variation of the incidence of liver tumors in mice and its confounding impact on the interpretation of carcinogenicity studies. A re-evaluation of the study came to the conclusion that HC Red No. 3 was non-carcinogenic and that the classification was not justified.

The classification of 2-amino-5-nitrophenol, i.e., “some evidence for carcinogenicity,” was due to a high incidence of pancreatic adenomas in male rats (63). In the study, the test substance was administered in corn oil. However, later NTP-studies revealed a similar increase in the incidence of the same tumor type in male rats when sunflower or corn oil was used as vehicle, resulting in the subsequent recommendation by the NTP to avoid vegetable oil-based vehicles in carcinogenicity studies. Therefore, the perceived carcinogenic potential of 2-amino-5-nitrophenol may have been due to vehicle-related factors.

An example of questionable conduct and interpretation of rodent carcinogenicity data was a study on PPD in combination with hydrogen peroxide alone (64). Although the combination of PPD and hydrogen peroxide alone is not used in hair dyes and known to be genotoxic in the absence of couplers, the study used weekly skin painting or subcutaneous injection of an aqueous solution containing 5% PPD, 2% ammonia and 6% hydrogen peroxide to groups of 10 male and 10 female rats for 18 months.

The authors reported an increase in mammary gland and uterine tumors in treated groups. Although the validity of the study had been rejected in 1991 due to insufficient number of animals and duration of treatment (65), a recent EU opinion considered the results as possibly supportive of a carcinogenic potential of PPD. However, in addition to the criteria listed by the SCC in 1991, the investigation had additional shortcomings. First of all, due to the absence of couplers the applied formulation was not representative for actual hair dyes. Second, although subcutaneous injection may be reasonably expected to result in a higher systemic exposure dose than topical treatment, the study results described a similar tumor incidence in subcutaneously and topically treated groups; therefore, a dose-response was absent. Moreover, the study reported no mammary tumors in control groups, although the spontaneous tumor incidence for mammary benign tumors

(fibroadenoma) in untreated rats of this age and strain is generally in the range of 12–45%, and the spontaneous incidence of mammary carcinoma in this strain ranges from 0% to 23% (66). Thus the zero-incidence of mammary tumors in the control groups of the study is highly implausible. In addition, the tumor nomenclature in the study was unclear, and included misclassification of non-neoplastic lesions as tumors. Finally, ethical aspects of repeated subcutaneous injections of a solution containing 2% ammonia may be questioned.

Considering current criteria for a weight-of-evidence approach (54) (Table 10), the study should be regarded as flawed and unacceptable as a serious evaluation of potential carcinogenicity of PPD, particularly when taking into account the evidence of 16 well conducted oral and topical carcinogenicity studies on PPD and PPD-containing hair dye formulations that were all negative. This view is supported by a recent review concluding that rodent carcinogenicity data from oral studies tended to result in overestimation of human cancer risk, whereas animal studies using the administration route that corresponds to the human exposure yielded more reliable results (67). Finally, concurrent with recent approaches for weighing animal

Table 10 Factors for Weighing Animal Tumor Evidence for Human Carcinogenic Risk

Increase weight	Decrease weight
Number of independent studies with consistent results	Few studies, inconsistent results
Multiple observations (species, sites, sex)	Single site/species/sex
Severity and progression of lesions, early-in-life tumors/malignancy	Benign tumors only
Dose-response relationship, lesion progression, uncommon tumor	High background of tumor incidence
Route of administration of human exposure	Route of administration unlike human exposure

(From Ref. 54.)

evidence for assessment of human carcinogenic risk and the recommendations of drug toxicology guidelines which recommend toxicology testing under the conditions of human exposure (68), the results of *topical* carcinogenicity studies on commercial hair dyes should be given a greater weight of evidence and relevance for the assessment of human cancer risk than studies performed via the oral route.

In conclusion, although some hair dye ingredients that are no longer used today were shown to possess carcinogenic potential in rodents after lifetime exposure to high, daily *oral* doses, there is no evidence that topical exposure may pose a carcinogenic risk. Therefore, the carcinogenic risk of hair dyes in humans under realistic use and exposure conditions appears negligible.

4.4.3. Human Exposure to Oxidative Hair Dye Ingredients

Given that the potential health risk of a chemical is a function of (a) its toxicity, (b) the potential human systemic exposure and (c) the dose-response of the relevant toxic effect, the toxicologic potential of a substance is only relevant when significant human systemic exposure occurs. For example, whereas chronic exposure to daily *oral* doses (at 0.24–0.5% in the diet) of 2,4-diaminoanisole (DA) to rats and mice caused tumors of the thyroid, skin and reproductive organs (61), topical application of a commercial formulation containing DA to mice and rats was non-carcinogenic (69). Thus the carcinogenic potential of DA is associated with the route of administration, possibly secondary to the magnitude and/or nature of systemic exposure.

The percutaneous absorption rate of oxidative hair dyes containing ^{14}C -DA in Rhesus monkeys was 0.032%, and 0.022% in humans, whereas that of ^{14}C -PPD-based dyes was 0.01% or 0.14% ^{14}C -equivalents, respectively (70). Overall, studies on the percutaneous absorption of other oxidative and direct ^{14}C -labelled hair dyes in humans and Rhesus monkeys revealed absorption rates of less than 1.0% for all ingredients tested (70,71). Taking the application rates of hair dye ingredients into account, the expected human systemic exposure dose would be in the range of 2–7 mg (0.03–0.12 mg/kg) per application. An even lower systemic exposure dose was confirmed by a recent study in human volunteers after actual hair dyeing; the human systemic exposure was estimated to be in the range of 340–700 µg (6–12 µg/kg) after topical application of 186–1014 mg PPD, corresponding to a systemic absorption rate of 0.04–0.25% (72). The order of magnitude of systemic exposure after a hair dyeing process was confirmed by a recent stringently controlled study in male volunteers after application of a dark-shade (highly concentrated) commercial hair dye formulation containing 1.31 g of ^{14}C -PPD. The study found a systemic absorption of $0.54\pm 0.25\%$ of the applied dose, corresponding to a systemic exposure dose of 0.09 mg/kg, taking into account the mean body weight of 78.5 ± 10.0 kg of the volunteers (73). Although the rate of systemic absorption in the study was slightly higher than the values measured in previous studies, this is not surprising, given the shorter hair length of males and the subsequently reduced absorption by the hair. Overall, all human exposure data suggest that the mean human systemic exposure to hair dye ingredients is in the range of 0.1–4 µg/kg/day, taking into account the intermittent (>30-day interval) nature of oxidative hair dye application.

An assessment of the human cancer risk for lifetime exposure to hair dyes containing DA (carcinogenic in rodents) resulted in a magnitude of risk of 6.1×10^{-8} to 4.9×10^{-9} for dark or light shades, respectively (74). Risks in this order of magnitude, i.e., less than 10^{-6} are considered to be negligible. This view was supported by a recent analysis of 139 substances positive in NTP carcinogenicity studies that suggested a linear correlation of the maximum tolerated dose (MTD) in animal tests with the (hypothetical) human exposure dose resulting in a negligible human cancer risk of 10^{-6} , i.e., the “virtually safe dose” (VSD). The correlation was approximately $\text{MTD}=\text{VSD}\times 740,000$; thus the numerical division of the MTD-value by the empirical factor of 740,000 corresponded to the magnitude of the human dose resulting in a lifetime cancer risk of 10^{-6} or less (75). Surprisingly, the results of this simple calculation were consistent within a factor of 10 with the results of conservative mathematical models for risk assessment and confirmed the empirical experience of 50 years of carcinogenicity testing: *potent carcinogens also tend to be highly toxic*.

Applying this rule of thumb, typical MTD-ranges in carcinogenicity studies of 100–1000 mg/kg/day would correspond to human VSDs in the range of 0.14–1.4 µg/kg/day. The fact that these values are consistent with the actual order of magnitude of the measured human exposure to hair dyes confirms the absence of a human health risk. However, NTP-tests used administration of daily, high *oral* doses. In contrast, human exposure to oxidative hair dyes are of far lower magnitude due to a number of modifying parameters associated with the use conditions of hair dyes in humans, such as the high affinity of hair dyes to the hair resulting in absorption, limited contact with the scalp, rinsing-off and others (Table 11) (76). It should also be noted that the magnitude of human exposure to hair dyes is at a comparable order of the “threshold of toxicological concern” (TTC) of 1.5 µg/person/day. Today, this threshold value is used in the risk assessment of food additive contaminants: human exposure below the TTC results in no significant risk to human health (77).

In summary, although some hair dye ingredients, such as 2,4-diaminotoluene, which are no longer used by the hair dye industry may have had a carcinogenic potential in rodents when given orally (111), they were non-carcinogenic when given topically alone or in hair dye formulations. Thus, while intrinsically *hazardous*, they were unlikely to pose a significant *risk* under their conditions of use. In addition, the order of magnitude of the actual human systemic exposure during a hair dyeing process suggest no or a negligible cancer risk, even for ingredients that were found to be carcinogenic in chronic oral rodent studies.

Table 11 Human Exposure Parameters to Oxidative Dyes During the Hair Dyeing Process

Parameter	Magnitude
Scalp surface	600 cm ²
Number of hairs/cm ² (scalp)	250
Total number of hair (scalp)	150,000
Mean hair diameter	0.06 mm
Average length	20 cm
Total surface of hair	60,000 cm ²
Ratio of hair to scalp surface in humans	100-fold
Quantity of hair dye absorbed on hair	1.25–5.6 mg/g hair
Percentage of hair dye rinsed off after dyeing	80–90%
Percentage of hair dye available for systemic absorption	<5%
Quantity systemically absorbed (man)	0.02–0.20%

(From Refs. 70,76.)

4.5. Reproductive Toxicity Endocrine Effects

In response to safety requirements in the EU, the Japanese Ministry of Health and Welfare or the US CIR, hair dye ingredients are routinely investigated for their potential reproductive toxicity, i.e., embryo-fetal toxicity and, if appropriate, male/female fertility and peri-/post-natal toxicity. Identification of any reproductive risk would preclude the use of an ingredient in hair dyes. A published study reported teratogenic effects of 2-nitro-*p*-phenylenediamine and 4-nitro-*o*-phenylenediamine after subcutaneous administration of high doses to mice, whereas 2,5-toluylenediamine was found to be non-teratogenic (78). However, the effects occurred at extreme multiples of the expected human exposure and the relevance of these studies to the human risk is debatable. The same ingredients were included in a topical multigeneration reproduction study on six commercial hair dyes that yielded no evidence of reproductive effects or systemic toxicity (59) (Table 6). A series of oral reproductive toxicology or embryo-fetal toxicity studies on commercial hair dyes found no evidence for reproductive effects (43).

Numerous epidemiological investigations have been published on the potential effects of hair dyes on human reproduction. Six out of seven different studies listed found no evidence for reproductive disorders or an increased risk for fetal malformation in hairdressers or their offspring, whereas a single investigation reported a slight association (43). The problem of some investigations may be illustrated by a recent study that suggested an increased incidence of low-birth-weight babies (LBWBs) in a population of Swedish hairdressers, i.e., 4.5% vs. 4.1% in a reference group (79). Further analysis of the data revealed that the incidence was borderline and significantly lower than the overall incidence of LBWBs in the total Swedish or Danish populations, i.e., 4.6% or 5.0%, respectively (80,81). In addition, the slight difference between hairdressers and the reference group may have been the results of physical aspects of hairdresser's work. For example, standing for more than 8 hr a day combined with physical work has been identified as a risk factor for LBWBs (82). Finally, the findings of the study are at variance with other epidemiological studies in the United States, the Netherlands and Canada, which found no adverse effect or a lower risk for stillbirth or LBWBs in hairdressers (43). In conclusion, there is no evidence that occupational or personal exposure to hair dyes adversely affects human reproduction.

Another recent contentious topic is the hypothesis of "endocrine disruption," i.e., environmental chemicals that may possess hormonal activity or affect endocrine systems of vertebrates. Initially, the definition of an "endocrine disrupting substance" addressed estrogenic, androgenic or anti-androgenic effects only; however, the definition was subsequently enlarged to include a variety of hormonal effects. For example, today the U.S. Environmental Protection Agency (EPA) defined an endocrine disrupting substance as "an exogenous compound that interferes with the synthesis, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior" (83), whereas the current European definition is "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations" (84). However, a final definition of the term "endocrine disruptor" is still under debate.

Resorcinol, an important ingredient (coupler) of oxidative hair dyes (Fig. 9) was included in a provisional list of materials that may pose a "high concern for further

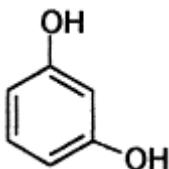


Figure 9 Structure of resorcinol (1,3-benzenediol).

evaluation of their role in potential endocrine disruption” (85). The basis for this classification was the high volume of production and use, potential human and environmental exposure and the potential effect of resorcinol on the thyroid. High parenteral doses of resorcinol may indeed affect the thyroid. This effect was reported in man in the 50s after medical use, i.e., after long-term topical application of resorcinol to ulcerated legs; the effect was confirmed in repeated, *subcutaneous* administration to rats at doses of 40 mg/kg/day and above (86,87), whereas no thyroid effects were observed in oral subchronic or carcinogenicity studies at doses up to 150 mg/kg/ day (88). However, adverse thyroid effects are a common finding in rodent toxicology studies; in contrast to higher mammals (dog, primate, man), rodents are known to be highly susceptible to develop thyroid hypertrophy/hyperplasia secondary to disturbance of thyroid hormone homeostasis (89). Many natural or synthetic inorganic or organic substances (iodine, perchlorate, thiocyanate anions, substituted phenols, thioamides, heterocyclic compounds, plants used in human nutrition) may affect the thyroid and are known to possess goitrogenic activity. For example, a high dietary intake of *Brassicaceae* spp. (Brussels sprouts, rape seed), turnips, millet, green tea, cassava, rutabaga, soy bean flower or walnuts has been reported to affect thyroid hormones and to be goitrogenic in many species, including man, higher mammals, rodents, and fish. However, it is debatable whether it is meaningful to qualify these common constituents of human or animal nutrition as “endocrine disrupters.”

Thus it is highly unlikely whether the effect of resorcinol on the thyroid after accidental human exposure or parenteral (*subcutaneous*) administration to rats has any relevance to human and environmental health, or justifies the label of *endocrine disruptor*. Whatever the definition, the above examples illustrate that, at present, the term *environmental endocrine disrupting substance* should be regarded as a poorly defined label, which has little relevance to human health.

The potential human systemic exposure to resorcinol from hair dyes was calculated to be on the order of 0.016 mg/kg per hair dyeing process. The estimated margin of safety was >1266-fold, when the potential systemic exposure dose was compared with the no-effect level of *in vivo* subchronic toxicity studies (90), or by more than an order of magnitude higher, taking into account the intermittent human exposure to hair dyes. The absence of a human health risk was confirmed by an epidemiological studies in occupationally exposed workers of the chemical industry, which showed no indication for adverse effects on the thyroid (91) and by the review of the US CIR Expert Panel, which concluded that resorcinol “is safe as a cosmetic ingredient in the present practice of use” (92).

In summary, the current safety evaluation schemes of hair dye ingredients safeguard the absence of risk to human reproduction under normal use as well as occupational exposure conditions. There is no evidence that “endocrine disrupting effects” from hair dye ingredients or other chemicals pose a risk to human health or the environment.

5. DISCUSSION

After this tour of highly technical details of hair dye toxicology, one may raise the pertinent question: What does it all mean? Are hair dyes dangerous or not, considering this mass of partly conflicting toxicological information? Is today’s approach to their safety evaluation scientifically justified?

First of all, let us consider the current “margin of safety” (MOS) approach. Although the MOS may be useful for a side-by-side comparison of the safety of different hair dye ingredients when tested under comparable conditions, it is by no means equal to safety, but rather represents a “surrogate endpoint” for safety. The relation of the MOS of a hair dye ingredient to the actual human risk is uncertain for the following reasons:

- Hair dye ingredients are not used on their own, but in combination with hydrogen peroxide and other substances, such as couplers.
- The NOAEL is a poorly defined parameter, which is increasingly being replaced by the more scientific concept of the Benchmark Dose (93).
- Results of *oral* toxicity studies, particularly when performed by single daily administration (*oral gavage*) have uncertain relevance for the potential toxicity after *topical* exposure.

Given that the potential for human exposure is generally determined by *in vitro* percutaneous absorption studies, their relevance is of pivotal importance for the assessment of hair dye safety. A study in human volunteers after application of hair dyes found an actual percutaneous absorption rate of PPD in the range of 0.04–0.25%; studies in Rhesus monkeys and man on ¹⁴C-PPD under normal use conditions reported absorption rates of 0.18–0.54%, respectively. In contrast, *in vitro* studies on this ingredient showed substantially higher percutaneous absorption rates ranging from 0.44% to 3.41%. Similarly, the *in vitro* percutaneous absorption rate of *p*-toluylenediamine (PTD) in human skin was in the order of 2.06–3.56% whereas studies with ¹⁴C-PTD in human volunteers suggested values as low as 0.33% (94).

A recent study in volunteers of a topically applied ¹⁴C-labeled UV-filter combined a parallel study on *in vitro* dermal absorption/penetration in human skin using identical conditions and formulations. The study found a theoretical human systemic exposure dose (SED) on the basis of the *in vitro* study that was approximately 30-fold higher than the actual, measured human systemic exposure (95). In a subsequent repeated-dose study on the same UV filter, even lower amounts were found to be systemically absorbed (96). These findings suggest that *in vitro* penetration data may result in an overestimation of the systemic exposure and the subsequent risk to human health.

Although recent EU guidelines agree that *some other application routes (dermal) might offer more relevance to the data*, the traditional safety assessment of hair dye ingredients in the EU has been largely based on the NOAELs of *oral* *in vivo* toxicity

studies, most of them performed by single daily (gavage) administration, according to current guidelines that *the investigation of potential toxic effects (subchronic toxicity, oral route) remains a necessity* (4). However, oral and topical administrations represent quantitatively and qualitatively different exposure scenarios: oral doses generally result in a systemic exposure profile that resembles that of an oral drug including high C_{\max} values (the maximum concentration of the substance in the blood) that may trigger adverse effects. In contrast, topical application tends to result in extended exposure of the organism to low blood concentrations secondary to the slow diffusion of the substance through the skin followed by uptake in the blood (97). Therefore, repeated-dose oral toxicity studies using single daily administration tend to produce disproportionately higher toxicity when compared with a corresponding topical dose, even in cases where topical application results in high systemic exposure. Moreover, orally administered substances may be detoxified by first-pass effects (extraction/metabolism by the liver), whereas this effect is less prevalent after topical administration. Conversely, topical administration may result in systemic exposure to metabolites secondary to metabolism in the skin (see above, *para*-aminophenol), in contrast to an oral administration that may cause systemic exposure to different metabolites formed in the liver.

Overall, given these quantitative and qualitative differences, comparison of topical with oral toxicity data is simplistic, unless pharmacokinetic data and the metabolism of the test compound are known and taken into account. Although topical toxicological studies allow application of only limited amounts of test compound to the skin, the hazard assessment of hair dye ingredients should be improved by the performance of *topical* repeated-dose toxicity studies or, if technically unfeasible, by performance of oral studies via the dietary route as opposed to daily gavage. Given the slower uptake of a substance administered in the diet, the resulting pharmacokinetic profile better resembles that of topical exposure; typically, dietary studies tend to yield higher NOAELs when compared to studies using single daily oral administration by gavage (98).

Experience from the pharmaceutical industry demonstrated that a reliable comparison of animal and human toxicity may be obtained from the area under the curve (AUC) of corresponding blood or plasma concentrations, given that the AUC is proportional to the quantitative exposure of the target organism or organ to the agent (39). Ideally, all repeated-dose toxicity studies should be accompanied by toxicokinetic evaluation allowing calculation of toxicokinetic-based safety factors, i.e., comparing the quantitative exposure of the test organism with the corresponding human systemic exposure or that of an appropriate *in vivo* model. For example, a recent oral vs. topical toxicokinetic study on ^{14}C -Lawson in rats at a dose of 4.9 mg/kg (this dose corresponds to the approximate NOAEL of a subchronic oral toxicity study in rats and the approximate human topical exposure dose during a hair dyeing process) under exaggerated topical exposure conditions yielded toxicokinetic-based safety margins (plasma AUC after oral dosing vs. AUC after topical exposure) of over 70-fold (99) (Fig. 6). Given that toxicokinetic-based safety margins of above 25-fold are recognized to reflect a high degree of safety, these values give reassurance that topical exposure under simulated use conditions poses no or negligible risk of systemic adverse effects.

In summary, the current MOS approach for safety evaluation of hair dyes is highly conservative and may result in overestimation of their health risk. Although it may be argued that overestimation offers additional consumer protection, one should keep in

mind that the MOS represents only a surrogate endpoint that is not identical or proportional to the safety of the tested substance.

Second, let us review the relevance of hair dye genetic toxicity and carcinogenicity studies. Given the large use of hair dyes and their chemical class, their ingredients have been subject to many investigations. Thus the current confusion about the safety of hair dyes may be partly due to the paradoxical fact that too much toxicological/epidemiological information has been produced on hair dyes and some of their ingredients. Toxicological studies tend to yield probabilistic answers that reflect biological variability and intrinsic differences in the test systems or protocols. In other words, the results of nearly all toxicological tests, particularly those from in vitro studies, yield a considerable rate of confounding results including false-positives. Thus when several *Salmonella* assays are performed on a substance in different laboratories under various conditions, some positive results may be expected a priori, simply on the basis of statistics, even when protocols adhere to standardized and internationally recognized guidelines.

However, when tests are conducted on reaction products and/or in the presence of other potentially mutagenic substances such as hydrogen peroxide, their results are likely to be even more variable due to additional confounding parameters such as the type and proportion of chemical reagents, their purity, the duration of the chemical reaction and other, unknown factors.

Similarly, when 18 (!) different in vivo carcinogenicity studies are performed on a substance such as PPD, their results may be reasonably expected to show variation, and isolated data suggesting a carcinogenic potential are not surprising. Taking the intrinsic variability and quality of toxicological studies into account, an overall safety evaluation of multiple and variable results must include a weighing of the evidence, i.e., the conclusion should focus on *pivotal data*, i.e., those that appear to be the most reliable and/or relevant. Some rules of thumb for grading the reliability of toxicological data are summarized in Tables 10 and 12. Using such a weight-of-evidence approach, many results of marginal studies may be simply discarded.

Finally, concerning the predictive power of genotoxicity tests, their "validation" refers to the ability to predict rodent, and not human carcinogenicity. Here we should consider the intrinsic limits of rodent toxicity and carcinogenicity studies. For example, a large-scale retrospective analysis of the concordance of animal and human toxicity of drugs showed a concordance rate of 71% for rodent (rat, mouse, guinea-pig) and non-rodents (dog, monkey, pig) studies taken together, a rate of 63% for non-rodent data alone, but a rate as low as 41% for rodent data alone (100). In other words, rodent toxicity does not correlate with human toxicity in six of 10 cases. Moreover, it has been accepted that rodent carcinogenicity studies represent a biological model with a particularly high degree of uncertainty. For example, the International Agency for the Research of Cancer of the World Health Organization (IARC) stated that *at the present time, a correlation between carcinogenicity in animals and possible human risk cannot be made on a scientific basis* (101). In at least half of the carcinogenicity tests performed by the NTP or published elsewhere, substances that were carcinogenic in rats were non-carcinogenic in mice and vice versa, even at maximum tolerated doses (102,108) (also see Table 7). Conversely, not all of the approximately three dozen known human carcinogens were carcinogenic in rodents, whereas some important human carcinogens had only borderline

carcinogenic activity in rodents. These data underline the truism that *all rats are mammals, but not all mammals are rats* (103).

Thus the question may be raised: If the carcinogenic potential of a substance in mice after lifetime *oral* administration at maximum tolerated doses has only limited relevance for rats, what is the meaning of this potential for a human population that is sporadically and intermittently exposed to minute topical doses of the same substance? Furthermore, what is the value of positive *in vitro* genotoxicity tests for human health risk when such tests have even uncertain relevance to predict rodent carcinogenicity? The answer is that we simply do not know. But the correlation is likely to be very weak. Indeed, *the dominant analytic difficulty is the pervasive uncertainty of carcinogenicity testing* (104).

Table 12 Criteria for the Evaluation of Toxicology Results on Hair Dyes for Their Relevance to Human Health

	Greater relevance	Less relevance	Example
Aspects of test species	Human data	Animal data	Genotoxic risk of hair dyes
	Large animal data	Rodent data	See Ref. 100
	In vivo data	In vitro data	Genotoxicity results
	Mammalian cell system	Bacterial test system	Genotoxicity results
	Cellular test system	Sub-cellular test system (DNA)	Genotoxicity results
	Actual toxicological endpoint tested	Test on surrogate endpoint	Genotoxicity tests for carcinogenicity
	Effect exists in humans (cancer; sensitization, irritation)	Endpoint uncertain in humans	Chemical photo-carcinogenesis; endocrine disruption
	Validated test	Non-validated test	SCE test
	Species known to be sensitive/specific for the chemical class	Test less specific/sensitive for test compound class	<i>Salmonella</i> assay on aromatic amines
	Administration route corresponding to human exposure (e.g. topical)	Different exposure route (oral, parenteral)	Subcutaneous or oral (gavage) NTP toxicity studies on hair dye ingredients
Protocol aspects	Test conditions correspond to human exposure	Different test condition (exaggerated exposure duration)	<i>Salmonella</i> assay on aged (>0.5 hr) hair dyes
	Test with commercial formulation	Test with pure ingredients	NTP carcinogenicity studies
	Well-characterized test article	Unknown purity/impurities present	Mutagenic activity of impurities

Negative (vehicle) control group present	No or invalid control group	Absence of hydrogen peroxide-treated controls <i>in vitro</i> genotoxicity tests
Standard test protocol	Non-standard protocol	Carcinogenicity studies on 10 animals/sex/dose
Standard test organism/animal strain	Less well-known organism/animal strain	–
Availability of historical control data	Absence of historical control data	–
Sufficient number of animals/cells	Low/inadequate number of animals/cells	Carcinogenicity studies with 10 animals/sex/dose
Complete description of methods	Inadequate description of methods	–
Greater relevance	Less relevance	Example
Test performed under GLP conditions	Non-GLP study	–
Results from chronic studies	Results from subacute/subchronic studies	PPD: effects in subchronic studies not seen in chronic studies
Aspects of toxic effect	Effect at doses at the order of expected human exposure	Calculi (urinary bladder) in carcinogenicity studies on Disperse Blue 1
	Effect increases with dose (dose-response)	Dose-response for liver tumors in mice (HC Red No. 3)
	Effect biologically and statistically significant	–
	Effect biologically plausible	In vivo genotoxicity of Lawsone
	Effect reproducible and confirmed	In vivo genotoxicity of Lawsone
	Effects in absence of excessive toxicity	In vitro studies on chromosome aberrations (Lawsone)
	Effect in both sexes, several species or strains	<i>N</i> -nitrosamines (all species) vs. thyroid tumors (rodents)

Unusual effect in test species or strain	Effect known to occur spontaneously	Mammary (SD rats) and testicular tumors (F344 rats)
Incidence/magnitude of effect exceeding historical control range	Incidence/magnitude of effect within the range of historical control data	Liver tumors in mice (HC Red No. 3)
Effect consistent with biologic activity and/or class effects	Effect inconsistent with known data	Micronucleus test results on Lawsons
Positive correlation of multiple effects	Isolated effect	Change in organ weight in absence of histopathological or biochemical changes
Compound-related effect	Procedure-related effects	Fore stomach lesions in rodents (oral administration of irritant materials)
Typical control data	Atypical control data	Absence of tumors in control animals
Test system responds to positive control substance	No/marginal response to positive control substance	–
Other Conclusion consistent with the results	Overinterpretation of the results	In vitro genotoxicity data for rodent/human carcinogenicity
Results published in a peer-reviewed journal	Publication in a less recognized, non-peer-reviewed journal	–
Author known as competent in the field	Unknown author/co-author	–
Independent experts agree with conclusion	Experts disagree with conclusion	–

It has recently been stated that the current application of rodent carcinogenicity data for risk assessment and subsequent risk management measures mainly serves to “provide an anxious public with the comforting illusion that risks are being looked after, while they cannot warrant that some of its most significant and costly regulatory actions are either beneficial or harmful” (103). Given that hardly any new *human* chemical carcinogens have been identified in the past 20 years, it has been suggested that identification of an ever-increasing number of rodent carcinogens and the subsequent measures targeted to reduce human exposure to substances labeled as carcinogenic played little or no role in the prevention of human cancer (105).

Moreover, although in vitro genetic toxicity tests are recognized to be valuable and inexpensive tools for screening rodent carcinogens, they certainly do not represent magic mirrors for identifying carcinogenic risks to humans. Therefore, when evaluating such data and, particularly when applying them to human risk assessment, we should always

keep the limitations of in vivo and, above all, in vitro tests in mind: after all, although man is not a big rat, he is even less a big *Salmonella*, mouse lymphoma or Chinese hamster ovary cell.

6. CONCLUSION

In conclusion, today's safety evaluation of hair dyes is a relatively crude tool, which tends to overestimate human health risk. The safety assessment of hair dye ingredients could be improved by performing appropriate toxicokinetic investigation and selecting appropriate administration routes. Concerning the actual human health risk of hair dyes, the facts speak for themselves: when commercial dyes have been tested under conditions resembling those of human use, the results of human, animal and in vitro studies indicated no evidence of systemic, genetic or reproductive toxicity, or carcinogenicity. Therefore, when taking all results into account and considering the weight of evidence, it is concluded that the use of modern hair dyes poses no or a negligible risk for human health.

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Hair Product Safety

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1. INTRODUCTION

The use of products aimed at cleansing, styling, conditioning, or beautifying the hair, or in modifying its color, dates back several thousand years and, in practice, this type of product has rarely been associated with serious health problems.

In the past few decades, however, their use has intensified considerably. Indeed, numerous ingredients (surfactants, colorants, polymers, silicones, etc.) obtained thanks to new technology, have meant that manufacturers have been able to develop more effective and more diversified products. Moreover, with improvements in living standards and life expectancy, these products are aimed, throughout the world, at more and more consumers.

Without waiting for regulations, it has always been the responsibility of each company that markets new cosmetic products to ensure that they meet the primary requirement “primum non nocere”. As stipulated in Article 2 of the European Cosmetic Directive 76/768/CEE (1) “they must not be harmful to human health when applied under normal or reasonably foreseeable usage conditions.”

Hair products, like all other cosmetic products, must present the highest safety level both for the consumers who use them and for the professionals (hairdressers) who apply them. This means that the product must not cause damage to human health in general or cause undesirable reactions locally.

Moreover, in recent decades, new regulatory requirements have arisen to protect our environment and reduce damage linked to human activities to a minimum. Hence, apart from the consumer health protection aspect, companies that put new cosmetic products on the market must also approach safety matters from another angle and evaluate the impact of these new products on the air, water, or soil, whether during manufacture, their usage or at the end of their shelf life. Safety assurance is a continual process that is developed along with the development of the product from its conception through to its marketing. It requires the attention of a large number of personnel:

- That of the *product development* project leaders who must ensure, from the outset, that the various ingredients they have selected for a specific foreseen use present no risks to the users or to the environment and who must only put forward products whose stability has been closely monitored and from which the risks of contamination have been carefully avoided.

- That of the *packaging* project leaders who must ensure that this maintains the quality and safety of the product throughout its use and averts, as far as is possible, the risks of inappropriate or accidental misuse.
- That of the *manufacturing* project leaders, who must manufacture products in accordance with good manufacturing practices and quality control.

A very special role in the overall procedures falls to the Safety Assessor who is required, prior to marketing, to issue his or her findings, on the basis of the information gathered, on the absence of risks with this new product with regard to human health under normal or foreseeable usage conditions.

When the product contains special ingredients that might present a danger under certain storage or usage conditions (the case of pressurized packaging, for example), it is essential that the consumer is informed of the nature of these risks and of the storage or usage conditions to be respected.

2. SAFETY IN USE

2.1. Potential Risks with Hair Products

The use of hair products in general can induce four types of potential risk to health:

1. The risk of skin irritation resulting from direct contact between the product and the scalp; this reaction may occur as soon as the first contact is made, particularly if the user has an exceedingly sensitive scalp or a pre-existent dermatosis. With products that are applied frequently, this irritation may only become evident after several successive applications, following failure of the natural skin barrier.
2. The risk of eye irritation linked to the fact that many hair products must be rinsed off after application and can thereby come into contact with the eyes. Eye safety testing is therefore of particular importance.
3. The risk of allergy (or contact hypersensitivity) as the result of recognition by the organism of a substance, called an allergen, to which the subject has become sensitized. Any further contact with this subject will trigger an inflammatory reaction at the site of application, capable of spreading to neighboring skin areas. Contact hypersensitivity may also be pre-existent on application of the product but equally it can be generated by repeated applications of a product. Linked to the individual sensitivity of the user and only affecting a small proportion of them, the allergic reaction is harder to predict. The evaluation of this risk must take into account not only the specific data obtained on each of the ingredients in the formulation, but also the possible interactions between them in the finished product.
4. A risk of systemic toxicity resulting from penetration through the skin or mucosa, or inhalation of various constituents of the hair product. This type of risk is closely linked to the nature and toxicological profile of the ingredients on the one hand, and to their ability to cross the skin barrier in the course of application of the product on the other. Evaluation of this risk is based on toxicological data on the ingredients and on the level of exposure by the user to these ingredients, taking into account their

concentration in the finished product, the amount of the latter in contact with the scalp and the duration of contact.

2.2. Classification of Hair Products Relative to the Level of Exposure

Prior to carrying out any assessment of hair product safety, it is necessary to determine the level and mode of exposure of the user. Several factors must be taken into consideration, notably the presentation and the mode and frequency of application. Taking these factors into account, hair products can be classified in four categories:

- *Technical hair products* that modify hair fiber permanently, to color or bleach or to modify its shape (hair dyes, bleaches, permanent waves, straighteners, relaxers, etc.). These products are essentially applied to the hair, they are always rinsed off and have only brief contact with the scalp. Generally, frequency of use is of the order of 4–6 weeks for coloring or bleaching and 3–6 months for perming or straightening.
- *Hair cleansing and conditioning products* that are rapidly rinsed off and thus are only briefly in contact with the scalp. They can be used very frequently however: these consist mainly of shampoos and numerous hair care products.
- *Hair dressing and styling products* that are not rinsed off, also used frequently but whose application is focused on the hair itself, with little contact with the scalp. Products in this category are those in the form of a gel, cream or mousse, and those delivered in sprays such as the aerosols and pump-action dispensers.
- *Scalp care products* that are not rinsed off and whose frequency of use is variable.

2.3. Assessment of Hair Product Safety

The evaluation of hair product safety begins with an in-depth knowledge of the composition of the product, its characteristics and its presentation. It must take into account the way in which it is used and the risks that it entails. Then it is based on two types of data: those concerning the ingredients and those available on the product itself. The assessor must also take into consideration the size of the population targeted, the type of expected users and, in particular, whether it is a mass-market product or one reserved for professionals. Finally, the assessor must ensure that acceptable usage instructions and precautions are present on the packaging.

2.3.1. Composition and Innovative Nature of the Product

After having identified the cosmetic category of the hair product, the characteristics and mode of use must be considered. Risk evaluation will depend upon whether the product is applied undiluted or diluted; it will obviously depend upon the recommended contact time and frequency of applications. Particular attention must be paid to the presentation; some professional products, i.e., products for hairdressers require a special method of preparation and the risks linked to this preparation must be clearly identified.

The next step is to scrutinize the composition of the product meticulously:

- ensuring that it conforms to the European or national regulations; notably verifying the absence of prohibited substances and compliance with usage conditions for substances

- governed by the positive lists, such as preservatives for example, or by the restrictive list (cf. Chapter 13);
- assessing the level of innovation; the requirements of safety evaluation differ in respect of a minor change in the composition, a major modification, or a totally new formulation concept.
 - identifying the ingredients corresponding to new chemical structures or those already known but used at a higher concentration or for a different purpose.
 - ensuring that the product stability and microbiological data are satisfactory.

2.3.2. *Ingredient Data*

The ingredients included in the composition of hair products are multiple and varied; whether synthetic substances or natural extracts, their characterization and the identification of any impurities must be determined with care. It is important to clearly identify the risks that could be linked to their physicochemical properties and chemical reactivity.

A thorough knowledge of the toxicological data of the ingredients of a hair product is an indispensable prerequisite in the evaluation of hair product safety, especially when new chemical substances, an ingredient used at a higher concentration in the same type of product, or the novel use of an ingredient are concerned.

The suppliers are the main source of data on the ingredients. They must satisfy the national and international (e.g., EU in Europe) regulations on chemical products and thus have available toxicological data on the ingredients they market since these data are required for the safety of workers who manufacture or handle them, as well as for their transport and labeling (2,3).

The scientific literature, databases such as Toxline and Medline, Cosmetic Ingredient Review reports, European Centre for Ecology and Toxicology of Chemicals (ECETOC) reports, safety records or sheets and the in-house experience and/or data can also shed light on the safety of numerous ingredients. Their use in product categories other than cosmetics is also an important source of data.

What Sort of Data Must Be Obtained? The EU Scientific Committee on Cosmetic and Non-Food Products (SCCNFP) has drawn up a list of information entitled “Guidelines for Cosmetic Ingredient Safety Testing” (4)

Whatever the type of hair product in which an ingredient is used, a minimum dossier of pertinent data is required for evaluation of the risk of skin irritancy, eye irritancy and sensitization, and to ensure there is no mutagenic potential. Moreover, this dossier must indicate the oral toxicity level, in the event of accidental ingestion of the product, for example, by young children. In the case of hair products remaining in contact with the scalp, data on phototoxicity, subchronic toxicity and cutaneous absorption must be available. For new ingredients entering into the composition of hair products in aerosol form, the supplier must be able to supply adequate data for evaluation of the risk of inhaling low levels, but frequently.

Technical products containing substances likely to react with the hair fiber demand very close examination. The use of a large number of these ingredients is regulated; for example, the thiols used in permanent waving or straightening, or hydrogen peroxide and ammonia used in bleaches and mixed with some coloring products.

Several colorants used in hair coloring products are also regulated; such is the case, for example, for paraphenylenediamine, the aminophenols or resorcinol and their derivatives. Due to their chemical structure and reactivity, the evaluation dossier on hair colorants must be supported by teratogenic data and those with a bearing on the risks of long-term usage (cf. Chapter 10, Safety of Hair Dyes).

Perfumes constitute a very special case; since every perfume is composed of a large number of ingredients, the suppliers are responsible for evaluating the safety of the mixture when used as recommended and for supplying a safety certificate for each perfume used.

2.3.3. *Finished Product Data*

The ingredients are obviously selected on the basis of their non-irritant nature at usage concentrations; however, it is necessary to ensure that their combination in the finished product is well tolerated by both the skin and eye. This appraisal has to be carried out prior to any human exposure. For many years, the only means of assessing the potential risks of a product was recourse to animal testing by standardized methods in accordance with regulatory requirements. Hence French regulations imposed the determination of the skin irritancy potential following both a single application or repeated applications of the product, and determination of eye irritant potential as well as the repair capacity over the course of 7 days following application. Research into alternative methods was undertaken by some leading members of the cosmetic industry in the 1970s. The first studies were carried out on organotypic culture models, followed by the emergence and progressive mastery of the first cell cultures, with which the impact of soluble products on cell functions could be studied in a culture medium. While improving the reliability of the cultures, the researchers tried to identify pertinent endpoints for the acquisition of data on the risk of toxic or undesirable effects. The results obtained were then compared with results *in vivo* in the categories of products compatible with these techniques and for which wide-ranging safety data were available.

A limited number of alternative methods have been validated by the European Center in charge of their validation (ECVAM) but it is nonetheless now possible to ensure that a product presents no risk to humans without using animal tests, thanks to the experience accumulated by the formulators and the advantage of the combined use of methods *in vitro* by the Safety Assessor and chosen for the complementary nature of the data they produce. Verification of product safety is thus based on tests *in vitro* on the one hand and clinical testing on the other, taking into account the mode and frequency of use of the product.

Choice of Tests Applied. If the new product is a simple variant of an existing product, or if the formula is simply composed of ingredients already used previously in similar products and at very similar concentrations, a simple comparative test *in vitro* of the earlier and new formulations will suffice for its use in consumer tests to be authorized.

If the new formulation corresponds to a new concept or if its composition is significantly different from that of earlier products, it will be necessary firstly to get confirmation by one or more appropriate tests *in vitro* that it yields comparable results to those of hair products in the same category, then the satisfactory results will be supported *in vivo* by controlled applications on a limited but pertinent number of volunteers. The

latter tests must be carried out under strict medical supervision, with full respect for ethical principles. Following this clinical evidence of good compatibility, the product can be distributed for tests on a larger scale, to confirm its acceptability to consumers.

2.3.4. *In Vitro Tests*

In vitro methods are used to compare the results of any new formulation with those already acquired for known formulations of the same type that are well tolerated. They are also very useful as a guide, at a very early stage, to the choice of new formulations with a view to achieving improved tolerability.

In vitro methods are useful tools to assess the risks of eye and skin irritation. However, there is still no test in vitro, to date, that can be used to evaluate the risk of allergy to cosmetic products. Some approaches exist, but are still at the research stage (5,6), or are of use mainly for the prediction of the sensitizing potential of ingredients of the same chemical family. The most promising methods of prediction of allergy induced by cosmetic products are probably those of reconstituted skin into which Langerhans cells have been introduced (7). Currently, the evaluation of the potential of a product to cause allergy is thus based mainly on knowledge of the ingredients and by making sure, in volunteers and under medical supervision, of the absence of allergen potential.

Conversely, evaluation of the irritant potential of a cosmetic formulation can be achieved using in vitro techniques.

Basic Principles of the Use of Tests In Vitro. In vitro tests are very useful tools in the comparison of similar products. The formulations to be evaluated must always be compared with similar formulations whose tolerability is known (the “benchmark” concept).

For example, when developing a new shampoo, the results obtained must be compared with those of shampoos in the same category (baby, antidandruff, etc.). The most relevant way is to carry out a comparative test involving a positive and a negative benchmark products. Using these methods, the effect of the products can be studied against a predetermined endpoint. The methods do not cover the whole range of mechanisms of irritation, so it is necessary to use several complementary methods in order to gauge the overall irritant potential, or to follow up with clinical tests in man when a single method in vitro has been used.

Due to this “specialization” of in vitro methods, to which certain technical limitations can be added, the methods suitable for the evaluation of one category of products may not be suitable for the evaluation of another category. The limitations are discussed further below.

Ideally, cosmetic products should be tested in accordance with the expected exposure conditions: diluted in the case of shampoos, undiluted but with short exposure times in the case of technical products such as those designed for perming or coloring. Indeed, any dilution or inappropriate exposure modifies the interaction between the product and the test matrix and can thus lead to erroneous conclusions. This means that protocols must be adapted to the categories of test products. This point is crucial to the implementation of tests in vitro.

Tests In Vitro to Be Used in Various Hair Product Categories. Technical hair products such as perms, bleaches, and dyes have a high degree of irritant potential. These

are products that are rinsed off and have a short contact time. The main advantage of in vitro methods is that eye irritant potential can be compared with that of products already on the market.

The in vitro methods suited to these products are essentially methods that use the isolated eye (8) or cornea (9,10). Cell culture tests are not very suitable because the products can exhibit an irritant effect depending on the pH, whilst culture media are buffered. Tests using hen's egg chorioallantoic membrane (HET-CAM) also appear to be unhelpful for such products in that this very fragile membrane is overly sensitive. For evaluation of tolerability by the scalp, tests on skin ex vivo or reconstructed skin models (11,12) are useful for checking skin compatibility prior to clinical evaluation. It should be mentioned that, for this type of product, specific protocols must often be drawn up that resemble or mimic actual usage conditions.

With hair care products (shampoos, rinsed conditioners, hair masks, etc., often rich in surfactant systems), alternative methods are the tools of choice in the evaluation of safety. All the eye irritancy tests may be used—cell cultures, HET-CAM, isolated eye or cornea, etc. (13–15)—on products designed to be diluted (shampoos, etc.). With undiluted products, the HET-CAM tests and those on isolated eye/ cornea are the most useful. For evaluation of tolerability by the scalp, tests on reconstructed skin model are preferred.

In the case of hair care and styling products, their form has to be taken into account. Sprays, aerosols and other vaporized products are unlikely to come into contact greatly with the skin: essentially such contact is limited to the forehead. However, eye irritant potential must be investigated.

The tests most suited to these products, which can be rich in solvents, are the eye/isolated cornea tests that have the advantage of accepting all kinds of undiluted products and are particularly suited to moderately irritant substances.

In the case of leave-on conditioners, masks or mousses applied to the hair, as well as scalp care products, in vitro tests are commonly used to evaluate skin compatibility. In this field, tests on reconstructed skin have been found to yield valuable data to predict skin irritant potential.

The Place of In Vitro Tests in the Safety Assessment of Cosmetic Formulations. In vitro tests have been the subject of numerous investigations to evaluate both their relevance and their reliability. Most alternatives to eye and skin irritancy tests have been included in pre-validation and/or validation programs. This has led laboratories to develop tests with a high level of reproducibility. Furthermore, most of these methods (cell cultures, HET-CAM, reconstructed skin models, etc.) have been found to have higher sensitivity than tests in vitro.

Formulation aids, screening tools. In vitro tests are the tools of choice to help in the development of new cosmetic formulations. These methods are used particularly to study the effect of the vehicle or formulatory environment on the irritant potential of an ingredient or a combination of given ingredients. They are also used to assess the irritant potential of a new ingredient within a formulated system. Finally they can contribute to the choice (screening) of the best formulation in the context of a new development. The tests in vitro are thus highly valued tools in the development of new products having a high level of skin and/or eye compatibility.

As an example, the results are shown below of a test on reconstructed skin model carried out in the context of the development of a new shampoo (Fig. 1).

The inclusion of "detoxifying" additives (modulators) in a surfactant base restored the viability of keratinocytes which indicate gentleness, i.e., a very mild shampoo.

Prerequisite prior to clinical tests. Besides the dossiers on each of the ingredients, in vitro tests contribute to the knowledge of the safety of formulations. These

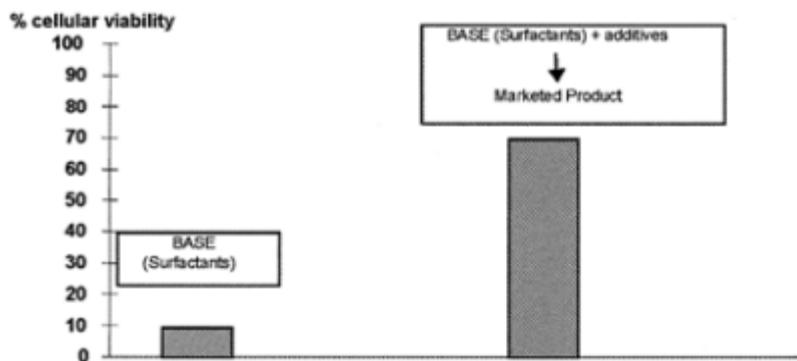


Figure 1 Effect of the inclusion of additives on the cell viability of reconstructed skin model to which surfactants have been applied (development of a child's shampoo).

tests can thus be essential tools in confirming eye and skin tolerability prior to final clinical monitoring. This is particularly important in the case of hair products when considering the risk of accidental contact with the eye.

Only an excellent awareness of the safety of products in the same category in the in vitro model considered can yield a fair evaluation (benchmarking). Most subcontractors specializing in these tests in vitro currently carry out this comparison and classify the product evaluated according to its category.

The cosmetic product dossier. When minimal changes are made to a formula (e.g., a change in a non-irritant ingredient), it should be sufficient to monitor how well the new formulation is tolerated, by comparison with the marketed product in an in vitro test.

When in vitro tests are carried out as a prerequisite prior to clinical tests and, in particular, in the context of assessing the potential effects of an accidental contact with the eyes, the inclusion of in vitro tests in the product dossier must also be envisaged. In any event, it is essential to ensure that the tests are carried out in accordance with good laboratory practices (GLP), as expected when these tests are performed by qualified subcontractors.

In vitro tests are an integral part of the evaluation of hair product safety. Their use answers both ethical considerations and the need for greater understanding of these products. Their reproducibility and sensitivity make them complementary and preliminary tools before any clinical evaluation of product tolerability.

2.3.5. Clinical Testing

After going through the toxicological profile of the ingredients and getting the results of in vitro tests, offering the necessary and adequate guarantees prior to tests in humans, the setting up of clinical tests constitutes the final stage in the evaluation of hair product safety before marketing. European legislation does not require clinical tests on cosmetic products and there are no official protocols recommended by the authorities. Nonetheless clinical test procedures have been the subject of studies and recommendations by the European cosmetic industry represented by COLIPA (Comité de Liaison de la Parfumerie) and by the European Commission through the SCCNFP.

The European cosmetic industry has considered the use of clinical tests to evaluate the safety and compatibility of cosmetic products and ingredients. Two guidelines have been published by COLIPA, one on finished products and the other on ingredients (16,17). They set out the ethical requirements to be met in the conduct of this type of test, stressing the prerequisite of toxicological evaluation of test substances. They also supply recommendations on the methodology to be applied for a scientifically pertinent clinical evaluation.

The SCCNFP has expressed its position in Appendices 11–13 of the “Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation,” dated October 2000 (4).

Appendix 11 (18) concerns the clinical testing of ingredients or mixtures of ingredients that are potential skin irritants. This type of test, designed to confirm the safety data obtained in animals or in vitro, may be necessary from both the scientific and the ethical point of view.

Appendix 12 (19) concerns tests of compatibility and acceptability. Compatibility is defined as the absence of secondary effects when a cosmetic product is applied to the skin or mucosa for the first time. The acceptability of the product corresponds to the degree of satisfaction of the consumer relative to his or her expectations when using the cosmetic product.

Appendix 13 (20) describes, in the case of cosmetic ingredients or mixtures of ingredients, the methods that are predictive of a skin sensitization potential. Only methods *in vivo* are included as no test *in vitro* has yet been validated. The SCCNFP expresses deep reservations concerning clinical tests in men designed to predict the skin sensitization potential of cosmetic ingredients or mixtures of ingredients, considering that the immunological basis and mechanisms leading to positive reactions in this type of test are insufficiently understood as yet and that it is not ethically acceptable to induce sensitization in volunteers with the added risk of triggering cross-reactions.

Clinical tests play an important role in the evaluation of cosmetic product safety. They contribute to verifying tolerability, to confirming compatibility with the skin and product acceptability. They help to ensure a more satisfactory and better-targeted market launch of the product. They supply information to the Safety Assessor prior to marketing. They can also be used to uphold claims of (high) tolerability by sensitive skin associated with the product.

Responsibility for the choice of tests to be carried out falls on the Safety Assessor of the company that markets the product or on the subcontracting test laboratory carrying out the investigation.

After having defined the appropriate tests, their sequence, the study protocols and the population required, clinical tests will usually be carried out by a clinical trial center depending upon the guarantees the centre can offer in terms of ethical considerations, the quality of the studies and the application of Good Clinical Practices. But the manufacturer retains responsibility in terms of the use of its products in clinical trials.

Clinical Trials Must Be Conducted in Accordance with the Principles of the Helsinki Declaration (1964) and its Modifications (21). They must also meet the requirements of the legal or regulatory authorities in the countries where they are carried out (thus, in France, clinical research is governed by law 88–1138 of December 20, 1988, published in the Official Journal dated December 22, 1988), called the HURIET law, and by the directives on its application.

Among the essential elements in terms of ethics are the informed consent of the volunteer and the approval by an Ethics Committee qualified to decide these matters.

A clinical test can only be carried out legitimately if it is scientifically justified. The safety of the volunteers must be guaranteed in terms of both the protocols and the cosmetic products or ingredients tested. A qualified and experienced toxicologist must evaluate their safety in the planned test. His or her approval is a condition sine qua non if the clinical trial is to be conducted. This procedure is one of the key elements in the management of clinical test safety in the field of cosmetic products.

The number of volunteers must be sufficient to meet the aims of the test. A test including too few volunteers, unable to yield a relevant scientific result, would thus be unacceptable, even on the ethical level. A test in which the number of volunteers was excessive would not be acceptable either. In practice, the population is chosen that will meet the requirements of the trial and its statistical validation.

One crucial point in the conduct of trials is to carefully select volunteers who are representative of the users to be targeted with the test product. The inclusion and non-inclusion criteria must be clearly defined, for the test to be ideally discriminatory while guaranteeing optimal safety of the volunteers.

The tests are generally carried out by specialized organizations having qualified personnel and suitable premises and equipment. They are conducted under medical supervision and, if necessary, with the participation of specialists (dermatologists, ophthalmologists, pediatricians, etc.).

2.3.6. Methodology

In general, the products are placed in contact with the skin and/or hair under usage conditions. In the case of compatibility studies, more experimental methods may be employed that offer varying degrees of maximization of the response.

In practice, the choice of type of test is usually based on experience with the evaluation of products in the same category and with a similar composition. If such experience is non-existent or insufficient, a progressive approach has to be made. The unoccluded application to the skin for a brief period (e.g., 20–30 min) of a product, perhaps diluted, represents the starting point of a progressive evaluation. If this first application gives rise to no clinical reactions, the product concentration and/or contact time may then be increased. The next step might be either several unoccluded applications to the same skin site, or a single application under a semi-occlusive or

occlusive patch. If this fails to yield an adequate discriminatory potential, the next step would be repeated application to the skin under semi-occlusion or occlusion. The repeated application may be followed by a rest period and a challenge test.

Finally, prior to a product launch, a usage test may be carried out, either monitored in a clinical trial center, or unmonitored, carried out at home by the volunteers. Usage tests furnish information on the acceptability and tolerability of a product during its use. Single applications, open or under a patch, determine the compatibility of the product with the skin. Repeated patch tests explore irritation phenomena further. After a pause followed by renewed exposure, the presence or absence of a sensitizing potential of the test substance under the test conditions can be verified.

Given the great variety of hair products, in terms of the composition and chemical reactivity of the ingredients, it is necessary to *implement protocols suited to each product category*. Products that are diluted and rinsed off, such as shampoos, must generally be tested at a dilution. The choice of the test system and the rate of dilution must generate sufficient skin reactions for the test to have a good discriminatory potential, while avoiding excessive reactions that would be unacceptable to the volunteers.

Products used undiluted fall into various very different cosmetic categories, such as unrinsed shampoos, scalp treatments, hairstyling aids, perms, non oxidation or oxidation hair coloring, hair straightening, or bleaching. Such products are generally tested undiluted. Trial conditions: application open or under a patch, patch type and form of occlusion and duration of application must all be defined in terms of the nature of the product and its potential aggressiveness toward the skin. The tier approach of testing is particularly crucial in the case of strongly reactive ingredients (perms, oxidation coloring, straightening, etc.).

Some products used sequentially may call for two successive applications to the same site so as to reproduce the usage conditions; for example, in the case of perms, application of the reducing agent and then neutralizing agent, each application being followed by water rinsing. With products requiring the mixture of two preparations or components prior to application, such as in the case of hair oxidation coloring or lightening products, it is the mixture that is used in the test.

However, many hair colorants, whether they contain oxidation colorants or direct colorants, cause coloration of the skin, which makes clinical evaluation of the results difficult or even impossible. In this event, it is necessary to decrease the colorant concentration in the test product or to study the formulation in the absence of the colorant. The result obtained with the vehicle alone can be strengthened by taking into account any available knowledge on the colorants contained in the formulation. It should be remembered that these tests take place during the last stage when the safety of a product has been sufficiently well established to permit its application in man.

The tests must be designed in such a way as for the results to be unambiguous and clearly interpretable. Questions likely to be raised may be, for example, how is the new product positioned by comparison with the product it must replace, with another brand product, with a product reputed to be well tolerated or, again, with the market leader?

On the methodology level, it is necessary to have one or more reference products at one's disposal to be able to relate the test results to the reality of product usage on the market.

In every possible case, the interpretation must have a comparative character. Moreover, it is most useful to have two reference marketed products, both having an historical record (see Sec. 2.4.) of tolerance, but one presenting low scores and the other higher scores in the clinical test under consideration. This yields a realistic scale of comparison with a low and a high reference products in the test. This approach consolidates and enriches the interpretation and the values expressed with the two reference or benchmark products can also shed light on any variability within a clinical trial center or between different centers.

The quality of the tests is conditioned by respect for ethical principles in clinical trials and the professionalism of the clinical evaluation partners—itself conditioned by a certain intransigence: no clinical trial can be conducted until the safety of the product has been cleared by a rigorous toxicological evaluation leading the Safety Assessor to the conclusion that the clinical trial can be conducted without risk to the volunteers. Clinical tests thus constitute a large and unavoidable part in the evaluation of hair product safety prior to its market introduction.

2.3.7. Final Safety Evaluation

The results of clinical testing complement the toxicological data already evaluated. On the basis of these elements, the Safety Assessor must determine whether the available data are relevant and sufficient and consider whether further testing of the ingredients or the finished product is needed.

The importance of the role and responsibility of the Safety Assessor should be stressed. He or she must have not only recognized qualifications but also sufficient experience of general toxicology and competence in the field of toxicology applied to cosmetic products. It is in the interest of the company to choose a responsible individual known for his/her ethical approach.

It is his or her responsibility, after having examined the overall data available and appraised their pertinence, to draw the conclusion that:

- the product is safe and requires no special precautionary measures,
- the product is safe as long as it is used in the appropriate container or as long as the usage precautions are explained in detail together with precise definitions of the mode of use,
- the data are inadequate and it is necessary to draw up complementary studies,
- the product is unsafe under the proposed usage conditions.

It is also his or her responsibility to record in writing the marketing viability and absence of risks “when the product is applied under normal or reasonably foreseeable usage conditions.”

2.4. Post-Marketing Safety Monitoring: Cosmetovigilance

The know-how of the formulators, respect for legislation and the whole range of data accumulated and studies carried out mean that the products offered to consumers have a high safety level. However, the marketing of millions of units of a new product, in various countries in which the population, habits, and climate each have their own

characteristics can engender a certain number of unexpected reactions or undesirable effects.

It is thus very important for all information concerning these reactions to be gathered promptly, for them to be analyzed, their origin determined, and to establish if there is a causal link between use of the product and an undesirable event. This is the role of cosmetovigilance.

In Europe, the legislation has fully recognized the importance of this monitoring since the sixth modification of the European Directive on Cosmetics requires that existing data on undesirable effects for human health be held at the disposal of the competent authorities. Without waiting for this regulatory requirement, most companies that market cosmetic products have already drawn up a structure to gather and process all information concerning actual or suspected undesirable effects arising from the use of a cosmetic product under normal usage conditions.

2.4.1. The Running of a Cosmetovigilance Department

All complaints received by telephone, fax, mail or e-mail, whatever the brand, its distribution, the country in which it is marketed, are recorded by local workers on an identical computerized system, then the data are transmitted and centralized in a single department for the company.

The personnel running this organization preferably consists of physicians trained in dermatology and/or toxicology with an in-depth knowledge of the formulae and technology used in cosmetology.

This threefold competence ensures that the clinical nature of the undesirable effect and the existence or absence of a relationship with any product can be readily identified. Each of these queries must be handled rapidly so that the maximum information on the clinical description of the symptoms, the timing, the simultaneous use of other products, the history, and the environment can be recorded.

To complement any lack of information, the individuals responsible for cosmetovigilance in various countries, in direct contact with the central department, can make contact with the consumer or the physicians in order to obtain additional information and supply any data required on the products concerned.

This meticulous questioning and the accumulation of knowledge about the product helps to determine whether the product was responsible for the reported effect or whether further investigation is required.

The method used to assess attributability is based on the official method of Bégaud et al. (22) adapted for cosmetics. This method is based on symptomatological, chronological and bibliographic data and on the results of appropriate complementary examinations: patch tests, for example, in the case of an allergic response. The undesirable effects can be classified as being of certain, probable, possible, doubtful, or no attributability.

A register of undesirable effects per annum is drawn up and at any time it is possible to check the number of undesirable effects recorded per market area.

Skin reactions constitute the largest section of health-related complaints made. Most of the reactions are presented as allergies but irritation seems to be by far the most common occurrence. It is always difficult to determine the origin of urticaria, even when

explored by the most highly trained teams of allergologists. Systemic allergic reactions are very rare.

Overall, only a very small percentage of cases is found to be attributable with certainty to the product investigated. This low percentage, relative to the number of units sold, demonstrates the very great level of safety offered by hair products.

2.4.2. *The Value of Cosmetovigilance*

Protection of the consumer's health is the prime aim of cosmetovigilance: in this way the problems of undesirable effects can be dealt with more effectively, more straightforwardly, by direct and rapid contact with the consumer and/or his or her physician. However, its value goes beyond this central aspect since it meets the real needs of the consumer, of the doctor in formulating a diagnosis and of the manufacturer as well:

- *For the consumer*, identification of the true problem and of the mechanism of an undesirable effect leads to its disappearance or failure to recur as appropriate measures can be taken such as elimination of the ingredient responsible. Moreover, replacements that are acceptable esthetically and without risk of trouble to the consumer can be suggested.
- *For the physician*, who obtains not only basic information on the ingredients of the product from competent experts, but also samples of the various ingredients so that s/he is in a position to perform further tests if required. This is very important to the extent that many of the ingredients are not found in standard batteries, for example, and a compatible vector and a non-irritant concentration have to be selected. The dialog can also reframe the undesirable effect observed in a wider context and provide the physician with data on any similar observations.
- *For the manufacturer*, cosmetovigilance constitutes an alarm system that enables undesirable effects of a new product to be compared at the historical and ethically acceptable level with reference products. Indeed, if a few sporadic cases of benign undesirable effects attributed with certainty to a given ingredient are acceptable on both the ethical and commercial level, a greater frequency to that which is acceptable for reference products must inevitably lead to rapid modification of the formulations.

A further gain by cosmetovigilance is that it provides complementary data to epidemiological studies. Thus the nature, extent and frequency of side effects related to a given ingredient over time can be closely followed and registered. An illustrative example can be brought out in hair dye products containing *p*-phenylenediamine (PPD), a known allergen. This type of products has been in existence for some 100 years, initially intended to hairdressers only, then available to general public as home-used kits in the last 50 years. They meet with growing demand and favor (see Chapter 9). In 1996, it was estimated that some 60% of female consumers in EU and North America used hair dyes compared with 40% in 1969. Since then, dyeing hair has steadily and often markedly increased in all parts of the world. Presently hundred millions of individuals worldwide use hair dyes on a regular basis.

From cosmetovigilance data, confirmed allergic reactions to PPD are currently rated as one per one million hair dye units sold (industry records). These results support epidemiological data from patch tests to PPD in dermatological clinics that have been

reviewed by Hall in Appendix I. Altogether, recorded data substantiate the efficacy of prevention measures developed and put in practice by manufacturers:

- *Labeling, warnings, instructions for use* (e.g., wearing gloves) to limit exposure on the packaging and enclosed leaflet of every hair coloring product.
- *Education and training of hairdressers.* Drawing up of guidelines for good practice and application of hair products for professional use strongly advising to carry out a sensitivity test 48 hr prior to hair dyeing with oxidation coloring product, not to apply on an individual previously sensitized or having experienced any skin reaction, even slight, to such type of product, not to apply on sensitive, irritated or damaged scalp and to use the product exclusively for hair dyeing with strict compliance with prescribed amount and contact time.

3. THE ENVIRONMENTAL SAFETY OF COSMETIC PRODUCTS

Apart from the need to ensure the safety of the user, the scientist developing cosmetic products must also take into account ethical concerns about the environmental impact of these products.

The scientist takes into consideration, on the one hand, the intrinsic risk with each ingredient based on its capacity to a hazard and, on the other, the potential for diffusion into the natural surroundings—the degree and nature of the *exposure* under normal usage conditions.

The characteristics of harmfulness to the environment of a substance are essentially based on the following three criteria:

- Persistence
- Bioaccumulation
- Toxicity (ecotoxicity).

3.1. Persistence

A substance is said to be “persistent” if its transformation or conversion into degradation products is sufficiently slow for it to remain present in the natural surroundings for a long time and to be widespread.

The persistence of a product is characterized by its half-life, or the time required for half the original concentration (or quantity) to disappear.

Various degradation phenomena can be involved such as photolysis, hydrolysis, or biodegradation (aerobic or anaerobic).

This depends upon the situation in which the pollutant is found (air, water, sediment, or soil).

In the aquatic medium, biodegradation is often the most common phenomenon.

Reference is often made to “ready” biodegradability, which can be defined as follows:

- Arbitrary classification of a chemical substance that has passed certain specific ultimate-biodegradation tests; these tests are so rigorous that it can be assumed that

such compounds undergo rapid and complete biodegradation in the aquatic environment under aerobic conditions (table of methods in Appendix II);

or to *intrinsic biodegradability* of which the definition is:

- classification of a chemical substance for which there is unequivocal proof of biodegradability (primary or ultimate) in any biodegradability test (methods in Appendix III).

If these tests are not conclusive, a simulation test must be carried out, reproducing the conditions existing in waste water treatment plants (method again described in Appendix III).

3.2. Bioaccumulation

The term “bioaccumulation” is defined as an organism’s enrichment with a substance via any and all of the absorption routes: the atmosphere, water, soil, and foodstuffs. More restrictively, “bioconcentration” is defined as an organism’s enrichment with a substance following exposure, of which water and/or air are the vectors. The bioconcentration level is defined by the “bioconcentration factor” (BCF).

3.3. Ecotoxicity

Ecotoxicity designates the capacity of a substance to provoke toxic effects on organisms present in the environment concerned, or on their offspring, such as lower survival, growth or reproduction rates, carcinogenicity, mutagenicity or teratogenicity, and prejudicial effects as a result of disturbance of the endocrine system.

Depending upon the duration of exposure and the target organism’s lifespan, ecotoxicity can be classified as follows:

- *Acute ecotoxicity*: lethal or sublethal toxicity resulting from intermittent or continuous exposure to a substance or mixture of substances for a period clearly shorter than the life expectancy of the organism in question.
- *Chronic ecotoxicity*: sublethal toxicity resulting from intermittent or continuous exposure to a substance or mixture of substances for a period close to the life expectancy of the organism.

Ecotoxicity is determined by tests carried out on approved organisms, principally algae and the *Daphnia* genus in the case of the aquatic environment (description of acute ecotoxicity tests in aquatic environment in Appendix IV and description of chronic tests in Appendix V).

3.4. Choice of Ingredients Formulating a New Product

The selection of ingredients in the process of developing a new product must take into account the following three criteria and the means by which the impact may be minimized:

- *Non-persistence*: A preference for readily biodegradable or even intrinsically biodegradable ingredients. If this should not be the case, other degradation phenomena must apply, such as photolysis or hydrolysis.
- *No tendency toward bioaccumulation*: European regulations consider as bioaccumulable any substance having BCF>2000, the result of a regulatory bioaccumulation test.
- *Lower ecotoxicity level*: For example, in Europe, to avoid being classed as ecotoxic, a substance must exhibit CL₅₀>10mg/L (see Appendix IV for definition of the CL₅₀)

Clearly, no ingredients should ever be selected that might present a profile persistent and bioaccumulable and toxic (PBT) or even a very persistent, very bioaccumulable (vPvB) profile (see Appendix VI for the European project for PBT or vPvB classification).

3.5. Risk Assessment

When the quantities of products exploited in a given geographic zone are known, it is possible to carry out an assessment of the risk to the environment posed by the products concerned.

The expression “risk assessment” designates the relationship between the predicted exposure on the one hand and, on the other, the inherent prejudicial effects. It goes through four major phases: determination of the hazard, evaluation of the reaction to doses, evaluation of exposure, and characterization of the risk.

Assessment of the risk posed by a substance must be carried out for the whole “life cycle” of the substance, from synthesis of the raw material through consumption of the product to final treatment of urban effluent, recycling or incineration and covering the three compartments:

- *aquatic compartment* receiving domestic and industrial effluents (directly or via waste water treatment plants),
- *terrestrial compartment* (soil) that undergoes agricultural spreading of the sludge,
- *atmospheric compartment* that takes up volatile substances.

The aquatic compartment includes surface, subterranean, and marine water. The sediments of fresh and marine water must also be included.

Risk assessment is expressed for each stage and each environment in the form of a ratio between:

- the predicted environmental concentration (PEC),
- the predicted no-effect concentration (PNEC)

It is assumed there is no predicted risk if PEC/PNEC<1.

A substance will thus be environmentally safe if the various PEC/PNEC ratios are <1.

If they are not, the determination needs to be refined or, if confirmed, a reduction should be made in the quantity used.

APPENDIX I**Review of Reported Epidemiological Data from Patch Tests to PPD in Dermatologic Clinics (Hall, 2003)***Prevalence of Positive Patch Test Reactions (Contact Allergy) to PPD in Unselected Eczema Patients*

Table 1 illustrates the prevalence of positive patch test reactions to PPD in dermatology clinics in Europe, North America, and Singapore. The studies were conducted

Table 1 Frequency of Positive Patch Test Reactions to PPD in Patch Test Clinics (226,779 Patients)

Country	Years	No. of patients tested	% Positive (mean)	Reference
Denmark	1975–1980	3664	1.25	Veien et al, 1992
Denmark	1973–1977	3225	1.3	Hammershoy, 1980
Denmark	1986–1990	6759	0.35	Veien et al., 1992
Sweden	1969–1980	8933	4.0	Edman and Moller, 1982
Sweden	1993–2000	21,840	1.52	Wahlberg et al., 2002
Bulgaria	1978–1990	8051	5.06	Krasteva, 1993
Germany	1992–1996	45,250	4.8	Uter et al., 1988
UK	1982–1998	26,706	2.5	Armstrong et al., 1999
Italy	1984–1988}	42,839	3.0	Sertoli et al., 1999
	1989–1993}		4.1	Gola et al., 1992
Italy	1999	8573	3.6	Lisi et al. 2001
Spain	1973–1977	4600	6.05	Romaguera and Grimalt, 1980
Switzerland	1989–1993	2014	4.9	Elsner et al., 1995
European Research Group ^a	1967–1968	4825	4.5	Fregert et al., 1968

Country	Years	No. of patients	% Positive	Reference
		tested	(mean)	
ECDGRG ^b	1989	2070	2.3	Dooms Goosens et al, 1989
The Netherlands	1995–1999	2058	3.1	Devos and van der Valk, 2001
The Netherlands	1995–2000	1701	2.87	Van der Valk et al., 2003
Canada	1972–1981	4190	7.3	Lynde et al., 1982
NACDG ^c	1972–1974	3216	6.1	Rudner et al., 1975
NACDG	1979–1980	2145	6.5	Lynde et al., 1982
NACDG	1984–1985	1138	6.9	Storrs et al, 1989
NACDG	1985–1989	2017	6.8	Nethercott et al., 1991
NACDG	1992–1994	3549	6.3	Marks et al., 1995
NACDG	1994–1996	3111	6.8	Marks et al., 1998
USA	1988–1997	927	5.0	Albert et al., 1999
Singapore	1984–1985	2471	3.2	Goh, 1987
Singapore	1986–1990	5557	4.5	Lim et al., 1992
Singapore	1998–1999	406	8.1	Goh, 2001

^a Denmark, England, Finland, Holland, Italy, Sweden, West Germany.

^b Belgium, Denmark, England, Finland, Germany, Holland, Ireland, Spain.

^c North America Contact Dermatitis Group.

between 1967 and 2000 and provide results from 226,373 patients with eczema undergoing routine investigation. The reaction strength (+, ++, or +++) was not specified and no information was given on the clinical relevance of the positive patch test reactions.

1% PPD-base in petrolatum was patched throughout, with the exception of the studies conducted from 1985 to 1988 where 0.5% PPD dichloride was used^a. Excluded from this review are individual case reports, studies where selection criteria and/or patch test procedures were not clearly described, studies on selected patient populations (e.g., only occupational) and studies on less than 1000 patients, with the exception of one study where 927 patients were tested (Albert et al. 1999) and one reported by Goh in 2001.

Frequency of Hair Dye Dermatitis in Unselected Eczema Patients

Information presented in Table 2 is a compilation of data reported by European and Japanese clinics for the period between 1970 and 2000. A total of 72,413 unselected eczema patients were either patch-tested with 1% PPD in petrolatum (A) or examined, with no mention of the patch test (B).

^aUntil 1984 PPD base was patch-tested at 1% in petrolatum as part of the ICDRG standard series. Between 1984 and 1989 it was replaced by PPD dichloride (0.5% in petrolatum) and thereafter the

patch test procedure was reversed and it continues until today in its pre-1984 form. Comparative studies, both in man and in the guinea-pig demonstrated diminished sensitivity of the 0.5% PPD dichloride patch (increased rate of false-negative reactions) compared with 1% PPD base (Dooms-Goossens et al. 1989).

Table 2 Prevalence of Hair Dye Dermatitis in Unselected Eczema Patients (72,413 Patients). Patch Test—Aided^(A) or Examination Only Based^(B) Diagnosis

Country	Years	No. of patients	Hair dye dermatitis (%)	Reference.
Italy ^(A)	1968–1983	8230	33 (0.40)	Angelini et al., 1985
Portugal ^(A)	1993–1998	1934	12 (0.62)	Antunes et al., 1998
Netherlands ^(A)	1995–1999	2058	10 (0.49)	Devos and van der Valk, 2001
Sweden ^(A)	1993–2000	21,840	57 (0.26)	Wahlgren et al., 2002
Japan ^(B)	1970	24,564 ^a	34 (0.13)	Ishihara et al., 1983
Japan ^(B)	1978–1980	13,787 ^b	17 (0.12)	Ishihara et al., 1983
Total		72,413	163 (0.22)	

^a Data from five large metropolitan hospitals in Japan, joint survey with the Japanese Ministry of International Trade and Industry.

^b Data from one Japanese University Hospital.

A total of 163 patients, i.e., 0.22% of the unselected eczema patients, were thus positively confirmed with hair dye-induced dermatitis.

Conclusion

Overview of clinical epidemiology of contact allergy to PPD (positive patch test reactions) in Europe and North America during the period of over 30 years (Table 1) shows a steady or diminishing rate of positive reactions to be considered within a general background of increased exposure to PPD resulting from the significant growth of hair dye use (containing PPD for the greater part) by women, from about 40% in 1969 to some 60% in 1996 (Industry data).

It can be noted from the presented data that the prevalence of confirmed hair dye dermatitis in the population of unselected eczema patients (Table 2) is less than one-tenth of the prevalence of contact allergy to PPD, based solely on the patch test data (Table 1).

APPENDIX II**Ready Biodegradability**

OECD Princ iple	Method	Inocu lum ^a	Bacteria per ml	Concen tration of tested substance	Anal ytical crite rion	End point	AFNOR	CEN	ISO	EEC 92/69	
301 A	DOC exhaus tion	Disappe arance of DOC AFNOR modified	15–30 mg/L MES	10^7 – 10^8	10–40mg DOC/L	Disappe arance of DOC	70% elimini nation	NF T 90–312	EN 27827	ISO 7827	C ₄ A
301 B	Carbon dioxide (CO ₂) release	Sturm modified	15–30mg/ LMES	10^7 – 10^8	10–20mg DOC/L	Respir ometry: CO ₂ release	60% CO ₂ / CO ₂ th	NF EN 29439	EN 29439	ISO 9439	C ₄ C
301 C	Respi rometry Oxygen (O ₂) cons umption	MITI modified	30mg/ LMES	10^7 – 10^8	100mg/L	Respir ometry: O ₂ consum ption	60% Odb /ODc				C ₄ F
301 D	Respiro metry O ₂ consu mption	Closed flask	Up to 5mL sec ondary effluent/L	10^4 – 10^8	2–110mg/ L5–10 mg ODth/L	Respiro metry: disso lved O ₂	60% ODb/ ODc	NF EN 10707	EN 10707	ISO 10707	C ₄ E
301 E	DOC exha ustion	OECD modified screening	0.5 mL secondary effluent/L	10^5	10–40 mg DOC/L	Respir ometry: DOC disapp earance	70% elimini nation				C ₄ B
301 F	Man ometric respiro metry	Mano metric respiro metry	15–30mg /LMES	10^7 – 10^8	100mg/L 10–40mg DOC/L 50–100 mg ODth/L	Mano Metric respir ometry: O ₂ consu mption	60% ODb/ ODc	NF EN 29408	EN 29408	ISO 9408	C ₄ D

^a Usually activated sludge. Only from a purification plant dealing mainly with domestic waste waters. DOC, dissolved organic carbon; ODth, theoretical oxygen demand; ODb, biological oxygen demand; ODc, chemical oxygen demand.

APPENDIX III

Inherent Biodegradability

OECD	Principle	Method	Inoculum	Bacteria per mL	Concentration of tested substance	Analytical criterion	Endpoint	AFNOR	CEN	ISO	EEC 92/69
302 A	DOC exhaustion	SCAS modified	1–4 g/L MES		>20mg DOC/L	DOC exhaustion	70% elimination	NF EN 29887	EN 27887	ISO 9887	Page 123
302 B	Release of DOC or ODc	Zahn Wellens EMPA	0.2–1 g/L MES		50–400mg DOC/L 100–1000 mg ODc/L	DOC or ODc exhaustion	70% elimination	NF EN 29888	EN 29888	ISO 9888	Page 99
302 C	Respirometry O ₂ consumption	MITI modified	100mg/L MES		30mg/L	O ₂ consumption	O ₂ /ODth %				

N.B. 1: "CONCAWE biodegradability test" proposed as OECD 302 D standard test.

N.B. 2: "OECD 302 A test" not currently accepted for environmental risk evaluation in EU.

Biodegradability—Simulation Trial

OECD	Principle	Method	Inoculum	Bacteria per mL	Concentration of tested substance	Analytical criterion	Endpoint	AFNOR	CEN	EEC
								ISO		92/69
303 A	Simulation of urban waste water treatment plant conditions	Aerobic treatment of waste waters: coupled units	Secondary effluent + soil + surface water		> 12 mg DOC/L ≥40mg DOC/L Optimal value: 20 mg DOC/L	Disappearance of DOC or ODc vs. control	% DOC or ODc vs. control			ISO 11733

References: OECD Guidelines for testing chemicals—Section 3: Degradation and Accumulation.

THOMAS H, *Surfactant and the Environment—An Overview*, special Publication Royal Society of Chemistry, 1999, 230, p. 23–39.

Stage: *Les essais d'écotoxicité et de la cancérogénicité des produits chimiques*, CSE Metz. JOCE N° L 383 du 29/12/92

APPENDIX IV**Acute Aquatic Toxicity**

OECD	Type of test	Recommended species	Test duration	Measured endpoints	Result
201	Algae Growth inhibition assay	<i>Pseudokirchneriella subcapitata</i> (previously <i>selenastrum capricomutum</i>) <i>Scenedesmus subspicatus</i> <i>Chlorella vulgaris</i>	72 h ¹	Growth rate and biomass	CE50 or CI 50 ²
202	Daphniae Instant standstill assay	Genus <i>Daphnia</i> (<i>Daphnia magna</i> , <i>Daphnia putex</i> ,...)	48 h	Standstill	CE50

¹Test duration is not set by OECD Guideline, it is defined based on controls' growth. In general, test duration is 72 h.

² According to OECD 201 Guideline, the results are expressed as CE₅₀ (growth rate) et CE_b50 (biomass); EU regulations take in account the lower value so called CI50.

Reference: OECD Guidelines for chemicals. Section 2: effects on biological systems.

APPENDIX V**Chronic Aquatic Ecotoxicity**

OECD	Type of test	Recommended species	Test duration	Measured endpoint	Result
201	Algae Growth inhibition assay	<i>Pseudokirchneriella subcapitata</i> (previously <i>selenastrum capricomutum</i>) <i>Scenedesmus subspicatus</i> <i>Chlorella vulgaris</i>	72 h ¹	Growth rate and biomass	CSEO or NOEC
211 ²	Daphniae Reproduction assay	<i>Daphnia magna</i>	21 d	Reproduction	CSEO or NOEC

¹Test duration is not set by OECD Guideline. It is defined based on control's growth. In general, test duration is 72 h.

² OECD 211 Guideline replace OCDE 202 Guideline, section II.

CSEO (concentration sans effet observé)=NOEC (No Observed Effect Concentration)

APPENDIX VI**PBT, vPvB^a Criteria**

P (Persistence)	B (Bioaccumulation)	T (Toxicity)
Half-life>40d fresh water		NOEC<0.01 mg/L

or>60d marine medium		Or CM 1 and 2R 1, 2 et 3
or>120d fresh water sediments	BCF>2000	Or T, R48 classification, or X _n , R48
or>180d marine medium sediments		
or>120d soil		(According to EU 67/548 Directive)
vP		VB
Half-life>60d fresh water and marine medium or>180d sediments or>180d soil		BCF>5000

^a EU regulation draft.

NOEC, Non-observed effect concentration; C=carcinogen; M=mutagen; R=toxic for reproduction.

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12

Evaluation of Product Efficacy

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The development of appropriate and reliable test methods for the evaluation of hair product efficacy represents a permanent challenge. The availability of such tests is needed for:

- The detection of the activity and potential advantages of a new ingredient,
- The assistance in the formulation of this ingredient and in the development of new products,
- The development of claim substantiation files.

Efficacy assessment of a hair product can be approached in two ways, which are in many cases complementary and indispensable to obtaining an understanding of the efficacy of a hair product:

- In vitro assessment using instrumental methods on hair swatches,
- In vivo assessment, either by instrumental methods on head, by sensory analysis, or by consumer testing.

1. IN VITRO ASSESSMENT

The availability of new ingredients and the development of hair products continue to grow. The products themselves are becoming more and more sophisticated and need evaluation methods, instruments, and test protocols that are more and more complex and relevant. In recent years, sophisticated tools have been introduced in hair research, product evaluation, and claim substantiation to achieve these challenges (1–4). One goal of these methods is also to simulate real-life conditions (5), and consumers must be able to perceive the measured effects (6).

Instrumental evaluation can be classified arbitrarily according to the major categories of effects sought:

- Style and style retention,
- Surface quality,
- Quality of the fiber,
- Color.

The starting point of in vitro evaluation of the performance of a hair product is the hair itself, either single fibers or in assembly (usually hair swatches). All the measurements

depend on the nature of the hair sample and hence its "cosmetic history." The first priority is therefore to standardize the hair samples on which the tests and studies are performed and control their quality. This standardization is established directly on the basis of knowledge of the users habits and thus the quality of hair found on the head (7–9).

1.1. Evaluation of Hairstyle

Together with its colour, the shape of the head of hair is the most visible property for the consumer, which both men and women like to change. Hairstyle has a very significant effect on overall appearance. It can be modified by many different kinds of product which are classified into two main categories according to the duration of the effect over time:

- A lasting change in style, e.g., by the use of a perm or a relaxer,
- A temporary change in style, e.g., by the use of a mousse or a spray.

The style of the head of hair depends on a wide variety of parameters and characteristics of the fiber including the angle of implantation of the hairs on the scalp, hair diameter and length of the hair, degree of natural curl, fiber flexion modulus, friction coefficient, and many others. Hairstyle is often related to the concept of hair body and volume and it has been suggested that it could be predicted from single hair characteristics (5), but it remains a tricky approach (5,10). Fiber to fiber interactions are one of the most critical parameters for determining style and most authors agree that only measurements made on hair swatches can properly predict hair style. As a result, methods developed on single fibers, although easier to interpret, are most often used for the screening of new ingredients such as polymers whilst tests conducted on swatches, closer to the perceived effect, are devoted to the evaluation of the final performance of products.

Due to the great variety of products aimed at modifying the style of hair (ranging from specifically formulated shampoos to hair setting and permanent waving products), and the diversity of the perceived properties contributing to define "style" (hold, rigidity, spring, stickiness, etc.), a huge number of methods have been described for evaluating style and style retention. Most often, they can be used irrespective of the type of hair styling product, but some of them are specific to one type of treatment, especially perm waves and relaxers. It is impossible to make an exhaustive description of all reported test methods within the scope of this chapter. Only the most often referred to will be considered according to the following classification:

- Measurement of the natural shape of hair,
- Evaluation of style retention by static methods,
- Evaluation of style retention by dynamic methods,
- Measurement of mechanical characteristics of fibers or swatches,
- Permanent waving and relaxing efficacy,
- Hair volume and body.

1.1.1. Measurement of the Natural Shape of Hair

Measuring the 3D geometry of hair curliness (the so-called crimp) of hair is quite difficult. The simplest method is to use a template (11) and to compare the hair

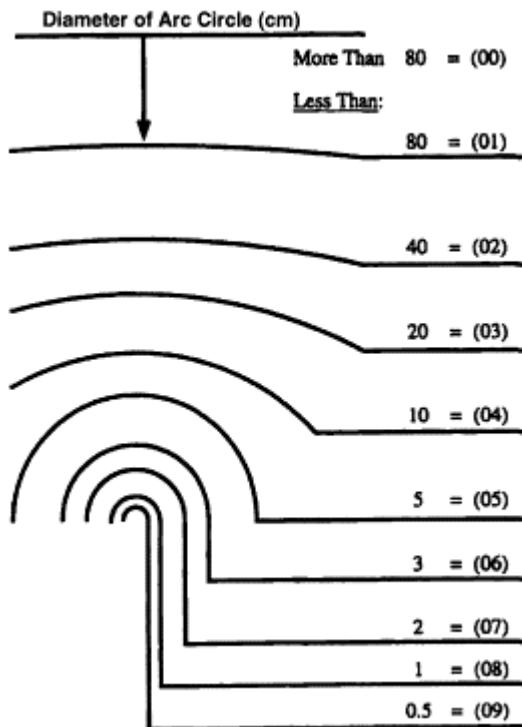


Figure 1 Hair curvature measurement template. (From Ref. 11.)

curvature to circles of known diameter (Fig. 1). Hair can thereby be classified as a function of its local crimp.

Instrumental methods were proposed to measure some geometrical parameters of hair fibers, such as diameter (12–15), local curvature and torsion (16), or the number of turns and the ratio between the natural and the stretched length of hair (17,18) (Fig. 10), but they all proved to be very difficult to run and the data obtained were difficult to use.

1.1.2. Evaluation of Style Retention by Static Methods

Since a long time, the reference method has been the curl retention test (10,19,20). Easy to run, it provides direct comparison of the performance of the products, whatever their type. The basic principle is simple: calibrated tresses of hair are wound round a curler of known size under given stress conditions and treated (Fig. 2) (21). After drying under controlled conditions, the curled tresses are unwound from the rollers, hung in front of a

graduated panel (Fig. 3), and allowed to relax in a chamber where the relative humidity (RH) and temperature are controlled. The increase in length of the tress vs. time provides relevant information on the effectiveness of a hair setting product. The level of curl retention is commonly expressed from Eq. (1), even though some authors proposed other ways to calculate this parameter (22):

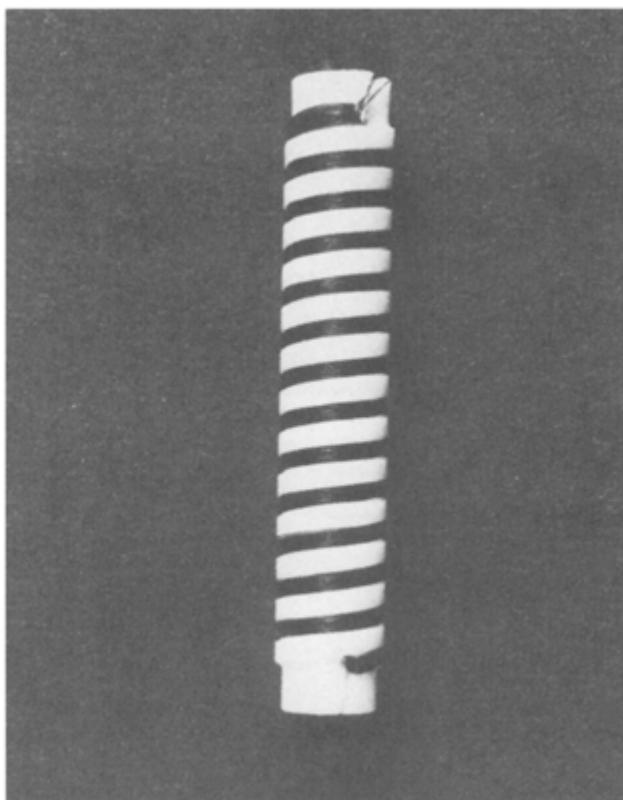


Figure 2 Hair tress wound round a curler for Static Curl Retention Test.
(From Ref. 21.)

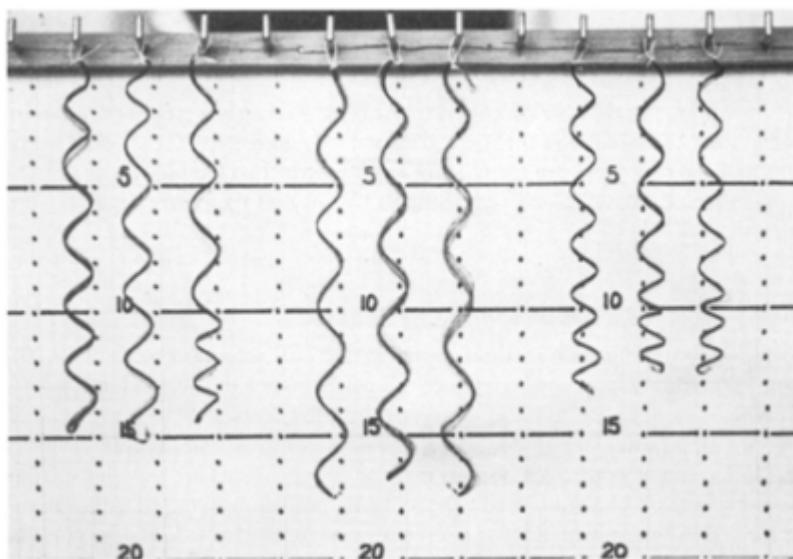


Figure 3 Curl retention test, unwound tresses after relaxation under controlled humidity. (From Ref. 21.)

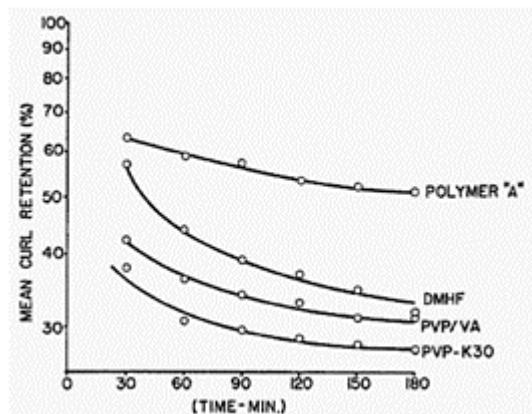


Figure 4 Curl retention at 90% RH as a function of time for several commercial hair spray polymers (from Ref. 20). PVP: polyvinylpyrrolidone; PVP/VA: polyvinylpyrrolidone/vinyl

acetate; DMHF: dimethyl hydantoin-formaldehyde resin.

$$\text{Curl retention (\%)} = \frac{(L - L_t)}{(L - L_0)} \times 100$$

(1)

where L is the length of hair fully extended, L_0 is the length of hair before exposure to controlled humidity, and L_t is the length of curl after exposure for time t .

This method has been applied to the assessment of all types of product. It can be also used to study and compare the effect of polymer solutions sprayed on the unwound tresses (Fig. 4). The method is often used in high RH conditions as water plays a major role in curl retention: relaxation phenomena are strongly accelerated at high RH, increasing the sensitivity and discriminating potential of the method. Many variations on the test procedure are found in the literature, including rollers of different diameters, the use of a pegboard instead of rollers, leading to a flat but undulating (zigzag) shape (23–25).

The curl retention test is a simple and efficient routine method but it requires repeated assays to overcome the variability in the procedure of sample preparation (stress applied when winding, superimposed spirals, etc.). The visual determination of length can be improved using image analysis techniques. The curl retention test has been compared with many other instrumental methods (26) and with sensory analysis. It appears to be the fastest and most reliable.

1.1.3. Evaluation of Style Retention by Dynamic Methods

Dynamic methods have been proposed to improve the static curl retention test by imparting motion to the swatches in order to account for real-life conditions. The simplest method to most closely reproduce the behavior of hair on head is the mobile

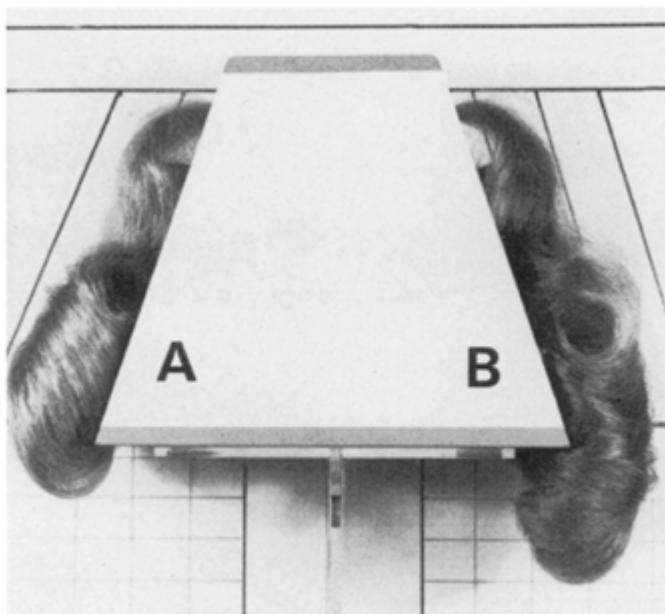


Figure 5 Dynamic hold test—result of comparison between swatches set after application of a setting lotion (A) vs. water set (control B). (From Ref. 21.)

wig test (27). Carefully calibrated swatches, treated in identical fashion except for the tested product, are set up so as to form the two halves of a mobile wig. The wig is stirred with an alternate motion for a given length of time in order to mimic the movements to which hair is daily submitted. The differences in appearance between the left and right wig halves indicate the degree of effectiveness of any kind of hair styling, setting or hold product compared with water set or benchmark product (Fig. 5).

Some authors suggest that the measurement of dynamic mechanical properties of treated swatches leads to an improvement in the evaluation of imparted style and retention performance. Caruso et al. (28) proposed a method called "curl bounce test" which involved the measurement of the oscillation amplitude, frequency, and damping of a curled swatch vertically dropped (Fig. 6). Busch et al. (29) allowed the end of a swatch, treated to form a hook, to freely oscillate and measured the oscillating amplitude, frequency, and damping. As in the former method, the mechanical characteristics assessed are aimed both at better describing the benefits afforded by the tested product and estimating other important parameters such as the elasticity or springiness of the curl to be measured. However, these methods are quite complicated and tedious compared with those previously described. Consequently, they are infrequently used and limited data are reported.

1.1.4. Measurement of Mechanical Characteristics of Fibers or Swatches

In the case of hair styling products (sprays, mousses, etc.), the styling effect is reached through deposition of a setting polymer that either glues the fibers or modifies their rigidity. Resulting modifications of the mechanical properties of single hair or bundles of hair has been used to predict the efficiency of styling polymers, and several methods described for measuring it.

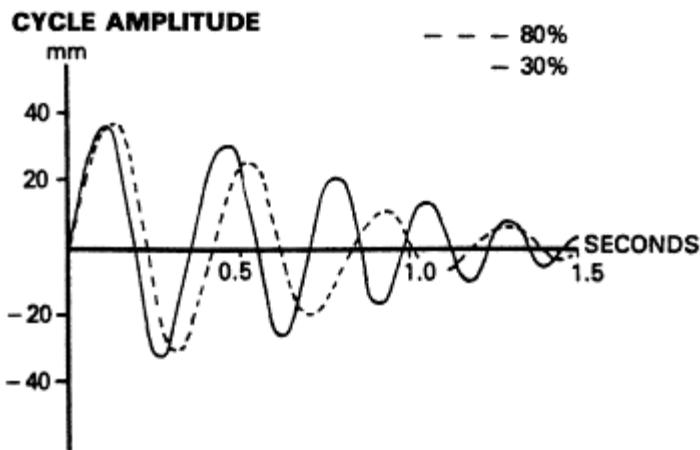


Figure 6 Bounce test—Influence of relative humidity (plain line: 30% RH; dotted line: 80% RH) on the frequency and amplitude of the swatch vertical motion. (From Ref. 28.)

Mechanical Properties of Fibers. The shape of hair is partly governed by its flexion properties. Mousses and other styling products act by spreading a rigid polymer onto hair, increasing the overall flexion modulus of the composite hair-polymer. A number of methods have thus been designed to measure the flexion modulus of fibers covered by a setting, film-forming polymer. Among them, the oscillating beam method proved to be the most sensitive and allowed polymers to be correctly ranked according to their perceived properties (30). Hair sprays work in a different way, by creating weld points between fibers. Wickett et al. (31) designed a method for measuring the adhesive strength of hairspray bonds by measuring the force needed to break a polymer bond made between two fibers (Fig. 7). This method showed that breakage generally occurred at the polymer-hair interface (adhesive junction). A key factor is therefore to achieve optimal spreading of polymer solutions on the hair. Several methods, based on the physicochemical characterization of the hair surface (32–34) have been used to evaluate this. A good correlation between this force and the efficacy of the spray was found.

Mechanical Properties of Bundles of Hair. As for single fibers, the idea is to obtain an evaluation of the bending properties of small swatches of hair treated by a setting

polymer. These tests are believed to give data that contain information on both the modification of the bending rigidity of the fibers and the increased resistance to mechanical stress generated by the junctions created between hairs. As a consequence, they are more difficult to interpret, but yield global results that better correlate with the styling performance of the products. All the methods are based on the same principle: a bundle (fiber array, swatch) of hair is treated with a polymer solution (or spray), then submitted to mechanical stress. The resistance of the bundle to the applied stress is a measure of the styling ability of the polymer. Applied mechanical stress can be:

Flexion. As described by Frosch and Vogel (35) and later by Jachowicz and McMullen (36), a straight swatch is treated, and its resistance to three-point bending measured. From the data obtained, information can be drawn on various parameters

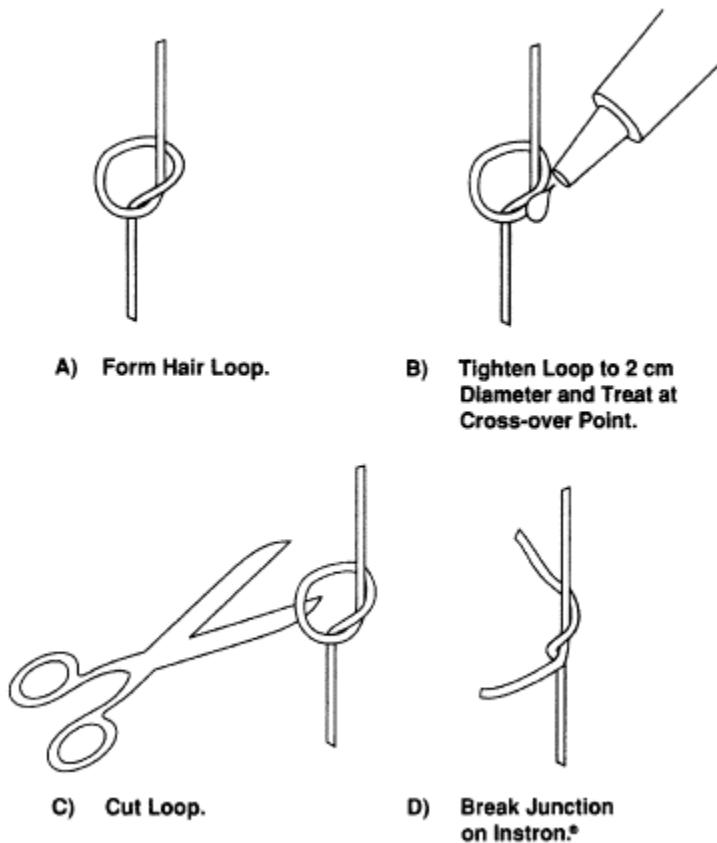


Figure 7 Illustration of the knot adhesion test method. (From Ref. 31.)

such as rigidity, flexibility, plasticity, etc. Their combination permits to design polymers that provide high levels of style and style retention.

Torsion. Bogaty (37) and Ganslaw et al. (38) showed that torsion forces were important in the setting of a head of hair. Twisted swatches were treated with polymer solutions and then allowed to untwist. The remaining twist after a certain period of time proved to be in excellent agreement with the results given by the curl retention method. The authors reported that this torsion procedure showed a better reproducibility and was able to bring out statistical differences between setting polymers where the curl retention test could not.

Composite stress. In this approach, hair assemblies are curled into one unique curl (omega loop) (36,39,40) or several curls (41), then treated with the polymer solution, and their resistance to compression was measured. Setting efficiency was evaluated by compression of the loop. The main advantage of these methods is that they also provide information on the aging of the polymer-hair composite when submitted to various cycles of compression and thus on the ability of the final product to sustain repeated stress. These methods also enable the measurement of the tack generated by a styling product during its drying.

1.1.5. Permanent Waving and Relaxing Efficacy

Permanent Waving. As permanent waves aim at modifying the shape of hair, all the previous methods can be used to assess their efficiency. Another approach is widely used (42). It consists of winding a thin tress of hair onto a small-diameter roller, then carrying out the perm process. The hair is then cut in order to obtain individual snippets of hair, more or less curled depending on the strength of the perm: the smaller the diameter of the snippets, the greater the efficacy of the waving solution. An ideal perm efficacy is obtained when the diameter of the snippets is equal to the diameter of the rollers. As in the case of the curl retention test, the evaluation can be made by imposing a 2D deformation on the hair, instead of a 3D curl (43), which makes the measurement easier.

Another approach (44) consists of carrying out the measurements on a tress of hair that more closely resembles the actual head of hair visually. Thus, a tress is wound round itself with a given number of turns (whorls). Maintained in this position at a given stress, it is treated with the test perm under specific temperature and time conditions. One of the ends of the tress is then freed so that the hair can unwind freely. The waving efficiency is obtained by determining the ratio of the number of whorls obtained to the initial number of whorls. The hold of the perm is evaluated by determining the ratio of yields before and after soaking the tress in hot water (Fig. 8).

Straightening. Relaxers require specific methods of testing, as it is not easy to measure the “uncurling” of hair. The fastest method merely compares the apparent length of curly hair before and after treatment. The straightening efficacy is considered satisfactory when the length after straightening approaches the maximum length of fully stretched hair (Fig. 9). Some authors (45,46) suggest measuring the length of hair after immersion in water followed by drying in order to take into

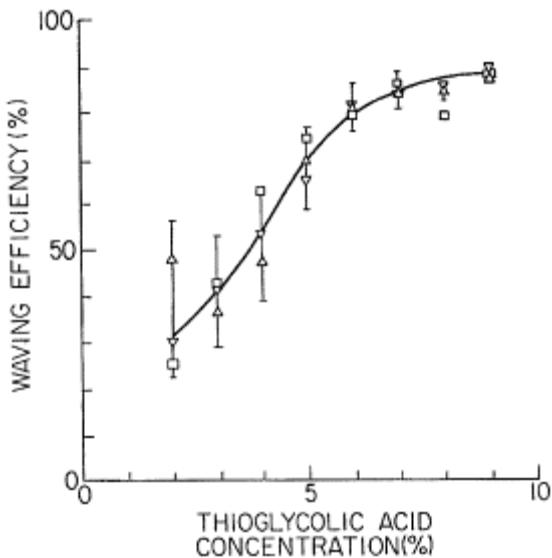


Figure 8 Waving efficiency as a function of thioglycolic acid concentration. (From Ref. 44.)



Figure 9 Hair swatch before and after application of a straightener.

account the persistence of the relaxing effect. This qualitative method is rapid but cannot give quantitative data on product efficacy. Thus, Hawkins et al. (47) proposed to express the straightening efficiency as the ratio of the lengths of several fibers from the swatch before and after treatment (Fig. 10).

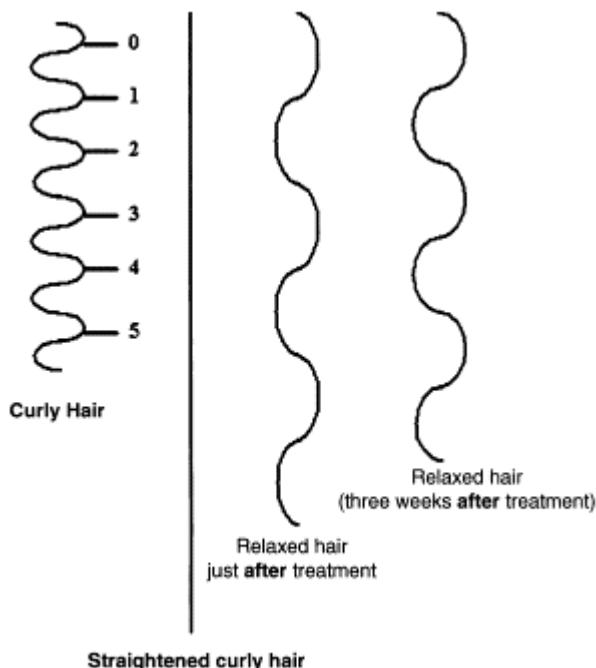


Figure 10: Diagram of the basic principle for characterizing the action of a straightening product according to the Hawkins' method. (From Ref. 47.)

1.1.6. Hair Volume and Body

The volume and body of a head of hair are two different concepts. But they are often related, the former being essentially visual while the latter is more tactile. Many factors (such as the density of hair per cm², the angle of implantation of hairs in the scalp, their diameter and suppleness or stiffness of hairs, the degree of curliness and friction between them) play an important role in the volume and body of a head of hair (10,48).

The most used method to evaluate the body is the “ring method” (49,50) which consists of passing a tress through tubes or rings of different diameters and measuring the force required using a traction instrument. This method encompasses several parameters at the same time: friction, body, and volume (51). Using this method it has been possible to demonstrate differences in compressibility between Oriental and Caucasian hairs, and the effect of treatment by a conditioner. Further progress was suggested by Robbins and

Crawford (52) using rings of decreasing diameter which can be extrapolated to measure the maximum diameter of the tress corresponding to zero force (Fig. 11). A range of organosiloxanes was studied using this test method so as to define those that provide the hair with greatest body/volume (53).

Another method using a pycnometer has been described (54), especially suited for measuring the increase in hair volume as a function of relative humidity. With the development of imaging techniques, several optical methods have been proposed,

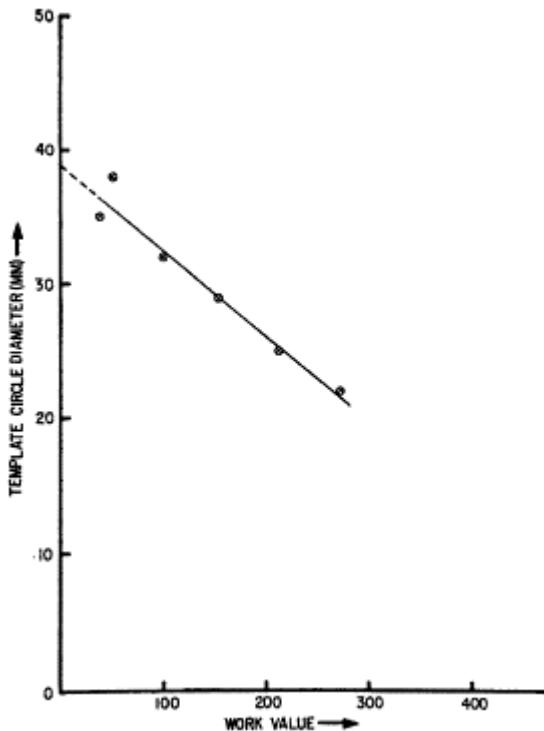


Figure 11 Measurement of hair body and volume according to Robbins and Crawford. Intercept of the line with y-axis is an estimate of the volume, its slope an estimate of the body. (From Ref. 52.)

either measuring the light flux intercepted by a swatch (55), or taking pictures with a video camera to evaluate the swatch area under different angles (56).

Using a greater volume of hair, a technique based on the use of a mobile wig has also been developed (27) in which the hair swatches are assembled on a mobile carrier performing 75 oscillations per minute.

1.2. Evaluation of Surface Properties

The surface of hair determines numerous cosmetic properties as well as the most immediate appreciation of the quality of the fiber. Indeed, the surface of hair is the most accessible parameter perceived by the consumer by merely touching the hair. A complete evaluation of the surface properties is thus pivotal especially since it is the first structure of hair to be exposed to external traumas (chemical, physical, and mechanical). Various approaches may be used to appraise the condition of hair surface ranging from different techniques of microscopic observation to the measurement of physical properties, e.g., tribology (from greek *tribein*: rubbing) or the instrumental assessment of more global cosmetic properties such as ease of combing, body or fly away.

1.2.1. Surface Observation

Observation of the surface is an essential step in the evaluation of hair properties. Aging and chemical treatments rapidly induce changes in the cuticle morphology and hair care and styling products generally act by the deposition of a more or less thin polymeric film on the surface of the hair whereby its properties are modified.

Scanning Electron Microscopy. A large variety of methods are now available to evaluate the condition of hair at the microscopic level. The most widely used technique is still the scanning electron microscopy (SEM). Contrary to conventional optical microscopy, SEM combines high resolution and high depth of field, allowing easy observation of the cuticle (4,57–60). Despite the prior necessary metallization of hair and observation under vacuum, SEM is a fast technique that gives high-resolution images of the hair surface. This has been used, for example, to classify hair according to its origin (61–63) or age (64), to characterize pathologies (65) or for forensic studies (66). SEM is also a very convenient tool for the study of weathered hair (the combination of repeated combing, brushing and sunlight exposure) (67). Significant modifications of the hair surface are clearly seen by scanning a fiber from root to tip (see Chapter 1, Fig. 5). Near the scalp, the hair has regularly edged cuticle scales. Further from the root, the cell borders become more and more irregular and the scales lift up. Toward the tip, significant cuticle thinning and erosion are observed even including its disappearance leaving the cortex totally exposed. SEM has also been used for investigating cuticle damage induced by cosmetic treatments (68–70) and to characterize the breakage profile of hair (71–73). Several studies of the morphology of various deposits on hair, including styling or conditioning polymers, have also been carried out with SEM (74–76).

A major drawback of SEM is the need to put the hair samples under high vacuum and to sputter a thin layer of metal over them. The morphology of the polymer deposits is also strongly modified by the vacuum (74) and it is impossible to look at hydrated samples with SEM, even though new techniques such as low voltage SEM or environmental SEM, allow the observation of samples without metallization or under a partial pressure of water vapor (77–79).

Confocal Laser Scanning Microscopy. Conventional light microscopy does not allow the observation of 3D samples, part of the image always being out of focus. Confocal laser scanning microscopy (CLSM) can be used to obtain sample images that do not have out-of-focus areas due to the specific optical arrangement (Fig. 12): a focused beam of light illuminates a small area of the sample and the reflected light passes through a

pinhole in the front of a detector, eliminating out-of-focus reflections. Focal plane images are generated by scanning the sample at different heights and the 3D surface of hair can then be reconstructed. CLSM gives high-quality images of the hair surface, allowing the observation of details comparable with those obtained with medium SEM resolution (Fig. 13) (80–82). The noninvasive confocal mode for imaging the hair surface in its natural environment enables repeated observations of the same field of view. It is therefore possible to compare the hair surface before and after a treatment by observing the same area of the fiber. For example, polymer deposits can be evaluated before and after shampooing, leading to an easy classification of their resistance to shampoos. Figure. 14 shows two types of experimental response. Polymer A, initially covering a large part of the surface (Fig. 14a), has been almost completely removed by the shampoo (Fig. 14b). Polymer B irregularly covers the surface (Fig. 14c) and two different patterns of deposit could be identified: smooth droplets corresponding to the polymer and rough features caused by dust particles. After shampooing (Fig. 14d), almost all of the dust particles disappeared but the polymer droplets remained at the same place with an identical pattern and distribution.

Atomic Force Microscopy. Though CLSM is very useful for global observation of the hair surface, its spatial resolution is limited to a fraction of micrometer

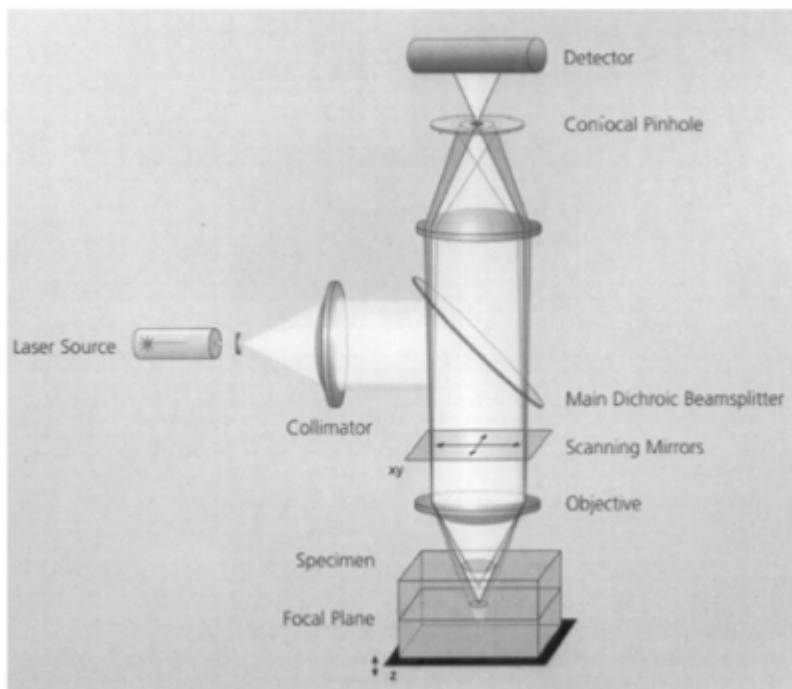


Figure 12 Diagrammatic drawing of a CLSM.

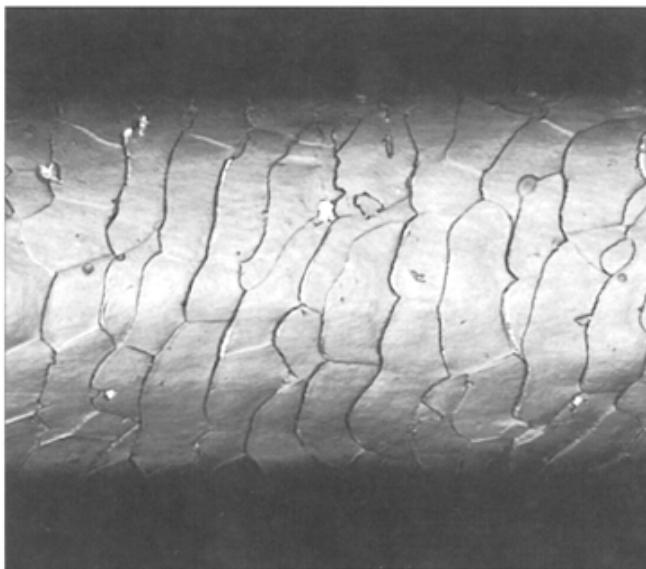


Figure 13 Surface of a virgin hair observed with CLSM.

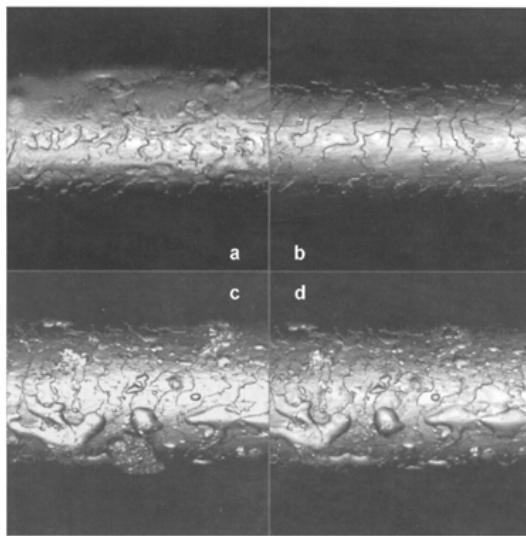


Figure 14 Observation of polymer deposit on the surface of hair with CLSM: (a) polymer A before shampoo; (b) polymer A after shampoo;

shampoo; (c) polymer B before shampoo; (d) polymer B after shampoo.

as all optical microscopy and does not allow the characterization of fine details, as SEM does. The availability of the scanning tunneling microscope (STM) and the later development of the atomic force microscope (AFM) (83,84) provided the scientist, and especially the cosmetic chemist (85,86), with tools which combine the advantages of optical (no specific preparation of the sample measurements made in ambient atmosphere or in liquid medium) and electron microscopes (very high spatial resolution, down to the nanometer). They even extend the possibilities offered by a microscope to various kinds of physical measurement and their easy use makes them suitable for routine observations. The principle of AFM imaging is amazingly simple (Fig. 15). A tiny sharp tip (radius of curvature between a few and 100 nanometers), mounted at the end of a cantilever spring, is scanned over the surface by piezoelectric scanners. A laser, focused on the reverse side of the cantilever, is reflected to a displacement photo-detector. While the tip is in contact with the sample, or when a force gradient is measured, the cantilever vertically bends and the laser spot at the photodiode shows a voltage offset. The surface profile of the sample is imaged by forcing the cantilever to follow the surface topography (contact mode). Maximum image size is typically $\sim 100 \times 100 \mu\text{m}$, but the scanning field can be reduced to achieve molecular resolution. Other measurements can be made with AFM, such as friction or viscoelastic properties (see below).

AFM offers the opportunity of obtaining true topographic information without any special constraint (no vacuum, no metallic coating). Figure 16 shows a typical AFM image of the hair surface. Even though the resolution is far from the ultimate spatial resolution that may be reached with AFM, fine details of the hair surface can be clearly seen, notably on the phase image. As the measurement of the topographic height profile is straightforward and quantitative, experiments based on the quantification of the scale step height give insight into the hair structure and its sensitivity to external parameters (physical or chemical). Most studies reflect a wide distribution of cuticle-step heights, with an average value of $\sim 300\text{--}500\text{nm}$ for human hair (87–90).

One of the most important factors affecting hair morphology and topography is water or air moisture, which is known to swell hair fibers. AFM demonstrates that swelling also affects the structure of the cuticle scales (Fig. 17). An increase by $\sim 12\%$ in the thickness of the cuticle cells of wool has been found after immersion of the fibers in water (87). In the case of human hair, the measurements of cuticle swelling

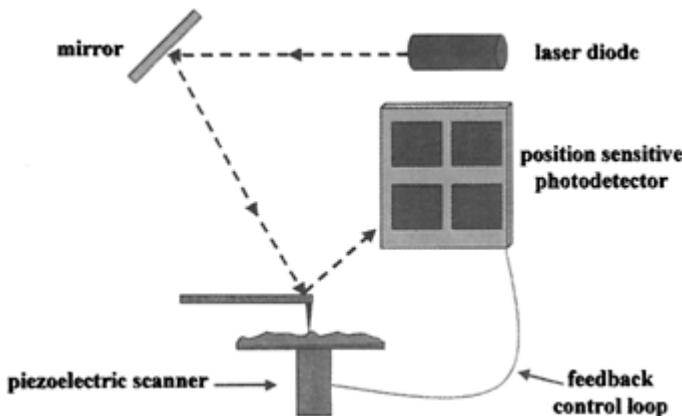


Figure 15 Diagram illustrating the principle of AFM.

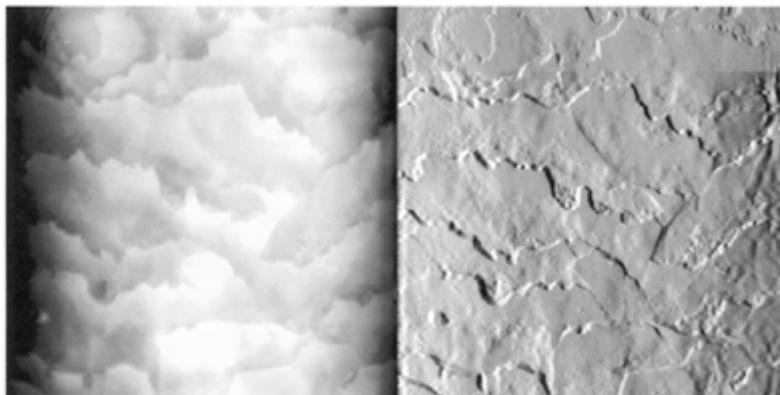


Figure 16 AFM images of the surface of a virgin hair. Left: topographic image; right: phase image, giving more details of the surface structure.

are more scattered, ranging from 13% (91) up to as high as 150% increase (88). In a relatively ingenious way, hair longitudinal expansion vs. humidity has also been measured by AFM (92). Finally volume expansion has also been observed as a function of temperature (89).

Beyond these purely topographic measurements, the possibility to achieve very high resolution leads to the observation of very fine details previously unseen with SEM under vacuum. Some specific features of the cuticle can be imaged, which is of great interest for the understanding and evaluation of the tribological properties

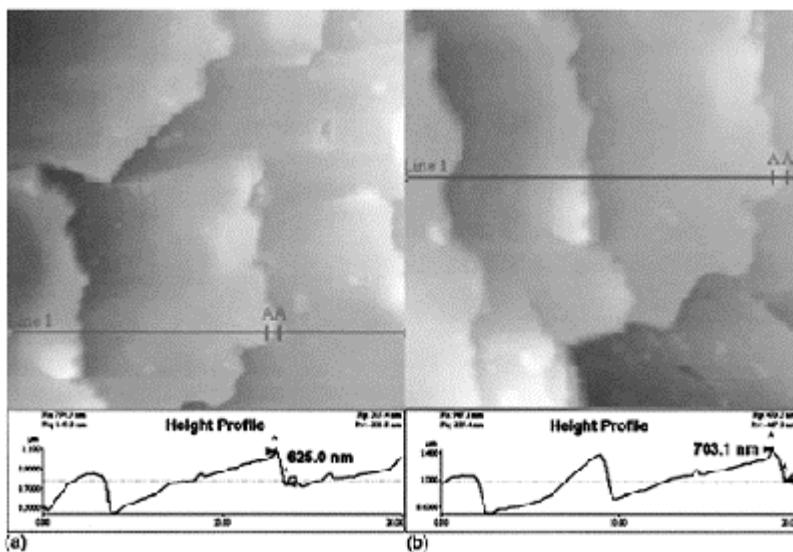


Figure 17 Observation of hair surface with AFM. (a) Dry state: the profile height is shown along the line, the height of the cuticle cell (AA) is 625.0 nm. (b) Same area of the same hair immersed in water, the cell is swollen (AA=703.1 nm).

of hair (see below). Fine analysis of the morphology of polymers or other ingredients deposited onto the hair surface has been carried out (Fig. 18). Even though the first observations lead to the conclusion that the hair surface was not smooth enough to obtain quantitative results (93), AFM proved to be an unrivalled technique for the observation and characterization of thin polymer films (94).

1.2.2. Tribological Properties

Tribology is defined as the study of interacting surfaces when they are rubbed one against the other: it includes friction, adhesion, lubrication, and wear phenomena.

Macroscopic Friction. Hair friction properties are among the most important properties of the hair surface. They not only affect combing and softness, but also the way the hairstyle lays, how it is dressed and its hold. The origin of frictional properties of hair probably lies in the epicuticle, the thin membrane surrounding each cuticle cell (see Chapter 1). The direction of the scales along the hair from root to tip gives it different friction properties depending on whether it is stroked from the root to the tip or from the tip to the root, the former being lower than the latter (95,96) (Fig. 19).

At the macroscopic level, friction is defined as the resistance between two bodies sliding over each other. The basic laws of friction developed by Amonton postulate that:

- The frictional force is proportional to the normal load N applied to the contact area and the proportional coefficient is independent of the load:

$$F = \mu N \quad (2)$$

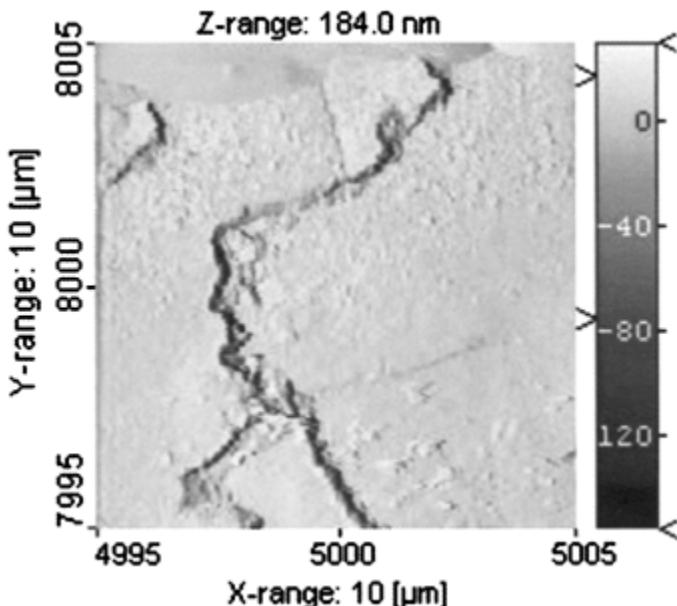


Figure 18 AFM phase image of a hair fiber treated with an anionic polymer solution. Hair coating by the polymer is inhomogeneous and appears as a fine granular layer.

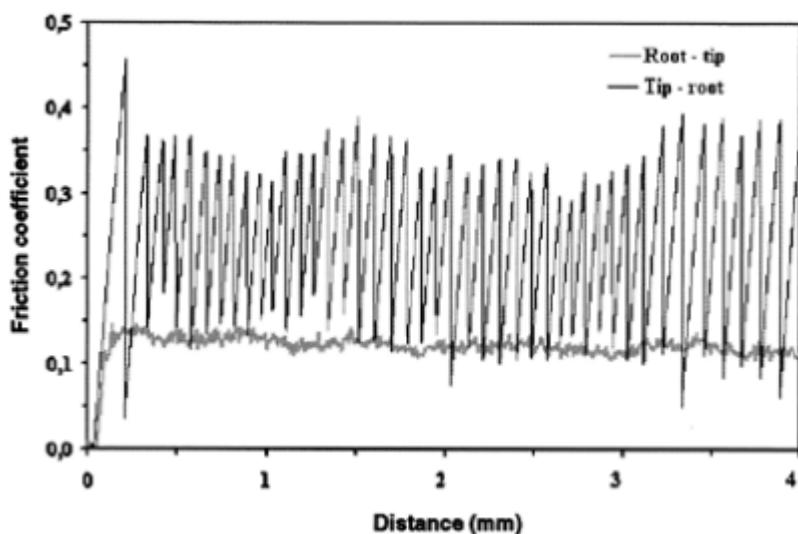


Figure 19 Friction coefficient of a virgin hair near to the root. Red curve: measurement from root to tip; black curve: from tip to root. (From Ref. 96.)

- The frictional force is independent of the contact area for a defined normal load:
 $F=AS$

(3)

where A is the surface area and S is the force relative to the unit area.

Friction properties can be measured on single fibers or on hair tresses, between fibers or between the hair and other materials. Studies aimed at the prediction of the behavior of a head of hair on the basis of friction measurements on isolated hairs have been carried out (5). The first measurements of friction forces were made on wool using a "lepidometer" (97). The fiber was attached with its tip to a torsion balance and rubbed between two plates, which moved anti-parallel to each other along the fiber (Fig. 20). This method has also been widely used on hair (98) and a good

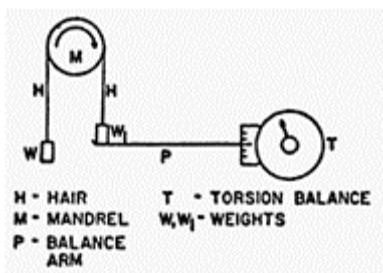


Figure 20 Diagram of a friction apparatus. (From Ref. 98.)

correlation between this method and the sensory evaluation of softness has been found (6). Major improvements of this test consist in increasing the contact areas between hair and the sliding surface (99) and the possibility to run such experiments in a liquid medium to simulate shampooing the hair (99,100).

Measurement of fiber-fiber friction is quite difficult and it is difficult to interpret the results due to the poor control of the contact area between the two fibers. A method using two twisted fibers (Fig. 21) has been described for textile fibers (101) and later used for hair (102,103).

Another approach giving a clearer picture of friction phenomena has been adapted to fibers. It involves a modification of Amonton's law taking into account the adhesion phenomena and the viscoelastic behavior of the material which leads to empirical equation:

$$F = aN^n \quad (4)$$

where F is the friction force, N the load applied, and a and n are the two coefficients related to the friction properties of the tested material.

Among fibers, studies based on this theory have been carried out on cotton (104), various keratin fibers including hair (105) and fabrics (106,107). Recently a study carried out on acrylic fibers showed that if the load imposed on the fibers was increased, the friction coefficient decreased but the stick-slip increased. Similarly, reduction in rubbing speed accentuated the stick-slip phenomenon (108).

The nature of the wiper has a strong influence on the friction properties measured on hair. For example, the friction coefficients can be in single or double figures depending on whether aluminum or a hard rubber surface is used (98), whereas Teflon yields the smallest friction coefficients (109). Some materials such as specific

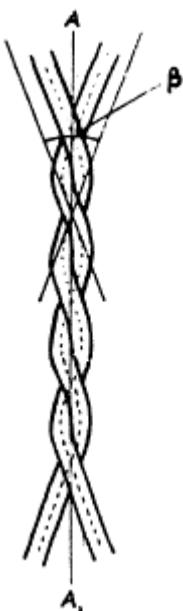


Figure 21 Measurement of fiber-to-fiber friction by twisting two fibers.
(From Ref. 101.)

rubbers offer a good concordance between friction measurement and the sensory evaluation of softness (95). For fabrics, an artificial polymer finger measuring 10×25mm has been designed and used to evaluate the feel (110,111).

Another approach based on quite a different principle involves the passage of an airflow or water flow through a tress and the measurement of the variation in pressure between the top and bottom of the tress (112). With that method it has been found that the softness parameter decreases with relative humidity.

When the hair is wet, its friction coefficient increases (98). Friction coefficient also varies with and many treatments tend to increase the hair's friction forces such as, for example, permanent waving, dyeing, or bleaching (103). Other forms of treatment commonly used in the fabric domain, such as chlorination, also lead to an increase in friction coefficient, particularly when acidic conditions are used (102). Conversely, some shampoos and conditioners achieve a reduction in friction coefficient, especially cationic surfactants or polymers and silicone derivatives, e.g., polydimethylsiloxane (PDMS) (Fig. 22) (113).

Nano-friction. As mentioned before, one of the advantages of AFM is the ability to retrieve more information about the physical properties of the surface studied. The first interest has been to explore the micro and nano-tribology (adhesion and friction) of hair surfaces as both provided a way to detect the presence of surface mechanical changes and the influence of active ingredients. It is believed that submicron structures play a dominant role in fiber-fiber interactions (105,114). AFM gives access to local friction and

thus enables one to make progress toward an understanding of both the hair surface and the molecular origin of macroscopic friction. The latter is, among others, a key factor when studying the effects produced by hair products such as hair setting, hair styling, conditioners, and shampoos.

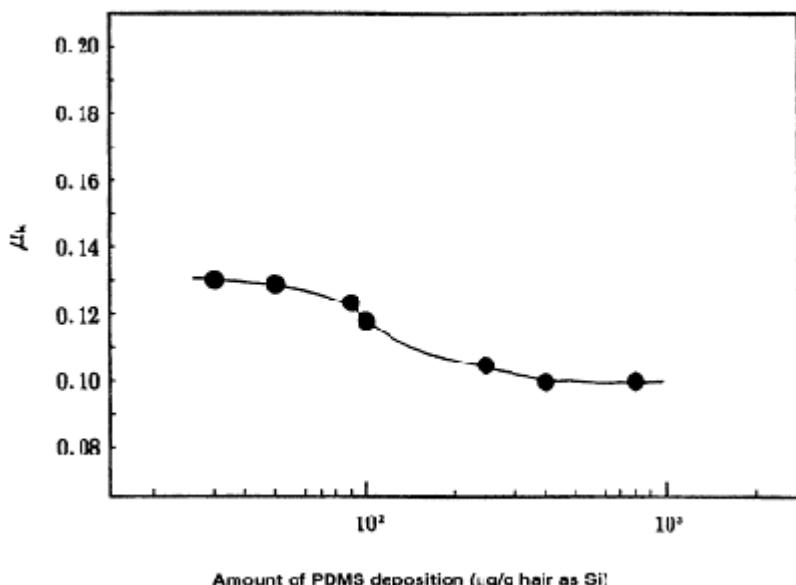


Figure 22 Effects of amount of PDMS deposition on the kinetic frictional coefficient of dry hair fiber. (From Ref. 113.)

The application of the friction mode of the AFM (sometimes called lateral force microscopy, LFM) is therefore of great use for these applications. When scanning a surface with the AFM tip, friction forces lead to a torsion of the cantilever supporting the tip. This torsion is measured by the displacement of the laser reflection on the detector in the horizontal axis. Friction forces can be analyzed in comparison with the simultaneously obtained topographic images.

Phillips and his colleagues at CSIRO (87) were the pioneers in the use of these techniques on keratin fibers by detecting the effects of a surface oxide treatment on wool through changes in the homogeneity of the friction force distribution on the surface.

As discussed in Chapter 1, the outermost layer of hair is composed of specific lipid structures (a monolayer of covalently linked fatty acids, especially 18-methyl eicosanoic acid) that are assumed to participate to a great extent in the friction properties of hair. Modifications of these structures by cosmetic treatments can be followed with AFM by analyzing the fine details of the friction contrast map (115), and the combination of topographic and friction maps leads to the observation of fine details that no other

methods can demonstrate, e.g., the different layers of the intercellular spaces and the components of the cell membranes (Fig. 23).

A variety of cosmetic products such as shampoos and conditioners can change the frictional characteristics of hair at the macro-level. It is clear that not only better quantification of friction forces and their dependence on rubbing speed and load, but also complete description of the phenomenon at the molecular level become necessary to progress in the understanding of hair fiber tribology, especially when studies are extended to cosmetic-treated hair. In this way McMullen et al. (116,117) examined the effect of load on the nano-friction behavior of several treated hair samples (Fig. 24). The friction seemed to follow Amonton's law [Eq. (2)]. A pseudo-friction

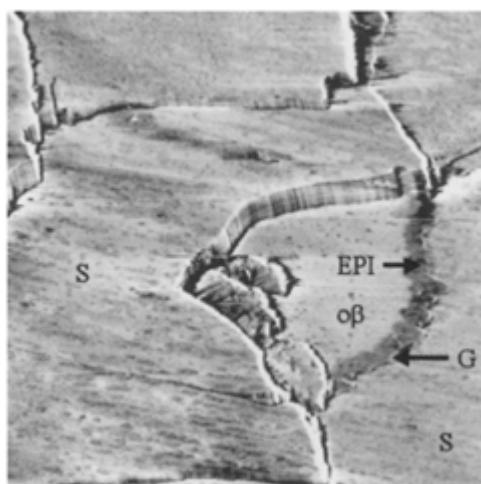


Figure 23 Lateral force microscopy image of a brown European hair surface previously treated with a 70:30 chloroform/methanol solution for 5 hr in order to extract the unbound fatty acids. The image shows a cuticle step in which part of the cuticle is missing (the original scale edge follows the path G). The original outer cuticle (S) is seen, as well as the surface of a newly exposed cuticle cell. The friction contrast reveals the presence of two layers hardly detected by topography: the epicuticle (EPI) and the supposedly covalently linked lipid

layer (outer β -layer, $\text{o}\beta$) of methyl eicosanoic acid (18-MEA). Field of scan: 10 μm . (From Ref. 115.)

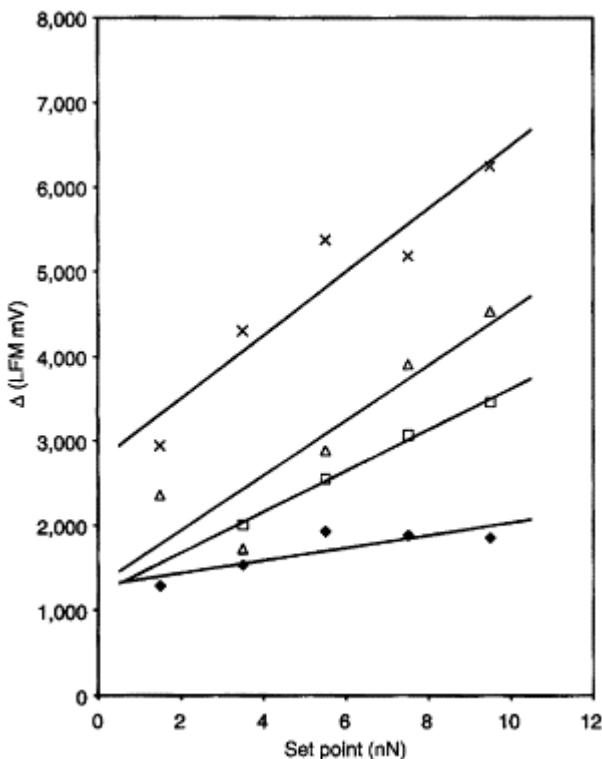


Figure 24 Effect of load on the friction behavior of several treated hair samples. (From Ref. 116.)

coefficient was drawn from the results allowing for a friction classification: the native fiber showed the lowest friction followed by bleached, solvent extracted and finally polymer treated hair.

Although a correlation between nano- and macro-friction results is not straightforward, several papers demonstrated that AFM friction results were in good correlation with dry combing forces (116,118).

Another key parameter of the frictional behavior of solids is the viscoelastic properties of the material. Thin films of polymers can modify the micromechanical properties of the hair surface and thus its frictional behavior. By using AFM equipped with nano-indentation device, Ruetsch et al. (119) demonstrated that polyquaternium-10 softened

the hair surface, which could explain, at least partially, the conditioning effect of this polymer.

Besides its exceptional and unique technical performance and capabilities, AFM is relatively easy to use and provides information that cannot be obtained with other techniques. Its use will thus be more and more important in the evaluation of cosmetic efficacy.

Noise Generated by Friction. Among the main cosmetic properties claimed is the softness of hair to the touch. This feature is difficult to measure objectively because it is the result of the combination of surface condition of a group of fibers. More general than measurement of friction coefficient of a single fiber, the evaluation of softness of a tress of hair is based on quantification of the friction of hair swatches (6,95).

When the hair is combed, the friction of the comb's teeth on the scales covering the fibers generates acoustic vibrations. The intensity of this noise varies depending on the surface condition of the hair, the presence of material on the fiber and the quality of the hair ends. The level of noise when combing is directly related to the softness or roughness of the fibers (120), and a microphone attached to a comb can easily record this "combing noise." The recorded sound is then calibrated using a sound-level meter whose analog output is connected to a tension integrator. The greater the noise recorded, the rougher the hair is. A study (121) has demonstrated the excellent correlation between noise level and hair softness (Fig. 25).

Dry and Wet Combing. The ease of combing for wet or dry hair is one of the main cosmetic criteria sought for a hair product. The methods for evaluation of combing ease, with operating conditions very similar to real-life usage, use either hand-held or automated instruments. However, whatever the method, the ease of combing is represented by the overall effort required when the teeth of the comb are passed through the swatch.

Typically, manual types of measurement are carried out using a dynamometric comb (Fig. 26) that records the efforts on torsion (122) or flexion (123) when it passes through dry or wet hair. This instrument has the advantage of being easily transportable for *in vivo* evaluations. Practical, easy to use, the dynamometric comb, however, is only used for screening purposes as the manual nature of the measurement leads to poorly reproducible results from one operator to another, hence the advantage presented by automated measurement.

The most widely used automated method of evaluation consists of pulling a swatch linked to a strain gauge through the teeth of a comb fixed to a frame. This method is used to study either wet or dry hair. There are several variants of this test. The Garcia method (124), based on the use of an extensometer is an improvement of the first device developed by Newman et al. (125). Kamath et al. (126) added another comb in order to separate the hairs from the start and thus define the contribution of each of the components of combing action: effort to distribute the hairs between the comb's teeth, hair/comb friction and the force linked to displacement of the swatch. Generally, the measurements are carried out in pairs, i.e., the results before and after

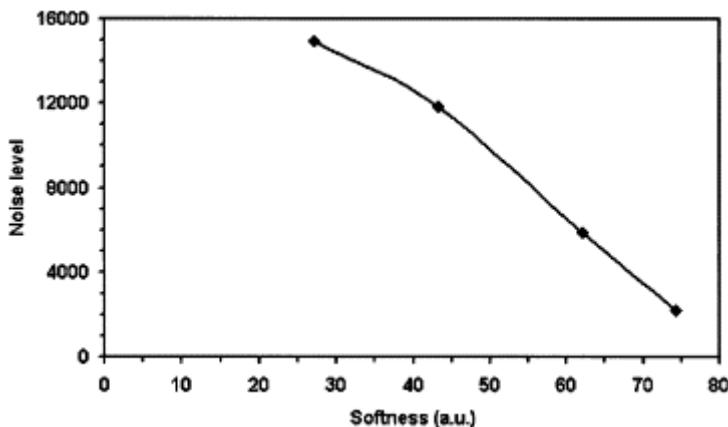


Figure 25 Correlation between the noise measured during hair combing and hair softness (sensory evaluation). (From Ref. 121.)



Figure 26 Swatch disentangling: evaluation by a tensile comb. The swatch of hair is submitted to standardized entangling then disentangled by means of a tensile comb recording a figure proportional

to the effort applied. The whole procedure is carried out on the same swatch before and after applying a given product to assess the disentangling effect. (From Ref. 21.)

treatment are compared. These methods are systematically used to assess the conditioning efficacy of cationic surfactants and polymers because they correlated well with sensory evaluation (127).

Several improvements were proposed in order to get results even more closely related to sensory perception. For example, Jachowicz et al. (128) suggested a card system fitted to the measurement sample during treatment in order to define, on a single swatch, the treated and untreated control areas. It is then easy to rapidly evaluate the effect of treatment on the ease of untangling or combing of treated swatches. Bauer et al. (27) designed a specific device for dry combing (Fig. 27), estimating that the speed of combing was too slow when using an extensometer. The ease of combing of the swatch is then represented by the sum of the efforts recorded in the course of passages of the combs. This method seems to present the best correlation to the perception of consumers, probably due to its relevance toward the combing habits of consumers. For example, subtle differences between specific shampoos applied after oxidative dyeing were clearly demonstrated (27).

UV exposure rapidly leads to significant alterations of the chemistry and morphology of hair surface. Combability measurements are thus efficient tools for estimating the level of damage resulting from photooxidation (129). For example, an increase in the combing force with the exposure time was observed (Fig. 28). This was interpreted as a photo-induced decomposition of the lipids of the epicuticle layer which play an important role in the surface condition and friction properties of hair.

1.2.3. Fly Away

In the course of combing or brushing, the hair is charged with a varying degree of static electricity (charges of opposite sign but equal magnitude are generated on one side on the comb or brush and on the other side on the hair surface). The magnitude

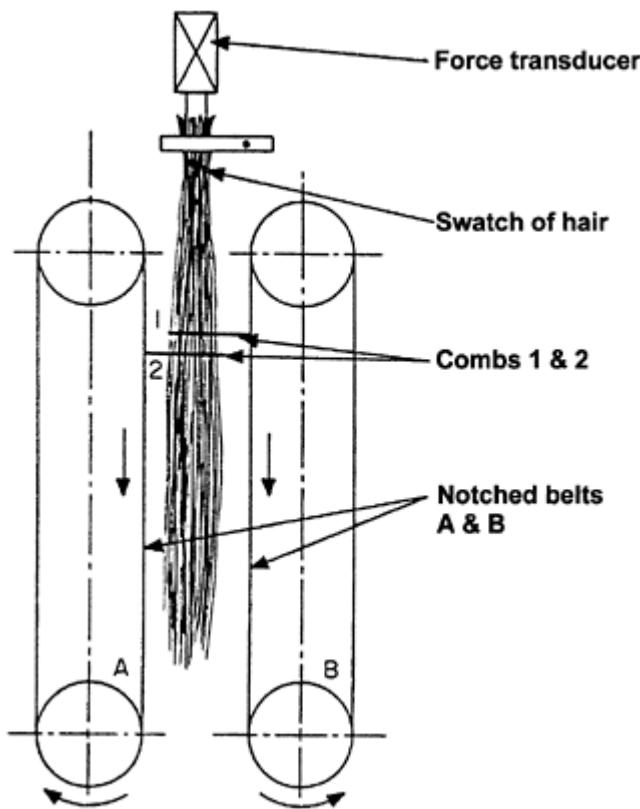


Figure 27 Diagram of automated apparatus for the measurement of dry combing work. (From Ref. 27.)

of the charges depends upon the surface quality of hair, the ambient relative humidity, or the cosmetic treatment it has undergone. When hair is electrically charged, the fibers are submitted to repulsive forces and the "fly-away" phenomenon appears. It makes the hair difficult, even impossible in extreme cases, to style. To attenuate the triboelectric effects, the application of appropriate hair products is required.

To evaluate static fly-away, the general principle consists of determining the electric charge generated during combing either directly on the hair or indirectly by measuring the charge borne by the comb, the hypothesis being that the ionic charges produced on the comb and on the hair are equal in number and opposite in nature. The effects can be evaluated using numerous methods. Whatever the method employed, the measurements are always performed under controlled RH and generally at room temperature.

After generating static charge on hair swatches by repeated combing or brushing, the "fly-away" can be evaluated either by measuring the volume of the swatch, which is increased by electrostatic repulsion between fibers (130), or by measuring the electric

charge generated on the comb (131) or on the swatch itself (132). For the rapid screening of new hair care or hairdressing formulations, a portable device has been described (133) which performs measurements on the hair directly.

The appearance of electrostatic charges on the hair surface being mainly due to the action of combing or brushing, an automated combing device (mentioned earlier)

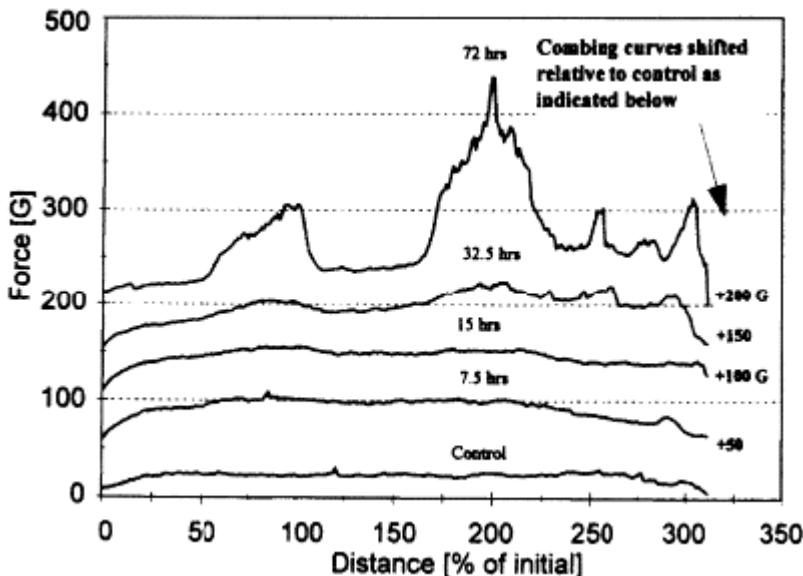


Figure 28 Combing curves of brown Caucasian hair after various irradiation times. Irradiations were through exposure windows at 56–106% and 162–212% relative displacement.
(From Ref. 129.)

has been developed to evaluate the combing property and in the same time the static fly away by the ionic charge on the comb developed during combing (27).

With a view to understand the triboelectric phenomena on the hair, Jachowicz et al. (134) studied the charges generated on hair by friction with various materials (gum, metal, plastic, etc.). The results showed that an antistatic effect was provided by a combination of two factors, an increase in surface conductivity and a decrease in the electrochemical surface potential gap between the rubbing material and keratin. Another work from the same authors showed that the triboelectric charge distributions generated by combing of hair stresses correlated with combing forces (135). Moreover, adsorption of long-chain quaternary ammonium compounds, cationic polymer and polymer-detergent complexes lowers the combing force by combining a decrease in the electrochemical potential, an increase in hair conductivity, and an increase in lubrication.

Major treatments (oxidation/reduction processes) induce only minor changes in the triboelectric charges and none in surface conductivity vs. virgin hair (134).

1.2.4. Shine

Definition. Shine is one of the most sought-after and most desirable cosmetic criteria for a hair product. Hair shine is not only limited simply to the attribute of beauty but also suggests hair cleanliness and good health. This is why it is important to evaluate the performance of hair products in terms of shine.

In general, the shine of an object is defined as being the surface attribute that gives it a shiny or lustrous appearance (136). More precisely, the shine of an object is proportional to its capacity for reflecting light. Unlike color, which is a spectral attribute, shine is a geometric attribute linked to the manner in which an illuminated

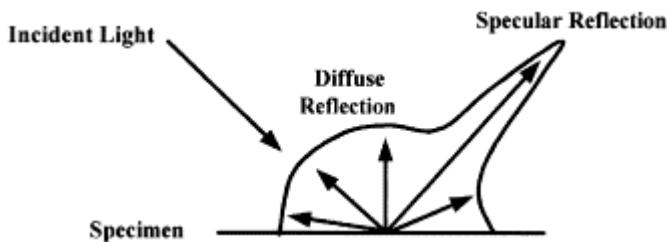


Figure 29 Diagram of geometrical distribution of light reflected by an object.

object reflects the light. When light falls on the surface of an object it is mainly reflected in two ways: reflection known as “specular” emitted at an angle opposed to the angle of incidence (as in a mirror) and the so-called “diffuse” reflection emitted in an isotropic manner in all directions (Fig. 29). The more intense the specular reflection, the shinier the object. Conversely, the stronger the diffuse reflection, the duller the object will appear.

Despite the absence of a universal definition of shine, these two reflection phenomena form the basis of all the physical parameters used to characterize shine (136). Shine can be characterized by the intensity of specular reflection (qualified as “specular shine”). It can also be characterized by the ratio between the intensity (or flux) of specular reflection S and the intensity (or flux) of diffuse reflection D when it will be qualified as the “contrast shine.” The latter, originally used to characterize the shine of fibers in fabrics (137), is today the most used parameter in the case of hair (138–140). Contrast shine is expressed by the following equations:

$$\text{Shine} = S/D \quad (5)$$

$$\text{Shine} = (S-D)/S \quad (6)$$

Although these two expressions are correlated (138), Eq. (6) is often preferred because it delineates the range of measurements between 0 and 1. The value 0 corresponds to a perfectly matt object (where $S=D$) and the value 1 to a perfectly shiny object (where $D=0$).

Thus, the evaluation of shine requires the determination of both S and D which is achieved by measuring the geometrical distribution (Fig. 30) of the light reflected by the hair (or the intensity of the reflected light relative to the angle of observation), generally called as the “reflectance indicator.”

Method. The gonioreflectometer measures the angular distribution of the light intensity reflected by an object when illuminated from a given angle (usually 30° relative to the normal axis of the sample) and with a given geometry. The first studies to characterize the optical properties of hair using a gonioreflectometer were carried out in the 1970s (141). Gonioreflectometer is not limited to shine characterization; it is also used to measure other optical properties such as hair transparency (139). Measurement can be carried out on individual hair fibers aligned in parallel or on a tress of hair, held under constant stress and perfectly aligned, misalignment being the main source of variability (139). The light source used is often a white light whose characteristics are close to those of daylight. A monochromatic laser source

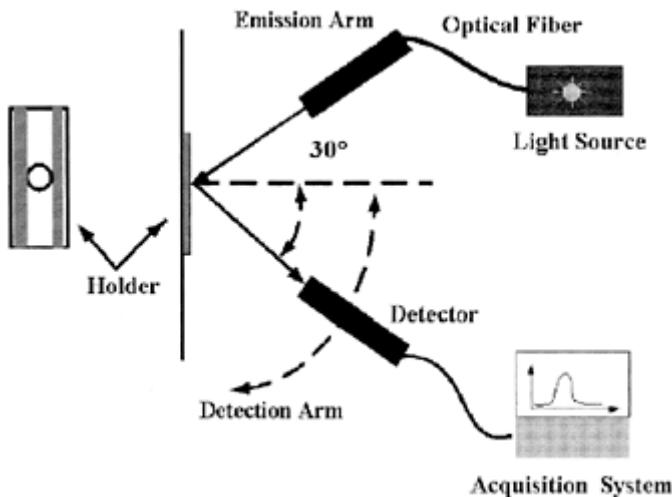


Figure 30 Diagram showing the principle of a Gonioreflectometer measurement.

can also be used (142,143). Due to the asymmetric structure of the hair surface, the reflectance curves are not the same depending on whether measurements are carried out with a tip/root or root/tip illumination angle (141).

Figure 31 shows the reflectance curves of two virgin hair of different color (dark brown and gray). These curves are the consequence of the reflection of light by the

various structural components of hair as illustrated in Fig. 32. They are composed of three main parts:

- *Specular reflection:* this reflection is emitted at the air/hair interface at an angle of approx. 23° – 25° . The deviation from the theoretical 30° angle is

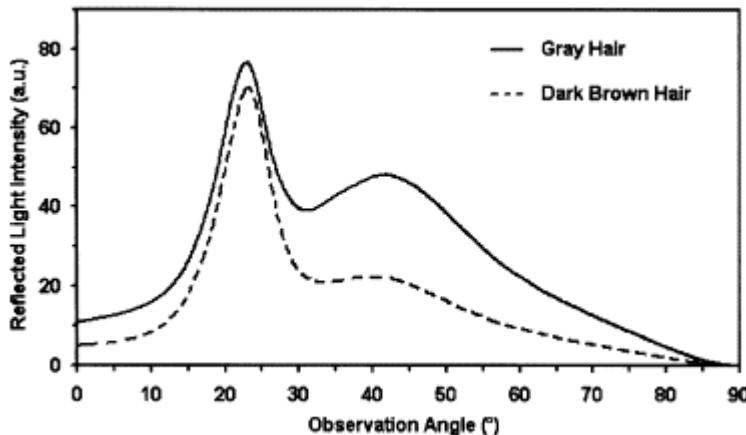


Figure 31 Distribution of light reflection as a function of the observation angles for two hair colors (dark brown and gray). These curves are obtained on an array of 10 individual natural hair fibers with an illumination angle of 30° .

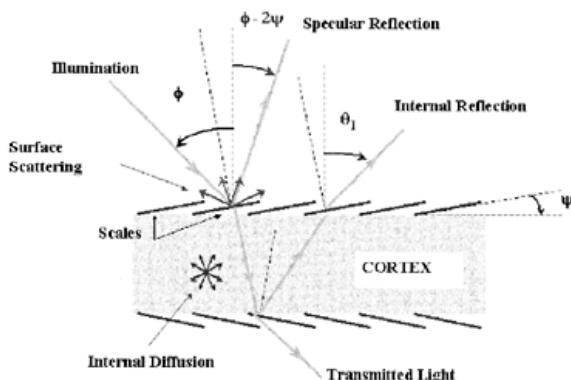


Figure 32 Diagram showing reflection of light from a hair fiber.

due to the lean of the cuticle cells and the difference corresponds to twice the angle of the scales (141).

- *Internal reflection:* it corresponds to the light reflected by the hair after having been refracted at the hair/air interface of the “front” side, then reflected on the “rear” side, before being again refracted on the “front” interface. The angle at which the internal reflection is emitted depends on the refractive index of the hair, the angle at which the scales are inclined and the angle of incidence (141). Moreover, the intensity of this reflection is highly dependent on hair color: the lighter the color, the greater the intensity of the internal reflection.
- *Diffuse reflection:* this reflection is the consequence of two phenomena: diffusion due to hair surface irregularities and internal diffusion within the hair fiber. The internal diffusion and hence the diffuse reflection depend on the lightness of hair.

Once the reflection curves have been obtained, they can be analyzed to yield the values of S and D . This analysis consists of (138):

- Calculating the flux corresponding to diffuse reflection (area under the straight line linking intensity at 0° to that at 90°), which corresponds to D .
- Calculating total flux (area under the whole curve), which corresponds to $S+D$.

The parameters S and D are thus obtained and the shine can be calculated using Eqs. (5) or (6).

Although the gonioreflectometer method of characterization described above is fairly satisfactory for the evaluation of hair shine as well as the benefits of hair treatments in terms of shine (144), it is of less interest when comparing the shine of two samples of different colors. Indeed, the shine parameters S and D , calculated using Eqs. (5) or (6), greatly depend on the color of the sample tested. Measured shine decreases when hair color is lighter (138,139). It also depends on the internal reflection, which is also color-dependent. The use of polarized light can be a way to partially reduce this dependence (140,145). Nonetheless, internal reflection seems to play a role in the rendering of shine and more generally in the appearance of hair color (146–148). Attempts have been made to define a parameter representing hair shine that takes internal reflection into account (149). Reich and Robbins recommended taking the width of the specular reflection peak into account (150).

More recently, image analysis techniques have been used to characterize shine on hair tresses (151,152). Nevertheless, the measurement principle remains identical to that of the gonioreflectometer. The image analysis method consists of taking an appropriate image and analyzing it in a way to determine the reflectance indicator as defined above. This indicator is then analyzed using Eq. (6) to determine the shine (152). Unlike the gonioreflectometry method, image analysis can be used to measure hair shine on a head directly (145,151,153).

Hair Treatment and Shine. Hair shine is affected by any chemical treatment (dyeing, bleaching, straightening, permanent waving) that acts on and modifies the hair surface. Furthermore, hair is damaged by everyday handling such as brushing, combing, drying, etc., as well as by daily pollution (sebum, dust, etc.) and exposure to sunlight (67). All these processes affect hair surface integrity and hence its shine. They generally result in an impaired and irregular hair surface and consequently a matt appearance (diffuse reflection increases while specular reflection decreases). For example, successive

bleachings of hair (with basic formulation containing no protective treatment) lead to a more and more impaired surface and thus a more and more matt appearance (154). Similarly, the presence of an irregular sebum deposit on the hair surface makes it less shiny, as shown by the results in Fig. 33 (152).

However, hair treatments that provide a regular, smooth, and reflective deposit on the hair surface of each hair will enhance the shine. This is the case with certain hair products containing silicone derivatives, giving the hair a shiny appearance as a result of smooth and reflective deposit of silicone on the hair surface. Similarly, a simple cleansing shampoo generally succeeds in eliminating dirt and pollutant deposits from the hair, so reducing diffuse reflection on the surface with a shinier

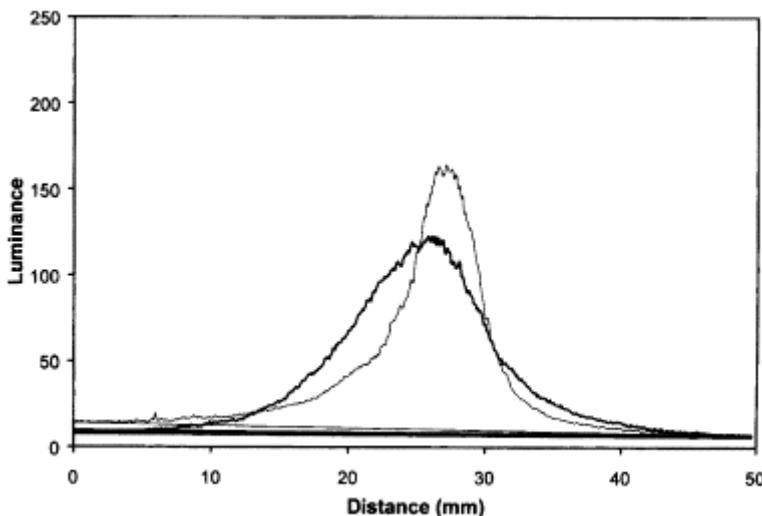


Figure 33 Light distribution curves of untreated hair and hair treated with synthetic sebum. (From Ref. 52.)

appearance as a result (154). Some cationic polymers can also be added to hair products in order to improve surface condition and thus impart more gloss (155).

Wettability. Despite the chemical composition of cuticle cells, mainly hydrophilic proteins, the hair surface is hydrophobic. It is likely to be related to the epicuticle covering each cuticle cell and its outermost, presumably covalently bound lipid monolayer. The hydrophobic properties of the hair surface are made even more pronounced by the presence of sebum. They however can be deeply altered by environmentally induced oxidative degradation of high sulfur cuticle proteins under sun exposure whereby highly hydrophilic groups are generated at the surface of hair. Moreover, mechanical damaging of the epicuticle or even of the cuticle can expose hydrophilic cortical regions. The wetting behavior of the hair can therefore be used to assess the extent of weathering and mechanical damage sustained by hair. Furthermore, the wetting behavior of hair is important to the formulation of hair care products,

particularly when they are sprayed on the hair. Droplets will perform differently whether hair surface is hydrophilic or hydrophobic.

In the case of hair, wettability was first evaluated qualitatively by microphotography (156). From the 1970s, the technique of choice to study this property has been the microbalance (157). Its principle consists in immersing a few millimeters of a short piece of hair in a liquid then removing them at a constant speed. The force opposed by the liquid during immersion and extraction is measured by a microbalance. It is linked to the angle of contact between the liquid and the hair by the following equation:

$$F = gp \cos \theta \quad (7)$$

where F is the force, g the tension of the liquid surface, p the perimeter of the hair, and θ is the angle of contact.

The influence of natural aging on the wettability of hair by water has thus been demonstrated (157). Between the root and the tip, on a length of 25 cm, the angle of contact at penetration increases from 72° up to 103° . At the root, the force exerted by water is negative, i.e., water rejects the hair while at its tip it is positive, in parti-

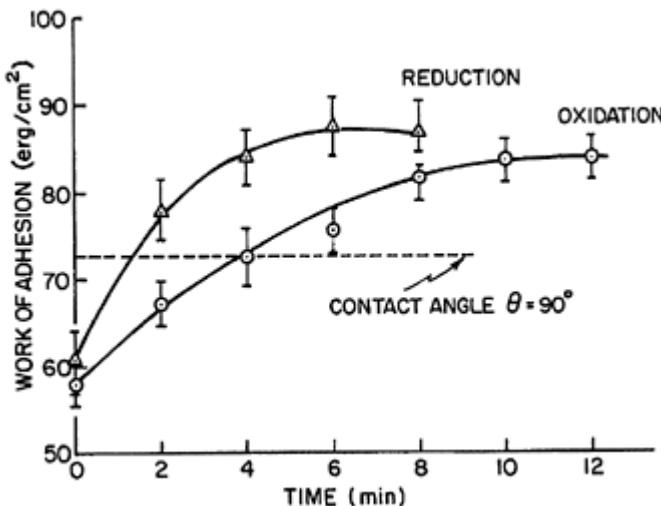


Figure 34 Effect of oxidation and reduction treatments on human hair fiber wettability. (From Ref. 157.)

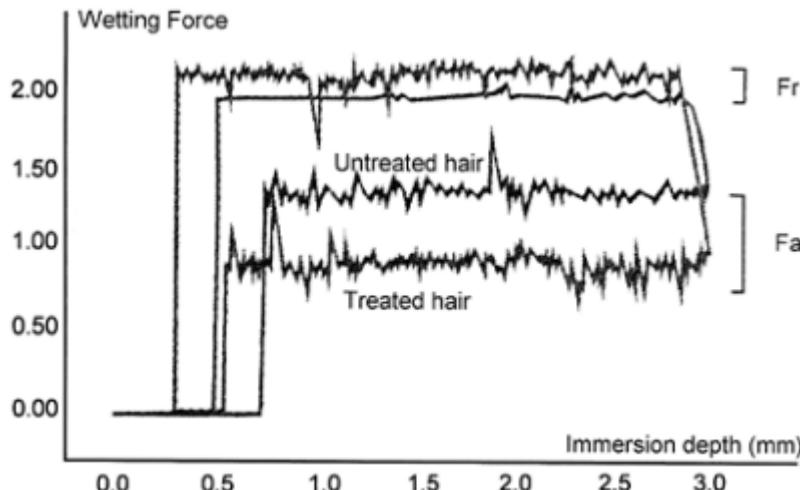


Figure 35 Effect of a ceramide-containing rinse-off product on the wettability of bleached hair. (From Ref. 166.)

cular if the cortex is bared. The effect of cosmetic treatments on wettability of hair is obvious (Fig. 34) (158–160). Chemical modification of the hair surface always induces the generation of charged chemical species (mainly sulfonates and carboxylic acids), which lead to an increase in the wettability of hair by water.

This technique has also proved useful in examining deposition of ingredients on the hair surface especially cationic surfactants or various kinds of polymers (161–164). The same types of measurements have been used to demonstrate the conditioning effects of a product containing guar polymers (165) and the beneficial effect of a deposit of ceramide in a rinse-off product (166) (Fig. 35).

The influence of the speed of immersion has also been studied: an increase in speed from 0.1 mm/min to 10–15 mm/min leads to a reduction in the wetting force (increase in the angle of contact) (167). In the same study, the difference between the forces at work on penetration and extraction was linked to surface roughness.

An improvement in this method has been suggested (168): instead of immersing the hair, it is passed through a thin film of water (Fig. 36). This device can measure the variations in wettability of a hair along the hair shaft and thus detect, as an example, uneven deposits of polymers on the hair surface.

1.3. Evaluation of the Integrity of the Hair Fiber

Assessing the integrity of hair fiber is mainly used to measure the impact of chemical treatments such as perming, bleaching, etc., or exposure to external factors such as sunlight, oxidative stress, etc., interacting with the hair structure. Test methods are mostly

based on the measurement of mechanical properties of hair or chemical analysis that give the most relevant information on the structural changes undergone by hair as a result of these treatments.

1.3.1. Mechanical Approach

Tensile Testing. The study of tensile properties by extensometry is most appropriate for the evaluation of oxidation (bleaching) and reduction (perms and straightening) treatments. Changes in the chemical bonds (cystine and peptide bonds) induced by

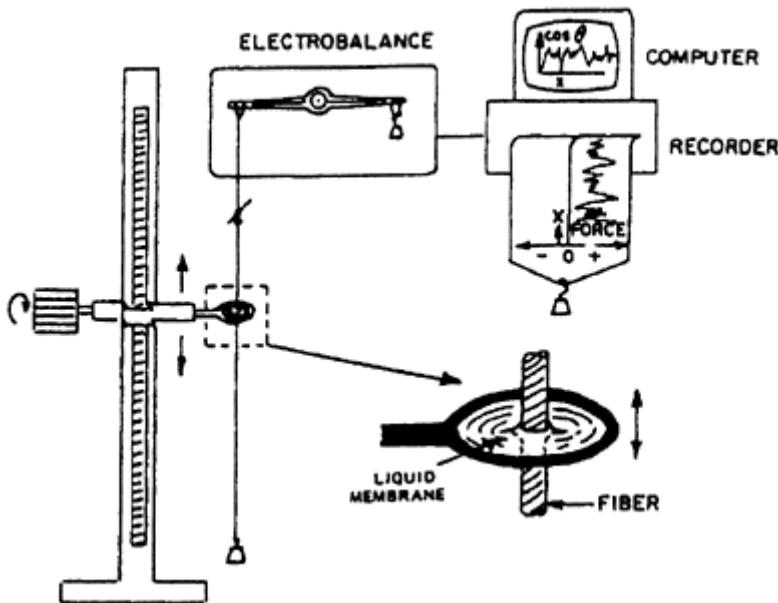


Figure 36 Experimental device for the measurement of fiber wettability by the liquid membrane method. (From Ref. 168.)

such treatments are produced at the heart of the cortex that plays a major role in the mechanical behavior of the hair (169). After application of an oxidizing or reducing agent, the Young modulus and the stretching force that causes hair rupture greatly decrease, while the elongation at break increases (Fig. 37) (170). In contrast, the oxidative color does not significantly change the tensile properties of the fiber (171).

The experimental conditions are partly determined by the type of hair product to be studied. The alteration of cystine bonds (by reduction) is mainly reflected by

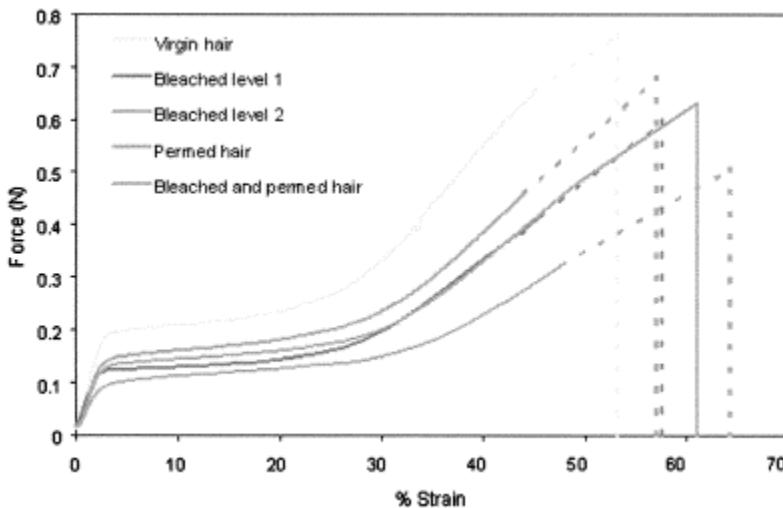


Figure 37 Load/elongation curves in water as a function of hair treatment (bleaching and perming). (From Ref. 170.)

the mechanical properties in water (164), while the modifications induced by the cleavage of peptide bonds (by alkali) are demonstrated by measurements of traction when dry conditions prevail (172). The tensile properties are also affected, but to a lesser extent, by other factors such as light, grooming.

Some hypotheses can be drawn about the influence of the fiber structure on its mechanical properties. For example, during the breakage of the very first disulfide cross-links, the main modification of mechanical properties is readily observed in the post-yield region of the stress-strain curve (Fig. 38) (173).

The kinetic aspects of reduction have been studied using a method based on relaxation under traction. The results are in agreement with the data from amino acid analysis (174) and demonstrate the influence of various parameters such as temperature, pH, or hair origin on the reducing efficacy (175,176). The rate and distribution of reduced sites within hair fibers as a result of the competition between diffusion kinetics of reducing agents and their reaction kinetics have been extensively studied (177).

Straightening, generally applied to African hair, which is by its nature less resistant to traction (breakage), significantly decreases the Young modulus and it leads to a notable increase in elongation and a decrease in force at break. The dynamics of the extensometry measurements on natural hair vs. treated hair, as shown in Fig. 39, prove that the method is particularly relevant for the evaluation of straightening treatments, on which few data have been published (178).

The stretching of the hair fiber in dry or wet condition leads to damage, mostly in the non-keratinized regions of the cuticle (endocuticle) and of the cortex (cell membrane complex), as demonstrated, for example, by the frequently observed separation of cortical

cells after breakage (Fig. 40). These phenomena mostly depend on the condition of the fiber, and can occur at extension levels well below the breakage, e.g., at about 10% at the tip and 20% at the root. This aspect is important since,

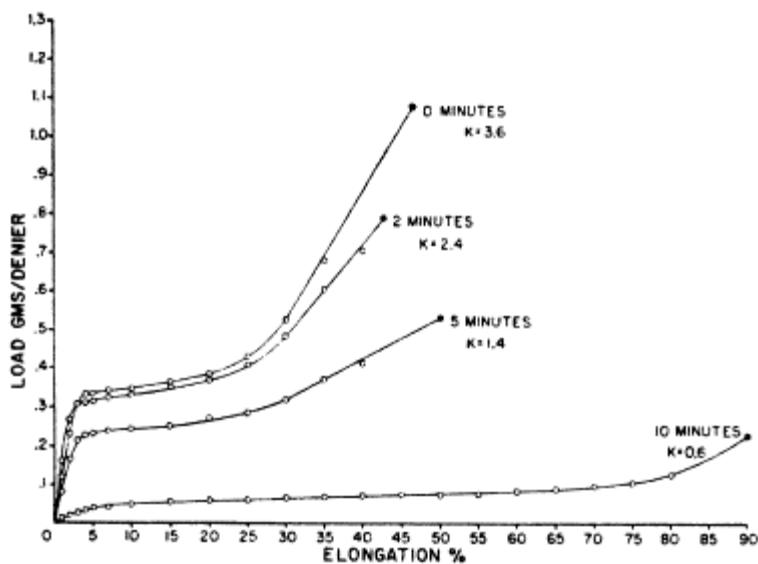


Figure 38 Load elongation curves of hair fibers treated with waving perming lotions for different times (K =value of the post yield slope). (From Ref. 173.)

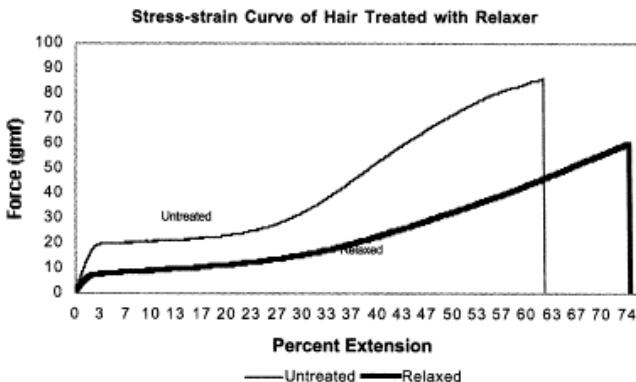


Figure 39 Load-extension curves of a virgin and a relaxed hair in water.
(From Ref. 178.)

during grooming conditions, the stretching of the fiber may reach 5–10% (179). Repeated handling added to other alterations has a major impact at the cosmetic level eventually leading to hair breakage. Many papers have described the pattern of hair fracture (72,73,180,181), diversely referred to as: smooth, step, fibrillation, and splitting fractures. These patterns vary with ambient humidity, chemical state of the fiber (previous treatments), and its shape (twisted or not). They are in fact explained by the composite nature of the hair material, integrating the intrinsic properties of each component of its structure. The “step” patterns are mostly observed at medium ambient humidity whereas the “smooth” fractures are found

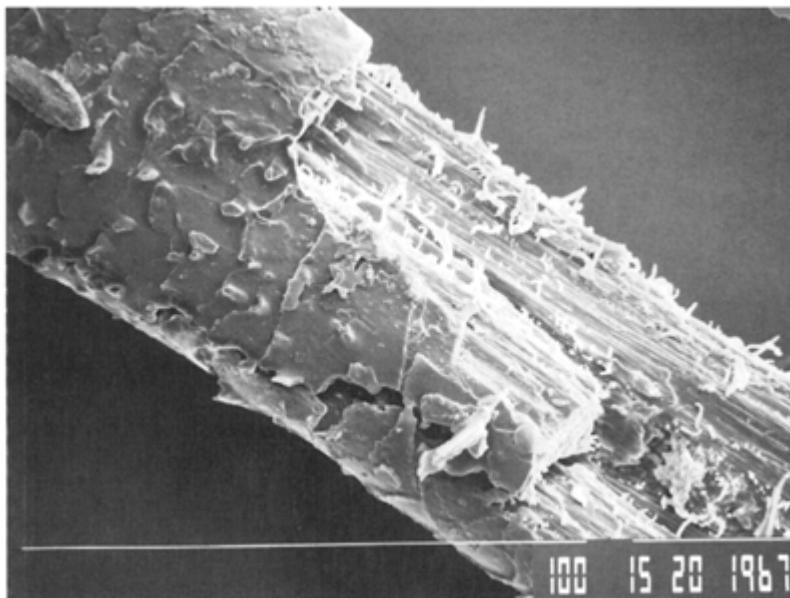


Figure 40 SEM picture of a broken hair. Dislocation and separation of cortical cells are clearly seen.

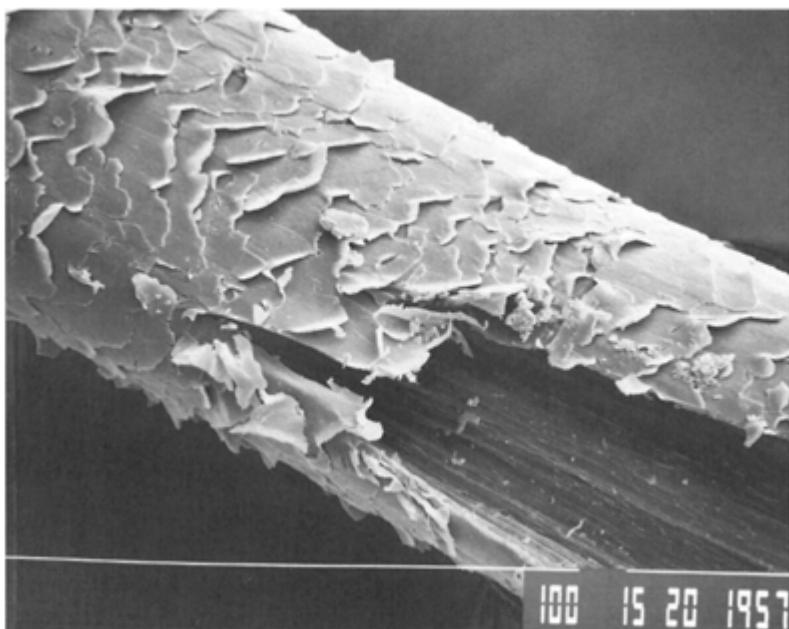


Figure 41 SEM picture of a broken hair showing separation of cuticle from cortex.

at low or high humidity conditions. Accordingly, separation between cuticle and cortex can be observed (Fig. 41).

Dynamic Mechanical Analysis. The mechanical properties can also be evaluated using a dynamic method. This method is very interesting because deformations are slight and therefore realistic relative to the conditions of everyday life. This technique is also very useful, and in fact the only one available, in following the evolution of mechanical properties during treatments applied to the hair, such as reduction (182,183) (Fig. 42). It thus gives unique information on the mechanisms of action of these treatments.

Bending Properties. When trying to define the concept of hair "body" (48), it is clear that the bending properties of single hair fibers play an important role. A model aiming at predicting hair body from these properties was established (184), and proved to be reasonably relevant. Moreover, considering that evaluation on single hair does not appear to be precise enough to gain overall information about the head of hair, the evaluation of bending properties of a group of fibers can provide a more general view of the end result on a head of hair.

One of the oldest techniques is built on the principle of the cantilever (185). The sample is fixed to one of its extremities, placed horizontally and submitted to a vertical charge. Measurement of the flexion amplitude (highest point) at a given distance from the fiber anchorage yields the flexion modulus. In this way various geometries of the section of the sample (circular, elliptical) can easily be integrated. Inspired by this test, a

vibratory method has been developed that can be used to study very slight deformations in flexion (30). This precise method cannot only be used to observe variations in flexion modulus at varying levels of relative humidity but can also be used to assess the effect of treatment applied (Fig. 43). One of the advantages of the method is the weak deformation that mirrors the real-life situation when a set of fibers is involved (i.e., in the course of moving the head).

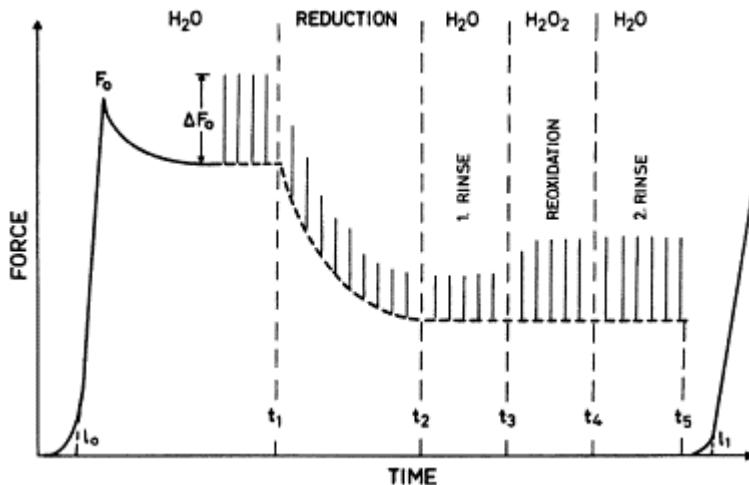


Figure 42 Idealized representation of an experimental curve obtained during static and dynamic extension testing of a hair fiber subjected to reduction/oxidation treatment. (From Ref. 183.)

Another very simple method to evaluate the stiffness in a bend mode for a single fiber (except for curly hair) was developed by Scott and Robbins (186,187): two weights of the same mass are fixed to each end of the fiber that bends, and the distance between each end of the fiber is directly related to the flexion modulus. Using this method, the influence of water on bending properties was investigated and reported results are consistent with other studies (Fig. 44). Moreover this method demonstrated a linear relationship between rigidity and cross-section, in direct proportion to the linear density (LD) of the fibers.

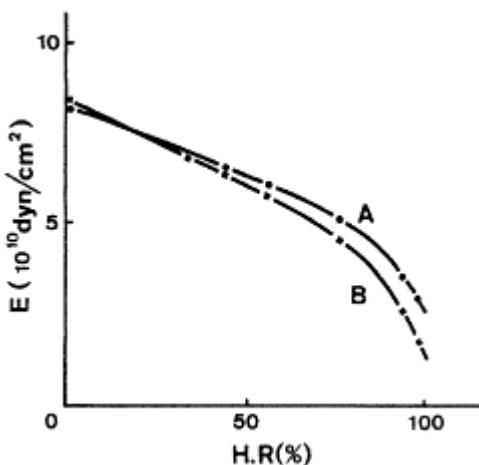


Figure 43 Relative bending modulus for virgin (A) and bleached (B) hair as a function of relative humidity. (From Ref. 30.)

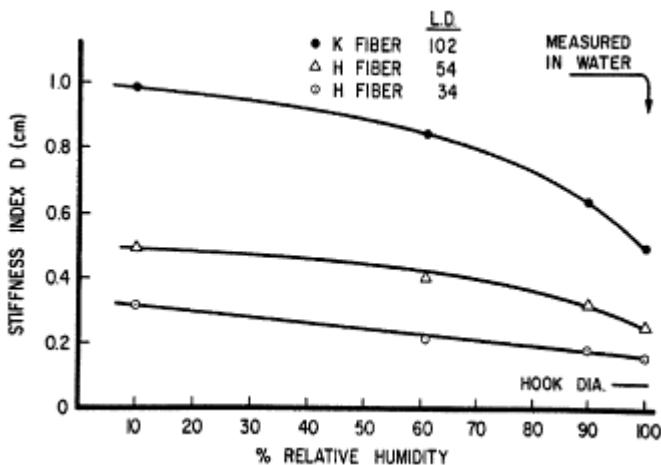


Figure 44 Stiffness index of different linear density hair fibers as a function of relative humidity. (From Ref. 187.)

Using this method, Wortmann and Kure (188) monitored bending properties of hair during the process of permanent waving. Taking into account the relaxation process and the viscoelasticity of the hair fiber, this study made it clear that the bending stiffness of reduced/oxidized fibers controlled the efficiency of permanent waving.

The previous methods were based on single hair. In contrast, a suppleness pendulum has been developed involving 39 fibers which are subjected to flexion simultaneously (189). A heavy mass released without initial speed bends a comb represented by the 39 hairs in parallel alignment and placed vertically. The energy dissipated by flexion of the fibers progressively slows the pendulum down. Thus, the greater the number of beats achieved by the pendulum before it stops swinging, the lower the rigidity of the hairs. The flexion modulus can be calculated from the geometry of the system and the diameter of the fibers (see Fig. 36 in Chapter 1). This technique is very convenient to evaluate the stiffening or softening of hair fibers after cosmetic treatments (Fig. 45) as well as to study geometrical effects:

- A relation is established between the diameter (hairs from various ethnic origin) and the number of strokes and this study (189) can be linked to the work published by Swift (184).
- Bleached hair appears more rigid at low relative humidity levels and is more affected by humidity than virgin hair.

Resistance to Fatigue. During combing, the hair is subjected to three types of mechanical stress: traction caused by the comb's teeth that pull the fibers, flexion around the comb's teeth or around neighboring fibers and abrasion induced by the friction between fibers and between fibers and comb's teeth. These types of strain are commonly called flexabrasion (Fig. 46).

An instrument, derived from the ASTM standard (190), is used to evaluate the effect of a hair product on the resistance of hair to flexabrasion. The principle of the test is as follows: the hair fixed at the root slides as it folds on a stretched steel wire of

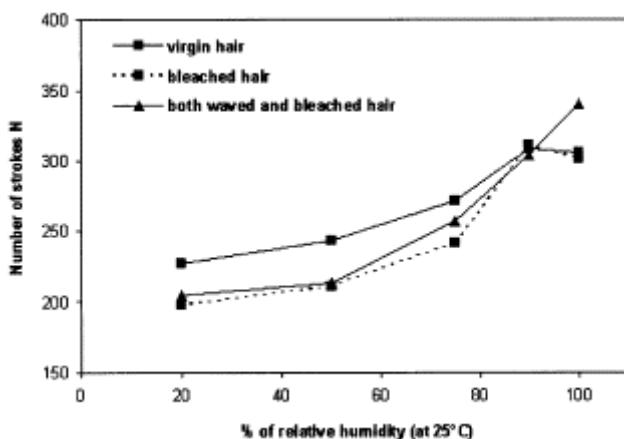


Figure 45 Pendulum strokes vs. relative humidity (at 25°C) for three different types of hair (virgin, bleached, and both waved and bleached). (From Ref. 189.)

small diameter and a small weight fixed to the other end of the hair ensures it remains stretched. The hair is thus lengthwise imparted a regular to-and-fro movement.

The fatigue of hair is characterized by the number of cycles before breaking. The distribution of the breakage time for a given population of hair obeys Weibull's law (191) (Fig. 47).

By rubbing on the wire, the outer covering of the hair appears to be a determinant factor in the resistance to fatigue. However, due to the traction caused by the tiny weight hanging from the hair, the cortical part also contributes to the resistance

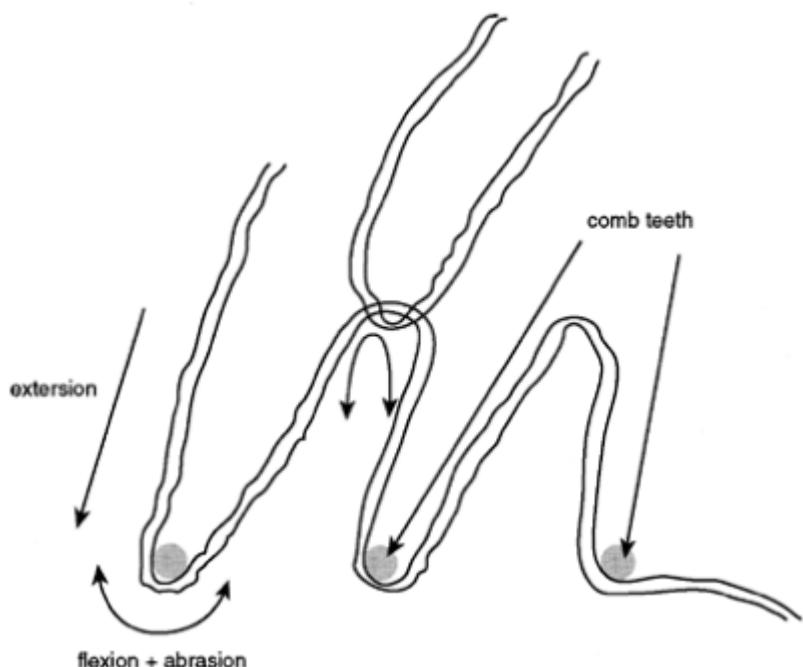


Figure 46 Stresses on hair during combing.

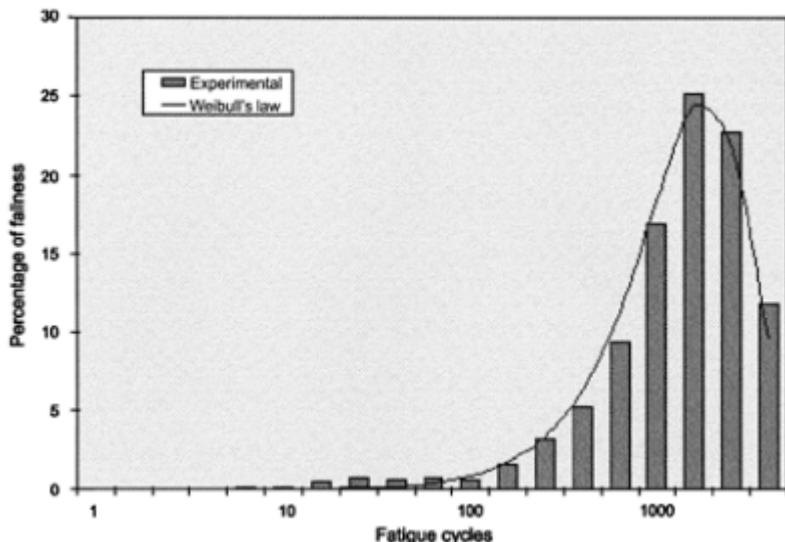


Figure 47 Distribution of lifetimes in the flexabrasion test on virgin hair.
(From Ref. 191.)

to flexabrasion. Weathering and chemical treatments reduce the hair mean lifetime. On the contrary, some strengthening agents whose penetration within the fiber has already been demonstrated can increase the fatigue resistance of hair even if the deposit on the surface is minimal (Table 1).

This method has been recently used for the evaluation of new ingredients (192). Swift et al. (193,194) improved it especially by developing a specific procedure for sample preparation. Using this protocol, hair strength reaches its highest at an RH level of 80% when hair is plasticized (Fig. 48). This observation is totally consistent with consumer experience and in vivo observations, where hair breaks more often when combed in a very dry atmosphere or when wet (193).

Other types of fatigue experiment, e.g. in tensile mode have also been carried out on treated hair (181,195). They also demonstrated that bleaching reduced survival probability (196).

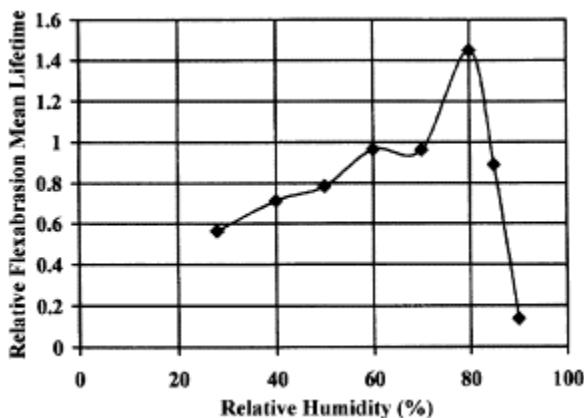
1.3.2. Swelling Properties

In the presence of vapor or liquid, the volume of hair increases due to adsorption (see chapter 1). While longitudinal swelling is weak, of the order of 1–2% in the case of virgin hair, the diameter may increase by as much as 15%. Compared with a reference hair, determination of swelling in diameter after hair treatment indicates

Table 1 Demonstration of the Strengthening Effect of α -Hydroxy Acids

Hair type	Number of cycles (mean value)
Virgin hair	2171
Two bleaches without post-treatment	791
Two bleaches and five shampoos ^a at pH 5	2025

^a Containing 4 g% citric acid. (From Ref. 191.).

**Figure 48** Effect of relative humidity on hair flexabrasion lifetime relative to that at 60% RH. (From Ref. 194.)

the impact of treatment on the hair structure. The initial speed of swelling is linked to cuticle porosity and cysteic acid content of the hair, while the maximum swelling is principally a function of the internal structure of the fiber (197–199).

In 1951, Eckstrom (200) used a microscope equipped with a micrometric eyepiece to establish the kinetics of swelling by simple observation. This method was used to study the behavior of hair in water, as well as in various solvents and reducing agents (201,202). Many optical methods have been developed [evaluation on the basis of photographs, use of polarized light (203) or laser beam diffraction (204)]. Today, many commercial instruments are available for measuring the variations of fiber diameter in liquid media, not only water, but also in reactive solutions mimicking cosmetic treatments. For example, Nothen et al. followed the swelling of fibers during the different steps of permanent waving (Fig. 49) and demonstrated that the swelling during intermediate rinsing was due to osmotic pressure (205).

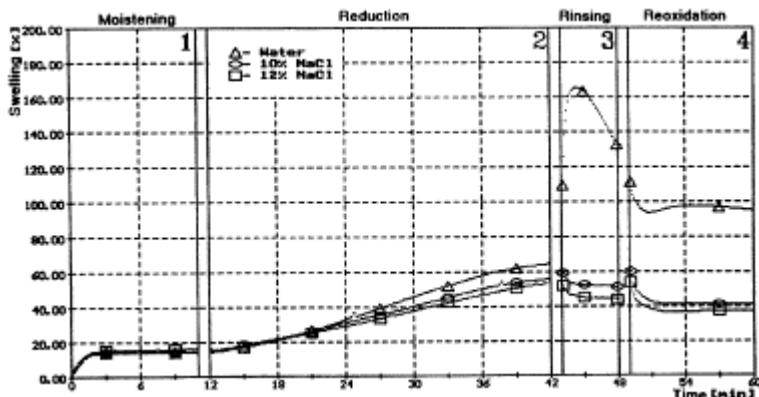


Figure 49 Osmotic swelling of hair during the intermediate rinsing of permanent wave process (reducer: sodium thioglycollate). (From Ref. 205.)

Table 2 Swelling Properties of Virgin, Permed, and Bleached Hair

Hair type/characteristics	Swelling rate (%/min)	Maximum of swelling(%)
Virgin hair	9.4	7.4
Bleached hair	26.1	10.1
Permed hair	17.3	9.9

(From Ref. 207.)

Measurements with contact overcome some drawbacks associated with the use of a microscope, i.e., a limited depth of field, difficulty in setting up, the effects of borders. A specific device has been developed for rapid evaluation of the swelling in different liquid media (206), and is appropriate to compare swelling of hair treated by various products in order to evaluate hair damage associated with these treatments (Table 2) (207). Klemm et al. (208) did equivalent experiments and derived from experience empirical equations allowing them to distinguish fibers treated by single or repeated perming or bleaching treatments.

1.3.3. Alkali Solubility

Alkali solubility was first proposed to evaluate wool damage after specific treatments (mainly oxidative treatments) (209,210) and has become an ASTM standard (211). The solubility of hair in an alkaline solution (0.1 N sodium hydroxide) gives an estimation of the oxidative damage to hair: the higher the level of oxidation of hair, the greater its

solubility in alkali (212) (Fig. 50). A linear relationship between alkali solubility and the level of cysteic acid in the hair has been found for oxidative treatments (213).

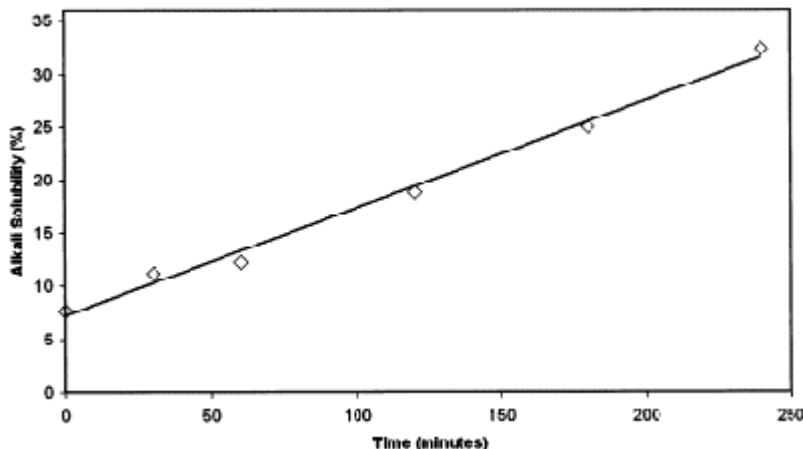


Figure 50 Relationship between alkali solubility and the bleaching level (expressed as time of treatment). (From Ref. 212.)

1.3.4. Birefringence and Polarization Microscopy

Crystalline, or molecular chain-sheet substances of a non-isometric nature, exhibit the optical property of birefringence when they are placed in a field of planepolarized light. This phenomenon, due to a condition known as optical anisotropism, results from the difference in refractive indexes of a crystal and its surrounding medium. The quantitative measurement of birefringence provides a sensitive evaluation of optical anisotropism. The cortical region of a human hair is optically anisotropic. The connection between mechanical properties and birefringence has been studied at different relative humidity levels (214). Birefringence of hair can also be altered following reducing or oxidizing treatment (215). More recently a device for online measurement of fiber birefringence has been developed (216). It consists in a double-beam detection method combined with an interference fringe device.

1.3.5. Thermal Analysis (Differential Scanning Calorimetry)

Thermal analysis, and especially differential scanning calorimetry or high pressure differential scanning calorimetry (HPDSC), is an accurate way to assess changes in the structure of materials. In the case of hair, DSC thermograms show specific enthalpic peaks attributed to the fusion or degradation of different structures, notably the intermediate filaments (IF) (217,218). Bleaching and perming treatments (Table 3 and 4) induce a decrease in both the IF fusion peak temperature (219), which is kinetically

controlled by the cross-link density of the matrix, and the peak enthalpy, which depends on the amount and structural integrity of the alpha-helical structure. It appears that HPDSC in water is an especially suitable method to determine the kinetics of damage in human hair resulting from cosmetic treatment. This method has been used to find new ingredients to protect the hair fiber from degradation (220).

1.3.6. Chemical Analysis as a Diagnostic Tool

All the modifications of the physical properties of hair are a consequence of changes in its chemical structure, whether these changes are due to a chemical modification of the hair itself or the result of a deposit or penetration of specific ingredients. Analysis of the chemical nature of hair is thus important to understand and evaluate the effects of hair treatments. A number of methods have been described to carry out

Table 3 Determination of Denaturation Temperature (Td) and Enthalpy (ΔH) for Hair After Repeated Bleaches

Number of bleachings	Td $\pm s^a$ (°C)	$\Delta H\pm s^a$ (J/g)
0 ^b	158.3 \pm 0.3	19.3 \pm 0.4
1	157.8 \pm 0.2	18.0 \pm 0.2
2	152.6 \pm 0.5	15.6 \pm 1.1
3	145.3 \pm 0.4	13.7 \pm 0.8
4	140.9 \pm 0.4	12.4 \pm 0.4
5	141.4 \pm 0.3	12.9 \pm 0.4
6	139.6 \pm 0.2	13.0 \pm 0.4
7	138.4 \pm 0.4	11.7 \pm 0.2

^a Standard deviation for fivefold measurements.

^b Untreated hair. (From Ref. 218.)

Table 4 Determination of Denaturation Temperature (Td) and Enthalpy (ΔH) for Hair Samples After Repeated Permings

Number of treatments	Time of perming treatment (min)	Td $\pm s^a$ (°C)	$\Delta H\pm s^a$ (J/g)
Start ^b	—	155.2 \pm 1.0	15.3 \pm 0.3
Bleach ^c	—	152.5 \pm 0.6	14.9 \pm 0.4
After one perm	10	152.9 \pm 0.6	11.3 \pm 0.4
	20	150.3 \pm 0.4	11.5 \pm 0.3

	30	148.7±1.1	10.2±0.6
After three perms	10	146.4±1.1	8.5±1.0
	20	144.0±0.9	7.4±0.7
	30	143.5±0.6	7.5±1.0
After five perms	10	136.7±0.7	3.2±1.0
	20	134.6±0.7	1.9±0.4
	30	121.3±2.0	1.0±0.3

^a standard deviation for fivefold measurements.

^b Untreated control hair.

^c One bleaching performed on control hair or after one or repeated perming treatment applied for different time periods. (From Ref. 218.)

rapid chemical analysis. We will focus here on the most important, either by their field of application or their regular use.

Amino Acid Analysis. The determination of amino acid composition of hair is a frequently used method for appraising the impact of cosmetic treatments or following various types of damage such as from UV (172). Commonly, this is done by acid hydrolysis of hair followed by a chromatographic separation and quantification of the various amino acids (221). Extensive studies have demonstrated that almost all cosmetic treatments and physical aging lead to changes in the amino acid composition of hair. Bleaching or oxidative dyeing induces a decrease in cystine content, balanced by an increase in cysteic acid and a slight reduction in tyrosine, threonine, and methionine (222–225). Permanent waving leads to an increase in cysteic acid, due to the oxidation of reduced keratin (222,226), and the presence of a mixed disulfide between keratin and the reducing agent (thioglycolic acid) (227). Alkali straightening induces the formation of huge amounts of lanthionine (and consequently a decrease in cystine) and of isopeptide bonds (lysinoalanine) (228). Weathering, and especially the combination of UV irradiation and water, induces chemical reactions in the hair, notably the cleavage of cystine bonds to form cysteic acid and also lanthionine, glycine, and isopeptide bonds (229,230).

Protein Analysis. As their constituent amino acids, proteins may undergo severe modification after chemical treatment. Extraction of proteins in a reductive medium followed by 2D gel electrophoresis separation allows the identification and quantification of the different proteins of hair (231). The yield of extraction in a reducing buffer is slightly increased after bleaching or perming (232–234). In contrast, the amount of extracted proteins is significantly reduced following alkaline relaxing of hair (232) or sun exposure (235), a consequence of protein cross-linking thus generated. Few modifications of the pattern of 2D gel electrophoresis of low sulfur proteins (intermediate filament proteins) have been observed in all these studies, contrary to the high sulfur and high glycine-tyrosine proteins (matrix proteins) that are affected by all the above treatments. Kon et al. (234), however, found a significant decrease in intermediate filament proteins when weathering and perming of hair were combined.

Protein analysis provides essential information to help us understand the structural changes produced in hair after cosmetic treatments. Unfortunately, the available methods do not yet seem to be accurate enough to give reproducible and comparable results.

Spectroscopic Analysis. A great number of spectroscopic analytical techniques are readily available. We will not undertake an exhaustive survey of these methods but focus on those that are among the most often used, or which seem to be the most appropriate for hair analysis. In general, none of these techniques on their own allows the scientist to get a complete description of the sample and several therefore have to be used in combination. For example, the mass (elemental or molecular) spectral character of the data generated by secondary ion mass spectroscopy (SIMS) complements the vibrational data provided by infra-red or Raman spectroscopy or the elemental and chemical shift information given by X-ray photoelectron spectroscopy (XPS).

FT-IR Spectroscopy. FT-IR spectroscopy in attenuated total reflectance (ATR) mode has been mainly used to study the oxidation of hair and, more precisely, its photodamage and the photoprotection afforded by different ingredients. Cysteic acid and other cystine oxides give well-defined absorption peaks (236–242). Diamond cell ATR, which enables to apply higher pressure on the sample and thus contributes to a better contact, provided more reproducible results than conventional ATR crystal (243).

FT-IR spectroscopy has also found some specific applications like the detection and quantification of cationic polymers on the hair surface (244) or the water content of hair and water-binding energy when measurements are done in the near-IR range (245,246).

IR and Raman Spectroscopy. IR and Raman spectroscopy both provide information on the chemical functions present in the hair. Each has specific advantages and drawbacks, and they have to be considered as complementary. For investigations of the cortex of hair fibers, Raman spectroscopy proved to be the most interesting technique. Despite the fact that use of unpigmented or weakly pigmented hair is needed because the fluorescence of melanin overlaps the Raman bands, Raman spectroscopy has very interesting characteristics compared with IR spectroscopy: water does not give a broad band; many well-defined bands correspond to the different oxidation state of cystine and to lanthionine; the amide band is better defined than in IR spectroscopy, which enables one to study changes occurring in the protein backbone structure as a result of treatments. Moreover, the availability of confocal Raman microscopes allows microscopic investigations of the chemical composition of the inside of hair without requiring to cut the hair and it offers the possibility to analyze specific proteins, lipids, and even water in the hair (247,248).

As for FT-IR spectroscopy, most of the studies have dealt with the evaluation of oxidation of hair, either due to bleaching or UV irradiation (249–251). The decrease in intensity of the disulfide band is accompanied by an increase in the intensity of the cysteic acid band (bleaching) or of the bands related to other oxidation derivatives such as cystine oxides (photooxidation). Reduction in hair during perming can also be followed by comparing the relative intensities of the disulfide and thiol bands (252) and the modifications to the protein structure are evaluated by following the alterations to the amide band (250).

Fluorescence Spectroscopy. Some amino acids of hair, mainly tryptophane and to a lesser extent tyrosine, have fluorescent properties. The relative amount of these amino acids can be evaluated by measuring the intensity of hair fluorescence. As they are sensitive to

oxidation and more specifically to photooxidation, the main application of fluorescence spectroscopy on hair is the evaluation of photodamage (239).

Weigmann et al. (253) also used fluorescence spectroscopy to evaluate the deposition of polymers on the surface of hair, by incorporating a fluorescent chromophore in the polymer and observing the deposition patterns by microfluorometry.

X-Ray Photoelectron Spectroscopy. X-ray photoelectron spectroscopy, also called electron spectroscopy for chemical analysis (ESCA), is strictly a surface analysis method. It gives elemental analysis on the surface of hair, which makes it less useful than FT-IR spectroscopy for analyzing organic material. XPS can, however, give additional information both on the oxidation state and on the chemical environment of surface atoms. Like FT-IR spectroscopy, it has been largely used for the quantitative evaluation of hair surface oxidation, by following the relative amounts of the different oxidized species of sulfur, especially after weathering of hair (237,254–257).

Another interesting application of XPS is the evaluation of the adsorption of cationic surfactants and polymers onto the hair surface (258–260), but with some limitations due to the similar elemental composition of the polymer and hair, and its relatively low sensitivity (79).

Secondary Ion Mass Spectroscopy. Secondary ion mass spectroscopy emerged as a technique of potential importance in surface characterization in the early 1960s (261). The principle is simple (Fig. 51): a primary ion beam is focused onto the sample surface to sputter secondary ions that are extracted from the surface and analyzed with a mass spectrometer as a function of their mass/electric charge ratio. The first systems (called dynamic SIMS or DSIMS) used high-energy primary ions and only allowed elemental analysis, which restricted their use to inorganic materials. In the 1970s (262), static SIMS (SSIMS) used low-energy primary ions. The secondary ions were then fragments of molecules, which allowed the analysis of organic materials (polymers), very often by acquiring complete mass spectrum using a time-of-flight mass spectrometer (TOF-SIMS). A primary ion beam can be scanned over the surface of hair in order to get an image of its chemical composition and recent progress in the design of SIMS has led to acquire images with a resolution as high as 30 nm (263).

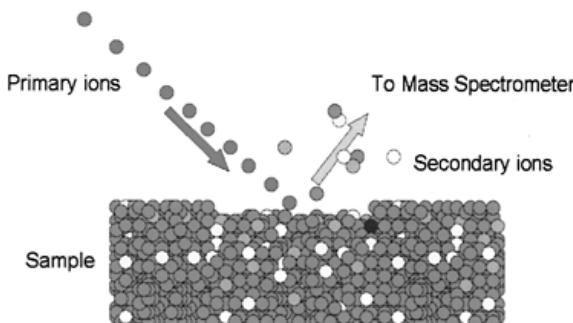


Figure 51 Principle of SIMS: SIMS reveals atomic mass composition of a given sample.

Even if it has not yet been widely used, SIMS has been applied in many different ways in hair chemical analysis, due to its versatility: the capability of acquiring high-resolution chemical maps and the detection of elements, of stable or radioactive atoms, of low- or high-mass molecules. The first reported use of SIMS on hair was the study on the adsorption and penetration of ceramide (166,264). Ceramide was found to be uniformly adsorbed on hair (Fig. 52) and, taking advantage of the ability of SIMS to erode the hair, it was demonstrated that ceramide penetrated between the cuticular cells (Fig. 53), which could account for the increased resistance to breakage it specifically imparts to hair.

Most of the studies with TOF-SIMS have dealt with the quantification of various ingredients (cationic polymers, surfactants, silicones, oils) onto the hair, in relation to their conditioning or protecting effect (69,79,265–267). Some of these compounds penetrate the hair (69,267). SIMS also proved to be very useful when combined with other techniques such as XPS for studying the lipid and protein composition of the hair surface, and its chemical or photochemical oxidation (266,268). The very high resolution offered by the latest generation of SIMS enables us to investigate the local composition of virgin or chemically treated hair. For example, the distribution of sulfur in the various structural domains of the fiber was analyzed, and specific regions with high (exocuticle) or low (medulla) levels of sulfur were detected (269) (Fig. 54). Labeling of reduced keratin with ^{14}C -iodoacetic acid and SIMS imaging of ^{14}C on hair cross-sections allows chemists to map the reduced regions of hair and hence to follow the penetration of reducers (270) (Fig. 55).

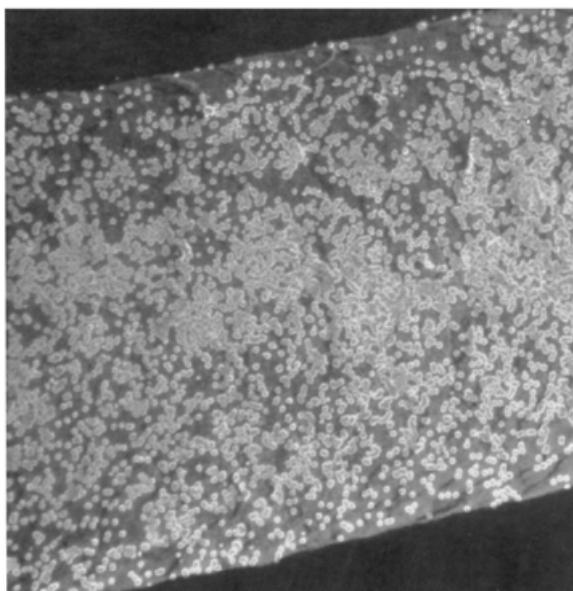


Figure 52 SIMS image of adsorption of ^{14}C -tagged ceramide onto hair surface. Green: $^{12}\text{C}^{14}\text{N}$ -signal

(proteins); red: $^{14}\text{C}^{14}\text{N}$ -signal (ceramide). (From Ref. 166.)

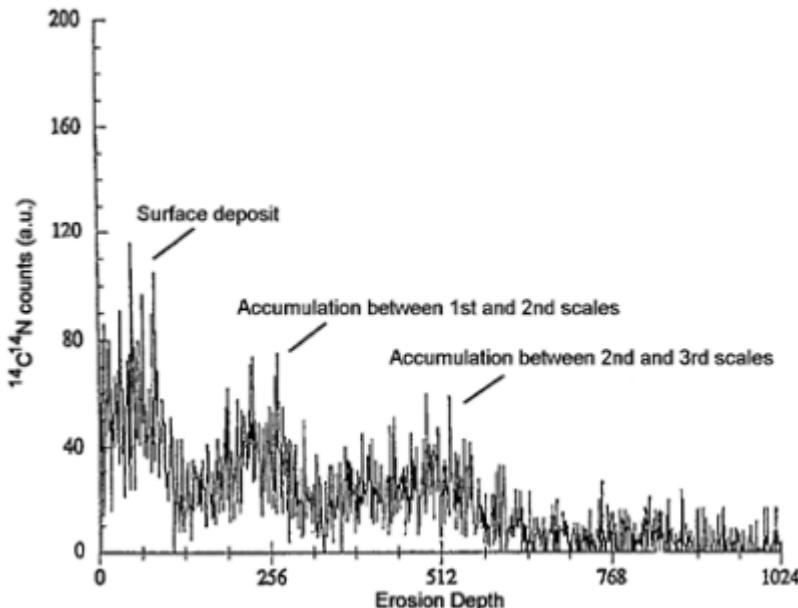


Figure 53 Penetration in the hair cuticle of ^{14}C -tagged ceramide; X-axis: erosion time (depth of analysis); Y-axis: $^{14}\text{C}^{14}\text{N}$ - signal (ceramide). (From Ref. 166.)

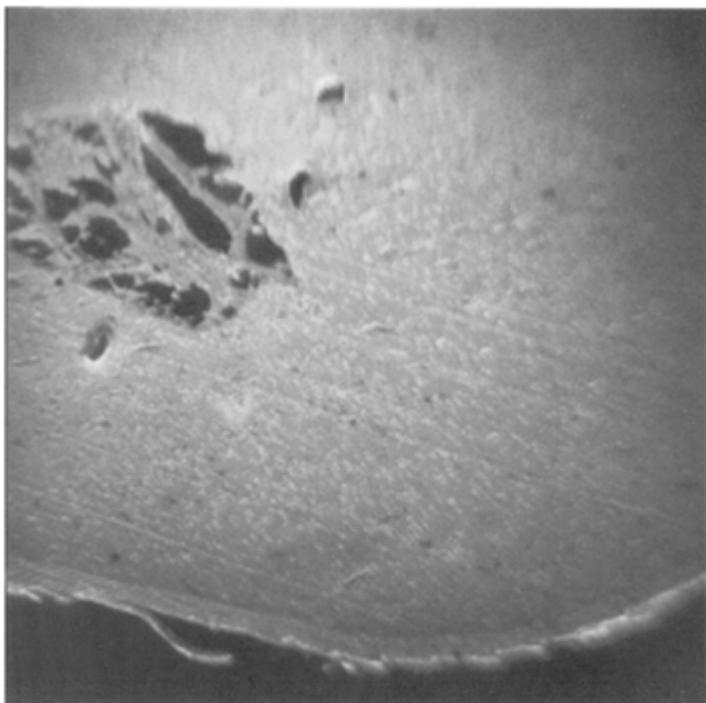


Figure 54 SIMS image of Sulfur distribution in hair. Green: $^{12}\text{C}^{14}\text{N}$ (protein); Red: ^{32}S (sulfur-rich proteins). (From Ref. 269).

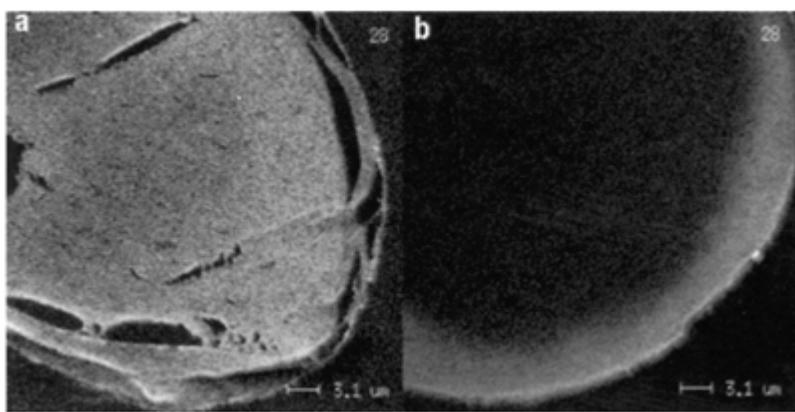


Figure 55 $^{14}\text{C}^{14}\text{N}$ map of cross-sections of ^{14}C -iodoacetic labeled

reduced hair: (a) ammonium thioglycollate reduced hair shows a uniform reduction; (b) trihydroxymethyl phosphine reduced hair shows a ring reduction. (From Ref. 270).

1.4. Surface Integrity

Many methods have been used and developed to evaluate hair surface integrity. Surface evaluation is always difficult to interpret; it is very important to present conclusions in relationship with the probes or tools used.

1.4.1. Surface Charge

The most exhaustive method to evaluate the electric charge of hair is to measure the ion exchange capacity of hair by chromatographic techniques using hair as the solid phase (271,272). This method is however difficult to carry out and easier techniques have been developed. Among them, the streaming potential technique gives access to the zeta potential of the hair surface and to its dependence on the composition of the solution. It thus provides useful information not only about the electrical charge of the hair surface, but also about the interactions of various cosmetic ingredients (surfactants, polymers) with hair (273,274). Jachowicz et al. (275–278) brought a significant improvement in the technique by coupling electrokinetic and permeability measurements. It is claimed to better simulate the cosmetic treatments and gives useful data on the amount of material adsorbed on or desorbed from hair as well as the kinetics of the exchange process.

The simplest method to evaluate the electrical charge of hair surface is based on the measurement of the absorbed quantity of a negatively or positively charged dye (279). With this method, the hair is classified according to its porosity and condition. Nine categories of hair are thus defined. The reliability of these tests was checked by performing them on the hair of 33 persons in a hairdressing salon.

1.4.2. Determination of Cationic Deposits on the Hair Surface

The cationic compounds included in hair products improve the hair surface condition and consequently its cosmetic properties (untangling, softness). Apart from the analytical techniques (described above), many indirect methods have been proposed to evaluate the distribution of cationic polymers adsorbed onto hair surface. One of the easiest relies on the fact that not all the cationic sites of the polymers are engaged in binding with hair and some therefore remain free to bind to anionic species, especially dyes. After immersion of treated and control hair swatches in an acid dye solution, followed by thorough rinsing, the amount of cationic compounds bound on or in the hair can be determined by colorimetric comparison of the two samples (280). The more the sample is colored, the higher the level (281). The most widely used dye is Red 80 (282). Relationship has been

established between cosmetic properties and the presence of cationic polymers onto the hair surface (283).

1.4.3. Cuticle Cohesion

The cuticle is made of about $0.3\text{ }\mu\text{m}$ thick overlapping scales (five to 10 scales on average for a normal hair which amounts to $1.5\text{--}3\text{ }\mu\text{m}$ cuticle thickness). Cohesion between these scales is ensured by the intercellular spaces between cuticle cells, also called Cell Membrane Complex (see Chapter 1). The quality of cuticle cohesion is a paramount criterion as it reflects cuticle condition and its fastness against external trauma.

Several methods for characterizing the cuticle cohesion have been suggested. Scraping the hair with a razor blade has been used, but this method is tiresome (284). In general the hair is subjected to vigorous stirring in a liquid medium using, for example, ultrasound (285,286) or mechanical stirring in water (57,287). Some chemical methods also exist, such as immersion of hair in formic acid (286), violent nickel-assisted decomposition of hydrogen peroxide (288), or enzymatic digestion of hair (289,290). These methods are generally combined with a high level of mechanical stirring and can deliver a significant amount of extracted cuticle (Fig. 56) (287).

The cuticle fragments are recovered by centrifugation. They are analyzed using various methods—simple weighing, observation under the microscope, analysis of size and/or weight distribution using a particle counter or chemical analysis of protein (291). Several treatments lead to modification of cuticular cohesion. Reducing and oxidizing treatments weaken the cuticle (290,291), as also do weathering, UV,

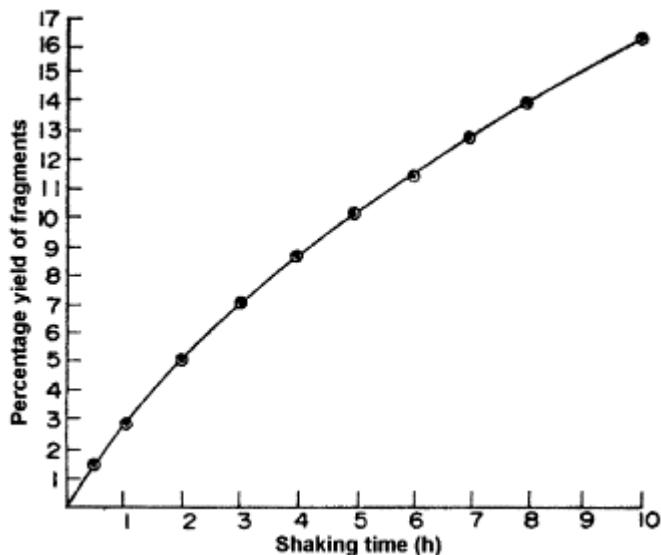


Figure 56 Amount of cuticle fragments recovered vs. stirring time.
(From Ref. 287.)

repeated combing, and shampooing (292). Specific treatments with, e.g., ceramide or cationic cellulose enhance cuticle cohesion (166,289).

1.4.4. Influence of Combing, Brushing, Hot Irons, and Methods to Characterize

As mentioned before, the hair surface is the first structure exposed to the daily traumas such as brushing, heat, UV, and chemical treatments. Many studies have shown alteration of the surface and, in the case of some specific agents such as hot irons, changes in cortex properties. On the hair surface, cycles of wetting and blow-drying applied on hair swatches are responsible of the formation of cracks in the hair cuticle (293). This, in association with cyclic mechanical stress (torsion), accelerates the formation of such cracks (294,295). They have also been observed in hair from people who commonly blow-dry their hair (Fig. 57). These effects have been shown either by general methods (previously described in this chapter) or by using specific and targeted techniques.

1.4.5. Protein Loss by Repeated Combing

The aim of this procedure is to quantify protein loss caused by repeated combing of a swatch of hair. It involves the collection of cuticle fragments dislodged from the hair in the course of combing and the quantitative evaluation of these fragments either by weighing them, by the measurement of turbidity (291,296) or by an assay for amino acids (Table 5). The method has shown that the greater the number of passages of the comb (between 25 and 200), the greater the protein loss. Depending on the extent of hair “sensitization,” significant differences are observed. As shown in Fig. 58, protein loss increases progressively with repeated treatments reflecting progressive damage and increased liability toward protein loss. During combing, bleached hair loses more protein material than virgin hair. In contrast, permanent waving has only

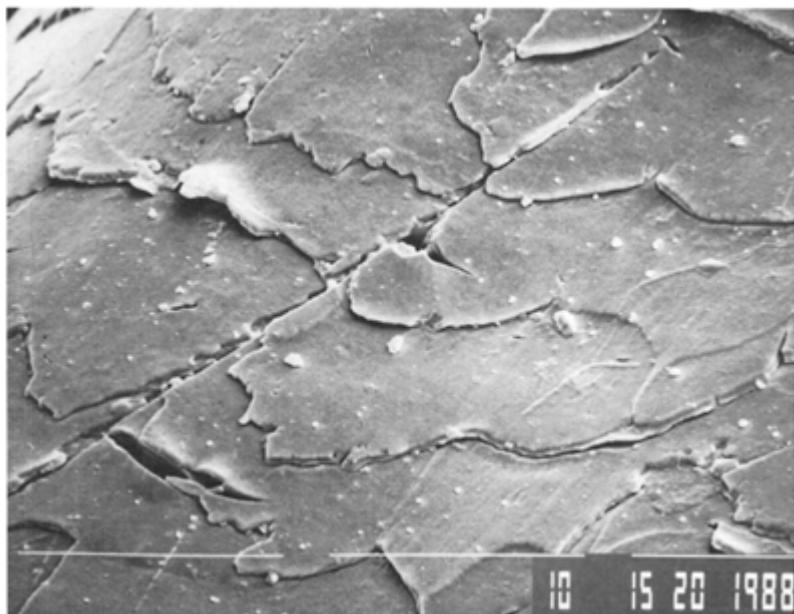


Figure 57 SEM picture of hair cuticle showing cracks due to repeated combing associated with heat-drying.

Table 5 Effect of Post-shampoo Brushing on Protein Loss from Oriental Hair.

Treatment	Number of Combing Strokes					
	Turbidity (optical density at 600 nm)					
	100	200	25	50	100	200
Shampoo A	0.189	0.376	0.226±0.01	0.433±0.03	0.944±0.07	1.965±0.17
Shampoo B	0.067	0.169	0.118±0.01	0.290±0.02	0.545±0.04	1.283±0.012

(From Ref. 296)

a slight effect on the rate of protein release. This method is sensitive enough to differentiate the protective effect of shampoos containing specific protecting agents (296).

1.4.6. Split-End Formation

Split-ends are one of the most obvious and frequent forms of damage to hair, as soon as it reaches a medium length. They are the consequence of the progressive lifting and loss of cuticle cells (154) due to cumulative treatments (297), handling (combing and brushing) (298–301), and weathering of hair. When the cuticle is totally removed, the cortex strength is lowered and lateral separation of cortical fibrils occurs (Figs. 59 and 60).

To study this phenomenon and to prevent the split-end formation, or to repair them, methods have been developed with a view to generate split ends on hair swatches and to study their prevention or repair under a light microscope (298,302).

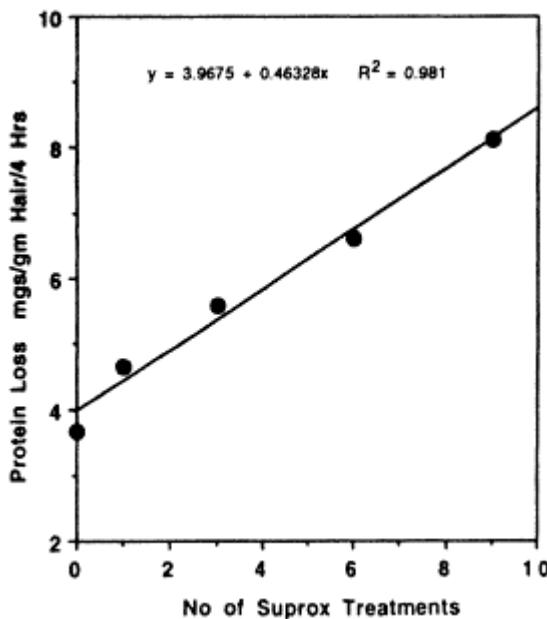


Figure 58 Protein loss from hair after various oxidation (Suprox) treatments.
(From Ref. 291.)

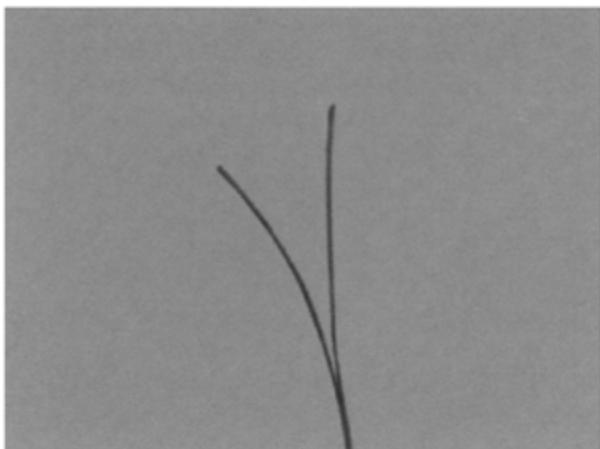


Figure 59 Optical microscopic observation of a split-end.

1.4.7. Breakage on Blow-Drying

In most cases, treating hair involves drying. Apart from natural drying, blow-drying is the most frequently used technique to give a temporary “style” to hair (303). When blow-drying, wet hair is subjected not only to ongoing strains from the brush (friction, abrasion, extension) but also to heat produced by the hairdryer. Repeated

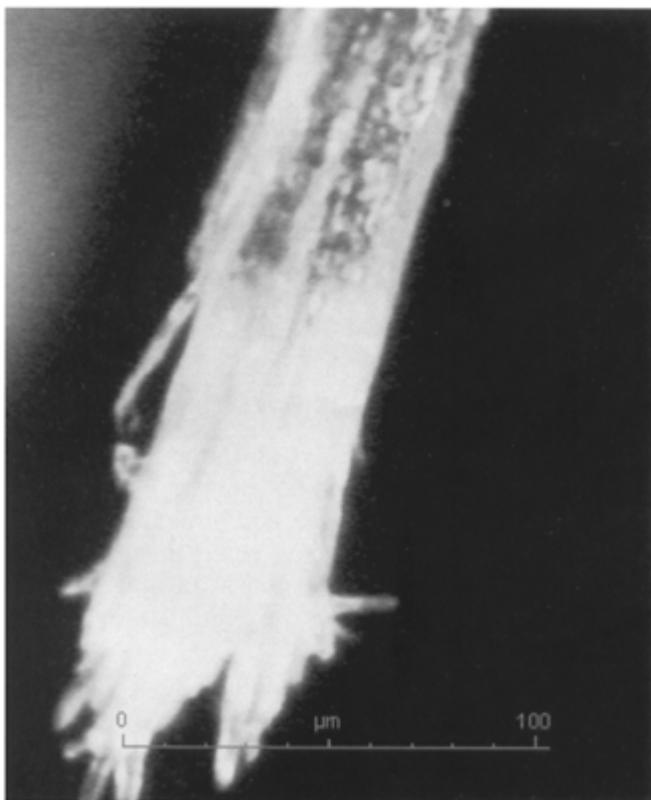


Figure 60: Fibrillation of the tip of a hair showing the beginning of a split-end.

blow-drying can thus cause erosion of the cuticle and generate cracks and tears characteristic of the combined action of mechanical effort and heat (293,304) and eventually lead to split ends (305,306). Blow-drying is also associated with considerable stress causing a high level of traction on the hair (303). In the case of "sensitized" hair, breakage may occur as the brush passes through the hair. Damage linked to brushing depends greatly on the nature of the hair, its surface condition and also the degree of "sensitization."

Rating the amount of hairs broken during blow-drying of a swatch of hair is a convenient method to assess overall damage (264). This very simple approach that adequately reflects real-life conditions, consists of fixing a swatch by the roots to a frame after having washed it, treating it with a hair product or leaving it untreated, and rinsing it (hair remaining damp). The swatch is then brushed for a given time from the root to the tip while being exposed to heat. At the end of hot blow-drying, broken hairs are recovered and weighed. The weight of hair fragments determines the degree of

impairment of hair and the efficiency of a conditioner in limiting hair breakage during blow-drying. The test thus characterizes in a general manner the integrity and surface condition of the hair.

1.4.8. Iron Process

The hot iron is now used in complement of other application of products especially to give long-lasting effects. The effect of a hot iron on hair condition is not as yet completely described. As the range of temperatures reached with an iron (approx. 120–200°C) is very different from the range reached with a blow dryer (approx. 60–90°C), the changes produced in hair condition are different but they affect a number of hair properties—surface damage, tryptophane degradation, discoloration (yellowing), and increase in combing work. The amplitude of induced alterations have not been fully estimated, but they increased as a function of exposure time to high temperature (307,308). Regarding mechanical properties, it has been shown that repeated thermal treatments using a curling iron in certain conditions leads to an increase in post-yield modulus and in an improved fatigue resistance. It might be related to structural modifications such as increased cross-linking due to thermally induced dehydration (309).

1.5. Color Measurement

Hair color is a consequence of a complex phenomenon based on the interaction of light with hair. Eye perception of hair color does depend not only on pigmentation peculiar to each hair fiber, but also to its shape, its volume, and of illuminating and observation conditions (136,310). Hence, color is a subjective perception that makes its measurement more difficult than objective properties such as length or mass. Characterizing and measuring color is however essential for many industries such as plastics, paints, textiles and, of course, cosmetics.

The key for a reliable color measurement is a standard color system where specifications are established in order to be able to describe a color, to produce a given color, to predict color, and also to calculate the difference between two colors. Applying it to hair, one should be able to describe the hair color (natural or artificial), to produce a given hair color by mixing dyes of different colors, to predict the final color, taking into account the original hair color, or also to be able, when dyeing, to describe the difference between the obtained and the desired colors as well as to evaluate the evolution of hair color due to weathering, shampoo, hair treatments, etc.

For hair color evaluation, two systems are commonly used, the Munsell Color Notation System and the CIE-LAB System (281,311). In both systems, color is described as a 3D parameter. Here below is a brief description of these two systems (312,313).

1.5.1. The Color Notation System

The Munsell Color Notation System. For centuries, color atlases have been created to yield a representation and especially a notation of colors in order to get an objective evaluation rather than a subjective assessment. One of the most commonly and widely

used is the Munsell Color Notation System, made by Munsell at the beginning of the 20th century (314). In the Munsell system, colors are arranged in a cylindrical coordinates system using three scales (Fig. 61) (312):

- Munsell *value* (*V*) or *lightness*, which indicates whether a color is dark or light, varying from 0 for a perfect black to 10 for a perfect white.
- Munsell *hue* (*H*), which constitutes the shade or the chromatic component of a color (red, green, blue, yellow, etc.). It varies in an angular interval from 0 to 100 with five equally spaced, so-called Munsell principal hues: R (red), Y (yellow), G (green), B (blue), and P (purple).
- Munsell *chroma* (*C*), which constitutes the saturation or the purity of a color. *Chroma* for all achromatic colors is 0. For chromatic colors, the more the color is different from the achromatic color of the same *value* (*V*), the highest the *chroma* (*C*) is.

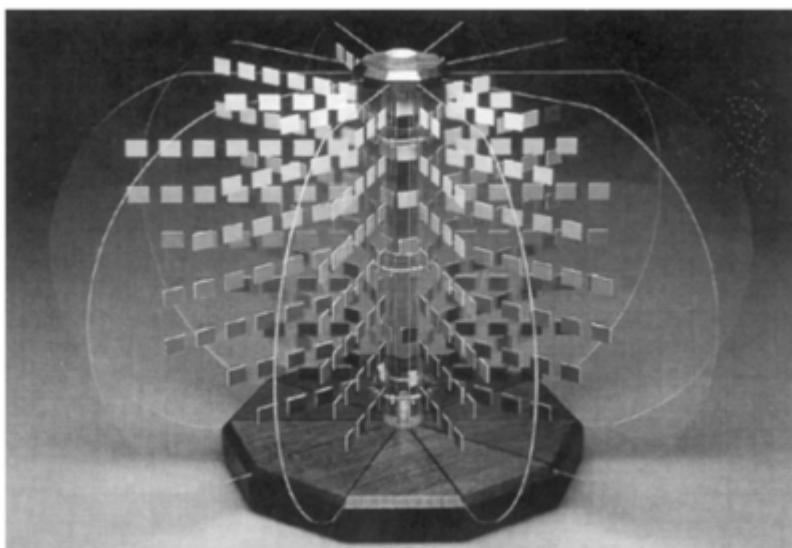


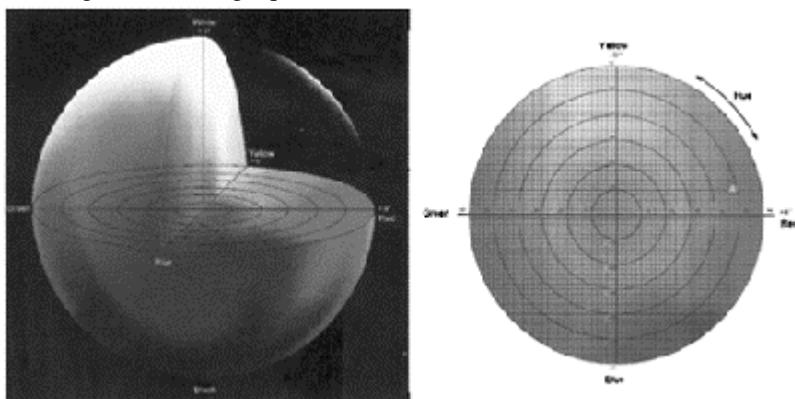
Figure 61 The Munsell Color Tree.
(From Ref. 312.)

The 1976 CIE-LAB Colorimetric System. The 1976 CIE-LAB system is the result of a continuous evolution of the first CIE set up in 1931, based on experimental observations and taking into account the visual perception and the sensitivity of the human eye. The 1976 CIE-LAB system is an approximate uniform color space where each color can be characterized by a 3D parameter, in cylindrical or rectangular coordinates. In the cylindrical coordinates, a color is described by L^* for *lightness* (Z-axis), C^* for *chroma* (radius coordinate), and h for the *hue* angle (angular coordinate). The transformation of these coordinates into a rectangular coordinates system gives the CIE-LAB system. Here, each color is described by L^* for *lightness*, a^* (the chromatic red-green component), and

b^* (the yellow-blue component). An illustration of this color space is shown in Fig. 62 (315).

The colorimetric coordinates (L^* , a^* , b^*) can be calculated from measured reflectance values, based on the color matching functions established by the CIE for a standard observer in the visible spectral region.

In the color field, one does not only need to measure color, but also to be able to evaluate color differences in a way similar to that of the human eye. Here is one of the main advantages of the 1976 CIE-LAB system. In the $L^*a^*b^*$ color space, the difference between two colors is simply given by the geometrical distance between the two color points in the space. The color difference, called in this case DE^{*76} , can be calculated using the following equation:



(8)

The 1976 CIE-LAB system is widely used in the industry to express color and color differences and the measurements are closely related to human perception. In 1994, an extension of the DE^{*76} formula was recommended by the CIE, where corrective factors have been introduced in order to correct for supplementary effects, especially related to *chroma* or the eye perception of color difference (316). This extension, known today as DE^{*94} is shown by the following equation:

$$DE^{*76} = \sqrt{[(DL^*)^2 + (Da^*)^2 + (Db^*)^2]}$$

Figure 62 Illustration of the 1976 CIE-LAB color system. Left: the CIE color space; right: horizontal cross-section (colors of the same brightness). (From Ref. 315.)

$$DE^{*94} = \sqrt{\left[\left(\frac{DL^*}{K_L S_L} \right)^2 + \left(\frac{DC^*}{K_C S_C} \right)^2 + \left(\frac{DH^*}{K_H S_H} \right)^2 \right]} \quad (9)$$

where S_L , S_c and S_H are weighting functions and K_L , K_C and K_H are corrective factors that are to be determined by experimental observations where material nature, viewing and assessment conditions are taken into account. For textile, which is the closest field to hair, the best concordance of DE^*_{94} results with those from visual assessment of textile samples is expected when $K_L=2$ and K_c and $K_H=1$ (312).

It is also important for color industries to determine the acceptability threshold, which dictates the DE threshold where two different colors are considered visually different. This acceptability threshold depends on the nature of colored material and on viewing conditions and can be also determined by experimental visual assessments. The acceptability threshold can be used to judge with color measurement the uniformity of color on dyed hair or the resistance of artificial hair color regarding washing and sunlight.

1.6. Color Measurement Instrumentation

Today, there are two main types of instrument for color measurement: tristimulus colorimeters and spectrophotometers. In both cases, a light source is used to illuminate the measured sample using a specific illumination and viewing geometry. The reflected light is then measured, analyzed and the tristimulus color coordinates are calculated. It is the way the reflected light is analyzed that differentiates the two categories of measurement. For the tristimulus colorimeters, reflected light is analyzed using three filters to match the differential spectral response of the eye. On the contrary, spectrophotometers make accurate measurements at many points across the visible spectrum. Both types of instrument, tristimulus colorimeters and spectrophotometers, are usually used in hair color measurement applications (311,317,318).

Using tristimulus colorimeters can be sufficient for a simple quality control, where one needs to determine whether the color of the measured sample is within a specified tolerance of a standard test sample. For match-prediction work, measurement of color under different illuminants, detection of metamerism, absolute color measurement or evaluating color difference against numeric standards, a spectrophotometer must be used. It is the reason why the spectrophotometer has become nowadays the main instrument for measuring color and it is also true for hair color measurement. Typical specifications for a spectrophotometer can be found in various books (312). Illumination and viewing geometry is one of the most important conditions to specify when measuring color. The most used today is the D/8 geometry, where the illumination is a diffuse light, usually D65, from an integrating sphere, the viewing beam is measured at 8° from the normal to the measured sample. This geometry allows a color measurement where the specular (gloss) component of reflection can be either included or excluded (Fig. 63). However, in the case of hair, the specular excluded mode is not really useful because the angle between the hair surface and the angle of view is not exactly 8° due to the inclination of cuticle scales on the hair surface. It should be the reason why no significant difference is observed between specular included and specular excluded color hair measurement (319). In

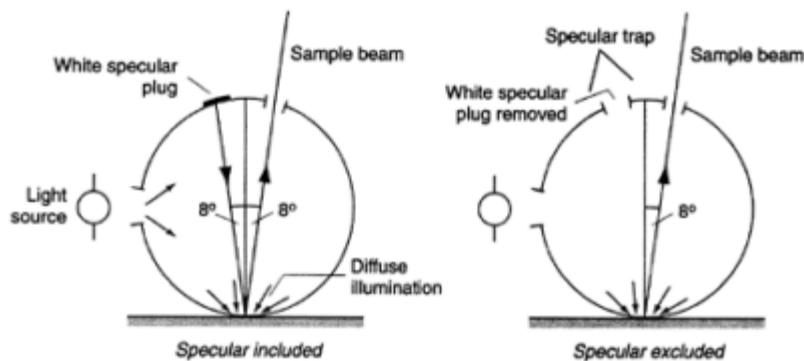


Figure 63 Illustration of D/8 specular included and specular excluded modes.
(From Ref. 312.)

some cases, color measurement under UV illumination can be done using spectrophotometers with UV filters allowing UV-included or UV-excluded measurements.

1.7. Measurement of Color Applied to Hair

Hair color measurement for laboratory application is usually carried out on hair swatches. The orientation and the alignment of individual hair fibers within the measured swatch and especially the difficulty to always reproduce the same configuration (orientation and alignment) are the main sources of variability when measuring hair color. Averaging a significant number of measurements on the same swatch is often necessary to get an accurate and reproducible hair color measurement. In addition, it is recommended to use the specular included measurement mode, as the specular excluded mode is not suited to hair as mentioned above.

1.8. Color Fastness Methods

Color measurement has been widely applied to the field of hair dyeing. Hair color evaluation is commonly used to select new dyes, to create new dyeing formulae, to reproduce an existing formula, to obtain a desirable color, or to study the impact of shampooing, weathering or other hair treatments (bleaching, weaving, conditioning, etc.) on hair color, whether artificial or natural.

1.8.1. Color Resistance to Washing

Resistance to washing is one of the fundamental characteristics of a hair dye. Temporary coloring must be removed by a single wash, semipermanent coloring must resist some six to 12 shampoos, and oxidation coloring is designed to be “permanent.”

The trial consists of evaluating the alterations of color after a given number of shampoos on hair swatches previously dyed with the product to be tested. The shampoos can be simulated using a special device, Ahiba Texomat (320) that reproduces in a standard manner the two actions of hair shampooing, viz. the friction and the foaming. Swatch color is evaluated before and after each trial and the color

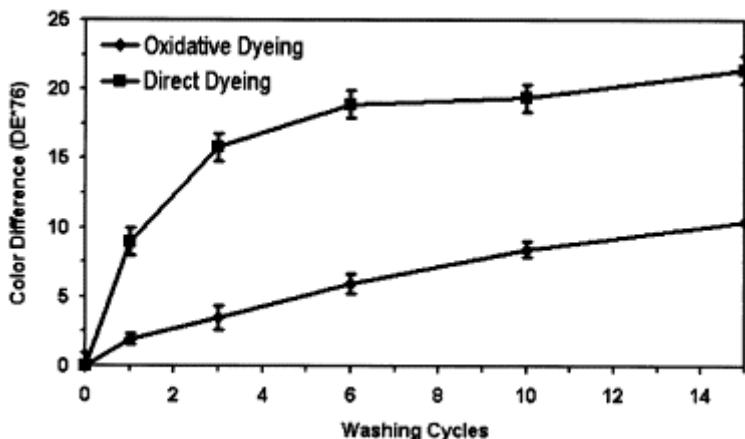


Figure 64 Color difference as a function of washing cycles in the Ahiba Texomat System; comparison of washing fading behavior of oxidation vs. semipermanent hair dyeing. (From Ref. 321.)

difference is then calculated using the CIE1976 color difference equation (DE^*_{76}). The greater the color difference, the more inadequate the tested product.

In Fig. 64, the evolution of hair color as a function of the number of shampoos is represented for two types of coloring, an oxidation coloring vs. a semipermanent coloring product. One can clearly see that the oxidation coloring is far more resistant than the semipermanent (321).

The ability of a specific shampoo formulation to protect hair color from alteration can also be tested. The assay can be carried out on similarly dyed hair swatches and the color difference is measured after a given number of washing cycles using different shampoos. The smaller the color difference, the most adequate the shampoo to keep hair color unaltered.

1.8.2. Fastness to Sunlight

This test is very similar to the wash testing with the only difference that the device for hair washing is replaced with a sunlight simulator. A sunlight simulator is a device that delivers radiation with a spectral distribution close to that of sunlight (in its visible and

UV ranges). The radiation power and the exposure time can be adjusted. Using special filters, the intensity of UV radiation can also be regulated and the assessment of light fastness can be completed by light/rain fastness by combining exposure to sun simulator light and repeated water swelling by automated spraying.

In Fig. 65, the evolution of hair color as a function of the received radiation dose is represented for two types of dyeing product, a black and a red shade. One can clearly note that the red shade is much less resistant to sunlight exposure than the black one (322).

1.8.3. Other Fastness Methods

In the same way, color resistance to some special hair treatments, such as permanent waving, or to other types of exposure such as sweat, especially in the case of

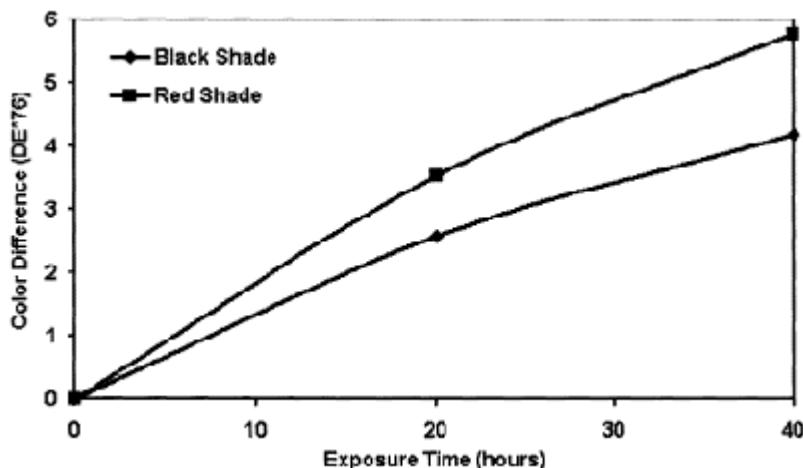


Figure 65: Color difference as a function of exposure time in a Xenotest Simulator at 45 Wm^{-2} of power; comparison of light fading behavior between two different shades.
(From Ref. 322.)

semipermanent dyeing, can also be performed. In the last case, artificial sweat is commonly used. Combined fastness tests involving simultaneous washing and light exposure could also be of interest. The presence of water during light exposure seems to increase significantly the extent of color degradation (323).

These tests are also applied to the evaluation of color protection provided by specific ingredients. Rinse-off or leave-on products are now developed in order to protect artificial hair color against washing and light exposure. Color measurement methods can, for example, be used to evaluate the effects of different surfactants (324).

It is sure that these tests will not exactly predict the real-life behaviour of hair dyes. For example, in the case of light fastness, it is impossible to simulate all the irradiation conditions that can exist worldwide (325). Continuous improvement of these tests and methods leads, however, to more and more accurate results that give the chemist a relatively precise idea of the performance of a given product.

2. IN VIVO ASSESSMENT: SENSORY ANALYSIS

The aim of these tests is to get an objective characterization of the products under development in real-life conditions, providing the formulator with the necessary data to achieve most adequate composition for targeted effects and users.

2.1. What Is Sensory Analysis?

Sensory analysis is defined as a whole group of methods that assess the characteristics of a product using the primary human senses:

- *feel* assesses hair softness, the wetting or sticky character of a hair spray when applied, etc.,
- *sight* appraises hair shine, volume, shade or highlights brought out by hair coloring, etc.,
- *smell* characterizes and rates the olfactory character of hair products, etc.,
- *hearing* gauges the sound of a hair mousse, the puffing of a spray, etc.

2.2. What Are the Principles of Sensory Analysis?

The sensory analytical methods developed lead to a *quantitative, objective description* of products based on evaluations made by *panels of trained experts*.

1. Sensory analysis can characterize a product in the course of various phases of its use, from release from its packaging to the end result on the hair. This characterization is achieved by means of a group of criteria designated as *attributes*. They are pertinent, discriminating, and have a precise definition.

Shampoos: important characteristics include the foam (onset, hold, unctuousness, softness, etc.), rinsing (ease, rapidity, etc.) the wet hair after rinsing (ease of untangling, overall appearance, etc.), shaping the dry hair (shine, volume, etc.).

Hair sprays and styling products: application is of importance (wetting effect, etc.), hair characteristics after application (shine, rigidity, hold, etc.), elimination, ease of untangling and the hair properties after disentangling (suppleness, dry feel, discipline, manageability, etc.).

Coloring products: the characteristics of the product applied are of interest (rapidity with which a homogenous mixture is obtained, consistency, ease with which hair becomes impregnated, soaking in, distribution through the hair, etc.), the wet and dry hair after rinsing (ease of untangling, suppleness, volume, etc.) as are the tinctorial results, brightness, etc.

2. *Evaluation* is performed by:

- either trained experts, e.g., hairdressers who directly evaluate the hair of volunteers,
- or individuals selected on the basis of their sensory acuity, who report their appraisal on their own hair.

In any event (individual or trained expert) the *judges* work under standardized conditions and have been trained in:

- the terminology (descriptors) and the associated modes of evaluation, so as to establish a common language and mode of evaluation within the panel.
- familiarization with an evaluation questionnaire that may consist of nonstructured continuous scales and/or scoring scales.

Importantly, this training is based on the use of reference products or benchmark products that are representative of the extreme limits of the intensity scales of the descriptors.

For example a shampoo that does not foam or foams poorly vs. a shampoo that foams copiously, a hair spray that does not wet or wets poorly vs. a hair spray that wets readily and so on.

In the case of each hair product, the greatest vigilance has to be maintained in keeping the evaluation framework, the benchmark products and the training of experts up to date, in relation to developments in the market, necessitating a permanent watch on competitors' products.

2.3. Examples of Reference Products

2.3.1. Abundance of Shampoo Foam

This is defined by the quantity and volume that it occupies. Products with opposing characteristics delineate the reference framework. It can be minimized to a lesser or greater degree depending upon the aimed objective.

2.3.2. Wetting by a Spray

This is assessed visually by the presence of droplets on the hair and tactiley by the wetness felt by the fingertips.

Training sessions complete the training process so that evaluators work under actual test conditions in a standardized environment that contributes toward diminishing the influence of external factors (products presented blind, homogenous rooms equipped with lighting that reproduces daylight, etc.). These training sessions also allow trial co-ordinators to determine the reliability of the panel. During the course of a year, it is worth checking that the panel of experts does not lose its acuity or mode of assessment and to proceed, if necessary, to renewed training.

2.4. What Might Be the Applications for Sensory Analysis in the Course of Hair Product Development?

2.4.1. *Sensory Analysis Is Mainly an Aid to the Process of Formulation*

Formulation scientists who develop new products on a daily basis need to test products on the human head to make progress in the developmental process. The aim of these tests is to provide an objective characterization of the formulations under real-life conditions, to confirm their suitability for the given purpose on various types of head of hair, to look at their flexibility or versatility, or otherwise reveal or identify potential limits or weaknesses. Based on collected observations, the formulator can adjust, fine-tune or modify the product composition, improve the quality/pleasure in use or extend the scope of use.

It may, for example, be of interest to evaluate one polymer compared with another in a shampoo, to study the impact of a new silicone on the cosmetic properties of a conditioner, to measure the repercussions, with a new hair dyeing product, on the tintorial and conditioning results when moving from a liquid to a gel form, to assess the influence of the type of actuator on the long-lasting hold capacity of a hair spray, to judge the innovative character of a new concept of formulation and so on.

These tests are carried out in the first place in the laboratory hairdressing salons where the products are applied to volunteers by hairdressing technicians.

The volunteers on whom these tests are performed present a wide variety of hair types. Firstly, therefore, the hair types corresponding to the target population have to be selected. Within the target group, there is always some variability. To achieve the clearest discrimination, applications are made to the half-head, comparing the test formulation to a reference formulation. The two are applied, randomly to the right or left side of the head, according to a protocol mirroring in-use conditions as closely as possible (amount of product, mode of application, waiting time, etc.).

In many cases, it may be important to monitor changes in certain characteristics over time. In a general way, it is also important to carry out duplicate or even triplicate applications combining different types of hair products, and particularly when the latter are marketed in the same product range.

The data obtained can be complemented by evaluations from a panel of trained users with the test products. These experts apply the product themselves according to their normal usage habits (quantity, procedure, etc.) and evaluate the product characteristics on their own hair. To compensate for inter-individual variability, some 30 volunteers participate in each study. An identity card of the product properties or *sensory profile* is established by taking the mean of the evaluations.

Using appropriate statistical methods (ACP, PLS, etc.) that map the results, the test products can be positioned relative to others and grouped in the “product universe,” so defining, with the positioning of products on the market, a state of the market and yield an image of the variability of the characteristics of the various ranges offered to consumers.

Sensory analysis is also a valuable tool for understanding, among the product characteristics, those that generate pleasurable reactions and satisfaction from the user such that they will encourage repurchase of the product. The objective data supplied by

the panel of experts can be compared with hedonic data gathered from users in response to questions such as: among these six hairdressing gels, which one do you prefer?

The correlation of the two sets of data can generate preference mapping, in which preferences related to user groups are explained by variables objectively characterizing the product.

2.5. Sensory Evaluation Among Consumers

In carrying out tests on hair products, two important elements must be taken into account (326–329):

- the limitations of each technique taken in isolation;
- the international dimension of the market.

The combined use of complementary test methods, such as those described below, carried out in several countries, is capable of:

- optimizing the pertinence of the data retrieved;
- taking into account cultural, climatic and physiologic diversity as well as the habits peculiar to the main geographic areas.

Such an international approach often requires adapting the method (questionnaire, scales, and protocol design) to the local cultures.

In the case of each of these methods, various protocols cover monadic tests (a single product) or comparative tests (comparison of several products). It is also possible to measure the interaction of products designed to be used together or successively (for example, shampoos and conditioners in the same product range).

2.6. Tests in Hair Salons

Products designed for professional use have to be evaluated in the context of a hairdressing salon.

A panel of salons is constituted. For each test, a sample is chosen from among this panel, the salons being selected depending not only on the type of information required, the type of product they habitually use, but also on the aptitude of their employees to evaluate the products presented to them. Judgment acuity and experience do indeed enter into the hairdresser selection process.

The researchers distribute the product. The hairdressers use it for a time span that varies in relation to the nature and frequency of use of the product, then they respond over several sessions to a questionnaire including open-ended and closed questions, in the form of face-to-face interviews with the researcher. Qualitative evaluations form a large part of the data thus retrieved.

2.7. Consumer Tests

2.7.1. *The Qualitative Approach*

The qualitative approach yields an initial perspective, it is exploratory in nature. The procedures are mainly centered on an in-depth study of the way thoughts and feelings are expressed and of the behavior of individuals.

Samples of 10–15 volunteers selected on the basis of their physical characteristics and their consumer habits are invited to apply the hair products in a bathroom either at home or in our facilities, in which they agree to be filmed. A detailed interview, conducted by a qualitative researcher, follows on immediately after usage of the product. Several successive applications may be involved, over the normal timespan of product usage.

Then a content analysis is carried out on the interviews and the videos showing each participant's mode of use. Such studies help, among other things, to focus attention on the usage properties of the product, on exploring the possibilities offered by new textures, on new approaches to formulation or new modes of application and in gaining a better understanding of users' behavior. However, quantitative testing must often validate these tests, which present the advantage of opening up new leads.

2.7.2. *The Quantitative Approach*

This approach aims at obtaining more substantial, reliable data using representative cohorts whose size is greater than 150 individuals. The product is blind-tested, only accompanied by instructions for use and a general name. Judgment is required of the product itself: that is why any interference with perception by the packaging or marketing communication is avoided.

A sample of individuals selected for their hair characteristics and their usage habits use a product for a given period, taking into account the normal usual pattern. The participants in the trial must be able to make several successive applications of the product. At the end of this period, they respond to a questionnaire including open-ended and closed questions.

The mode and the site of reception of the responses can vary. The interview can take place in the tester's home, his/her place of work or an office set up for this purpose. The questionnaire can be presented "off line": face-to-face or by telephone; the subject may also be requested to complete the questionnaire independently (self-administered questionnaires) on paper or "online" via Internet, a computer, etc. Collected data are then processed using specific software for statistical analysis.

2.7.3. *Mixed Techniques*

It can be worthwhile to gather, very early in the development of a product, the opinion of future users and compare it with that of the panel of experts. This is why techniques that combine various approaches (qualitative and sensory) have been drawn up.

A restricted sample of volunteers recruited on the basis of their hair characteristics and their consumer patterns of behavior is invited to use the product. An expert evaluates their hair, the volunteers apply the product, they complete a self-administrated

questionnaire including open-ended and closed questions, and then an expert again evaluates the condition of their hair.

These tests, whose response time is short, provide rapid preliminary information combining two points of view on usage by the public on products in the course of development.

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13

The Regulation of Cosmetic Products

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1. INTRODUCTION

Regulation is generally considered a boring matter and, from a static point of view, it is. But if you consider it from a dynamic perspective, it may be a real adventure. Striving toward international harmonization of regulations has been the main objective of the last decade and it will continue. The legislator has a heavy responsibility in this context, but industry and consumers share it. The first part of this chapter will be devoted to a review of the last four decades and the search for harmonization of cosmetic regulations.

The second part will not describe the main national regulations, as it would normally do, because they will be considered in an evolving context. Whereas the 1986 Edition of the present book could usefully describe the main features of many existing regulations in a relatively stable legislative environment, using a similar approach today would entail the risk of becoming obsolete before the ink was dry. Those interested in the national regulation texts are given some references or addresses of Internet sites in the Appendices at the end of the present chapter.

Thus, the second part of the chapter is devoted to four main points: the definition of cosmetic products, their placing on the market, their labeling and rules applying to their ingredients. Moreover, some attention is given to American OTC drugs and Japanese Quasi-Drugs.

As regulations usually do not deal separately with “cosmetic products” and “hair products,” except when some of the latter are specifically regulated, regulations covering cosmetics in general are presented.

To help the readers find their way among all those obscure acronyms which populate the regulatory literature, their meanings are given in the Appendices as well as some useful definitions.

The reader's attention is drawn to the fact that many other regulations can have an impact on cosmetic products, e.g., regulations relating to chemicals, the environment, biocides, aerosols, volatile organic compounds (VOCs), advertising, packaging, occupational protection, etc. They are not discussed here.

2. THE EVOLUTION OF COSMETIC REGULATIONS

During the 1960s, some 10 countries had laid down regulations governing cosmetic products. These had been adopted in each of the countries concerned in the interest of

public health to ensure the safety of users and to avert, case by case, any undesirable reactions observed during the use of products on the market. International trade exchanges being rare at the time, each country had its own products and consequently, its own regulations.

With the growth of national markets, more and more States became aware of the need to regulate cosmetics. They drew on either the most developed cosmetic regulations, the American and European, or on their own experience in other fields such as pharmaceuticals. The first system, specifically devised for cosmetic products, is what is named today an “in-market control system”: manufacturers and importers can freely introduce cosmetic products on the market under their own responsibility and authorities are in charge of controlling the products on the market. The second system, generally devised by pharmaceutical administrations and thus derived from drug regulations, is a “pre-market control system”: before introducing the product on the market, the manufacturer has to submit a file to the control authorities for each cosmetic product and to wait for an authorization. With all those new regulations, at the turn of the century, cosmetic products, and among them hair products, were subject to a 100 different sets of regulation throughout the world.

The situation has been changing recently, mainly under the influence of the World Trade Organization (WTO), set up in 1995 to follow up the General Agreements on Tariffs and Trade (GATT): this organization promotes the creation of free trade areas (FTAs) and in this context many States, particularly in Latin America and Asia, entered the process of harmonizing their cosmetic regulations. Before examining the current situation, attention will be given to the first attempt at harmonization of cosmetic regulations that began around 1960 in Europe. Thus, when considering the current evolution, it will be important to bear in mind the steps taken during this first harmonization process and to assess the results obtained, the time needed and the effort invested.

2.1. The Experience of European Harmonization

The harmonization of cosmetic regulations began in Europe in 1957 with the signing of the Treaty of Rome that established the European Economic Community (EEC). After the disruption of the Second World War, six countries (Belgium, France, Germany, Italy, Luxembourg, and The Netherlands) sought to guarantee a lasting peace in Europe based on a single market. The movement toward harmonization had been launched and, under its impetus, cosmetic regulation underwent rapid and continuing change.

In the constructive spirit of the time, the six Member States (MSs) thus undertook to remove the regulatory technical barriers to trade in order to promote the free circulation of goods throughout the territory of the Community. Cosmetic Industry was required by the EEC Commission to make a proposal for a Cosmetic Directive that was to be applied uniformly, after implementation in the national regulations of each of the Member States. In order to speak with one voice, in 1962, Industry set up a professional association based in Brussels and named COLIPA (“Comité de Liaison des Industries de la Parfumerie,” recently renamed “The European Cosmetic Toiletry and Perfumery Association”). COLIPA had five members at that time, i.e., the national associations of cosmetic

producers of the six MSs: France, Germany, Italy, the Netherlands, Belgium, and Luxembourg, the two last countries having the same association. The work could begin.

Fifteen years of laborious discussions between the European Commission, the MSs government representatives and Industry as well as interested parties such as consumers, were needed before the Cosmetics Directive 76/768 could finally be published in the EEC Official Journal, on September 27, 1976. The text, partially provisional, contained numerous imperfections but at least it existed. The Directive was imposed on the MSs with the requirement that it be implemented into their national regulations within a fixed time. In the meantime, Industry had to comply with the national regulations.

It may be useful to know how the European Commission and the MSs overcame certain "insuperable" disagreements because such conflicts are bound to happen in all harmonization processes. A first approach was the mutual recognition of national regulations which did not require the modification of existing regulations. In the case of mutual recognition, a product complying with the regulation of the country of origin is accepted for marketing in the destination country by the authorities and vice versa. For example, the mutual recognition agreement was applied when the authorities accepted the various systems of denaturation of ethyl alcohol enforced in the different MSs.

The second approach excluded from the harmonized regulation certain contentious points with national requirements being maintained until a consensus was eventually found. A solution of this type was adopted for the regulation of certain ingredients. It was thus decided to list a dozen ingredients—or families of ingredients—in Annex V whereby they were "excluded from the scope of the Cosmetics Directive" so that the existing national requirements could be maintained in force in the MSs. Over the years, harmonized decisions were reached for all listed ingredients and Annex V no longer contains any single substance of interest to the cosmetic industry. Annex V is therefore likely to be removed one day.

In the early years following its publication, the Cosmetics Directive was implemented in a somewhat confused way by most of the MSs. Some of them delayed implementation or applied it in an erroneous manner, others refused to implement certain requirements because they considered their national system preferable and did not give up the hope of having it adopted at the Community level. It was, for example, the case in France which had to face a tragedy in 1972 when 32 babies died after application of a talcum powder containing a far too high concentration of hexachlorophene as a result of a confusion between two ingredients during the manufacturing process. In applying the EEC *status quo* rule, a MS cannot adopt a national regulation in a field where a Directive is under discussion. But at that time the public concern was so great that the Community let the French Authorities enact the most stringent cosmetic regulation ever. Published in 1975, one year before the EEC Cosmetics Directive, the "Loi VEIL" contained, among others, a series of obligations including the "product dossier," the professional qualification of the manufacturer, etc. Some of these requirements were eventually adopted at the Community level with the Sixth Amendment of the Cosmetics Directive in 1993.

The prevailing confusion was increased further by the continuing enlargement of the European Community, that became the European Union (E.U.) after the Maastricht Treaty signed in 1992, extending successively from six to nine then 10, 12, 15 and today 25 Member States (cf. Appendices). The annual updates of the Cosmetics Directive, while eminently desirable to keep up with technical progress, added to the muddle owing

to their uncertain implementation by the MSs. Manufacturers and importers had to be alert to the publication in each country of successive implementations that sometimes modified each other.

Considering that such a confused regulatory situation existed not only in the cosmetic field but also in numerous other fields, the Commission, the administrative body of the E.U., charged with overseeing the correct application of European Directives in the Community, proposed "the Single European Act." By this Act, adopted in 1986, the MSs took the decision to put the Common Market into full practice from January 1993. Moreover, they provided the Commission with more power to carry out its surveillance more efficiently.

As far as cosmetics were concerned, the Commission undertook a complete revision of the Directive. The "Sixth Amendment" (Directive 93/35 published on June 23, 1993), introduced numerous new requirements intended to satisfy all the MSs. This included Full Ingredient Labeling, based on the U.S. model, the Inventory of cosmetic ingredients, "product information" to be held readily accessible to the Competent Authorities, the prohibition of animal testing for the evaluation of the safety of cosmetic products and of ingredients intended exclusively for cosmetic purpose, etc. After the enforcement of the Sixth Amendment, the Cosmetics Directive has been at last rigorously applied in the E.U., more than 30 years after discussions had started.

The structural changes that accompanied the setting up of the European Union in 1993 have had a direct impact on the evolution of the Cosmetic Directive: The European Parliament, from now on elected by universal suffrage, became co-decider with the European Council, instead of being consulted only. This considerably enhanced the pressures from various lobbies and decisions are now often taken more on political than technical criteria, with sometimes little consideration given to practical feasibility. It is in this context that the Cosmetic Directive has again undergone a fundamental transformation with the publication of the "Seventh Amendment" (Directive 2003/15) on March 11, 2003. Among numerous other requirements, this Amendment broadens the full ingredient labeling to 26 perfume ingredients that may cause allergenic reactions, and requires the indication of the period of time after opening for which the product can be used without any harm to the consumer, on the packaging of cosmetics with a minimum durability of *more than 30 months* (sic!). In addition, part of "product information" must be made available to the public. Moreover, the Seventh Amendment confirms the progressive ban on the marketing of cosmetic products when themselves or their ingredients have been the subject of animal testing "in order to meet the requirement of this Directive;" it also bans the performance of such an animal testing on the E.U. territory, both measures being subject to specific deadlines and complex conditions. Discussions are ongoing to determine solutions to these obligations in practice and to assess their international consequences.

By way of conclusion to this first experience of harmonization of the laws relating to cosmetic products in several countries, it seems useful to emphasize not only the obvious positive aspects, but also certain significant drawbacks which may endanger the whole regulatory harmonization process in the future. On the positive side, a single regulation applies today in 15 and soon 25 E.U. MSs, clearly facilitating trade exchanges within the largest single market all over the world. What is more, due to its frequent updating, the Directive strives to keep up with technical progress and to take into account the evolution

of scientific knowledge. High-quality products, taking advantage of more recent discoveries, are rapidly and simultaneously made available to a very large number of consumers by a responsible industry, under the control of competent authorities which cooperate to control the market in order to safeguard public health.

However, one should be cautious in respect of a system in which all questions, whether raised on scientific grounds or resulting from political lobbying or media interest, often give rise to new regulations. In the E.U., cosmetic products, which the authorities recognize as not posing a real threat to public health, have nonetheless been the subject of 45 specific Directives since 1976 including the basic Cosmetics Directive, seven Directives for Amendments, 32 Directives for Adaptation, seven Directives on official Analytical Methods, and a few other general texts. And if the national implementations of those directives are to be considered—and they must be—it means that industry has had to adapt their products to more than 600 new regulatory texts in 30 years. This proliferation as well as the increasing complexity of the harmonized texts has reached previously unheard-of heights. Yet it does not take into account either the many directives relating to the adjacent fields which may have indirect consequences on cosmetic products (aerosols directive 75/324/EEC, biocides directive 98/8/EC, color additives directive 95/45/EC, dangerous substances directive 67/648/EEC presently under fundamental rediscussion, packaging waste directive 94/62/EC, pharmaceutical products directive 2001/83/EC, medical devices directive 93/42/EEC, general product safety directive 2001/95/CE, liability for defective products directive 85/374/EEC, etc.) or the Guidelines and numerous opinions stated by the Scientific Committee on Cosmetic and Non-Food Products (SCCNFP), which will be discussed further in this chapter under the section on Ingredients.

Aware of this problem, the Commission adopted in 1996 a program intended to develop a more reliable and user-friendly Community legislation in order to improve the regulatory environment in which business operates. Named the SLIM operation (Simpler Legislation for the Internal Market), it concerns more than 20 policy sectors including the cosmetic products field whose regulation has been reviewed during the first phase of the program. The review team published a first Report in 2002 which plans the codification of the Cosmetic Directive, but the SLIM exercise will need some further work to take into consideration the Seventh Amendment which introduces major modifications to the Cosmetics Directive. Among the ideas expressed by the SLIM team, the suggestion to use Regulations instead of Directives for all future modifications of a technical nature has the advantage that a Regulation is enforced directly in all MSs without needing further implementation. Whatever the result of the SLIM review, the complexity of the E.U. Cosmetics Directive has become so great that it cannot be proposed as an international model anymore. It is the reason why the Florence Principles, which will be described later, have been established.

Since 1985, biennial "Mutual Understanding" meetings have been organized by the Industry where the three main regulatory frameworks were in force: E.U., United States, and Japan. The objective was to attempt to harmonize the three corresponding cosmetic regulations by regular contact with the authorities. While mutual understanding has been strengthened by these contacts, they have had nearly no effect on regulations in E.U. and the United States. In the first case because the authorities are overwhelmed by the efforts at harmonization within the Community, in the second due to a lack of political impetus

which we know is essential to progress regulatory harmonization. After a few years, the term “harmonization” was dropped in favor of “alignment,” the administrations only being prepared to resolve differences that did not require changes to legislation.

2.2. Japanese Cosmetic Deregulation

The surprise came from Japan which had the most obtuse cosmetic regulation: introducing a product on the market required a long and costly registration process, not just for the product but for all its ingredients that had not yet been approved in the type of cosmetic product envisaged. In the absence of known official lists of allowed ingredients, the authorities had to be consulted case by case to discover whether an ingredient had already been approved and thus had a good chance of being accepted. The difficulties were so great that the American Cosmetic, Toiletry and Fragrance Association (CTFA) ended up establishing a “Gray List” of substances having already been approved in certain cosmetic products in Japan. This unofficial list had been drawn up on the basis of confidential information supplied by CTFA members.

The situation began to improve from 1986 with yearly publications of the standards of approved cosmetic ingredients. Then, the authorities adopted an automatic approval system for already accepted ingredients named the Comprehensive Licensing Standards of Cosmetics by Category (CLS). This system was initially based on 35 exhaustive lists of ingredients, each being allowed in one category of cosmetic products. A product complying with the corresponding CLS had only to be notified. Some years later, the CLS system was simplified again by reducing the number of cosmetic products categories to 24 and ultimately 11.

In 2001, within the framework of WTO negotiations, the Japanese authorities proceeded to what was named the “Deregulation” of cosmetic products. Since then, legislation has been based on an in-market control system, better adapted to the fast evolving cosmetic products market: the manufacturers can freely put their products on the market, under their own responsibility, unless they contain ingredients subject to approval and not included in the three positive lists. The new law imposes new obligations on Industry, in particular Full Ingredient Labeling and “product information” to be held at the disposal of the authorities. It should, however, be noted that Cosmetic Deregulation does not apply to product categories which are considered as quasi-drugs in Japan in particular antidandruff products, permanent waves, and oxidation hair coloring products.

2.3. Harmonization in Free Trade Areas and the Florence Principles

By 1995, the industry realized that it would be better to aim its efforts at harmonization of those regulations in the process of change, especially those in countries taking part in Free Trade Areas (FTA). FTAs have been flourishing all around the world under the WTO influence and, like the E.U., their members have to rid themselves of regulatory trade barriers and harmonize, including among other sectors, the cosmetic field. Thus, the Mutual Understanding Event which took place in Florence in 1998 was open for the first time to all the regulatory authorities interested in cosmetic regulation.

To prepare for Florence meeting, COLIPA and the CTFA set up a working group to examine the advantages and disadvantages of the existing cosmetic regulatory systems and to propose principles on which to base a cosmetic regulation likely to both guarantee public health and to meet the needs of the authorities, health workers, consumers, and Industry. These principles were presented to the authorities from 35 countries, under the name of Florence Principles. They were generally well received and still serve as a reference in discussions on the development or harmonization of cosmetic regulations.

2.3.1. The Florence Principles

CTFA & COLIPA: Framework of principles for cosmetic regulation harmonization—1998

- *Industry is responsible for the safety of its products on the market,*
- *Authorities are responsible for the control of the market (in-market control),*
- *One universal definition of cosmetic products based on that of E.U. Directive, with six areas of application, six functions, and an illustrative list of cosmetic products,*
- *One formula world-wide, ingredients being regulated, if necessary, on a case by case basis, after a scientific assessment of the risks. Positive lists not supported.*
- *Labeling providing adequate information to all interested people (consumers, regulatory bodies, health personnel, etc.). If full ingredient labeling is required, INCI names should be used.*

Two years later, more than 50 countries participated in the Malta Mutual Understanding Event and some presented an assessment of their progress in the regulation of cosmetic products. The progress toward harmonization in the Andean Community was particularly noteworthy, with the setting up of a system of mutual recognition for the registration of cosmetic products. According to the Andean decision, the registration of a cosmetic product made in the country of manufacture or of first importation was recognized by the other MSs of the Andean Community. Originally limited to cosmetics produced in the Andean countries, the mutual recognition is now also applied to imported products according to the Decision no. 516 published on March 14, 2002. The submission of a file is always required, but only in the first country of production or importation of the Community.

At that time, the four MERCOSUR and the 10 ASEAN countries were on the brink of turning their *pre-market approval system* into an *in-market control system*. This change was supported by the presentation made during the Event by the Argentinean Authorities: they presented the results of an experiment which had been conducted during a 16-month period in their country. It consisted of a comparison of both systems, applied in parallel: the in-market control of cosmetic products was found to be far more effective than the pre-market control of dossiers. Their conclusions were that the pre-market approval system was “efficient...on paper only!” and that it did not help to prevent the presence of unapproved, illegal, or even dangerous products on the market.

Industry which shared this point of view added another reason: a pre-market control system was not adapted to cosmetic products principally because of the long delays needed to obtain an approval—frequently more than 1 year at that time—which hindered and is detrimental to an industry subject to the pressure of fashion.

2.4. Other Cosmetic Regulations

We cannot end to this overview of changing cosmetic regulations without considering briefly the 100 or so many countries which are also in the process of implementing, modernizing, developing, modifying, and increasing their cosmetic regulation. In fact today, all countries with a developed cosmetic market feel the need to regulate these products and the great majority of them modify their regulations frequently to keep up with product and legislative evolution.

Among those countries, special reference should be made to those where the regulatory situation is the most difficult, e.g., China where the Industry has to report to several administrations applying divergent rules, South Korea where importers and local industry are treated in different ways.

In the majority of countries, progress is being made toward more modern regulation to protect public health without unnecessary paperwork and delays, letting consumers benefit quickly from advances in science and technology. At the same time, countries have started to explore another way to control cosmetic products through the development of an international standard.

2.5. ISO Standardization

In July 2000, the first steps in the development of an ISO standard relative to cosmetic products were undertaken. The countries involved were mainly those without any specific legislation in the field. Today, some 40 countries have declared an interest in a cosmetic standard and among them, 15 participate actively in the discussions and testing. The items presently under discussion are the following:

- microbiology guidelines,
- labeling,
- good manufacturing practices (GMP),
- preservatives,
- nitrosamines,
- terminology.

New items can be suggested at each annual meeting of the Technical Committee (TC 217).

ISO standards should be updated every 3 years and all interested parties can participate in the discussions: Industry, authorities, consumers, academics, etc., 140 countries are ISO members and thus potentially interested in these standards.

Countries which do not have any cosmetic regulation will have the possibility to use the standard for the control of cosmetic products. The countries which already have cosmetic regulation can ignore the standard, given that legislation always takes precedence over a standard. The last solution is to integrate the standard, or parts of it, into the national regulatory framework so that it becomes mandatory.

One can easily understand that great attention should be paid to ISO discussions in order to ensure the greatest possible compatibility of the standard with the existing regulations. It would be worrying to see new barriers to trade set up in a field already overloaded with regulatory problems.

3. MAIN RULES CONCERNING HAIR PRODUCTS

3.1. Definition of Cosmetic Products

It is not easy to give an overall definition of a cosmetic product in the disparate and ever-changing regulatory environment which has been described above. Even though discussions have been ongoing in recent years with a view to harmonizing the regulations in a large number of countries, the rules relating to the definition of cosmetics have taken diverging routes in one region from another. All countries or regions have, however, taken as a model one of the three main sets of regulation: that of the European Union considering two types of products (cosmetics and drugs) and that of the United States and Japan with a third, intermediate type of products between cosmetics and drugs, named OTC drugs (Over The Counter drugs, i.e., drugs sold without prescription) in the United States and Quasi-drugs in Japan. Note that those intermediate categories have nothing in common as they cover different kinds of product, subject to different rules. These three main definitions will be examined in detail and their respective areas of influence described so as to reach an overall view of the present international situation.

3.1.1. European Union

The Cosmetics Directive 67/768 defines the cosmetic products in a fairly precise way. In its initial “Whereas...” preamble, it clearly delimits the field of cosmetics from that of pharmaceuticals, exclusively aimed at the cure, mitigation and prevention of diseases, and especially those intended to be ingested, inhaled, injected or implanted in the human body. Article 1 then provides a definition based on six areas of application and six purposes of use: if a product is presented as being intended to be placed in contact with one of these areas, to exert one of these purposes, it shall be considered as a cosmetic product. Article 2 states that cosmetic products “must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use.” The cosmetic product may not therefore present a risk to the consumer, whatever the benefits provided, unlike drugs for which a risk/benefit ratio can be taken into consideration. To be more precise, Annex I supplies an illustrative (i.e. nonexhaustive) list of products to be considered as cosmetics in all the E.U. Member States.

Directive 76/768—Article 1.1

A “cosmetic product” shall mean any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition.

Directive 76/768—Annex I Illustrative list by category of cosmetic products

- *Creams, emulsions, lotions, gels and oils for the skin (hands, face, feet, etc.).*
- *Face masks (with the exception of chemical peeling products).*
- *Anti-wrinkle products.*
- *Tinted bases (liquids, pastes, powders).*
- *Make up powders, after-bath powders, hygienic powders, etc.*
- *Toilet soaps, deodorant soaps, etc.*
- *Perfumes, toilet waters and eau de Cologne.*
- *Bath and shower preparations (salts, foams, oils, gels, etc.).*
- *Depilatories.*
- *Deodorants and anti-perspirants.*
- *Hair care products:*
 - hair tints and bleaches,*
 - products for waving, straightening, and fixing,*
 - setting products,*
 - cleansing products (lotions, powders, shampoos),*
 - conditioning products (lotions, creams, oils),*
 - hairdressing products (lotions, lacquers, brilliantines).*
- *Shaving products (creams, foams, lotions, etc.).*
- *Products for making up and removing make-up from the face and eyes.*
- *Products intended for application to the lips.*
- *Products for care of the teeth and the mouth.*
- *Products for nail care and make-up.*
- *Products for external intimate hygiene.*
- *Sunbathing products.*
- *Products for tanning without sun.*
- *Skin whitening products.*

Despite this illustrative list of products falling within cosmetic definition, an inevitable degree of uncertainty surrounds some products verging on the frontier between cosmetic and drug—the so-named borderline products—on which the Member States must decide case by case if they are cosmetics or drugs. An example is hair products that claim to induce hair growth or regrowth or treat certain scalp disorders (seborrhea, etc.). MSs make their decision on the status of a product taking account not only of the composition of the product but also its presentation, labeling, advertising, and the state of scientific knowledge. These elements do not avoid interpretations that can differ from one country to another, given local customs, language differences, and national sensitivity.

The European definition in Directive 76/768, thanks to its relative simplicity, its relevance to the functions normally fulfilled by cosmetics and its precision, has been adopted as the model recommended in the Florence Principles described above. It has become valid in some 50 countries in Africa, Latin America and Asia and notably those countries that have already taken steps toward the harmonization of their cosmetics legislation (cf. Appendices):

- the 15 Member States in the European Union,
- the 10 States in the process of joining the European Union,

- the four Member States of Mercosur,
- the five Member States of the Andean Community,
- the 10 Member States of ASEAN. Those countries have introduced this harmonized definition in their draft Cosmetics Directive which should be implemented into national laws in the coming years during which current legislations remain in force.

Other countries have also adopted the European definition, among whom are Algeria, Egypt, Morocco, Saudi Arabia, and Switzerland with the probable addition of Russia in the near future.

Finally, the Central American Common Market (CACM), should be mentioned as having recently undertaken discussions with a view to harmonizing their national cosmetics legislation and envisaging adoption of the European definition so making it applicable throughout the whole South and Central America.

3.1.2. United States

Hair products considered to be cosmetics in Europe are generally also classified as such in the United States. However, those that exert a “physiological” action on the human body enter into the class of so-called OTC drugs, i.e., drugs that may be freely purchased without a medical prescription.

Cosmetic products are defined in the Federal Food, Drug and Cosmetic Act of 1938—Section 201 (321) (i) as follows:

The term “cosmetic” covers Articles to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance, and the ingredients entering into their composition,

while the OTC drugs are defined in the same F.D.C. Act, Section 201 (321)(g)(1) under:

The term “drug” covers

Articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man and animals

Articles (other than food) intended to affect the structure or any function of the body of man or other animal and the ingredients entering into their composition.

The rules applied to these two product categories are very different: cosmetics remain under the responsibility of the Industry which retains the initiative to a large extent, while OTC products must be formulated, tested, manufactured and labeled according to precise rules laid down in Monographs specific to each product type. These Monographs have been laboriously drawn up over more than 30 years and are published in the Federal Register with a view to their inclusion in the Code of Federal Regulations Title 21.

Two of these OTC Monographs specifically concern hair products:

- Dandruff/seborrhea/psoriasis products—CFR 21 part 358. Final monograph dated December 4, 1991 updated January 28, 1994
- Hair growth/hair loss products—CFR 21 part 310. Final monograph dated July 7, 1989

These reference documents specify the composition and, in particular, the active substances approved; if any...because no active ingredients have been approved yet for hair growth activity. The conditions of use are also specified in the Monographs as well as the warnings to be included on the packaging, the approved claims and the methods to be used to prove the efficacy.

Furthermore, all OTC products with a Monograph in application must follow extremely strict packaging and labeling requirements described in a general Monograph on OTC labeling Requirements published in 1999.

Canada has enacted an OTC regulation based on the American system.

3.1.3. Japan

As in the case of the United States, Japan distinguishes two categories of products among those considered to be cosmetics in the European Union: cosmetic products and quasi-drug products that are intermediate between cosmetics and drugs. But the analogy with the American OTC stops there, because the quasi-drugs do not include the same products as those classified OTC in the United States (cf. Table 1) and the requirements concerning both categories are different too.

The difference between a cosmetic and a quasi-drug product is based on the intensity of the action exerted on the human body, a very subjective notion and hence tricky to handle.

Table 1 Comparison of the Status of Some Hair Products: E.U./United States/Japan

Product	E.U.	United States	Japan
Antidandruff	Cosmetic	OTC	Quasi-drug
Depilatory	Cosmetic	Cosmetic	Quasi-drug
Permanent wave	Cosmetic	Cosmetic	Quasi-drug
Oxidation coloring	Cosmetic	Cosmetic	Quasi-drug

Here is a translation of the Japanese official definition of cosmetic products:

The term “cosmetic” signifies any article intended to be used on the human body by mean of rubbing, spraying or by other similar application for cleaning, beautifying, promoting attractiveness or altering the appearance of the human body and for keeping the skin and hair in good condition, provided that the action of the article on the human body remains mild.

Cosmetic products are divided into 11 categories, one of them corresponding to hair products, i.e., products designed for use on the hair and scalp, in particular shampoos, setting lotions, hairstyling products, and temporary coloring products. The latter can be formulated with coal tar cosmetic colorants, i.e., synthetic organic dyestuffs, listed in the MHW Regulation no. 30 (1966), no. 3 (1967) and no. 57 (1972). These products must be presented as cosmetics and must not contain active ingredients that cause them to fall within the quasi-drug category. It is useful to compare the above definition of cosmetics with that of the quasi-drugs in which a large number of hair products is found:

Quasi-Drugs are products designed to exert a moderate action on the human body or products that have the following purpose:

- 1—prevention of foul breath or body odor,
- 2—prevention of prickly heat sore or the like,
- 3—prevention of hair loss or hair restoration or depilation,
- 4—prevention of harmful insects.

They may also be products designed to avert acne, roughness, chapping or itching or to disinfect the skin or oral cavity. They are divided into 13 categories that include the following types of hair product:

- 4—hair growth products,
- 5—depilatories,
- 6—oxidative hair dyeing products
- 8—medicated cosmetics (antidandruff shampoos and rinses, disinfectants),
- 9—permanent wave products,
- 10—insect repellents.

South Korea and Taiwan are among the countries that took inspiration from the Japanese quasi-drug regulations. China, which is in the process of drawing up a cosmetic regulation framework seems also interested in creating a third category between cosmetics and drugs.

3.1.4. Status Comparison of Certain Hair Products: E.U. vs. United States vs. Japan

Table 1 compares the status of hair products which are considered as cosmetics in E.U. and OTC drugs or quasi-drugs in the United States and Japan. It is important to note that the presence of a drug-type ingredient or an overly pharmaceutical presentation is capable of tipping any cosmetic products into drug, OTC or quasi-drug category.

The differences between the definitions in the three main sets of regulation can be explained by historical reasons and there is little hope of a world-wide definition of cosmetics ever being established, even though the European model tends to take precedence in countries keen to modernize their legislation. In fact, these definitions and their interpretations are enshrined in local habits and may express a reluctance to change or a different perception of things which, by its very nature, is not amenable to harmonization.

3.1.5. Borderline Products

A borderline product is a product whose function, presentation, claims, and/or composition is at the border between different types of product, principally between cosmetic and pharmaceutical products. Authorities have to decide to which category a product belongs on a case by case basis, taking into account, among other criteria, local customs and sensitivity, language, etc. These criteria cannot be generalized thus no general rule can be established to solve the matter.

Nevertheless, two documents can help to define a more precise border to the cosmetic field: the Annex I of the E.U. Cosmetics Directive provides an illustrative list of products considered as cosmetic in the 50 countries or so using the Directive definition.

The second document entitled “Comparative Study on Borderline Products and Borderline situations in various European Countries” was published by the Council of Europe as a starting point to converge toward a more common view on borderline products. The experts emphasize the fact that a cosmetic product can have a main function but also one or several secondary functions at the border with other fields including pharmaceuticals, biocides, medicinal devices, food additives or general consumer products. The classification of a particular product will depend on its function, presentation, claims, mode of application and composition. The study provides a set of criteria to help the evaluation of borderline products and situations.

As we have already seen, those countries such as the United States and Japan which have a third category of products between cosmetics and drugs differ as to the types of product they put in it and as to how they regulate them. This lack of coherence suggests that a three-category system is not able to provide a global solution to the problem of borderline situations and, even if it may allow stronger claims, it creates two confused and confusing borderlines instead of one.

3.2. Placing Hair Products on the Market

As previously seen, there are roughly two types of regulation governing the introduction of a cosmetic product onto the market: *the pre-market product approval system*, based on pharmaceutical regulations, and the *in-market control system*. Those two systems will be described successively, beginning with the in-market control system which is part of the Florence Principles, already in application in the three main regulations above described (E.U., United States and Japan) and now being adopted by an increasing number of countries.

For effective in-market control of cosmetic products, the authorities need to identify which companies manufacture or import cosmetic products in their country and under what conditions. For this purpose, they put in place a variety of administrative procedures: declaration by the responsible company of the manufacturing premises, notification of the types of cosmetics produced or imported, distribution licenses, free sales certificates, etc.

As the company is responsible for its products, it can introduce them freely on the market, without prior approval by health authorities as long as the product presents no risk to be harmful to consumer health. Product safety has thus to be carefully checked before marketing. All the data collected for the safety assessment should be kept in file, regularly updated, available to control authorities for inspection in the company premises.

In the event of a user complaint or for the purposes of an enquiry into the safety of certain product categories, the control authorities can request access to and review the information they need about the marketed product.

From its side, the pre-market approval system is still in application in numerous countries, especially in those countries where cosmetic products and drugs are under the control of the same administrative body. In this case, the placing of a cosmetic product on to the market requires prior approval. To obtain it, the manufacturer must submit an application file for examination by the authorities and wait for their approval before launching the product. The type of data, extent, the delay necessary for the examination, the additional requirements, etc., all vary depending upon the country concerned. Moreover, the registration number is often requested on the label which results in other complications and delay. One can easily imagine that launching a product simultaneously on an international basis can be a true nightmare in these countries.

The information usually required, whether submitted to the authorities in the context of a registration procedure, or to be available at the company in the in-market control system includes:

- composition of the product (qualitative, quantitative or frame formula)
- physicochemical and microbiological specifications of the finished product
- physicochemical and microbiological specifications of the ingredients
- safety certificate and, sometimes, toxicological test reports
- free sale certificate proving that the product complies with the legislation in the country of manufacture
- analytical methods for the control of certain ingredients
- proof of the effects claimed
- stability test results
- Good Manufacturing Practices certificate
- method of manufacture
- samples, packaging, inserts

Apart from this procedure, many countries require that health services in charge of emergencies, generally poison control centers, be sent a description of the product or even product formula, so that they can operate rapidly and efficiently in the event of a misuse of the product, the most common example being product ingestion by children. As the formulae of cosmetic products are often complex, ever changing and confidential, the work of these centers would have been difficult if some professional associations (CTFA, CTPA, etc.) had not provided short and adequate information in drawing up data sheets by product type. More recently, the European association of manufacturers (COLIPA) has worked with the European Association of Poison Control Centers and Clinical Toxicologists (EAPCCT) to draw up 112 "frame formulae" of which 29 correspond to hair products. The company has to declare the frame formula corresponding to its product, adding specific elements if necessary.

3.2.1. European Union

In E.U. Member States, cosmetic products can be placed on the market without prior approval, under the responsibility of the manufacturer or the importer who is required to determine beforehand that the product is safe under normal or reasonably foreseeable conditions of use.

The company concerned must notify the competent authority of the address of the place of manufacture or of initial importation into the Union and, in certain countries, of the types of product to be put on the market.

For monitoring purposes, the responsible company must keep readily accessible to the competent authorities a set of data, constantly updated, on each product at an address specified on the product label. The data must include safety assessment by a qualified person, proof of the claimed effect(s), data on reported undesirable effects and data from animal testing which is on the verge of being banned in E.U. for cosmetic products and their ingredients.

Through its association, COLIPA, the Industry has published guidelines to help in product safety assessment, in the performance of certain toxicological tests without using laboratory animals and in the evaluation of product efficacy. It has also provided for recommendations on the management of microbiological quality, good cosmetic manufacturing practices, etc. (cf. Appendices).

3.2.2. The United States

Hair products can be either cosmetics or OTC products, or even both, depending on composition, claims, presentation, etc. Hair cosmetic products, which are the most common category, will be discussed first, followed by a brief look at the few hair OTC product categories.

A cosmetic product can be introduced on to the American market without prior approval, under the responsibility of the manufacturer or the importer, who is nevertheless encouraged to participate in the Voluntary Cosmetic Registration Program drawn up by the professional association, CTFA, in 1972 and published in the Code of Federal Regulations (CFR)—Title 21—Part 710, etc.; this program consists of the registration to the Food and Drug Administration in charge of cosmetic products of the manufacturing sites, the ingredients used and product composition. The fourth and last part of the program which concerned declaration of product experience on the market was abandoned in 1997 because the FDA could not afford to process the information gathered.

As far as the manufacture of cosmetic products is concerned, the FDA is in charge of site inspection and its Good Manufacturing Practice Guidelines are available on their Internet site as part of the FDA Cosmetic Handbook. On its part, the CTFA published its own Manual on the subject as well as many other recommendations in the legal and technical fields.

The Company placing a product on the market is responsible for verifying its safety under normal conditions of use by all the means at its disposal. If the company fails to meet this requirement, for example, if it uses an ingredient whose dossier has been classified “insufficient data” by the Cosmetic Ingredient Review experts, the product

labeling should include the phrase "Warning—The safety of this product has not been substantiated" (CFR—21—740).

Unlike cosmetics, OTC drugs (e.g., antidandruff products, products for hair growth and/or hair loss prevention, etc.) can only be marketed if they meet the general rules applying to OTC drugs (labeling, presentation, manufacture, etc.) and, in addition, rules laid down in the specific Monograph of the corresponding product category (CFR—21—Part 700 et seq.) Production must take place at a site approved for the manufacture of drugs and regularly inspected by the FDA. It must be carried out under pharmaceutical GMP. However, it is possible to manufacture OTC products destined for the American market abroad, as long as FDA approval of the manufacturing premises has been obtained.

Among the Monographs relative to hair products that have been published, that one concerning antidandruff products (21 CFR—Part 310 and 358) lays down precise rules on composition (positive list of active ingredients) and on specific labeling, including approved claims.

Obtaining the approval for a new OTC active ingredient in the United States has been a quasi-impossible challenge for it required that the substance has been present for several years on the American market. Hence, the only procedure has consisted in filing a New Drug Application as if the submitted ingredient was a new therapeutic agent, a procedure out of reach of most manufacturers of cosmetics. In 2002, the FDA established a new procedure called "Time and Extent Application" whereby the product may be accepted if data are produced that prove it has been marketed in five different countries and for at least 5 years in one of them. Obviously, an exhaustive safety dossier should be submitted (OTC active ingredients. Time and Extent Application—Federal Register—January 23, 2002—June 11, 2003).

3.2.3. Japan

Before introducing a hair product on the Japanese market, the first step is to decide whether this product will be a cosmetic or a quasi-drug product. Depending on the decision taken, the requirements will be very different. We will consider the situation of cosmetic products first and then the regulation of quasi-drug products.

Concerning the regulatory framework relative to cosmetic products, it was extensively amended in 2001 and a degree of "deregulation" was instituted. The new system has reinforced the responsibility of the Industry, previously protected by the approval granted by the authorities to every single cosmetic product and ingredient.

From now on, while it is still necessary to get a professional license before manufacturing and marketing a cosmetic product, it is no longer necessary to register each cosmetic and its ingredients, prior to placing it on the market. In return, as in the European Union, the responsible company (manufacturer, importer or distributor) must hold at the disposal of the competent authorities a set of data proving the safety of the product. The professional association JCIA (Japanese Cosmetic Industry Association) has published various technical Guidelines to help the Industry to gather the data that must, of course, be constantly updated.

Quasi-drugs are a category of products which contains, among many others, antidandruff products, permanent waves and, oxidative hair coloring products. Their

manufacture and importation is subject to a pre-market registration process and the company must have a professional license, issued after inspection of manufacturing sites, ingredient storage areas and Quality Control (QC) laboratories, these being placed under the responsibility of a qualified person.

Quasi-drug products are subject to a pre-market registration process. The application dossier must contain, among others:

- the formula as well as the function of each ingredient,
- the method of manufacture,
- the finished product specifications,
- the instructions for use,
- the testing method for the active ingredient(s),
- claims of activity that must correspond to a quasi-drug category,
- stability testing and recommendations for storage and shelf-life, where appropriate.

If the concentration of the active ingredient varies by more than 10% at room temperature over a period of 3 years, an expiry date must be mentioned on the label. To determine this, accelerated aging tests (6 months at 40°C and 75% relative humidity) are accepted by the authorities.

If the product only contains already approved ingredients—including active ingredient(s)—authorization takes around 3–6 months. If not, it is advisable to ask the authorities whether the active ingredient or additive might have already been approved in a product which another company has submitted. It would then be sufficient to add the ingredient specifications to the existing dossier and the timespan will be the same as in the former case. Conversely, when the product contains a new active ingredient or component, very extensive procedures have to be followed, with the production of a toxicological dossier, and, at best, a time span of 18 months can be expected.

3.2.4. Other Countries

After this quick description of the main three regulatory frameworks, now all based on the responsibility of the manufacturer, i.e., the introduction of cosmetic products onto the market without prior approval and their in-market control by the authorities, we are going to quote some countries or regions where the pre-market registration system is still in practice.

In Latin America, the regulatory situation is changing rapidly in the cosmetic field, where harmonization is envisaged at two levels: within Mercosur, the Andean Community and the CACM, but also at the whole sub-continent level.

The *Andean Community* is proceeding with the harmonization of national cosmetics regulations. The mutual recognition of cosmetic products registrations prior to the introduction of the product on to the market is today a reality, i.e., approval given by the country in which the product is first launched or first imported is valid for the five Member States. The submission of a dossier by a locally approved pharmacist is mandatory. The manufacturing sites are subject to license and the Andean Good Manufacturing Practices (GMP) must be respected. It is important to avoid any claims relating to therapeutic activity.

The *Member States of Mercosur* are harmonizing their cosmetic regulations and are progressively implementing in their countries the resolutions that have been already adopted. Companies must be registered and must conform to local GMP. The products are subject to registration, under the responsibility of a pharmacist. A body of inspectors is being established so as to ensure that the market and the companies that sell cosmetic products are monitored.

CACM: discussions are under way with a view to drawing up a community text starting from widely differing national situations.

In Asia, apart from Japan reviewed above, most regulations are currently evolving. In some countries such as China and South Korea, it is extremely difficult to gain a clear vision of the situation, given the extent to which things are left unsaid, and the local interpretations.

In many countries, hair products such as coloring products, permanent waving, anti-hair loss or antidandruff products are considered as "special cosmetics" or as quasi-drugs, with all that implies in terms of restrictive obligations (China, Taiwan, Korea, some ASEAN countries, etc.).

The 10 ASEAN countries have recently drawn up a Cosmetics Directive that envisages the replacement of the current pre-market product approval required by most Member States with a simple notification: there will thus be no need to await approval before launching a product, but notification must still be backed up by a substantial dossier. The text, not yet in force, states that manufacturing establishments must continue to be approved and that the GMP recently issued must be complied with. Application of this Directive will no doubt take several years during which the current divergent rules will continue to be in force.

In conclusion, let us consider the merits of the two procedures in force for the market introduction of a product:

- The *in-market control system*, based on the responsibility of the manufacturer and the control of products on the market by the health authorities, facilitates business activity in that no administrative delays hamper the introduction of new cosmetic products on to the market. Moreover, it helps the inspectors to exert a more efficient product control since they have at their disposal a constantly updated information within the company premises.
- The *pre-market control procedure* is based on the approval of every single cosmetic product before its market introduction. From the Industry point of view, it represents not only a considerable administrative burden but it also imposes variable and sometimes large delays to the launching of the products, hampering simultaneous international introduction of products on to the market. It creates discrepancies between neighboring countries practising different systems. Concerning Authorities, the pre-market control system is over consuming in terms of personnel, especially if we consider its inadequacy to protect the consumer, as it was shown in Florence. As far as consumers are concerned, the pre-market system deprives them, for an indeterminate period of time, of the benefits of technical and scientific advances in terms of safety, performance, and comfort.

Under these conditions, it is not surprising that the system of registration is dwindling: it has been abolished in Japan (products classified in the cosmetic category only) and will

soon be abolished in the 10 new Member States of the European Union. It is also currently being simplified in Latin America and in the 10 ASEAN countries.

3.3. Cosmetic Product Labeling

Labeling is an essential factor in the development of a hair product because it must not only enable the consumer to make an informed choice but also provide enough information for it to be used correctly and, ultimately, if required, to refer back to the manufacturer. Labeling has other functions as well, such as helping the authorities to check products on the market and the health services to identify any problems.

The rules to be followed in cosmetic product labeling come not only from specific cosmetic regulatory requirements but also from more general regulations concerning consumer protection, waste products, aerosols, etc. Even if all these regulations have to be taken into account we are mainly concentrating here on the rules which come from cosmetic regulations. They have been the subject of many discussions and attempts at international harmonization in recent years. Indeed, many consumers or potential purchasers are also travelers who are likely to buy cosmetic products at the airport or in the countries they are going to visit. Many consumers are well informed internet surfers used to buy cosmetic products on the web, whatever their country of manufacture. These people must be provided with products bearing understandable and somewhat standardized labeling. With a view to harmonizing, the Industry has drawn up an international nomenclature of cosmetic ingredients (INCI) and the ISO Technical Committee 217 is working on an international standard related to the labeling of cosmetic products.

Here we are going to consider, item by item, what constitutes cosmetic product labeling today on the basis of the main rules applied in a large number of countries and those with special characteristics. Some reference to past practices will help to better understand the differences that still exist within all these regulations.

Name and address of the company responsible for marketing the product: This is usually the manufacturer or importer, but sometimes also the distributor or even the packaging company which can complicate matters, e.g., as far as responsibility for the product is concerned. In order to simplify the labeling, the address may sometimes be abbreviated, as long as the firm can be easily identified. In the E.U., in the event of multiple addresses, the one at which the product information is to be made available to the control authorities must be underlined.

Country of origin: this concept is not always easy to interpret, especially when the manufacturing process includes several stages carried out in different countries (mixtures of preparations, dilutions, filling, packaging, etc.).

Nominal content: this information is generally not required for small packs, free samples or single-application packs. The weight or volume should be expressed using the international metric system. In the United States, imperial units can still be used for some years, beside the metric system units.

Use-by date: the requirements concerning a date to be labeled on the product are highly disparate. Most countries—50 or so, including the United States, Australia, Canada, New Zealand and India—do not require this kind of information, counting on the good sense of the consumer to dispose of any product that is past its best.

Some 20 countries, mainly European, insist upon a “best before” date if the product durability is less than 30 or 36 months. The E.U. has just supplemented this requirement with a period after opening, surprisingly imposed on products whose durability *exceeds* 30 months; it will be labeled after a logo showing an open jar.

An expiry date is required in some 20 countries but mainly on OTC-type products (United States), quasi-drugs (Japan), “medicated cosmetics” or cosmetics whose durability is less than 30 or 36 months (Japan).

Finally, Nicaragua, South Korea, and Turkey require a date of manufacture with an additional date of the type of those mentioned above.

Such disparity calls for efforts toward harmonization which are being done at ISO level. There is also a need for the development of an accelerated aging method to justify the dates that must be labeled.

Precautions in use and warnings: In addition to the information freely supplied by the cosmetic manufacturer, many regulations require certain mandatory information.

In the E.U., this information is generally related to the presence of regulated ingredients. In the United States, it is highly recommended to follow the labeling recommendations of the Cosmetic Ingredient Review (CIR), but there are also some compulsory warnings related to some product categories or types of packaging: foam baths, coal tar hair dyeing products, aerosols or products whose safety has not been established (cf. section on Ingredients). In some Latin America countries such as Brazil and Mexico, there is a variety of mandatory warnings that apply to specific cosmetic categories.

In the field of hair products, special attention must be paid to the labeling of professional products such as hair coloring products, straighteners or relaxers, permanent waves, etc., because professional use presuppose much longer skin or inhalation exposure than a private use.

Instructions for use: this item is rarely the subject of a requirement because it is probably considered to be the responsibility of the supplier of the product.

Recommendation for a sensitivity test: In the United States, such a recommendation must be labeled on oxidation hair coloring products so that a sensitivity test is carried out prior to the first use, to detect any sensitivity to “coal tar” colorants. In the E.U., such a requirement has existed for many years, but the opinions of dermatologists vary, some considering that the touch test increases the risk of sensitization by multiplying contact with the substance. The requirement for a sensitivity test was finally removed from the Cosmetics Directive in 1992. It is thus now up to the supplier to take responsibility for this matter.

Batch number: a batch number is generally required. That will be helpful to identify products to be quickly recalled from the market in case of problem.

Function: Mentioning the function of the product is mandatory unless it is clear from the presentation of the product.

Composition of the product: The idea of labeling the composition appeared in the United States at the beginning of the 1970s. Indeed, labeling was part of the CTFA Voluntary Program which requested a full ingredient labeling (FIL) on the outside packaging of cosmetic products. In order to make the FIL understandable to the average consumer, a simple nomenclature intended to avoid the problems of synonyms was necessary. CTFA carried out the work in 1973 and submitted its Cosmetic Ingredients Dictionary to the

FDA for approval. The nomenclature became official in 1977, as part of the cosmetic labeling program which became no longer voluntary after its publication in the Code of Federal Regulation.

In the E.U., it was only in 1993 that the labeling of the ingredients on the outer packaging of cosmetic products was required. COLIPA and CTFA worked together to review the Dictionary which became the International Cosmetic Ingredient Dictionary (ICID). The ICID contains thousands of ingredients whose name is given according to the International Nomenclature of Cosmetic Ingredients (INCI), now recognized on an international basis: The INCI system has been adopted or is on the process of being adopted not only in the already mentioned Free Trade Areas (E.U., Mercosur, Andean Community, ASEAN, etc.) but also in many countries such as Australia, Canada, Israel, Mexico, Russia, Saudi Arabia, South Korea, and, more recently, Japan where INCI names are accepted but must be written on the packaging in Japanese characters.

The ICID is regularly updated (8th edition in 2002) and contains all the cosmetic ingredients available on the market, at least in the United States and E.U. Ingredient suppliers of all countries can obtain an INCI name by providing the CTFA, which continues to administer the system, with the technical information needed for characterization of the ingredient. The inclusion of a compound in the ICID does not indicate that the ingredient is authorized in a given country.

The INCI nomenclature still requires some harmonization efforts: the United States and E.U. are seeking a solution to the double nomenclature that still exists for colorants, plants extracts and trivial names, the United States using English while European linguistic history led to use the Color Index (CI) for colorants, the Pharmacopoeia Latin for common names and the international Linnean botanical classification for plants. Another anomaly that is detrimental to INCI universality is that a few rare countries such as Mexico and Brazil have felt the need to “translate” this nomenclature that is, by its very nature, untranslatable.

The FIL is required to help consumers at the time of purchase to avoid product containing ingredients to which they may have been sensitized. It is therefore generally only required on the outer packaging of the product.

The ingredients present at a concentration greater than 1% are listed by decreasing order of weight; then come in any order those contained at a concentration lower than 1%, followed by colorants. Some chemicals do not need to be mentioned, e.g., ingredient impurities.

“Parfum” and “aroma” are considered as ingredients as a whole; however, the E.U. has just issued a requirement that 26 of their constituents that are known to be allergenic should be listed by their INCI names in the FIL if above 0.001% in leave-on products and 0.01% in rinsed-off products.

For decorative products marketed in several color shades, it is possible to mention on the packaging of every product of the range all the colorants used as ingredient in the range of products, preceded by the terms “May contain” or the sign “+/-.” In the E.U. this possibility is not accepted for hair coloring products in all the MS.

Registration number: it is sometimes required on the label in countries where cosmetic products need a pre-market approval. As the number is known at the end of the registration process only, this obligation delays further the placing of the product on the market.

Language: Most countries require that the majority of compulsory labeling be expressed in their national language(s) especially those items related to consumer safety. An outer packaging or a leaflet is often needed to include the translations.

As a result, the number of compulsory items that have to be put on a cosmetic product packaging may actually hinder the transfer of useful information. Moreover, it is not infrequent to be obliged to add a redundant packaging for the sole purpose of increasing the surface area to accommodate the compulsory labeling.

3.3.1. OTC Drug and Quasi-Drug Products Labeling

So far we have only dealt with the labeling of cosmetic products. Things are very different when entering the domain of American OTC drugs that include, in particular, antidandruff products. OTC drugs are the subject of very stringent labeling rules described on the one hand in the OTC Drug Labeling Regulation published in 1999 and on the other hand prescribed by the Monograph, if it exists, specific to the type of OTC concerned.

Labeling of OTC products is much more austere and takes up a lot more room on the packaging than that required for cosmetics: the Regulation specifies a single format entitled "Drug Facts" with standard subtitles concerning the active agents, the use, warnings, instructions for use, contraindications, etc. A minimal print size is specified. Small packs are subject to special rules.

The composition must be mentioned, listing in alphabetical order the U.S. Adopted Names (USAN) and concentrations of the active ingredients. The other ingredients appear, clearly separated, in the FIL order required by the cosmetics regulation.

As noted earlier, OTC products must bear an expiry date except if their durability exceeds 3 years.

In Japan as well as in South Korea or Taiwan, a great number of mandatory warnings must be included in the labeling of oxidation hair coloring products considered as Quasi-Drugs. As in the United States, an expiry date is required on the product except if its durability exceeds 3 years. The composition does not have to be labeled on the packaging, but more than 130 ingredients must be mentioned when present.

3.4. Regulations Regarding Ingredients

The need to avoid potential health problems associated with the use of hair coloring and hair waving products, was the reason behind the first regulatory measures known to apply to cosmetics in the 1950s. The discrepancy in the legal requirements concerning the same substance adopted by different countries over the years can be explained by the fact that cosmetic products were different from one market to the other due to the rarity of international trade exchanges at that time and to the fact that there was nearly no contact between the scientific and regulatory authorities in charge of cosmetic products in the different countries. Thus, for 30 years, regulation of cosmetic ingredients developed separately leading to such complexity that companies intending to sell their products on the international market faced a real challenge. Boric acid can be quoted as an extreme example of this situation with 26 different national cosmetic regulations in force by the end of the 1980s. This situation frustrated the R&D laboratories who had to devote a

large amount of time and effort to adapt their products to each market's requirements. On top of that, came the difficulties due to the positive lists which were under development in the E.U. This led the Industry to organize Mutual Understanding meetings to bring together the American, Japanese and European authorities and Industry with the objective to get some harmonization in cosmetic regulations. Today these efforts toward harmonization are beginning to bear fruit; taking boric acid as an example again, the number of regulations to comply with world-wide has been reduced from 26 to around 10.

Despite this favorable evolution, the discrepancies between maximum concentration allowed in different countries for the same ingredient will not disappear completely in the future, even if the authorities were to make harmonized decisions, for the simple reason that the concentration chosen may not be the highest concentration without undesirable effect but the concentration *requested by companies* without undesirable effect. If another company should need a higher concentration it would make a new application with new toxicological data for the requested concentration.

The regulatory systems governing the composition of cosmetic products are generally based on lists of ingredients either prohibited, or permitted for a given cosmetic function, or subject to certain qualifications. These lists are named negative, positive, or restrictive lists.

A *negative list* contains substances or families of substances whose use is banned in cosmetic products. Certain elements of a family can be granted an exemption thus authorizing cosmetic use on certain conditions; these ingredients are generally put on the restrictive list of the regulation concerned. The presence of a substance on a negative list only prevents its intentional incorporation in cosmetic products; it does not prohibit traces, i.e., extremely small amounts, unintentionally introduced in the product via its ingredients and unlikely to be harmful to consumer health. *Sola dosis fecit venenum*.

A *restrictive list* includes ingredients whose use in cosmetics is subject to various qualifications: product type, field of application, concentration, purity or pH criteria, warnings to be put on the label, etc.

Positive lists per function: Positive lists are restrictive lists of substances having the same function. The difference between a restrictive and a positive list is that a positive list is exhaustive, i.e., substances which are not on the list cannot be used for the given function; moreover, the positive list regulates only the main function of the product, the other uses or secondary functions being allowed unless otherwise regulated.

Many national cosmetic regulations contain positive lists corresponding to the three main functions:

- Colorants intended to color the skin or the actual product. Forty national regulations contain this type of list generally based on both the E.U. and the U.S. lists,
- Preservatives primarily intended to inhibit the development of micro-organisms. This type of list is found in around 30 national regulations, nearly all of them being based on the E.U. list,
- UV filters specifically intended to protect the skin from the harmful effects of solar radiation. Around 30 national regulations include this type of list.

Other types of positive list for cosmetic product ingredients are in practice in a few countries, such as: hair oxidation coloring agents in Mexico and China (Japan, South

Korea and Taiwan have such a positive list but hair oxidation dyeing products are quasi-drugs) hair waving active ingredients in Japan, South Korea and Taiwan as quasi-drug components; anti-dandruff agents and antiperspirants in the United States and Canada as OTC ingredients; vitamins (Taiwan); anti-aging in South Korea as cosmeceuticals; skin whitening agents in South Korea. It should be noted that, in some countries, the presence of ingredients of these lists could cause the product to be considered as an OTC, quasi-drug or “medicated cosmetic” product whatever the concentration.

For a new substance to be included in a positive list per function, the Industry has to submit a file with toxicological data to the competent national authorities. The procedure can take several years, especially for OTC or quasi-drug ingredient.

3.4.1. Discussion on the Positive List Issue

An efficient cosmetics regulation must contain a list of prohibited substances as well as a list of substances whose use is subject to various qualifications. But a positive list does not appear to be the right way to regulate cosmetic products, even if it is a easy-to-handle type of regulation for official bodies as well as for the formulators, which is probably the reason why it is so widespread internationally.

To give an example, two positive lists of cosmetic colorants were established in the United States in 1956 and in the E.U. 20 years later. The first one was part of a regulation common to food, drugs and cosmetics, and may be well adapted to food and drugs. Today, it contains nearly the same 62 colorants as twenty or thirty years ago, far less than the E.U. list, and each batch of certain color additives should be submitted to the FDA for a certification whose usefulness is not obvious to everybody. Why such excessive scrutiny for those unproblematic substances among 8000 ingredients or so? The second list was adopted in 1976 in the E.U., not as the best system to regulate cosmetic colorants but as a shaky compromise to close difficult discussions. It contains 157 colorants which were allowed 30 years ago and have not been reviewed since then. Another example is the positive list of preservatives: if a preservative is considered to be potentially harmful because of its biological activity, why should it be regulated in cosmetics for its main function only and not for its other uses at higher concentrations or its secondary functions?

During the discussions of the Sixth Amendment to the Cosmetic Directive, a major point of discussion was the drawbacks of the positive list system:

- may give a false sense of security, in particular for the formulators and those responsible for placing a product on the market who may give credit to the conditions of use allowed by an outdated regulation,
- long delays for getting a new ingredient listed postpones the availability to consumers of ingredients offering a higher degree of safety and/or efficacy,
- delays approval reducing the validity of patents,
- reduction in the number of preservatives allowed potentially increases the exposure of consumers to the remaining ones. Microbiologists have been drawing attention for many years to the fact that the continuous reduction in the number of preservatives at their disposal makes their task more and more difficult, e.g., in a market demanding natural products whose preservation is often difficult. This problem is of particular concern in the E.U. where fewer and fewer ingredient manufacturers are prepared to

search for new, safer and more efficient cosmetic preservatives due to the impending ban on animal testing and the requirements of the Cosmetics, Biocides and Dangerous Substances Directives.

Without modifying the fundamentals of the above three-list ingredient regulation, a solution might be to move toward a two-list system with the usual negative and restrictive list, the last one containing all the substances allowed with restrictive conditions, including ingredients from the present positive lists with their function(s) specified as is in practice in Switzerland.

3.4.2. Ingredient Regulation in the European Union

The Cosmetic Directive 76/768 regulates the use of ingredients following the three-list system described above. The lists are reopened on a yearly basis and have been updated some 32 times since 1976, in consideration of new data or scientific advances.

Originally, the legislator took its inspiration from the rules governing food additives and pharmaceuticals and decided on a mixed system comprising a negative list banning medicinal and dangerous substances (Annex II), a restrictive list (Annex III) and three positive lists containing cosmetic colorants (Annex IV), antimicrobial preservatives (Annex VI) and UV filters intended to protect the skin from harmful UV rays (Annex VII). A special Annex V had to be added to put in the “substances excluded from the scope of the Directive” pending a harmonized decision by MSs. As previously stated, this last annex should disappear soon.

For an ingredient to be listed in one of the Annexes, a Directive for Adaptation to Technical Progress is needed. The decision is taken following a scientific assessment of the risk associated with the ingredient used in a product under “normal or reasonably foreseeable conditions of use,” on a case-by-case basis, by the Scientific Committee on Cosmetic and Non-Food Products (SCCNFP). To help in the drawing-up of application files and to explain its methods of evaluation, the SCCNFP has established Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation which are updated in real time on the European Commission’s internet site (cf. Appendices). After its publication in the E.U. Official Journal, the Directive providing for a new ingredient to be listed still has to be implemented into each national regulation to become effective. Note that 30 or so many non-E.U. countries that have adopted the E.U. ingredients lists have neither obligation nor deadline to implement their adaptations, so harmonization remains somewhat theoretical.

In 1993, it was finally decided to stop developing new positive lists and instead, to provide the SCCNFP with the information needed to have a clear view of the composition of cosmetic products. This new transparency was based on:

- an Inventory of cosmetic and perfumery ingredients, regularly updated by Industry, out of which the SCCNFP could select potentially harmful ingredients to be regulated,
- the composition listed on the products with Full Ingredient Labeling.

The three existing positive lists were maintained, but it was decided to speed up their updating.

The Inventory of cosmetic and perfumery ingredients contains some 10,000 ingredients; it was published in every E.U. language in the Official Journal in 1996 to

serve as a basis for the International Nomenclature of Cosmetic Ingredients (INCI). This nomenclature provides the ingredient names to be used for labeling ingredient composition of cosmetic products on the market. Since then, updates of the Inventory have been delayed due to the need for translation. It does not concern INCI which is an international nomenclature and by its very nature untranslatable, but other data present in the Inventory. To escape from this pitfall, the Commission placed the 1999 Inventory update on its Internet site and renounced for the time being any official publication. The European professional association, COLIPA, is planning to post a version online so that it can be updated as frequently as needed. In the meantime, the International Cosmetic Ingredient Dictionary issued by the American CTFA and regularly updated on its site can be used: it contains all the cosmetic ingredients used in the United States, the E.U. and some other countries.

Annex II: Substances Banned in Cosmetic Products. The list of “substances which must not form part of the composition of cosmetic products” drawn up in the 1970s was largely inspired by the “Tableau des Substances Vénéneuses” which, in France, restricted certain substances to medicinal use. Thus, most of the 361 substances prohibited by the 1976 Directive have never been used in cosmetics. This is not the case with ingredients that were prohibited later on, as knowledge about the toxicity of ingredients increased with the availability of more relevant safety data. Today, Annex II contains 1130 substances and will be regularly supplemented not only due to possible bans of cosmetic ingredients but also as a result of the systematic evaluation of chemicals under the Dangerous Substances Directive (DSD). In practice all substances classified CMR 1 & 2, i.e., Carcinogenic, Mutagenic or toxic for Reproduction in humans or in animals will be banned automatically for cosmetic use. CMR 3 ingredients, i.e., those where there are concerns about the same hazards, will also be banned, except following favorable risk assessment by the SCCNFP. This link between Cosmetics and the DSD is somewhat inconsistent because DSD classification is based on the concept of “hazard,” an intrinsic property of a substance, whereas the Cosmetics Directive is based on the more realistic approach of “risk,” i.e., its ability to be harmful in conditions of use.

As indicated earlier, the Directive prohibits the intentional addition of substances listed in Annex II but tolerates traces in a cosmetic product if technically unavoidable in Good Manufacturing Practices and if they are unlikely to be harmful to consumer health.

For the record, some measures taken on the grounds of environmental concern may also affect hair products, e.g., the prohibition of chlorofluorocarbons (CFCs) such as HCFC 11, 12, 22 and 142b which are known to have a depleting effect on the ozone layer, as well as 1,1,1-trichloroethane. Moreover, regulatory developments relative to volatile organic compounds (VOCs) will also have to be monitored (see Chapter 5).

Annex III: List of Substances which Cosmetic Products Must Not Contain Beyond the Qualifications and Conditions Laid Down. Annex III can stipulate any type of restriction deemed necessary for the safe use of a substance in cosmetics: product categories or type of users (professional users or children for example), pH, labeling, maximal concentration of use, purity, incompatibilities, precautions in use, warnings, etc.

Annex III currently contains 156 cosmetic ingredients of which 96 are regulated permanently (part 1) and 60 are regulated provisionally (part 2). Twenty-six perfumery ingredients have recently been added to this Annex.

Although the Directive does not address cosmetic categories as such, some hair products are covered by the requirements due to their composition. This is the case notably with hair coloring, permanent waving, straightening and antidandruff products of which the active ingredients are, for the most part, regulated in Annex III, e.g., thioglycollic acid, its salts and esters, ammonia, sodium and potassium hydroxides, hydrogen peroxide, some antidandruff agents as well as 66 oxidation and semipermanent hair colorants.

As far as hair colorants are concerned, the initial intention of the Directive was to draw up a positive list. In practice, authorities recently decided to put those ingredients in Annex III pending their evaluation by the SCCNFP. Industry supplied related safety dossiers and a review is ongoing. The Commission has threatened to propose the ban of all hair colorants whose safety dossiers have not been submitted.

Annex IV: List of Coloring Agents Allowed for Use in Cosmetic Products. Annex IV contains 157 colorants usually named "cosmetic colorants" that are authorized for various fields of application:

1. Coloring agents allowed in all cosmetic products,
2. Coloring agents allowed in cosmetic products except those intended to be applied in the vicinity of the eyes,
3. Coloring agents allowed exclusively in cosmetic products intended not to come into contact with the mucous membranes,
4. Coloring agents allowed exclusively in cosmetic products intended to be in brief contact with the skin.

Lakes and salts of the colorants listed that can also be used in the products concerned are mentioned. All these colorants are allowed in hair products on the given conditions, but hair coloring agents are expressly excluded from this Annex and the majority of them can be found in Annex III.

Even though the references given to purity criteria of food colorants have not been updated for a long time, the formulator will be well advised to observe the spirit of the text that requires the use of a food quality colorant if such exists.

Annex VI: List of Preservatives which Cosmetic Products May Contain. Annex VI contains an exhaustive list of 55 preservatives allowed in cosmetic products "for the primary purpose of inhibiting the development of micro-organisms" as stated in the preamble of this Annex. This preamble is worthy of study since it limits the ambiguity attached to positive lists: it specifies, for example, that substances that are not on the list, notably many essential oils, can contribute to the preservation of cosmetics as a secondary function. It indicates also that "substances marked with the symbol (+) may also be added to cosmetic products for other specific purposes apparent from the presentation of the product, e.g. (...) antidandruff agent in shampoos." These so-called "other uses" of preservatives are in the process of being regulated via Annex III.

Annex VII: List of Permitted UV Filters which Cosmetic Products May Contain. Annex VII hardly concerns hair products since the filters listed are those used for protection of the skin. However, attention is drawn to its preamble which specifies that the 26 filters listed "may be added to other cosmetic products under fixed limits and conditions." Even if this remark was initially aimed at products for daily skin protection

as opposed to sunscreens, it was not specified that hair products were not concerned, this usage having not been addressed. Legal ambiguity remains.

The preamble also specifies that other filters may be used for the purpose of protecting the product. This use requires much smaller concentrations than the protection of the skin and the legislator has not considered it necessary so far to impose qualifications for this use.

Analytical Methods. Harmonized analytical methods are needed in order to control cosmetic products throughout the E.U. in the same way. The Member States have therefore undertaken the development of a method for each of the ingredients regulated in the Cosmetic Directive so as to verify its presence and determine its concentration if needed. Seven Official Analytical Method Directives were published between 1980 and 1996 (cf. Appendices)

3.4.3. Ingredient Regulations in the United States

At first glance, the regulations that govern cosmetic ingredients in the United States may look rather surprising: they include a positive list of cosmetic colorants which has not been updated for ages, a regulation which requires FDA certification batch by batch for harmless cosmetic color additives, a hair dyes exemption that limits the regulating power of the Food and Drug Administration (FDA), scattered regulations for a few cosmetic ingredients, some of them being enacted by the Environment Protection Agency (EPA) or by certain States such as California or Texas (orders on VOCs). Nothing showing consistency at first sight. But when looking a bit further, one easily understands that the masterpiece of the system is not by Regulation but the Cosmetic Ingredient Review (CIR), a remarkable work that represents an invaluable collection of scientific data and formulation advices deserving world-wide interest. After a brief review of the regulations quoted above, we will focus our attention on the interest, advantages and authority of the CIR.

From the regulatory point of view, cosmetic products and their ingredients, with the exception of cosmetic colorants, can be freely sold or used under the responsibility of Industry without prior approval by the FDA. Nevertheless, the companies can participate in the Voluntary Reporting Program set up by the Industry Association CTFA and the FDA at the beginning of the 1970s, and can register their formulations at the FDA on a voluntary and confidential basis.

A few substances considered as dangerous are prohibited in cosmetic products or subject to qualifications in use via the Code of Federal Regulations (21 CFR 700–11 to 24 and 240), or have even been voluntarily discontinued: these ingredients are methylene chloride, CFCs, bithionol, halogenated salicylanilides, chloroform, vinyl chloride, hexachlorophene, mercury compounds, AETT, musk ambrette, 6-methylcoumarin, nitrosamines, and dioxane.

Concerning colorants, different scenarios should be considered depending on their chemical composition or on their cosmetic use:

- *Cosmetic color additives* can be used in cosmetic products only if they are listed on the positive list and in line with the purity criteria laid down for most of them (21 CFR 70–71–74–80–81–82). The first part of the positive list contains 34 color additives and many lakes subject to FDA certification: *Each batch* has to be submitted to the FDA

for analytical control. The second part contains 28 color additives, for most of them mineral or vegetable pigments, which are exempt from certification. Their field of application, and sometimes their concentration, is regulated, e.g., eye area, externally except eye area, oral cavity products, etc. Bismuth citrate, henna, and lead acetate can be used as “scalp hair dyes” only.

- **Hair dyes:** The so-called “coal tar” hair dyes are synthetic organic compounds. While being subject to the general regulations of cosmetic products within the FD&C Act, they are not subject to FDA approval due to the so-called “Hair dye Exemption.”

Indeed when this Act was drawn up in 1938, the manufacturers of hair dyes and the hairdressers were alarmed by the fact that some hair dyes could be considered to be adulterated, and consequently prohibited, if they gave rise to allergic reactions. After long discussions between the FDA and Industry, the decision was taken to exclude hair dyes from the scope of Article 601(a), which states:

a cosmetic shall be deemed to be adulterated...if it bears or contains any poisonous or deleterious substance which may render it injurious to users under the conditions of use prescribed, or, under such conditions of use as are customary or usual.

provided that the products bore the following warning label:

Caution—This product contains ingredients which may cause irritation on certain individuals and a preliminary test according to accompanying directions should first be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

and the labeling provided for adequate directions of use for such preliminary testing.

This exemption inevitably raises discussions each time a hair colorant is in question. For example, at the end of the 1970s, when the use of 2,4-diaminoanisole was queried by the scientific community because of its carcinogenic potential (cf. Chapter 10), its use in hair coloring products was discussed at length and it was finally banned by most of the national cosmetic regulations. In the USA, due to the Hair Dyes Exemption, the FDA had no power to ban its use. It thus got round the problem by requiring the mandatory inclusion of the following clause in the labeling:

Warning—Contains an ingredient that can penetrate your skin and has been determined to cause cancer in laboratory animals.

However, given the pressure exerted by consumers and by the legal profession in the United States, such dissuasive measures turn out to be as effective as a ban. This also applies to the recommendations of the CIR described below, more credible in this country than in any other.

Cosmetic Ingredient Review. The establishment of the CIR set up by CTFA in 1976, has completely changed the cosmetic ingredient regulatory perspective in the United States replacing the previous case by case approach intended to prevent health problems

already encountered on the market by a continuously comprehensive review of the safety of some 8000 ingredients used on the American market.

Procedures were put in place in order to ensure objectivity and expertise: Funded by the Industry the CIR is nevertheless carried out step by step by an Expert Panel including independent experts in toxicology, dermatology, chemistry, and pharmacology. Their work is regularly presented and discussed in public meetings attended by consumers, Industry and FDA representatives. Priorities are set using a weighted formula taking into account frequency of use, biological activity, concentration, type of product, customer complaints if any, etc. In order to avoid duplication of work, the review is postponed for substances object to other ongoing reviews. On the contrary, any substance put into question by reliable scientific studies is examined without delay. Moreover, reports that are more than 15 years old, or for which there is new information available, are reopened and amended by the Panel if deemed necessary.

The safety evaluation of an ingredient begins with a bibliographical search, on the ground of which the CIR staff prepares a Scientific Literature Review summarizing the available data. This SCL is made available for public comment for a 90-day period, with a request for published or unpublished missing data. After discussion at team level and public meetings, the Expert Panel issues a Tentative Report at disposal for another 90-day comment period. In the end, a Final Report taking all the comments and data into consideration is released for distribution and publication in the open scientific literature (International Journal of Toxicology).

The Panel conclusion can be: (1) safe as used, (2) safe with qualifications, (3) unsafe or (4) insufficient data to support safety. The uses referred to are the American uses declared to the FDA within the framework of the voluntary declarations of product composition or come from specific Industry inquiries. They are given in detail at the beginning of each report.

The CTFA publishes an annual Compendium containing abstracts, discussions and conclusions resulting from the CIR. Today, more than 1000 Reports are available concerning a large number of ingredients, given that many of these Reports concern families of substances. Roughly 90% of the reviewed ingredients are safe as used' in cosmetic products, a third of them being subject to qualifications; 1% were found to be *unsafe* and not recommended for use in cosmetic products; lastly, 9% of the evaluated substances have insufficient data to support their safety. In this case, the substance is not banned but it cannot be used in a cosmetic product unless it is labeled "The safety of this product has not been substantiated" or unless the company has the required toxicity data at its disposal. As previously stated, such a dissuasive measure turns out to be as effective as a ban in the United States; but if a company is able to obtain the missing data, it does not have to wait for a lengthy authorization procedure.

Scientific and Regulatory Affairs people should be aware of the importance the CIR may have in the future as a basis for harmonization of regulations, recommendations and standards of cosmetic ingredients. Indeed, the CIR constitutes an efficient tool in supplying a comprehensive review, regularly updated and publicly available, of the safety of cosmetic ingredients. The work is done according to well-established priorities, formalized procedures, using all available scientific expertise and taking into account all data, comments and feedback. It provides a relevant and reliable resource at the disposal

of regulatory authorities as well as Industry and consumers and its conclusions should be considered as a reference world-wide.

SCCNFP Opinions and CIR Reports on the safety of cosmetic ingredients are often compared and their conclusions sometimes differ. Indeed these two bodies have neither the same objectives nor the same way of working which may explain discrepancies now and then. The SCCNFP is the scientific advisory body of the E.U. Commission in the field of cosmetic products. It is responsible for providing scientific advice for the drafting and amendment of Community rules. So the SCCNFP contents with answering precise questions from the Commission on queried ingredients or with looking at one function of the ingredients in the case of a positive list. In a near future, it will also have to advise the Commission on all substances classified CMR 3 within the framework of the Dangerous Substances Directive.

Concerning the way of working, the provisions enacted and applied by the European Commission to set up Scientific Committees in the field of consumer health impose the principles of excellence, independence and transparency. The independence principle de facto excludes experts of cosmetic products, academics included, and hinders communication between the SCCNFP and the Industry so that data, questions and answers have to pass indirectly, undiscussed through the Commission. In very infrequent occasions only, experts may be invited to working group meetings.

To sum up, the CIR strives to provide a comprehensive, realistic and upto-date view of the safety of cosmetic ingredients whereas the SCCNFP, whatever its excellence, only contributes case-by-case to the E.U. regulation of cosmetic ingredients.

OTC Drug Products. OTC drug products, e.g. antidandruff products, cannot contain other active ingredients than those classified in category I as “generally recognized as safe and effective” in the related OTC final Monograph for the type of product. Before introducing a product on the market, the company must declare the name of the active ingredients to the FDA and has to update this information twice a year. Attention should be focused on the fact that the colorants allowed in OTC drugs are not the same as those allowed for cosmetic products.

3.4.4. Japanese Ingredient Regulation

The regulatory situation for ingredients differs as to whether they are used in cosmetic products or in quasi-drug products.

As far as *cosmetic products* are concerned, we have already discussed the evolution of the Japanese regulation toward an in-market control system which was implemented in April 2001 as part of what has been called “Deregulation” (cf. above).

Beforehand, the regulation was based on an overall positive list of cosmetic ingredients, i.e., only the approved ingredients could be used in cosmetic products and it was very difficult to know whether they were approved because the official list was incomplete. Thus the Ministry had to be approached, ingredient by ingredient and, in the event that they had not been previously approved for another company, extremely lengthy and expensive procedures had to be undertaken to get approval.

This opaque situation gradually became clearer thanks to the Japanese authorities, stimulated by the efforts at harmonization organized under the umbrella of the WTO: they began by publishing, in English, the specifications of approved ingredients. Then

they set up the Comprehensive Licensing Standards system (CLS) consisting of positive lists including all the ingredients approved for use in certain types of cosmetic product. They then grouped similar categories to reduce their number from 35 to 24 and finally 11 in 1999. In 2001, they progressed to "Deregulation," modernizing the system from top to bottom.

Today, the use of a cosmetic ingredient does not need approval unless its function is covered by one of the three positive lists which concern, as in the E.U., cosmetic colorants, preservatives, and UV filters. The regulated substances are distributed among five lists of ingredients which are not really new since all the ingredients they contain have already been approved under the same conditions. However, the lists have undergone a change in presentation, encompassing:

- a negative list containing the 30 ingredients banned in cosmetics, among which are hydrogen peroxide, formaldehyde, and methylene chloride,
- a list of ingredients subject to various qualifications,
- a positive list of UV filters designed to protect the skin and the hair from UV radiation. The filters listed are those that appeared in the earlier CLS list of ingredients approved for use in sunscreen products,
- a positive list of preservatives that brings together those previously included in all the CLS lists. They can be used in the categories of products corresponding to the CLS on which they were previously listed,
- a positive list of the 137 "coal tar" colorants already approved.

The rules which the ingredients of the *Quasi-Drug products* (QD) have to comply with are very different and highly constraining and stringent compared to the ingredients of cosmetic products. The regulations governing QD ingredients have hardly changed during the past decades which means that they remain extremely restrictive and burdensome: in fact quasi-drugs can only contain ingredients approved for the type of product concerned and this applies to any ingredients of the product whatsoever, including active substances and any other ingredient as well. Moreover the only lists of approved ingredients known until now concern oxidation hair coloring and permanent waving products. For other products, the Ministry has to be approached case-by-case. For antidandruff products and those promoting hair growth, it remains necessary to apply to the administration concerned and submit files on the active ingredients. For the status of other ingredients, the former CLS lists can still be used, subject to the qualifications laid down.

To conclude, it can be said that the efforts at harmonization of the regulations of cosmetic ingredients are beginning to bear fruit but they must be continued.

Whereas some differences between labeling or administrative procedure rules are acceptable due to custom, perception, administration structures, etc., no differences should arise where the regulation of cosmetic ingredients is based on science. The national authorities that do not have specific regulations governing cosmetics tend more and more to rely on and accept the ingredient lists from the countries whose regulations seem to have a scientific foundation, i.e., the Annexes of the E.U. Cosmetic Directive and the American list of cosmetic colorants. But some of them are positive lists with all the problems described above.

For the future, the CIR reports could help to progress toward global harmonization of the situation of cosmetic ingredients in providing valuable recommendations in line with the evolution of the scientific knowledge. There is, after all, no need to reinvent the wheel.

4. APPENDICES

4.1. Abbreviations, Acronyms, e-Addresses

Andean Community	Bolivia, Colombia, Ecuador, Peru, Venezuela
ASEAN	Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam
CACM	Central American Common Market
CCTFA	Canadian Cosmetic, Toiletry & Fragrance Association
CFR	Code of Federal Regulations (USA)
CIR	Cosmetic Ingredient Review (CTFA)
CLS	Comprehensive Licensing Standards of Cosmetics by Category
CMR	Carcinogenic, Mutagenic or toxic for Reproduction (DSD classification)
COLIPA	European Cosmetics, Toiletry & Perfumery Association
CFSAN	Center for Food Safety & Applied Nutrition (FDA)
CTFA	Cosmetic, Toiletry & Fragrance Association (United States) http://www.ctfa.org/
CTPA	Cosmetic & Toiletry Products Association (UK) http://www.ctpa.org.uk/
DSD	Dangerous Substances Directive (E.U.)
EAPCCT	European Association of Poison Centres & Clinical Toxicologists
EEC	European Economic Community
EPA	Environmental Protection Agency, USA
E.U.	European Union, i.e., Austria, Belgium, Denmark, Eire, Finland, France, Germany, Greece, Italy, Luxembourg, The Netherlands, Portugal, Spain, Sweden, United Kingdom. From May 2004: Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, Slovakia, Slovenia
FDA	U.S. Food & Drug Administration
FIL	Full Ingredient Labeling
FIP	Fédération des Industries de la Parfumerie (France)

FTA	Free Trade Area
GATT	General Agreement on Tariffs and Trade
ICMAD	Independent Cosmetic Manufacturers & Distributors Association
ICID	International Dictionary of Cosmetic Ingredients (CTFA)
IKW	Industrieverband Körperpflege- und Waschmittel e.V. < http://ikw.org/ >
INCI	International Nomenclature of Cosmetic Ingredients http://www.iso.ch/
ISO	International Organization for Standardization
JCIA	Japanese Cosmetic Industry Association < http://www.jcia.org/ >
MERCOSUR	Argentina, Brazil, Paraguay, Uruguay
MHW	Ministry of Health & Welfare (Japanese Koseisho. Replaced by MLHW)
MLHW	Ministry of Labour Health & Welfare (Koseirodoshō, Japan)
MSs	Member States
OTC Drug	Over-the-Counter Drug (sold without prescription)
QC	Quality Control
SCCNFP	Scientific Committee on Cosmetic & Non-Food Products (E.U.) http://europa.eu.int/comm.food/fs/sc
VOC	Volatile Organic Chemical
WTO	World Trade Organization

4.2. European Union

A—The Official Journal (<http://europa.eu.int/eur-lex/>)

B—The Commission (<http://www.europa.eu.int/comm/dgs/health>)

The Council Directive 76/768/EEC of 27 July 1997 on the approximation of the laws of the Member States relating to cosmetic products, EC Official Journal L262–27.09.1976 modified, amended and completed by:

- Directives for Amendment: 79/661, 82/368, 83/574, 88/667, 89/679, 93/35, 2003/15,
- Directives for Adaptation: 82/147, 83/191, 83/341, 83/496, 84/415, 85/391, 86/179, 86/199, 87/137, 88/233, 89/174, 90/121, 91/184, 92/8, 92/86, 93/47, 94/32, 95/34, 96/41, 97/1, 97/45, 98/16, 98/62, 2000/6, 2000/11, 2002/34, 2003/1, 2003/83, 2004/87, 2004/88.
- Texts for application of Articles: Directive 80/1335, 82/434, 83/514, 85/490, 93/73, 95/32, 96/45 (Methods of analysis)—Commission Decision 96/335 (Inventory and common nomenclature of cosmetic ingredients)—Directive 95/17 (Non-inclusion of ingredients in the labeling)—Commission Directive 2003/80 (Symbol of durability), 2004/94 (Annex IX).

CosmetLex, The rules governing cosmetic products in the European Union, 1999: <http://pharmacos.eudra.org/F3/cosmetic/CosmLex>

Volume 1—Cosmetics legislation—Cosmetic products (1999)

Volume 2—Methods of analysis—Cosmetic products (1999)

Volume 3—Guidelines—Notes of guidance for testing of cosmetic ingredients for their safety evaluation (SCCNFP)(1999)

SCCNFP—Opinions, Guidelines and Minutes (<http://europa.eu.int/comm/food/fs/sc>) The Commission Web site contains all the Opinions of the Scientific Committee on Cosmetic and Non-Food Products related to cosmetic ingredients as well as the Notes of Guidance for testing of cosmetic ingredients for their safety evaluation, updated after each SCCNFP meeting.

C—COLIPA (<http://www.colipa.com/>)

- Cosmetic Frame Formulations—EAPCCT & COLIPA, January 2000
- Good Manufacturing Practices (1994)
- Guidelines on Microbial quality management—MQM, August 1997
- Cosmetic ingredients: Guidelines for percutaneous absorption/penetration 1997 (Food Chem Toxicol 37 (1999) 191–205)
- Guidelines for the safety assessment of a cosmetic product, August 1997
- Cosmetic product test guidelines for the assessment of human skin compatibility, 1995 (Food & Chemical Toxicology 34 (1996) 651–660)
- Test Guidelines for the assessment of human skin tolerance of potentially irritant cosmetic ingredients, 1997
- Cosmetic products information requirements in the European Union—Amended guidelines for the cosmetic industry based on article 7A of the Sixth Amendment to the Cosmetics Directive, 1995
- Mutual Understanding 1998—Global Cosmetic Regulatory Harmonization: An Impetus to the Development of Export Markets. Proceedings of an international Cosmetic Industry Congress, April 22–23, 1998 Florence, Italy
- Mutual Understanding 2000. Toward global harmonization of cosmetic regulation. Proceedings of an international cosmetic industry conference. Malta, April 2000
- Guidelines on ingredient labeling in the European Union for the Cosmetic Industry, 1995.
- Guidelines for the evaluation of the efficacy of cosmetic products, 2nd ed., 2001
- The Cosmetic Directive of the European Union—Changes introduced by the 6th Amendment, 1995
- The Seventh Amendment to the Cosmetics Directive: Technical guidance document on its implementation, 23 June 2003
- INCI—Moving forward towards global harmonization for cosmetic ingredient labeling, 1995
- Framework of Principles for Cosmetic Regulation Harmonization (CTFA & Colipa), June 1998

D—E.U. Member States: main cosmetic regulations

Belgium <http://www.fgov.be/>
<http://www.just.fgov.be/cgi/welcome.pl>

France <http://afssaps.sante.fr/>
<http://www.legifrance.gouv.fr/>

Law n°98-535 of the 1st July 1998—Renforcement de la Veille Sanitaire Journal Officiel 2 juillet 1998, Decree 2000-569 of the 23 June 2000.

Germany	http://bgvv.de/ http://www.bundesanzeiger.de/ Verordnung über kosmetische Mitteln dated 7 October 1997 as amended
Italy	BGBI. IS 2410. Law n°713 of the 11 October 1986 as amended specially by Decree of 17 August 2000.
Luxembourg	http://www.etat.lu/memorial
UK	The Cosmetic Products (Safety) Regulations 1996—SI 1996 n°2925 as amended and specially by S.I. 2000 n°2765

4.3. JAPAN (<http://mhlw.go.jp>)

- Pharmaceutical Affairs Law n°145 of 10 August 1960 as amended, especially by the Pharmaceutical Publication n°990 of 29 September 2000 (Cosmetic Deregulation) and the Notification n°1339 dated 28 December 2000 (Cosmetic Claims)
- MHW Notification n°110 of March 1994 on Comprehensive Licensing Standards of Cosmetics by Category as amended
- Pharmaceuticals & Cosmetics Division, Pharmaceuticals Affairs Bureau, Ministry of Health & Welfare—Guide to Quasi-Drug and cosmetic regulations in Japan—Yakuji Nippo Ltd., 1992 and Supplement, 1994
- Principles of cosmetic licensing in Japan, 2nd ed., Yakuji Nippo Ltd., 1989
- Guidance for Cosmetic Safety Evaluation, Yakuji Nippo Ltd., 2001
- The Japanese Standards of cosmetic ingredients, 2nd ed. & Supplements—Yakuji Nippo Ltd., 1985, 1992,
- The Comprehensive Licensing Standards of Cosmetic by Category, Yakuji Nippo Ltd., Part 1 (1986)—2 (1987)—3 (1988)—4 (1989)—5 (1990)—6 (1992)
- Good Manufacturing Practice regulations of Japan, 4th ed., Yakuji Nippo Ltd., 1992
- Quasi-Drug Registration Requirements in Japan, 1st ed., Yakuji Nippo Ltd., 1990

4.4. UNITED STATES

<http://www.fda.gov/>
http://www.access.gpo.gov/su_docs/help/hints/fr.html

- Federal Food, Drug and Cosmetic Act 1938 as amended 1976: VI-Cosmetics—VII-Color additives
- Fair Packaging and Labeling Act (FPLA)—CFR Title 15

FDA-CFSAN (<http://www.cfsan.fda.gov/>)

- Code of Federal Regulations—Title 21: A-Color Additive, D-OTC, G-Cosmetics
- Cosmetic Handbook for Industry, 1992
- Cosmetic Labeling Manual, October 1991
- Cosmetic Good Manufacturing Practice Guidelines

CTFA

- Labeling Manual, 7th ed., 2001
- Microbiology Guidelines, 2001
- Quality Assurance Guidelines, 1992
- Framework of Principles for Cosmetic Regulation Harmonization—(CTFA & Colipa), June 1998

4.5. SOME AUTHORITIES

Andean Community	< http://www.comunidadandina.org/ >
Australia	< http://health.gov.au/ >
Brazil	< http://anvisa.gov.br/ >
China	< http://www.moh.gov.cn/ >; < http://aqs iq.gov.cn/ >
Malaysia	< http://bpfk.org/html/index.htm >
Philippines	< http://www.doh.gov.ph/ >
Thailand	< http://fda.moph.go.th/ >
International	< http://www.europeanchamber.com.cn/ >

4.6. COSMETIC PRODUCERS ASSOCIATIONS

Belgium	< http://www.fedichem.be/ >
Canada	< http://www.cctfa.ca/ >
Denmark	< http://www.spt.dk/ >
European Union	< http://www.colipa.com/ >
France	< http://www.fipar.com/ >
Germany	< http://ikw.org/ >
Japan	< http://www.jcia.org/ >
Norway	< http://www.klf.no/ >
United Kingdom	< http://www.ctpa.org.uk/ >
Sweden	< http://www.ktf.se/ >
Thailand	< http://thaicosmetic.org/ >
United States	< http://www.ctfa.org/ >

4.7. MISCELLANEOUS

Biocides (E.U.)	< http://ecb.jrc.it/ >
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CEFIC	< http://www.cefic.org/ >
Council of Europe	< http://www.coe.fr/soc-sp >
ECETOC	< http://www.ecetoc.org/ >
EPA	< http://www.epa.gov/ >
FEA	< http://www.aerosol.org/ >
IFRA	< http://www.ifraorg.org/ >
ISO	< http://www.iso.ch/ >

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14

Skin Tests

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1. INTRODUCTION

Skin tests are used both to predict and minimize the risk of irritant and allergic reactions to hair care products and to detect irritant and allergic reactions in consumers or those working in the hair care industry.

1.1. Quantitative Risk Assessment

This requires identification of the intrinsic irritation and sensitization potential of a chemical formulation. It requires assessment of all potential hazards and exposures (1,2) both from anticipated use and misuse. This risk assessment should include a prediction of the number of cases of irritation or sensitization that will occur in certain situations of use and/or volumes of sales.

2. PREDICTIVE TESTS FOR IRRITANTS AND ALLERGENS

Estimating the relative strength of a hazard allows one to make a risk assessment, which can then be used in risk management. Predictive tests for irritants and allergens form one component of risk assessment.

2.1. Predictive Testing for Irritants

In general, in humans, one cannot predict the response of one irritant from the response to another. The response is complex. Currently, there are several in vitro methods available to assess the corrosive potential of substances (3,4). Traditionally, the primary assessment of in vivo irritation potential has been the Draize test (5). It is, in general, a poor method for assessing chronic irritant dermatitis potential in humans (6).

Because of this, several exaggerated or repeat exposure models have been developed but, with the realization that testing for humans on humans is more relevant, various tests have been developed to test mild potential skin irritants in human volunteers. So far as possible, tests should try to mirror actual exposure and therefore, for many products, the most appropriate test is that of exaggerated or repeated exposure to the final formulation and/or use concentration of the chemicals to which the population is going to be exposed (7,8).

Response to irritants depends both on the type of skin and formulation of product. Changing the formulation even slightly can alter the relative hydrophilic/hydrophobic balance and the irritant potential of the formulation. Since there is genetic heterogeneity any population sample should be representative of those who will be exposed and should reproduce as closely as possible the likely conditions of use and situations of exaggerated exposure. Single 4 hr and single 48 hr occluded patch tests will provide some assessment of the potential acute irritancy of a product following enhanced contact with the skin. They will not, however, replicate the exposure pattern of an irritant such as a detergent where, in normal use, exposure is likely to be repetitive and where both frequency of exposure and rate of skin recovery may be important variables (9,10). Repeat insult patch testing and cumulative irritancy patch testing to finished products are variations developed to try to assess this (11). Patches containing a standard quantity of material are applied consecutively for 24–48 hr repeatedly for several days varying between 10 and 21 days with readings taken 1 hr after removal of the patch.

This is best performed as a comparative test using the formulation to be tested and a product whose irritancy profile is well known from previous testing and consumer tolerance data. It involves many volunteer visits and an experienced and consistent observer to record the results. A similar system may be used with the repetitive open application test (ROAT) or use test (12).

It is important also, to realize that “reactive” or “sensitive” individuals who may constitute only a small proportion of the normal population need to be included in any test population. Therefore, if one wants to test for neurosensory irritation, one would needs to have a panel of patients who react in this way and test relevant products on these individuals’ facial skin or scalp since these are normally the only sites that exhibit this type of sensitivity. In other situations, such as testing detergents for mildness, human repeat exposure tests such as repetitive hand washing or repetitive exposure of forearm skin including a population with atopic skin disease might be more appropriate. In other situations a human patch test or repeat patch test might be appropriate.

2.2. Testing for Subjective Sensory Irritation/Neurosensory Irritation (13)

Stingers and smarters and other patients with sensitive skin can be identified by questionnaire (14). Such individuals can be invited to participate in a sensitive skin panel. The lactic acid stinging test or the methanol/chloroform or 90% alcohol tests can also be used to identify those who react in this way. Some of these “stingers” may have increased trans-epidermal water loss and impaired barrier function (15) and others will show increased Doppler perfusion following application of methyl nicotinate (16). In our experience the capsaicin test was superior to the lactic acid test.

2.3. Predictive Tests for Sensitization Potential in Animals

Classically, the Magnusson and Kligman guinea-pig maximization test (17) or the Buehler occluded patch test (18) have been used. Test animals are exposed by topical or intradermal exposure or by a combination of the two, sometimes with adjuvant to enhance immune response. Subsequently, the animals are re-exposed to the chemical or

product at the maximum non-irritating concentration with subsequent reading of responses against non-sensitized controls. The sensitizing potential of the chemical or formulation can be assessed on the basis of the number of animals sensitized. Such test results have proven to be historically generally reliable in predicting the sensitizing risk of chemicals, but have not been particularly good at assessing the relative potency of the hazard. In practice, the end point is the frequency of response, rather than the strength of response.

Because of this, several more quantitative tests have been developed including the mouse ear swelling test (19) and the local lymph node assay (20,21). The latter test measures the proliferation response in the local lymph nodes of sensitized animals following re-exposure to allergen (22). It can also be used to measure the relative potency of allergens.

2.4. Predictive Testing for Immediate-Type Reactions

A guinea-pig ear swelling test (23) has been described.

2.5. Predictive Testing for Sensitizers in Humans

Predictive tests of sensitization in humans are more problematic, because of the potential consequences of inducing sensitivity in volunteers. For this reason, animal tests are still required for any new chemical to be used in products for humans although work is continuing on finding non-animal substitutes. As with irritancy testing, it may be more helpful to compare the response with other known sensitizers. Substances can then be shown to be more or less sensitizing than other known products or chemicals. This allows a risk assessment to be made.

The degree of contact allergy can be assessed either by the strength of the patch test reaction or by assessing the lowest concentration to which a sensitized individual reacts (24). The degree of sensitivity is clinically relevant (25–27). The dose-response curve is also important.

2.6. Exposure

The amount of allergen per surface area is a key factor for induction and elicitation of allergic contact dermatitis (28,29). Experimental clinical exposure studies may form the basis for regulation of allergens in the future (30).

2.7. Experimental Human Models to Confirm Sensitization

The simplest tests involve a single open test or repetitive open or provocative use tests. Such tests may be undertaken either on normal or previously affected skin. They are referred to by various terms including open test, use test, provocative use test and ROAT (31). Open exposure tests are suitable for finished products or for ingredients in standard vehicles at non-irritant concentration. Such products or ingredients can be applied to 1–5 cm² of skin on the arm twice daily for up to 2 weeks in patients known or suspected to be allergic to one of the ingredients (32). Such ROATs provide more clinically relevant

patterns of exposure. A positive test may present as itching, follicular papules or diffuse redness with or without infiltration and vesicles. The tests should be compared against either vehicle alone or against some other appropriate control. Readings should be based on both the extent and strength of response. False-negative and false-positive (irritant) responses occur (33).

2.8. Use Test for Rinse-off Products

This is a variation on the above test giving a relatively small degree of allergen exposure, because of the dilution factor and short duration of exposure when using shampoos or other rinse-off products. Studies can be designed with product containing allergen vs. control product without allergen in a sensitized population (34).

2.9. Finger, Hand, Forearm Immersion Tests

There is a need for experimental models that provide quantitative exposure assessments. The finger immersion model (35) and the hand/forearm exposure models allow one to replicate normal use patterns. Exposure levels for some substances can be measured in the skin and nails (36) and could also be used for hair.

The elicitation threshold for allergy will vary not only according to the sensitivity of the individual being tested, the test method and exposure ($\mu\text{g}/\text{cm}^2/\text{surface area}$), but will also vary according to barrier function and “normality” of skin area being tested, and according to the frequency of exposure, presence or absence of occlusion and presence or absence of inflammation or previous reactivity at the site being tested (Table 1) (37).

3. TESTING FOR DELAYED-TYPE ALLERGY

In vitro tests for allergy are only at the level of hazard identification (38). Techniques include chemical structural analysis, structural-activity relationships and in vitro assessment of cellular responses. At present, none of these predicted tests are fully established.

3.1. Diagnostic Patch Testing

This is a well-established technique for diagnosing type IV (delayed) hypersensitivity (allergic contact dermatitis). It makes use of the fact that circulating sensitized T lymphocytes (memory cells) will home into and initiate an allergic/inflammatory response when re-exposed to an allergen even when this allergen is applied to previously unexposed skin.

Table 1 Concentration Threshold for Reactivity to Formaldehyde in Formaldehyde-Sensitive Patients in Different Experimental Exposure Tests

Method	Threshold (ppm)
Repeated (1 week) exposure on normal skin	300
Repeated axillary exposure	150
Finn chamber patch test	150
Repeated patch testing in same area	30
Hand eczema skin immersion (40 min) one patient	0.2

(From Ref. 37.)

Table 2 ICDRG Readings

?+	Doubtful reaction; faint erythema only
+	Weak positive reaction: erythema, infiltration, possibly papules
++	Strong positive reaction: erythema, infiltration, papules, vesicles
+++	Extreme positive reaction: intense erythema, infiltration, coalescing vesicles
-	Negative reaction
IR	Different types of irritant reaction
NT	Not tested

(From Ref. 39.)

Appropriate concentrations of allergen need to be used to avoid non-specific irritant responses and to maximize the chance of eliciting allergic reactions. The patch test employs occlusion to enhance penetration of allergen through normal skin and is standardized in respect to the amount (concentration, volume and surface area) of allergen used and as regards both timing and "reading" of reactions (Table 2) (39,40).

Patients with a history of suspected allergic contact dermatitis are re-exposed to the suspect allergens under controlled conditions (Figs. 1 and 2). A limited number of allergens are available with precise amounts of allergen per surface area in hydrophilic gel (True test) (41). Most patch test allergens are made up in petrolatum at standard concentrations. Most patch test reactions are read on day 2 (after 48 hr exposure) and again at day 3–5 (normally day 4) (42). The 8 mm Finn chamber (Epitest, Helsinki, Finland) is the most commonly used patch test system, but other systems such as the A1-test and van der Bend chamber also exist. All are mounted on acrylate-based tapes, e.g., Scanpore (Norgess Plaster, Kristiansand, Norway). Allergens are available from both Trolab Hermal (Rhinebeck, Germany) and Chemotechnik (Malmo, Sweden). Sometimes allergens have to be made up at appropriate concentration (usually in petrolatum, occasionally in water or other solvent) with samples of the product ingredients being

supplied by the manufacturer (43). Controls are necessary for non-standard allergens. Many products that are intended for leave on use can be tested "as is" but both false-positive and false-negative results can occur.



Figure 1 Application of patch tests.

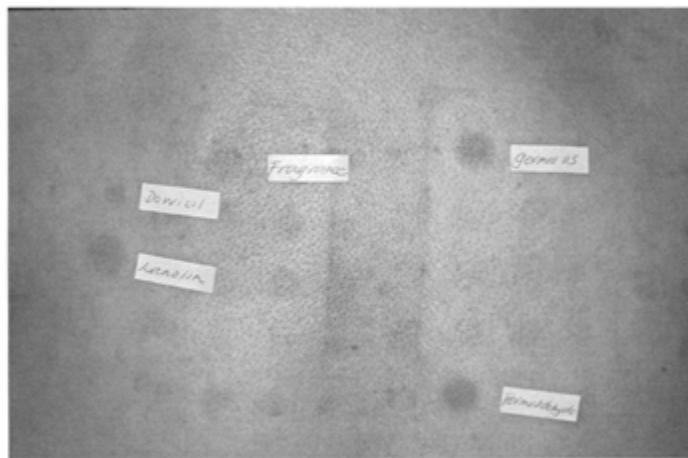


Figure 2 Positive allergic reactions as seen at day 2–4.

For liquids, patch testing is usually undertaken with 15 µL of the allergen at an appropriate concentration applied to a filter paper inside the test chamber.

The preferred test site is the upper back or upper outer arm. The position of tests can be marked with marker pen or with 15% dihydroxyacetone solution.

When testing with potential allergens, one must discriminate between "pure" allergens to confirm the actual allergen causing the reaction and "actual exposure" allergens when

the chemical represents the actual chemical to which the patient was exposed, since sometimes, the allergen may be an impurity, metabolite or degradation product.

3.2. Patch Testing for Suspected Allergic Reactions to Hair Products

In most cases a suspicion of allergic contact dermatitis will have arisen because of an association in time and site of dermatitis, coupled with known or suspected exposure to an established allergen. Patients with suspected adverse reactions to hair care products need to see a dermatologist and, once the acute reaction has settled, to be patch tested to a standard series (44,45) plus a hairdressing series (Table 3) plus any pro-

Table 3 Amersham Hairdressing Series

Substance	Concentration/vehicle
<i>p</i> -aminodiphenylamine	0.25 pet
<i>o</i> -nitro- <i>p</i> -phenylenediamine	2.0 pet
<i>p</i> -Toluene diamine sulfate	1.0 pet
Resorcinol	1.0 pet
Ammonium persulfate	1.0 pet
Hydroquinone	1.0 pet
Ammonium thioglycollate	2.5 aqu
Glycerol thioglycollate	1.0 aqu
Zinc pyrethione	0.5 pet
Hydrogen peroxide	3.0 pet
Pyrogallol	1.0 pet

Table 4 Recommended Concentrations for Testing Hair Products

Products	Test concentration and vehicles
Bleach	Ammonium persulfate 1% petrolatum
Depilatory	Thioglycollate 1% petrolatum
Hair dyes	2% aqueous
Permanent wave solution	Glycerol thioglycollate 1% (freshly prepared) aqueous
Shampoo	Soap or detergent 1% aqueous
Straightener	Do not test (NaOH)
Leave on product	As is

(From Ref. 43.)

duct or ingredients that are suspected at appropriate concentrations. The product can be tested as is, if it is not irritant and if the product is designed for leave-on application on the skin. Otherwise products will require appropriate dilution, again at an appropriate concentration (Table 4) (43).

As the commonest allergen is usually the hair colorant, it is recommended to include the actual dye used by the client. This will not only confirm or refute allergy, but can also be used to identify any cross-reacting sensitivities. Patients should be informed of the result and given advice about any future risk. In a series of 88 hair dye-sensitive patients, Cronin (46) found 94% positive to paraphenylenediamine (PPD), 45% positive to paratoluene diamine and 31% to ortho-nitro PPD.

3.3. Interpretation

In many cases, the assistance of a dermatologist may be needed to help differentiate between constitutional, irritant, or allergic eczema. Patch testing also needs to be undertaken by someone with appropriate training and expertise. It is an aphorism that any fool can apply a patch test, but that it takes many years of experience to be able to interpret them correctly. The interpretation of patch test results is by no means straightforward, especially when it comes to differentiating between allergic and irritant responses (Figs. 3 and 4) and determining relevance.

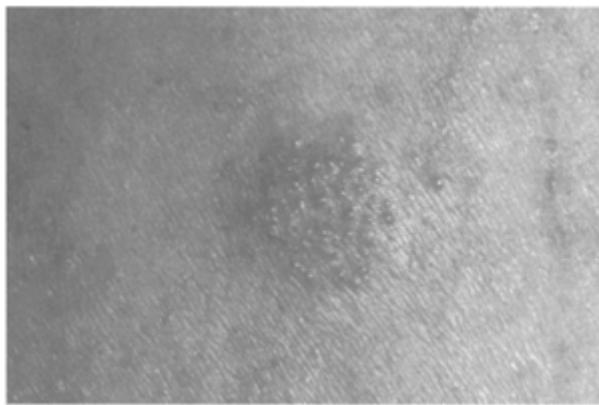


Figure 3 Typical allergic patch test reaction.

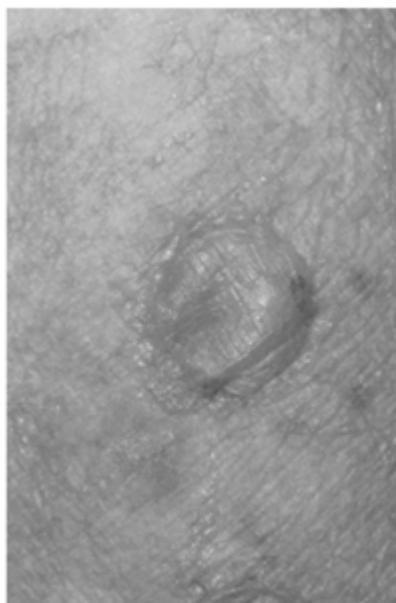


Figure 4 Irritant (bullous) reaction to a product or chemical applied at an inappropriately high concentration.

Where new suspected allergens are concerned (47), there may need to be initial open tests (so long as the product can be safely applied to the skin) with subsequent patch testing of product and ingredients (at various concentrations and possibly in different vehicles). Testing in controls (non-exposed individuals) to exclude irritancy will also be necessary. Repeat open application tests may also be needed. Sometimes, structure-activity relationships or animal testing can predict potential allergens. Chemical analysis may also sometimes reveal unsuspected allergens or impurities.

Stronger reactions and reactions occurring at lower concentrations are more likely to be relevant and reproducible. Erythema only or weak follicular or papular reactions are particularly difficult to interpret. There is no morphological way to distinguish between weak allergic and weak irritant responses. Histology and immunocytology is also of very limited value in separating those reactions that are clinically confusing. Where there is doubt the tests may need to be repeated with an increased range of allergen concentrations or with ROAT tests in both exposed persons and controls.

3.4. Cross-Reactivity

Individuals sensitized to one chemical may on occasion react to other similar or closely related chemicals. This is likely to be due to allergy to a shared part of a molecule or a common metabolite. An individual sensitive to paraphenylenediamine may not only react to the dye itself, but also to other substances with an amino group in the para position,

such as PTD and PAP, azo dyes, local anesthetics and sulfonamides. Many cross-sensitivities reported in the past may, however, have been due to impurities in the test compounds (48).

3.5. Relevance (49)

Differentiating irritant from allergic reactions and evaluating the relevance of reaction is the most difficult part of the patch test procedure, since this requires knowledge of potential sources of exposure and linking this with both time course and sites and patterns of eczema. For many allergens, there are lists that provide the most common sources of exposure. For cosmetics and some medicaments there may be ingredient labeling available. For many household and industrial allergens, the only information available may be through contacting manufacturers or by chemical analysis of a product. Sometimes the allergy is clearly the cause of a patient's current episode of dermatitis (current relevance); sometimes it may relate to past exposure/ previous episodes of contact dermatitis (past relevance). At other times no relevance can be found (unknown or latent sensitivity). In this latter group, the possibility of non-specific irritant reaction needs to be excluded.

3.6. False-Positive and False-Negative Reactions

These may occur especially when testing with non-standard preparations. Appropriate concentrations may vary significantly according to vehicle, size of patch test chamber, duration of exposure and presence or absence of occlusion. Standardization of test procedures deals with many of these variables, but patch testing is still a compromise between finding a concentration of allergen that detects most cases of clinically relevant allergic contact dermatitis, whilst minimizing the number of non-specific irritant reactions (Tables 5 and 6). This has been well documented for chromate (50). When there is only one strong reaction and several weaker reactions or when there are many positive reactions (angry back) it is normally recommended to retest the weaker reactions separately at some later stage.

Some allergic reactions only seem to occur with repeated exposure or when the allergen is reapplied to previously sensitized sites or when there are additional enhancing factors, such as occlusion, sweating, friction or damaged skin or when there is co-exposure to an irritant (51). For these various reasons and because testing is undertaken on normal rather than affected skin, contact allergens are usually tested at greater than normal usage concentration.

3.7. Potential Complications of Patch Testing

If patch tests are performed to internationally accepted standards then the risk of an adverse reaction is minimal. When testing non-standard substances, start at 0.01–0.1% on easily accessible skin. This way the patient can remove symptomatic patches

Table 5 Causes of False-Positive Patch Test Reactions (Irritant)

-
- Tested substances irritant
 Too high concentration of allergen
 Vehicle in which allergen is tested is irritant
 Patient with excited or hyperactive skin, e.g., those with active or recent eczema
 Uneven dispersal of allergen
 Compound irritancy, e.g., irritant only when two or more marginal irritants are combined
 Mechanical irritation/pressure
 Artifact
-

Table 6 Causes of False-Negative Reactions

-
- Too low concentration of allergen
 Poor penetration of allergen
 Inappropriate vehicle
 Insufficient volume applied
 Inadequate occlusion
 Too short duration of exposure
 Delayed reactivity, e.g., neomycin, corticosteroids
 Hypo-reactivity of test site
 Prior exposure to UVL
 Concurrent or prior exposure to topical steroids
 Concurrent use of oral steroids or other immunosuppressants
 Missed allergens
 Impurity (not present in “pure” test compound)
 Allergen is a metabolite
 Compound allergy
-

easily. Increase subsequently to 1% if no reactions were obtained at lower concentrations in a range of subjects. The commonest complications of patch testing are given in Table 7.

3.8. Flare Up and Re-test Reactivity

Flare up of former allergic contact dermatitis sites and patch test reaction sites are sometimes seen following re-exposure to allergen. This may occur either because of locally retained allergen (52), which may remain in the skin for up to two weeks or it may be due to specific memory T-cell retention at these sites, which may persist for several months (53,54).

3.9. Open Test for Delayed-type Reactions

An open test is the application of product or ingredient or commercially available allergen (at appropriate concentration and in an appropriate vehicle) to normal skin without occlusion. Any substance applied in this way should be one intended for use on the skin, scalp or hair and any inherent irritancy should have been excluded prior to use. If in doubt one should check the pH and consult the manufacturer for advice in light of their standard toxicological tests and, if necessary, start with very low

Table 7 Complications of Patch Testing

Irritant reactions, e.g., from non-standard preparations
Flare up reactions at previously affected sites
Patch test sensitization, especially with plant allergens, woods, PPD
Allergens applied at too high a concentration
Too many closely related allergens tested simultaneously
Pigmentary change including depigmentation with phenols
Scarring (rare)
Persistent reactions, e.g., gold and mercury salts
Anaphylactoid reactions (very rare)

concentrations in controls. The application site should be checked at regular intervals during the first 2 hr and then again over the next few days. This test is often undertaken in advance of patch testing with products or substances with which one is unfamiliar. Unknown substances and caustics should never be applied to the skin.

3.10. Use Test or Repeat Open Application Test (31,55)

These tests are intended to mimic the actual use conditions of a product intended for use on the skin. They may be undertaken on normal or previously affected skin. They are often used to investigate the clinical relevance of a positive patch test reaction. ROAT tests are normally applied to a 5 cm area of skin on the back or upper inner arm twice daily for up to 15 days (or until there is a positive reaction). Although there may be some qualitative differences, and although ROAT tests may become positive sooner in cases of allergic reaction, they do not completely differentiate between allergic and irritant

response, but they do confirm that the actual product can cause dermatitis under normal or exaggerated use situations.

4. PHOTO-PATCH TESTING

Photosensitive reactions can be caused by cutaneous exposure. They may be either photo-allergic or phototoxic. Some compounds may cause both types of reaction. In some cases the mechanism still remains uncertain.

Photo-patch testing is primarily a tool for assessing cutaneous contact photo-allergy. The clinical spectrum of disease due to cutaneous photosensitivity may include acute phototoxic or photosensitivity reactions, chronic actinic dermatitis and persistent light sensitivity in association with contact dermatitis or photo-contact dermatitis. Clinically, it may be difficult to differentiate between these diseases and sometimes more than one condition may co-exist.

4.1. Contact Photo-Allergens (56)

Classically these have, in the past, included halogenated salicylanilides (57), musk ambrette (58) and 6-methyl coumarin. Recently, most cases of photo-contact dermatitis have been related to sunscreens and fragrances.

Photo-contact allergens, once identified, are usually removed from the market. Most new ingredients will have been screened for potential photosensitivity and contact photosensitivity and this is therefore an uncommon problem now, at least in western Europe. Some patients, previously sensitized, continue to exhibit persistent light sensitivity even in the absence of current allergen exposure.

The photo-patch test series, therefore, is constructed not only to identify photo contact allergens, but also to detect those allergens that may mimic photosensitivity.

Various series of allergens for photo-patch testing have been published by the Scandinavians (59), the Germans (60), and the British (61).

4.2. UVA Dose

Most photo-allergens react to UVA 315–400 nm. In general 5 J/cm^2 or half median erythema dose for UVA (0.5 MEDUVA) is the recommended dose used for testing (range 1–15 J). The dose for very photosensitive patients will need to be much lower and these patients should be light tested before they are photo-patch tested. At 5 J/cm^2 phototoxic reactions may occur (62). For some substances, a dose of UVA as low as 1 J/cm^2 may suffice (63). Patients being photo-tested usually also have standard patch tests applied to the standard European battery and to a cosmetic/facial series. The two photo-patch test series are applied in parallel with one series exposed to UVA after 48 hr and with the other series completely protected from light. Additional substances may be added to include any suspect ingredients (at appropriate concentration) and any other possible contactants (products).

Patch test readings are undertaken as for any other patch test at both 48 (2-day reading) and again 2 or 3 days later. A reaction occurring in both “exposed” and

“control” panels indicates simple contact allergy, a reaction occurring only on the exposed panel indicates photo-allergy (or photo toxicity). A reaction in both panels but greater on the exposed side may represent both allergy and photosensitivity.

Sometimes some variation in technique or reading is required (60) as well as testing dilutions to help differentiate phototoxic from photo-allergic reaction (64).

5. SPOT TESTS AND CHEMICAL ANALYSIS

5.1. pH

Since strong acids or alkalis can damage the skin, for any non-standard aqueous chemical or product that is to be applied to the skin, assessment of pH can be done with pH paper. Strongly acidic or alkaline solutions should not be applied to the skin. Sometimes the product or allergen can be “buffered”, but even products or substances with a neutral pH can be irritant to the skin.

5.2. Spot Tests

These are particularly useful in detecting the presence of formaldehyde (65). Even the manufacturer may not be aware that it is already present in many detergents that have been transported from the manufacturing facility. Other useful spot tests include the dimethylglyoxime test for nickel (66).

Other techniques to identify the presence of an allergen in a product include thin layer chromatography, gas chromatography, high-performance liquid chromatography, atomic absorption spectrophotometry, inorganic spectrophotometry, UV spectrophotometry, infra-red spectrophotometry, mass spectrophotometry and nuclear magnetic resonance spectrophotometry. Readers are referred elsewhere for further details (67).

5.3. Testing for Immediate-Type Reactions

In immune-contact urticaria the allergen is usually a naturally occurring peptide or protein derivative. Protein additives in products such as hair conditioners include wheat, soya, and silk. Natural rubber latex in gloves used by hairdressers for protection from wet work and chemicals also causes immune contact urticaria. Persons sensitized to natural rubber latex may have an allergy to cross-reacting fruits such as banana, lychee, kiwi, etc. Furthermore, they are at risk of reactions from many rubber items in everyday use and in health care environments.

5.4. Open Application Test (Open Patch Test or Skin Provocation Test) for Immediate Hypersensitivity

The open application test can be used to elicit both immune contact reactions and non-immune contact reactions (68). The tests can be undertaken on normal or previously affected skin (69). Sometimes, it is necessary to test on the cheeks since some reactions have only occurred on the face (70).

Open tests in humans involve applying 0.1 mL of the suspected allergen/ product over a 3-cm² area on the volar aspect of the forearm. The skin is assessed at 20-min intervals over 1 hr. A ROAT can also be undertaken (71). Wheal and flare reactions are regarded as positive and erythema alone as equivocal. Positive reactions will appear in 15–20 min and may last several hours. Sometimes a vesicular eczematous response is seen rather than the usual wheal and flare.

For suspected non-immunological contact urticaria (NICU) 0.1–0.3 mL of the test substance is applied to 1 cm² area of skin. The cheeks, neck or upper back are the most sensitive sites (72). Non-immunological reactions tend to develop more slowly than allergic ones. A 30-min observation period is usually sufficient but observation up to 1–2 hr may be required (73). Positive results can be assessed by redness, edema, wheal and flare or by laser Doppler. Testing needs to be undertaken also in controls.

5.5. Rub Test

This is in effect an exaggerated open application test with suspect material gently rubbed into normal or affected skin.

5.6. Scratch Test (74)

Again, this is an exaggerated form of open test and interpretation of results may be difficult. A 5-mm scratch across the surface of the skin (avoiding causing any bleeding) is made by lightly stroking with a hypodermic needle with the subsequent application of suspected allergen.

5.7. Scratch-Patch Test

This is performed as the scratch test but the suspected allergen is placed in a Finn chamber to give an occlusive effect for 15 min. Again, interpretation of results is difficult.

5.8. Chamber Test

This test is undertaken with occlusive Finn chambers alone in the absence of any trauma to the skin. It seems that some type I allergies are best detected by prick testing and others by occlusive chamber test. Some patients react to one or the other or both (75,76).

5.9. Prick Testing

This is the quickest and most convenient way to detect immunoglobulin E (IgE)-mediated allergy. It is normally conducted only in hospitals where medical support is available. There are a large number of commercial allergens available but raw ingredients of protein material can be used. A drop of each allergen is applied to the skin 3–5 cm apart, usually on the volar aspect of the arm. The skin barrier is penetrated with a special lancet, e.g., Dome Hollister Steer prick test lancet. Histamine is used as a positive control with saline or base solution as negative control. At 15–30 min the diameters of the wheals are

measured (longest diameter and diameter perpendicular to it). Reactions greater than 3 mm or at least 50% greater than the diameter of the histamine control are regarded as positive (77).

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15

Adverse Reactions to Hair Products

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1. INTRODUCTION

The range of physical and chemical treatments used on hair is now very large and new treatments are continually being introduced. Nater and de Groot (1) list seven categories of hair product that may cause adverse effects on the skin (Table 1).

Reactions to hair products may range from subjective neurosensory irritation through transient erythematous or contact urticarial reactions, to irritant and allergic reactions or caustic burns when chemicals are applied inappropriately. Damaging effects may be seen on the hair shaft and on the skin. Rarely, severe reactions including anaphylaxis may occur.

2. HAIR TRAUMA

Traumatic injury to the hair may be either physical or chemical.

2.1. Physical Trauma

Excessive traction or physical trauma may not only cause temporary hair loss and hair breakage but, if continued over a long period, will also lead to permanent hair thinning. Different hairstyles lead to recognizable patterns of hair loss: either marginal, linear, frontal, or fronto-parietal. Tight braiding, hot combing (2), waving, excessively tight rollers or tight hairstyles are usually to blame. Traction alopecia occurs readily in those predisposed to androgenetic hair thinning where prolonged tension may induce follicular inflammatory changes leading to scarring alopecia.

2.2. Chemical Trauma

Substances used for permanent waving, straightening, and bleaching of hair disrupt the cross-linking chemical bonds in the protein molecules of hair keratin. When used for too long, too frequently, or at excess concentration, they will cause loss of elasticity with the hair becoming brittle and breaking easily (Fig. 1). The skill and experience of the hairdresser in the correct use of the chemical solutions and a knowledge

Table 1 Hair Chemicals Causing Adverse Reactions on the Skin; % Positive to Product (or Any Ingredient) on Patch Testing in Hospital Clinics

Shampoo	2.4–9.9
Hair conditioner	0–3.9
Hair spray	1.5–4.9
Permanent wave	0.5–5.2
Hair straightener	0–3
Hair colors	2–14.9
Growth promotion	0.3–1.6

(From Ref. 1.)

of the client's hair is essential to prevent such accidents, which can normally be regarded as errors of technique. The roots and base of the hair are not normally damaged in such situations and the hair will grow out normally over subsequent weeks or months leaving no permanent damage.

Patterns of hair damage that can be seen under the microscope include trichorrhexis nodosa (3), where the cuticular cells become disrupted allowing cortical cells to splay out and form nodes following hairdressing procedures (3–5). Some congenital hereditary defects of the hair shaft predispose to damage. The pattern of trichorrhexis nodosa is normally proximal in Negroes (at areas of greatest trauma) and distal in other races. There may be other incidental clinical findings including dryness, dullness, and brittleness of hair, in particular when applying hydrogen peroxide on hair previously colored by metallic salts.

Other changes that may be seen include trichoclasis (fracture of hair) and trichoptosis (split ends). Both may occur as a result of excess weathering and trauma to hair. These changes may be seen in both congenital and acquired fragile hair conditions. "Bubble hair" has also been reported following cosmetic trauma (6), particularly if the hair dryer is too hot or held too close.



Figure 1 Damage to hair following use of permanent waving solution.

3. BIRD'S NEST HAIR

Mild degrees of irreversible tangling and matting (7) of hair is quite common. Sudden severe matting has been reported under the title of "Bird's nest hair" (8) after use of a cationic detergent shampoo. In this situation the hairs are welded together in a viscous mass and have to be cut off. This process of "felting" can be reproduced experimentally. It is more likely to occur when undiluted shampoo is applied direct to the hair. There is no evidence that the subjects affected have especially susceptible hair and they can often use the same shampoo again without the same phenomenon developing.

4. COLOR ACCIDENTS

Although color rinses only temporarily attach to the outside of the hair shaft, unexpected reactions may occur if the hair shaft has been damaged by prior permanent waving, bleaching or following exposure to sun and sea water. This may make the hair more permeable and an anthraquinone or azo dye may then penetrate into the hair and produce an unexpected color (9). The majority of unexpected coloring, however, is the result of metal salts such as copper turning the hair green (Fig. 2). This occurs particularly in those with blonde hair, after exposure to chlorinated pools, algaecides or when new copper piping has been fitted in the home (10,11). These deposits are not removable by shampooing, but sequestering agents, such as EDTA, can be used. Chinoform (iodochlorohydroxyquine) may also cause white hair to turn red in sunlight. Photo-

pigmentation may also occur around the scalp margins if photosensitizing or phototoxic chemicals are applied to the hair.

5. ADVERSE REACTIONS ON THE SKIN

Irritant reactions may be subjective, transient, non-eczematous, eczematous or, rarely, may include chemical burns (Table 2). Allergic reactions may be immediate or delayed (Table 3).



Figure 2 Green discolouration of hair.

Table 2 Spectrum of Irritant Responses

Subjective	Stinging, smarting, burning, etc.
Transient	Non-immune contact urticaria
Non-eczematous	Dryness, irritant reaction
Eczematous	Irritant contact dermatitis acute/chronic
Caustic	Chemical burns

Table 3 Allergic Responses

Type I	Immediate, IgE-mediated responses
Type IV	Delayed lymphocyte-mediated responses

5.1. Skin Penetration

Both irritants and allergens must penetrate through the stratum corneum and into the skin to have an effect (12). Following application, substances may be metabolized or undergo changes in concentration and structure during their passage through the skin. The skin barrier may change with time and may be influenced both by duration of exposure and by previous treatment. On the scalp there is an alternative pathway for penetration through

the hair follicles and sweat glands and this pathway may be more important for molecules exhibiting relatively slow rates of percutaneous absorption. The skin on one site, e.g., the backs of the hands, may be much more vulnerable to irritants than skin elsewhere, e.g., the palms, due to variation in thickness of the stratum corneum.

In order to be absorbed, a compound must be released from its formulation, encounter the skin surface, penetrate the stratum corneum, and diffuse through the epidermis into the dermis. The principle factors affecting diffusion are the concentration gradient, the path length, and the diffusion coefficient. At any step in this process, the compound may bind to another compound or may be metabolized. Metabolism probably plays a significant role in determining the fate of many topically applied compounds.

The stratum corneum is the main barrier to percutaneous absorption. It is composed of 80% proteins and low molecular weight polar compounds with 20% intercellular lipids (ceramides, cholesterol, and long chain free fatty acids). The lipid content forms an important part of the skin barrier with small non-polar (lipophilic) molecules penetrating more easily. Any disorder of keratinization may adversely affect barrier function. Once an irritant has overcome the skin barrier it facilitates its own penetration and this amplifies the damage (13). The influence of the carrier medium on percutaneous absorption is complex. In general, it depends on the physicochemical interaction between the compound and its carrier, and the carrier and the skin surface. Both higher concentrations of a substance and a vehicle that is more damaging to the stratum corneum, will result in a higher than expected penetration.

Irritants produce both inflammatory and non-inflammatory toxic reactions, through direct effects on the nerve endings (as with some neurosensory irritation) or by means of keratinocyte damage, and release of inflammatory mediators and cytokines. T-memory cell function is not involved. Different chemicals cause varying irritant responses (Table 4). There is often marked inter-individual variation (14).

Table 4 Factors Affecting Irritant Responses

Nature of the substance
Concentration
Frequency of exposure
Environmental conditions
Site
Individual predisposition, e.g., atopy, pre-existing skin disease

Other than for chemicals at the extremes of pH, predicting irritancy potential is difficult. For low-level irritants, repetitive exposure tests are often necessary.

The scalp is relatively resistant to irritant damage, because of its relatively thick epidermis and horny layer, and rapid epidermal turnover. The forehead and scalp margins, however, may be more at risk and those with pre-existing eczema or dermatitis will also be more susceptible to irritant reactions. Irritant reactions often present initially with symptoms of burning, soreness or "tightness" of the scalp or scalp margins, with more extreme exposure leading to erythema, edema, exudation, and damage to the hair. Misuse of thioglycollates, alkali-based relaxers, bleaching preparations, and heat are the

main problems. Irritant dermatitis normally affects only the skin that has been in direct contact.

5.2. Subjective Sensory Irritation (Neurosensory Irritation)

Subjective sensory irritation only affects certain individuals. It is the most common type of irritant reaction to cosmetics, toiletries, and hair products (15), especially as regards products used on the face, neck, and scalp. Symptoms include immediate and delayed stinging, smarting, and burning. Delayed stinging normally occurs 1–2 min after application, peaks at 5–10 min, and fades by 30 min. There is little or no inflammation and the response is best considered as a form of pain response. There is significant inter-individual variation but reactions are usually reproducible. Chemicals known to have caused this type of reaction are listed in Table 5. Penetration may be one important factor. An increased trans-epidermal water loss can be detected in some stingers (17) and increased Doppler perfusion has been noted following application of methyl nicotinate (18). Tests include the lactic acid stinging test, the capsaicin test (19), and ethanol/methanol or chloroform/ethanol tests. The frequency of this type of reaction in the general population for the scalp is shown in Table 6 (L’Oreal data).

Table 5 Chemicals Known to Cause Stinging

Immediate stinging	Delayed stinging
Alcohols (100%)	Phenol 1% in ethanol
Acids: ascorbic, acetic, citric, sorbic	Salicylic acid 5% in ethanol
	Resorcinol 5% aqueous
	Propylene glycol 100%
	Lactic acid 5% aqueous
	Sodium hydroxide 1.3% aqueous
	2-Ethoxy ethyl- <i>p</i> -methoxycinnamate 2% in ethanol

Table 6 Frequency of Subjective Sensory Irritation of the Scalp

Question	% Total	% Male	% Female
Do you regard yourself as having a sensitive scalp?	23.5	25.4	23.35
Do some hair products make your scalp itch, sting, or smart?	30.6	23.00	31.6

5.3. Transient Irritant Responses

Non-immune contact urticaria (NICU) and other non-immunologic immediate reactions (NIICRs) of the skin are inflammatory reactions occurring within seconds up to 1 hr. They normally disappear within minutes to a few hours. No prior sensitization is required. There is variable susceptibility amongst individuals (20). Intensity varies

according to concentration, vehicle, site, area, substance, and mode of exposure. Reactions range from the subjective (such as itching), through erythematous and urticarial reactions to generalized urticaria. Both non-immune and immunemediated contact urticarial reactions may develop into a more persistent eczematous reaction when the stimulus is repeated. The contact urticarial syndrome, with local and more generalized urticaria, conjunctivitis, rhinitis, asthma and, sometimes, anaphylaxis has also been reported (21). Chemicals reported to give such reactions include ammonium persulfate (22), formaldehyde, alcohols, benzoates, sorbates, camphor, nicotinic acid esters, tar extracts, and some fragrance materials (23).

Non-immune contact urticarial reactions are not inhibited by antihistamines, but are blocked by non-steroidal anti-inflammatories and there is also some inhibition following exposure to ultraviolet radiation and topical anesthetics. Prostaglandin D₂ is thought to be an important mediator (24). There is often a degree of tachyphylaxis, i.e., reduced response on repeated exposure.

Substances can be tested for their non-immune contact urticarial potential using the guinea-pig ear-swelling test (25).

5.4. Irritant Reaction

Sometimes a low level of repetitive exposure to irritants leads only to an irritant reaction with somewhat monomorphic dryness or redness of the skin. This is normally seen on the hands of those frequently exposed to water, detergent or solvents (Table 7). Sometimes this reaction persists almost indefinitely, as in some hairdressers. At other times, hardening may develop and the reaction resolves. At other times, the condition progresses to an irritant contact dermatitis. A mixture of

Table 7 Substances Causing Chronic Irritant Dermatitis

Water
Surfactants
Emulsifiers
Alkalies
Acids
Solvents including alcohols and propylene glycol
Oxidizing agents including hydrogen peroxide
Reducing agents including thioglycolate
Plant extracts including citrus

Table 8 Endogenous Factors for Development of Irritant Contact Dermatitis

-
- Individual susceptibility, e.g., atopy/atopic status
 Pre-existing eczema/dermatitis and/or active eczema elsewhere
 Recent “deconditioning” exposure, e.g., prior damage to skin barrier
 Other constitutional factors including impaired barrier function
-

Table 9 Exogenous Factors for Development of Irritant Contact Dermatitis

-
- Inherent irritancy of substance
 Amount of irritant in contact with the skin
 Duration of contact
 Presence/absence of occlusion
 Body site
 Mechanical factors, e.g., friction
 Climate, season, humidity
-

endogenous and exogenous factors normally determines whether irritant dermatitis develops. These are listed in Tables 8 and 9.

5.5. Acute Irritant Contact Dermatitis

This is often the result of a single overwhelming exposure to an irritant. The clinical presentation may vary from just some surface dryness/wrinkling, through transient erythematous, urticarial, or eczematous responses to caustic burns. Histologically, the appearances may be very variable (26).

5.6. Chronic (Cumulative) Irritant Contact Dermatitis

This normally develops as a result of repetitive exposure especially to surfactants, solvents and wet work.

5.7. Chemical Burns

These are normally induced by highly alkaline or acid compounds, e.g., sodium hydroxide. These agents can cause severe damage to the skin. The reaction usually develops within minutes to some hours, often with painful erythema, wealing, vesication, blisters, sores, and skin necrosis which may lead to scarring, hyper and hypo-pigmentation, and hair loss.

6. THE ROLE OF EPIDERMAL CELLS, CYTOKINES AND OTHER FACTORS IN IRRITANT AND ALLERGIC CONTACT DERMATITIS

For many years it has been known that lymphocytes and Langerhans' cells (LCs) have a central role in the induction and elicitation of allergic contact dermatitis (27). It is only more recently that it became clear that keratinocytes and the immune system also play an important role in irritant contact dermatitis (28,29). It has now been shown that keratinocytes can produce a variety of cytokines and other factors including IL-1, IL-6, IL-8, IL-12, IL-19, granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF- α), and transforming growth factor beta (TGF- β). Keratinocytes can also be induced to express major histocompatibility complex (MHC) class II molecules, cell adhesion molecules (CAM), and intercellular adhesion molecules (ICAM-1). Expression of these molecules, in conjunction with the release of cytokines, upregulation of E-selectin, and vascular cell adhesion molecules (VCAM-1) on dermal endothelial cells makes the keratinocytes an important cell in the induction and maintenance of inflammation within the skin.

Langerhans' cells compose 2–5% of the epidermal population and are located in the suprabasal layers of the epidermis. This dendritic cell expresses CD-la and MHC class II molecules. It has the capacity to pick up antigen then migrate to proximal lymph nodes where the antigen is presented to T lymphocytes. LCs also secrete cytokines such as IL-1 β , IL-6, IL-10, IL-12, and TNF- α required for induction of allergic contact dermatitis and immuno-surveillance of skin. Dermal antigen presenting and immune-modulating cell subsets exist which include macrophages and dendritic cells.

T lymphocytes in the skin are involved in the regulation of the inflammatory process, and in allergic contact dermatitis. In allergic contact dermatitis there is upregulation of E-selectin encouraging T helper cells with cutaneous lymphocyteassociated antigen (CLA) surface marker to migrate to the skin.

6.1. Differentiation Between Irritant and Allergic Contact Dermatitis

There are only minor differences histologically and immuno-histologically between allergic contact dermatitis and irritant contact dermatitis (28,30,31). The key difference is the requirement for prior sensitization and immunologic memory in cases of allergic contact dermatitis with an initial production of IL-1 β from LCs being the early initiating event in allergic contact dermatitis. Irritants exert their effect through direct cellular damage to keratinocytes and other cells leading to synthesis and release of cytokines, upregulation of MHC class II and cell adhesion molecules, and infiltration by immunocompetent and inflammatory cells.

Different irritants and different concentrations of the same irritant may have varying effects, both on the cells in the epidermis and on cytokine release. Proinflammatory cytokines include IL-1 α , IL-1 β , and TNF- α . Irritants may damage the stratum corneum both through hydration and disorganization of the lipid membrane structure. Damage to the skin barrier may last for some days or weeks (32).

7. MECHANISM OF ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis is a T-cell-mediated immune response that involves the activation of allergen-specific T cells (33). Most contact allergens are small lowmolecular weight (<400 Da) chemically reactive molecules (haptens). There is both a sensitization and an elicitation phase. After initial contact with an allergen sensitization may develop after a period of some days or weeks, after many months or years or not at all. Once sensitization has occurred, then re-exposure to the allergen will normally elicit an allergic contact dermatitis within 1–2 days (may be up to 14 days with large/poorly absorbed or weak allergens).

Induction of allergic contact dermatitis involves the following phases:

1. Penetration and binding of allergen to protein in the skin.
2. Hapten-induced activation and migration of antigen-presenting cells (LCs) through the lymphatics to the paracortical area of draining lymph nodes.
3. Recognition of allergen-modified LC by specific T cells leading to activation and IL-2-induced proliferation of allergen-specific T cells.
4. Systemic propagation of specific T cells into the efferent lymphatics and blood, with receptor molecules facilitating migration into peripheral tissues.

Elicitation of allergic contact dermatitis follows renewed contact with allergen by antigen presenting cells and specific T lymphocytes leading to release of local cytokines and chemokines, release of proinflammatory mediators, recruitment of further inflammatory cells, and amplification of the local reaction.

7.1. Pro-Haptens

Some potential allergens require activation by light (34), or metabolic conversion or oxidation, e.g. *p*-phenylenediamine has to be oxidized and transformed to its reactive double-ring metabolite Brandrowski's base (35). Increasingly, it is recognized there may also be an important part played by genetic polymorphism (36).

8. IMMUNOLOGIC CONTACT URTICARIA

Less commonly, an immediate, IgE-mediated hypersensitivity (type I hypersensitivity) occurs, mostly to proteins and occasionally to other chemical haptens. IgE antibody is manufactured by hapten-specific B lymphocytes and sensitization may be through the mucosa or through the skin. These IgE antibodies bind to high-affinity FCE receptors on the surface of mast cells and basophils. The reaction is TH2 lymphocyte-dependent and leads to release of histamine and leukotrienes, including prostaglandins, platelet activating factor, substance P, and other agents. Clinically, the reaction leads to contact urticaria, rhinitis, conjunctivitis, asthma and, on occasion, to generalized urticaria and/or anaphylaxis—the contact urticaria syndrome. The most common causes of immunologic contact urticaria are listed in Table 10. In clinical practice, hydrolyzed proteins and natural rubber latex (37) are the most important. Cross-reactivity between various protein groups is common.

Sometimes it is difficult to differentiate between immune and non-immune contact urticaria. For some substances, such as ammonium persulfate, the mechanism remains uncertain or at times both mechanisms may be relevant. Persulfates can

Table 10 Causes of Contact Urticaria

Animal products including hydrolyzed protein
Food including fruit, vegetables, grains, nuts, seeds, sea food, etc.
Fragrances and flavorings
Drugs and medicaments
Metals
Plants and plant products including wheat, soya, silk, chamomile, henna, natural rubber latex
Biocides and disinfectants
Enzymes
Miscellaneous including ammonium persulfate, paraphenylenediamine

cause direct histamine release with contact urticaria and asthma, irritant contact dermatitis, and allergic contact dermatitis (22,38).

9. COMMON CAUSES OF IRRITANT AND ALLERGIC CONTACT DERMATITIS RELATING TO HAIRDRESSING SHAMPOOS

These are in contact with hair and skin for a relatively short time and are then washed off. All surfactants are potentially irritant to varying degrees but, in practice, they are normally only a problem in those who are particularly susceptible, e.g., those with atopic or seborrheic eczema or in situations of excessive usage. Even those with susceptible skins usually tolerate shampoos without problems. When there are problems, it is usually the hands, neck, arms, face, and upper trunk that are affected. Irritancy may present as aggravation of facial seborrheic or atopic eczema, as hand dermatitis or as a discoid pattern of atopic eczema on the arms and upper trunk. In general, the now rarely used cationic shampoos (Chapter 3) are more irritant than anionic, and both amphoteric and non-ionic shampoos have lower levels of irritancy. Consumers may develop eczema of the neck and scalp margins, or on the hands and face when sensitized to one or other ingredient in shampoos, most commonly preservatives or fragrance (Figs. 3 and 4).

Shampoos contain several potential allergens including preservatives (which may already be present in the surfactant) and fragrance along with various "natural" additives, such as hydrolyzed protein, natural triglycerides, plant extracts, waxes, phospholipids, vitamins, etc. Even surfactants may sometimes sensitize. The amphoteric surfactant, cocamidopropylbетaine, is recognized as having caused a number of cases of allergic contact dermatitis in both hairdressers and in the general population (39). The components responsible for allergic reactions may be the final compound itself, or, one of

the substances used in its synthesis (40) which may be present as an impurity such as amidoamine. Although the concentration of biocides in shampoos is low, their frequent use and co-exposure with irritants increases the risk of developing allergic contact dermatitis. The current situation as regards biocide sensitivity as detected in dermatology patch test clinics in Europe is shown in



Figure 3 Allergic contact dermatitis to preservative in shampoo.



Figure 4 Allergic contact dermatitis to fragrance in shampoo.

Table 11 (ECDRG 2000 preservative results). Low concentrations of a biocide, however, may be tolerated in rinse-off products, even by those known to be sensitized (41).

10. PERMANENT WAVING

Traditional perming solutions rarely cause allergy, but may cause irritation, especially if left on the scalp for too long. Dermatitis may sometimes be severe, but is often patchy, relating only to those areas of excess exposure. The reaction usually develops within a couple of days, often after some hours, and presents with itching, burning, redness, swelling, and oozing or crusting. In mild cases, only redness and scaling may be seen.

Waving (or straightening) can be achieved by a variety of techniques. A temporary effect can be achieved using just water and heat, or steam in conjunction with rollers but, for more long-lasting effect, it is necessary to induce physical and chemical changes in the hair. The hair is softened by loosening the keratin cross-linkages, re-aligning them, and the process reversed by means of hardening. Thiol-based reducing lotions, e.g., thyoglycollates are used for the first step. Then, after thorough rinsing, the imparted shape is made permanent by applying dilute hydrogen peroxide solution. A typical "cold" wave lotion contains ammonium thioglycollate plus ammonia or mono-ethanolamine. The salts of thioglycolic acid are most commonly used, since the acid itself is a stronger irritant and sensitizer and needs to be neutralized to be effective as a reducer. The main problem, in recent years, has been due to an ester derivative, glyceryl monothioglycollate. Allergy, however, more often occurs amongst hairdressers rather than their clients (42) and the incidence of problems has

Table 11 European Preservative Patch Test Data:
Average% +ve from 16 Countries

Parabens	0.6
Formaldehyde	2.2
Quaternium 15	1.2
Imidazolidinyl urea	0.7
Diazolidinyl urea	0.9
Methyl/chloro isothiazolinone	2.4
Methyldibromoglutaronitrile	3.6

(From Ref. 65.)

reduced since its withdrawal from several European markets. There has been a report of glyceryl monothioglycollate causing contact urticaria (43).

Shaping of hair is normally undertaken with rollers. Skill is required both in use of rollers and in application of the perming solutions.

The reversal of the hair softening process and reformation of disulphide bonds (neutralization) may occur naturally with time (12 hr) but is then usually incomplete, making hair more prone to breakage. The process can be speeded up through crosslinking by the use of hydrogen peroxide, sodium perborate, percarbonate or, in the USA, sodium or potassium bromate solutions.

Permanent waving is potentially damaging to keratin, especially at high pH (44) and there needs to be scrupulous avoidance of contamination of both the skin and the eyes

with the perming solution. Overheating with traditional perming solutions or excess duration of application of chemicals will not only damage the hair, but may also cause irritant dermatitis on the hairline and neck, or the scalp. "Hot combing" is also potentially damaging to hair and may even lead to scarring alopecia. Hair straightening using alkalis, such as soda or potash also requires rigorous avoidance of skin contamination. Neutralizing/oxidizing agents may also cause irritant dermatitis and damage to the hair if not thoroughly rinsed off.

11. PERMANENT DYES

Permanent hair dyes are known to be allergenic. Unfortunately no suitable alternative has been found and, therefore, a degree of sensitization and allergic reactions over the years has been tolerated. Paraphenylenediamine-type hair dyes normally cause type IV (delayed) allergic contact dermatitis reactions, but may also rarely cause type I (immediate) type reactions.

11.1. Immediate Type I Reactions

These may cause itching and local urticaria (contact urticaria) or more generalized urticarial reactions including angio-edema with swelling of face and eyelids. Sometimes there may be asthma-like symptoms and, rarely, even anaphylactic shock. The reaction usually develops within a few minutes and subsides in a few hours. Reactions have been reported with several hair dye compounds (45). There has been the occasional fatality (46,47). A dab test or prick test with the dye, or dye plus oxidant, is normally positive within 20 min.

11.2. Delayed Reactions

Type IV allergic contact dermatitis develops within hours or days of dye application. In its mildest form, there may be just some itching, redness, scaling and vesiculation (eczema) of the ears and scalp margins (Fig. 5), neck (Fig. 6), and/or scalp. The reaction may be severe (48) with considerable edema of face and eyelids (Fig. 7), or a florid eczema of the face and scalp. Rarely, the reaction may become generalized. Systemic steroids are usually required. Exudation causes crusting and matting of the hair. It may take several weeks for the allergic reaction to settle; partly because of its intensity, partly because of residual dye/antigen remaining on the hair. Some patients will continue to react to their dyed hair, even after several shampoos (personal observation). Frequent washing will help remove any residual unbound dye.



Figure 5 Allergic contact dermatitis to PPD.

Residual dye can be neutralized using Schuellers mixture (sodium chloride 15%, hydrogen peroxide 20 vol).

Patients are left sensitized to "para" dyes and sometimes may also react to other "para" group chemicals such as caines (topical local anesthetic), and azo dyes (49,50).

Contact dermatitis of the scalp itself is less common, which contrasts with the relatively high penetration rate of chemicals at this site. Most reactions occur on the adjacent skin. Occasionally, reactions may be seen in a sensitized partner (connubial or consort dermatitis). Rarely, PPD may cause erythema multiforme-like systemic reactions (51). Dye applied to eyelashes, eyebrows (52), moustaches, or beards (53) can also cause sensitization. Temporary black "henna" tattoos, which have contained high or even illegal concentrations of PPD have also caused sensitization (54,55).



Figure 6 Allergic contact dermatitis to PPD.



Figure 7 Allergic contact dermatitis to PPD.

12. ALTERNATIVE DYES

Once sensitized to one of the permanent dyes, a patient or client is unlikely to be able to use any other permanent or semi-permanent dye safely. The only alternatives are pure natural henna, camomile, metallic dyes, or other hair coloring product not based on oxidative dyes. Work is ongoing to develop F&DC dyes but these currently give only limited color options and do not permanently cover gray hair.

13. PREVENTION

Concern about the sensitizing potential of PPD led to it being banned in certain countries in Europe. However, the EU now allows its use up to a concentration of 6% with appropriate hazard labeling. In reality, most commercial hair dyes contain 2% or less.

Interestingly, since PPD was reintroduced into Sweden, there has been no increase in the overall prevalence rate of sensitivity to permanent dyes, possibly because the related hair dye (PTD) was no more or no less sensitizing (55).

Open, dab or touch test with unmixed colorant to an area of skin behind one ear is recommended 48 hr prior to dying of hair for those previously exposed. A recent study confirms that, at least, for the darker shades, this is an effective way of identifying sensitized individuals (56).

14. INCIDENCE

The frequency of sensitization from permanent hair dyes does not seem to have increased over recent years in spite of steadily increased usage. However in cultures where black hair is the norm and where gray hair is less acceptable, a higher frequency of PPD sensitization is seen (57). Most positive patch tests to PPD are clinically relevant and most sensitized persons have to avoid further contact with permanent hair dyes. There are occasional individuals who, although sensitized, seem to be able to tolerate contact with hair dye (58).

15. SEMI-PERMANENT DYES

Semi permanent dyes, such as *ortho*-nitro-paraphenylenediamine are less reactive with hair proteins and less well bound to hair. They are, therefore, more easily washed out and less allergenic. Para-toluenediamine is said to be less likely to cause allergy than paraphenylenediamine. Patients allergic to paraphenylenediamine will, however, often also react to the semi-permanent dyes. Some patients, however, will react only to one or the other (although the risk of sensitivity broadening to include other dyes is increased). Tone on tone coloring is a variant of oxidative coloring using PPD and PTD at a lower pH resulting in the absence of lightening and limited damage to the hair. It is permanent and thus resists more frequent shampooing. Anthraquinone and azo dyes used as color rinses and in color mousses rarely cause sensitivity (59).

16. VEGETABLE DYES

Henna is widely used in the Middle East and Asia, both as a hair and skin dye. Its active ingredient (lawsone) is an extremely rare allergen. Both type I (immediate) and type 4 allergic dermatitis-type reactions have been reported. "Black henna" always contains an oxidative (permanent) dye. Camomile has also been used as a hair dye. The active agent, apigenin or 4,5,7-trihydroxyflavone is obtained from the flower heads of *Anthemis nobilis* (*Roman camomile*) or *Matricaria camomile* (*German camomile*). Allergy to camomile is reported, but not from its use in hair dyes.

17. METALLIC DYES

Cases of allergy to metallic dyes are extremely rare. One patient has been reported, who was allergic to lead acetate used as a color restorer for gray hair (60). Hair breakage is the main side effect and occurs when oxidants such as hydrogen peroxide are subsequently applied.

18. BLEACHES

Bleaching is undertaken not only for those who want blonde hair, but also to decolorize hair prior to a change in hair color. Bleaches are based on hydrogen peroxide/ammonia mixtures. Persulfates, particularly ammonium, sodium, and potassium persulfate are added for stronger lightening or bleaching. They penetrate the hair and oxidize the melanin in the hair shaft to a colorless compound. They are capable of causing damage to the hair if applied for too long or in too high a concentration (61). Bleached hair tends to be dull and brittle.

Any prolonged skin contact with persulfate can cause burning and redness of the scalp as an immediate irritant reaction. This may progress to an acute dermatitis with exudation, crusting and matting, or breaking of hair (22). This is normally an irritant reaction with negative prick tests and patch tests.

More frequently, there may be transient urticarial-type reactions following contact with persulfates (62). This may involve simply some redness and itching of the scalp and face, sometimes associated with swelling of the face and eyelids. Occasionally, the reaction is more generalized with redness and itching all over the body and with vulval irritation. Affected individuals often complain of drowsiness and may even fall asleep. A few complain of shortness of breath and wheezing. In the most severe situations there may be collapse and unconsciousness with patients being admitted to hospital. Recovery takes place over a few hours and is usually complete.

The signs and symptoms suggest a histamine effect and, indeed, the reaction can be blocked with antihistamines. No specific immunological (IgE) mechanism has been found. Persulfates are not potent histamine liberators in vitro (63). The reaction is usually classified as a contact urticarial reaction of unknown mechanism.

19. OTHER MATERIALS

Mineral oils and brilliantine have, in the past, caused pigmentation and pomade acne. Complaints of dermatitis from hair lacquer are not uncommon, but can rarely be proved. A large number of other ingredients known to be at least potentially dermatitic have been used in hair preparations. Cases of contact dermatitis have been described from some of them, e.g. monoethanolamine (64). In some cases of dermatitis or other adverse reaction, there may have been pre-existing dermatitis of the scalp. This may have been aggravated by hair treatment. Similarly, some clients will attribute varying degrees of hair loss or changes in texture of hair, to hair treatment. Normally, contact dermatitis of the scalp recovers and does not have any permanent effect on hair growth. Nevertheless, some

individuals claim the loss of hair or a reduction in the quality of hair. This is often difficult to prove without evidence of the previous state of the hair.

19.1. Ocular Effects

Accidental contamination of the eyes from shampoo or other hair products may produce inflammation and/or damage to the eye. Normally an irritant conjunctivitis will resolve with time following simply eyewashes. If any chemical hair product gets in the eye, it should immediately be washed out with copious amounts of water and, if necessary, the person should seek advice from an emergency department, or an ophthalmic casualty department. Acute swelling of the eyelids may temporarily interfere with vision. Sometimes a product can be washed out of the hair into the eyes by rain, swimming or profuse sweating. Most hair products will, therefore, usually have had pre-marketing laboratory tests to minimize any risk of ocular irritation.

19.2. Secondary Effects

If individuals develop an allergic reaction as a result of using hair dye, they may become secondarily sensitized to a variety of other substances (cross-sensitization). Contact with any of these cross-reacting substances may then cause dermatitis. Occasionally contact dermatitis from hair dye may occur in the partner of a person who has dyed their hair.

Scarring is not a normal consequence of even the most acute allergic contact dermatitis. It may, however, occur if there has been secondary infection or following severe burns or skin necrosis from caustic chemicals.

19.3. Systemic Effects

Persulfate bleaches are readily absorbed through the skin and can produce local and more generalized urticaria, rhinitis, conjunctivitis, asthma, and on occasion anaphylactic shock. Hair dye can also be absorbed through the skin. The less skilled the technique the more absorption is likely to occur. Other systemic effects and issues relating to potential carcinogenicity of hair dyes are discussed in Chapter 10.

19.4. Unsubstantiated Effects

There have been various unsubstantiated claims about adverse effects following exposure to hair products. These may include emotional, neurologic or other systemic effects. There have been anecdotal reports of nephritis, jaundice, meningeal hemorrhage, fever, and fetal death, but so far there is no medically accepted proof linking such effects with the use of any hair product.

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16

Occupational Disorders in Hairdressers

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1. DEFINITION

An occupational dermatitis is a dermatitis that occurs wholly or partly due to a person's work. Skin disease accounts for approximately 30% of all occupational diseases and most of this presents as hand dermatitis. The incidence of occupational skin disease is 0.5–0.7 cases per 1000 workers per year in the Netherlands (1) and is estimated at 5–19 per 100,000 full-time workers per year in Bavaria (2). Age and sex are not themselves risk factors but are associated with different patterns of exposure. Therefore women, who have a greater exposure to wet work at home and at work as hairdressers, nurses, cleaners, etc., have a greater incidence of hand eczema (3).

Occupational dermatoses may include contact urticaria, protein contact dermatitis, and both irritant and allergic contact dermatitis. Contact dermatitis accounts for 90% of cases (4,5).

In several European countries, statutory collection of employment and occupational disease data allows better monitoring of the rates of occupational disease. Hairdressing has the highest risk of occupational contact dermatitis (6,7) (Table 1). For example, the rate in Northern Bavaria for hairdressers was 97.4 per 100,000 workers compared with 7.3 in health care workers (8) although there was an observable decline in incidence rates over a 10-year observation period (8– 10). In the UK, there is a voluntary reporting system from dermatologists (EPI-DERM) and from occupational physicians (OPRA) (11) and this also showed hairdressers to be the group most affected by occupational dermatitis (120/100,000 per annum). In general, irritant contact dermatitis from wet work, detergents, and irritant chemicals is commoner than allergic contact dermatitis (although both not infrequently co-exist). Atopy, especially those with past or present eczema or hand eczema, predisposes to irritant contact dermatitis (12). Common irritants are listed in Table 2.

As can be seen, many such agents are encountered regularly in hairdressing. Although there is no common European regulation on working conditions, Germany has introduced legislation on wet work under The Technical Rule on Dangerous substances 531 (13). In this wet work is defined as exposure of the hands for

Table 1 Incidence of Contact Dermatitis per 1000 Persons per Year by Occupation

Hairdressers	11–24
Cooks, bakers	6–10
Mechanics	3.4–6.6
Fish and Meat industry	3.9
Cleaners	2.5
Nurses	1.5

(From Refs. 6,7.)

Table 2 Common Irritants

Water
Surfactants
Emulsifiers
Alkalies
Acids
Solvents including alcohols and propylene glycol
Oxidizing agents including hydrogen peroxide
Reducing agents including thioglycollate
Plant extracts including citrus

more than 2 hr a day in a wet environment, wearing occlusive gloves for this time or having to clean the hands frequently and intensively. The regulation demands that assessment of the hazard is made and protection, advice, and medical preventive examinations are provided.

2. SITES

Hands are most commonly involved in occupational contact dermatitis (Figs. 1 and 2); sometimes also the wrist, forearms, and face/exposed skin sites.

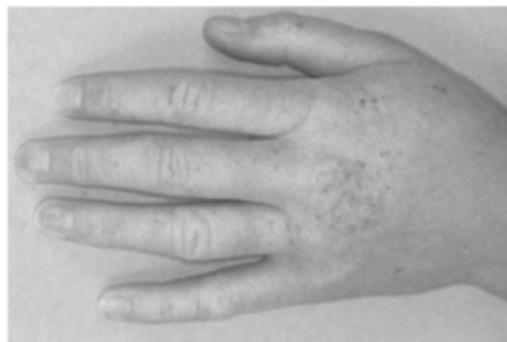


Figure 1 Discoid and patchy hairdressers hand dermatitis. Constitutional, irritant and allergic factors may co-exist.



Figure 2 Allergic contact dermatitis from glyceryl monothioglycollate in perming solution.

3. DIAGNOSIS OF CONTACT DERMATITIS

To establish a diagnosis of contact dermatitis, one needs to relate both the primary site of dermatitis to known or expected exposure to a recognized irritant or sensitizer. The timing of episodes of dermatitis is also important. Deterioration of dermatitis during the working week with improvement over weekends or during holidays is suggestive of occupational contact dermatitis.

Whilst irritant dermatitis is normally restricted to sites of exposure, allergic contact dermatitis may spread to secondary sites or may present in a volatile or air borne distribution (4). Allergens of particular importance in hairdressing are listed in Table 3. Diagnosis is assisted by knowledge of other cases of dermatitis occurring among other

workers and by knowledge of the risk of contact dermatitis in hairdressers as well as sources of allergens and irritants in their work environment.

4. INVESTIGATION

Patch testing is the key investigation as regards diagnosis of allergic contact dermatitis. Patients are normally tested to a standard series of allergens that will pick up approximately 90% of sensitivities. This must be supplemented by appropriate additional occupational series such as the hairdressing series (Table 4) and with appropriate dilution of products used in the workplace.

Table 3 Common Potential Allergens in Hairdressing

Preservatives
Hair dyes
Perming solutions
Fragrance
Nickel
Rubber chemicals and natural rubber latex
Animal and plant extracts

Table 4 Recommended Hairdressing Series

Paraphenylenediamine	1% pet
Nitrophenylene diamine	1% pet
<i>p</i> -Toluene diamine sulfate	1% pet
Resorcinol	1% pet
Ammonium persulfate	1% pet
Glyceryl mono thioglycollate	1% aq or pet freshly prepared
Patient's own hair dye	As is

Sometimes tests of immediate sensitivity may also be required for plant and animal extracts, ammonium persulfate and natural rubber latex in rubber gloves.

5. MANAGEMENT

A visit to the place of work may be helpful (14). A period off work or with the individual employed in a different, no-risk area may also help to clarify the role of occupational

factors. Constitutional factors predisposing to the development of dermatitis (7,15) need to be assessed along with hobby factors and exposures from an individual's personal environment. A suggested approach would include:

1. Identification and, if possible, avoidance or substitution of the allergen to which the person is exposed (16).
2. Use of "closed systems" for mixing bleaching and dyeing products.
3. Use of vinyl instead of rubber gloves, regular use of hand cream or after work emollient and possible use of barrier creams.
4. Treatment of dermatitis, usually with topical steroids.
5. Treatment of secondary infection (if present).
6. If possible, a period off work to allow the skin barrier to recover.
7. Aim to return the person to work (if possible). Otherwise to consider retraining or transfer to alternative employment.

6. PROGNOSIS

The prognosis of those cases of occupational contact dermatitis referred to a specialist is that of persistence in more than 50% with improvement in half of these (17). Ten percent of patients develop persistent post-occupational dermatitis (18). Education improves prognosis (19). In general, the prognosis is better for those with acute (recent onset and short duration) dermatitis rather than those with chronic and established dermatitis. It is also better for those with clearly defined allergies who can be moved to an allergen-free environment. Although this is difficult in a salon, some hairdressers who have problems with perming and dyeing agents can change to only cutting undyed hair. In hairdressing, it is usually the apprentices who undertake much of the hair washing and who may be exposed to the strongest irritants and allergens. Partly this is the nature of the work; partly dermatitis occurs through inexperience and lack of training or lack of care in respect of avoidance of known irritants and allergens (20). Employers can very easily find replacement workers and pressure of customers, lack of knowledge and interest and "disposability" of the work force means that manufacturer's safety precautions are often disregarded. Even when apprentices were followed closely in a study, the incidence of hand eczema rose to 55% by the end of the 3 years apprenticeship (9). Atopics, and those with pre-existing nickel (metal) allergy, are particularly at risk and have a worse prognosis.

Some allergens are so potent that, once sensitized, a hairdresser can never again tolerate even minimal levels of contact with the allergen. This was particularly so with glyceryl monothioglycollate ("acid" or "two part perms"), which, because of its protein-binding potential, became fixed not only to hair but also to towels and brushes, etc.

7. PREVENTION

Those with present or past atopic eczema and past or present hand eczema are at high risk of developing chronic irritant hand eczema and should be discouraged from taking up hairdressing as a profession (21). Those with pre-existing allergy to nickel (metal) may

also be at increased risk (22,23). The dimethylglyoxime spot test can be used to identify nickel items in hairdressing salons and the offending items can be replaced with a suitable alternative. Those known to be already allergic to one or more hairdressing chemicals should also avoid an occupation in hairdressing (24).

Vinyl or plastic gloves should be worn when handling potentially sensitizing chemicals such as perming solutions or dyes. It is usually impractical to routinely wear gloves for washing hair and customers and salon owners often object. Care must be taken to avoid spills and these must be wiped up while wearing gloves with disposable cloths.

8. SPECIFIC RISKS IN HAIRDRESSING

Dryness of the skin of the hands, chronic irritant reaction and low-grade chronic irritant dermatitis from chronic wet work and the irritant and degreasing effect of shampoos is almost universal. Those with the greatest exposure and intensity of exposure will be most at risk of developing contact dermatitis as will those who are already predisposed to the development of eczema. Those with an already damaged stratum corneum skin barrier and pre-existing low levels of inflammation will be at increased risk of developing a secondary contact dermatitis from the many potent allergens present in the workplace. These include PPD, PTD, glyceryl monothioglycollate, ammonium persulfate, nickel, and other dyes.

Glyceryl monothioglycollate has been a significant cause of allergic contact dermatitis that gave rise to large outbreaks of contact dermatitis among hairdressers in Europe (25) and in the United States (26) in the early 1980s. Contamination of the workplace due to spillage when mixing or applying the solution to the rollers meant that it was impossible for sensitized personnel to avoid contact (27). Patch testing to this allergen has to be done with a freshly made preparation in either water or petrolatum. Occasionally, patients allergic to glyceryl thioglycollate will react to ammonium thioglycollate used in "hot" perm procedures. Glyceryl monothioglycollate perming agents have been withdrawn from several European countries with a marked reduction in sensitization rates (28). Ammonium thiolactate (ATL) has been used as an alternative. Difficulties have been experienced in patch testing to ATL as it is unstable and has to be freshly prepared, and it is irritant. The recommended concentration is 1% aqueous (29).

Para-phenylenediamine (PPD) is a colorless compound that acts as a primary intermediate in hair dyes. It is oxidized by hydrogen peroxide and then polymerized to a color within the hair by a coupler. It remains an important allergen in both clients (30) and hairdressers (25,31–33). The rates of sensitization vary from country to country and are higher in countries where dark hair is the norm with positive patch test rates as high as 32% in a study from Spain (34) and 18% from a study in India (35). Cross-reactions or co-sensitivity to other hair dyes such as 2-nitro-4-phenylene diamine, 2,5-diaminotoluene sulfate, and *para*-aminophenol, may occur. It is common that persons sensitized to PPD are also allergic to these similar compounds. The risk of sensitization probably relates to the quantity of active intermediates such as quinones that penetrate the skin (36).

Most cases of PPD allergy are due to contact with hair dyes, although some may be sensitized by other sources. Likewise patients who are allergic to PPD may therefore also sometimes react to benzocaine, procaine, sulfonamides, PABA sunscreens, azo and

aniline dyes, anthraquinone and some antihistamines (37). Although hair that has been dyed is thought to be non-reactive once dried and polymerized, individuals who are already sensitized may still react when patch-tested to dyed hair. The likelihood is increased when the oxidant/coloring precursor ratio has not been followed.

The most important measures to reduce risk of sensitization include restricting the concentration at which PPD can be used, combined with the effective removal of excess dye from recently dyed hair, the use of protective gloves and adequate education of hairdressers and consumers.

Ammonium persulfate is able to cause both immunological and non-immunological immediate-type reactions—urticaria, rhinitis, conjunctivitis, and asthma (38), and can also cause delayed contact dermatitis type reactions (39).

Preservatives are used in all aqueous and oil in water preparations to prevent growth of bacteria and molds. The standard patch test series will detect allergy to many preservatives such as formaldehyde and formaldehyde releasers such as quaternium 15, imidazolidinyl urea, diazolidinyl urea, and other preservatives including methyl-isothiazolinone/methyl-chloro-isothiazolinone (MI/MCI), methyldibromoglutaronitrile (MDBGN), and parabens. Other preservatives such as 2-bromo -2-nitropropane-1,3-diol, DMDM hydantoin and chlorphenesin will need to be added as necessary. There developed an unacceptable rate of sensitization to MI/MCI in the early 1980s in some countries of Europe and the maximum permissible level was reduced to 15 parts per million. However, the rate of positive patch tests remains at about 2.5% on average for Europe, as does formaldehyde. The commonest preservative allergy at present is methyldibromoglutaronitrile which has a European average sensitivity rate of 3.5% with wide variations between different countries of Europe with higher levels in Italy (6%) and lower levels in the UK (1.4%) (40). Low concentrations of preservative in rinse-off products may be tolerated even by those known to be sensitized (41).

Rubber chemicals which are used as accelerators in thin rubber products such as rubber gloves and highlight caps cause delayed-type hypersensitivity. The natural rubber latex protein allergens can cause an immediate-type contact urticarial reaction and this type of allergy has increased over the past 20 years due to increased production and use of cheap disposable rubber gloves. Although the highest level of sensitivity has occurred in health care workers, hairdressers have also been affected (42,43). There is always the possibility that persons sensitized to natural rubber latex proteins may develop anaphylaxis.

Fragrance is the second commonest allergen in Europe after nickel. Approximately 1% of the general population is sensitive to fragrance (44). In contact dermatitis clinics between 6 and 14% of patch-tested patients are found to be allergic to fragrance (45). In some countries, such as Germany and Denmark the percentage of patch-tested patients sensitized to fragrance materials is rising (46). Occupational contact however is rarely significant (47), but has been reported (48).

Cocamidopropylbetaine, a “no tears” surfactant, used in many shampoos has caused a certain amount of contact allergic dermatitis in hairdressers and customers (49), principally due to dimethylaminopropylamine and amidoamine contaminants in cosmetic grade product (50,51).

Antioxidants, such as butylated hydroxytoluene (BHA), butylated hydroxyanisole (BHA), gallates (dodecyl, propyl and octyl), and tocopherol (Vitamin E) are used in oily products

to stop them from going rancid. A case of cross-sensitivity to BHT and BHA from a hair dye product tertiary butylhydroquinone has been reported (52).

Hydrolyzed protein compounds cause immediate-type reactions and are present in many shampoos and conditioning agents. They include collagen, keratin, elastin, wheat, milk, soya, almond and silk, and are often amalgamated with a quaternary ammonium-type conditioning agent (53).

Occupational asthma is mainly caused by ammonium persulfate in the form of bleaching powder (54), and is an IgE-mediated immediate-type response. It accounted for 90% of respiratory disease compared with 17% of hand eczema in a Finnish study of hairdressers (55). Other studies have found that other airborne fumes and smoking contribute to occupational asthma (56).

Trauma to the hands may arise from chemicals handled and trauma in combination with softening agents may cause koilonychia of the nails (57). Hair may become embedded in the web of the hands (58) or at more distal sites that become contaminated with hair clipping (59).

Repetitive strain injury of wrist, elbow, neck and shoulders was a cause of hairdressers leaving their profession in a Finnish study (60).

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17

Hair Shaft Abnormalities

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This section brings together some distinctive conditions, both hereditary and acquired, that have characteristic and diagnostic hair shaft changes.

1. PILI TORTI

Literally translated, the term “pili torti” means hair twisting. This sign can occur as a specific genodermatosis with no other demonstrable abnormality (1), as one component of many complex ectodermal syndromes, and as an acquired sign of focal scarring alopecia.

1.1. Hereditary Pili Torti

Many authorities limit the term pili torti to hereditary cases in which the hair shaft is flattened and shows multiple 180° twists microscopically (Fig. 1A,B) and no other abnormality. Such cases typically show autosomal dominant inheritance. In many cases the hair shows no propensity for breakage, growing to a normal length; others break easily and have been termed “dystrophic pili torti.” Specific 180° twists may be a sign of many genetic syndromes, including Menkes’ syndrome, a genetic fault of copper metabolism, and Bjornstad’s syndrome, comprising twisted hair, follicular skin atrophy, and facial tumors.

1.1.1. Clinical Findings

The abnormality may be present from birth but only appears abnormal when the first coarse-pigmented hairs occur. If dystrophic, the fibers may break easily. More typically the hair grows to a satisfactory length but may be rather unkempt and give a “sparkling” or spangled effect to the eye with reflected light. Most cases affect only the scalp.

No effective treatment is known, but hair of a satisfactory length can be maintained if cosmetic and physical trauma is kept to a minimum.

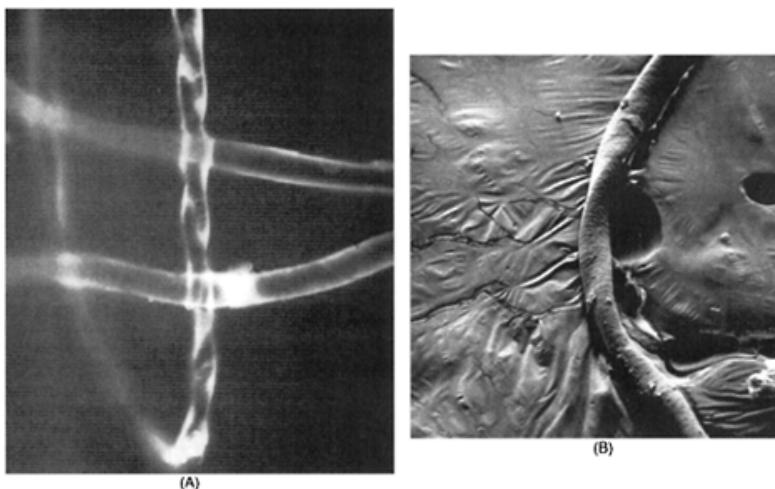


Figure 1 Pili torti, light (A) and scanning (B) electron micrographs.

1.2. Acquired Pili Torti

Many acquired conditions that cause focal follicular scarring may give *irregular* twisting of the hair within or adjacent to the scarred area. Such findings may be seen in a variety of scarring conditions, including pseudopelade (Brocq), discoid lupus erythematosus, and follicular lichen planus.

2. PILI ANNULATI

Pili annulati, also known as *Ringel Haare* or *Ringed Hair* (2), is a condition determined by an autosomal dominant gene, and in any one pedigree many individuals may be affected in each generation. It only rarely presents as a medical problem, since most affected individuals know of the entity from other family members, and because the hair appears entirely normal apart from close inspection.

2.1. Microscopy

The hairs show alternate light and dark bands, the latter being the abnormal sections in which the hair cortex is filled with multiple small irregular air spaces; these are very clearly seen by scanning electron microscopy (SEM) of transverse sections (Fig. 2). SEM and transmission EM show that in the affected bands the surface cuticle is rather "cobblestoned" with individual cells thrown into folds. There is no abnormality or melanin pigmentation in this condition.

To the eye, scalp hair shows alternate light and dark bands (a "sandy" appearance). Rarely, nodes of trichorrhexis nodosa type may be seen. When the hair is wetted or oiled, the changes are difficult to see.

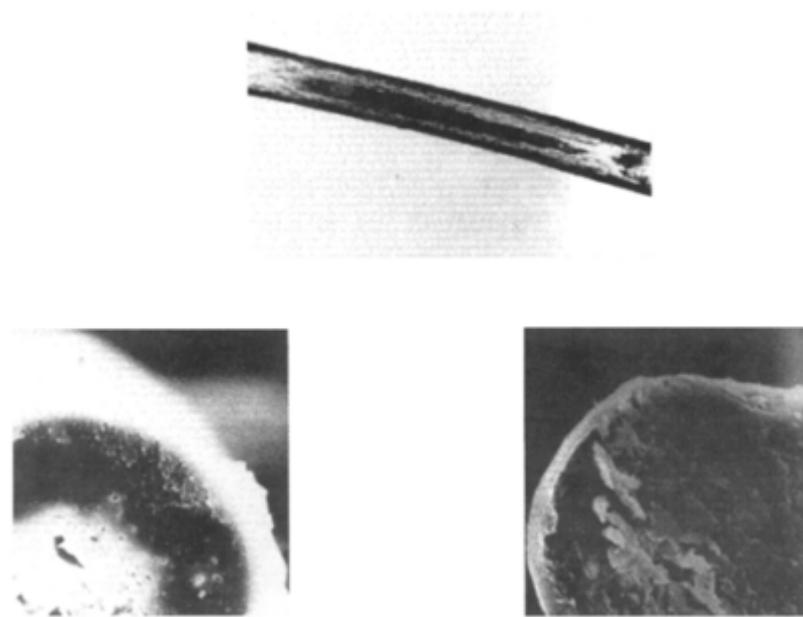


Figure 2 Pili annulati, showing light microscopy of an abnormal band (top) and scanning electron micrographs of transverse sections through a normal band (right) and an abnormal band (left).

2.2. Prognosis

The condition is permanent but shows no tendency to hair fragility. Destructive cosmetic treatments such as bleaching and permanent waving tend to enhance the tendency to fragility and must be kept to a minimum.

3. WOOLLY HAIR

This abnormality exists as a localized nevus, all recorded cases having been on the scalp, or as a hereditary condition in which the whole scalp has the appearance either of sheep's wool or negroid hair in Caucasian individuals.

Generally the hair behaves normally throughout life and withstands cosmetic procedures as for normal hair.

Woolly hair exists from birth but may only become visible when coarse terminal hair develops. Acquired progressive kinking may mimic woolly hair, but differs in only developing after puberty, having a male preponderance and being followed in most cases by androgenic alopecia.

4. UNCOMBABLE HAIR SYNDROMES

Under the heading uncombable hair syndromes are grouped cases in which the hair grows to a significant length but remains unkempt and "sticks out."

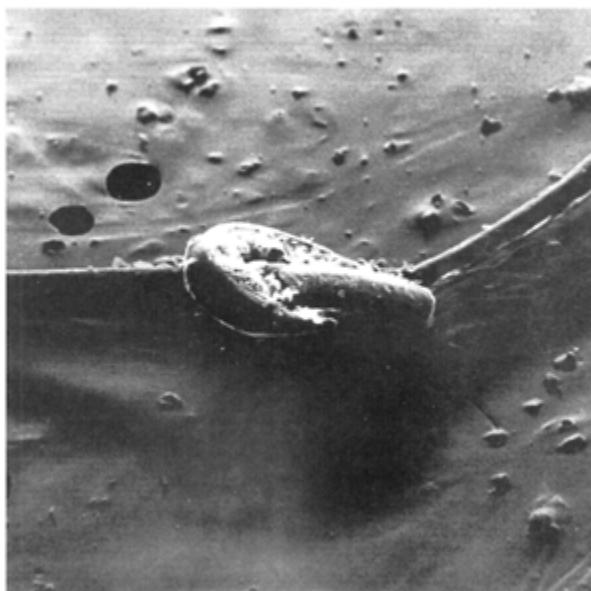


Figure 3 Trichonodosis: a single knot.

Localized areas of scalp hair rarely exist in this form as straight hair nevus. Usually only one small area of the scalp is affected.

Syndromes with hair twisting may give an uncombable appearance but are easily diagnosed by hair microscopy.

The *Cheveux incoiffables* syndrome described by Dupré et al. (3) is characterized by hair fibers that have a longitudinal depression and are triangular in cross-section—*pili trianguli et canalliculi*.

5. TRICHONODOSIS

Trichonodosis literally means knotting of hair (Fig. 3). It is an acquired condition. Knotting occurs most commonly in negroid hair and longer hair that is curly. The condition is of significance cosmetically, since combing and brushing tend to pull out knotted fibers: those that remain tend to weather quickly and fracture.

6. WEATHERING OF HAIR (INCLUDING TRICHLORRHESIS NODOSA)

All scalp hair fibers undergo some degree of cuticular and secondary cortical breakdown from root to tip (4), the "rough" imbricated cuticular surface having a high coefficient of friction. The more brushing and combing are carried out and cosmetic procedures, such as permanent waving and bleaching, tend to enhance the process. Particularly in women who overdo these cosmetic procedures, the enhanced breakdown may give rise to the fissuring and fracturing with node formation termed trichorrhexis nodosa.

In trichorrhexis nodosa, the hair is fragile, lusterless, and shows many punctate white nodes along the shaft.

Light microscopy shows cuticular damage appearing dark with transmitted light. Nodes consist of transverse cuticular fissures through which cortical cells protrude (Fig. 4). The tip of most affected hairs shows exposed longitudinal cortical cells giving the characteristic "paint-brush" tips (Fig. 5).

Trichorrhexis nodosa may be seen in childhood in many genetic syndromes in which the hair is intrinsically fragile and weathers excessively in response to normal frictional forces. This is seen most prominently in the syndrome of trichothiodystrophy, a complex neuroectodermal syndrome in which the hair is sulfur-deficient. More specifically there is high sulfur protein deficiency, which is particularly absent from the A-layer of the cuticle and the matrix of the cortex. Consequently, the hair weathers and breaks within millimeters of leaving the scalp.

7. MONILETHRRIX

Monilethrix, or hereditary beading of hair, is microscopically the most distinctive and easily diagnosed of the hair shaft defects (5). It is usually transmitted as an autosomal dominant trait and typically has associated with it widespread follicular hyperkeratosis.

Microscopically, individual hairs look beaded along their length, the narrow so-called internodes being the abnormal segments (Fig. 6). Hairs with very narrow internodes rarely attain a length of more than a few centimeters before breaking. Hair length varies from a few millimeters to several centimeters. The most pronounced beading is seen on the scalp, although terminal hair anywhere on the body may exhibit the phenomenon. At sites of follicular hyperkeratosis, hair tends to be absent.

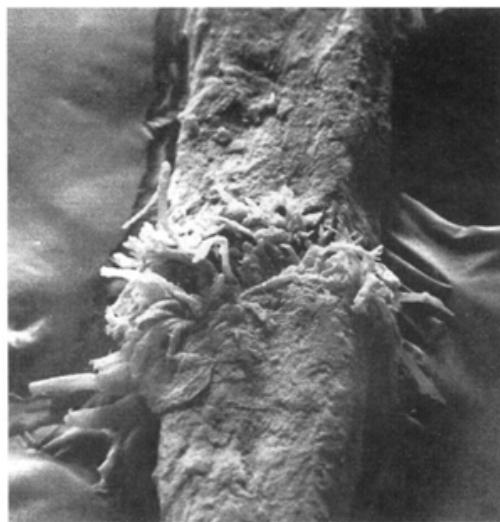


Figure 4 Trichorrhexis nodosa. A single node on one hair shaft (scanning electron micrograph).

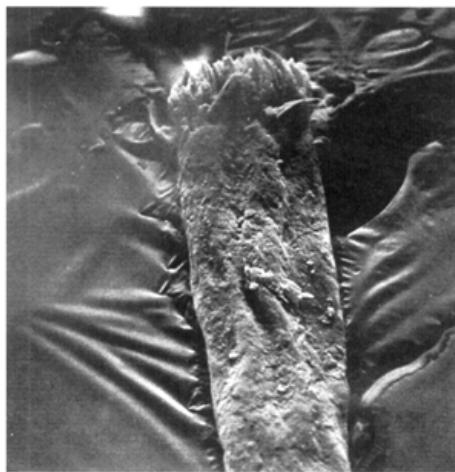


Figure 5 Trichorrhexis nodosa. A hair fiber with a "paint-brush" tip (scanning electron micrograph).

The mechanism of node formation has been extensively studied, but no clear pathogenesis has emerged. It has been suggested that the root sheaths intermittently

harden in a wide and narrow concentric ring around the moving softer hair cells, which consequently produces beaded hairs on hardening. The length and frequency



Figure 6 Monilethrix showing characteristic beading (scanning electron micrograph).

of the nodes bear no relationship to any specific period of linear growth, e.g., no diurnal variation.

No specific treatment is known, but of interest are the anecdotal cases in which pregnancy improved hair length and decreased node size. Oral aromatic retinoids have been used, which inhibit the follicular hyperkeratosis and enhance hair growth without in any way reducing node formation.

8. NETHERTON'S SYNDROME

This is a rare genetic syndrome in which a persistent scaly eruption, ichthyosis linearis circumflexa, occurs together with fragile hair. Microscopically, it shows a variety of changes including nodes of trichorrhexis nodosa and others resembling bamboo cane nodes, together with sections of hair in which invagination appears to have occurred prior to hardening in the follicle.

The clinical state may remain undiagnosed if hair microscopy is not conducted.

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18

The Hair Pigmentation Unit and Hair Graying

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Our visual appearance mostly relies on our skin and hair colour, and perceived variations between human subgroups can be ascribed to the different levels and types of pigments, namely melanins, involved. The hair colour results to a large extent from the presence or absence of melanins in the cortex of the hair fiber, melanins being end products of a complex biochemical pathway called melanogenesis, involving tyrosine as the initial substrate (see Chapter 1). This melanin production takes place in specialized cytoplasmic organelles called melanosomes produced by neural crest-derived pigment cells called melanocytes. A characteristic feature of the human hair melanocyte as compared with the epidermal melanocyte is its permanently activated state throughout the growing phase, i.e., anagen, of the follicle, probably supported by a specific regulatory network, as yet not fully understood.

1. ORIGIN OF HAIR COLOUR

The large variety of hair colour results from the presence of variable amounts and mixes of different kind of melanins in the hair cortex. The melanocytes responsible for hair melanin content are located in the bulb of the follicle. The body of these melanocytes lies on the basement membrane surrounding the dermal papilla (DP) at its apex (Fig. 1) while their dendrites extend to the precortical keratinocytes, allowing melanin transfer to the future cortex of the shaft. This tripartite organization defines the hair pigmentation unit. In the hair bulb one melanocyte interacts and fuels five keratinocytes with melanosomes. By contrast in the epidermal pigmentation unit, one melanocyte interacts with 35 viable keratinocytes.

The melanogenesis process involves at least three enzymes namely tyrosinase, 5,6-dihydroxyindole carboxylic acid (DHICA) oxidase (TRP-1: tyrosinase-related protein 1) and dihydroxyphenylalanine (DOPA)-chrome tautomerase (TRP-2: tyrosinase-related protein 2) (Fig. 2). These melanogenic enzymes are the products of tyrosinase gene family expression which specifically takes place in melanocytes (1,2). Tyrosinase is considered as the rate limiting enzyme of melanogenesis resulting in the synthesis of eumelanins (black to brown pigments) and pheomelanins (yellow

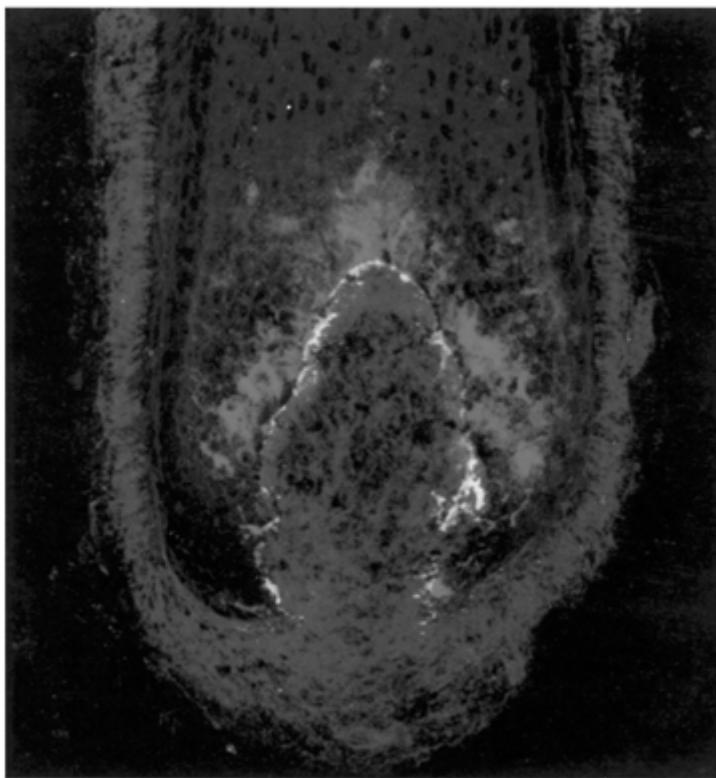


Figure 1 The hair pigmentation unit as seen by laser confocal microscopy. Cryosection was processed for triple immunolabeling: melanocytes (M), epithelial cells and mesenchymal compartment are visualized in red, blue, and green, respectively. Hair bulb melanocytes (M) are located on the basement membrane surrounding dermal papilla (DP) and transfer melanin granules to precortical keratinocytes (pcK).

to red pigments). This enzyme is endowed with the particularity of catalyzing three different reactions in the melanin biosynthesis pathway including tyrosine hydroxylation to produce DOPA as the first step. On the contrary, TRP-1 and TRP-2 seem to be specifically involved in eumelanin biosynthesis (3). Furthermore, it is considered that a

decrease in TRP-1 and/or TRP-2 activity might be sufficient for up-regulation of pheomelanogenesis (3). The expression of these melanogenic enzymes appears to be regulated at the transcriptional level (1,4). It has been well demonstrated, for example, that tyrosinase and TRP-1 gene expression is activated by microphthalmia gene product (MITF), a basic helix-loop-helix-leucine zipper (bHLH-LZ) transcription factor whose expression is restricted to a few types of cell and is enhanced in response to cAMP signaling pathway stimulation (4).

2. DYNAMICS OF HAIR PIGMENTATION UNIT

2.1. Embryogenesis of the Follicular Pigmentation Unit

The neural crest origin of pigment cells was demonstrated in amphibian, avian, and mouse embryos (5). The neural crest is a transient and pluripotent cell population that develops at the time of neural tube closure. Neural crest cells from the truncal

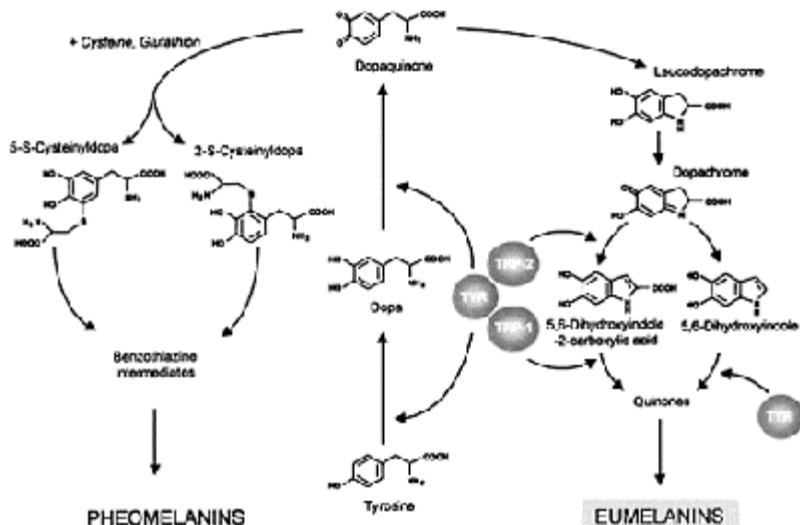


Figure 2 Melanogenesis pathway according to Oetting. (From Ref. 1.)

neural crest become committed melanoblasts under the influence of several factors including fibroblast growth factor 2 (FGF-2), mainly secreted by mesenchymal cells (5), Wnt-3A and bone-morpho-protein (BMP-4) (6). Melanocytes develop from these melanoblasts migrating along pathways characterized by the presence of numerous extracellular matrix components such as fibronectin, laminin, collagen, and tenascin, under the control of growth factors. It is believed that human mela noblasts enter the dermis and are already present in the epidermis at about 7 weeks estimated gestational

age, 2 weeks before hair morphogenesis takes place (7). They then migrate within the growing hair follicle.

During this complex set up, melanoblast survival and migration are under the control of a series of factors, in particular (i) stem cell factor (SCF) and its receptor c-kit which all appear as key factors for melanoblast entry into the epidermis and subsequent migration into the hair follicle, acting as surviving and chemokinetic factors (8–10) and (ii) endothelin-3, a potent mitogen for melanoblasts, together with its receptor B (Ednrb) (11,12). Interestingly, the hair DP appears to be an important source of local SCF production (13). Indeed either disruption of SCF/c-kit and /or endothelin-3/Ednrb function is responsible for an alteration of hair colour as evidenced by genetic mutations of each of the genes coding for those proteins (14–16), the most characteristic of which is the c-kit mutation associated with piebaldism (16).

2.2. The Hair Growth Cycle and Melanin Unit Recycling

In human scalp, hair follicles display a cyclical activity, each cycle being characterized by three successive phases, namely anagen, catagen, and telogen with clearly distinct durations (17). Anagen is the growth phase during which the hair fiber is being produced for a 3-year average period. Catagen is the involution phase with a 3-week mean duration, a short phase involving an abrupt and early cessation of matrix cell proliferation and arrest of inner root sheath (IRS)-specific protein synthesis (18). Telogen is the resting phase with an average 3-month duration. Thus, one characteristic feature of the hair cycle is that hair follicles undergo successive cycles of growth, tissue regression, and regeneration. As a consequence, the hair follicle melanocytes must somehow follow a similar process through the hair cycle stages, but the origin of melanocytes present in the bulb after human hair regeneration is still not fully understood. Indeed, in addition to the bulbar area, the upper outer root sheath (U-ORS) of the human hair follicle also contains melanocytes, but in an inactive state with regard to melanogenesis (19). Both bulbar and ORS melanocytes can be identified by the expression of the premelanosomal protein pMel-17. We have shown at the cellular level that in human pigmented hair follicles, the expression of tyrosinase and TRP-1 was detectable from anagen phases III/IV to VI, only in those melanocytes which were located in the bulb (20). During the catagen phase, the two evaluated melanogenic enzymes were no longer detectable although melanocytes were still present in the bulbar area. The epithelial column of the catagen follicle and the capsule of the telogen follicle also contained inactive melanocytes as evidenced by pMel-17 labeling. At the induction stage of a new anagen hair follicle, some melanocytes were committed to cell division, but only when located in the nascent bulb, close to the dermal papilla (Fig. 3). Thus melanogenic proteins are differentially expressed in bulbar as compared with ORS melanocytes, in relation to their activation state in the anagen hair follicle (Fig. 4). While bulbar melanocytes are pMel-17, tyrosinase and TRP-1 positive, ORS melanocytes are pMel-17 positive

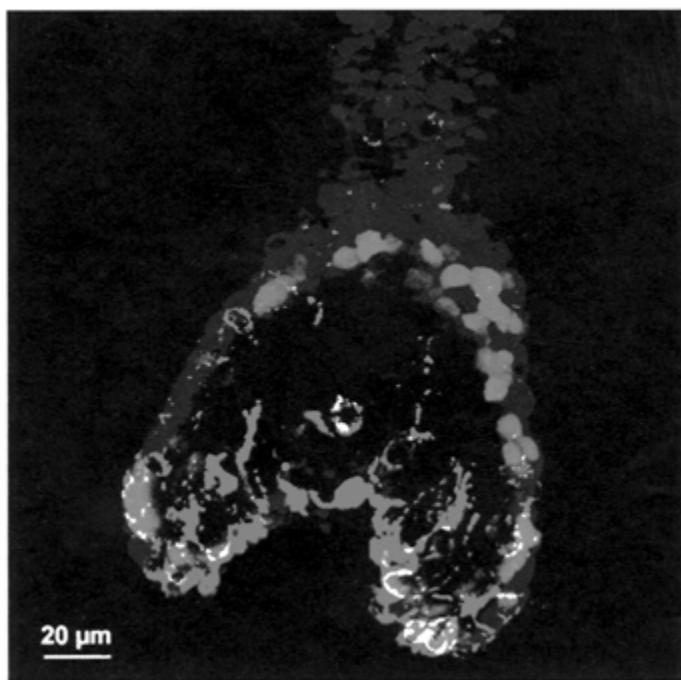


Figure 3 Proliferation of bulbar melanocytes at the onset of the anagen phase. An anagen II human hair follicle was isolated, processed for triple immunolabeling and analyzed by laser confocal microscopy. Proliferating cells (Ki67+) are stained in green, while keratinocytes and melanocytes are stained in blue and red, respectively. Note the presence of yellow cells in the nascent bulb.

but both tyrosinase and TRP-1 negative (20). Our results underline the close relationship between melanogenesis and hair cycle and suggest that in humans, melanogenesis is restricted to the anagen hair follicle not because of the regulation of tyrosinase activity but because of melanogenic enzyme expression, e.g. tyrosinase and TRP-1. Furthermore the fact that, in the new developing anagen hair follicles, cell division commitment and both tyrosinase and TRP-1 expressions were only observed in those melanocytes located in the nascent bulb, suggests a highly region-specific melanocyte stimulation in the early anagen phase. Altogether our observations suggest that hair melanogenesis is driven by

local, intrinsic hair follicle inducers, melanocyte stimulation being coupled with the anagen phase induction.

The question asks whether a melanocyte precursor population exists in the hair follicle has been raised for a long time (19). Immunohistochemical studies on anagen human hair follicles suggested that depending on the marker used, namely dendricity, p-Mel-17 or c-kit expression, this precursor population could be located either in the bulge area (21) or in the lower infundibulum (22). From our analysis throughout the hair cycle, it emerged that melanocytes observed in the telogen capsule were probably not different from those observed in the U-ORS of anagen hair follicle, since the catagen phase led to ORS regression until the permanent part of the follicle was reached. In fact, it has been shown that both the telogen capsule and U-ORS contained amelanotic melanocytes that could be stimulated. In that respect dopa oxidase-positive melanocytes were observed in the telogen capsule when exposed to x-rays (23), while U-ORS melanocytes express melanogenic enzymes under proper culture conditions

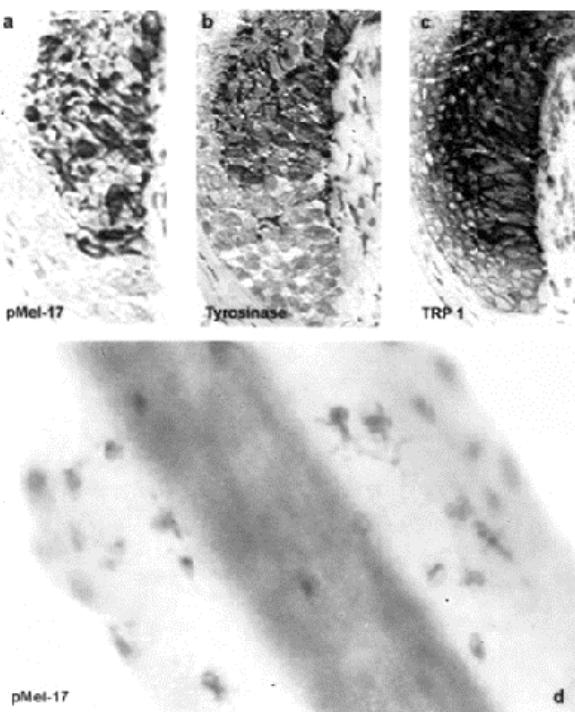


Figure 4 Differential expression of melanogenic proteins in hair follicle melanocytes. Cryosections of pigmented hair bulb (a–c) and the whole mount of a hair follicle (d) were immunolabeled to detect pMel-17

(a,d), TRP-1 (b) and tyrosinase (c). Active melanocytes in the bulb express the three melanogenic proteins (a–c) while inactive melanocytes located in the ORS only express pMel-17 (d).

(24). Thus, the U-ORS melanocytes of human anagen hair follicle could be considered as the melanocyte reservoir for successive hair generations, since hair appears as an independent unit with regard to its pigment cell population (Fig. 5).

3. REGULATION OF HAIR PIGMENTATION

Although some 21 genes have been identified as being involved in pigment cell formation and function (2,25), little is known about the regulation of human hair

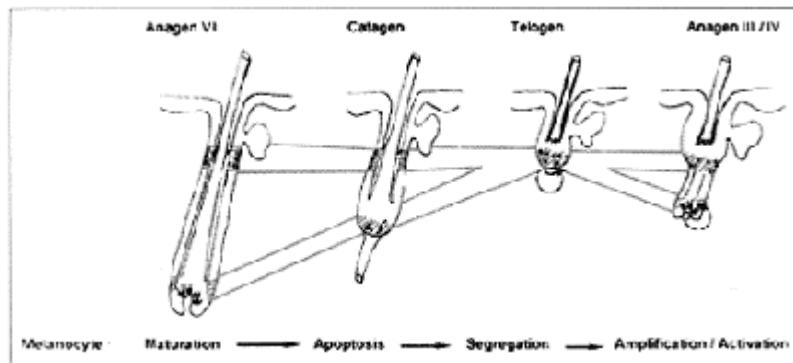


Figure 5 Schematic representation of the pigmentation unit recycling throughout the hair cycle. Red: quiescent melanocytes (reservoir); black: active melanocytes. The blue arrow indicates the recruitment-activation process which takes place at the telogen-anagen transition.

pigmentation. Indeed, events controlling either the number and localization of melanocytes in the hair bulb or melanogenesis activity or melanin transfer do have a strong incidence on hair colour. It is thus likely that the expression of proopiomelanocortin (POMC) gene products together with the MC1 receptor (MC1-R), the product of *extension* locus and cognate receptor of α -MSH, play a role in regulating hair pigmentation. MC1-R is a seven trans-membrane receptor coupled to a G α protein,

which upon binding of POMC-derived ACTH, α -MSH or β -MSH peptides, activates adenyl cyclase (26,27). Subsequent cAMP production stimulates melanocyte proliferation, melanogenesis and dendrite formation (28). Interestingly, accumulation of POMC products is found predominantly in the ORS follicular keratinocytes in the scalp rather than the overlying epidermis (29). Furthermore, mRNAs coding for POMC as well as MC1-R were found to be predominantly expressed during the anagen phase, in relation to tyrosinase activity, in mice skin (30). Altogether these observations suggest that the activity of the local POMC/MC1-R axis plays a role in the physiological regulation of anagen-associated hair pigmentation. This is confirmed by the facts that (i) loss-of-function mutations at the MC1-R are associated with a switch from eumelanin to phaeomelanin production, resulting in a red or yellow coat colour (31) and (ii) the major part of red-haired individuals are compound heterozygotes or homozygotes for up to five frequent loss-of-function mutations of the MC1-R gene (32). Apart from this POMC/MC1-R axis, a growth factor/cognate receptor network including FGF-2, hepatocyte growth factor, SCF, and endothelins (11,33) is likely also to be important in post-natal hair follicle pigmentation and in regulating melanocyte survival, proliferation and differentiation in a paracrine way.

4. HAIR GRAYING

The loss of melanin content in the hair fiber is a natural manifestation of aging leading to apparent hair whitening. While it is considered that a normal incidence of hair graying can be observed as early as 20 years of age in Caucasians and 30 in Africans (34), it has been reported that on average, in a cohort of Caucasians (35), 50% of people had at least 50% gray hair when 50 years old. This graying incidence occurs irrespective of sex, hair colour, and initial content of melanin (35). However, the molecular and cellular origin of hair graying remains unclear to date. It has been shown that tyrosinase activity was decreased in the pigmented hair bulb of older people as compared with middle age (36), while gray hair underwent a reduction in the amount of melanin granules (37). Altogether, these observations suggest that hair graying follows a decrease in melanin synthesis in the bulb. However, the reason for this reduction in melanin synthesis is not clear. In this respect, it has been shown that tyrosinase was either absent (36) or present (38) in depigmented hair bulb. In agreement with a possible persistence of tyrosinase, it has been shown that gray hair still contained a few active melanocytes (39). However, the presence of melanocytes throughout the graying and whitening process remains a controversial issue since (i) gray hairs apparently contain a normal number of melanocytes (37), (ii) white bulbs are totally devoided of melanocytes (37), and (iii) depigmented hair bulbs still contain melanocytes (38). On the contrary, white hair follicles still contain inactive melanocytes in the ORS (40,41). These latter findings suggest that hair graying could be due to impaired recruitment of melanocytes during the hair cycle. On the contrary, the observations by Magnani et al.(38) suggest that graying is not caused by a loss of melanocyte but rather originates from failings in the later steps of melanogenesis and/or melanin intracellular transport along the dendrites.

Thus, although hair graying is a very common phenomenon characterized by loss of pigment in the hair fiber, the events that cause and control natural hair whitening with age

are still deeply unclear. In particular each of the three main factors that affects hair colour, namely melanocyte number and location, melanogenesis activity and melanin transfer, has been suspected to be involved in natural graying.

5. CONCLUSION AND PERSPECTIVES

Together with the hair follicle, the hair pigmentation unit undergoes cyclical renewal. Although its general structure and function are rather well characterized, the regulatory pathways controlling (i) the cyclical recruitment of melanocytes from its reservoir at the time of hair regrowth, (ii) the melanogenesis activation, (iii) the balance between eu- and phaeomelanin synthesis, (iv) the melanosome transfer to precortical cells, as well as (v) the understanding of the mechanism whereby hair turns gray, are just starting to be deciphered. This domain of research remains an open field today, and one might expect genetic and genomic approaches to bring new insights and shed new light on the fascinating process of hair pigmentation.

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19

Alopecia

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1. INTRODUCTION

In clinical practice, when a patient consults a dermatologist about hair loss, their condition may or may not lead to an alopecia. Alopecia is inevitably preceded or accompanied by hair loss, whereas hair loss may not progress to alopecia.

Each day, an average of 30–80 hairs in the final telogen phase are lost. At the same time, 30–80 new hair cycles begin, so that the number of hair on the scalp remains constant (100,000–160,000 on the whole scalp of an adult). In the northern hemisphere, seasonal variations have been described with a peak in hair loss during the Spring and the months of August and September (1,2). This seasonal variation must be taken into consideration when interpreting trichograms and the hair loss intensity during these calendar periods. These variations also explain the increased number of consultations for hair loss during late summer.

Significant hair loss is defined as a loss of more than 100 hairs per day. An effluvium, which constitutes a significant hair loss, can be telogen (as is the case during a post-partum telogen effluvium) or an anagen effluvium (as observed during anti-mitotic chemotherapy) or a mixed anagen and telogen effluvium (as during the chronic effluvium associated with alopecia areata and as also seen during the acute phase of androgenetic alopecia).

Alopecia is defined as a decrease in hair density, whatever the body area concerned. The term “alopecia” comes from the Greek, “alopex,” meaning “fox mange.” Alopecia being then defined as a reduction in hair or fur density, it can be assumed that in the time of Hippocrates foxes were widely affected by mites, the probable cause of their fur loss.

There are many types of alopecia, varying according to their topography, evolution, and etiology (3,4). Generally, two main types of alopecia are described: diffuse alopecia involving the whole scalp, and localized alopecia involving only part of the scalp. Diffuse alopecia is categorized according to whether it is acute or chronic. Local alopecia is categorized according to the type of injury: scarring or non-scarring. The classifications of alopecia are presented in Figs. 1 and 2.

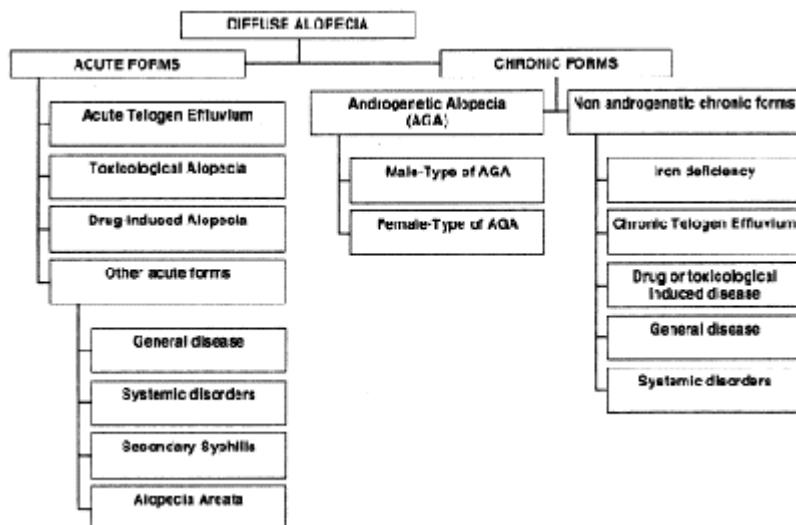


Figure 1 Diffuse alopecia.

Among the wide variety of alopecic conditions encountered, the main one is androgenetic alopecia (AGA) which will be reviewed first in this chapter, followed by other manifestations of alopecia.

2. ANDROGENETIC ALOPECIA

Androgenetic alopecia is a frequent condition. Considering the psychologic consequences in some young men and in females (which are often overwhelming), AGA needs to be seriously considered (5).

More than 90% of hair loss consultations in men are motivated by AGA, which affects 20% of males aged 20 years and 50% by the age of 50 years. In both males and females, the incidence of alopecia increases with age. In females the prevalence of androgenetic alopecia (AGA_F) is lower, with 10% of them being visibly affected prior to the menopause and 40% after the menopause, between the ages of 50 and 60 years (6).

2.1. Clinical Aspects

2.1.1. Common Pattern

A family history is present in 80% of cases and the mode of transmission is multigenic. The existence of a family history on the mother's side is a poor-prognosis factor. When a woman is affected by AGA_F the condition is observable before the age of 30 in 54% of her male progeny and 23% of her female progeny (7).

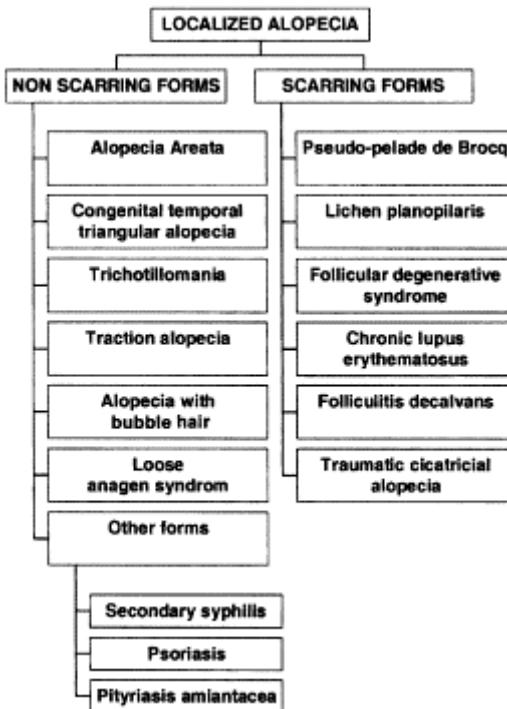


Figure 2 Localized alopecia.

Diagnosis is usually straightforward because AGA generally occurs after puberty and its topography is unmistakable. It develops slowly, over 15–30 years. Hair loss precedes a reduction in density by several years. The earlier its onset, the greater the severity of the condition.

During the course of AGA, a reduction in hair density is accompanied by a reduction in hair diameter (8,9). Terminal hairs are replaced progressively by intermediate hairs, followed by vellus hairs. AGA is often accompanied by increased seborrhea of the scalp.

Recently, it has been reported (10–12,42) that hair diameter diversity (HDD) and peri-pilar signs (PPS) are associated with AGA. HDD is defined as a combination of hairs of different diameters in the same area of the scalp with a ratio thin hair/ thick hair, of more than 20% (Fig. 3). HDD is observed at all stages of AGA, but during the initial AGA stage, it appears that the mean hair diameter has already decreased. Histological data demonstrate that HDD is associated with miniaturized follicles.

PPS are characterized by changes in the appearance of the scalp around the hair follicle ostium. There are two different kinds of PPS: (i) peri-pilar cupules, (ii) peri-pilar halos (Figs. 4 and 5).

2.1.2. In the Male (AGA_M)

Initially, alopecia affects the parieto-frontal regions on the one hand and the vertex on the other (Figs. 6 and 7). The anterior frontal edge recedes and the vertex tonsure

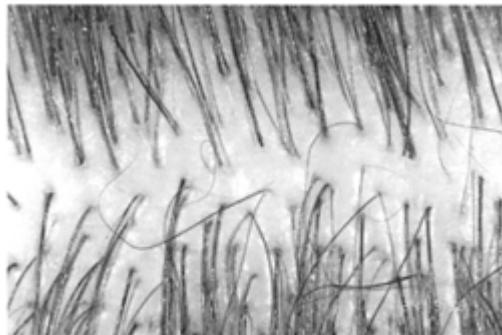


Figure 3 Hair diameter diversity during androgenic alopecia. Note the thin hair which denote the involvement of hair follicles in miniaturization process. (Photo: L’Oreal Recherche)

increases in a centrifugal fashion. The alopecia progressively spreads, leaving only the occipital area and the lower parietal areas unaffected. The incidence of AGA_M is 25% at 25 years, 40% at 40 years, and 50% at 50 years.

Various classifications of the progressive stages have been proposed. The classification currently recognized internationally and used in clinical trials is that of Hamilton (13) modified by Norwood in 1975 (14). This classification includes seven types and five variants (Fig. 8). In certain cases, men can develop androgenic alopecia that resembles the female type of AGA (Fig 21). Savins classification applicable to men and women is more precise (15). It measures density from 1 to 8 on three areas of the scalp: frontal, medial, and vertex (16).

2.1.3. In the Female (AGA_F)

The apex of the crown is affected by alopecia but the condition is more diffuse, extending over the whole central region of the scalp with a progressive reduction in density of the median area combined with exposure of the scalp at the temples

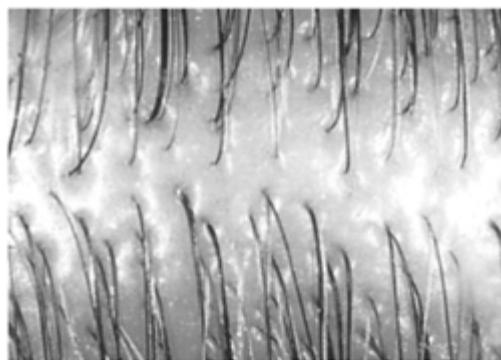


Figure 4 Peri-pilar cupules. Note the stuffing aspect of the scalp around the hair emergence (more visible at the periphery of the slide). This sign is associated with a high level of perifollicular and dermal cellular infiltrates. (Photo: L’Oreal Recherche)

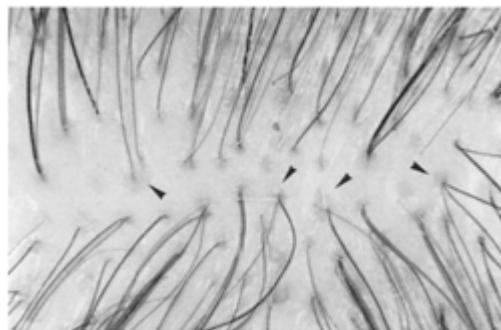


Figure 5 Peri-pilar halos. Note the colored spot around the hair emergence. This sign likely denotes likely the visibility of the follicle from the surface of the skin, as the consequence of follicle ascending into the deep dermis. (Photo: L’Oreal Recherche)



Figure 6 Male androgenetic alopecia: score IIIa on Hamilton scale. (Photo: Dr. Reygagne)

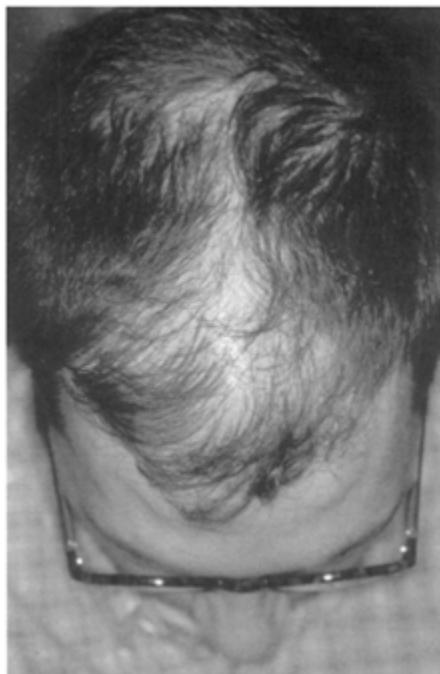


Figure 7 Male androgenetic alopecia. (Photo: Dr. Reygagne)

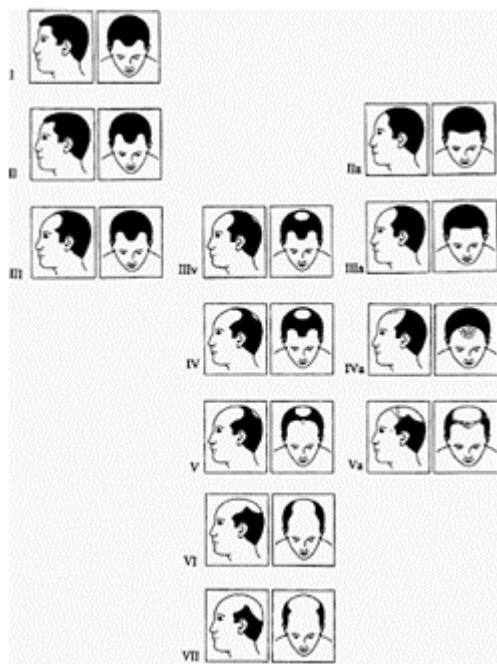


Figure 8 Hamilton scale.

and vertex, usually not affecting the anterior frontal strip. The differential diagnosis between this and telogen effluvium can be difficult (17,18). Vellus hair is rarer than in the male. The alopecia may be combined, particularly during periods of aggravation, with burning sensations or discomfort that resemble the reactions of a sensitive scalp; such discomfort, sometimes painful, may increase when the hair is moved, which is known as trichodynia.

The most recognized classification of AGA_F is Ludwig's one which defines three-stages (19):

Stage 1: Moderate alopecia with a slight widening of the central parting that does not affect the anterior frontal strip of 1–3 cm. This stage represents the very great majority of women presenting with AGA_F (Fig. 9).



Figure 9 Female androgenetic alopecia: score 1 on Ludwig scale.
(Photo: Dr. Reygagne)

- Stage 2: More extensive alopecia with considerable widening of the central parting, not affecting the anterior 1 cm frontal strip (Fig. 10).
- Stage 3: Almost complete alopecia of the whole vertex, leaving a very thin anterior frontal strip. This stage is exceptional (Fig. 11).



Figure 10 Female androgenetic alopecia: score 2 on Ludwig scale.
(Photo: Dr. Reygagne)

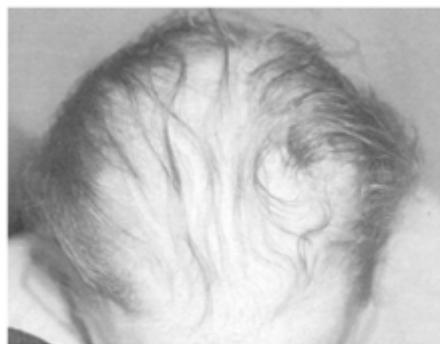


Figure 11 Female androgenetic alopecia: score 3 on Ludwig scale.
(Photo: Dr. Reygagne)

Two other topographical presentations of AGA_F are possible:

- Predominant medio-frontal alopecia not affecting the anterior frontal strip. Exposure of the scalp increasing from the vertex forwards the anterior frontal strip, this having

been described as the “Christmas Tree sign” (20). This sign (Fig. 12) may be observed in the course of AGA_F at early or advanced stages.

- At the end of puberty, a physiological recession of the anterior frontal strip with bitemporal recession is observed in 80% of young women (21). An AGA_F of male topography is possible, especially after the menopause. This calls for investigation into pathological androgen production if the AGA development is sudden or if hirsutism is present.

AGA_F may be triggered or aggravated

- by androgens,
- by an androgenic progesterone,
- by an estropogestergenic contraceptive with an androgen phase,
- by a non-androgenic progesterone with a strong antigenadotropin effect hence reducing ovarian estrogen synthesis,
- in the course of a postpartum telogen,
- after terminating contraceptive treatment,
- during the premenopause and menopause,
- or, finally, under corticosteroid treatment.



Figure 12 Female androgenetic alopecia: Christmas tree sign. (Photo: Dr. Reygagne)

In the female, hormone levels need only be tested if the condition is combined with menstrual disorders or other signs of hyperandrogenism. Dysthyroidism and iron deficiency must be excluded. A telogen effluvium due to iron deficiency is possible in the female, either on its own or in addition to AGA in the event of blood ferritin levels lower than 40 ng/mL (22). Moreover, in the event of AGA_F, treatment with minoxidil or cyproterone is of little use if the blood ferritin level remains low (23).

2.2. Pathophysiology

Much progress has been made in understanding of the pathology of AGA over recent years. It consists of an accelerated aging process (8,24) in the course of which many hereditary, hormonal, vascular (15), and inflammatory factors also play a role, as well as chronological aging.

Histologically, the number of hair follicles remains within the norm for a long time in both men and women. Cycle after cycle, the follicles produce ever finer hairs until they have no cosmetic benefit (26). The follicles migrate within the dermis upwards, then become miniaturized; some are fibrotic. The increase in the number of vellus and telogen follicles and the decrease in the number of terminal and anagen follicles are best demonstrated by horizontal histological sections (27).

In any particular scalp area, not all the follicles regress at the same pace.

Frequently, a moderate perifollicular lymphocytic infiltrate can be observed surrounding the hairs and appendages in the affected areas.

Androgenetic alopecia is dependent on both genetics and hormones. The role played by androgens in hair loss was recognized very early on by Hamilton (28), who observed that men castrated before puberty did not develop baldness as long as they were not given testosterone treatment (28,29). Therapeutic castration of older subjects arrested the balding process, but without leading to hair regrowth.

In both sexes, the androgens are the hormones that exert the greatest influence on hair growth. In men, androgens are of testicular origin while in women they are of ovarian and adrenal origin. Androgens increase the growth rate and caliber of hairs in some androgen-dependent areas of the body (beard, face, armpits, pubis, breast, abdomen, medial thigh, back, and shoulders in men) and they accelerate hair renewal and the transformation of terminal hairs into vellus hair in androgen-dependent areas of the scalp. Thus androgens can cause either loss or growth of androgen-dependent hairs. Genetic factors are responsible for the variable sensitivity of the follicles to androgens. Within the hair follicle, testosterone (T) is transformed by an enzyme, 5 α -reductase (5AR), into dihydrotestosterone (DHT). DHT then binds to a cytosolic receptor and then a nuclear receptor. The biological effect produced by DHT is a slowing down of the anagen phase. DHT is the most potent androgen, its affinity for androgen receptors is five times greater than that of testosterone. 5AR activity and the density of cytosolic receptors are genetically determined. Their excessive expression may induce a severe or early AGA_M. Conversely, aromatase restricts AGA_M by transforming testosterone into 17 β -estradiol. Aromatase activity is weaker in males than in females; however, in the male, it is higher in the occipital region than in the frontal region (30).

Regulation of the hair cycle by DHT may exert its effect through induction of apoptosis by activation of caspases (proteolytic enzymes expressed in the hair follicle), notably caspase3, higher levels of which are found in the external epithelial sheaths of subjects with alopecia (31).

With regard to 5AR, two types have been identified: 5AR1 that is specifically expressed in the skin and sebaceous glands and 5AR2 that is mainly expressed in the prostate gland and, to a lesser extent, in the frontal area of the scalp or, according to some reports, at the vertex where its expression would be stronger in men predisposed to AGA_M (30). By immunohistochemical staining, 5AR2 has been localized to the inner epithelial root sheet in the proximal areas of the follicle (32,33), also in the internal part

of the outer epithelial root sheet, the stratum granulosum of the infundibular epithelium and in the sebaceous duct (33). Other authors claim that the messenger RNA of 5AR2 is only found by PCR in freshly dissected follicles (34) and, more specifically, within the papilla or dermal follicular sheath (irrespective of whether the follicles are from alopecic or non-alopaeic areas of scalp), and when messenger RNA of 5AR2 has also been localized in cultures of the dermal fibroblast papilla or fibroblasts of the dermal sheath (35).

The presence of lymphomonocytic infiltrates was observed very early on in around 50% of patients affected by AGA (36) which suggests that the AGA_M pathology is not strictly and directly linked to androgens (36). This infiltrate has also been described by Whiting (27) and activated T-lymphocytes and macrophages have been detected more specifically in the upper third of the follicles in affected areas (37). During periods of progression, numerous proinflammatory cytokines play a role in the regulation of the hair cycle (38).

It is interesting to note that peri-pilar cupules (localized swelling of the scalp around the hair ostium) which are observed on the scalp during AGA have been linked to peri-follicular infiltrates (39). These signs are observed mainly in women with AGA and in men during the first stages of AGA. These data support the hypothesis that lymphocytic infiltrates in the dermis and around the follicle are involved in the initial steps of the biological process of AGA.

Furthermore, the evolution over time of peri-pilar cupules and peri-pilar halos in men and women reveal that cupules decrease as halos increase (Fig. 13). These observations clearly suggest that lymphocytic infiltrates precede follicular ascending into the dermis.

Regarding genetic patterns of AGA, it has been recently published that AGA_F is associated with a polymorphism of the androgen receptor (40).

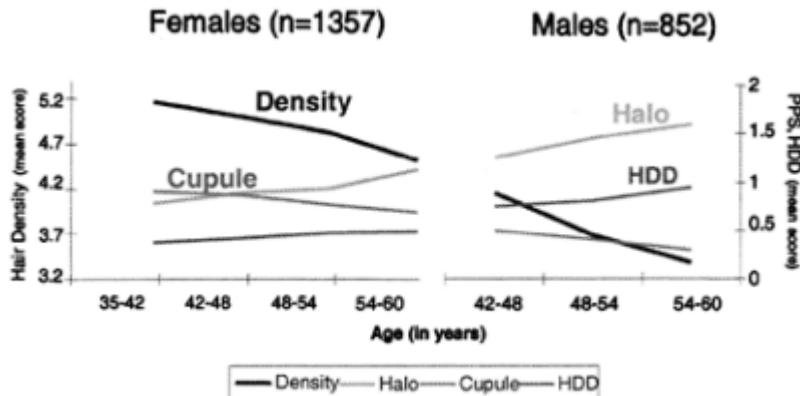


Figure 13 Evolution of hair density, peri-pilar signs and hair diameter diversity according to age and sex.

The above data support the hypothesis of an accelerated aging of the scalp during AGA. The hairless gene implicated in recessive autosomal transmission papular atrichia

is not implicated in the development of AGA_M: the heterozygotes of a hairless gene mutation do not develop greater AGA_M than unmutated healthy homozygote subjects (41).

3. OTHER TYPES OF ALOPECIA

In the event that diffuse hair loss is not secondary to androgenic alopecia, the etiology can be multifactorial: medicinal, hormonal, nutritional, and systemic. Of primary importance is to evaluate the extent of loss and its chronology and to eliminate the possibility of physiological or seasonal hair loss. Once the real degree of hair loss has been established, the cause must be investigated; either the cause can be suppressed and regrowth is then the rule, or it cannot be suppressed and treatment merely suspends the end result.

Finally, some cases of diffuse alopecia, especially in the female, may reflect a combination of etiological factors, hence the importance of a thorough etiological investigation.

Before considering other causes of alopecia, it should be borne in mind that some patients present with a “false alopecia” due to:

- a sudden awareness of physiological hair loss,
- genetically determined fine hair,
- genetically determined sparse hair.

These consultations for false alopecia can often be triggered by authentic episodes of acute hair loss, giving rise to initial, and then long-term, anxiety on the part of the patients.

In every case, the patient must be reassured pointless treatment avoided, and any underlying anxiety or depression dealt with. Examination of the scalp using a Dermatoscope® (42) or a trichogram (43) may suffice to reassure the most anxious of patients.

3.1. Diffuse Alopecia

3.1.1. Acute Diffuse Alopecia

Acute alopecia (44) is usually secondary to a telogen effluvium (loss of hair in telogen) and sometimes to anagen effluvium (loss of hair in anagen). It can occur suddenly, within a few weeks.

Acute Telogen Effluvium Reactions. The event responsible for the telogen effluvium precedes the alopecia by 2–3 months. The alopecia is sudden and affects especially the temples and around the ears. It is reversible in 4–6 months. Etiology takes the following forms:

- childbearing, spontaneous or induced abortion; breastfeeding delays hair loss postpartum but can lead to slower regrowth;
- prolonged high temperature greater than 39°C for whatever reason;

- surgical operation or general anesthesia;
- acute hemorrhage, acute nutritional deficiency, sudden weight loss;
- serious accident, bereavement, extreme stress (psycho-affective) shock.

In all these cases, regrowth is spontaneous and total; a whole year is required to restore the original hair volume in the female. Vitamins or sulfur-containing amino acids can promote regrowth and reassure patients (unpublished data). Topical minoxidil should be avoided during an acute effluvium as it is capable of initially accentuating the hair loss due to the fact that it may speed up shedding of hair in telogen. Later, when the acute phase is over, minoxidil can accelerate regrowth.

Alopecia of a Toxic or Iatrogenic Nature. Too often overlooked, investigation must systematically include the possibility of such applications by recording in detail the drugs administered and by taking into account the likelihood of any accidental or professional exposure to a toxic substance. Toxic or toxin-induced alopecia is usually limited to the scalp but the eyebrows, armpit hair, pubic hair, and any other body hair, can be affected.

Toxic or Toxin-Induced Alopecia. The main toxins inducing alopecia are as follows:

- Thallium continues to be used as a pesticide in some countries. Contamination of food stocks has been responsible for minor epidemics. Its use for criminal purposes has been reported. Alopecia occurs 10–20 days after exposure to the poison. Associated neurological symptoms are suggestive.
- Arsenic poisoning is accompanied by a palmoplantar exfoliative dermatosis, hair loss, nail lesions, sensorimotor polyneuritis, and digestive, cardiovascular and hematological signs.
- Boric acid is contained in many pharmaceutical preparations: eyedrops, gynecological topical solutions, spermicides, antiseptics, etc. It is also used as a herbicide, fungicide, and bleaching agent. Acute cases of poisoning combine digestive signs, hyperthermia and convulsions followed by diffuse erythema and diffuse alopecia.
- Chloroprene is used in the synthetic rubber industry and in the synthesis of neoprene; the workers involved are now well protected and cases of chloroprene alopecia are no longer observed.
- Numerous plants containing cytostatic substances are responsible for alopecia of anagen type: colchicums (colchicine), cantharidin, etc.
- Radiotherapy is known to cause dose-dependent reversible or irreversible alopecia within about 2 weeks. Alopecia may be irreversible if scar tissue is produced which usually occurs with irradiation dose greater than 60 Grays.

Drug-Induced Alopecia. Drug-induced alopecia (45) is much more common than toxin-induced alopecia.

Some drugs almost consistently induce alopecia and the mechanism by which they induce this is well understood (Fig. 14). Others have occasionally been thought responsible for alopecia but the observations are often isolated or poorly documented. Table 1 presents the drugs known to cause alopecia.

Before attributing an alopecia to a drug, it is advisable to verify that the time (lapse) to onset is consistent and that the mechanism of the alopecia is compatible with the action of the incriminated drug.

The most severe forms of alopecia are of course those observed with antimitotics (Fig. 15). Any of these can induce an anagen effluvium, but the incidence and severity depend

upon both the dosage used and the nature of the products themselves. Adriamycin, the other anthracyclines and taxol, for example, cause rapid



Figure 14 Toxic induced alopecia (Depamide). (Photo: Dr. Reygagne)

and total alopecia in more than 90% of patients. These cases of alopecia are completely reversible; the patients must be informed and, above all, reassured.

The prevention of chemotherapy-induced alopecia can be attempted with a cooling hood rather than with the earlier inflatable device that has now been abandoned.

Other Acute Forms of Alopecia. Numerous acute general or systemic disorders can be responsible for sudden alopecia:

- autoimmune disease (lupus erythematosus, dermatomyositis, systemic sclerosis, etc.),
- Hodgkin's or non-Hodgkin's lymphomas,
- infectious diseases,
- vasculitis (polyarteritis nodosa, other vascular disorders),
- acute anemia,
- dysthyroidism, other acute endocrine disorders.

In all these cases, alopecia is not the only danger signal and the etiology is readily established by the whole range of other symptoms.

- Secondary syphilitic alopecia can occur between the third and eighth month after the chancre (Fig. 16). It is usually heterogeneous, patchy, formed of multiple small and incomplete alopecic areas, but it can also take on the appearance of an acute diffuse alopecia. Blood tests for syphilis (TPHA, VDRL) are always positive.

- Alopecia areata (Fig. 17) is normally manifested by hair loss in circumscribed areas and readily diagnosed, but it can sometimes result in diffuse hair loss that is more difficult to diagnose. Personal or family history, progression, patchy effect, ophiasis or around the ears, hair that is disfigured or forms an “exclamation mark,” involvement of the lashes, eyebrows or body hair or associated nail abnormalities provide clues to the diagnosis.

Table 1 Drugs Inducing Alopecia

Group I: Frequent Alopecia	Group II: Occasional Alopecia	Group III: Rare Alopecia
<i>Antimitotics</i>	<i>Anticoagulants</i>	<i>Antidepressants</i>
Anthracyclins	Coumarine	Imipramin, desipramin
Actinomycin D	Phenylindione and derivatives	Maprotilin
Bleomycin	Heparins	Fluoxetin
Cyclophosphamide, Chlorambucil	Dextrans	Paroxetin
Methotrexate, 5-fluorouracile		
Vincristine, Vinblastine		
VN26, VP16		
BCNU, CCNU		
Hydroxyurea		
Paclitaxel		
Colchicin		
Cisplatin		
Mitomycin		
<i>Interferons</i>	<i>Anticonvulsive</i>	<i>Beta blockers</i>
Interferon alpha	Hydantoin	Propanolol
Interferon α 2a, α 2b	Carbamazepine	Metoprolol
	Sodium valproate	Nadolol
		Timoptol
<i>Retinoids</i>	<i>Antithyroïdiens</i>	<i>Hypocholesterolemiants</i>
Vitamine A (overdose)	Carbimazol and derivatives	Clofibrate
Isotretinoin	Thiouracil and derivatives	Fenofibrate
Etretinate		Clinofibrate
Acitretin		
<i>Heavy metals</i>	<i>Protease inhibitors</i>	<i>Neuroleptics</i>
Lithium	Indinavir	Phenothiazins

Bismuth	Nelfinavir	Haloperidol
Gold	Ritonavir	
	Saquinavir	
<i>Other medications</i>	<i>Other drugs</i>	<i>Other drugs</i>
Anabolic steroids	Corticosteroids	Albendazol-Allopurinol
Danazol	Oestroprogestatives	Amiodarone-Bromocriptine
Testosterone	Androgenic progestative	Captopril-Cimetidin
Aromatase inhibitors	Reverse transcriptase “inhibitors”	Clomid-Chloramphenicol Dixyrasine-Enalapril Ethambutol-Ethionamide Fluconazol-Gentamicin Ibuprofen-Indometacin L -Dopa, methyldopa Methysergide-Naproxen Nitrofurantoin-Piroxicam Proguanil-Sulfalazin Terfenadine-Verapamil

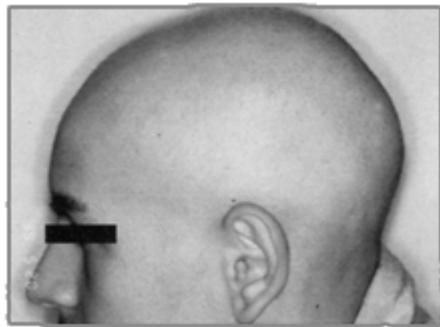


Figure 15 Antimitotic induced alopecia. (Photo: Dr. Reygagne)

3.1.2. Chronic Non-Androgenetic Diffuse Forms of Alopecia

Chronic diffuse alopecia in the male takes the form of androgenetic alopecia in the great majority of cases. Diagnosis is usually straightforward as it occurs after puberty and the topography is typical. In females affected by chronic diffuse alopecia, androgenic alopecia is also the most frequent diagnosis but others can be considered: iron deficiency,

or dysthyroidism in the first place, and also nutritional deficiency or an endocrine, inflammatory or idiopathic (chronic telogen effluvium) syndrome. Clinical observation will determine the laboratory tests that may be needed. In the absence of clinical orientation, and faced with a female non-androgenetic diffuse alopecia, blood testing is required including hematogram, ferritin, and thyreostimulin (TSH) levels (46).

Non-androgenic chronic diffuse alopecia is sometimes difficult to detect in women. The bitemporal recession can be marked or the effect may be most noticeable at the apex of the crown but the hair loss is usually diffuse, affecting also the back and sides of the scalp.



Figure 16 Secondary syphilis. (Photo: Dr. Reygagne)



Figure 17 Alopecia areata. (Photo: Dr. Reygagne)

The etiology usually takes the following forms:

- drug or toxin,
- iron deficiency,
- malabsorption or malnutrition,
- dysthyroidism, systemic disorders,
- renal or hepatic insufficiency,
- chronic telogen effluvium.

Chronic telogen effluvium (CTE) is an entity of unknown etiology characterized by Whiting (47,48); it mainly affects middle-aged women having, or having had, a good head of hair. Differentiating it from AGA_F is sometimes difficult. Its progress fluctuates, hair loss is diffuse, sudden and recurrent, often seasonal, but hair renewal occurs in between the episodes of effluvium and the hair density remains almost normal. The traction test is positive over the whole scalp area and there is no miniaturization toward vellus hair. During hair loss phases, the trichogram can demonstrate a significant and diffuse telogen effluvium over the whole scalp area, not just limited to the AGA_F region.

Telogen effluvium due to iron deficiency is a differential diagnosis to be eliminated by blood ferritin assay.

Treatment of CTE depends upon vitamin treatment or treatment with sulfur-containing amino acids over a period of 2–3 months (unpublished data). Iron deficiency, a recent episode of stress or a drug-induced etiology must be eliminated and these patients must be reassured and the fact that CTE does not progress toward true baldness must be explained.

3.2. Localized Alopecia

3.2.1. Non-scarring Localized Alopecia

Alopecia Areata. Alopecia areata affects 2% of the population. Its etiology is as yet unknown but it is probably an autoimmune disorder, however psychological factors could represent triggering factors. There is a family history in 20% of cases. Recent studies have shown the importance of the predominance of class II HLA groups. The antigens DQ3, DR4, and DRw11 predispose to alopecia areata. Conversely, the antigen DRw52a protects from alopecia areata. The antigens DR4, DR11, and DQ7 predispose to total or universal alopecia areata (see “Other Acute Forms of Alopecia” under Sec. 3.1.1).

The diagnosis of alopecia areata is simple, the patches are smooth, the scalp is not scarred and the hair follicle orifices remain visible; at the periphery there are dead hairs

and fractured "exclamation mark" hairs; the traction test at the periphery is positive when the condition is progressive.

In a child, such events are often severe with total or universal hair loss. Ophiasis with hair loss around the nape of the neck often has a poor prognosis and can spread more readily than patchy alopecia. The alopecia can affect the lashes and eyebrows as well as other areas of body hair. Nail signs are visible in the most severe forms. The first stage consists of punctuations in the nail plate. In the most severe cases, longitudinal striations, sometimes deep, are evident.

Histology demonstrates a reduction in the number of terminal hairs, with an increase in the number of hairs in telogen and in catagen. Numerous follicles are miniaturized; a lymphocytic infiltrate occurs around the bulbar and suprabulbar region (usually greater around terminal follicles). Here again, horizontal section yields a better picture of these changes.

Localized forms of alopecia areata often regress spontaneously. The use of corticosteroids by the topical or intralesional route represents the main initial therapeutic strategy.

Congenital Temporal Triangular Alopecia. This form of localized alopecia can be bilateral and symmetric but the asymmetrical or unilateral forms are those most frequently encountered. Diagnosis may be delayed, as some forms are not detected until puberty. The triangular patches are usually the site of very fine vellus hairs. This condition remains unchanged throughout a person's life. Treatment is not necessary but if the demand for it is strong, topical minoxidil 2 or 5% applied twice a day, morning, and evening can be offered. If the improvement after 6 months' minoxidil treatment is inadequate, micrografts may be an option.

Trichotillomania. This is a common localized form of alopecia most commonly seen in childhood. It is often limited in extent and the prognosis in childhood is good. Hairs that have been broken or pulled out repetitively have different lengths. The scalp is normal. There is no inflammation, erythema, squames, or atrophy. The hair resists traction with tweezers which serves to differentiate it from alopecia areata. The prognosis of trichotillomania in an adult is more serious rather than in a child. There are more extreme forms and psychiatric treatment is essential in such cases. Trichotillomania applied to eyelashes usually involves the upper lid, as those on the lower lid are difficult to hold. Histologically trichomalacia is noted, with hairs broken within the follicles, intrafollicular hemorrhages, numerous hairs in catagen, and an absence of inflammatory signs. Hair counts show a normal number of terminal hairs, a low number of vellus hairs and an increase in the number of hairs, in catagen and telogen.

Traction Alopecia. Traction alopecia is very common among African or Caribbean patients. It starts with alopecia in a marginal band predominantly of the fronto-temporal areas and the anterior temporal area (Fig. 18). It is aggravated by bunches, plaits, pony tails, excessive hair handling, and by relaxing treatment.

Alopecia with Bubble Hair. This form of alopecia is secondary to the use of excessively hot hairdryers or curling/straightening tongs that are too hot. The hair deformed by the bubbles is fragile and breaks readily, causing irregular alopecic plaques.



Figure 18 Traction-induced alopecia.
(Photo: Dr. Reygagne)

Loose Anagen Syndrome. It is most often seen in subjects with pale or deep blond hair. The hair shows little growth and these children do not go to the hairdresser. The hair is easily removed by traction, without pain. The diagnosis can be confirmed by trichogram: the hairs are all of the anagen type and deprived of their sheath, with bent anagen bulbs. The differential diagnosis is alopecia areata. There is no treatment, pulling the hair should simply be avoided; the appearance improves with age.

Other Causes of Non-scarring Localized Alopecia. Secondary syphilis can give rise to diffuse alopecia or small patches of incomplete alopecia. Thick psoriasis and pityriasis amiantacea are also capable of inducing reversible alopecia. Alopecia secondary to infection (Fig. 20), hair shaft abnormalities, genodermatoses, or acquired dermatosis affecting the scalp will not be dealt with here.

3.2.2. Localized Cicatricial Alopecia

Alopecia is said to be cicatricial when there is replacement of hair follicles with fibrosis (49). This is therefore a permanent alopecia that, once established, can only be corrected by surgery (hair implant and/or scalp reduction).

The same clinical appearance characterizes the final stages of cicatricial alopecia of whatever cause: atrophic areas of the scalp where the hair shaft orifices have disappeared are sometimes accompanied by sclerotic areas. At this stage, the histological appearance yields little or no information, hence the need for biopsy of a recent, progressive lesion.

There are numerous causes of cicatricial alopecia that can be separated into two groups:

- i. Primary alopecia where the hair follicle is the specific target of the destructive process: such as found in Brocq's pseudo-pelade, lichen planopilaris, follicular degenerative syndrome, chronic lupus erythematosus, balding folliculitis, keloid acne, or dissecting cellulitis of the scalp.
- ii. Secondary alopecia in which the hair follicle is destroyed in a non-specific manner, such as in sarcoidosis, scleroderma, neoplasms, and trauma. In this group there is a very large number of causes, congenital or acquired. Diagnosis is achieved by

histology and treatment consists of attention to the causative disorder (whenever possible).

The main causes of localized cicatricial alopecia are the consequences of a specific destructive process of the follicles (primary alopecia).

Pseudo-Pelade of Brocq. Described for the first time by Brocq in 1885 (50), its existence is not universally recognized. Some consider it to be an autonomous entity, others believe it to be a cicatricial stage common to various diseases such as lupus erythematosus, lichen planus, scleroderma, sarcoidosis etc.

The classic appearance is stereotypical, consisting of multiple small alopecic areas that are discreetly atrophic, sometimes slightly erythematous in the active state, disseminated on the scalp, especially at the vertex, developing in a centrifugal manner, often confluent "like footprints in the snow." It mostly affects adults but rare pediatric cases have been reported. Progression is often slow and gradual over the years, with alternate periods of aggravation and remission.

Histology at the initial inflammatory stage shows a moderate or minimal lymphocytic infiltrate, generally superficial but capable of involving the middle third or even the complete follicle (51). Perifollicular fibrosis is secondary; it starts and predominates in the middle-third of the follicle.

At the cicatricial stage, all the follicular structures have been destroyed and there only remains a fibrous band and the arrector muscle.

Direct cutaneous immunofluorescence is negative. The absence of specificity of clinical and histological signs in pseudo-pelade of Brocq (that can also be encountered in lichen planopilaris and cutaneous lupus of the scalp) makes this entity a diagnosis by elimination.

Since this disorder evolves slowly over many years and the plaques are multiple, monitoring is problematic. It requires charts and standardized photographs.

Lichen Planopilaris (LPP) or Follicular Lichen Planus. This represents follicular involvement in the course of lichen planus (LP). It is the most frequently encountered cause of primary cicatricial alopecia, in our experience, but LPP only represents 1.8% of cases of alopecia (50). One in two cases of LPP is not accompanied by any lesion outside the scalp area (52). It occurs most often in females after the age of 30 years (52). The most classic clinical form appears as a follicular hyperkeratosis with a purple perifollicular erythema visible around the edges of the cicatricial alopecic plaques whose distribution on the scalp although preferentially median. The patches spread centrifugally; they are mutually confluent and the areas of cicatricial alopecia are readily atrophic.

Papular forms are extremely rare on the scalp (51). Histological examination yields evidence in the initial stage of a rather superficial, perifollicular lichenoid infiltrate surmounted by an orthokeratotic hyperkeratosis organized as corneal plugs in the follicular orifices. Invasion of the basal membrane or keratinocytic necrosis is variable and the lymphocytic infiltrate is less dense than in the course of ordinary LP.

At a late stage, the appearance is indistinguishable from that of other types of cicatricial alopecia.

Direct cutaneous immunofluorescence is an important element in the diagnosis when it is characteristic: with labeling of the cells of a globular shape with anti-IgM serum or,

less often, by anti-IgG, IgA, C3 antibodies, or fibrinogen (53). This characteristic image was found in 64% of the cases reported by Mehregan et al. (52).

Its evolution is chronic, of varying severity, interspersed by remissions that vary in duration.

Two clinical forms are individually noteworthy:

i. *The Graham Little syndrome*: This combines a cicatricial alopecia of the scalp with spinous lichen planus like lesions of the body and non-cicatricial

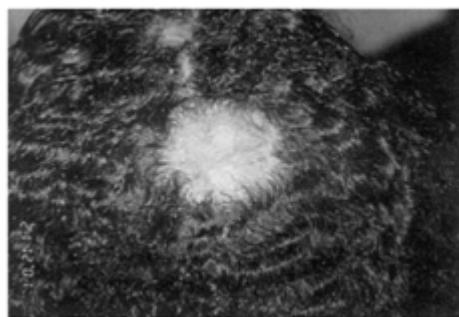


Figure 19 Cutaneous lupus erythematosus. (Photo: Dr. Reygagne)

alopecia of the armpits and pubis. This entity is not universally recognized as a manifestation of lichen planus (54).

ii. *Post-menopausal fibrosing frontal alopecia*: This is a fairly stereotypical clinical picture (55,56) whose characteristics are as follows:

- Onset in post-menopausal patients (mean age: 69 years).
- Fronto-temporal cicatricial alopecia, whose coronal topography is very different from that of female androgenic alopecia.
- Presence of follicular hyperkeratosis and perifollicular erythema at the scalp border during the active stage.
- Eyebrow depilation, sometimes complete, in 80% of cases.
- Generally, an absence of lichen planus signs elsewhere.
- Histological appearance of lichen planopilaris with a fibrosing cicatricial evolution.
- Evolution that is gradual and progressive over the years.
- Generally, local or systemic corticosteroids or synthetic antimalarials have proved ineffective.

The Follicular Degenerative Syndrome (FDS). Initially known as the “hot comb alopecia,” it consists of an anamnecological entity rebaptized FDS by Sperling et al. (54,57). This is a fairly frequent cause of cicatricial alopecia among the black population in the USA (53,54).

The clinical appearance is that of a cicatricial alopecia beginning at the vertex, of progressively centrifugal extension affecting mainly black subjects. Histology shows an

abnormally low-lying disappearance in the follicle of the internal epithelial sheath, sometimes associated with an infiltrate of mononuclear cells.



Figure 20 Tinea capitis (trichophyton mentagrophytes). (Photo: Dr. Reygagne)

Although such premature destruction of the epithelial sheath is detectable in very inflamed hair follicles, in FDS it is observed in the normal scalp in non-inflammatory hair follicles (54). The cause of FDS is unknown but the exclusive causal role of chemical or mechanical attack linked to African-American and Caribbean hairdressing techniques appears to have been ruled out (57).

Cutaneous Lupus Erythematosus (CLE). Scalp CLE is a frequent cause of cicatricial alopecia (Fig. 19). The scalp is affected in 50% of patients with CLE (53) often but not always accompanied by facial lesions (53).

Clinically, the typical appearance is that of one or several alopecic plaques that may or may not be accompanied by erythema (sometimes telangiectatic), hyperkeratosis with squames that are either dry and diffuse or follicular surrounding the plaques, atrophy, dyschromatic areas (hypo- or hyperpigmented), and dilation of the follicular orifices.

Histology serves to confirm the diagnosis when the condition is typical, showing an orthokeratotic hyperkeratosis often organized into follicular plugs, an atrophic epidermis, a vacuolar degeneration of the basal layer, a perifollicular lymphocytic infiltrate along the whole length of the follicle, periappendagal, perivascular, and sometimes interfollicular. Immunofluorescence, positive in 90% of cases, demonstrates bands of granular deposits along the length of the dermal/epidermal junction consisting mainly of IgG and IgM, and also of complement.

Balding Folliculitis can lead to severe cicatricial alopecia. Its treatment is problematic; histologically destruction of follicles is observed with usually superficial pustules surrounded by an inflammatory infiltrate rich in polymorphonuclear leukocytes, succeeded at a late stage by cicatricial fibrosis.

Traumatic Cicatricial Alopecia. Such cases are secondary to thermal or chemical burns, trauma, or prolonged pressure.



Figure 21 Male androgenetic alopecia, female type. (Photo: Dr. Reygagne)

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20

Seborrhea

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1. INTRODUCTION

Sebum production by the sebaceous glands is a natural process which is beneficial to the hair. However, any excess in the sebum load leads to unpleasant cosmetic and esthetic effects, and in some cases fuels specific disorders. Greasy hair looks dull and moist, and lies in thick, flat masses that are difficult to comb. Hairs are impregnated and weighed down with sebum which makes them adherent and flattens them on the scalp. Greasy-haired people notice that, after wiping or even washing, the sebum coating is quickly restored, a fact responsible for coining this condition "seborrhea" (from the Greek rheos, river). Seborrhea strictly refers to lipid production and excretion secondary to sebaceous gland activity. Most seborrheic subjects have both a greasy scalp and a greasy forehead, but some divergence between these locations may be found.

Sebum is associated with several properties in the human body. It is a vehicle for odors involved in sexual and social attraction. Volatile compounds known as pheromones are secreted by mammalian skin from apocrine sweat glands and sebum. The individual scents of each human being, which are easily detected by dogs even on clothes, are a clear illustration of this phenomenon. Similarly, the newborn child can recognize a mother's odor on her body and on her clothes. The reciprocal situation seems to be valid during the first weeks of life. The recognition curve overlaps with that of sebaceous gland secretion in the newborn.

Sebum is fungistatic to some dermatophytes. Ringworm of the scalp caused by *Microsporon* or *Trichophyton* species is indeed only seen before puberty when sebum production is almost absent. Sebum also shows bacteriostatic properties. The high squalene content of sebum would constitute, when reabsorbed, an important substrate for cholesterol and vitamin D synthesis by the epidermis. Sebum also provides vitamin E, alpha-melanocyte-stimulating hormone (α -MSH) and various other compounds to the stratum corneum. Elasticity and cohesion of stratum corneum cells are somewhat related to sebum level. Sebum also protects the skin against damage from acid solutions. Similarly, sebum protects the hair shaft. Combing and hairdressing generate frictional effects on the cuticle cells. Lipids tend to diminish the intensity of friction. As a result of depletion, oligoseborrheic subjects have hair whose ends are devoid of sebum with concomitant cuticular alterations as a result. Skin and hair surfaces are primarily hydrophobic and paradoxically are made more wettable by sebum components such as free fatty acids (1). It is indeed important to notice that hair and skin surface wettability is

a significant factor involved in various protective functions including ecosystem preservation, smoothness, resilience and barrier to various xenobiotics.

1.1. Seborrhea in Cosmetology

Seborrhea may be a matter of great concern to the affected individual. It is also of interest both to the dermatologist and cosmetologist, with numerous avenues of exploration open to the investigator, depending on the level of complexity required. Seborrhea seldom involves equally the whole scalp. Forehead, temples, and vertex are most affected while parietal and occipital areas are affected less. Even with extreme seborrhea where the whole hair is involved, the latter areas are usually less affected. This finding is of physiological importance. Androgenic alopecia presents the same pattern, which shows that the pilosebaceous unit has a different sensitivity to causal pathomechanisms depending on scalp area.

Observing the skin under ultraviolet light reveals subclinical patterns of epidermal melanization (2). Typically, a speckled perifollicular pattern is present on the face and the scalp of seborrheic individuals, in particular those with androgenic alopecia (Fig. 1). The perifollicular melanotic pattern is obvious when actinic-related melanosis is not yet present. In addition, this perifollicular aspect is not seen in children. It is also absent in the non-seborrheic parts of the body, even in sun-exposed areas. We have postulated a hypothesis that the subclinical perifollicular epidermal melanosis on seborrheic skin is the result of melanocyte activation by α -MSH produced in follicular infundibulum (3).

Greasy hair is both dull and darker (Table 1). It has lost its natural luster and darkens somewhat when wet, a feature particularly noticeable with fair hair. This condition is accompanied by adverse effects on hold. When hair is straight, seborrhea decreases its volume and flattens all hairstyles. Naturally curly hair exposed to rain become unmanageable. Therefore, sebum appears to have the same effect

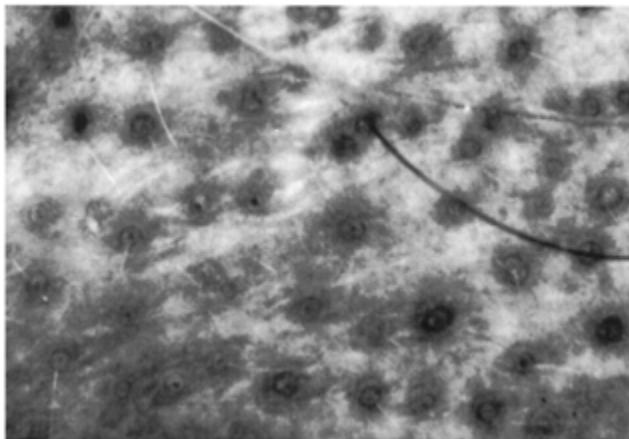


Figure 1 Infraclinical spotty perifollicular melanosis in androgenic

alopecia. Appearance under ultraviolet light illumination using a Visioscan.

Table 1 Clinical Signs of Scalp Seborrhea

Hair condition

- Darkened
- Dull and lacking luster
- Moist and greasy look
- Stuck in thick tresses
- Greasy fingertips on paper
- Limp, flattened with naturally uncurly hair
- Disorderly locks with naturally curly hair

Possible associated signs

- Pruritus of the scalp
- Dandruff in hair, on the scalp and on clothes
- Facial seborrheic dermatitis

as a humid atmosphere on hair hold. In all cases, seborrhea weighs down hairs by sticking them together in thick and unequal, disorderly tresses. When feeling these tresses, it is difficult to separate hairs and their limpness is very perceptible. Grease is left on the fingers, and even more easily on a piece of paper, which becomes translucent. This can be used as a simple test to differentiate between sweaty and greasy hair. On heating, an aqueous stain disappears within minutes by evaporation, while lipids remain. It should be noted that when sweating continues for more than 1 hr, it may be associated with increased seborrhea. Other features of scalp and facial skin may be found together with direct consequences on the hair, which help diagnose seborrhea in a subject whose hair has just been shampooed.

A scaly scalp dermatitis is often present in seborrheic subjects and is sometimes associated with pruritus or slight discomfort in the hair root during the days preceding a shampoo. While these symptoms disappear with regular shampooing, dandruff become more visible, since scales are no longer stuck to the scalp by sebum, and they are more easily shed on to the clothes and pillow.

2. STRUCTURE AND DEVELOPMENT OF THE SEBACEOUS GLAND

Human sebaceous glands develop between weeks 13 and 15 of gestation appearing as outpouchings of cells from the follicular primordia. They appear in the same sequence as the development of hair, following a cephalocaudal sequence. The 14-week-old fetus

possesses mature glands in the scalp and face where follicles are present. Elsewhere on the body, sebaceous glands appear at various times after the hair follicles are formed.

The mature sebaceous gland is a holocrine-lobulated gland which is distributed throughout the skin except on the palms and soles. Apart from specialized sites such as the eyelids and prepuce, sebaceous glands open indirectly onto skin surface via the hair follicle (Fig. 2). Three types of pilosebaceous follicles are recognized according to the volume of the sebaceous gland and the size of associated hair. They are accordingly termed terminal hair follicle, vellus hair follicle and sebaceous follicle. Terminal hair follicles are present on the scalp and beard region in men. The hair shaft is thick and the sebaceous gland is of medium or large size reaching about 1 mm^3 . Vellus hair is found over the entire body surface except in the palms, soles

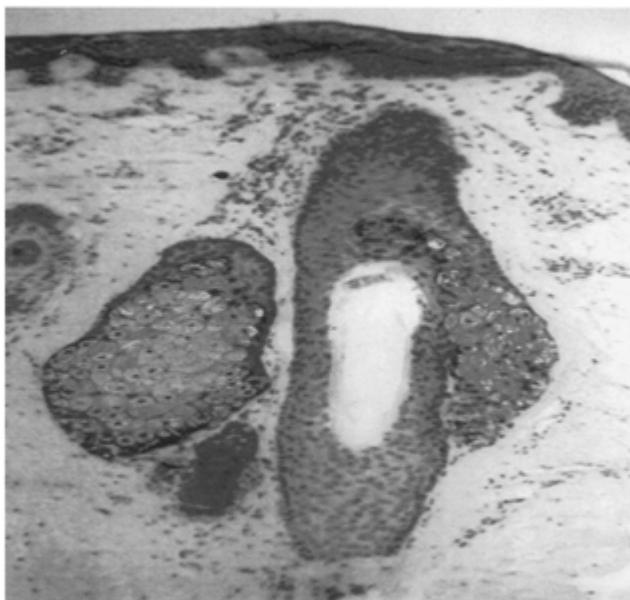


Figure 2 Sebaceous gland attached to a hair follicle (epithelial membrane antigen immunostaining).

and areas with terminal hair follicles. In this type of follicle, the hair shaft is short and thin, and the sebaceous gland is tiny when present. The sebaceous follicle is only found in humans, particularly on the face. The gland is quite large whereas the hair shaft is miniature and does not reach the skin surface.

The density of sebaceous glands differs from site to site on the body, being highest on the face and scalp followed by the back, chest, abdomen, arms, and legs. The face contains $300\text{--}1500\text{ glands/cm}^2$, the scalp about $300\text{--}500\text{ glands/cm}^2$, and other sites present 100 glands/cm^2 or less.

Sebaceous glands produce sebum by a holocrine process beginning with the proliferation of basaloid undifferentiated cells located at the periphery of the acini (4) as well as in transglandular walls (5). As the sebocytes mature, they increase in size. They become enriched in the so-called epithelial membrane antigen, and their organelles change significantly (Table 2). They move toward the ostia of the glands while enlarging up to 150-fold in volume during lipid synthesis (Fig. 3). Dyes for neutral lipids (Oil Red 0, Sudan III) stain these foamy cells intensely. Finally, the cell wall ruptures, the lipid content and the cellular remnants form the sebum discharged into the sebaceous duct and to the pilosebaceous infundibulum. The overall transit time of sebocytes within the gland takes about 2–3 weeks (4,6). It takes a further week or so for the sebum to reach the skin surface.

The pilosebaceous infundibulum is a reservoir which may contain a considerable amount of sebum (7). This reservoir influences the rate of sebum delivery to the surface of the stratum corneum which itself may be considered as a sponge trapping part of the sebum and eventually resorbing lipids (8). In these respects, the bulk of lipids present on the skin surface depends on so many variables that it does not directly reflect the metabolic events taking place in the glands themselves.

The size of sebaceous glands is likely to be variable with time. One glandular acinus may empty while another enlarges and then shrinks in turn. Such a mechanism

Table 2 Characteristics of Sebocytes at Different Stages of Maturation

Organelles	Peripheral undifferentiated cells	Differentiating cells	Mature sebocytes
Nuclear/cytoplasmic ratio	High	Lower	Low
Free ribosomes	High	Abundant	Sparse
Mitochondria	Numerous	Abundant	Numerous
Golgi apparatus	Small	Large	Sparse
Sebum vesicles	Absent	Variable	Large and numerous

implies a dissociation between sebum production and excretion processes. In normal individuals, sebaceous glands are further submitted to complex rhythms of sebum excretion (9). Dietary habits have no influence on sebum production although fasting selectively decreases squalene synthesis, and starvation has been reported to abate sebum production (10).

The activity of sebaceous glands affects the amount and nature of microorganisms found in the infundibulum and at the skin surface (11). The sebum-enriched pores provide lipophilic microorganisms and parasites including *Propionibacterium* spp., *Staphylococcus epidermidis*, *Malassezia* spp. and *Demodex* mites with an aerobic environment (Fig. 4).

2.1. Lipid Composition of the Sebum

Skin surface lipids originate from two sources, the keratinizing epithelium and sebum. The composition of lipids from these two origins differs greatly. Large amounts of sebum may be collected by washing the scalp with lipid solvents such as petrolatum ether, diethyl ether, hexane or methanol/chloroform, which are subsequently evaporated. Biochemical analysis and high-performance thin layer chromatography (HPTLC) reveal the presence of diverse-specific components. Native sebum is made of triglycerides, wax esters, squalene, and cholesterol esters

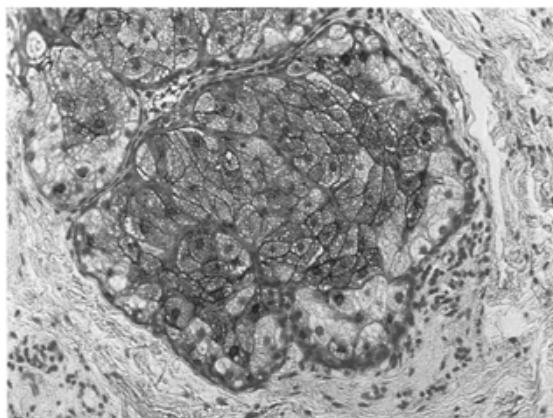


Figure 3 Sebaceous lobule showing the maturation of sebocytes.

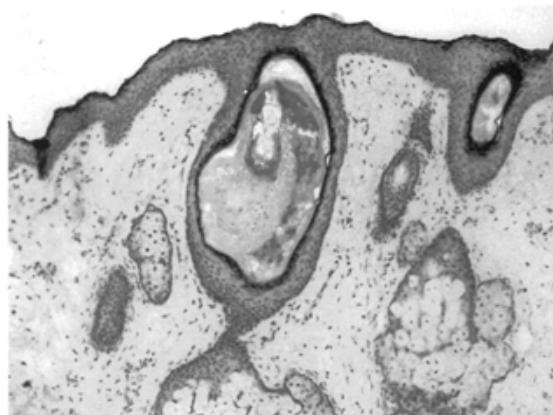


Figure 4 Enlarged infundibulum enriched with many bacteria highlighted by immunostaining.

(Table 3). In mature sebaceous cells, vacuoles almost fill the cytoplasm and contain two components, clearly visible on electron microscopy. One is opaque, cloudy and osmium-positive and is probably enriched in squalene. The other component is translucent and osmium-negative reflecting the presence of saturated lipids.

Synthesis of the various components of sebum implies two different pathways including (1) squalene synthesis via the classical mevalonate and farnesyl pyrophosphate route (2) and fatty acids and wax esters synthesis. Cholesterol is only present in trace amounts in natural sebum because it is related to the structural elements of the cell rather than to sebum itself. Indeed sebocytes do not process the necessary enzymatic equipment to synthesize cholesterol from squalene. Wax esters or squalene are generally used as an indicator of sebum excretion to differentiate sebum from epidermal lipids.

The fatty acid component of the triglycerides is varied, being either saturated or unsaturated, either branched or not, and showing straight hydrocarbon chains with even or odd numbers of carbon atoms. Wax esters contain the longest chains, the C₁₆ and C₁₈ fatty acids being most commonly found. Indeed, fatty acid synthesis involves successive additions of two carbon atom sequences through binding on acyl

Table 3 Average Lipid Composition (%)

Component	Native sebum	Epidermis	Skin surface	
			Forehead	Scalp
Triglycerides	57	15	30–40	30
Wax esters	25	0	22–25	22
Squalene	15	7	12	12
Cholesterol esters	3	23	7	3
Diglycerides	0	5	2	1
Free fatty acids	0	20	16–25	31
Ceramides	0	30	2	1

carrier protein. When acetyl CoA is involved as the precursor, the resulting fatty acid chains are straight, with an even number of carbon atoms. When propionyl CoA is the precursor, the straight chains are iso-branched and contain an even number of carbon atoms. Otherwise, when α -methylbutyryl CoA is involved as precursor, synthesis produces anteiso-branched chains with an odd number of carbon atoms. Such synthesis takes place independently of mitochondrial metabolism. Fatty acids included in the wax ester component have a distinct profile from those in the triglyceride fraction.

The diversity and complexity of sebum fatty acids make them clearly distinct from those released by adipocytes, where all the chains are straight, with an even number of carbon atoms, and sometimes having a double bond between C₉ and C₁₀. In contrast when present, this double bond is located at the C₆–C₇ binding in sebum. Once synthesized, the fatty acids of sebum are incorporated into three distinct fractions. The first ones are used to produce esters of free cholesterol of structural origin. Another significant fraction is reduced to C₁₄–C₂₄ fatty alcohols, further esterified with fatty acids to generate

polyunsaturated and branched wax esters. Lastly, the third and major part of fatty acids is used to build the triglyceride component. When reaching the region of the excretory duct, the mature sebocytes disintegrate under the influence of lysosomal activation with consequent hydrolysis of the membranes and structural elements. The synthesized lipids are thus released.

During its transit up to skin surface, the sebum composition is altered by oxidative processes and biodegradation, partly induced by specific microorganisms (12,13). Indeed, triglyceride hydrolysis by bacterial lipases gives rise to free fatty acids, and mono- and diglycerides. At the skin surface, epidermal lipids are admixed with the sebum forming a spotty or continuous lipid film covering the stratum corneum (Table 3). In adults, the relative contribution of epidermal lipids to extractable surface lipids is insignificant in areas rich in sebaceous glands, but it can affect surface lipid composition on the limbs and trunk away from the midline. On the scalp and forehead, for example, epidermal lipids are present at 5–10 µg/cm² of skin whereas sebum is present at 100–700 µg/cm².

Quantitative variations in sebum production with the ingestion of drugs (retinoids, antibiotics, etc.) are often associated with subtle changes in its molecular composition (14) which in turn may alter the cornification of the infundibulum and be a key factor for comedogenesis (15,16). Other changes in sebum composition have been ascribed to specific diseases such as AIDS (17). These findings await confirmation.

The complexity and changes in the composition of lipid mixtures forming the sebum may explain some variability in its physical properties. Sebum viscosity varies among subjects. Under normal indoor conditions (20–22°C), it ranges between 0.6 and 0.8 poise (18). Viscosity increases below 30°C, and complete solidification occurs below 15–20°C (18). These features relate to scalp temperature, which is about 33°C in a thermally balanced patient, but it may fall below 20°C under cold or windy conditions. In such situation, sebum spread on hair is probably in a viscous or solid state. However, it probably remains fluid in the follicular duct and in the sebaceous gland.

2.2. Sebaceous Gland Volume and Activity

Some information about the glandular activity can be obtained from biopsies and histological examination. Cross-sectional area of the gland measured by planimetry and image analysis reflects its volume. However, data interpretation has to be carried out under strictly defined conditions. Gland volume is a function of four parameters including the germinative compartment size, the proliferation rate, the duration of the differentiation phase, and the final volume of the sebocyte. Any change in these parameters leads to different effects on the global size of the gland. Increased mitotic activity leads to an enlarged gland. Any increase in differentiation time results in gland reduction as it discharges earlier, and the lobule shrinks under the pressure of neighboring tissues. Thus, senile sebaceous hyperplasia can be observed concomitantly with a reduced proliferation rate and considerable lengthening of the sebum transit time, which results in marked glandular hypertrophy accompanied by a considerable fall in sebum production. As a consequence, except in some particular situations, measurements of sebum production should normally provide a better indication of sebaceous activity than gland volume determinations.

2.3. Sebum Excretion and Spreading

Basically, one should distinguish sebum produced at the scalp surface and sebum accumulated on hair shafts. Indeed, most of the available information on sebum excretion comes from studies performed on glabrous skin. Sebaceous gland dynamics involves four distinct components which are sebum production (a secretion rate function), storage (a volume function), surface output (a delivery rate function), and stratum corneum permeation (an influx rate function). The oily appearance of skin and hair results from an excess of sebum excretion, spreading and interaction with sweat and with skin and hair surfaces.

On the scalp, sebum appears partly as discrete droplets emerging from follicular outlets, and partly as a surface coating. The droplets are unevenly spread on the hair. In normoseborrheic subjects little or no sebum is found up to the first centimeter of external hair or on the tip. In seborrheic subjects, the whole hairshaft appears to be fully coated with sebum. The lipid coating along the hairshaft decreases progressively from root to tip although the tip may appear greasier through contact with neighboring hairs (19). On the skin surface, sebum is usually accumulated in the follicular funnels from where it flows out. Sebum permeates the superficial layers of the stratum corneum, but a homogenous film is only found in seborrheic subjects. Sebum does not migrate along the hairshaft (19,20). Hair whose distal end is dipped into sebum does not become coated by lipid. A drop of sebum left on hair for some days does not move or spread. However, sebum migration occurs between two hairs or in a swatch, due to capillary forces (20). A characteristic feature of greasy hair is the formation of tresses whose average intershaft distance is reduced. Capillary forces do not extend beyond about 16 cm, showing an initial refatting rate of 2–3.5 mm/min (20). For the whole hair, the lipid flow reaches about 22 mm/hr during the first 30 hr in a subject where 900 mg of sebum are collected 60 hr after a shampoo (21). To sum up, the density in sebaceous glands is lower in the scalp than in the forehead, but the hair surface is available for spreading of sebum. Consequently, the true surface coated by lipids may be far larger, taking into account that the whole surface of the hair is about 6–8 m² or even more vs. a 500–700 cm² scalp surface.

2.4. Methods of Sebum Excretion Measurement

Methods for the subjective evaluation of hair greasiness have been proposed based on tactile and visual scales (22). Their correlation with an overall rating into five classes (very dry, dry, medium, greasy, very greasy) is often satisfactory. These assessments are particularly useful for the appraisal of sebum-controlling products.

Measuring sebum excretion gives better objective evidence of greasy skin and hair. Over the years, a wide variety of ingenious methods have been developed for measuring the amount of sebum excreted at the skin surface and on the hair (23). Indeed, scalp and hair sebum counts must be distinguished because they differ significantly. Estimating the amount of scalp sebum needs either a miniature sampling method or the hair must be shaved 24–30 hr before measurement. There is apparently no effect from hair removal on sebum excretion. A multi-pronged approach may be necessary to assess hair and scalp greasiness accurately.

During the past decades, the amount of sebum present on the skin surface has been measured using several non-invasive methods based on solvent extraction, cigarette paper pads, bentonite clay, photometric assessment, and lipid sensitive tapes. Nowadays, only the two latter methods are routinely used. Indeed, the others tended to give inaccurate results unless considerable care was taken to avoid the many sources of potential error. The recognized value of the current techniques is the result of both reproducibility and sensitivity of various samplings on a given skin site for a given subject. A reproducibility of about 10% and a sensitivity threshold in the 5 µg range of lipid amounts are usually considered to be satisfactory for *in vivo* non-invasive methods (24).

Sebum production and secretion in the gland itself can hardly be evaluated. In contrast, sebum excretion at the skin surface after its transit within the storage and delivery units corresponding to the infundibulum reservoir can be conveniently measured using specific non-invasive techniques (23–26). Several methods were landmark steps designed to evaluate certain parameters quantifying the sebum bulk and rheology. It should be kept in mind that the components of excreted sebum can be partly trapped as for any xenobiotic by the stratum corneum (8,27). Hence, part of the sebum is free inside the infundibulum and at the skin surface while another part permeates onto the stratum corneum before eventually being metabolized and further resorbed.

2.5. Photometric Techniques

The photometric ground glass technique was a major advance in technology in the early 1970s as easy and reproducible collections of sebum were obtained for the first time (28). The basic principle relies on the fact that opalescent glass or sapphire plate of a given opacity to light becomes translucent when its surface is coated with lipids. Compared with the other techniques, the photometric procedure is time-saving, highly reproducible and does not require specially trained scientific staff.

The first accurate optoelectronic apparatus, called Lipometer® (L'Oréal, Paris, France), afforded direct readings of the collected lipids in µg/cm² (29–33). The total amount of sebum produced is obtained by successive sampling from the same area of skin. Four samples were sufficient for dry skin but at least six were required for greasy skin. In the latter case, however, the instantaneous production of a follicular droplet did not allow a complete degreasing effect. It is possible to eliminate such a source of error by taking into account the first four samplings only.

The amount of lipid collected decreases with the order of sampling, following an exponential law, which implies the existence of a constant ratio between the amount of lipid sampled and the amount present on the skin before sampling.

The first sample, which gives a higher value for the lipids, is correlated with the total amount of sebum, and is therefore an acceptable index of this parameter. However, this mathematical approach may not be valid in the case of very high sebum levels, where the first sample collects more than the saturation value of the glass slide.

The Sebumeter® SM810 (C & K Electronic, Cologne, Germany) is a commercially available device which has gained popularity (34,35). In contrast with the Lipometer, sebum is absorbed onto a piece of opaque plastic strip instead of being adsorbed on a sapphire plate. The measuring probe consists of a 0.1 mm-thick matted plastic strip on a roller which is manually rewound for each measurement. The probe is pressed against the

skin surface with a built-in spring delivering a constant pressure at each measurement. Pitfalls may arise from skin microrelief and roughness which impair the close contact between the probe and the stratum corneum. After the probe has been in contact with skin surface for 30 s, measured by an internal timer, it is placed back into the main unit of the Sebumeter. Transparency of the plastic film is measured by a photocell after emitted light has passed twice through the strip. The result is evaluated by a microprocessor using an internal standard. It is acknowledged that the measures are a good reflection of the actual amount of lipids present on the strip. In fact, it is estimated that an average of about 40% of total skin surface lipids is absorbed with one sampling. The digital read-out displayed as $\mu\text{g}/\text{cm}^2$ gives the estimated total amount of lipids on the skin. However, this extrapolation may be inaccurate when seborrhea is intense because there is a saturation effect of the plastic strip. The sebum amount can also be expressed as a value relative to an oil-saturated standard instead of absolute values in $\mu\text{g}/\text{cm}^2$. These relative data are equally useful for the comparison of different treatments since they avoid complex calibration procedures involving solvent extraction of lipids. Nonetheless, in order to get valid data, it is necessary to take several samples within a given area so as to avoid problems associated with the heterogeneity of sebaceous gland activity.

The Lipometer and the Sebumeter do not give the same values for the amount of sebum on the face (23). Each of the devices has some advantages over the other. The Sebumeter is more user-friendly as there is no need to clean the probe after each evaluation. Calibration of both devices must be performed on a regular basis.

The above-mentioned techniques of sebum sampling yield a single global estimate of the casual total lipids present on a given surface of the skin at one point in time. The test area is always large compared with the size of the ostia of sebaceous follicles. Thus, any difference between the activities of individual sebaceous follicles remains impossible to evaluate. A few overactive sebaceous follicles releasing a significant amount of sebum have a disproportionately large effect on measurements.

2.6. Tape Techniques

Almost 15 years ago, progress was made simultaneously by two research teams who introduced a method using standardized hydrophobic lipid absorbent tapes (36–38) made of an opaque, open-celled, microporous polymeric film. Since its introduction, it has always been considered as complementary to and as useful as the photometric method (23,24,39–42). It has largely superseded solvent extraction and gravimetric methods. In most studies, sebum excretion is evaluated on the forehead. Ideally the tape material has to be affixed to the skin by gentle pressure ensuring the elimination of air bubbles. When the sebum absorbent tape is placed on a skin area that can be moved by muscles, the investigator should periodically check that the uniform contact between tape and stratum corneum is maintained throughout the test.

Two proprietary tapes are currently available. One type is the regular Sebutape[®] (Cuderm Corp., Dallas, USA) characterized by the presence of an adhesive coat on one side of the tape designed to adhere tightly to the horny layer. Such an adhesive coat impairs the swift penetration of sebum into the tape. The other type of lipid-sensitive tape is designed to be applied for only a very short time to the skin surface without any

adhesive coating. These commercially available tapes are the Instant Sebutape[®] (Cuderm Corp.) and the Sebufix[®] (C & K Electronic).

Depending on the protocol design, the skin may be prepared prior to the timed collection by removing sebum from skin surface. The collection time should be determined according to the type of tape. With the regular Sebutape, the adhesive interposed between the lipid sensitive film and the skin is a limiting factor to the transfer of lipids. This may be of importance when the rate of sebum excretion is low and/or when the duration of the test is short. On the contrary, a saturation effect occurs on regular Sebutape when evaluating intense seborrhea during a test period beyond 1 hr. The amount of sebum collected over several hours is in fact lower than the addition of hourly sebum collections during a similar cumulative period of time. This is associated with confluence of lipid droplets and inaccuracy in identifying each spot as a single sebaceous follicle. These features are the main reasons why sebum samplings longer than 1 hr should be avoided when using regular Sebutape (43). When using one of the uncoated tapes, a contact time of 30 sec to 1 min is appropriate.

During the test, each follicular outlet (enriched in sebum) pours out lipids which fill pores in the tape rendering it transparent at each site. The size of the clear spots is proportional to the amount of sebum delivered. The number of spots reflects the number of sebum-rich follicular ostia. These parameters can be evaluated by visual inspection alone. Looking at samples against a black background in reflection mode results in a black and white pattern that can be assessed using an ordinal scale. The method allows one to obtain a rough, but reasonable estimate of skin greasiness without requiring sophisticated equipment. Better quantitative evaluation is achieved using computerized image analysis (23,38,39,41,42,44), transmission photometry (40) or reflectance colorimetry (45,46).

Image analysis is the most sensitive and accurate method (Fig. 5) as offering the possibility of recording the number and size of individual spots and calculating the mean and total area of spots (TAS). The free sebum content of the follicular reservoir is conveniently estimated using Sebufix tapes affixed onto the head of a recording videocamera working under ultraviolet light illumination (Visioscan VC 98[®]; C & K Electronic). Computer-assisted image analysis provides proper readings (47).

A photometric evaluation obtained by measuring the intensity of light transmitted through the samples is an alternative rapid approach (40) and is roughly equivalent to the TAS value obtained by image analysis. A variant is represented by reflectance colorimetric assessment of the samples placed against a colored background (45,46). However, these overall quantitative approaches lose one of the main benefits of the lipid-absorbent tape method, namely the evaluation of sebum excretion of each individual sebaceous follicle.

A similar quantitative method was designed aimed at collecting data at any time during which the tape remains applied onto the skin. The principle relies on the measurement of the modifications in color of the tape that occur when it becomes transparent. It shifts the natural "white" color of the tape to a color closer to that of the skin itself (46). Reflectance colorimetry is conveniently expressed as DE*ab

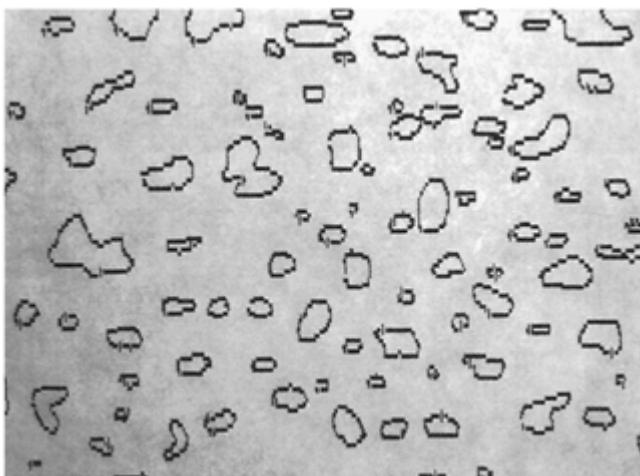


Figure 5 Image analysis of a lipid-sensitive tape applied to a shaved area of the scalp.

or calculated following the variation in the so-called individual typology angle (ITA°)(48,49). The benefit of such an approach is the ability to obtain multiple measurements without removing the tape, and therefore to explore the kinetics of sebum output.

There are some limitations to the proper interpretation of the data. Some are specifically related to the material itself. Sebum spots are subject to changes in their size and transparency depending on storage time and temperature. At 20°C or so, they should be evaluated at a defined time after removal, preferably within 24 hr. When immediate evaluation is not possible, storage in a freezer at -30°C is advisable.

2.7. Combined Methods

When using lipid-sensitive tapes alone, the interpretation of the number and size of lipid droplets with regard to the sebaceous glands may be difficult. In fact, it is not valid to ascribe a single follicular outlet to each spot, particularly when the latter is large. In order to solve such uncertainty a two-step method was designed (38). Before removing the sebum-sensitive tape from the forehead, its outlines are delineated on the skin with an ink mark. In a second step, a follicular biopsy using a cyanoacrylate-coated polyester film (Melinex O; ICI Plastic division) is harvested from that site. The follicular biopsy (50) which is an extension of skin surface biopsy (51) is a good way of studying follicular casts and microcomedones (16,50,52–54). The ink marks of the outline of the sebum-sensitive film are present and visible on this material. The skin surface stripping and the corresponding lipidsensitive tape are then exactly superposed using the ink imprint as an adjusting mark. The material is then examined under the microscope before processing in a computerized image analyzer. This method allows simultaneous assessment of the size

of lipid droplets and that of the corresponding follicular ostia and microcomedone (Fig. 6).

A surrogate method relies on examination using a videocamera working under ultraviolet light illumination (Visioscan VC98®; C & K Electronic). A frame designed to precisely attach and locate the camera is first affixed onto the test site.

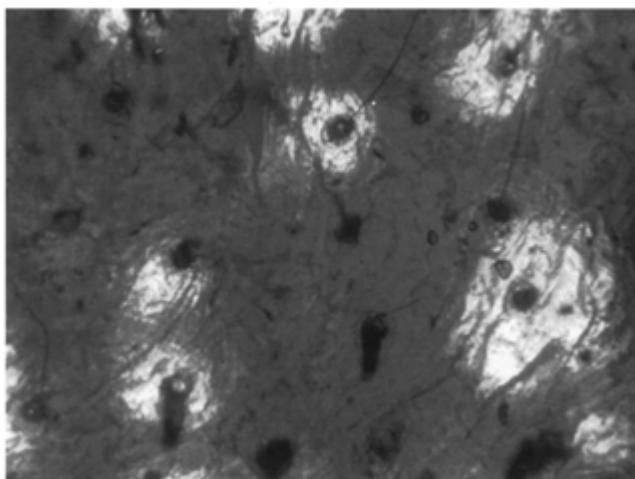


Figure 6 Combination of cyanoacrylate skin surface stripping with a lipid-sensitive tape showing the hair follicle openings enriched or not in sebum.

The appearance of the skin surface and follicular outlets is recorded. In a second step, a Sebufix is interposed between the stratum corneum and the camera. The picture of lipid droplets is recorded after a 45 sec collection. The comparison of both pictures identifies sebum-poor and sebum-rich follicles (47).

2.8. Quantitative Parameters of Sebum Excretion

The diverse sampling methods of sebum provide information on a series of specific parameters. As a rule, the values obtained for these parameters may differ from subject to subject by a 10-fold coefficient but each value is representative of a given subject in the same environment.

2.8.1. Sebum Casual Level

Sebum casual level (CL) is defined as the lipid amount present at equilibrium when the skin surface remains untouched for several hours (12,28,29). It is an estimate of skin

greasiness. For practical reasons, most researchers record CL after a 4-hr lag time following a controlled removal of the sebum film from the skin surface. As it is not certain that the measurement reflects a plateau value, CL is not recommended as a single parameter for in-depth studies of the sebaceous system. CL is believed to be a constant value for each normal adult. In contrast, interindividual variations are large as shown by CL ranging from 100 to 700 $\mu\text{g}/\text{cm}^2$ on the forehead of healthy subjects (12,25). Similar wide variations are found on the scalp (32).

2.8.2. Sebum Excretion Rate

The sebum excretion rate (SER) refers to the amount of sebum excreted by a given skin area during a defined period of time (12,28,55–58). The duration of the defined collection period is important because SER progressively decreases over the first hours after degreasing the skin (59). SER in the first hour of sampling usually ranges from 0.5 to 2.5 $\mu\text{g}/\text{cm}^2/\text{min}$ on the forehead. On the scalp, it varies from 0.1 to 0.8 $\mu\text{g}/\text{cm}^2/\text{min}$ (32,60–63). TAS values yielded by the lipid-sensitive tape method represent a surrogate for SER evaluations.

As a rule, SER is correlated with CL (12). Irrespective of the method employed, a linear relationship is commonly found between four successive 1-hr SER and TAS measurements at least in the medium range of seborrhea severity (43). The correlation is lost when the sebum output is either very low or quite high which indicates that these parameters are related to the delivery of the pool of sebum already secreted and stored in the outer portion of pilosebaceous duct (7). Thus, it is very clear that an initial 3–4 hr collection is a measurement of sebum excretion rather than sebum secretion.

2.8.3. Sebum Replacement Time

The sebum replacement time is less frequently used because it is difficult to reach precise figures. It refers to the time needed to recover CL after removing sebum from skin surface. It has been reported to take about 4 hr in subjects with normal sebum excretion (12).

2.8.4. Density in Sebum-Enriched Reservoirs

The number of spots over a lipid-sensitive tape is a rough indicator of the density of follicular reservoirs enriched in sebum. Such a figure is usually lower than the number of sebaceous glands present on that area of skin. This information can be confirmed by staining cyanoacrylate follicular biopsies for lipids. The sebum delivery at a given ostium may represent a clue for the presence of an actively secreting sebaceous gland. It may also represent the site of a follicular reservoir passively filled by the sebum coming from the skin surface. It is clear, using the combination of lipid sensitive tape and follicular biopsy, that many follicular ostia neither store sebum nor are a route for sebum outflow (44). It is also possible that one single droplet corresponds to the merging of several smaller ones. The size of the follicular outlet on the skin surface is not correlated with the presence or absence of sebum (44,47).

2.8.5. Instant Sebum Delivery

SER and TAS decrease almost linearly during the first hours of collection time. Calculating the regression line for the cumulative data at 1 hr intervals allows one to extrapolate a theoretical value for instant sebum delivery (ISD) at T_0 . Such a parameter is supposed to reflect the spontaneous leakage of free sebum from follicular reservoirs (45,64). ISD is not always correlated with SER. Similar information to ISD is provided by the Sebufix tape applied to the skin for a few seconds.

2.8.6. Follicular Excretion Rate

The slope of the above-mentioned regression line between cumulative TAS over time has been coined follicular excretion rate (FER). It is a measure of the delivery rate of sebum from the follicular reservoirs. FER is not infrequently related to the first-hour TAS value although physiological influences and some topical compounds may interfere with such a relationship (45,64–67).

2.9. Secondary Physical Parameters Influenced by Seborrhea

The dull or darkened color of hair, the limpness, and the difficulty in hair manageability are all criteria that can be measured by refined physical methods. With a goniometer and polarized white light, the inclination angle of cuticle cells to the fiber axis can be determined as a measurement of their reflectance and therefore of hair gloss (68). Hair bounce (69), combability (70,71), and even hair volume may be assessed (72). The tendency to adhere in tresses and the mean spacing between individual hairs can also be measured (20).

The mechanical properties of hair are altered by increased hydration as a result of coating by fat or the adhesive effect of sebum. Dullness may be related not so much to the total amount of sebum on hair, but to changes in lipid composition. An increase in wax esters, unsaturated fatty acids and monoglycerides, and a decrease in cholesterol esters has been reported to occur in seborrhea (22). The surfactant properties of monoglycerides should facilitate sebum flow. More C₁₈ unsaturated fatty acids and an increased ratio of 8-octadecenoic acid to 9-octadecenoic acid characterize greasy hair, producing a decrease in viscosity (73,74).

2.10. Factors Affecting Sebum Excretion

2.10.1. Physicochemical Regulation

SER and FER are influenced by the physicochemical characteristics of sebum. Environmental and skin temperatures, and the balance between the sebum molecular components affect the viscosity and rheology of lipids at the skin surface (55,67,75). This could explain in part chronobiological variations in SER and FER including seasonal fluctuations (76) as well as the influence of the ovarian cycle (77,78) and perhaps other chronobiological rhythms of unknown periodicity (9). A circadian rhythm has been found

(55,79) with sebum output being maximal in the mid morning, and minimal during late evening and early morning hours.

The width of follicular ostium may greatly influence sebum rheology. There is an inverse relationship between the fluid flux and the fourth power of the radius of the tube in which the fluid passes through. Hence, variations in corneocyte accumulation and swelling at the lips of follicular outlets, as may occur during the ovarian cycle or after occlusion, may influence sebum rheology (77,78,80,81).

Skin surface energy phenomena result from molecular interactions. They are involved in sebum and sweat dispersion. Sweating is indeed an important confounding factor in rating sebum excretion. Even in dry-skinned patients, intensive or continuous sweating increases the CL. The sebum of seborrheic subjects appears as an oily and homogeneous fluid barely emulsified with water at ordinary temperatures. However, in some instances, it can be emulsified with sweat though it may take several hours. A drop of sebum labeled with a lipid stain enters a drop of sweat stained with methylene blue by means of pseudopodia to finally engulf it. Microscopic examination reveals a W/O or an O/W emulsion according to the relative volumes of both phases. Blue droplets contrasting on a lipid background or the reverse situation, depending on the time of sampling, before or after sweating, may be seen microscopically by spraying methylene blue and Sudan red onto the skin *in vivo*, followed by sampling of the sebum with a glass slide. The sweat of different subjects does not exhibit identical emulsifying properties.

The theory of a continuous sebum excretion is opposed by the concept of a discontinuous excretion with a feedback control through the CL. Some experiments have shown that SER declines progressively as CL regains its initial value. From this observation comes the hypothesis of a feedback mechanism controlling sebaceous excretion by the lipid film on the skin surface. This concept was strengthened by the fact that the amounts of sebum collected at constant time intervals seem to increase with the number of degreasing procedures. Sebum excretion stops spontaneously even in highly seborrheic patients if the area, although isolated, remains uncovered. However, the plateau effect in excretion kinetics is only apparent and is due to the spreading of sebum over a larger surface, or to its permeation into the upper layers of the stratum corneum. It is concluded that the initial phase of sebaceous secretion is likely to be continuous or fluctuating as a result of a combination of chronobiological rhythms. Any feedback mechanism from CL could only affect the sebum excretion thus modifying sebum storage rather than sebum production.

Shampoos dedicated to greasy hair are commonly used as sebum-controlling products. Some chitosan-derived molecules hinder sebum coating of the hairshaft. Particles of dry shampoos adhere to hair, retain lipids and exert electrostatic forces repulsing the hairshafts. Frequent regular shampoos do not increase the sebum output on the scalp and do not influence the sebum coating of hair. In contrast, cationic polymers and silicone oils used in some shampoos facilitate sebum spreading. Regreasing studies on scalp and hair show general differences according to the level of greasiness. The amount of hair sebum is typically lower than scalp sebum. Scalp CL recovers completely after 1–4 days following hair washing, at least for greasy hair types (32,63) and remains fairly constant in the following days. Scalp SER has been reported to progressively increase until 24 hr after shampooing when it reaches its maximum value (60).

2.10.2. Hormonal Regulation

Human skin, in particular the sebaceous glands, receive, produce and coordinate activation and inactivation through various molecular signals. The physiological mechanisms involved belong to the endocrine, paracrine, juxtacrine, autocrine and intracrine hormonal repertoire. In the endocrine function, hormones produced by established distant endocrine organs reach the target sebocytes through the blood circulation. In the paracrine function, hormones act locally on cells other than those by which they are produced. In juxtacrine functions, hormones produced by one cell interact directly with the receptors present on the closest neighboring cell. In autocrine functions, hormones act on the cell from which they have been released. In the intracrine function, hormones are activated within the cells in which they are produced, by binding to nuclear receptors. In this context, the sebaceous gland fulfills all requirements for being one of the key tissues in the skin with regard to being one of the largest independent peripheral endocrine organs.

Sebocytes are capable of synthesizing cholesterol which is a substrate for steroid hormone synthesis. The same cells also convert the circulating androgen steroids dehydroepiandrosterone (DHEA) and androstenedione to testosterone and further to 5- α -dihydrotestosterone (5 α -DHT). The two latter hormones can exert intracrine, paracrine, and endocrine activities. Thus, their effects are located both in the sebaceous gland itself and in the hair follicle, and beyond.

The outer follicular root sheath produces proopiomelanocortin (POMC) derivatives such as the adrenocorticotrophic hormone (ACTH) and α -MSH (82). Productions of ACTH and α -MSH are upregulated by ultraviolet light and interleukin (IL)-1 and downregulated by tumor growth factor β (TGF- β) and corticosteroids. It is assumed that α -MSH controls the androgen effect and acts rather specifically on lipogenesis. In keratinocytes, α -MSH stimulates cell proliferation. Follicular keratinocytes also produce corticotropin-releasing hormone (CRH).

Sebocytes contain a series of hormone receptors. The so-called serpentine membrane receptors encompass the CRH receptors types 1 and 2, and the melanocortin receptors MCR 1 and 5, both showing affinity for ACTH and α -MSH (82,83). CRH receptors in human sebocytes can be regulated by several downstream hormones, including testosterone, estrogens, and growth hormone (GH).

Another family of membrane receptors includes the GH, insulin and insulin-like growth factor (IGF-1) receptors. GH is supportive for increased sebum production (84,85). It stimulates sebocyte differentiation and also enhances the effects of 5 α -DHT on sebaceous lipid synthesis. Insulin also significantly stimulates sebocyte proliferation and differentiation especially in combination with GH. IGF-1 and GH may also control in part hair growth and hair patterning in man (86).

Two families of nuclear receptors, namely the steroid and thyroid receptor families, are also present in sebocytes. The first group is important because sebaceous gland development requires androgens. However, androgens alone have no discernible effect on stimulating sebocyte differentiation in *in vitro* culture systems (87,88). The very role of androgens in promoting lipogenesis has been questioned (89).

Free testosterone and 3 α -DHT are considered to exert a major boosting and dose-related effect on sebocyte proliferation and sebum secretion in man (25,90–92). The levels of the type 1 isoform of 5 α -reductase are significantly higher in sebaceous glands

than in other skin structures (93,94). Cells of the infundibulum are also reported to be sensitive to the same hormones. In women, the most important androgen is Δ^4 -androstenedione, which is produced by adrenal gland and ovaries. It may be converted into testosterone, but it possesses some intrinsic androgenic activity. 5α -Androstane- 3β , 17β -diol is a potent androgen and is the main metabolite of testosterone in back and scalp skin. Other androgen precursors of purely adrenal origin such as DHEA explain sebaceous development in the fetus, after birth, and in the prepubertal years. DHEA can be converted into androstenedione and testosterone inside the sebocytes. By contrast, conversion of DHEA sulfate to DHEA only occurs with the assistance of monocytes exhibiting steroid sulfatase activity.

The amounts of circulating androgens are important to consider. However, the local production of sex steroids provides autonomous control adjusting sex steroid metabolism according to body area. Facial and scalp sebocytes are particularly involved in this mechanism.

The glucocorticoid receptor is another nuclear steroid receptor present in sebocytes. Glucocorticoids have been reported to stimulate sebocyte proliferation (95) and differentiation in the presence of other growth factors (82). However, and in contrast with these biological effects, sebum output at the skin surface is decreased after topical applications of corticosteroids (96).

The second group of nuclear receptors, namely the thyroid receptor family, encompasses different soluble receptors in sebocytes. They correspond to the thyroid hormone receptor isotype β 1, the estrogen receptor β , the retinoic acid receptors (RAR) isotypes α and γ , the retinoid X receptors (RXR) isotypes α , β and γ , and the peroxisome proliferator-related receptors (PPAR) isotypes α , δ , and γ . PPAR ligands augment the androgen stimulation of sebocyte differentiation (97).

Estrogens exert the opposite effect of androgens but with a much weaker potency. However, any decrease in size and excretion of sebaceous glands is only achieved with high doses of estrogens that are non-physiological in women and feminizing in men. It is thus unlikely that normal estrogen levels play a role in inhibiting sebaceous gland activity. Following estrogen suppression, administration of testosterone restores sebum secretion. Estrogens at sebum-suppressive doses have been shown to reduce plasma and urine levels of testosterone. They also inhibit gonadotropin-releasing hormone synthesis and 5α -reductase activity.

There is no relationship between the growth rate of vellus hairs and sebum excretion (98,99). In contrast, the intensity of seborrhea and severity of evolving androgenic alopecia are often related (61). In this condition, GH and IGF-1 might play a role.

2.10.3. Neuropeptide Regulation

A neurovegetative nerve plexus surrounds the sebaceous gland, but acetylcholine and epinephrine do not seem to influence sebum secretion. Sympathectomy has no effect on the level of surface lipids. A localized neurological lesion may conversely induce seborrhea in the involved site. Several types of neuropeptide receptor are present in sebocytes. They include the μ -opiate receptor which binds β -endorphin, the vasoactive intestinal polypeptide (VIP) receptor, the neuropeptide γ receptor, and the calcitonin gene-related peptide (CGRP) receptor (83).

Psychotropic drugs, often dopamine inhibitors, increase sebaceous secretion considerably. The same observation is found in Parkinson's disease perhaps due to high α -MSH serum levels. During treatment with levodopa, seborrhea decreases, but the drug is inactive on sebaceous excretion in normal subjects.

2.10.4. Age Effect

As a consequence of the diversity of hormonal signals, sebum excretion varies according to age (23,100–106), gender (100,101,106), pregnancy (107), and climacteric period (47,108). At birth, sebaceous glands have reached a fair size in response to maternal androgens. Sebum levels in the newborn are equivalent to those found in adults. The amount of surface lipid may be lower in girls than in boys at birth, but undergoes a large increase between days 3 and 6 of life, followed by a fall to levels below those in boys. Sebum excretion may remain high until the end of the first month of life (104). Clinical signs of seborrhea can be observed including the seborrheic helmet of the newborn, accompanied by selective hair shedding at the forehead, temples and vertex, simulating androgenic alopecia. Seborrheic dermatitis of the face is frequently associated. From the second to sixth month, the sebaceous glands shrink to form small structures and sebum excretion decreases rapidly to a very low level. Then, it remains stable until mid-to-late childhood (about 8 years of age) and accounts for the often dry appearance of the scalp in young children. Considerable interindividual variations exist. Sebaceous excretion increases with the onset of puberty well before the expression of other sexual signs. Greasy hair is one of the first signs of adolescence. Girls are slightly ahead of boys at this stage, but at age 16 the masculine prevalence reappears and persists throughout life. Adult levels may be reached at mid-puberty, but full sebaceous gland maturity is not obtained in some individuals until late second or third decade of life.

During the menstrual cycle, sebum excretion increases in the premenarchal phase. In women, sebum excretion then remains fairly constant from adolescence until the menopause, when excretion may slowly decrease (47) but then shows little change after the seventh decade. In men, sebum excretion remains unchanged until the age of 80 years. Although surface lipid levels fall with older age, sebaceous glands enlarge, possibly as a result of reduced cell turnover. The decrease in sebum secretion is correlated with an increase in cholesterol concentration in the surface lipid film, which implies a higher relative contribution from epidermal lipids.

At any given age in men and women, both sebum excretion and secretion rates differ between individuals over a wide range. In addition, there is a huge overlap between the data obtained in both sexes. Hence, it is not the amount of circulating androgens but rather the receptivity of the target tissues that accounts for inter-individual differences in sebum excretion. It is clear that additional factors are likely to be involved.

SER and FER fall rapidly after birth and remain low during infancy (105,106). They slightly increase at adrenarche when the adrenal cortex begins to secrete androgens. At the dawn of puberty they increase markedly in response to gonadal activation. The onset of skin and hair oiliness reaches maximum values at about the time of achieving full physical stature. Values remain high in men until the eighth decade. In women, the rates remain unchanged until the menopause. During the climacteric period, seborrhea may either increase or steadily decrease with age. Using the lipidsensitive tapes, it is possible

to demonstrate distinct patterns according to age and physiopathological conditions (106). The size of follicular reservoirs and pores shows no tendency to shrink with age.

SRSE obtained from the bentonite clay method provides different information. This parameter peaks at around 20 years of age, and steadily declines in both men and women over the lifespan (100). The decrease per decade is approximately 23% in men and 32% in women.

A series of endocrine imbalances (91) and drug treatments aiming at direct or indirect hormonal effects also affect the activity of the sebaceous apparatus (47,96,108–112). Additionally, acne and androgenic alopecia are two distinct hormone-dependent disorders which affect many individuals without any evidence of an underlying endocrine disease. Both are typically associated with greasy skin (61,113–116).

The most potent inhibitor of sebum excretion is the synthetic retinoid 13-*cis*-retinoic acid or isotretinoin, which at oral doses of 0.1–1 mg/kg/day inhibits sebum production by up to 80% within 6–8 weeks. Isotretinoin reduces cell renewal and lipid formation in the sebaceous gland. Isotretinoin reduces not only SER but also the follicular reservoir, and both remain significantly suppressed for up to 1 year after therapy.

3. CONCLUSIONS

Our knowledge of sebaceous gland physiology and sebum rheology on skin, scalp, and hair fiber has made considerable progress, leading to the discovery of new antiseborrheic agents. This breakthrough was made possible through the development of qualitative and quantitative methods for measuring the amounts of sebum on skin, hair, and scalp, and its lipid composition.

Seborrhea on the scalp or forehead may be an isolated phenomenon, or may be part of a complex system of multiple disturbances. As an interesting index of neuroendocrine physiology, it plays an increasing role in biology, mainly due to the reliability of its measurement.

One of the most challenging tasks in supporting a claim about skin and hair greasiness is providing credible supporting data. A multi-pronged approach using a series of parameters and measurements is often recommended. Trials must be conducted on selected panelists. The relevance of the data depends on the inclusion criteria for eligible subjects. Ethnicity, age range, gender, adequate skin profile and test area on the body must be defined and appropriate for the purpose of the study. The environmental conditions including season, relative humidity, and temperature should always be controlled. Care should be taken to avoid any chronobiological effect. Despite clever experimental designs, it should be stressed that panelists perception, clinical gradings and biometrologic measurements do not always match. Whatever the parameters under investigation, strictly controlled experimental procedures are required.

Several objective methods have been devised for measuring the greasiness of the skin, most of which involve the collection of sebum once it runs off the sebaceous machinery. Preconditioning of the skin by prior removal of sebum from the skin surface is a common procedure. The part of sebum present inside the follicular reservoir can also be ignored by measurements. Uncontrolled depletion of the sebum pool impedes collection of reliable data. Hence precise methodologic procedures are mandatory to collect sound

information. Skin and ambient temperature affect sebum rheology. A number of physiological parameters modulate sebum excretion and some of them are responsible for chronobiological variations.

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21

Dandruff (Pityriasis Capitis Simplex): Of Yeasts and Men

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1. INTRODUCTION

Dandruff, a scaly disorder of the scalp, is a benign affliction shared by about 45% of the human population, irrespective of sex and ethnicity. Paradoxically, some textbooks on Dermatology even ignore it.

In fact, viewed primarily as a source of esthetical disturbance and a problem of cosmetic nature, it is hardly a disease, neither life-threatening, nor a source of pain, although itching is its almost permanent companion.

Although it may be considered trivial from a medical standpoint, dandruff is a fascinating field in skin research as a mosaic composed of physiological, microbiological and immunological components. In brief, it depicts a remarkable, if not unique, model of the cutaneous microecology.

Within the history of medicine, this affliction has been a perfect illustration, for centuries (1–3), of the difficulties of researchers of integrating, with time, the progress or emergence of new scientific areas such as microbiology or immunology.

In addition, the morphological classification of skin diseases by the masters of dermatology, contributed to the ambiguities. The precise distinction between dandruff, psoriasis, eczema, seborrhea, etc., was blurred by academic and authoritative statements. The 20th century, mostly the second half, conferred to dermatological research a more experimental and pragmatic approach, leaving dogmas behind.

The questionable ranking of dandruff as an inflammatory disorder illustrates such conflicts between facts and assumptions, with regard to the old Latin academic definition, i.e., *rubor, calor, dolor, tumor*. Scalps affected by dandruff are rarely red, painful or swollen, etc., and the pioneering histological descriptions of Sabouraud (1) shed new light on the inflammatory process at the tissue level. Although clinically silent, inflammation was demonstrated through the scattered presence of “squirting capillaries,” one of the main underlying features of dandruff.

The concomitant emergence of microbiology, still in its first uncertainties at the eve of 20th century, brought a truly new vision on cutaneous physiology. Skin then became regarded as a tissue facing chronic assaults from a microbial world, alternatively perceived as commensal or invader. In the dandruff scenario, on the scalp-stage, a resident yeast from *Malassezia* sp. (formerly *Pityrosporum*) plays a central role that has for a long time been disputed.

With time, improved methods, new approaches, etc., and in this century, we now have a much clearer portrait of this multi-factorial affliction. *Malassezia* is confirmed in its role and a large arsenal of adequate, simple and efficient treatments is available: eradicating or controlling the yeast on the scalp is the key. The following pages aim at describing the multiple criteria that confer to dandruff a particular status within dermatological and cosmetic research.

2. HISTORICAL BACKGROUND: DOGMAS VS. FACTS

2.1. Semantic/Clinical

The visible desquamation of the scalp, as scales and flakes, has a strong and logical semantic connection with corn and flour. From the Greek *pityron* (bran), creating later the generic pityriasis, to the Latin middle-age *porrigo* (likely the origin of “porridge”) to the more recent Italian *forfora*, etc., most of the terms refer to the vegetal envelope to which flakes are compared, the modern French *pellicules* making no exception. In English, the very word dandruff (initially *dandriff*, from dander) took over from porrigo in the 18th century: the abnormal desquamation suggests that scalp is angry—a popular image, as viewed later in these pages, which is somewhat realistic.

For centuries, this form of pityriasis was included in the various desquamative disorders (psoriasis, ichtyosis, eczema, etc.). It needed the talents of the 18th century masters such as Lorry, Plenk, Willan (1), etc., to give dandruff (or *porrigo*) not only scalp specificity, but also to distinguish it from the future Unna’s seborrhoeic eczema (seborrhoeic dermatitis). The clinical criterion of inflammatory-redness then became a strong guide in the classification of skin diseases.

It is only in the late 19th century that both the origin and nature of the scales received some attention. Although some suspected their cellular origin, Hebra described them as “concretions from the oily glands of the head.” Such a statement, from a so reputed master, had a strong impact and led to a new dogma: dandruff=seborrhea.

It was adopted and perpetuated by Hebra’s pupils (Kaposi, Duhring, etc.) and was continued, up to “1999”: in many studies (4,5) and handbooks (Merck Index), some anti-dandruff (in fact anti-fungal) ingredients are still registered as “anti-seborrheic.”

2.2. Microbiology

Rivolta (6), Malassez (7) and, later, Sabouraud (1) first described the high rate of colonization of dandruff scalps, by a bottle-shaped microorganism (Fig. 1). Initially regarded as cryptogenic, it was soon proposed as the causative factor, in line with the Pasteurian revolution. Such a thesis raised immediate opponents (Audry, Darier, etc.) and the following decades have witnessed for a debate of the “Chicken-Egg” duality. Mostly because normal, healthy scalps were found (1) to harbor the fungi (8), the two schools were regularly debating the basic question: was *Pityrosporum ovale* (PO, was the name initially given by Sabouraud) the primary cause of abnormal desquamation, or was the colonization by PO a consequence of a highly desquamative (and natural) condition of the scalp? As outlined by Shuster (3) in 1984, technical biases and

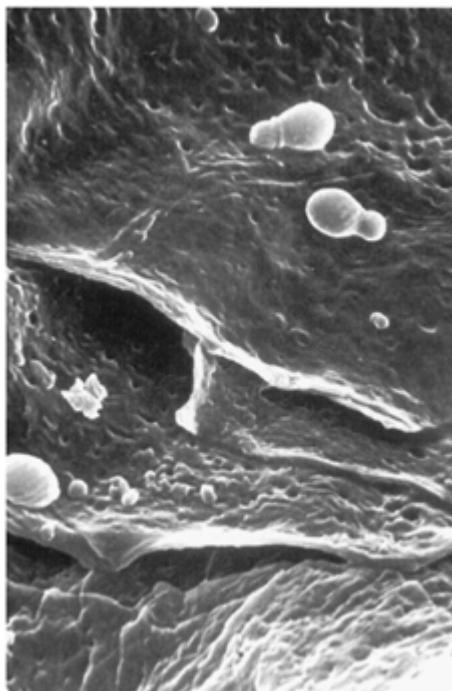


Figure 1 *Malassezia* on a dandruff scalp. SEM photograph, following a scalp replica.

misleading interpretations too often complicated this two-faced question. In addition, an industrial issue probably amplified it. The growing demand from the market prompted industrial research to provide answers: but which molecules should be developed: anti-fungal or anti-mitotic to slow down the elevated scalp turnover?

An anti-dandruff agent (5), Zinc pyridine thione (ZPT) (still quoted in hand-books as anti-seborrheic) was in the 1960s and 1970s, at the heart of this dilemma: it was alternatively shown to be anti-fungal and anti-mitotic. This had an immediate consequence on the legal side: as far as an anti-dandruff action required an antimitotic effect on the epidermis, it was a typical drug action, according to the FDA's criteria. From that event, anti-dandruff agents were therefore registered as such and included by the FDA in its various OTC drugs monographs.

To make a long story short, the consensus today is rather unanimous and clear, as developed later in these pages: the “founding fathers” Rivolta, Malassez and Sabouraud were basically right. The various active ingredients proposed and used over decades, albeit having very different chemical structures, share one common property: they all control and prevent the development of the yeast, *in vivo*.

3. DANDRUFF: FROM CLINIC TO TREATMENT

3.1. Clinical Methods

Dandruff is anything but monomorphic. The degree of scaling varies from very slight to intense or severe among subjects. Intra-individually, it fluctuates seasonally (worsens in winter) and psychological stress amplifies it. This can be assessed through visual scoring under standardized procedure (9,10). Irrespective of the range internally used, it should follow the general rules for clinical assessment, in particular:

- Necessity to well train the technician(s) involved in the scoring procedure.
- Due to the physical elimination of the scales by the act of shampooing, the visual assessment should be recorded at constant times post-shampooing, i.e., 2–3 days. Our own experience gives preference to a 2-day period, as a compromise between sufficient scaling restoration and a loss from external factors (combing, pillows, etc.) during this period.
- Since scalp scaling, in dandruff, is not a specific criterion and distinction between dandruff and seborrheic dermatitis is sometimes equivocal (see below), a careful examination of the subject by a physician, prior to any enrolment in a study, is highly recommended. In many occasions, with regard to the intense use (voluntary or not) of anti-dandruff shampoos in the western world, it is preferable to adopt a pre-wash-out period, by giving a bland shampoo to be used for at least 2 weeks prior to the start of clinical observations.
- The whole scalp should be examined, although dandruff does not involve the whole surface to the same extent, showing some foci of scaliness. In most subjects, the vertex is the scaliest area, whereas the nape is less involved and possibly scale-free. The half-head procedure (treated vs. non-treated), though valid since dandruff shows lateral symmetry (11), is tedious and may cause bias when comparing products due to possible cross-diffusion.
- A questionnaire for recording self-assessments and related parameters (itching, tolerance, stress, medications, etc.) may be very useful. Figure 2 illustrates the increased frequency of itching with clinical scores of severity.

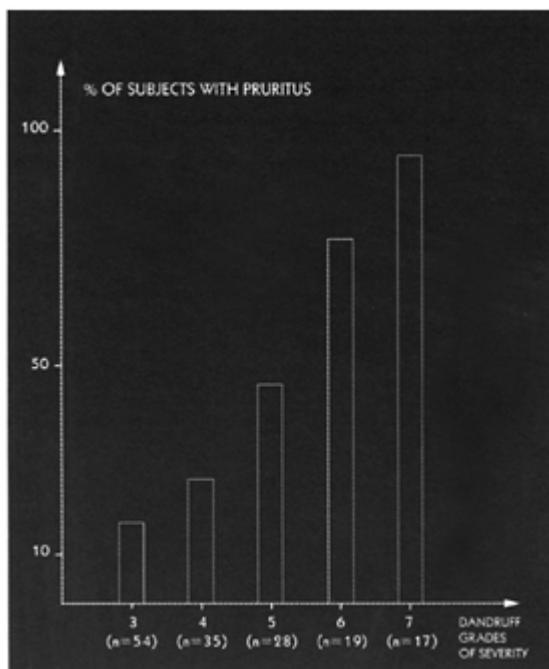


Figure 2 Frequency of itching/pruritus among subjects of various grades of dandruff severity (3: mild to 7: severe).
n=number of subjects per class.

3.2. Link with Seborrhea (See Chap. 20) (11) and Epidermal Lipids

The human scalp is a sebaceous-rich region (12,13), where daily amounts of sebum delivered to the scalp and hair surfaces are in the gram range. Many authors believed in a strong connection between dandruff and sebum (*Pityriasis steatoides*), supported by clinical and experimental evidence (14,15). Sebum is a nutrient for the resident scalp flora (see below) and puberty (which initiates a tremendous increase in sebum secretion) is a prerequisite for the onset of dandruff. Sampling sebum from a scaly surface gives a poor yield, it generates bias and may lead to conflicting results (16,17). However, when dealing with any dandruff study, it is recommended to record data on sebaceous physiology, either clinical or experimental. The Liège group failed to demonstrate a significant difference in the sebum excretion rate (SER) of the scalp in non-dandruff vs. dandruff-affected volunteers. SER appeared significantly higher in androgenic alopecia only (17).

This data suggests that we should abandon the old clinical definitions (1) relying on the greasy aspect of the flakes (*Pityriasis sicca*, *steatoides*, etc.). In the etiology of dandruff, if sebum is needed as a factor, it is unlikely to be a sufficient factor per se.

As far as the regreasing process of hair is concerned, it is a common observation that achieving a successful treatment of dandruff often leads to an increased coating of the hair fibers by sebum. Consumers often declare that they now have greasy hair. Such problems are very likely related to the "sponge" effects of both squames and stratum corneum. Both elements being decreased (squames become absent or rare with a less oil-absorbing, and thinner stratum corneum), the fraction of sebum they were previously trapping (see Sec. 3.4) then becomes available to the hair surface.

With regard to epidermal lipids, a recent study (18) indicated clear changes in dandruff, in both the amount and relative composition. It also confirmed both the inflammatory-induced dyskeratinized (loose) stratum corneum and a previous paper showed that the altered ultrastructure of the dandruff horny layer was improved by an efficient ZPT treatment (19).

3.3. Objective Methods

3.3.1. Global

Irrespective of the technique used, the initial and basic question is to determine what should be measured. Although apparently trivial, it is of a high importance. In fact, technical bias was introduced, in the past, as a result of single-cell counting from a scrub of the scalp surface (20,21). These were reviewed by Shuster (3). It is nevertheless true that dandruff-affected scalps produce many more single cells (about twice) that of non-dandruff scalps (22), reflecting the higher epidermal turnover evidenced by histology.

Since dandruff refers to increased scale production, scales should logically be the principal objects of measurement. One of their simplest definitions lies in their visible aspect (Fig. 3), i.e., a size at least above normal visual acuity (around 50 µm). Invisible single cells (corneocytes) are generally within a 30–40 µm-size range.

Numerous methods have been proposed and few are comparable with regard to what is really measured. As an example, vigorous brushing above an aluminum sheet followed by weighing does not completely fulfill the condition: though remarkably useful, a precision balance remains a blind tool.

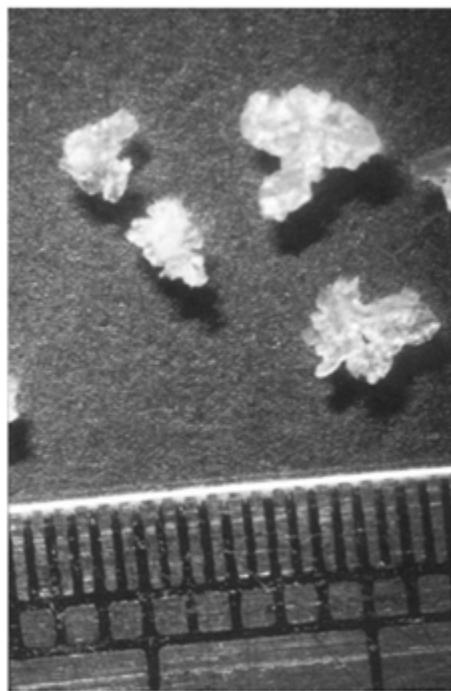


Figure 3 Squames collected from shampoo, following rinsing and drying.

The various methods range from brushing/collecting the scales (9), vacuuming the scales (23,24), video recording (25), scrubbing the scalp surface (20), and collecting through shampooing (22). Most methods of collection are generally followed by scale-weighing, whereas some others aim at determining the size of the scales using photodensitometry or image analysis (25,26).

We prefer the latter (22,27) for its specificity (only scales are retained, not single cells), simplicity and practicability. In addition, it fulfils ethical rules, represents a "natural" act and possibly a service to the volunteer under investigation.

Practically (Fig. 4), a framed nylon screen mesh ($100\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$) is inserted at the bottom of a washing sink and collects, during a standardized shampooing procedure (bland shampoo, known amount applied, time, etc.), scales and hairs (or debris). Following thorough rinsing with distilled water and gentle stove-drying overnight, scales are separated from hairs, using tweezers, and kept for further analysis (weighing, imaging, dislocation, etc.). As an example, Figs. 5 and 6 illustrate the kinetic of scale production with time of collection and its correlation with scores of severity. Table 2 summarizes the values of some parameters in both dandruff and non-dandruff subjects.

It is very difficult, if not impossible, to determine the accuracy of these methods when routinely used. For three main reasons:

- Apart from the shampooing extraction technique showing that two successive shampoos nearly remove nearly all the scales (22), few data are available about the respective yields of other proposed methodologies.
- From scalp to shoulders, pillow, comb, etc., the natural loss in scales is uncontrollable.
- Inter and intra-individual variations may be high and spontaneous.

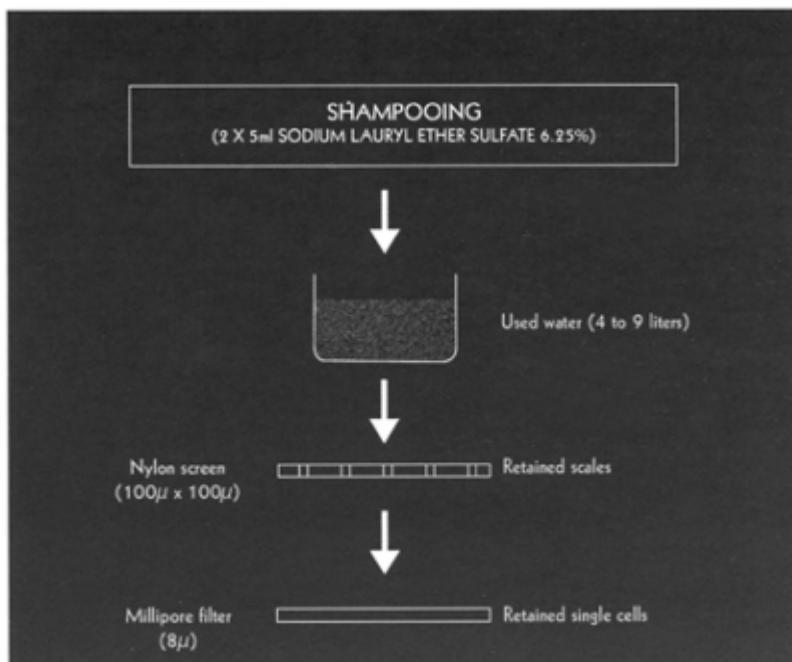


Figure 4 Scheme of the process for collecting scales and single cells.

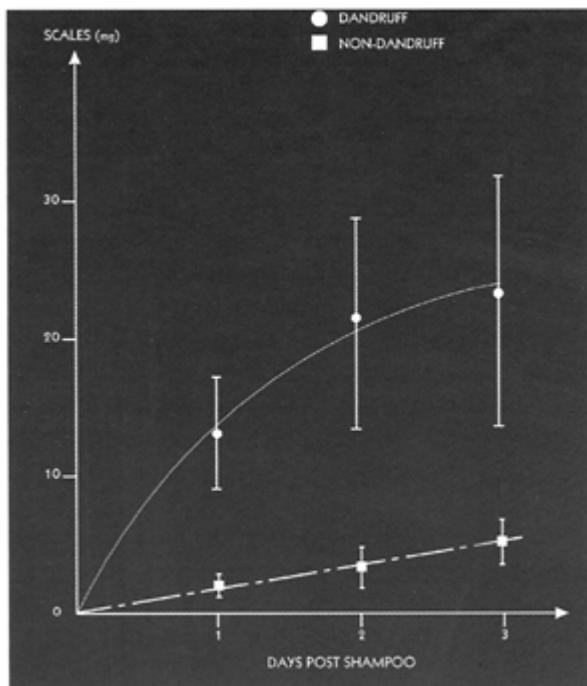


Figure 5 Kinetic of squame production at various times following initial shampooing.

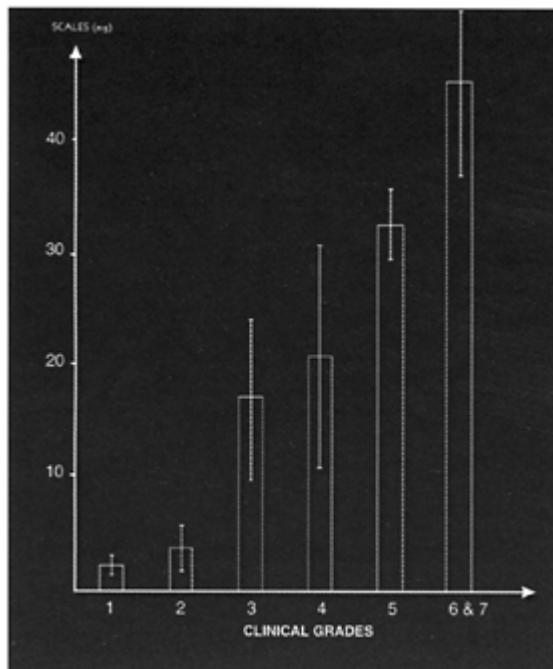


Figure 6 Relation between scale production (on a 2 days collection post-shampooing) with various grades of dandruff severity (1 and 2: non-dandruff, 3: mild dandruff, to 6 and 7 severe).

Despite these restrictions, when properly used and controlled, the above methods overcome to a large extent the limits of mere clinical grading. They finally provide useful tools for the selection of active ingredients and the assessment of final product efficacy.

3.3.2. Focal

Apart from scrub technique used for single-cell counting, few focal quantitative techniques have been developed in the past. It is only recently that this subject has received an innovative approach from the Liège group (28,29). Basically, sampling with adhesive tape (D'Squame[®]) onto the vertex, followed by immediate staining (Neutral Red), allows counting under the microscope the living yeasts and their density in number per mm² of scales onto which they adhere.

This simple and clever technique shows how scale density correlates with dandruff score and quantifies the residual effect of an anti-dandruff treatment: the “cut-off” point,

i.e., the yeast density from which dandruff becomes clinically evident, is estimated at about 200/mm².

Although it collects only the outermost fraction of the microorganisms, it appears a promising technique, not only for its quantitative aspect, but also for its help in the scoring procedure. In addition, the sampled material may be used to analyze, at the same time, the scales (number, size, etc.) through image analysis.

A development of tape stripping technique has been reported (30), elegantly showing the various patterns of inflammatory mediators among different scalp conditions (normal, dandruff, seborrheic dermatitis).

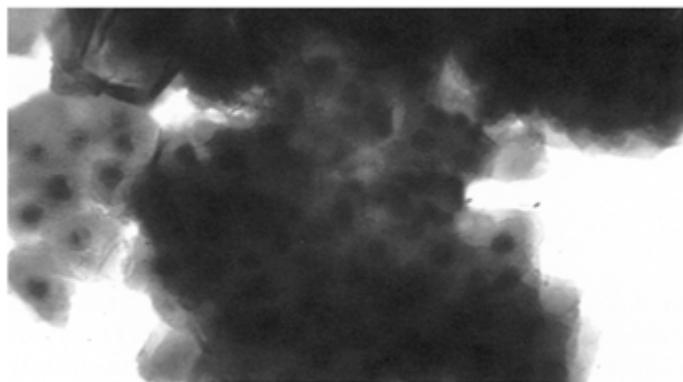


Figure 7 Squames (Giemsa stain) under optical microscope view.
Remnant nuclei (parakeratosis) can be revealed.

3.4. Cytology

As aggregates of hundreds or thousands of corneocytes, scales are visible objects (Fig. 3).

Adequate staining (e.g. May-Grünwald) reveals (Fig. 7) a high percentage of residual nuclei within the cells forming a scale. This percentage can be calculated following a rather complete dislocation of the scale to single cells, using sonication (22) and further staining and counting. This allows one approach to evaluate the parakeratotic component of the scales which probably represents the underlying subepidermal inflammatory process (31). The parakeratotic index parallels the dandruff grade (Fig. 8).

Additionally, scales may be weighed and submitted for solvent extraction. Such a procedure reveals a high content of lipids (10–40%) with a clear sebum profile (32). This confirms a previous paper (33) where scales and stratum corneum were compared with “sebum-sponges.”

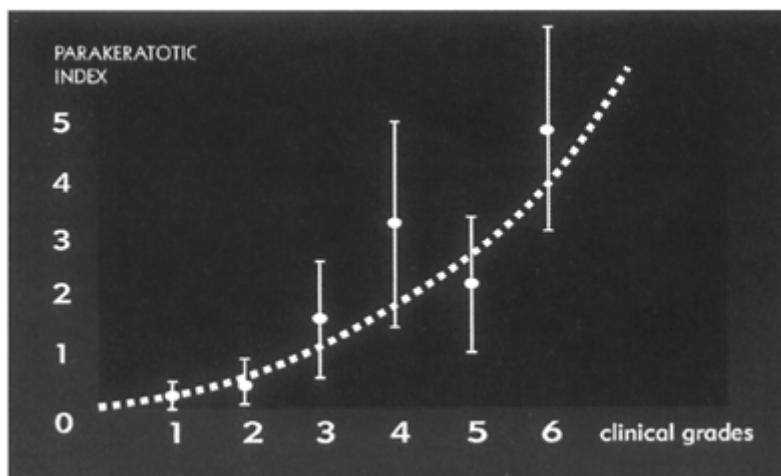


Figure 8 Variation of the parakeratotic index (mg of nucleated cells/scalp/2 days) with various grades of dandruff severity (1 and 2: non-dandruff, 3: mild, to 6: severe).

The aqueous extracts of scalp scales show neutrophil chemotactic properties, as elegantly studied by Kikuchi et al. (34). More cleavage products of the complement pathway (C5) are found in the extracts of scales from dandruff subjects than in non-dandruff subjects, suggesting that activation of this complement pathway may be involved in dandruff etiology.

3.5. Histology

The histological descriptions of dandruff-affected scalps (and seborrheic dermatitis as well) have been extensively documented by scientists such as Sabouraud (1), Pinkus and Mehregan (35), Ackerman and Kligman (31). The latter and his team adequately completed the description by comparing with non-dandruff scalps. They all address the same picture: the epidermis of dandruff scalps shows patterns of hyperkeratosis and parakeratosis, with underlying "squirting capillaries." Although non-clinically evident, a sub-epidermal inflammatory reaction (Fig. 9) is therefore confirmed.

However, the latter shows heterogeneity since the presence of scattered foci of inflammation was previously noted by Sabouraud (1), seeing "here and there, foci of inflammatory reactions amongst a landscape elsewhere serene."

Hence, the perivascular accumulation of mononuclear cells may be intense in some sites, and absent elsewhere. It is found, too, in normal, non-dandruff scalps but appears quantitatively more frequent in dandruff. Comparing the two populations suggests that no scalp shows normal desquamation, implying the presence of a gradient in inflammatory foci from non-dandruff to dandruff scalps and, possibly, seborrheic dermatitis. Such a

gradient is consistent with the parakeratotic data and scale production, as shown in Table 1.

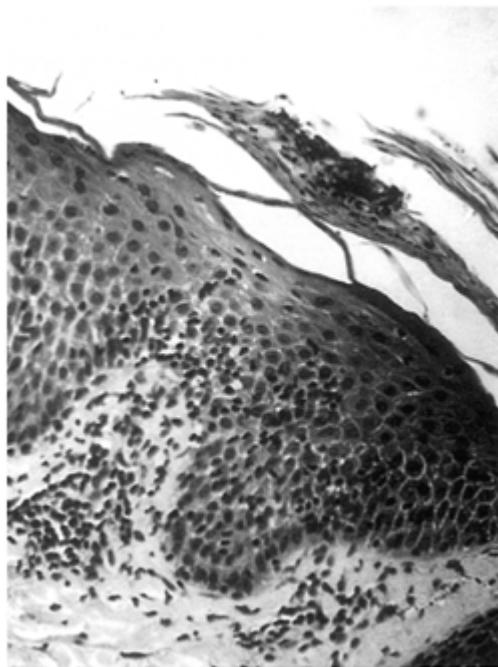


Figure 9 Histological section of dandruff scalp (H & E staining). Dermal inflammatory infiltrate is clearly seen. Squame formation/detachment is visible.

Table 1 Gross Patterns of Scale Production, Inflammatory Index, and Single Cells of Dandruff (All Grades Included) and Non-dandruff Scalps

	Dandruff	Non-dandruff
Scale production (mg/2 days)	33.2 ± 17.3	8.1 ± 5.3
Parakeratotic index (%)	18.4 ± 7.4	3.9 ± 2.1
Average single cells production ($\times 10^6/2$ days)	2.1 ± 1.3	0.9 ± 0.3

The epidermal turnover of dandruff scalp, as estimated through 3H -thymidine labeling, is about twice that of non-dandruff (31) with a noticeable heterogeneity. Some sites show intense labeling, others weak labeling. Accordingly, the sites of scale formation might

reflect the locations of uneven distribution in both turnover and maturation of keratinocytes.

Observations on the stratum corneum, always difficult due to external sources of bias (desegregated when cut), indicate a higher number of layers (average 30 vs. 12 in non-dandruff), but poorly organized, loose, with clusters of parakeratotic corneocytes. It is worth noting that examining the stratum corneum in dandruff is much better, and more simply carried out through surface sampling (using cyanoacrylate or tape stripplings). These techniques (28,29) provide correct and non-invasive ways of qualitative and quantitative determinations.

In summary, the dandruff-affected scalp, as compared with the non-dandruff scalp, results from increased foci of epidermal inflammation. These foci create, locally, a strong increase in the epidermal turnover, which, in turn, affects the maturity of keratinization, leading to the formation of scales/clumps, composed of parakeratotic cells.

3.6. Microbiology

Any study on dandruff implies a collection of microbiological data. Although the microbiological analysis of scalp flora is not per se technically difficult, obtaining a representative sample of the scalp flora is still a field where improvement is needed. For two main reasons:

- Scalp, unless bald, is not an easily accessible surface.
- Scalp flora thrives on both skin surface and within the follicle, i.e., in 3D. It is reasonable to assume that surface microorganisms, as previously shown (14), represent only a minor fraction of the whole population. Therefore, any surface sampling, e.g., from adhesive tape, contact plating, cotton swabs (36), etc., only provides an index of microbial colonization. Technical bias often occurs when the method of sampling may not lead to a representative collection.

Today, the most quantitative sampling method available remains the scrub-technique of Williamson and Kligman (37). Basically, a small sterile glass cylinder is held on the scalp surface, and 2–4 ml of a buffered surfactant are poured and scrubbed through a Teflon policeman, for a constant time. Both physical friction and diffusion of the fluid help to recover a high yield of microorganisms.

From the fluid, aliquots and dilutions in specific media are used for further selection and counting the various members of the resident scalp flora. This technique helped to show that:

- The colonization of any scalp by commensal flora is high, ranging from 10^4 to 10^7 per cm^2 (22,38). A triad of large families normally composes these resident microorganisms: *Propionibacteria* sp., *Micrococci* sp. and *Malassezia* sp. (formerly *P. ovale* and *P. orbiculare*, see later) yeast. These three gram-positive populations possess, in common, a high lipid-oriented metabolism: sebum is their preferred “fuel” (14,39,40).
- The relative proportions between these three families is scalp-specific, as compared with other sebaceous rich-regions, *Malassezia* sp. account for 45% of the whole population in normal scalp, increasing up to 75% in dandruff conditions (average $9 \times 10^5/\text{cm}^2$), reaching 85% in seborrhoeic dermatitis (38).

3.7. Etiology of Dandruff

Today, the etiology of dandruff seems rather clear: a majority of studies, using the methodologies described previously (29),(41–44), show that eradicating or controlling the yeast *Malassezia ovalis* (MO) is the most important (and simplest) strategy for treating dandruff. Using various techniques, both different protocols and active ingredients (see treatments), all indicate that applying effective anti-fungals leads to the following events, chronologically:

- Itching fades from the second or third application.
- Scale production and parakeratosis decrease in parallel (28,42).
- The yeast becomes rare or absent from cultures or in microscopic examinations (28,42).
The bacterial population seems otherwise not affected by the anti-fungal action.
- Stopping treatment restores, in about 2–3 weeks, the initial situation and MO recovers its former levels of scalp colonization (28,40,42).

This set of converging data altogether support the role of MO as the causative agent in the inflammatory reaction found in dandruff. Irrespective of the mechanisms involved, the chain of events, in dandruff scalps, could be briefly summarized into three successive steps, as follows: MO→1) INFLAMMATION→2) PARA-KERATOSIS→3) SCALE FORMATION.

Further considerations should be given to the fact that MO has clear antigenic properties (see below). Since non-dandruff subjects harbor comparable amounts of MO on their scalp, they probably differ from people with dandruff in terms of their immune response. Step 1 implies that MO would stimulate what is referred to as a host response mechanism (HRM), i.e., the various individual immune and neuroimmune (itching) sensitivity/responses to an aggressor. In this way, MO might be better defined as the main agent of dandruff rather than its cause. In the chain of events shown above, steps 2 and 3 are the ultimate stages of an inflammatory reaction classically seen in other desquamative disorders (eczema, psoriasis, sunburn, etc.) even though the actual mechanisms and clinical pictures differ.

In such a scheme, step 2 leaves room for a pure anti-inflammatory action (e.g. corticoids), which has proven benefits but is normally not justified, since using too potent drugs for such a trivial affliction, which have well-known side effects on a routine basis. Eradicating the primary cause of the problem, providing a safe anti-fungal action, would be one's first choice coming from a common sense.

However, this general scheme still leaves some doubt. It does not explain why a low-grade scaling (comparable with that of non-dandruff scalps, i.e. 2–5 mg/scalp/2 days) associated with a low parakeratotic index, still persists when dandruff has resolved and MO has vanished from cultures.

Some possibly associated hypothesis may be put forward:

- The anti-fungal agents do not eradicate the deep portion (follicle) of the living yeast's and a minimal inflammatory reaction is maintained.
- Other non-fungal agents may lead to a minimal chronic irritation such as Demodex (45), sebum-derived products (46), sunlight coupled to the presence of follicular photosensitizing agents such as porphyrins synthesized by *Propionibacteria* spp. (47).

- Physical and chronic irritation of the scalp brought about by daily routine “insults” (hard brushing, over-shampooing, hair frictions (48)).
- Neuroimmune influence (stress).

3.8. Dandruff and Hair Loss

It is a common observation, shared by many clinicians, that natural hair loss is amplified in dandruff (49). Counting hairs trapped by the screen, in the shampoo technique, confirms this observation. On a 2-day collection, non-dandruff scalps lead to a 50–100-hair loss whereas those affected by dandruff range from 100 to 300, although a positive correlation with clinical grades of dandruff cannot be drawn.

Since these hairs are mostly telogen (hair debris apart), dandruff probably alters the hair cycle. This seems to confirm experimental work on alopecia (50) where topical application of anti-fungal and bactericidal agents, resulted in a clear decrease in inflammatory infiltrate and T-cell accumulation in the isthmus of the follicle, and a concomitant normalization of the hair cycle. In dandruff, a comparable scenario is likely, the foci of inflammation appearing as a plausible chronic source of disturbance (which mechanisms?) of the hair bulb and its cycle.

3.9. The *Malassezia* sp. Revisited

The last 10 years have witnessed a significant progress in the knowledge of this microorganism, which has been reviewed and summarized by two groups of researchers (51,52). Breakthroughs occurred at various levels, clinical, microbiological, immunological, DNA sequencing, etc. *Malassezia* is now recognized as a potent vector of various afflictions among which dandruff is certainly the more trivial. Its main features are summarized in Table 2.

Historically (1), it was given many successive names (*Cryptococcus psoriasis*, *Torula vulgaris*, Bottle Bacillus of Unna, *Pityrosporum ovale* and *orbiculare*, etc.). Today, *Malassezia ovalis* and *Malassezia furfur* is the official taxonomy, although subgroups, through DNA ribosomal imprints, may be defined from wild strains (globosa, restricta, etc.) (53). It is very likely that various strains, with various sensitivities to anti-fungal agents, may be found (or selected by) on human scalps.

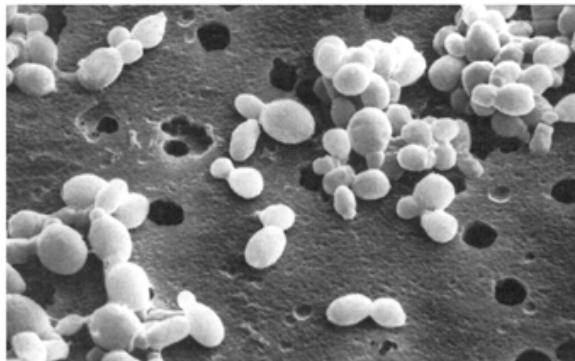
This yeast family is an anthropophilic fungus that lives in the yeast phase in two possible shapes (oval or round, Figs. 10 and 11), which are representative of different stages of the cell cycle. Its transformation into a mycelial form (hyphae), leading to *M. furfur* (as found in *Pityriasis versicolor*), is influenced by the environmental medium (54–56). *Malassezia* sp. can activate both classical and alternate complement pathways (57), *M. ovalis* and *furfur* are both antigenically and genetically identical (58–60). The strict dependence of MO on lipids, in vitro and in vivo, illustrates its high prevalence in sebaceous rich regions and its favorite sites of colonization (scalp, nasolabial folds, ears, etc.) (61) closely map those of dandruff and seborrheic dermatitis. With regard to dandruff, only one, among hundreds of scalp replicas

Table 2 Main Features of the *Malassezia* sp.

Criteria	Comments	References
Sites of colonization	Sebum-rich regions (face scalp, aisles of the nose, ears) $10^4\text{--}10^7/\text{cm}^2$. Settles at birth	(14,15,35,61)
Prevalence	>90%	(14)
Shape	Dimorphic (yeast/MO and mycelial phase/ <i>M. furfur</i>)	(54)
Culture in vitro	Tedious. Possible in lipid rich media only	(62,63)
Antigenicity-pathogenicity	Proven. Mal f 1 (36 kDa) as a major allergenic protein. Opportunistic pathogen	(58,71,72,75)
Sera antibodies against <i>Malassezia</i> sp.	Specific IgG and IGM found at early ages. Increase with ageing	(73,74)
Primary role in	Dandruff, seborrheic dermatitis (pityrosporosis), pityriasis versicolor and folliculitis	(3,44,70–72)
Secondary role in	Acne, atopic dermatitis, psoriasis	(72,80)
Systemic effects	Fungal infections. Lethal cases reported in premature newborns	(76,77)
Source of nosocomial infections	Proven. Catheters as major reservoirs	(78,79)

(Silflo® followed by MEB) we have examined, showed the presence of *M. furfur* on one follicle in one subject (free from seborrheic dermatitis) (Fig. 12). Apart from this unique case, only round and oval forms, at different stages of budding, have been found on dandruff scalps.

The relationships of MO with lipids (sebum) are strong and complex. In vitro, its culture strictly requires exogenous source of fatty acids in the medium (62,63). A high lipid-oriented metabolism of *Malassezia* can be demonstrated in vitro and in

**Figure 10** *Malassezia ovalis* (culture).

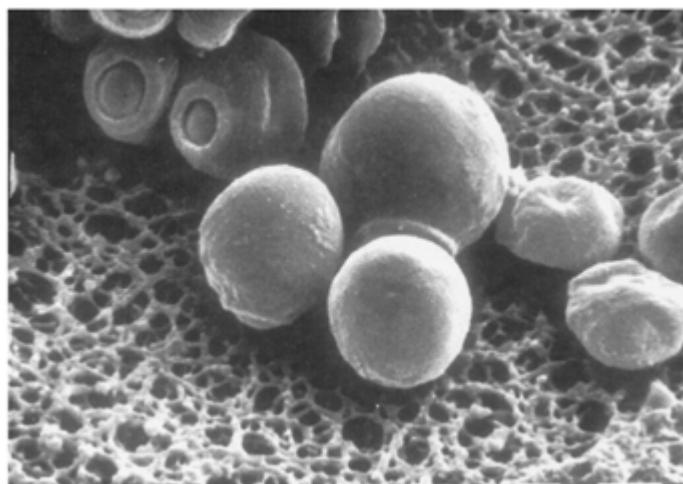


Figure 11 *Malassezia orbiculare* (culture).



Figure 12 An exceptional presence of *M. furfur* on a dandruff scalp (SEM photograph following scalp replica). Note the complex scaffold built by hyphae around the pilo sebaceous ostium. (Courtesy: G.Loussouarn, L’Oreal R&D, Clichy.)

vivo: lipase and lipoxygenase, (64–66) activities are high. Production of azelaic acid (67), and volatile fractions (lactones) were recorded both in vivo and in vitro (68,69).

3.10. Mechanisms of Pathogenicity

Many authors have viewed dandruff as a mild form of seborrheic dermatitis. In the latter, inflammation and redness become clinically evident and intense scaling involves other sites (eyebrows, ears, nasolabial folds, etc.).

Even today, the precise immune mechanisms are not fully understood. Although seborrheic dermatitis has a prevalence rate of 30–55% in HIV-positive subjects (control population 1–3%) (70), its onset has not been shown to be related to an altered immune response (71). According to the authors, the pathogenic potential of *Malassezia* might be transmitted through its metabolites (toxins, lipase, etc.).

Giving a priority to antigenic properties would confer to dandruff a possible definition as a slight but chronic allergic-type reaction to the yeast (72–74). Expression and complete cDNA sequencing of one antigen (a 36-kDa protein) from *Malassezia* sp. has been reported (75).

Irrespective of these different “mechanistic” approaches, most studies agree about both the key role of *Malassezia* in the development of the above afflictions and its necessary eradication as a common strategy of treatment. As illustrated in Noble’s book (14), the human scalp and its disorders have been, for long, linked to the nature of the scalp, acting as a reservoir of transient pathogenic microorganisms but more rarely related to resident flora.

Malassezia is now described as an opportunistic pathogen, which is involved in many other diseases, nosocomial infections included (Table 2). Its crucial reliance on exogenous lipids make catheters a potential source of spread (76–78), leading on occasion to rapid and fungal septicemias that may be lethal to premature newborns (79–81).

3.11. *Malassezia* sp. and Animals

Anecdotally, one of the first suggested close associations between *Malassezia* and desquamation came in 1925, when Weidman, at the Wien’s Zoo, isolated a yeast strain from a highly desquamative rhino (82). Belonging to *Pityrosporum* sp., it received the name of *P. pachydermatis*. However, it is not a lipophilic strain.

With regard to experimental models on animals, an important observation was reported in 1980 by Drouhet et al. (83), from the Institut Pasteur in Paris. On different species (guinea-pigs, mice), of hairy and nude phenotypes, topical applications of a fresh, living culture of *M. ovalis* incorporated into a lipid rich emulsion, led to a rapid and intense erythema, desquamation, followed later by sudden hair loss in the hairy species.

Although it clearly confirmed the inflammatory potential of the yeast, this may be not the most important finding: surprisingly, erythema and desquamation never occurred in the nude animals. It is regrettable that this work received too little attention. Today, these results still remain unexplained.

3.12. Treatments

3.12.1. Forms

These are simple. Basically, an active anti-fungal agent (listed in Table 3) is, most of the time, incorporated in a shampoo base. Although non-rinsed lotions are available and effective, the shampoo form is much preferred by the consumer since it offers a convenient and rapid “two in one” action (84–90). Since dandruff scalps are subjected to chronic irritation, the cleansing surfactant(s) should obviously be of optimal compatibility (see Chapter 3) (84–90).

Active ingredients are generally introduced from 0.5% to 2.5% and the complete shampooing procedure leaves an active residue onto the scalp in the $\mu\text{g}/\text{cm}^2$ range (91). The latter is sufficient to induce a rapid reduction in itching and scaling by the second or third shampoo. Used on a regular basis, i.e., twice a week, most treatments suppress the symptoms.

However, the global efficacy of these shampoos is modulated by four factors related to the consumer’s habits (see Chapter 3) (41):

Table 3 List of the Possible Ingredients for Treating Dandruff and Their Legal Status (Note: Despite their different structures, all have anti-fungal properties. Forms and concentrations given here correspond to the most common commercial products.)

Active ingredient	CAS	Cosmetic use	Form/concentration (%)
Amphotericin B	30652–87–0	No	L/0.25
Ciclopirox	29342–05–0	Yes	SH/0.5–1
Climbazole	38083–17–9	Yes	SH/0.5–1.5
Ketoconazole	65277–42–1	Yes	SH/1–2
Nystatin	1400–61–9	No	L/500,000 Units
Piroctone olamine	68890–66–4	Yes (CEE, JP) No (US)	SH/0.5–1
Selenium disulfide	7488–56–4	Yes ^a	SH/1
Tars	8007–45–2	No in CEE and JP yes in US	SH/0.5–5
Zinc pyrithione	13463–41–7	Yes	SH/0.7–1.5

^a Depending on the concentration. In most countries, concentrations above 1% require prescriptions. Sh, Shampoo; L, Lotion.

- The frequency of shampooing. By physically eliminating the scales (niches for yeast proliferation), the act of shampooing is of a great help. Ultimately, a bland shampoo, used intensively (e.g., once a day or more) will eliminate scales (22), although it does

not “treat” the condition. This is obviously more evident when an anti-dandruff shampoo is used, leaving residual active ingredient on the scalp surface. When shampooing frequency is kept constant, the efficacy is generally dose-dependent (0.5% vs. 1% or 1% vs. 2%) in respect of the active ingredient included in the shampoo base, but this effect plateaus rapidly.

- A pause (of some minutes) between wash and rinse is sometimes suggested. Although it does slightly increase the global efficacy of the shampoo, consumers in real-life conditions rarely follow such procedures.
- The amount of shampoo applied. In “real-life” conditions, this ranges from 4 to 30 g, irrespective of the hair volume to be cleaned (92). This obviously has a strong (positive or negative) impact on the active residue left on the scalp surface.
- Cessation of treatment: most anti-dandruff shampoos show a lingering effect, i.e., a delay (1–2 weeks) from which, when treatment is stopped, scales begin to reform. This lingering effect (29) is both a function of the active ingredient (its intrinsic anti-fungal activity) and of the first three criteria mentioned above.

In addition to the anti-fungal ingredient, some anti-dandruff shampoos include a “keratolytic” agent (0.5–2%), usually salicylic acid, to help scale desegregation. Coal tars are also used but are, now, no longer allowed in many countries, including the EU and Japan.

3.12.2. Active Ingredients

Table 3 lists all the ingredients previously tested and found active against dandruff, although some (Amphotericin B, Nystatin) are not commercially used for this purpose. A new ingredient (27) is presently still in a prospective phase.

Despite their structural differences, all the agents listed share in common a clear anti-fungal property.

Such activity can be detected and quantified in vitro through routine procedures, i.e., on solid or liquid medium (determination of the minimal inhibitory concentration, MIC). According to the *Malassezia* isolates tested, MIC’s of the various compounds vary from 0.001 to 10 µg/ml (93–95). This huge range (10,000-fold or even larger when expressed in molar equivalents) is not reflected in the in vivo situation: at 1%, all the most commonly used agents (ketoconazole, piroctone olamine, selenium sulfide, zinc pyrithione) exhibit only minor differences in their respective efficiency, in real life conditions.

In vitro testing, although necessary, is certainly not sufficient to explain and predict a reliable anti-dandruff activity in vivo. Other factors (solubility, amount deposited on scalp, adherence to stratum corneum, etc.) are important for modulating the global anti-fungal action (29,96) and its residual effect.

Some agents of well-proven efficacy, show no or weak solubility (e.g. ZPT, selenium sulfide) and are formulated as micro-suspensions. Today, there is no rationale explanation to such a paradox, which implies that solubility is not a prerequisite condition for fungicide or fungistatic activity. Acquired resistance by *Malassezia* toward these agents have not been reported.

For some of them, the mode of action has been documented. For example, azole derivatives (ketoconazole, climbazole, etc.) inhibit ergosterol synthesis by *Malassezia*,

therefore leading to membrane structural defects (97–99). Others are less clearly explained, although hydroxy-pyridones (piroctone olamine, ciclopirox), and ZPT which have chelating properties, might act as inhibitors of cell membrane transport (100). The precise mechanism of action of selenium sulfide largely remains to be documented.

3.13. Legal Aspects

Table 3 mentions a general legal status about the use of these ingredients. It cannot reflect the extreme variation from country to country, in the various legislations. Some forbid, some authorize (e.g. Tars), some limit the use by concentration, others by the distinction between a rinsed product and a leave-on, etc. with, in many aspects, an obscure rationale.

They all are safe and efficient. Widely used by millions of people over decades, their use as cosmetics has been proven to comply with these two *imperata*. With regard to normal use, no side effect has been recorded.

Additionally, with time, it appears clear that eradicating Malassezia and preventing its re-colonization in the long term (constant and repeated applications for years) does not lead to any side effects.

Anti-dandruff agents have been shown to be efficient in controlling a flora that induces, in about half of the population, slight but unpleasant damage to the scalp epidermis. Basically, the principle of such an action is strictly similar to that brought about by cosmetic anti-microbials in deodorant products.

4. CONCLUSION

The increasing use of anti-dandruff preparations over the last three decades follows both individual and social logic. Individually, they bring both esthetic gains and comfort, with regard to itching. In our societies, flakes on shoulders have for a long time been negatively perceived, socially. Today, it is a problem that is overcome and achieved by efficient, pleasant and easy to use preparations. It is likely that the next three decades will witness a constant drop in the prevalence of dandruff.

From a microbiological point of view, the last 10 years have brought significant progress in the knowledge of a yeast, for too long ignored. Together, cutaneous research, immunology and epidemiology have revisited the consequences of its proliferation. Since the early works of Pasteur, Fleming, etc., the human scalp has been confirmed as a potent reservoir of various microbial transfers. Nosocomial infections, which are currently a serious problem, are the ultimate consequence of such exchanges. As compared with the now-classic hygienic hand washing, “clean” scalps, or at least scalps less prone to induce troublesome conditions, are one aspect of the many simple ways for improving both individual and social health.

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22

Dermatitis and Eczema of the Scalp

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The term “dermatitis” is used to denote a cutaneous inflammation producing a minimal level of redness, whereas “eczema” implies in addition specific epidermal damage visible microscopically as intercellular edema with sites of cell breakdown and subsequent vesicle formation. These can grow to a large enough size to be apparent to the naked eye. They also produce fissures in the epidermis, which allow the tissue fluid to flow to the surface, seen as oozing and crust formation. Itching is always associated. Eczema is a non-specific syndrome, the cause of which may be constitutional, bacterial, irritative, or allergic.

Four main types of dermatitis can be observed on the scalp: seborrheic dermatitis, *tinea amiantacea*, atopic dermatitis and contact dermatitis; the latter is dealt with in Chapter 14.

1. SEBORRHEIC DERMATITIS OF THE SCALP

The condition owes its name to its location on seborrheic areas, namely the scalp, face, and mid-thoracic areas. On the scalp it particularly affects infants under 3 months and adults between 18 and 40 years of age. It is virtually absent in children, presumably related to the absence of sebaceous secretion during this period of life (1). Its milder form, without redness, named *pityriasis steatoides* (greasy dandruff), is common in adults, especially in males.

1.1. Clinical Feature

In the dermatitis stage, the scalp appears red, scaly, occasionally edematous, giving a burning sensation upon scratching but without itching (Fig. 1). In some places, a whitish fatty coating can be seen. The scales may form plaques stuck to hairs. This is especially frequent in infants (cradle cap). The lesions often form sharply marginated curvilinear confluent oval patches that tend to overlap onto the forehead and form the so-called *corona seborreica*. In the affected areas the hair gets thinner, and extensive hair loss may take place. In severe cases the hair loss is diffuse on the vertex and frontal area, but spares parietal and occipital regions, thus mimicking androgenetic alopecia (Fig. 2). However, unlike the latter, the alopecia of infant

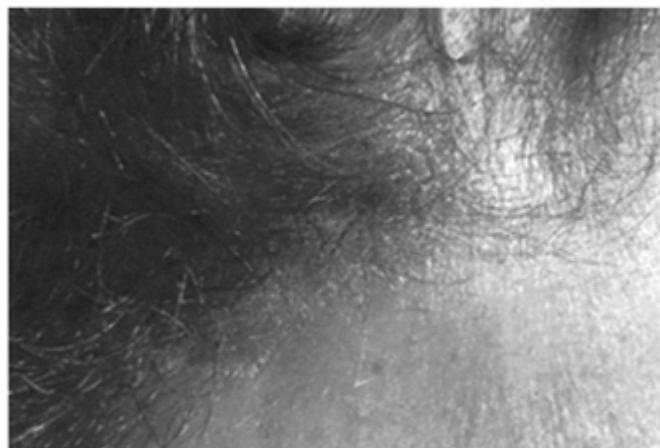


Figure 1 Seborrheic dermatitis of adult scalp: scaly patches and sparsity of hair.

seborrheic dermatitis disappears after the third month of life and following treatment of the disease. In adults with dry skin the disease may occur but usually takes a milder feature (*dermatitis seborrheica sicca*). In patients with AIDS the involvement is usually widespread.

Eczema may complicate severe cases; it is heralded by itching, oozing, and crusting. It is usually microbial (mostly staphylococcal) in nature, and in infants gives rise to retroauricular and cervical lymph nodes.

Severe cases show lesions beyond the scalp. Eyebrows, eyelids, nasolabial folds, and pinna of the ear are affected, with rarefaction of eyebrow hairs and loss of eyelashes. In infants the disease, if untreated, may spread to the whole body (Leiners-Moussous's erythrodermia); although the baby does not scratch the condition is life-threatening. In adults the mid-chest (Fig. 3) and mid-back areas typically show so-called petaloid (petal-shaped) lesions or pityriasisiform (fine squames) often circinated (arc-shaped) patches bearing a resemblance to *pityriasis* (*tinea versicolor*). As in the latter disease (which is related to the same agent) there may be localized



Figure 2 Transient alopecia following seborrheic dermatitis in infant.

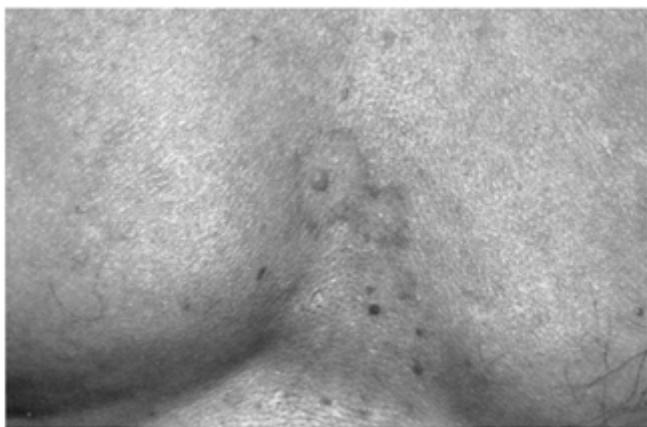


Figure 3 Midchest seborrheic dermatitis.

loss of pigment. Follicular lesions (*pityrosporon folliculitis*) are often found scattered throughout the seborrheic regions, namely the whole thorax and shoulders.

Seborrheic dermatitis may be associated with intertrigos (flexural areas with redness and oozing). In infants retro-auricular, intergluteal, and genito-crural areas are mostly affected (the so-called bipolar dermatitis); the clinical examination should always include a search for latent complications, otitis media, and urinary infections, even when the baby remains otherwise healthy. In adults the external auditory canal, upper part of the retro-auricular area, umbilicus, submammary folds, perianal area, and the glans penis fold are the main locations.

Two common diseases, atopic dermatitis in babies and psoriasis in adults, may be located at their onset at the site of a scalp seborrheic dermatitis. In the former case, scratching and eczema appear by the third month of life, a time when the seborrheic dermatitis should start to regress. Concomitant eczema of the cheeks usually supports the diagnosis. Psoriasis at onset takes on the same clinical appearance as seborrheic dermatitis, but typical lesions at other sites, especially elbows, may help in the diagnosis.

1.2. Causes and Mechanisms

The causative agent of seborrheic dermatitis and pityriasis steatoides is *Pityrosporon ovale* (*Malassezia ovale*) (2,3), a non-pathogenic yeast resident on the normal scalp (4). The proliferation of *P. ovale* appears to be necessary for the onset and persistence of the dermatitis, as evidenced by the fact that healing can be obtained by antimycotic medication alone. The disorder was experimentally reproduced in guinea-pigs (5) by applying a mixture of artificial human sebum, bacteria, and *P. ovale* to the skin for a week. Drouhet et al. (6) confirmed these results, and the determinant role of *P. ovale*. Lober et al. (7) attempted to reproduce the same experimental seborrheic dermatitis in human subjects by applying a suspension of *P. ovale* to the forearm over a period of 10 days. Only two subjects reacted, both with a previous history of seborrheic dermatitis of the scalp. Control subjects without a history of seborrheic dermatitis tolerated the yeast suspension perfectly well. *Pityrosporon ovale* is lipophilic and can be cultured in vitro in media enriched in lipids. This explains why it is found only in seborrheic areas in humans, and why seborrheic dermatitis usually develops in greasy skin. This has been found to be true even in infants (personal data) as given in the following:

Month of life	Optical density ^a	
	Healthy controls (number)	Seborrheic dermatitis (number)
1 ^b	74±12 (23)	58±14 (8)
2	73±11 (15)	79±12 (13)
3	89±9 (21)	79±12 (7)

^a Sebum amount assessed by the photometric method (see Chapter 20) using ground glass slides (the higher the sebum level, the lower the optical density).

^b 0.001<P<0.01.

Diseases associated with hyperseborrhea (e.g., parkinsonism) also foster the occurrence of seborrheic dermatitis.

However not all hyperseborrheic subjects have seborrheic dermatitis, and some individuals with low sebum levels can develop *dermatitis seborrheica sicca*. This emphasizes the role of the subject's predisposition: a small decrease in resistance to *P. ovale*, often associated with a slightly decreased immune defense against bacterial infection. This may account for the frequent occurrence of intertrigos. In addition, all diseases associated with alteration of the delayed immune response, e.g., AIDS, promote the appearance and augment severity of the seborrheic dermatitis.

In summary, two factors foster seborrheic dermatitis: seborrhea and an individual predisposing factor, probably of immune nature. The disease is not contagious because *P. ovale* is found on every normal skin.

1.3. Treatment

Treatment of seborrheic dermatitis of the scalp is primarily based on antimycotic medication applied topically following a mild shampoo. Amphotericin B, mycostatin, and mostly imidazole derivatives all work rapidly when used once or twice daily. Preparations containing alcohol should be avoided because they are too irritant. Ketoconazole is often better tolerated owing to its anti-inflammatory properties. In case of irritation, application frequency should be reduced and soothing lotions or watery creams with zinc oxide, bismuth carbonate, or calamine alternately used. Severe or protracted cases merit systemic antimycotic treatment. In very minor cases and in simple dandruff, shampoos with octopirox, zinc pyrithione, or selenium sulfide (all are fungicidal) may be useful; they should be left on the scalp for several minutes before rinsing.

Local corticosteroid therapy induces a dramatic response due to its anti-inflammatory and antimitotic effects. But it favors the development of infection and reduces the immune response locally. It has been responsible for many cases of aggravation and cutaneous atrophy. For these reasons it is better avoided, even if this means waiting longer for a result, and it should be used only in association with antimycotics.

Whatever the treatment, *P. ovale* cannot be fully eliminated from the scalp because of hiding places located in the hair infundibula and other sites of the skin surface, and because of rapid contamination emanating from other individuals. It follows that a permanent cure cannot be achieved and that minor and appropriate regular treatment must be continued for months or years depending on the propensity of the disease to recur.

2. PITYRIASIS AMIANTACEA

The disorder (also wrongly named *tinea amiantacea*) primarily affects children and young adults.

2.1. Clinical Feature

The scalp shows white, dry, and scaly plaques within which the hair shafts are trapped and stuck. On lifting the hairs by their ends, large squames wrapping the hair shafts unstick, laying bare the pinkish, moist surface of the scalp beneath. Hairs may be lost.

The disease is frequently associated with retro-auricular intertrigo (the fold looks red and oozing). Cervical lymph nodes may be enlarged. Screening for auricular or urogenital infections must be carried out.

2.2. Causes and Mechanism

The disease is mostly due to streptococcal infection, fostered by conditions that favor it: poor hygiene or lowered resistance to infection.

2.3. Treatment

A careful daily cleaning of the crusts with a mild shampoo should be followed by application of an antiseptic lotion or cream (e.g., containing phenyl mercuric acetate or borate, trichlorocarbanilide, or chlorhexidine), or preferably an ointment containing an antibiotic reserved for topical use (e.g., fucidin). A systemic antibiotic therapy is not always necessary. Careful disinfection of the entire skin is essential, including the preferred locations: flexures, auditory canals, interdigital spaces of hands and feet, and perineum. This precaution is aimed at preventing relapses.

3. ATOPIC DERMATITIS OF THE SCALP

This common disorder is sometimes called atopic eczema when vesicles and oozing prevail. It involves a personal or familial predisposition (named “atopy”) and is frequently associated with asthma or allergic rhinitis (often manifested as hay fever). It can begin at any age but especially in infants from 3 months onward, and is rare in people over 50. The condition is rarely restricted to the scalp; in this case it is (or has been) often triggered by a seborrheic dermatitis.

3.1. Clinical Feature

In most cases the scalp shows dispersed excoriated and oozing vesicles, tiny crusts, and sparse scales over a pinkish skin; there is no hair loss. Usually, the patient has a history of recurrent eczematous lesions affecting other areas, especially the face in infants or limb flexures in adolescents and adults. This is a typical feature. A personal or family history of respiratory problems or frequent sneezing is helpful in diagnosis.

The condition is recurrent. With time the lesions may become localized mostly on the nape and evolve into a poorly demarcated pink, thickened, and excoriated plaque. At the periphery a few elevated papules are seen, with excoriations leaving tiny crusts. There may be some hair loss or broken hairs on the plaque. Itching is often severe.

When this type of lichenification is the sole residual lesion, only a history of other locations, or respiratory symptoms may differentiate atopic dermatitis from an essential lichenification. The latter is named *lichen simplex chronicus* or “circumscribed neurodermatitis,” an old term that refers to the nervousness of the subject sometimes induced by the itch. Pediculosis (lice) may mimic the disease, but there is no plaque, and itching and excoriated lesions often spread down the upper part of the back. The diagnosis is made by finding lice or mostly nits.

3.2. Causes and Mechanism

Atopy is a disorder characterized by skin and mucous membrane hyperirritability and immunologic imbalance.

In addition to a predisposition to bronchial, nasal, and sometimes ocular mucous membrane irritation, the skin shows a lower itch threshold, and any irritant compound or disorder may precipitate or help maintain the dermatitis. In infants with seborrheic dermatitis the onset of atopic dermatitis is heralded by pruritus. Atopic dermatitis is deeply sensitive to and aggravated by an overgrowth of *P. ovale* and by latent *Staphylococcus aureus* in the skin flora.

The atopic condition is also characterized by an immunologic imbalance between immediate and delayed hypersensitivity. It is switched toward immediate IgE-dependent reactions at the expense of delayed lymphocyte-mediated reactions. As a consequence of the former, the patients are prone to immediate-type allergic (anaphylactic) sensitization to some proteins called "atopens" (mites in the house dust, air-borne pollens, pet's hairs, some foods). The main clinical signs are urticaria, seasonal rhinitis, and asthma. They are associated with an increase in immunoglobulin E blood levels and eosinophil count. Conversely atopic patients suffer from decreased T4-Th1 lymphocyte-mediated reactivity responsible for the skin's lower resistance to bacterial, mycotic, and viral (especially herpes) infection.

Other biological defects are associated with the disease. The main ones are:

- Abnormalities in essential fatty acid metabolism: hyper-production of proinflammatory compounds from arachidonic acid (a normal compound of the cell membrane), and a deficiency in $\Delta 6$ -desaturase activity leading to abnormal stratum corneum intercellular lipid, increased stratum corneum permeability to water and skin dryness.
- Abnormalities in neuropeptide release and metabolism within the skin responsible for hypersensitivity to itch and scratching (8). The latter alone is capable of generating eczematous lesions.
- A decrease in inflammation-triggered, epidermally produced antiseptic peptides (LL-37 cathelicidins and HBD-2 defensins) which leads to incapacity of the skin to kill the invasive staphylococci ever-present in atopic skin (9). This is a consequence of abnormal immune reactivity.
- A tendency to vasoconstriction of skin vessels (cold hands and abnormal pharmacologic reactivity)

To date there has been no overall theory that can account for the clinical symptoms, the biologic defects, and the genetic predisposition. But some years ago it was found that a too rapid degradation of intracellular c-AMP, a compound responsible for switching-off cell activity present in several cell types, including immunocompetent cells, might explain at least the imbalance in the immune system response and the abnormality in the reactivity of skin blood vessels.

3.3. Treatment

Three aspects should be addressed: clinical signs, trigger factors, and the disease background.

3.3.1. Treating Clinical Signs

In the case of exudative scalp dermatitis, daily non-irritant (non-ionic) shampoos followed by zinc oxide or calcium dicarbonate ointments are the most appropriate topical treatment.

When the scalp is no longer oozing but dry and thickened, local corticosteroid therapy usually achieves dramatic results but can only be used for a short period of time and at intervals, since there is a risk of aggravation through infection, corticoresistance and intolerance to any topical product rendering the condition intractable. Topical tacrolimus should have a similar beneficial effect with fewer drawbacks. The best tolerated and effective local treatment consists of tar and tar derivatives in shampoo, lotion, or cream form.

Covering the scalp with a bandage may be necessary to prevent painful dehydration of the skin surface, mostly when it is oozing.

Owing to skin irritability, antiseptics are usually poorly tolerated. Consequently the mandatory disinfection of the scalp and other sites against *S. aureus* (even in absence of overt infection) requires the systemic administration of antibiotics, sometimes for several weeks. For the same reason, treating a concomitant seborrheic dermatitis by systemic antimycotic therapy may be necessary.

Bathing with fresh water is often source of painful stinging and burning sensations and of pruritus, whereas bathing in sea water is well tolerated. This is because the osmotic gradient between skin and water decreases in fresh water whereas it rises in salt water.

Solar radiation or PUVA therapy is often beneficial when the scalp is dry and thickened.

3.3.2. Preventing Trigger Factors

On the scalp, these are seborrheic dermatitis, a heavy *S. aureus* carriage, irritant shampoo, hair styling (bleaching, coloring, perming, etc.), too frequent and hot showers, and abundant sweating.

Down-filled pillows, an irritant environment and house pets should be avoided. Even in patients with atopic dermatitis not involving the scalp, special scalp hygiene is required: infrequent shampoos, use of shampoos with cade oil or tar products, and periodic antiseptic treatment. This must be carried out with the utmost care to avoid irritation and to monitor subjective reactions.

3.3.3. Tackling the Background to the Disease

High doses of Primrose oil (because rich in gamma-linolenic acid) have been claimed to reduce the recurrence rate.

If anaphylactic reactions have been detected to respiratory or digestive "atopens," their avoidance is mandatory. Desensitization may be useful as far as pulmonary atopens are concerned.

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23

Scalp Dermatoses

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1. INTRODUCTION

This chapter does not pretend to cover, in an exhaustive or even schematic fashion, all the disorders that can have repercussions affecting the scalp. Such an enterprise would require on its own an entire volume equivalent to the present one. The aim here is just to trace the major conditions most frequently encountered at this site, whether inflammatory or neoplastic, with apologies in advance to the reader for any overlapping of topics dealt with in other chapters such as those dealing with alopecia, seborrhea, or dandruff conditions.

2. INFLAMMATORY DISEASES

2.1. Lupus Erythematosus

Lupus erythematosus (LE) is an inflammatory disease falling within the scope of “collagen” or “connective tissue” diseases, terms sanctioned by use but unacceptable since there is no evidence that collagen is primarily at fault. In such a group of diseases including LE, scleroderma, and dermatomyositis, the whole connective tissue of the organism may represent only a preferential target for an inflammatory process, with an increased turnover of collagen but without any molecular defect in the protein structure.

“Autoimmune diseases” is a more suitable term, since these disorders and particularly systemic LE (SLE), constantly show immunological abnormalities, including a high incidence of non-organ-specific humoral antibodies. This brief pathogenic survey provides a better understanding for a possible diffuse expression of these disorders. LE may involve various organs or systems according to the severity of the illness, ranging from a chronic type to a systemic form. The chronic type, the most commonly encountered form of which is discoid LE (DLE), mainly affects the skin, whereas organ dysfunctions (renal abnormalities, joint involvement, abdominal pain, lymphadenopathy, and/or splenomegaly, pleurisy, pericarditis, pyrexia of unknown origin, psychiatric disturbances, etc.), or abnormal serologic findings prevail in SLE.

Nevertheless, evidence based on genetic data has been produced that DLE is a separate disorder and not a benign variant of SLE (1,2), although common clinical, pathological, and biological criteria make the relationship between these two conditions conspicuous.

2.1.1. Pathology

Microscopic cutaneous changes of LE vary according to the type of clinical lesion from which the biopsy is taken and reflect the visible skin alterations (Fig. 1). They are usually more obvious and diagnostic in the chronic than in the systemic form.

The various clinical types of the disease all share a basic histological pattern, the salient features of which are (1) liquefaction degeneration of the basal cell layer of the epidermis with disruption of the dermal epidermal junction; (2) patchy lymphocytic infiltrate, particularly around appendages, which may be atrophic; and (3) fibrinoid degeneration of the connective tissue.

Other less characteristic changes, mainly found in DLE, consist of thinning of the epidermis with hyperkeratosis and plugging of the follicle ostia, thickening of the basement membrane of the epidermis and sometimes of small vessels, which may also be dilated and undergo fibrinoid degeneration.

Beside routine stains with hematoxylin and eosin (HE), a periodic acid-Schiff stain (PAS; Hotchkiss-MacManus reaction; stains glycogen and mucopolysaccharides and therefore the basement membrane) may be useful to show thickening of the basement membrane area, chiefly around hair follicles, a suggestive pattern in an otherwise indefinite histological picture. Less frequently and only in areas of pronounced hydropic degeneration of basal cells, the PAS positive subepidermal basement membrane zone may be fragmented or even absent.

An elastic fiber stain (acid alcoholic orcein) reveals significant differences between several types of alopecia: patterns of elastic fibers in the perifollicular and interfollicular dermis are helpful in differentiating idiopathic pseudopelade of Brocq from pseudopeladic states secondary to certain diseases including LE (3).

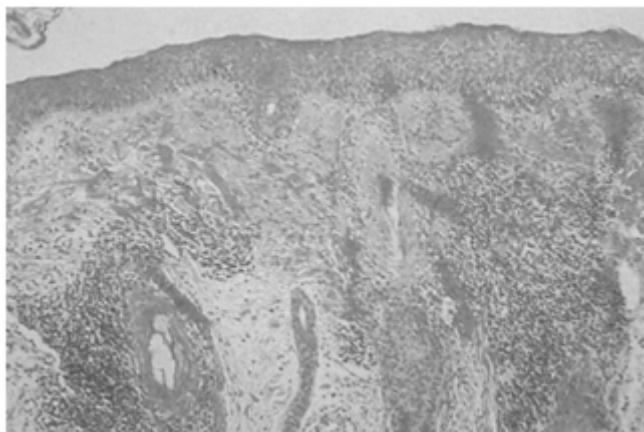


Figure 1 Lupus erythematosus (LE).
Dense lymphocytic infiltrate
surrounding skin appendages.

2.1.2. Immunohistology

Direct immunofluorescence (DIF) techniques show deposits at the dermal epidermal junction of immunoglobulins (Ig), mainly IgG and to a lesser extent IgM and IgA, together with complement (Fig. 2). The latter consists in a group of factors present in fresh serum, including an extensive series of glycoproteins and protein inhibitors responsible, among other important immunological functions, for the lysis of anti-body-coated (sensitized) bacteria and cells.

Schematically, DIF is performed using snap-frozen tissues, cut in a cryostat at about -20°C, dried, and stained without fixation. Staining is carried out in tissue sections by the addition of fluorochrome-conjugated antibodies to the substances to be detected (i.e., Ig and complement in such circumstances), and washing to eliminate the unfixed labeled antibodies.

DIF is positive in more than 90% of skin lesions of DLE and SLE (4), as well as in the uninvolved skin in active SLE if the biopsy samples are taken from sun-exposed skin, usually the dorsum of the wrist (5). Positive DIF of clinically normal unexposed skin is present in only about 50% of cases and correlates well with the most severe forms of lupus renal disease (6).

Although DIF examination has become an important diagnostic and even prognostic (6) test in SLE, it remains nonspecific since "false-positive" reactions with a "lupus band" are reported with rosacea, facial telangiectasia, dermatomyositis (7), and primary systemic amyloidosis (8). Conversely, DIF studies sometimes fail to demonstrate any lupus band in involved skin of otherwise proved LE, e.g., LE gyratus repens, a particular type of LE with slowly migrating gyrate patterned lesions (9).

Besides the lupus band, which may be homogeneous, granular, or in larger subepidermal clumps (4), LE lesions may show Ig deposits with or without complement, in or around the blood vessels. Whether these deposits, which have been taken to be toxic immune complexes, play a part in the pathogenesis of skin lesions in LE has been disputed for a long time and still remains puzzling (10).

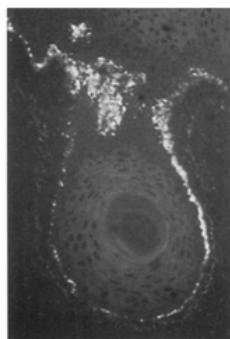


Figure 2 LE: Direct immunofluorescence of involved skin showing granular lupus band at the

dermal epidermal junction and surrounding a hair follicle.

2.1.3. Clinical Features

The hair and scalp abnormalities seen in LE are not specific to it but may be shared by other "collagenoses." Some patients display clinical and immunological overlap, making impossible a clear-cut diagnosis and so attesting to the sometimes hazy borderline between the different diseases of this group.

One basic feature common to all types of LE is that ultraviolet light may provoke or at least exacerbate skin lesions related to this condition.

Systemic Lupus Erythematosus.

Hair. Nonscarring alopecia is a common and characteristic finding in SLE, present in at least 50% of cases during the acute phase of the disease (11). It may be patchy or consist of diffuse shedding of hair, with or without underlying skin lesions. The hair is frequently coarse, dry, and without luster, resembling that seen in hypothyroidism. In one-third to one-half of the acutely ill cases, increased fragility of the frontal hair produces a receding hairline with short broken-off hairs, which tend to stand out in an unruly fashion. These hairs produce a disheveled appearance. Such a pattern represents the so-called "lupus hair," a valuable diagnostic sign that is evident even when the patient is observed from a distance (11,12).

The most frequent type of alopecia in SLE is a diffuse one, patients then complaining about hair loss following the combing of hair. Hair may become so sparse that the scalp is readily visible through it. If the scalp looks normal, this condition may be misdiagnosed as a diffuse form of alopecia areata. Although total alopecia has not been observed in LE, such a situation is most distressing to women.

Some patients with autoimmune diseases develop canities (13), which also temporarily resemble the classical patchy hair regrowth of alopecia areata, another condition with autoimmune connotation, Ortonne et al. (14) observed progressive depigmentation of the coat in C₃H mice thymectomized at birth. According to these authors a causative relationship probably exists between thymectomy and pigmentary defect, thus providing a pathogenic clue to possible changes in hair color in patients with autoimmune disorders, since thymic failure probably represents a basic feature of the immunological state of these conditions.

Structural disturbances in the hair shaft in the form of trichorrhexis nodosa and hair cortex fissures are seen in SLE and in dermatomyositis. In some cases the structural disturbances of hair shaft are reminiscent of the effect of antimetabolites (15).

Scalp. Scalp involvement may accompany hair lesions in SLE. In nonscarring alopecia scalp changes mainly consist of pure or scaly, diffuse or circumscribed erythema. Chronic discoid alopecia may follow patches of erythema and produce scarring alopecia.

Cicatricial or scarring alopecia is a rare feature of SLE, but may be present in cases in which the systemic phase has been preceded, or is accompanied by chronic DLE lesions. In a large series (12), the latter were found to represent the initial manifestation of SLE in slightly more than 10% of cases and to occur during the course of the disease in almost one-third of the patients. However, such percentages may be questioned, since the

incidence of the transformations between chronic DLE and SLE varies with the definitions used in classification and the patient groups studied.

Chronic Lupus Erythematosus. DLE lesions may be encountered in SLE but remain, most of the time, symptomatic of the chronic form of the disease. Usually chronic discoid alopecia occurs as well-defined erythematous patches covered with fairly adherent scales, the undersurface of which shows horny plugs pulled out from dilated pilosebaceous ducts. When not covered by scaling, these horny plugs can be

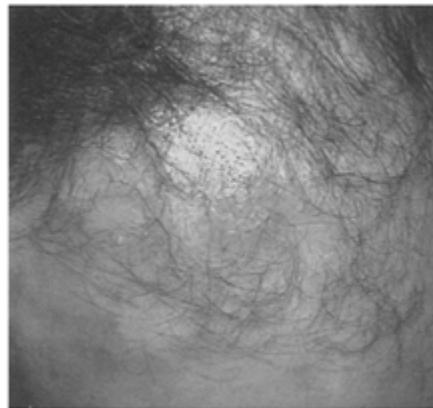


Figure 3 Discoid LE plaques.

seen by direct examination. The surface is then rough to the touch because of the follicular plugging. If hyperkeratosis is marked, a warty lesion results, a not uncommon type of DLE of the scalp.

If not treated DLE plaques progress concentrically, leaving a whitish pearly atrophic and telangiectatic center, the flattening of which may give rise to annular lesions (Fig. 3). At this stage, diagnosis is easily suggested by follicular plugging, erythema, and telangiectasia in association with scarring and alopecia. If treated, plaques flatten with more or less scarring. More frequently, a scarred area with a border of hyperpigmentation is left (Fig. 4). The so-called pseudopelade is a progressive cicatricial alopecia occurring without clinically evident folliculitis. It begins without any subjective symptoms. Initial small adjoining plaques, usually on the vertex,

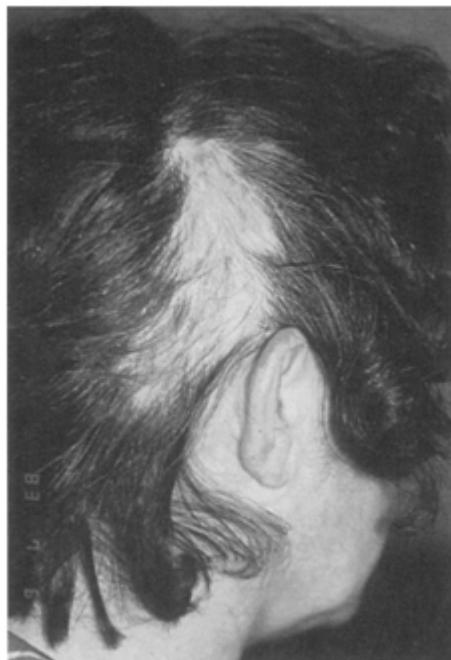


Figure 4 LE: Pigmented scarring alopecia.

merge to form a visible bald patch. The course is extremely variable usually with slow extension of the process. Pathological changes are not specific, showing an infiltrate of lymphocytes around the upper two-thirds of the hair follicles, sparing the bulb. Later, the epidermis is atrophic and the dermis sclerotic (Figs. 3 and 4).

Pseudopelade is a morphological syndrome and not an etiologic entity. It is best regarded as a pattern of follicular response to a wide variety of insults known or unknown. The former include lichen planus, lichen spinulosus, LE, and scleroderma, since typical lesions of these conditions may be associated with pseudopelade, either on the scalp or anywhere on the skin.

2.1.4. Treatment

Skin lesions of SLE settle down on systemic treatment together with general measures that play a large part in successful management: patients should be warned against excessive exposure to sunlight and helped by a sunscreen cream. According to the severity and course of the disease, different therapies are required separately or in combination, nonsteroidal and steroid anti-inflammatory agents, antimalarial, and immunosuppressive drugs.

Patients with DLE lesions may benefit from topical and systemic treatment with sun protection. Applications of a steroid cream and/or CO₂ dry ice represent two basic local measures. Intralesional corticosteroid injections are helpful in resistant cases. Oral antimarial drugs (chloroquine sulfate or hydroxychloroquine) have completely replaced the older parenteral treatments with gold and bismuth. They still represent the first line management of DLE lesions. Combined with antimarial drugs, retinoids may help patients with hyperkeratotic lesions. For cases which do not respond to antimarial drugs, thalidomide has proved remarkably effective in suppressing lesions. However, it is associated with potential, severe side effects (teratogenicity, peripheral neuropathy) which limits its use. β-Carotene, dapsone and clofazimine (Lamprene) are occasionally used in the management of DLE.

2.2. PSORIASIS

Psoriasis is a common genetically determined disorder of the skin, the spectrum of which may include a more or less severe arthritis. It consists of well-defined red plaques covered with a characteristic silvery scaling. The course is unpredictable, capricious, often chronic, with alternating relapses and remissions.

The condition affects both sexes at any age although it is rather uncommon in the first 2 or 3 years of life. Its prevalence is still difficult to assess because of few large-scale and reliable surveys. There is some racial variation. The pathogenesis of psoriasis is a multifocal problem. It includes (1) disorders of keratinization; (2) increased turnover time of epidermis; (3) decreased rate of epidermal chalones, substances which control epidermal growth; (4) local impairment of the adenylyl cyclase-cyclic AMP complex; (5) abnormal capillary dilation, which may represent the fundamental fault; (6) biochemical disorders with increased production of certain cytokines, and (7) enhanced polymorphonuclear chemotaxis.

Some circumstances or states are known to provoke the initial attack and subsequent recurrences: e.g. various acute or chronic infections, mainly of streptococcal origin, stress, and chronic alcoholism.

2.2.1. Pathology

The changes in psoriasis are essentially epidermal. They consist of acanthosis (i.e., an increased number of suprabasal cell layers), elongation of the rete ridges, a reduced

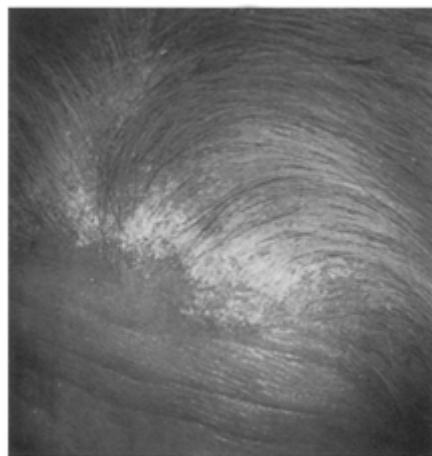


Figure 5 Psoriasis.

or absent granular layer, a considerable hyper- and parakeratosis (i.e., persistent nuclei at the horny layer level), and the presence of micropustules in the upper epidermis (Munro "micro-abscess"). The dermis shows some papillary edema with dilated and tortuous capillaries together with a mild mononuclear infiltrate.

2.2.2. *Clinical Features*

Psoriasis frequently involves the scalp (Figs. 5 and 6). Its clinical aspect is rather monomorphic at this site, so that it may be confused with other entities, e.g., seborrheic dermatitis or tinea amiantacea (Fig. 7), the ill-defined nosology of which makes the problem even more misleading. This emphasizes the need to perform a thorough examination of the whole integument in search of associated psoriatic lesions to confirm the diagnosis, in all patients complaining of redness and scaling of the scalp: apart from the latter, psoriasis may involve any part of the body but develops in preferential sites, e.g., nails, where the most frequent changes are pitting, discoloration,



Figure 6 Psoriasis.

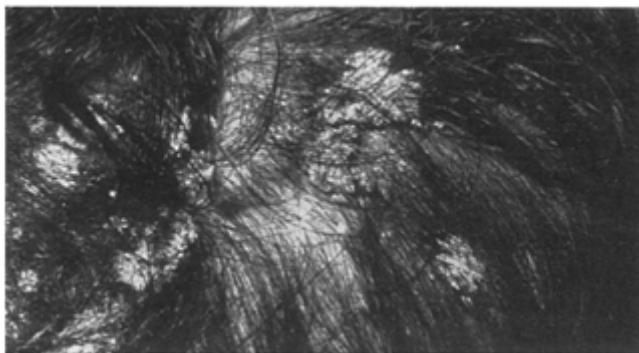


Figure 7 Pityriasis amiantacea: masses of sticky silvery asbestos like scales.

subungual hyperkeratosis, and onycholysis; palms and soles, genitals, elbows, knees, flexures, and sacral region.

The morphology of the eruption may adopt different patterns, at times mixed in the same patient: Guttate psoriasis describes the shower of small lesions scattered over the body, with little scaling except in the scalp. This once more emphasizes the monomorphous aspect of psoriasis in this site, whatever the clinical picture of the disease is elsewhere. This type of psoriasis particularly affects children and young adults following acute streptococcal infections. The most common form is nummular psoriasis consisting of palpable discs and plaques of varying size, well-defined, bright pink, or red in color with a variable amount of silvery scaling.

Repeated scratching of plaques makes the lesions turn white, giving an opaque appearance with mica-like scales. The complete removal of psoriatic scales gives rise to small bleeding points by erosion of the thin suprapapillary epithelium.

Scalp involvement has remarkably little effect on the hair, but some reduction in hair density may be seen (16). Extensive hair loss occurs in psoriatic erythroderma, a form of the disease involving the whole integument. Shuster (16) noted three types of alopecia in psoriasis (1) hair loss confined to the lesions, (2) acute telogen hair fall, and (3) follicular destruction giving rise to scarring alopecia, the least-common form.

Hair shaft involvement in psoriatic hair was emphasized by Shuster (16) and more extensively investigated using scanning electron microscopy by Wyatt et al. (17). These authors found micropits in cuticle cells in both normal hair and from unaffected as well as affected skin of patients with psoriasis compared with controls. Hairs growing from psoriatic plaques are significantly thinner than those elsewhere in psoriatic subjects and normal controls.

Despite hair shaft abnormalities, the rate of hair growth is normal in psoriasis (18). In kinetic studies of the follicle, using an in vitro technique in which thin tissue slices were exposed to tritiated thymidine, a raised labeling index was only found in the upper part of the external root sheath (19).

2.2.3. Differential Diagnosis

When isolated and in the absence of any relevant personal or family history of psoriasis, scalp involvement can be distinguished from tinea or pityriasis amiantacea, since the latter shows bundles of hair flattened by masses of sticky silvery, asbestos-like scales, whereas hair usually comes through thinner psoriatic scales (Fig. 7).

Nevertheless, clinical features are not always so clear-cut between these two conditions, nor are, in any case, their respective nosologies. Pityriasis amiantacea was named by Alibert (1832) "Fausse teigne amiantacée," or asbestos-like pseudo-ringworm. This is why it is often referred to as tinea amiantacea. The word "pityriasis" was subsequently substituted for "tinea" to avoid confusion with a ringworm infection.

Pityriasis amiantacea may be a pattern of eczematous reaction distinguished by its particularly well-developed scaly component. It represents a morphological entity in response to trauma or infection, usually of streptococcal origin. It may also be observed as a particular clinical pattern of the seborrheic state, lichen simplex, or psoriasis of which it may be the initial manifestation.

Frequent clinical evidence of overlap or associated symptoms connect seborrheic dermatitis and psoriasis. Seborrheic dermatitis is probably a genetically determined condition affecting both hairless and scalp areas and involving a skin resident yeast, *Pityrosporon ovale* or *orbiculare*. In the scalp, it ranges from the very common pityriasis simplex, i.e., more or less greasy dandruff, to more severe forms such as pityriasis steatoides consisting of large greasy and yellowish scales combined with redness and itching of the scalp. Neglected cases may evolve into very scaly areas indistinguishable from pityriasis amiantacea.

Seborrheic dermatitis may be difficult to differentiate from psoriasis, even more so since the latter is one of the diseases exhibiting the so-called "Köbner phenomenon": these conditions are elicited at the sites of preexisting skin insults, e.g., scar, sunburn,

vaccination, trauma, operation wound, or skin disease. Thus, in the case of an underlying seborrheic state, a misleading seborrheic patterned psoriasis may occur whenever associated.

2.2.4. Treatment

Topical therapy for scalp psoriasis requires proper vehicles to convey active drugs in hairy areas, so excluding greasy ointments. Salicylic acid, a keratolytic agent, is helpful to remove the scales. Anti-inflammatory drugs, namely corticosteroids remain widely used being very convenient. Drugs able to reduce the increased epidermal turnover such as chrysarotin, a tree-bark extract, have now mostly passed into disuse due to the benefits of vitamin D analogs. Tars, which were very effective albeit difficult to use, are no longer available in consumer products in Europe.

Very rarely, topical or intralesional cytostatic therapy is proposed. Topical fungistatic drugs effective again scalp yeasts such as selenium sulfide, ketoconazole or 1% terbinafine may be helpful in managing an underlying seborrheic dermatitis.

In widespread lesions, systemic treatments may be helpful. They include steroids, cytostatic drugs, and vitamin A acid derivatives. Such therapies must be carefully used, since psoriasis frequently relapses when these drugs are withdrawn.

UV phototherapy is widely used in all types of psoriasis. It consists either of UVB irradiation or depends on the interaction of a psoralen, a photoactive furocoumarin derivative, and long-wave ultraviolet light (UVA). This treatment is even more effective when used together with vitamin A acid. It may be used with topically applied or systemic psoralen according to the area to be treated.

Photochemotherapy of the scalp may be hampered by the hair, which prevents the ultraviolet light from reaching the involved scalp unless sparse and/or short enough.

2.3. Lichen Planus

Lichen planus is a relatively common disease of the skin and mucous membranes. It is now believed to represent a clinical pattern of reaction in response to various causes, mainly infectious, autoimmune, psychogenic, toxic, and genetic.

Among the suspected mechanisms, autoimmune and toxic theories are most commonly involved in the pathogenesis of lichenoid eruptions, which show all or most of the features of lichen planus, following graft vs. host disease and the administration of a wide range of drugs.

2.3.1. Pathology

The histology of a typical recent lesion of lichen planus is characteristic. The early changes are located in the basal layer of the epidermis, the cells of which undergo liquefaction necrosis and fibrillar change leading to the formation of so-called colloid bodies, easily recognizable since they stain homogeneously pink with eosin.

The rest of the epidermis shows thickened horny and granular cell layers, while the rete ridges tend to flatten out, at least in the center of the lesion.

Secondarily to these epidermal events, the dermis presents a dense infiltrate of mononuclear cells, mainly lymphocytes with a few histiocytes, close up against the epidermis, sometimes invading it so that the outline between epidermis and dermis becomes blurred.

Melanophores may be present in large numbers, having picked up pigment that has been dropped from the basal cell layer, providing a histological clue to the evolution toward pigmented lesions.

Periodic acid Schiff stains show more fragmentation of the basement membrane area than in lupus erythematosus, thus distinguishing between these two conditions, the histological pictures of which may be very similar.

2.3.2. Clinical Picture

The typical lesions are polygonal violaceous shiny flat papules, which may be quickly modified by scratching since itching is a fairly consistent feature, although occasionally absent. Lichen planus can affect any part of the body surface but has a characteristic distribution, mostly the front of the wrists, the genitals, the lumbar area, and the oral mucosa. Scalp involvement was reported as relatively uncommon in this condition (20), but its incidence may be higher, since in the quoted series they excluded patients in whom pseudopelade was the only manifestation of possible lichen planus.

Pseudopelade may represent one type of clinical pattern of lichen planus on the scalp, resulting from total destruction of the follicles, the ultimate stage of insult caused by the aggressive mononuclear infiltrate.

Atrophic and hairless patches not only persist but may even continue to appear or to extend for months after the typical skin lesions have faded, so making the diagnosis of the causal condition very difficult.

Typical perifollicular violaceous papules, together with erythema and scaling are more often seen in lichen planopilaris, a variant in which the disease process involves the follicles more selectively. These lesions show follicular plugging, an intermediate stage before scarring alopecia (Fig. 8).

2.3.3. Treatment

Topical or intralesional steroids may be helpful but only when active inflammatory changes are present. Possible causative drugs or chemicals must be removed. When

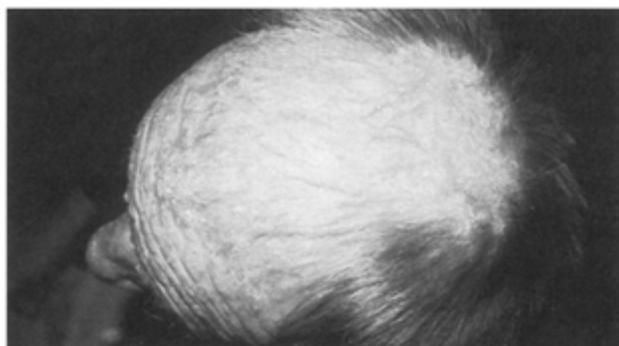


Figure 8 Lichen planus.

systemic treatment is required, steroids are more frequently replaced by retinoids and even PUVA therapy.

Scarring alopecia is permanent and may require plastic surgery to replace the hair defect.

2.4. Acne Necrotica

Acne necrotica as described by Boeck is also called acne frontalis or Hebra's acne varioliformis. This entity is of confused nosology, and therefore nomenclature since its pathogenesis remains obscure.

Acne necrotica occurs in adults between the ages of 30 and 50, much more frequently in males than females. It was initially believed to represent a particular type of acne, on the basis of (1) a possible similar appearance and (2) the possible growth of staphylococci from the lesions, at a time when acne was thought to be a microbial folliculitis.

Acne necrotica may be regarded as a pattern of folliculitis in response to staphylococci, even if cultures are not constantly positive, with hypersensitivity to staphylococcal antigens, which is held responsible for the necrotic reaction on the basis of strongly positive skin tests.

2.4.1. Pathology

The histological findings are those of a necrotic process involving the whole follicle and the adjoining cutis together with vessel thrombosis and peripheral inflammation. At times, gram-positive cocci are demonstrable. At a later stage, destruction of the follicle and scarring can occur.

2.4.2. Clinical Picture

Acne necrotica mainly involves the forehead and the temporal areas. It occasionally progresses backwards, reaching the hair margin and the contiguous frontal areas of the

scalp. The midline chest and back and the cheeks and nose are much less commonly affected.

The initial lesion is a follicular pinkish papule, 2–5 mm in diameter, often umbilicated and itchy, soon turning into a pustule, the desiccation of which gives rise to a very adherent hemorrhagic crust. After 3 or 4 weeks this crust separates, leaving a depressed, varioliform scar.

The lesions progress by successive attacks of a few weeks each, usually few in number at any one time. If not efficiently treated the disease may last for months or years.

A miliary form of acne necrotica may precede or follow more typical lesions, but more often occurs alone. It consists of itchy follicular vesicles, scattered over the scalp, becoming pustular and necrotic.

Typical lesions are rarely observed; they are often removed by scratching. Patients simply complain of pruritus, and local examination shows only nonspecific crusted excoriations. Stress is often incriminated in periodic recurrences of this condition.

2.4.3. Differential Diagnosis

Acne necrotica is easily distinguished from papulonecrotic tuberculide and tertiary syphilis on the basis of clinical criteria. In the miliary form the other classic itching conditions of the scalp have to be ruled out, i.e., pediculosis, dermatitis herpetiformis, and chickenpox, the former by searching for lice and/or egg capsules, and the others by evidence of suggestive lesions elsewhere.

2.4.4. Treatment

Attacks of acne necrotica are best controlled by topically and/or systemically administered broad-spectrum antibiotics. A low-maintenance regimen of oral antibiotics, just as in acne vulgaris, is usually effective and necessary to prevent relapse. Possible stress factors must be detected and treated.

2.5. Pityriasis Rubra Pilaris

Pityriasis rubra pilaris is an erythematous squamous disorder characterized by follicular plugging, perifollicular erythema tending to become confluent, and palmoplantar hyperkeratosis and pityriasis capitis (21). Such a definition emphasizes scalp involvement as one of the main features of this relatively rare condition.

Both sexes are equally affected, and the disease may occur at any age, with two peaks, one small in the first decade and a higher incidence between 40 and 60. There is no convincing evidence of any genetic factor but an autosomal dominant transmission with low penetrance and variable expressivity has been suspected in several pedigrees. Sporadic cases also exist, developing at a later age, now considered as familial cases with a lower or more delayed expression.

The etiology remains unknown, and some evidence suggests that juvenile and adult forms are not identical. Several studies have demonstrated an increase in tritiated thymidine labeling, i.e., an increase in the cell production of the epidermis (22,23). However, although increased, cell kinetic parameters are less than in psoriasis (22). As

expected, nail growth is above normal as well. Direct immuno-fluorescent studies with the main types of immunoglobulin and complement fraction conjugates are negative; HLA typing does not seem to differ significantly from a control population (22).

2.5.1. Pathology

The striking changes are hyperkeratosis with patchy parakeratosis, acanthosis, foci of spongiosis, and basal liquefaction. The changes are most marked when erythema is maximal and best seen in samples taken from areas of confluent erythema (21). The follicles are distended by horny plugging and surrounded with a mononuclear cell infiltrate of the adjoining cutis.

2.5.2. Clinical Picture

Griffiths (21) suggested a clinical classification based on the age of onset, clinical appearance, behavior, and prognosis. He distinguished five groups with their approximate frequency, as follows:

Type I	Classical adult	55%
Type II	Atypical adult	5%
Type III	Classical juvenile	10%
Type IV	Circumscribed juvenile	25%
Type V	Atypical juvenile	5%

The elementary lesion is common to all types. It consists of a follicular keratotic papule with an erythematous halo. The erythematous areas usually coalesce to involve the interfollicular skin, giving rise to scaly erythematous patches. The erythema may have a bright, red, orange, or yellowish hue, according to the involved areas.

Patches may coalesce to become erythrodermic with scattered islands of normal skin, except perhaps in type IV, which has no tendency to progress beyond the initial sharply demarcated areas of follicular hyperkeratosis of the knees and elbows (21).

The nails may show longitudinal ridging and subungual hyperkeratosis. Although nonspecific, scalp involvement is an important feature, since it is fairly constant and at times represents the initial manifestation of the disease. The changes may simulate seborrheic dermatitis, psoriasis or pityriasis amiantacea, but more often the scaling on the scalp and face is fine and powdery. In most cases, there is no reduction in hair density, except in type III, in which sparseness of the scalp is sometimes present.

The course of the disease is variable according to the type: about half the patients remit completely within 3 years. In others, the course is chronic with relapses and remissions of variable duration.

The prognosis in classical adult cases is good, more moderate in the circumscribed juvenile type, and with little or no tendency to remit in the atypical juvenile form (21).

2.5.3. Differential Diagnosis

In the early stages of the disease or in the erythrodermic form, the diagnosis may not be obvious. In all cases, the major diagnosis to rule out is psoriasis, especially follicular psoriasis.

Pityriasis rubra pilaris must also be distinguished from other follicular keratotic processes, e.g., follicular eczema, follicular ichthyosis, lichen planopilaris, or even dermatomyositis with generalized spinulosis (23a), probably due to marked degenerative smooth muscle myositis of the arrector pili muscles (24).

In cases with initial and isolated scalp involvement, the clinical pattern is nonspecific and can be diagnosed only when suggestive lesions appear elsewhere.

2.5.4. Treatment

Symptomatic topical treatment is provided by simple emollients such as yellow soft paraffin (21). Topical application of 1% amino-nicotinamide cream, a potent antagonist of nicotinamide, may also be useful (25,26).

Oral vitamin A (150,000–300,000 IU daily) has been used for several decades but is less effective than toxic doses (1 million IU per day for 5–14 days) (27). Such a regimen achieves total desquamation within 14 days, the skin remaining erythematous for several months before assuming a normal color.

Peck et al. (28) reported some success with oral isotretinoin, but it is less effective than in lamellar ichthyosis, or Darier's disease, two other keratinizing dermatoses (29). Other retinoids such as Etretinate may be helpful. However, the effects of retinoids on this condition remain unpredictable. Overall, cytostatic drugs (methotrexate and cyclosporin) seem less effective in Pityriasis rubra pilaris than in psoriasis. Phototherapy is known to induce flare up of this condition, but occasional success has been reported.

2.6. Lichen Simplex Nuchae

Circumscribed lichenification, chronic lichen simplex (Vidal), neurodermatitis (Brocq), or circumscribed prurigo (Darier) all refer to the most classical type of lichenification. The usual sites are the nape of the neck, the scalp, the upper thighs, and the genitals, although any part of the body may be affected.

Lichenification describes a pattern of response of predisposed skin to repeated rubbing. The rubbing may be primary or initiated by any itchy condition. However, secondary lichenification may become self-perpetuating after the initiating dermatosis has remitted.

Not all individuals are able to develop lichenification. Such a pattern of reaction has been considered as a characteristic feature of the atopic state (30), but not all atopics can lichenify, and lichenification may affect individuals with no past or present history of atopy. Emotional tensions often act as a triggering factor in susceptible subjects and may ensure the perpetuation of the dermatosis.

Lichen simplex is rare before puberty, the peak incidence being between 30 and 50 years. Women are affected more often than men.

2.6.1. Pathology

The pathological changes may vary with the site and duration. Constant changes include hyperkeratosis, acanthosis, and lengthening of the rete ridges. Foci of spongiosis and parakeratosis are sometimes present. A mixed chronic inflammatory cell infiltrate is usually seen in the upper dermis, which may show some fibrosis in very chronic lesions.

2.6.2. Clinical Features

Itching (pruritus) is the predominant symptom in all types of lichenification. Its severity is set by the patient at a level out of proportion to the objective signs of the disease. Progressively, the involved areas become pink and then thickened, slightly edematous and grained, with numerous scattered shiny, small papules. Later, the redness tends to subside, and the plaque then appears pigmented and scaly, thickened with exaggeration of the normal skin markings, giving rise to a square pattern of the affected surface. Surrounding the central plaque is a zone of small lichenoid papules, and beyond this an indefinite brown or yellowish area slightly thickened and pigmented, merging with adjoining normal skin.

Lichen simplex nuchae occurs as a single plaque of lichenification on the nape of the neck, often progressing upward onto the occipital scalp. Less frequently, other parts of the scalp are involved, sometimes with a misleading clinical appearance, since the presenting sign may be localized breaking of hair associated with underlying pruritus and scaling.

2.6.3. Differential Diagnosis

Lichen simplex nuchae may mimic psoriasis, since it is often very scaly. In such instances, typical lesions of psoriasis should be sought in other sites. Once established, the diagnosis of lichenification must be carefully investigated for a possible cause, chiefly allergic or irritant reactions to hair cosmetics.

2.6.4. Treatment

Primary lichenification requires psychological assessment to help the patient in reducing tensions. Sedatives are often helpful to reduce the scratching habit. In the case of secondary bacterial infection, a topical and/or systemic antibiotic may be temporary prescribed. Local applications include steroid anti-inflammatory agents and shampoos with salicylic acid to remove the scales and wood/coal tar to reduce the lichenification process. Intralesional steroids may be required in resistant cases.

2.7. Radiodermatitis

Ionizing radiation has been used in therapeutics since the beginning of the century. Despite little initial understanding of dosimetry, x-rays were soon widely used on an empirical basis to treat both benign and malignant skin diseases as well as internal malignancies, so that dermatologists may still be faced with the late effects of over-dosage.

X-rays are part of the electromagnetic spectrum and were the first known form of ionizing radiation. Several other types have been used since, e.g., β -rays, produced by the decay of radioactive substances, α -rays, which are particles being the nuclei of helium, and γ -rays, which are electrons given off by certain radioactive isotopes or produced by linear accelerators.

Besides numerous types of skin tumor, including warts, x-rays were employed to manage skin diseases as diverse as lupus erythematosus, lupus vulgaris, and acne. X-ray epilation for the treatment of scalp ringworm used to be the treatment of choice, but fell into disuse with the introduction of griseofulvin in 1958. The frequency of scalp radiodermatitis is related to the widespread use of x-rays in the past to treat common infectious conditions of this area.

However, radiodermatitis of the scalp may also occur as a consequence of skin damage following the treatment of internal or cutaneous malignancies.

The way ionizing radiations lead to skin and hair damage is not yet fully understood. Skin insult is dependent on the total dose, whatever the radiation technique used, regardless of the time over which the dose was delivered. Such a statement accounts for the relatively high frequency of radiodermatitis following x-ray epilation, since the most frequent mistake in carrying out this treatment was to overlap the demarcated fields of the scalp, all ideally equally irradiated, so that certain areas received double the intended dose (31). Hair damage in x-ray epilation, or as a side effect in other circumstances, depends on the high susceptibility of anagen hair to radiation, since cells with a high mitotic index are more radiosensitive than differentiated cells.

2.7.1. Pathology

Radiodermatitis may present either as an acute reaction or as a delayed effect.

In acute radiodermatitis there is edema and relative rarefaction of connective tissue beneath the epidermis. This may be accompanied by flattening or loss of epidermal rete ridges. Capillaries are congested and their endothelium hypertrophic. Hemorrhage and thrombosis are often seen. By the third or fourth post irradiation day, the basal cell layer of the epidermis shows progressive degeneration and the prickle cell layer displays abnormal mitoses, pyknotic nuclei, micronuclei, dispersed chromatin granules, and swollen cytoplasm (32). At the same time, subtle changes may be found in the DNA-RNA structure of epithelial cells using special stains (32).

Chronic radiodermatitis is characterized by fibrosis, which partially or completely occludes deeper vessels, superficial small vessels being telangiectatic. These occlusive vascular changes account for possible necrosis. The epidermis is generally atrophic with loss of adnexae such as hair follicles and sebaceous glands. In places, the epidermis may show some acanthosis and dyskeratosis and proceed to squamous or basal cell carcinoma.

2.7.2. Clinical Picture

Acute radiodermatitis consists of a primary erythema, which may become apparent within 5–11 hr and usually subsides within 24 hr (33). A second erythema usually appears within 10–14 days of radiation and reaches its maximum at about 20 days (33). With moderate doses, it begins to fade after about 3 weeks, leaving a pigmented and

partially or completely epilated patch of skin corresponding to the exposed area, epilating and subepilating doses producing dystrophic changes in hair as early as the fourth day after exposure.

Regrowth normally appears after 2 months, but in the case of accidental over-lap of two contiguous fields, the doubly irradiated area may show permanent epilation and delayed chronic radiodermatitis. Such a clinical course including primary and secondary or "threshold" erythema is achieved with quite a variable dose, the reaction tending to be more intense in patients with fair skin. This dose is about 3.5–4.0 Gy at 100–120 kV and may be in the range of 1000 rad at higher voltages (1000 kV or more) (33).

Late sequelae of chronic radiodermatitis may include any or several of the following: blanching, mottled pigmentation, telangiectasia, fibrosis, atrophy, keratosis, relative avascularity, necrosis, or cancer (33). They become apparent only after a period of months or years. Patches of cicatricial alopecia are constantly associated with the poikilodermatos changes of late radiodermatitis.

Radiation-induced degenerative changes may be minimal at first, but they inexorably progress throughout life. Extensive necrosis may develop, the healing of which is uncertain and time-consuming to achieve, sometimes requiring total excision of the involved area, to obtain sound granulation tissue. These changes are aggravated by degenerative changes from excessive sun exposure and aging.

Radiation-induced skin cancers consist of basal and squamous cell carcinomas, the former predominating in a ratio of about 4:1 (33). The interval between radiation therapy and the development of skin cancer may range from a few months to several decades, but according to a large series (33) the interval is rarely less than 10 years.

2.7.3. Treatment

There is no available treatment for chronic radiodermatitis and its related alopecia. Malignant tumors arising in radiodermatitis should be excised.

3. FUNGAL AND PARASITICAL INFECTIONS

3.1. Fungal Infections

Scalp ringworm (tinea capitis, tinea tonsurans) is an infection of the scalp with invasion of hair shafts by a ringworm fungus or dermatophyte belonging either to the genus *Microsporum* or *Trichophyton*. These fungi grow in keratin and may colonize and parasitize all types of keratinized structures, i.e., the horny layer of the skin, the nails, and the hair.

Epidermophyton is a dermatophyte but it never invades the hair. Conversely, most species capable of invading hair, even those with a predilection for the hair shaft, may also invade glabrous skin and nails.

Microsporum and *Trichophyton* may infect man in three different ways: both genera include species whose natural host is man and others that occur in one or more animal species, parasitizing man accidentally. The former are called anthropophilic and the latter zoophilic species. The third type of contagion involves at least one species, *Microsporum*

gypseum, normally a soil fungus belonging to geophilic species, and from which man and animals may acquire the infection.

Whatever the species involved, dermatophytes originally parasitize the stratum corneum often after minor trauma. Gradually progressing from the point of inoculation toward and then through the follicular orifices, they reach the neck of the hair bulb where keratinized structures of the follicular wall disappear and then invade the hair shaft. Only hairs in the growing (anagen) phase are attacked; the dermatophyte extends upward at the rate at which the hair grows. Dermatophytes may invade the hair shaft according to three different patterns:

1. In the ectothrix type the hair shaft is initially invaded in mid-follicle. The intrapillary hyphae (i.e., the individual parts of the interwoven mass of fungal filaments called mycelium) grow inward, toward the bulb of the hair, to burst out and grow in a tortuous manner over the surface of the hair shaft. These external hyphae produce small rounded-off or spherical arthrospores (i.e., vegetative structures, characteristic of the parasitic phase of the fungus that arise following modification of the hyphae) for species belonging to the microsporum type, and large arthrospores arranged in straight chains for some species of the trichophyton genus.
2. In the endothrix type, hyphae fragment into arthrospores that remain entirely confined within the hair shaft.
3. The favus type, due to *Trichophyton schoenleinii*, displays broad hyphae and air spaces within the hair shaft, but arthrospores seldom develop.

Beyond its physiopathological and mycological interest, such a classification provides some clues for the understanding of the degree of hair strength according to the responsible genus. In the case of intense parasitism as in endothrix and ectothrix types, the hair shaft breaks easily. This represents a basic clinical feature of scalp ringworm, accounting for the respective generic Latin and French designations "tinea tonsurans" and "teignes rondantes."

Conversely, in the favus type, the other clinical features of which will be further outlined, the less important mycological attack on the hair shaft correlates well with the clinically less-damaged hairs, which may continue to grow to considerable lengths.

Except in rare instances, mainly in women, scalp ringworm exclusively affects children. In the absence of available effective treatment in the past, scalp ringworm spontaneously subsided and healed at puberty. There is no satisfactory explanation of such a course and the possible fungistatic effect of sebum, the production of which increases at puberty, has not been fully established.

Boys are more susceptible than girls to scalp ringworm. The protective effect of the traditionally longer hair of girls compared with boys has been put forward to explain such a difference.

3.1.1. Clinical Picture

From the clinical point of view, the host behavior to a ringworm infection depends both on the degree of inflammation or suppuration and the pattern of distribution on the scalp. This behavior depends on the host's immune state, which may itself be modified by current therapy, whether prescribed for the infection or for any associated disease, the

nutritional status of the patient, and the special properties of the strain of dermatophyte concerned. Such variables indicate that almost any species of dermatophyte can be associated with any of the ringworm syndromes. Nevertheless, each species or group of species produces the same basic clinical features. Basing the clinical description of the scalp ringworm on the fundamental different types of host behavior and the responsible species or genus of dermatophytes should be of a great help.

The clinical appearance of scalp ringworm may or may not be inflammatory, the latter being roughly divided according to the genus of fungus, i.e., *Microsporum* or *Trichophyton*. Favus will be described independently because of its special features and course.

Noninflammatory. This subgroup corresponds to the “teignes tondantes” of the French nomenclature (cropping ringworm): it differs from tinea tonsurans, which encompasses the whole ringworm group.

As outlined previously, one distinguishes between noninflammatory ringworm of both *Microsporum* and *Trichophyton* genera. Both give patches in which the hairs are broken off a few millimeters above the scalp. The size of these plaques vary according to the genus involved. Since Sabouraud's time, generations of dermatologists have been taught the following adage, “a small spore, a large patch; and a large spore, a small patch.”

Microsporum. The clinical aspect is easily recognizable. It consists of single or multiple large plaques, rounded or irregular, seldom exceeding 5–7 cm in diameter (Fig. 9). When multiple, these patches may merge, giving rise to polycyclic lesions. All the hairs in the patch(es) are, without exception, broken off at about 5 mm, and when pulled out show a frosted-up like appearance of their base.

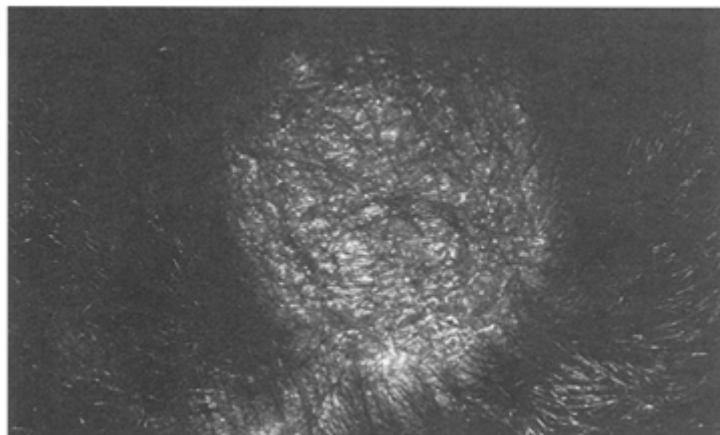


Figure 9 Large plaques with broken off hairs due to *Microsporum* genus infection. *Microsporum canis*.

Fungi of the genus *Microsporum*, when growing in hair, give a light green fluorescence under Wood's light. The effective use of this technique requires some experience: the

examination must be performed in a fully darkened room using a lamp emitting ultraviolet light. Scale and exudate, which sometimes cover the involved hair, may screen the fluorescence or give a parasitic actinic fluorescence that must be recognized and ignored. When initially covered, the fluorescence may be progressively unmasked as the lesion is gently and patiently cleaned.

As well as the use of Wood's light and microscopic examination of infected hairs to look for fluorescence and to clarify the invading pattern of the fungus, a culture on Sabouraud's medium of infected hairs, scales, exudate, or pus should always be carried out, since precise identification of the species is essential for epidemiological purposes.

The anthropophilic fungi of *Microsporum* genus such as *M. audouinii* as well as the African or oriental species (*M. langeronii*, *M. ferrugineum*, *M. rivalieri*) give this typical form of scalp ringworm. However, in Europe, children are now almost exclusively infected with zoophilic fungi of *Microsporum* genus, e.g., *M. canis* (Fig. 10), which particularly parasitizes kittens and puppies. These fungi more often show



Figure 10 Small circinated erythematousquamous patch of the occipital hairline due to *Microsporum canis*.

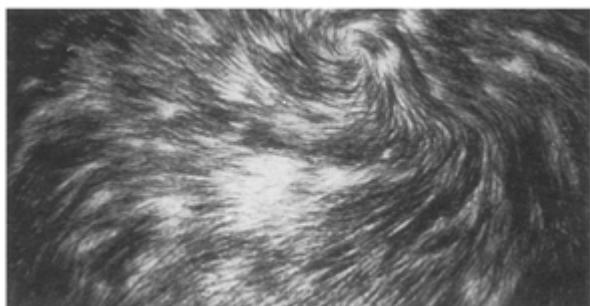


Figure 11 Noninflammatory ringworm *Trichophyton*.

an inflammatory clinical picture and are more frequently associated lesions of the glabrous skin (Fig. 10).

In ringworm due to one of these zoophilic species, the scalp surrounding the patch(es) may show some degree of scaling and inflammation although the covering hair looks normal. The only way to know if such areas are parasitized is to perform direct microscopic examination of the scales: indeed a culture is of no real value, with a high frequency of positive results, since the fungus may have been secondarily scattered from the patch(es) to the adjacent zones owing to local manipulations.

Trichophyton. Noninflammatory ringworm of *Trichophyton* genus is exclusively due to anthropophilic species. The classical lesion produced by these species is "black-dot" ringworm in which numerous small irregularly shaped plaques no more than a few millimeters in diameter, scattered over the whole scalp, are studded with "black dots," i.e. hairs broken off at the scalp level or just emerging from the scales (Fig. 11). However, the clinical appearance is very variable, to some extent correlated with the duration of the infection.

Scaling without loss of hair or with only occasional broken hairs may provide the only evidence of infection (Fig. 12), whereas in long-standing infections large plaques of cicatricial alopecia may dominate the picture.

The involved *Trichophyton* species include *T. violaceum* in North-African children and *T. soudanense* in young black Africans. It is often difficult in the latter case to distinguish between ringworm of *Microsporum* and *Trichophyton* genera, and they may be associated (*T. soudanense* and *M. langeronii*). All these imported infections remain only slightly contagious in Europe, since school epidemics usually involve no more than three or four children.



Figure 12 Noninflammatory ringworm *Trichophyton*.

These species do not fluoresce in Wood's light and when pulled out for examination, the infected hair may break at two levels, the neck of the bulb and just above the scalp. Such a phenomenon was noticed by Sabouraud, who thought it was responsible for the granulomatous reaction described by Majocchi with *T. violaceum*, because of the partial retention of hair shafts within the dermis (Figs. 11 and 12).

Trichophyton rosaceum and *T. rubrum* are very common species affecting glabrous skin and nails but very rarely cause scalp ringworm.

Inflammatory Ringworm. This starts as a scaly area, quickly extending to several centimeters in diameter (Fig. 13). About the 10th or 15th day, the lesion suddenly undergoes marked inflammatory changes with thickening and swelling. Severe pustulation develops, the pus initially rising from the follicular ostia. This pustulation occurs as a reaction to the fungus and not as a result of secondary bacterial infection.

The lesion is painless, but lymphadenopathy is frequent, and malaise and slight fever may occur. The red pseudo-tumorous lesion studded with large follicular pustules, so-called agminate folliculitis, finally turns into a raised boggy mass of pustules or kerion (Fig. 16), which represents the most severe inflammatory pattern of reaction to fungal infection. Spontaneous cure may take months, the lesion starting to fade in a few weeks. In such cases, the kerion heals but causes cicatricial alopecia. Complete recovery is best achieved by early effective therapy.

Several species may cause severe inflammatory lesions leading to kerion formation, but virtually all belong to the zoophilic type, so explaining the prevalence of such lesions in a farming environment. *Trichophyton mentagrophytes* is the most common of these species. It normally infects cows, invades hairs according to the ectothrix type, and a distinctive feature of this dermatophyte is to grow on a heart-brain medium at 37°C.

The morphology and course of infections with *T. verrucosum* are identical to those with *T. mentagrophytes*. *Trichopyton ochraceum* produces a less acute clinical picture.

Other species may incidentally lead to inflammatory ringworm sometimes turning into true kerions; as with endothrix infections (*T. violaceum*, *T. soudanense*, *T. tonsurans*), *M. canis*, and *M. gypseum*. The reason for an inflammatory, suppurative course with such fungi is most likely related to the underlying immune state of the host. The more inflammatory the lesion is, the shorter its course and the stronger the resultant immunity. **Favus.** Causal fungus, *T. schoenleinii*, a relatively common dermatophyte in the Middle East and South Africa may be found sporadically in Pakistan, the North

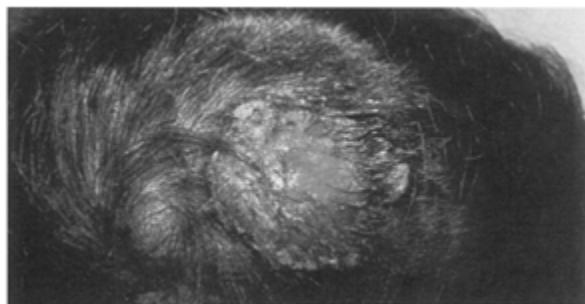


Figure 13 Kerion.

American continent, Western Europe (including France and the United Kingdom), and Australia.

This exclusively anthropophilic fungus gives rise to readily recognizable lesions, the most classical of which is the scutulum: it starts as a yellowish concretion of a hair follicle and enlarges to form a perifollicular cup-shaped disc, a few millimeters to 1 cm in diameter, firmly stuck at its center to the underlying scalp and centrally pierced by the

hair that may carry on growing to considerable lengths. Enlarging, adjacent scutula may merge together to form crumbling masses of yellow crusts.

Less-distinctive changes may be seen such as patchy pityriasisiform scaling and/or crusting. Under Wood's light, affected hairs show a dull greenish fluorescence along their entire length.

When diagnosed and treated early, this type of tinea capitis heals without sequelae. In longstanding cases with a clinical picture of well-formed scutula and/or widespread crusting covered with opaque and sparse hairs, recovery is not complete despite therapy and results in diffuse or patchy cicatricial alopecia.

Favus starts during infancy and, when neglected, may persist indefinitely. But early diagnosis and treatment will be completely successful and prevent such permanent cicatricial alopecia.

3.1.2. Differential Diagnosis

The differential diagnosis of tinea capitis includes all conditions capable of causing alopecia, scaling, broken hairs, or folliculitis, singly or in any combination. This means that almost all the conditions that may affect the scalp should be considered since the above signs represent most of the basic features of any scalp disease.

In the child the commonest sources of error are traumatic alopecia, alopecia areata, pityriasis amiantacea, and impetigo.

In traumatic alopecia, which is a habit tic called trichotillomania, the hairs are twisted and then broken, but remain of normal texture and the scalp looks normal.

Any suggestion of folliculitis requires samples to be taken for myco-bacteriological examination. Apart from conditions of patently infectious origin, there is a group of chronic types of folliculitis that induce balding, usually chronic and fluctuating in intensity, in which *Staphylococcus aureus* plays a possible, if not probable, role, not so as an infective agent but in the aggravating role of a co-factor, seizing the opportunity of a state of hypersensitivity to the host's bacterial antigens or to a specific local immunodeficiency. Other aspects described in this context are: Quinquaud's depilating folliculitis or folliculitis decalvans, typically occurring in middle-aged women, often in a known seborrheic context which may be sensitive to the combination of rifampicin and fusidic acid. It is not unlike "tufted" folliculitis, which may be the primary cause, where the tufts of hair have a nevoid character, or secondary if these have resulted from post-inflammatory cicatricial fusion of the follicles. The appearance is that of a "doll's hair" implant, sometimes observed at the periphery of plaques in pseudopelade and representing a potential common denominator in all cases of idiopathic inflammatory folliculitis of the scalp. In the localized forms, surgical excision may resolve the problem.

Dissecting cellulitis of the scalp or Hoffmann's *perifolliculitis abscedens et suffodiens*, more inflammatory, more suppurative and more strongly expressed, affects young men, often black, aged between 20 and 40 years, in a disease context evocative of the acne triad (acne conglobata, suppurative hidradenitis, and pilonidal sinus). Apart from local treatment, other forms of treatment include systemic anti-biotics, isotretinoin, zinc supplements, and corticosteroid injections.

Dermatitis papillaris capillitii, also known as fibrous or keloidal folliculitis, of the posterior hairline affects black subjects and presents in the form of keloidal lesions surrounding tufts of hair shafts following a pustular inflammatory phase.

Erosive pustular dermatitis of the scalp combining erosion and superficial pustules is limited to elderly women.

Eosinophilic pustular folliculitis may be misdiagnosed when it exclusively involves the scalp. It then mimics a relapsing impetigo readily responding to sulfones.

Forms of storage disorders can cause alopecia by choking the follicle with a coating of mucin or amylase, accompanied by minor local inflammation. The diagnosis is made on the basis of the clinical picture and above all a biopsy with specific stains.

Alopecia areata may show some erythema, and although of itself not a scaly condition, it may coexist with pityriasis capitis. Exclamation-mark hairs may add to the confusion when not distinguished from the broken off hairs of tinea capitis.

Seborrheic dermatitis is usually more diffuse than scalp ringworm, but in pityriasis amiantacea, the changes are often localized. However, in the latter condition, fasciculated unbroken hairs are flattened by asbestos-like scales.

In impetigo, a streptococcal and/or staphylococcal infection that may complicate pediculosis of the scalp, the hairs look normal but crusting may cause confusion with inflammatory ringworm.

Each time the diagnosis of ringworm is under consideration, the scalp should be examined under Wood's light, and abnormal hairs, scales, crusts, pus, or other exudates should be investigated by microscopy and culture.

3.1.3. Treatment

The treatment of scalp ringworm is based on both the mechanisms of infection and the ecology of the fungus concerned.

Oral griseofulvin, a fungistatic drug, is a product of several species of *Penicillium*. It is active against dermatophytes but not against yeasts or bacteria. Resistant strains of dermatophytes are unusual. Griseofulvin accumulates in the keratinized structures, rendering them resistant to further invasion by the fungus. Treatment must therefore be continued as long as the infected keratin is not replaced by resistant keratin. Griseofulvin is nontoxic, but side effects such as headache, nausea, gastric disturbance, and photosensitivity may occasionally occur. The standard dose for adults is 0.5–1 g daily, and for children from 10 to 20 mg/kg daily. The use of griseofulvin has largely been superseded in most countries by more recent drugs, mainly terbinafine but also itraconazole, except perhaps in tinea capitis. Terbinafine is a member of the allylamine antifungals. In contrast with griseofulvin, it is fungicidal rather than fungistatic and is effective in vitro against dermatophytes in addition to other fungi. The dose for adults is one 250-mg daily tablet; 125mg tablets exist, but are not available in all countries. Side-effects are uncommon, either quite benign and reversible such as nausea, loss of taste and skin rash, or exceptionally hepatic reactions or agranulocytosis. Despite terbinafine potency against most of the fungi involved in tinea capitis, including trichophyton species, it is not licensed for this indication in several countries. Itraconazole belongs to the triazole series. Its effects are similar to Ketoconazole, but without associated risk of hepatotoxicity. It is active in vitro against the main superficial fungal pathogens as well

as a wide range of fungi causing deep infections. It has a strong affinity for keratin-containing tissue. Although active against dermatophytes and effective in tinea capitis, it is not licensed in most countries for this indication. Side effects are not commonly encountered with this drug.

In noninflammatory tinea capitis, shaving is required, either local or total, respectively, for circumscribed or diffuse ringworm. The shaving is ideally performed twice, in order to eliminate the largest possible amount of infected hair, the first time when the treatment is initiated, the second time 3 weeks later when the infected hair shafts from the infundibula come through.

Topical fungistatic creams or ointments, may be beneficial but cannot achieve complete healing by themselves.

In addition to the removal of infected hairs, systemic drugs and topical drug applications, kerion lesions may require a short course of systemic corticosteroid therapy, in order to minimize local inflammation and so limit or even prevent late sequelae. When undiagnosed, kerion was sometimes surgically excised as if it was a bacterial abscess. Such a therapy is positively inadvisable, and of no interest other than delaying the final healing by producing skin defect.

Public health measures include identification of the source of infection to prevent further dissemination, and the responsible species by cultures. If the species is anthropophilic, family and school contacts must be examined and all infected individuals isolated until they have been successfully treated. When zoophilic species are concerned, school exclusion is not necessary, but the source of infection may be difficult to trace.

3.2. Pediculosis

The parasite concerned in pediculosis (Fig. 14) is the sucking louse, a dorsoventrally flattened, wingless insect of the order Anaplura (Fig. 15). Two lice species may parasitize human beings, *Pediculus humanus* and *Phthirus pubis*. Both are highly host specific. *Pediculus humanus* occurs in two distinct populations, *P. humanus capitis*, the head louse, and *P. humanus corporis*, the body louse. The morphological differences between head and body lice are slight, but these two types display marked physiological differences although they interbreed freely.

Phthirus pubis or crab louse not only differs in its characteristic location on the body but is so distinctive that it cannot be confused with other species.



Figure 14 Pediculosis. The parasite is clearly visible.

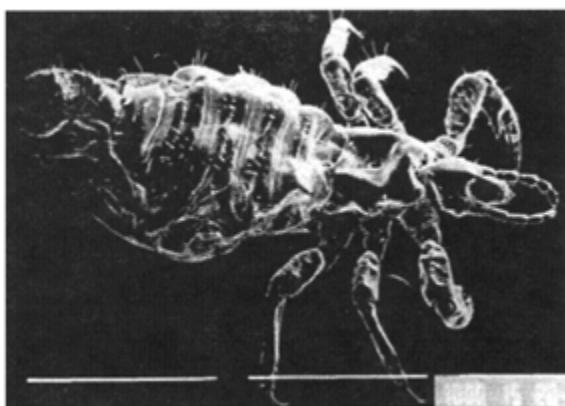


Figure 15 *Pediculus humanus capitis* ($\times 37.5$).

Except in wartime and during similar disasters causing overcrowding with unsanitary conditions, *P. capitis* is the most common type and has a worldwide distribution.

The adult *P. humanus* is a grayish-white insect (Fig. 15), about 3 mm long, the male being slightly smaller than the female. Their legs are adapted for grasping hairs. In the course of her life-span, about a month, the female lays seven to ten eggs daily. The eggs hatch in about a week, and the nymphs require a further 8 days to reach maturity. Nymphs and adults of both sexes suck blood and inject their saliva in the process.

Pediculosis capitis is mainly seen in girls and women. The relatively low incidence in adult males is said to be related to the smaller total weight of hair, but other factors may be involved. The incidence used to be higher during infancy, but no age is immune and infestation may remain frequent in adult life.

3.2.1. Clinical Picture

The presenting sign is often itching, the severity of which depends on the host immune response to the salivary antigens of the louse, but it is seldom absent.

The affected hairs become lusterless and dry, but the diagnosis is made by finding the lice, or more often of oval-lidded white capsules, known as "nits," firmly cemented to the hair shaft (Fig. 16). These eggs must not be confused with hair casts, which are yellowish-white accretions of epithelial cells and keratinous debris surrounding the hair shafts over a length of a few millimeters, and probably occurring as a result of the failure of the internal root sheath to disintegrate above the level of the sebaceous duct. These hair casts are usually recognizable, since they slide freely along the hair shafts.

Capsules are easily seen under Wood's light, and such a rapid examination is worthwhile whenever a large population has to be screened.

Complications such as furunculosis and impetigo, especially at the nape of the neck, are common. Secondary bacterial infection is initiated by scratching, and in such instances, the cervical lymph nodes become enlarged and the general health may be affected. Impetigo of the scalp should never be an acceptable diagnosis until pediculosis and scalp lesions of chickenpox have been ruled out.

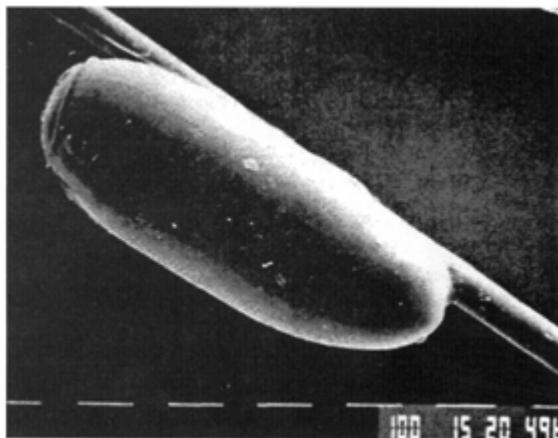


Figure 16 Nit: oval-lidded capsule cemented to the hairshaft ($\times 112.5$).

3.2.2. Treatment

The treatment of pediculosis is simple, since effective parasiticides are available, such as hexa-chloro-cyclohexane (666 or gammexane), dichloro-diphenyl-trichloroethane (DDT) and various synthetic or natural pyrethrroids.

The chosen preparation, powder or emulsion is rubbed into the scalp daily for 2 or 3 days with thorough washing of the hair before each application.

Such treatment kills the lice and the nymphs but does not affect the eggs. In order to avoid a new infestation when the eggs hatch, the same treatment is repeated 8 days later.

At that time the remaining nits are easily removed with a fine-toothed comb following a rinse with warm vinegar.

Lice resistant to gammexane or DDT are being increasingly encountered. In such cases, Malathion 0.5% in alcohol has been used successfully. For severe secondary infection a systemic antibiotic should be given.

4. TUMORS OF THE SCALP

4.1. Introduction

In its widest sense, a tumor is any localized increase in tissue volume. Growth may be of a proliferative cellular nature, giving rise to true tumors, but such a definition also includes growths that are not proliferative in the strict sense, such as cysts and certain localized inflammatory conditions, or even certain types of dysembryoplasia.

There is a priori no reason why any type of tumor potentially affecting the skin should not affect the scalp. On the contrary, no type of pathognomonic tumor of this region exists.

However, there is evidence of affinity for the scalp in certain conditions and the cephalic localization of some tumors appears therefore to be, if not pathognomonic, at least characteristic, their emergence at other sites being particularly surprising for the clinician. We will just take as an example the cylindroma, a tumor of either apocrine or eccrine origin to which we shall refer later.

So, rather than an exhaustive catalog of all the proliferative events that can occur in the scalp region, we shall limit ourselves to a description of the lesions with known affinity for this region, or tumors that are encountered as often on the scalp as at any other site on the skin, in particular those exposed to the sun, where epithelial carcinomas or pre-epitheliomatous lesions are of particular interest.

The very characteristics of the scalp, i.e., by definition, the presence of hair on the one hand and its site on the other, which tends to hamper the patient's view of any local pathological sign and so precludes any principle of self-monitoring, represent two frequent causes of delayed diagnosis that can sometimes have an adverse effect on the prognosis.

In view of their supposed or proven origin, the most common proliferative conditions affecting the scalp can be divided into four groups: benign or malignant epithelial tumors, benign or malignant connective or mesenchymatous tumors, lymphomas and pseudolymphomas and, finally, tumors of the pigmentary system.

4.1.1. Benign Tumors and Dysembryoplasias of Epithelial Nature

These lesions enter into the category of those that must not be overlooked, not only due to their high incidence but particularly due to their propensity to attack the scalp in particular; this fact is explained by their adnexal origin, the histogenesis of such lesions involving skin appendages, i.e., hairs, sebaceous glands, eccrine or especially apocrine sweat glands, these structures being reproduced with varying degrees of differentiation.

Epidermal (Pilar) Cysts. These cysts contain a semi-liquid keratin that is often malodorous, the outer wall reproducing the external epithelial sheath at the level of the follicular isthmus. Their development involves an overgrowth of the external epithelial sheath due to a genetically determined structural abnormality. Indeed, a family history of these cysts is noted in around 75% of patients, with a dominant autosomal mode of transmission being presumed.

These epidermal, or trichilemmal, cysts are observed on the scalp in 90% of cases. They are commonly known, incorrectly, as sebaceous cysts, a largely hackneyed term for lesions encountered almost exclusively in the context of a dominant autosomal infection, sebocystomatosis, for which the scalp is not in fact a site of predilection.

The evolution of epidermal cysts is benign but their excessive growth is esthetically displeasing due to their emergence between neighboring strands of hair and even more so due to the fact that the affected site is usually balding. The subcutaneous post-traumatic rupture of the cysts causes an inflammatory reaction preventing subsequent enucleation and requiring considerable surgical intervention, sometimes with substantial tissue loss. Excision is therefore normally recommended.

The proliferative trichilemmal cyst, with the same origin as the epithelial cyst, represents a tumor of moderate malignancy, as it hardly has any tendency to self-limitation. It afflicts elderly women, often affecting the scalp but also found in other areas such as the back.

The sebaceous or verruco-sebaceous hamartoma, described by Jadassohn, is usually a single desembryoplasia, sited on the scalp in around two-third of cases. It is a lesion present at birth in the form of a buff plaque with blurred edges, often unnoticed at an early stage, glabrous, with a round, oval, or linear shape.

During physiological development of the sebaceous glands at puberty, these plaques thicken, generally acquiring a mammillated surface, sometimes masked by a variable degree of keratotic tissue, placing them in the category of an associated epidermal hamartoma.

In one-third of cases, in adulthood, benign tumors, generally sudoriparous, apocrine or pilar but also sebaceous, or malignant tumors, essentially basal cell carcinomas, develop at the base of these hamartomas, thus justifying their prophylactic surgical excision, otherwise often indicated for esthetic reasons. A particular form is represented by the syndrome of sebaceous hamartoma (Schimmelpenning-Feuerstein-Mims syndrome) associating an extensive linear sebaceous hamartoma with severe skeletal and visceral malformations.

The syringocystadenoma papilliferum or *fistulovegetative verrucous hidradenoma*, though rare, deserves mention given its peculiar clinical presentation that may be misleading if it fails to be recognized: it consists in fact of a small alopecic plaque, slightly to very crusty, often moist and sanguinolent due to sero-hemorrhagic oozing through a small fistulous orifice that requires detection. It is an organoid hamartoma, and is often associated with the Jadassohn hamartoma, deriving from the most distal portion of the apocrine excretory duct. It occurs essentially on the scalp, surprisingly sparing the axillary and perineo-gluteal regions where the apocrine sweat glands are physiologically concentrated.

The cylindroma is one of the most common benign tumors of the appendages. In 90% of cases it occurs on the scalp and predominantly affects females. Some inherited cases have

been described, indicating dominant autosomal transmission. Clinical presentation is of a pink or red nodule, from one to several centimeters in diameter, hence easily detected. There may be a single or multiple lesions, constituting Poncet-Spiegler tumors, that may cover the whole scalp and have become known as "turban tumors." The combination of multiple cylindromas with multiple trichoepitheliomas, or even other appendage tumors such as spiradenomas reflects a genodermatosis of dominant autosomal transmission and constitutes a "symptom signal" calling for further examination for a potential underlying malignant neoplasia (34).

4.1.2. Pre-cancerous Lesions

One of the purposes of the head of hair is certainly protection from the sun. Advanced androgenic alopecia allows maximal actinic irradiation since it occurs at an optimal angle of incidence. The pre-cancerous lesions, i.e., pre-epitheliomatous keratosis or actinic keratosis, are observed with particular frequency in balding subjects. The clinical picture is one of brown or gray squamokeratotic patches, ill-defined, often erosive and bleeding readily when carelessly scratched.

Histological examination is sometimes performed to distinguish them from ordinary lentigenes, seborrheic warts or Dubreuilh melanosis, which are actually very rarely encountered on the scalp, even in severely balding subjects.

Under the microscope, they have the classic intra-epidermal appearance, with hyperkeratosis, atypical nucleocytoplasmic bodies and a non-specific dermal inflammatory infiltrate.

In the medium or long term, they evolve usually as spinous cell rather than basal cell carcinomas and histological examination is also able to differentiate a simple actinic keratosis from that which is already micro-invasive in view of the presence of dermal epithelial growths. Treatment consists of cryotherapy and, in very extensive forms, the use of local treatment with 5-fluoro-uracil or imiquimod may be envisaged, as well as dynamic phototherapy.

4.1.3. The Carcinomas

The basal cell carcinoma is a very common tumor, of uniquely local malignancy since it has no propensity for hematogenic or lymphatic dissemination. On the contrary, its local development is unlimited and tends to destroy any tissue encountered, the bones of the skull proving to be no barrier to its spread and there are historical reports of its reaching the meninges.

Histologically, the basal cell carcinoma combines a fibrous stroma with cellular islets resembling cells of the epidermal basal layer and of the hair follicle.

This tumor is derived from an undifferentiated and pluripotent germinative epithelial cell. Given its stromal dependency on the one hand and the capacity of the basal cell carcinoma for differentiation towards any type of skin appendage, its pathogenesis may involve the defective reproduction of embryological differentiation mechanisms.

Actinic factors play a predominant role in the onset of basal cell carcinomas. For the reasons already outlined, it is easy to understand that in the event of chronic or prolonged

alopecia, this type of tumor readily affects the scalp, either de novo or via a pre-epitheliomatous lesion such as an actinic keratosis.

However it is not exceptional for the onset of basal cell carcinomas to be observed beneath a dense head of hair.

Among the numerous clinical presentations of this lesion, the high incidence should be noted of the superficial basal cell carcinoma, capable of reaching a large dimension, and of the ulcerative forms, these two presentations being capable, moreover, of combining to give rise to pseudo-cicatricial areas that can be misleading. The clinical diagnosis will be helped by the presence, always to be looked for, of translucent papules with a pearly appearance, the common denominator of virtually all the forms of basal cell carcinoma and hence having a particularly high diagnostic value.

On the scalp, apart from the actinic keratoses, sebaceous or verruco-sebaceous hamartomas represent, in adulthood, a preneoplastic lesion within which the basal cell carcinoma may develop in the form of a small nodule or an unusual pigmented area, another classic clinical feature of this type of tumor.

The onset of basal cell carcinomas on radiodermatitis should be mentioned, as it was a classic phenomenon at the time when radiotherapy was used as a means of therapy in ringworm of the scalp. Of course, this treatment has been outdated for decades, i.e., since effective antifungal agents became available.

On the treatment level, the creation of an alopecic area caused by the elimination of these lesions is to be avoided, by undertaking expansion of non-balding skin areas prior to any excision. As far as the superficial and extensive forms on the balding scalp are concerned, other techniques such as the local application of 5-fluoro-uracil, or of imiquimod, or even CO₂ laser vaporization may be considered.

The spinous cell carcinoma constitutes the second most frequently encountered cutaneous neoplasia after the basal cell carcinoma. It also more readily affects areas chronically exposed to sunlight and, unlike the basal cell carcinoma, it possesses a degree of metastatic potential, initially and primarily lymphatic, but also visceral.

The spinous cell carcinoma is a malignant tumor of the keratinocytes. Just like the basal cell carcinoma, its onset is either de novo or by invasive evolution of a pre-existing actinic keratosis. Onset on a verruco-sebaceous hamartoma is much more exceptional than is the case with basal cell carcinomas.

The treatment of spinous cell carcinomas involves surgical excision, monitoring of satellite lymph glands and, in the event of palpable potentially metastatic adenopathy, associated lymph node dissection as well as radiotherapy and/or chemotherapy where appropriate. Very fortunately, in current practice, detection is usually achieved sufficiently early to allow evolution to be stifled by simple cutaneous excision in sano.

The appendage carcinomas, i.e., those reproducing skin appendage structures, to an extent in an imperfect manner, on the scalp, are essentially sited within the verruco-sebaceous hamartomas of which they represent a complication in the same way as the basal cell or, much more rarely, the spinous cell carcinomas.

Among the sudoriparous, sebaceous, or pilar appendage carcinomas occasionally encountered on the scalp, the microcystic appendage carcinoma, the tricholemmocarcinoma, and the sebaceous carcinoma can be cited.

The Merkel cell tumor, which has a predilection for the cephalic region, is sometimes found on the scalp in the form of a domed sooty-brown erythematous lesion. This is a

highly malignant lesion with a strong metastatic capacity, the treatment of which necessitates extensive surgery and radiotherapy.

The secondary carcinomas form a more fundamental chapter in the tumoral pathology of the scalp, this being an area of predilection for the emergence of cutaneous metastases of deep visceral cancers. Whether a single nodule, with a varying degree of inflammation, or the efflorescence of several lesions is concerned, biopsy or excision/biopsy is required on principle and as the first treatment of choice in the event of doubt in the clinical diagnosis. The diagnosis may be suggested straight-away where a visceral neoplasia is known to exist; these lesions can be, in particular, very revealing, hence their central diagnostic importance.

More deceptively, these metastases may be the cause of localized multifocal alopecia of the sclero-atrophic, pseudo-cicatricial type, suggesting, in a misleading way, a process of inflammatory origin or a storage disease (*alopecia neoplastica*). The seat of the skin metastases may depend upon the site of the primary tumor: thus, the scalp is more likely to be implicated in tumors of the oral cavity or larynx (35).

4.1.4. Conjunctival or Mesenchymatous Tumors

The scalp is not a preferred region of onset of tumors affecting the mesenchyma, whether a benign lesion or a malignant sarcoma. However, two types of tumor are exceptions to this rule: the subaponeurotic lipoma in the case of benign lesions, and the hemangioendothelioma in the case of malignancies.

The frontal subaponeurotic lipoma presents as a fatty tumor that does not develop at the expense of the subcutaneous fat, but beneath the epicranial aponeurosis, in the thickness of the frontal muscle, or beneath the muscle in the epicranial space separating the periosteum from the aponeurosis (36). The territory of its emergence is sited between orbital borders and the frontotemporal hairline. Clinically, it presents in the form of a dome-shaped cutaneous contour the lesion appearing poorly mobile between the finger and thumb given its deep-seated position in a narrow compartment. This tumor affects notably elderly males and treatment consists of surgical excision.

The malignant hemangio-endothelioma or *angiosarcoma* is a highly malignant tumor of endothelial origin, consisting of a lesion of the angiomatous type of which there exists three forms: the angiosarcoma without associated lymphedema of the scalp or face of the elderly male (37) that is of interest to us here; the iatrogenic lymphangiosarcoma or constitutional chronic lymphoedema (Stewart-Treves syndrome) and, finally, the post-radiotherapy angiosarcoma.

The angiosarcoma of the scalp presents as one or several "wine-colored" plaques, adorned with papules or nodules showing a special propensity for ulceration or bleeding. Its prognosis is dire given the likelihood that it will metastasize and only a particularly early diagnosis offers any hope of effectiveness of extensive surgery.

4.1.5. Lymphomas and Pseudolymphomas

The development of lymphomas on the scalp, whether or not they are epidermotropic, like the development of leukemic lesions on the skin, does not represent notable tropism

for this area, or any other particular characteristic, failing that of the capacity to induce alopecia.

Like Civatte (38), we will mention in this chapter the epitheloid hemangioma or angiolympoid hyperplasia with eosinophilia, or again the histiocytoid hemangioma or Kimura's disease.

This condition has its onset in the third or fourth decade, with a clear female bias, in the form of angiomatous nodules of the scalp and nape, sometimes associated with satellite adenopathy and blood eosinophilia (37). This nodule has a tendency for ulceration and may recur after surgical excision, although it does not metastasize.

Under the microscope, numerous vascular spaces can be seen in the two large hyperplastic endothelial cells, these structures being bordered by a large inflammatory infiltrate composed principally of lymphocytes, eosinophils and histiocytes, sometimes evolving into lymphoid follicles.

4.1.6. Histiocytosis X

Histiocytosis represent a malignant proliferation that cannot be classified among the leukemias or lymphomas. The existence of histiocytosis X in early childhood, Letterer-Siwe disease, justifies its inclusion within this chapter, given that it is frequently and typically detected on the scalp, not in the form of a tumorous proliferation but, deceptively, of an inflammatory pseudo-dermatosis, closely mimicking a seborrheic dermatitis, with papulo-squamous, crusty and purpuric lesions. The particular context of a child presenting with impairment of its general state of health and with hepatosplenomegaly, a concomitant eruption characterized by papulo-squamous, crusty and sooty-brown, micropurpuric erythema together with, if necessary, a biopsy, determines the diagnosis.

4.1.7. Tumors of the Pigmentary System

This section is obviously dominated by the melanoma. Like other malignant lesions, the problem here is again the impossibility of self-monitoring on the one hand and the fact that the hair masks the malignant pigmentary lesions whose prognosis depends to an extent on its depth (Breslow index) as well as the degree to which the dermis has been invaded, both elements directly dependent upon the timespan to diagnosis.

All types of nevus may be encountered on the scalp, a fairly classic form being the achromic and exophytic pure nevus, sometimes evoking a multidigitate papilloma without its hyperkeratotic component. Histological examination shows deep dermal nevus proliferation of neuroid differentiation. The classic pigmentary nevus is also encountered, as well as the atypical naevus, clinically characterized by its absence of relief, its blurred edges, its dimensions greater than 0.5 cm in diameter and its chamois or pink coloration. Cases of juvenile Spitz nevus are encountered more rarely and are sometimes difficult to differentiate under the microscope from melanoma.

A particular form is the cerebriform or cerebelliform nevus of the scalp (39), an amelanotic nevo-cellular nevus that gives rise to an alopecic plaque of encephaloid appearance consisting of whirling lobules, separated by deep furrows. While impressive, this lesion is nonetheless entirely benign. It must not be confused with the puckered

pachyderma with pachyperiostitis, identified by Touraine, Solente and Golé, which combines diffuse hypertrophy of the bony and soft tissue of the extremities, the formation of apparently greatly thickened skinfolds on the scalp and, here again, a cerebriform appearance. This condition in its primary dominant autosomal form of variable expression and the paraneoplastic secondary forms can only manifest itself on the scalp, then taking the name of *cutis verticis gyrata*, difficult to classify, exceptionally paraneoplastic but rather derived from predisposing genetic factors and local inflammatory factors.

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24

Hair Transplantation

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In recent years, considerable progress has been made in esthetic restoration of different types of permanent hair loss and particularly of male and female androgenetic alopecia (AGA). A review of the different operating techniques and a better evaluation of their reliability help to define precise surgical indications.

The terminology of the autograft technique has evolved in parallel to the new concepts. The term transplantation of "follicular units" or micrografts is actually preferred to that of hair transplantation.

Surgical indications depend upon numerous parameters such as size and location of the alopecia patch, sex, age, ethnic origin, scalp and hair characteristics, and progression of the disease. Choice of the most appropriate treatment for each individual case is facilitated by a dynamic, multifactorial classification taking into account all these criteria (1).

1. HISTORY OF SCALP AUTOGRRAFTS

- In 1930, Sasagawa first reported a genuine method of hair implantation (2).
- In 1939, Okuda described for the first time principles of autografts in the surgical correction of scalp, eyebrow, and moustache alopecia (3).
- In 1943, Tamura described female pubic hair reconstruction by single hair transplantation (4).
- Fujita, in 1953, reported an eyebrow reconstruction by punctiform hair grafts in lepers (5).
- In 1959, Orentreich (6) published an original method of harvesting and transplanting cylindrical autografts using a cylindrical instrument called a "punch" which takes out hair plugs in a plug measuring 3–4 mm in diameter. This punch was driven by an electrical motor. Removal of each hair plug from the occipital donor zone with a punch scrupulously parallel to the hair bulb, was followed either by closure with a suture or left as it is.

The insertion of each transplant was preceded by the removal of a plug at an appropriate oblique angle from the frontal or the occipital recipient area. This is done with an instrument 0.5 mm in diameter smaller than the one used on the donor site (7,8).

The key points of this procedure, which prevailed for 25 years in the treatment of male-pattern baldness, should be specified:

1. The positive aspects are:

- high hair-density is achieved if three or four successive sessions are performed on the same scalp area (9);
- it gives an appropriate oblique angle for the emerging hair, adapted to each head of hair.

2. The negative aspects are:

- a tufted appearance or a “doll’s head appearance,” that is unsightly and rather artificial-looking (10);
- an ugly “honeycomb appearance” of round scars in the occipital donor area.

These negative points have led most practitioners to turn to other procedures for the treatment of androgenetic alopecia.

- In 1980, Marritt proposed the use of micrografts to complete the visible frontal lines (11).
- In 1985, Bouhanna published the micrografting technique and recommended it in 90% of males and 100% of females with androgenetic alopecia who are good candidates for hair transplantation surgery (Fig. 1).

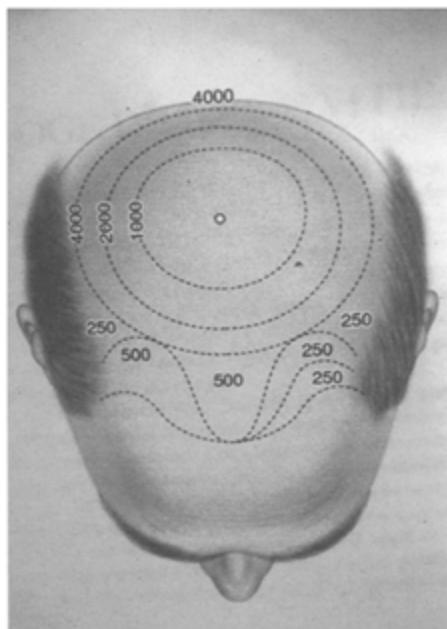


Figure 1 Average number of hairs and distribution with micrografts for a case of male baldness.

2. DESCRIPTION OF THE TECHNIQUE

2.1. Principle

Hairs from around the crown in males or from the median occipital region in females retain their capacity for normal growth once they have been harvested and implanted in the bald area (Figs. 2–4). The technique consists of dissecting tiny strips of scalp into small fragments. Each fragment bears either one to two hairs (micrografts) (12) or two to three hairs (minigrafts) (13).

2.1.1. *The Follicular Unit*

Defined by a natural entity, the follicular unit comprises one or more hair follicles with attached sebaceous gland and perifollicular adjacent structures. Hair follicles are naturally grouped together, sharing their appendicular structures and forming an anatomical unit (Fig. 5). This is easily identifiable on close examination of the scalp with a magnifying glass or in histological sections. The number of follicles in one unit varies from 1 to 5, combining terminal (1–3) and immature (1–2) hairs. As with hair density and follicular unit density, the number of hair shafts per follicular unit varies from one ethnic group to another (Table 1) (14,15).

2.2. Objectives

The process seeks to recreate the natural emergence of two to three hairs through each pilosebaceous orifice (Fig. 6) (*pili bigemini* or *trigemini*) which is known as the follicular unit (16):

- to cover bald scalp patches in order to obtain an attractive and natural hair look (17),
- to put the finishing touch to the frontal hairline adjacent to cylindrical grafts,
- to cover progressively balding areas (18),

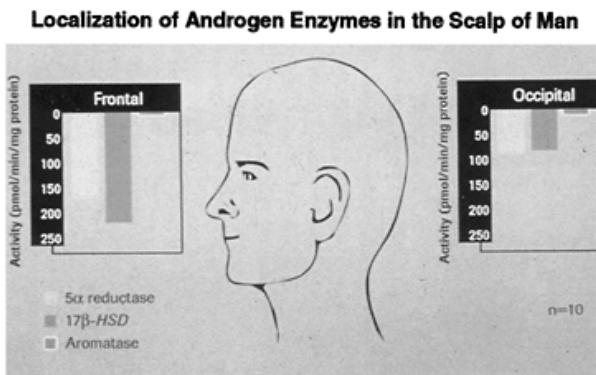


Figure 2 Site of androgenetic enzymes on male scalp. (Courtesy of Sawaya.)

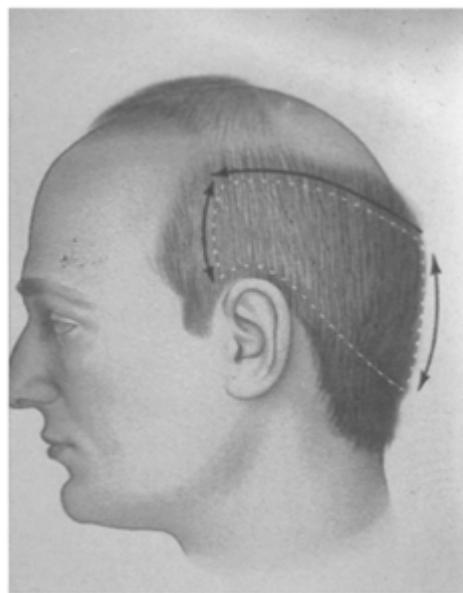


Figure 3 Diagram of the donor area in men.

- to correct the unnatural appearance of the anterior scarring line of certain skin flaps,
- to hide any visible fronto-temporal scars following a face-lift,
- to increase density in areas of sparse hair growth (eyebrows, beard, pubis).

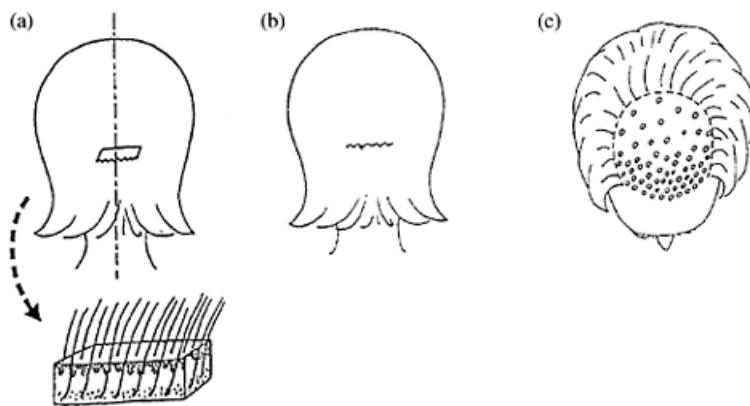


Figure 4 Diagram representing donor and recipient areas in women (a) median occipital donor area (b) linear scar after suturing do not area (c)

recipient area with the implanted micrografts.

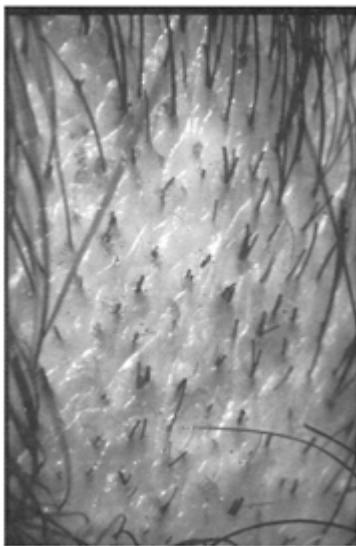


Figure 5 The natural emergence of two to three hairs through each pilosebaceous osticern (follicular unit).

2.3. Description of the Technique

Preparation, disinfection and anesthesia of the donor and recipient sites are similar to those usually undertaken in dermatological surgery. Disinfectants based on inflammable chemicals are to be avoided on the scalp. Hairs are either cut very short with scissors or electric razor, or they are left long.

2.3.1. Preparation of the Patient

The patient is advised to keep his hair fairly long (4–5 cm) to cover the donor area. In order to limit peri-operative bleeding, no acetylsalicylic acid or non-steroidal anti-inflammatory drugs should be taken during the week before surgery. Moreover, vitamin K antagonists should be avoided for 3 days prior to operation. Vitamin E and its derivatives should not be taken 3 weeks before operation. The prophylactic use of antibiotics (erythromycin or cephalosporin) remains controversial. They are sometimes administered 1 day before surgery and the course of treatment is continued for 4–7 days. Premedication with diazepam 10 mg, 30 min prior to surgery, produces patient's relaxation and also reduces peri- and post-operative bleeding (19).

The pre-operative work-up generally includes an electrocardiogram, a blood count, coagulation tests (prothrombin level, activated cephalin time), blood sugar, HIV, and hepatitis B and C tests.

Table 1 Ethnic Variations in Donor Area Density

	Caucasians	Asians	Africans
Follicular units/mm ²	1	1	0.6
Mean hair density/mm ²	2	1.7	1.6
Follicles/unit area	2	2	3

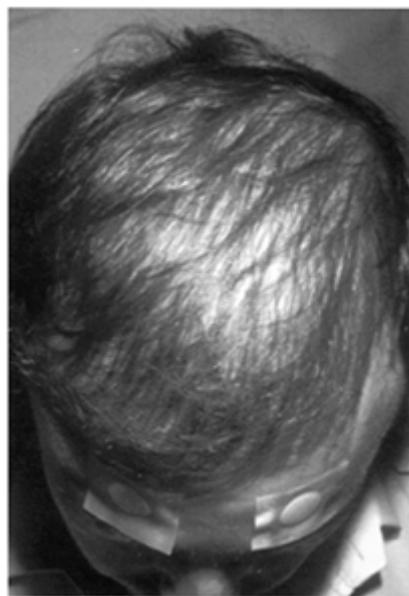


Figure 6 Prior application of EMLA anesthetizing cream allowing painless nerve block anesthesia for the whole frontal area.

2.3.2. Local Anesthesia

The scalp is anesthetized using:

- either a local anesthesia with a tumescent 1% lidocaine intradermal injection around the bald and hairy areas in a shape of frame,
- or through nerve block of the frontal and occipital nerves. A prior application of EMLA cream (20) has rendered these injections virtually painless (Fig. 6).

The percentage of damaged follicular unit grafts through dissection varies from 10% to 18%, thanks to the infiltration of the donor site, the use of a multiblade scalpel and graft dissection under a back-lit microscope (17,21). However, according to Arnold (22) the number of intact follicles that can be used may be much greater. Section of an implant above the point of insertion of the arrector muscle is said not to affect the success of a graft or hair growth. Therefore, the actual loss of follicular units during the dissection process may vary from 4% to 7%.

2.3.3. Donor Area: Preparation of Minigrafts and Micrografts

A number 11 blade is used to harvest a horizontal strip 10–20 cm long and 10–20 mm wide from the donor occipital area previously infiltrated with saline solution 12. The strip size depends upon the number of micrografts and minigrafts sought. The multi-blade instrument described by Vallis often facilitates harvesting of strips (Fig. 7). The number of blades and their spacing varies to yield two to five strips. The angle of penetration has to be constantly adapted and in particular in the lateral zones where the bulbs axes vary. Harvested strips must include the deep layers of hypodermis.

Closure of the donor site borders is achieved with a running suture of absorbable single thread 3/0 or with skin staples. The strip is placed on a compress in a Petri dish containing saline solution that lays on a deep-frozen container. Segmentation is carried out by cutting the fragments on a sterile tongue depressor with a no. 11 blade, or with a microtome (19).

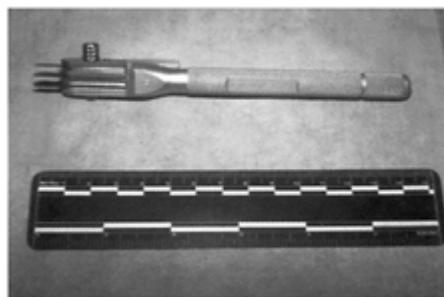


Figure 7 Multiblade scalpel for the harvesting of two to five strips, which will be cut into minigrafts and micrografts.

The strip is held by the epidermal edge with a fine pair of forceps and cut parallel to the bulb into minigrafts, micrografts, and follicular unit grafts (Fig. 8a–c). Follicular units of one to four hairs are clearly visible under frontal head magnifying glasses.

On average, 200–1000 grafts are obtained in this way. Every single graft is freed from any debris of hair shaft or of hypodermis before being placed in the recipient dish filled with refrigerated saline solution (Fig. 9).

Depending on the number of hairs in each graft, they will be placed in one of four compartments of a Petri dish. Prior removal of epidermis from micrografts seems to have been abandoned by most authors.

2.3.4. Preparation of the Recipient Area

The scalp area destined to receive the grafts must have been outlined with a skin pen. During the dissection by three assistants of scalp strips into follicular units or into minigrafts and micrografts, the surgeon performs holes and incisions on the anesthetized recipient site (Fig. 10).

Perforation. This stage varies from one author to another. Most authors perforate the scalp skin with an 18 or a 16 gauge Nokor needle (19,23–25) (Fig. 11).

Others prefer to perform the incision with a microsurgical blade mounted on a round handle (Fig. 11) while others make perforations using punches measuring 1, 1.25, 1.5, 1.75, or 2 mm in diameter, activated manually or with a motor (Fig. 12).

In fact, usually, perforation with a Nokor needle or a “spear point” blade is preferred for the implantation of micrografts of one or two hairs and a lancet incision (25), or a perforation by punch for minigrafts of three to four hairs.

Dilation. This stage is not wholly indispensable. It has more or less been abandoned. The dilation of orifices prior to implantation is achieved with dilators of various forms and materials.

Implantation. As soon as each minigraft or micrograft is cut, it is placed in a Petri dish containing normal saline solution. Each fragment is carefully selected and then inserted into the perforations or incisions after they are freed from hypodermal debris or keratin. Most authors hold the fragments with very fine jeweler’s forceps (Fig. 13).

Each minigraft or micrograft is delicately maintained throughout its length with the forceps and then inserted into the recipient holes.

A minigraft or a micrograft can sometimes have a tendency to pop out just after insertion. Several factors are behind this: cobblestoning, overstretched skin, very close perforations, too small or too large grafts relative to the orifice size, slight

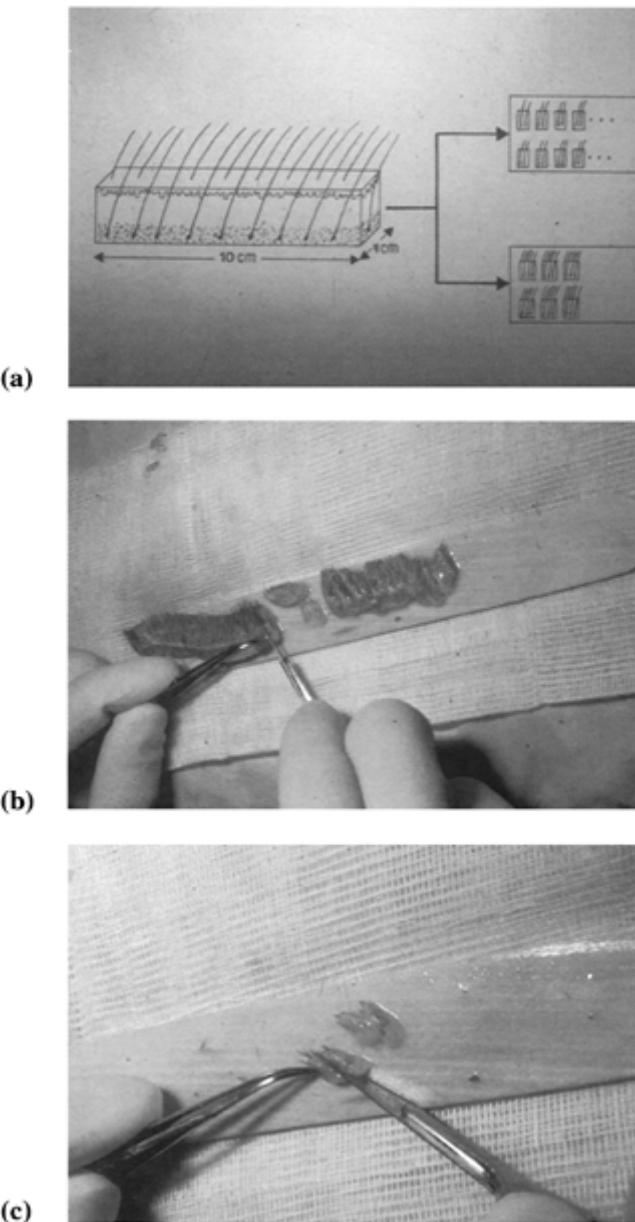


Figure 8 Precise dissection of each strip into (a) minigrafts, (b) micrografts, and (c) follicular unit grafts.

bleeding at the base of the incision. In order to avoid such inconvenience, the choice of micrografts and instruments and the preparation of the receptor site are extremely important (18,26).

Selection of Minigrafts and Micrografts. It is currently feasible to transplant in one session several hundred to one thousand minigrafts or micrografts. In the front, fragments used to form the hairline will have one hair (perforation with an 18 gauge Nokor needle), then two hairs for the second row and then three hairs (1–1.25 mm punch or no. 69 blade) for the remaining bald area.

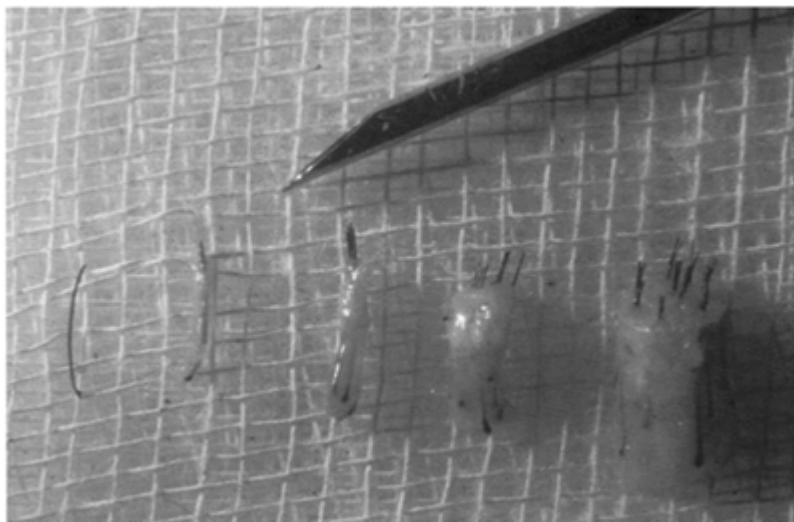


Figure 9 The appearance of each dissected and cleaned micrograft as compared with large cylindrical grafts on the right.

2.3.5. Post-operative Course

At the end of the session, a gentle compression is performed for 3 min using compresses soaked with normal saline solution. All the grafts are checked with a frontal head binocular glass to ensure that insertion has been satisfactory and that hair direction is appropriate.

The patient will remain in the reclined position for 1–2 hr, after which a further check of the implantation site must always be made. A dressing can be applied for

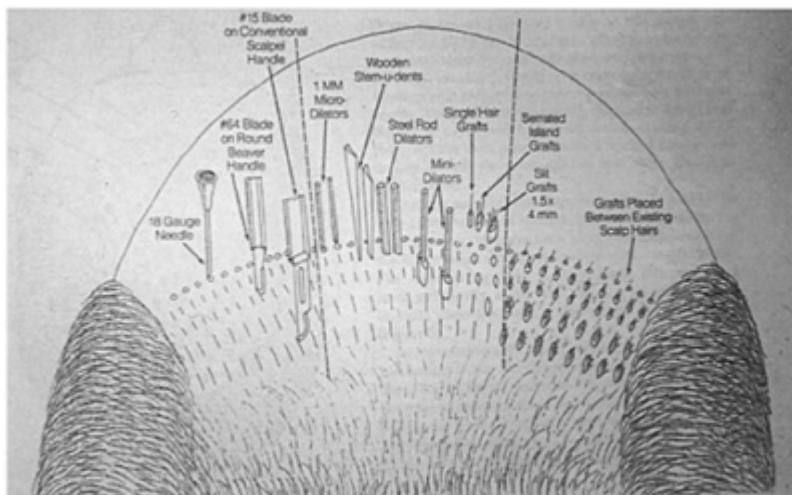


Figure 10 Diagram of the incisions or perforations on the outlined recipient zone (From Swinehart and Griffin.)

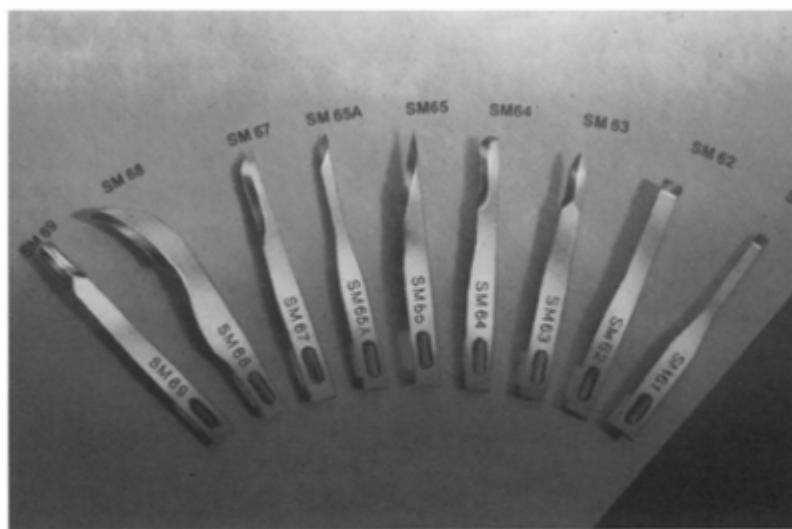


Figure 11 Microsurgical instruments and blades for perforation of the recipient zone.

24 hr. An antiseptic shampoo will be done gently 2 days later. Staples or sutures are removed 8–15 days after the operation.

Hair Graft Evolution. Each graft, whatever its shape or size, evolves in a stereotypical manner. Between day 8 and day 12, crusts that have formed will detach.

Minigrafts and micrografts transplant technique is extremely reliable. However, a foreign body reaction may occur due to the accidental insertion of skin debris

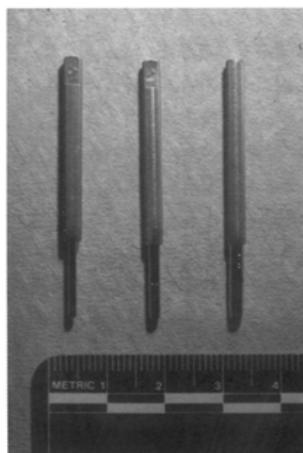


Figure 12 1, 1.25, and 1.5-mm punches.

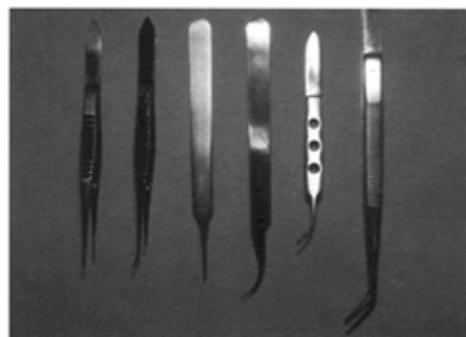


Figure 13 Various types of microsurgical and jeweler's forceps for manual insertion of micrografts.

or of a second micrograft in the same orifice. This small, exceptional complication should be treated in the same way as a flare-up of juvenile acne (27).

Post-operative edema in the frontal region and a transient hyper- or hypoesthesia in the donor and recipient areas have sometimes been reported.

Effects of Minoxidil on Hair Grafts. Usually, 2–4 weeks after hair transplantation, crusts on the grafts become detached, carrying with them the transplanted hair shaft. However, although this phase is transient with regrowth occurring some 3 months later on average, it is esthetically unacceptable.

A personal study has shown that local application of minoxidil 2% solution before and after transplantation, appears to maintain the growth of part or of all hairs present in more than two-thirds of the grafts. Moreover, the loss of less than half of the transplanted hairs in less than one-third of grafts has been noted (15,28).

Therefore, the pre- and post-operative applications of minoxidil 2% or 5% solution seem to prolong the anagen stage of a large number of transplanted hairs.

Long-hair Graft Transplantation. This technique, that was published in 1989, permits graft transplantation of varying size and shape, where hairs are left unshaved (21).

With this long-hair grafting process, patient's hair can have an almost natural appearance immediately after surgery. The patient can resume his work 24–48 hr later.

In this way, the esthetic problem of crusts and hair tufts following the transplantation of minigrafts may be avoided.

Indications for this technique are the same as for conventional short hair grafts. For the densification of sparse growth areas, a shaft length of 2–3 mm is normally sufficient. In order to obtain an optimal growth of the transplanted hairs, the patient should apply a minoxidil solution (5% in males and 2% in females) to the recipient zone, for 6 weeks prior to transplantation. This local treatment is continued for 3 months following surgery.

Progress made with this long hair technique, can take one of two forms: (1) hair loss at week 2 and hair regrowth occurring 3 months after surgery, (2) preservation and growth of some or all transplanted hairs.

New Micrograft Instrumentation. Various instruments or devices have been tested over recent years:

- The multiblade scalpel can be used for the simultaneous harvesting of three to five strips. The gain in speed does not seem to have an adverse effect on the strip quality except in the presence of a stiffened scalp or the pronounced curvature of curly hair roots (12–19,23,24) (Fig. 7).

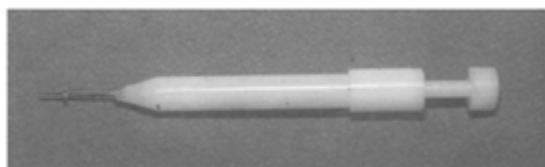


Figure 14 Disposable Choi's inserter of micrografts.

- The Choi inserter helps inserting micrografts one by one. This instrument is of little use in scalp hair transplantation (29). However, it may be useful for the eyebrows reconstruction (Fig. 14).

- The multiblade instrument for harvesting minigrafts is useful in the absence of assistants trained in manual dissection.
- An automated apparatus or Calvitron has not, so far, demonstrated a better advantage over the conventional manual technique in terms of rapidity, reliability, asepsis or esthetic quality.
- The single use Markman's device, for the simultaneous perforation and insertion of micrografts, appears to be of some value (Fig. 15).
- The new CO₂ or Erbium lasers have been tested for the preparation of recipient sites. The aim of the process was to vaporize the skin over a 1–2 mm predetermined area. Results obtained were, however, uncertain and contradictory. According to most authors, burning of neighboring zones weakens the chances for successful grafting and is likely to damage the roots of earlier grafted hairs (Fig. 16).

Megasessions with Micrografts (Fig. 17a,b). It is currently possible to harvest from the occipital donor area, under local anesthesia, up to 3000 hairs in a single session, generally leaving a discrete horizontal scar (1,30,31). However, this type of session requires a big team of assistants.

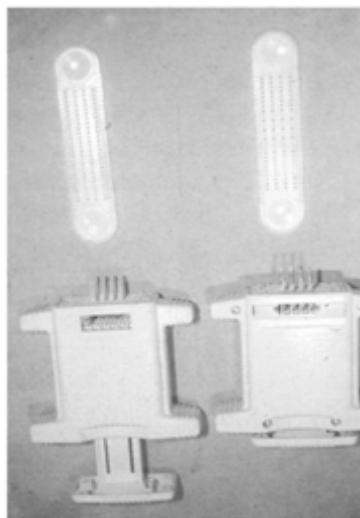


Figure 15 A disposable Markman's micrografts implanter.

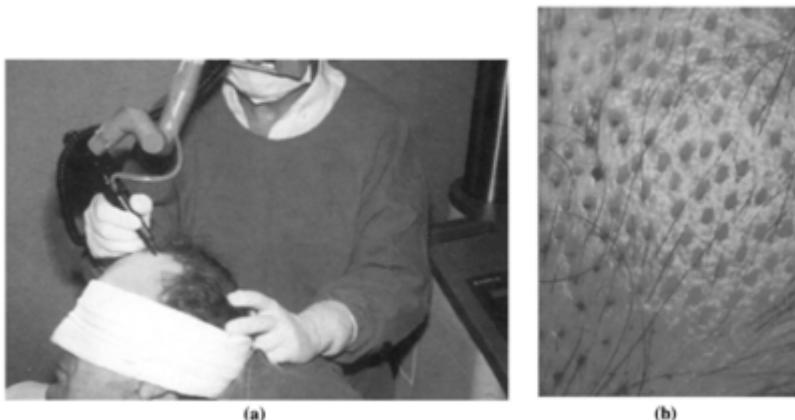


Figure 16 (a) Preparation of the recipient area using CO₂ laser. (b) Recipient area with implanted micrografts after laser perforations.

Some Figures on Minigrafts and Micrografts. The donor area: After the age of 60, 50% of males have an androgenetic alopecia (AGA) stage III–VII according to the Hamilton-Norwood classification and after the age of 80, 20% of them present a AGA stage VII (32). Eighty percent of males have an adequate donor area and the number of hairs left in the donor site is at least 12,500 (28).

Evaluation of the total number of implants. Evaluation of the number of sessions needed for the full restoration of a head of hair depends partly on the number of grafts implanted in one session and more specifically on the total number of implanted hairs. Some authors consider it to be the number of transplanted hair shafts while others consider it as the total number of follicular units containing one or more hair follicles. Measurement of the various hair and scalp parameters, according to the multifactorial classification of AGA (1), is a valuable tool for this evaluation.

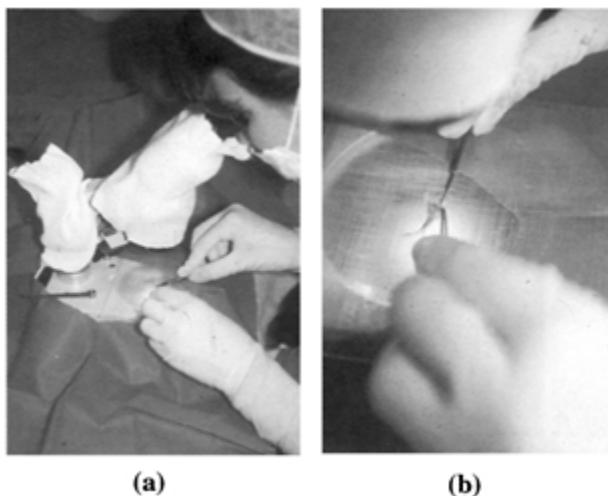


Figure 17 Use of the stereomicroscope for precise micrografts dissection.

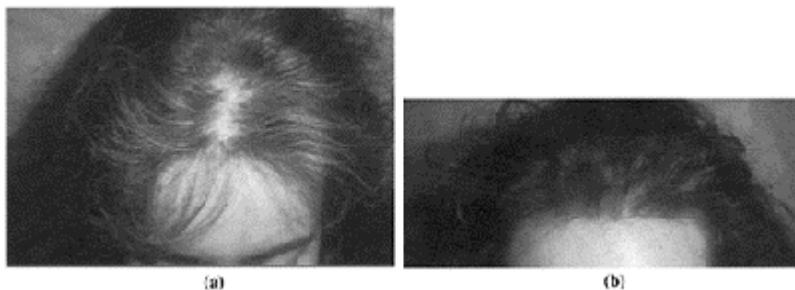


Figure 18 Ludwig type 1 alopecia in a 22-year-old female: (a) Before and (b) after densification with one session of minigrafts and micrografts.

3. SURGICAL THERAPEUTIC INDICATIONS

3.1. Indications in Women

Female androgenetic alopecia is characterized, in the majority of cases, by a diffuse hair loss from the forehead to the vertex, with a preservation of the frontal hairline (10). Sometimes it is characterized by a diffuse temporo-occipital thinning (Ludwig's

classification, 1977) (Fig. 27a). Here, only the median occipital region is maintained (Fig. 18a,b).

The use of minigrafts is recommended. Depending upon the Ludwig pattern alopecia one to three sessions of 200–300 minigrafts (two to four hairs) each, are performed on the front and crown regions, or on the thinning hair parting (Figs. 19–22).

3.1.1. Post-Cervico-Facial Facelift Alopecia and Scars

Hair loss and scars after a face-lift can sometimes be unattractive and justify the reconstruction of the pre-temporal region and the anterior frontal hairline. To match existing hairs, the transplantation must be particularly fine and should follow the natural direction and obliqueness of the hair growth pattern (Fig. 23a–c).

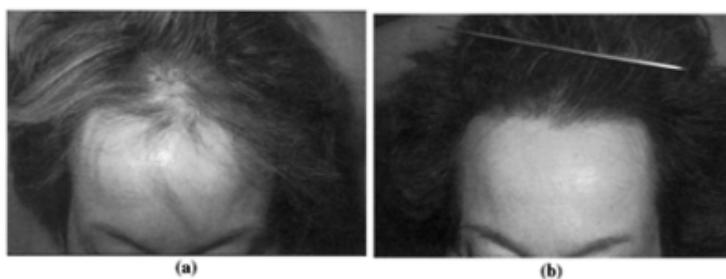


Figure 19 Ludwig type I alopecia (female, 45 years): (a) Before and (b) after densification with one session of minigrafts.

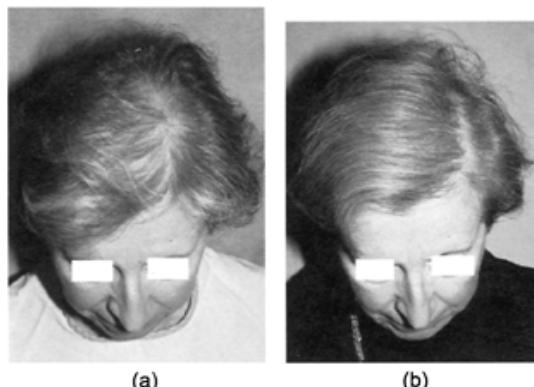


Figure 20 Ludwig type II alopecia (female, 67 years): (a) Before and (b)

after one session of minigrafts for densification

3.1.2. *Traction Alopecia*

Repeated traction on hairs may be of psychotic origin (such as trichotillomania), but it is more often of cosmetic origin (brushing, rollers, plaits, attachment of prosthetic hair pieces). This can lead to alopecia. Balding areas are essentially located on the vertex and the fronto-temporal regions. Hair transplantation can only be recommended after the complete and definitive cessation of any traction and in the absence

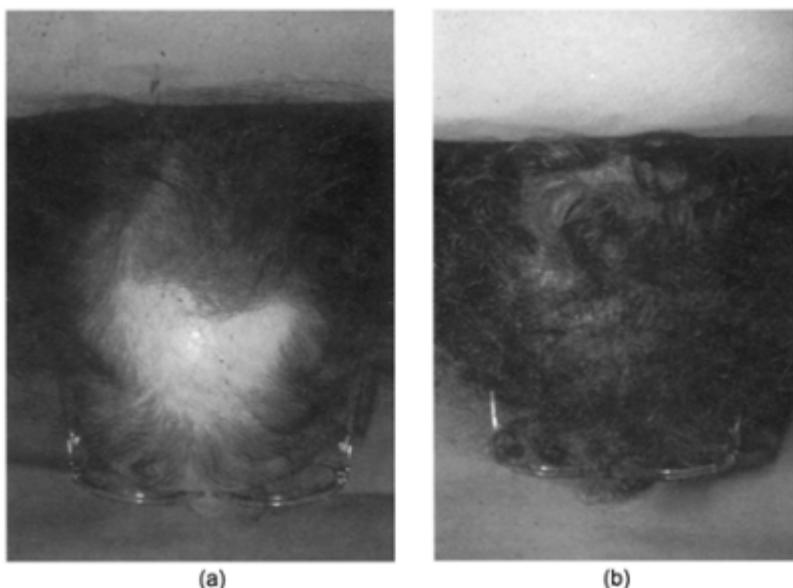


Figure 21 Ludwig type II alopecia (female, 55 years): (a) Before and (b) after two sessions of minigrafts and micrografts.



Figure 22 Ludwig type III alopecia (female, 55 years old): (a) Before and (b) after three sessions of minigrafts for densification.

of regrowth despite the stimulating effect of minoxidil solution (2% in the female and 5% in the male) (Fig. 24).

3.1.3. Eyebrow Alopecia

Causes of eyebrow alopecia are essentially post-traumatic or due to repeated plucking. The reimplantation will again be very fine, either hair by hair or by harvesting hairs from the unaffected eyebrow. The direction these hairs will take is obliquely upwards from the median part toward the apex of the eyebrow and obliquely downwards from the apex to the tail of the eyebrow. Design, form, and thickness of the eyebrow must be carefully considered and adapted to the needs of each individual (Fig. 25).

3.1.4. Pubic Alopecia

It is interesting to note that the development of the hair micrografts technique was the work of a Japanese physician, Tamura (4), on the replacement of pubic hair. As for scalp hair, esthetic reconstruction of pubic hair area must take into account

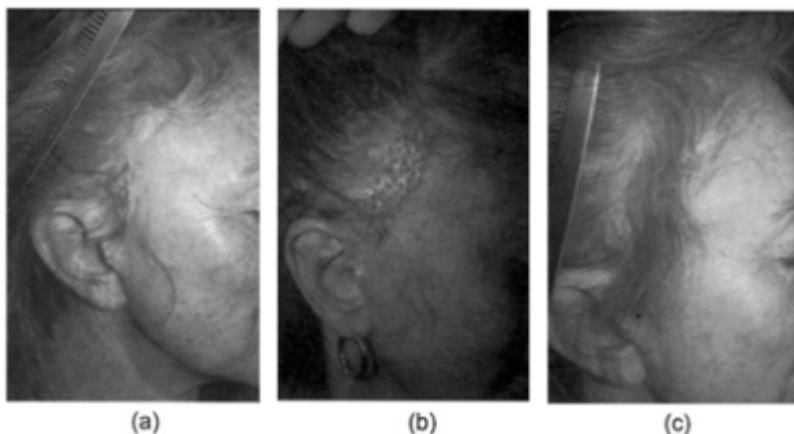


Figure 23 Micrografts correction of post-facelift scars: (a) Before correction; (b) crusts formed at the base of each micrograft just after insertion; (c) final appearance 9 months later.

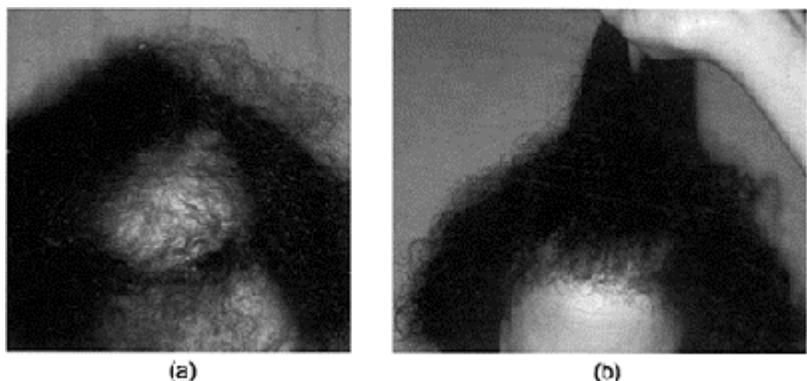


Figure 24 Traction alopecia in an African woman: (a) Before and (b) after implantation of minigrafts.

various parameters including: age (menopause), ethnic origin, hair color and hair shape (Fig. 26).

3.2. Indications in Men

The indications in men are more complex and depend upon various hair and scalp parameters. These were integrated into a multifactorial classification that permits the surgeon to draw the surgical indications of every patient.

1. The various parameters of Bouhanna's multifactorial classification of androgenetic alopecia (Fig. 27d) include (32):

- Stage of hair loss according to Bouhanna's simplified classification (14) (Fig. 27b) or to the seven types of the Hamilton-Norwood classification (Fig. 27c).
- Caliber, shape, and color of hair.
- Scalp mobility and thickness.
- A harmonious balance between the final result desired and the age and mental attitude of the patient on the one hand and the surgical techniques currently feasible on the other hand.



(a)

(b)

Figure 25 Eyebrow alopecia in a woman aged 45 years: (a) Before and (b) after implantation of micrografts.

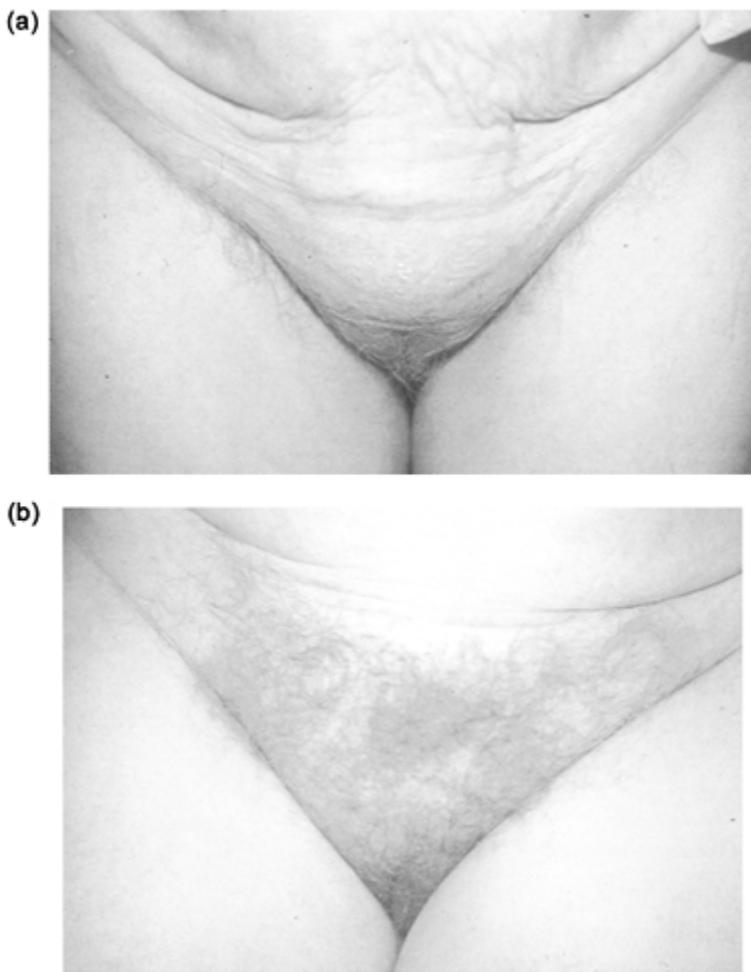


Figure 26 Pubic alopecia: (a) Before and (b) after implantation of micrografts in one session.

2. Indications depending upon the various stages of evolution (14) (Fig. 28):

a. Stage I affects the frontal recession:

- Can be corrected by transplanting 500–1000 hairs in one or two sessions.

b. Stage II implies complete frontal hair loss:

- Can be corrected by transplanting 1000–2000 hairs in two sessions, finishing the anterior frontal line with micrografts (Fig. 29a,b),

c. Crown alopecia:

- Can be corrected by the transplantation of 1500–2000 hairs in 2 sessions.

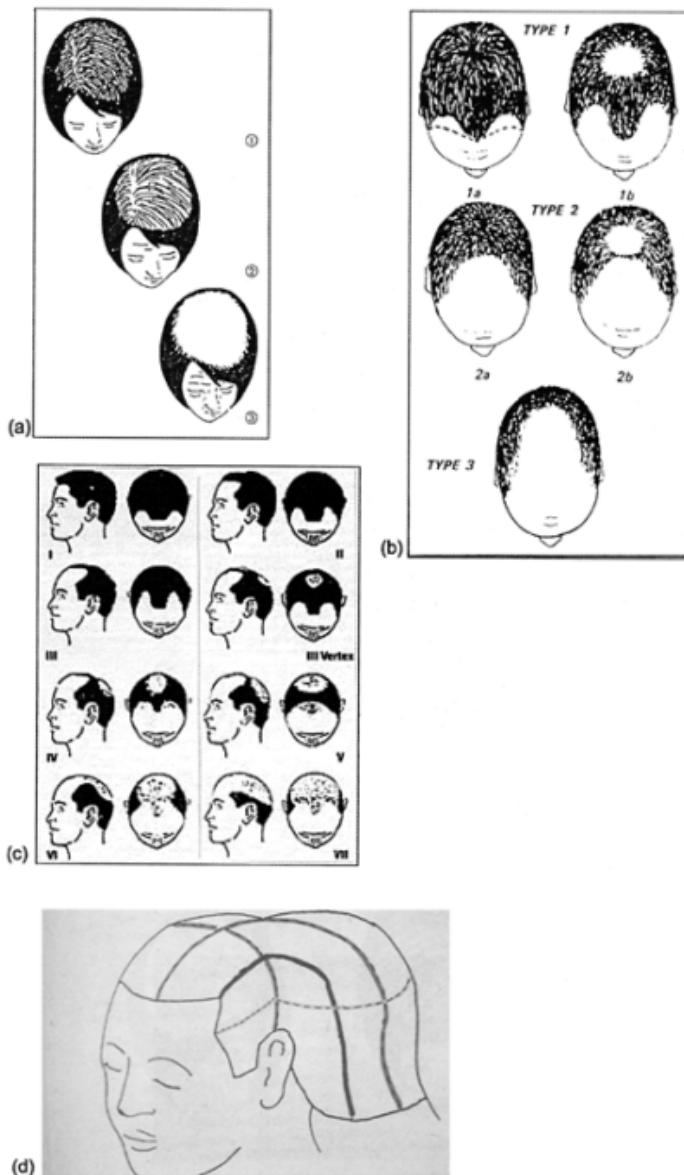


Figure 27 Classifications of androgenetic alopecia: (a) Simplified classification of female AGA (Ludwig,

1977); (b) Simplified classification of male AGA (14); (c) Classification of male AGA (Hamilton, 1941); (d) Multifactorial classification of male and female AGA. (From Ref. 24.).

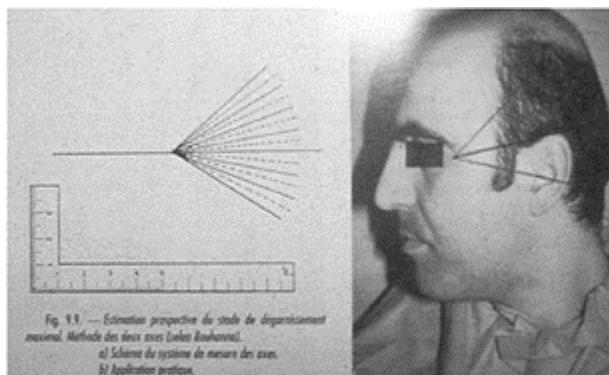


Figure 28 Diagram enabling estimation of the number of hairs required to yield an adequate density, depending on the affected site.

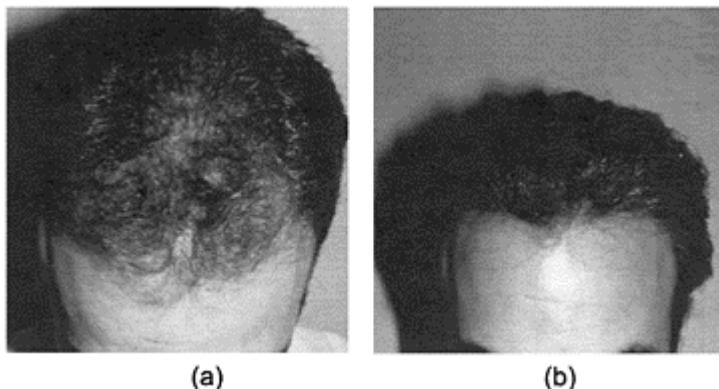


Figure 29 Male alopecia of the front and vertex: (a) Before and (b) after being treated by two sessions of minigrafts and micrografts (patient aged 28 years).

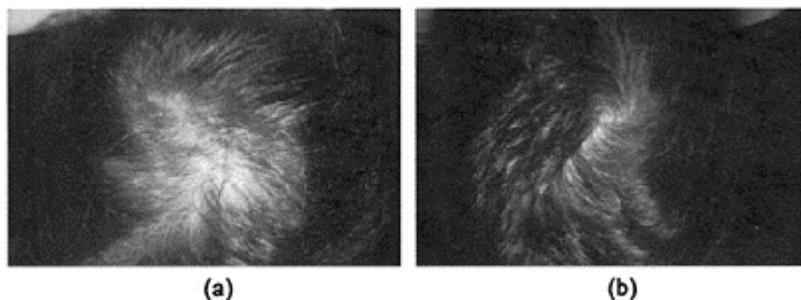


Figure 30 Crown alopecia in a 50-year-old male: (a) Before and (b) after two sessions of implantation with minigrafts and micrografts.

- or, more rarely, by repeated crown reductions complemented by hair transplantation (Fig. 30a,b).

d. Stage III

- Normally it should be simpler to describe the surgical indications in cases of baldness at this stage when only a circle of hair persists, rather than in earlier stages at which it is impossible to predict baldness evolution. Moreover, patient expectations and psychological profile must be carefully analyzed for the success of hair restoration surgery.
- Progressive transplantation of 4000–6000 hairs in two or three sessions should yield an adequate head of hair (Fig. 31a,b).

3.3. Ethnic Indications

These are difficult to define as numerous subjective factors may interfere, such as rites and religious customs as well as fashion, ranging from long hair to shaven heads.

For practical reasons, we shall only take into account the objective factors of hair and scalp as described in multifactorial classification of AGA (32) (Fig. 32a,b).

3.3.1. Asian Hair Grafts (18)

Dissection of strips into very fine micrografts under the microscope is essential for the success of hair grafting (27,29). Hair shafts of these thick and dark hairs are much more likely to give rise to a “doll’s hair” appearance. However, preparation of micrograft segmentation is facilitated by straightness of the hair roots. The recipient site preparation is usually done using a Nokor needle (Fig. 33a,b).

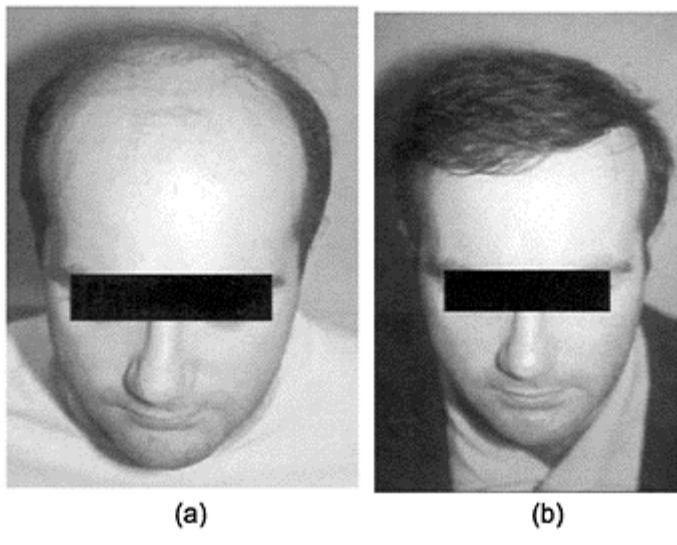


Figure 31 Male, 50 years, Hamilton stage III AGA: (a) Before and (b) after two sessions of minigrafts and micrografts.

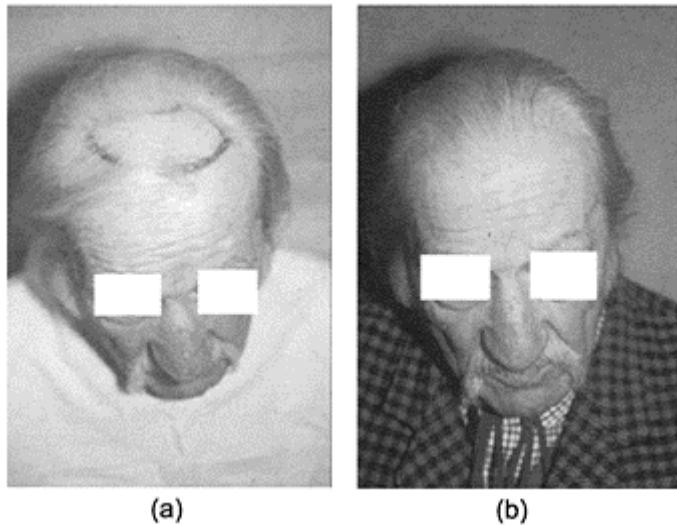


Figure 32 Male, 85 years, Hamilton stage IV: (a) Before and (b) after one

session of implantation with minigrafts and micrografts.

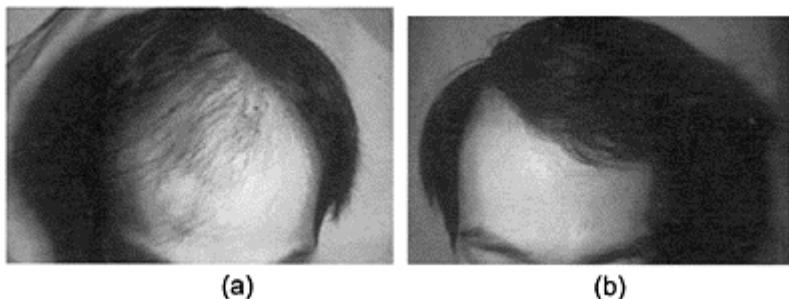


Figure 33 Correction of frontal alopecia in an Asian patient: (a) Before and (b) after one session of minigrafts and micrografts transplants.

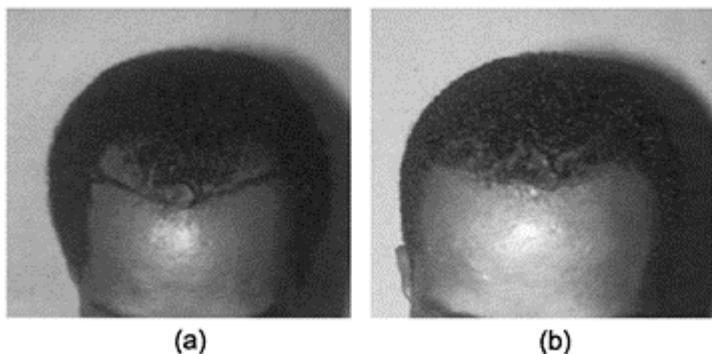


Figure 34 Correction of frontal alopecia in a patient of African American origin (21 years old): (a) Before and (b) after one session of minigrafts and micrografts transplantation.

3.3.2. Curly Hair Grafts, Particularly in Africans, African Americans, and West Indian Patients (19)

To achieve an esthetic result, it is not necessary to seek perfect graft miniaturization (20,27). Segmentation into minigrafts is difficult to achieve and one should avoid damage to the hair bulb of these incurving hairs (Fig. 34a,b).

The bed of minigrafts is mainly formed with small punches measuring 1.5 cm in diameter and with microsurgical blades (33).

4. CONCLUSION

Surgical correction of male and female baldness has been considerably improved by the precision and sharpness of new techniques and the capability of implanting large numbers of hairs during one session.

Seven key points are very important for the success of hair restoration surgery:

- The evaluation of the patient's psychological profile.
- Clarification of indications and an explanation of the pros and cons to the patient.
- Determination of the esthetic results, taking into account the probability of the continuation of the balding process.
- The rejection of unreliable or insufficiently monitored techniques.
- The choice of the procedure requiring the simplest post-operative care.
- Thorough awareness of complications to be readily avoided or rectified.
- Examination of the patient on an annual basis to check the extent to which the balding process may have progressed and to determine whether or not a complementary session may be necessary.

In scalp surgery, learning the technique is merely based on watching it followed by repeated practice. Determination of the precise indications is more difficult. Mastering all the techniques should allow each individual case to be appropriately treated.

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25

Hair: A Psychoanalytical Perspective

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1. INTRODUCTION

La chevelure vol d'une flamme... Occident de désirs.
(Mallarmé)

(Hair, like a soaring flame...an object of desire.)

Humanity remained close to nature during the first millennia of history, commonly known as the Stone Age, and nature blessed humans with abundant hair. Hair was left unmolested by comb or scissors, for these tools had yet to be discovered! Hair flowed to the shoulders, and a bushy beard lengthened the faces of men (1).

Centuries later, as early as can be traced, written documents bore witness to the desire shown by men and women alike to please by means of their natural ornament—hair. As evidence, there is the “Harris” papyrus and the d’Orbigny” papyrus (2), dating back approximately 20 centuries B.C. What do they, speaking from across the ages, reveal about our distant forebears?

The Harris papyrus is a brief poem:

My heart is filled anew with your love although (only) half of my temple
is braided. I run to seek you,...(Alas) it has come undone. Bah! I will don
a wig and will always be ready.

The “d’Orbigny” papyrus, written slightly later, contains what must be the oldest and one of the most celebrated seduction scenes in literature in which hair plays a major part. It is extremely relevant, and the translation goes as follows:

Now, several days later, when they were in the fields, he sent his younger brother, saying to him: “Go quickly and bring us seed from the farm.” He found the wife of his eldest brother, Anubias, arranging her hair and said to her: “Rise, and get me some seed. I must return quickly to the field because my brother is waiting from me. Be quick.” She replied to him: “Go yourself, open the storehouse and take what you want. Do not make me leave my hair unfinished.” So the young man went into the stable to get a large jar, because he wanted to take back much seed with him. He

filled it with barley and wheat and went out with his load. She asked him: "What does your burden weigh?" He answered: "Three sacks of wheat, two of barley, five in all: this is what I have on my shoulders." Then the woman said: "How strong you are! I admire your strength every day." She wanted to know him in the way that women know men, rose and held him, saying: "Come, let us spend an hour in bed. It will be to your profit, for I will make you beautiful clothes."

When Anubias came back home that evening, she gave him another version of what happened:

When your brother came to get seed, he found me alone and said to me: "Let us go spend an hour in bed, put on your wig."

(No doubt the large, ornamental wig worn by Egyptian women over their natural hair on great occasions.)

Both texts illustrate the fact that elaborately arranged hair or an ornamental wig were part of the preparations of a woman expecting to engage in lovemaking. Hair, whether natural or artificial, was intended to elicit male recognition of feminine charm and desirability.

It was far from coincidental that the goddess Hathor, so completely erotic in nature, was called "the lovely haired one," or that the goddess Rmpt was described as "the lovely haired one who dispenses love and brings rejoicing to her brother." In Chapter 115 of the version of the "Book of the Dead" as it existed from the Middle Empire, the idea of seduction was represented by a bird-trap set by a woman with beautiful hair!

If a perfect hairstyle constitutes a sure means to make oneself seductive, then the ever-ready wig can easily be seen as the sign of increased desire and, to an even greater extent, of immediate sexual availability. And the idea of sexuality is only a step away from that of life itself.

The Greeks and Romans thought that life resided in the hair, probably because it does not decay after death (a tuft of hair belonging to Queen Tyi, thirteenth century B.C. can be seen in the Cairo Museum).

The amazing passage from the Aeneid (Book IV, toward 700) is as follows:

Persephone—the goddess of Death—had not yet taken the blond hair from his head: she had not yet decided the hour of the hero's death.

And further on:

In the same way that the flamines (priests) remove a tuft of hair from between the horns of the sacrifice and threw it on a pinewood fire lit on the altar, Persephone cut a hair from the dying sacrifice consecrated to the powers of the underworld.

1.1. It Is impossible to Be More Explicit!

The tangible evidence of the death warrant of the hero or victim to be immolated in appeasement of the gods was the tearing out or cutting of the hair. Symbolically, too, hair

represented power, divine, and human alike. Thus, the heads of dethroned Merovingian kings (long-haired kings) were shaved.

In the Bible, God promises the mother of Samson (whose name means “fighter”) that she will bear a mighty son. He tells her that “the razor will never pass over his head because he will be consecrated to God.”

Traditionally, divine beings were thought to have superb heads of hair, for gods were thought to be eternally young, exempt from the ravages of old age. Those in power—kings, generals, emperors—also dreamed of possessing eternal youth. Their hair proclaimed their dignity: a wealth of luxuriant curls, harmoniously framing the face, arranged in a style worthy of a divinity. Caracalla, a Roman emperor, had his hair arranged like that of Alexander the Great, thus affirming his ambition to conquer the world. Conimodus, another Roman emperor, wore his bright hair as a divine aureole, and Gallienus in imitation, sprinkled his hair with gold powder (3)!

Hair symbolized supremacy, distinction, freedom, immortality, its loss created a feeling of draining strength, weakness, degradation, and disgrace. The satirists of antiquity exercised their sharp wits mercilessly at the expense of the bald. Ovid's celebrated distich is often quoted:

*Turpe pecus mutilum, turpis sine gramine campus
et sine fronde futex et sine crine caput*

(Shameful the mutilated herd, shameful the field without grain, and the forest
without the foliage and the head without mane.)

In wars of centuries past, the tribute that the conquerors exacted of defeated warriors was that their heads be shorn. This was also the sign of infamy used by justice to brand criminals or, in the custom of Germanic peoples, an adulterous wife.

Throughout the history of the human race, hair has always been viewed as something precious and irreplaceable; it deserved lavish care and attention, like any other fine adornment. Leaving all notions of disorder aside, the influence exerted by hair on the psyche is visible quite simply in the fluctuating moods of a woman in her everyday life and is reflected in the writings of the modern philosopher Karl Jaspers (4) where he reviews the significance of a mental pathology not only by its cause, but also of crucial significance to patients, by its emotional significance—what the pathology represents at a deeper, personal level. For example, a woman who has just been to her hairdresser generally exhibits a certain tendency to be euphoric. She is clearly less vulnerable to the depressing effects of daily problems. She may be totally free of vanity and coquetry but perfect hair makes her feel more attractive and thus increases her self-confidence; she can have a rosier outlook on life.

Independent of hair care and embellishment, there are some natural characteristics of hair that can affect the psyche, sometimes negatively. Many women's constitutional tendency to experience depression or anxiety is focused on their feelings about their hair. They channel their feelings into worry about hair color, shape, or length. Some are wild

with despair over being redheads, others find their hair too frizzy. Others claim that their hair is too straight, or too curly. They see in these perfectly normal characteristics a morbid significance and develop inferiority complexes, or they feel themselves to be ridiculous or even disgusting. This comes close to being a psychopathologic problem.

With the case of a pretty young woman in psychotherapy in a psychiatric environment, we enter the clinical realm. This young woman became schizophrenic after her mother had her long, magnificent, curly locks cut off against her will when she was 17 years of age.

Hair is midway between nature and culture, between skin and clothing. It is an adornment that is at the limit of something visible and something hidden, a fundamental dichotomy in the functioning of the Eros.

There is a relationship between the psyche and hair in all areas of hair loss: diffuse alopecia, alopecia areata (AA), androgenic alopecia (AAG), and age-dependent or occasionally imaginary hair loss. If hair falls out for no known reason, sometimes quite early in men, the effect of the narcissistic self-concealment behind the patient's complaints must be recognized (Fig. 1). This will vary in extent, according to the emotional and social experience of each individual. One patient, herself a doctor,

19 oct 1963 23 rd fr. night	(166) 98+128	15 oct. 1963 pm	(14.14) 1964 95
ma 2 oct. 24 th 2m. 34 th	100 30+71 63+114 100	16 oct. 1963 pm	94
f. 3 oct. 25 th 1m. 35 th	1063 50+121 100	17 oct. 1963 pm	76 = 13+30
f. 4 oct. 26 th 2m. 36 th	(167) 100+111	18 oct. 1963 pm	71+16=87
f. 5 oct. 27 th 3m. 37 th	118	19 oct. 1963 pm	60+24=84
f. 6 oct. 28 th 4m. 38 th	66	20 oct. 1963 pm	58+35=93
m 7 oct. 29 th 5m. 39 th	100	21 oct. 1963 pm	60+24=84
ma 8 oct. 30 th 6m. 40 th	100	22 oct. 1963 pm	62+24=86
ma 9 oct. 31 st 7m. 41 st	100	23 oct. 1963 pm	60+24=84
ja 10 oct. 1 st 8m. 42 nd	100	24 oct. 1963 pm	60+24=84
ja 11 oct. 2 nd 9m. 43 rd	100	25 oct. 1963 pm	60+24=84
ja 12 oct. 3 rd 10m. 44 th	100	26 oct. 1963 pm	60+24=84
ja 13 oct. 4 th 11m. 45 th	100	27 oct. 1963 pm	60+24=84
ja 14 oct. 5 th 12m. 46 th	100	28 oct. 1963 pm	60+24=84
ja 15 oct. 6 th 13m. 47 th	100	29 oct. 1963 pm	60+24=84
ja 16 oct. 7 th 14m. 48 th	100	30 oct. 1963 pm	60+24=84
ja 17 oct. 8 th 15m. 49 th	100	31 oct. 1963 pm	60+24=84
ja 18 oct. 9 th 16m. 50 th	100	32 oct. 1963 pm	60+24=84
ja 19 oct. 10 th 17m. 51 st	100	33 oct. 1963 pm	60+24=84
ja 20 oct. 11 th 18m. 52 nd	100	34 oct. 1963 pm	60+24=84
ja 21 oct. 12 th 19m. 53 rd	100	35 oct. 1963 pm	60+24=84
ja 22 oct. 13 th 20m. 54 th	100	36 oct. 1963 pm	60+24=84
ja 23 oct. 14 th 21m. 55 th	100	37 oct. 1963 pm	60+24=84
ja 24 oct. 15 th 22m. 56 th	100	38 oct. 1963 pm	60+24=84
ja 25 oct. 16 th 23m. 57 th	100	39 oct. 1963 pm	60+24=84
	551103	498	69+56=125
			55+57=112

Figure 1 Facsimile of an envelope submitted by a female patient giving a detailed countdown of hairs collected each day on her pillow.

and having terminal intestinal cancer, said to her dermatologist: "Doctor, do something; I don't want to die bald."

In cases of diffuse hair loss or of alopecia areata, the dermatologist is often the first to be consulted, because the alopecia dominates clinically and the psychic origins are as yet unsuspected (latent depression, sexual dissatisfaction). If the organic checkup turns out to be normal—no deficit, no infection, no mycosis, and no known heredity (the trichogram is normal)—then the dermatologist must look for a depressive state with its trio of characteristics: troubled sleep (difficulty in getting to sleep, waking up at 2 a.m., difficulty in falling asleep again), a slowdown in activity and memory, with anxiety and abnormal emotional responses. This second type of check-up makes it easier to ask additional questions about the patient's occupational and emotional life, and the subject of sexuality, in an effort to make a more accurate diagnosis.

If failure prevails in these three areas—occupational, emotional, and sexual—then the help of a psychiatrist is necessary. The doctor's role will here again be to listen and prepare the patient for a consultation with a specialist. It is up to him to present its usefulness to the patient; if this is done correctly, then, in the vast majority of cases, the patient will agree.

The causes of alopecia areata totalis and universalis are still poorly understood (5). If time is taken to search for them, a stressful event is sometimes uncovered, occurring 1–6 months prior to the onset of the first patch of alopecia areata (6–10). And not just any old stress (short for distress)—it may consist of:

- bereavement: the loss of a loved one, even a pet;
- separation: joining a new school, moving house, hospitalization of the mother, the arrival of a sibling for one who has been the center of attention, the break-up of a relationship; being uprooted (roots!) followed by becoming withdrawn. This was the case with a young woman, a teacher from Ireland, happy with her French husband, also a teacher, and their son, also happy with being a mother, but nostalgic for her country and her warm and united family, and above all for her mother “whom she misses” (separation).
- a death-related fear, as in the case of the woman who caught her 2-year-old son on the sill of an eighth-floor window. Two months later, all her hair fell out. Or that young Algerian whose father had forbidden her to attend university, which she entered despite him: her hair fell out in the shower 3 months later...to the extent that she had to wear a wig when she consulted the therapist. Or that other Algerian, arriving to spend her holidays in Algeria, on learning that a crime had been committed next door.... Two months later, she suffered from alopecia. The same thing with a soldier buried for 3 days under ruins during the Algerian war: he had been the only one to escape alive, miraculously, but with no hair! And then that 45-year-old woman returning from a trip to Egypt and losing all her hair 2 months later—the trip had only reminded her of death and her depressive condition was exacerbated on her return.

Contemporary research using psychological and sociological enquiry (11) has demonstrated the importance of understanding stresses (life events) in a personal context: what is stressful for one person may be little problem for another—this context must be taken into account. However social deprivation—living conditions, a confidante, support with a young family, seem important to all no matter where they live. Childhood experiences also bear upon the expression of later psychopathology.

Stress due to an excessive emotional stimulus, related to death, will have threefold repercussions on the organism:

- On the *nervous system*, which integrates the external world to the internal world via the brain (hypothalamus, hypophysis, mesencephalon) and nerves and, consequently, the skin and hair—for all are derived from the ectoblast (or ectoderm). Local changes in the peripheral nervous system at the level of dermal papilla or bulge region may play a role in the development of AA as a result of neuropeptide release (12). A decrease in calcitonin gene-related peptide (CGRP) and Substance P (SP) expression in the scalp of patients with AA has been reported (13,14). CGRP has a potent anti-inflammatory action (15) and SP is capable of inducing hair growth in mouse (16).
- On the *endocrine system*, a biochemical system, i.e., the same embryologic origin, through hormones secreted at the time of stress—catecholamines, norepinephrine, dopamine—sometimes to the point of exhaustion (17). Might dopamine be necessary for hair regrowth? Some antidepressants stimulate its production and depression is present in the patient suffering from alopecia areata (18).
- On the *immune system* (19), the recognition of “self.” It allows the organism to maintain its “self” by reacting against any antigen recognized as foreign, to neutralize it and thus, via the lymphocytes, to defend itself from the non-self. This control system may be affected in some subjects or in some families, even to the extent that it no longer recognizes the “self.” In the case of alopecia areata, the hair is expelled like a foreign body. It is no longer recognized as being part of oneself which reflects an organ-specific disease directed against the hair follicle. An impairment of T-cell functions leading to an increase in helper T-cells (CD4) and a decrease in suppressor T-cells (CD8) has been suggested (20,21). The resulting increased ratio of helper to suppressor cells was found to be correlated with the amount of hair loss in AA (22). The immune-mediated nature of AA pathogenesis (23) is supported by positive effects obtained with treatments using immunomodulatory agents (24–26) and genetic associations between AA and HLA class II antigens (27,28).

According to Haour and Crumeyrolle-Arias (29), the mechanism leading to the onset of alopecia can be pictured by considering the close relationships between these three systems endowed with a highly cooperative and sophisticated communication network weakened and shattered by the affects of suffering related to a previous death; nervous, endocrine and immune systems interact via proteins called messengers; the message that they bear is picked up by other proteins called receptors which recognize them specifically, e.g., neuropeptides produced by nerve cells can interfere with follicular keratinocytes inducing the release of proinflammatory cytokines IL1 α , IL1 β , TNF α which are potent inhibitors of hair growth (30). At the time of loss, if the patient had earlier set these three systems in motion—a painful past at the affective level—a reproduction and exacerbation of these exchanges between messengers and receptors would occur, as in a Pavlovian reflex.

In the last few years it has been recognized that some of these messengers and receptors are common to the three systems—nervous, endocrine, and immune (31). The study of interactive signaling and receptors makes sense of all these phenomena that, until now, have been shrouded in mystery!

So, in reaction to a pain perceived like death, registered by the nervous system but recalling earlier depressive states, what happens at the level of the brain's limbic system, the seat of motivation and emotion?

The brain is capable of responding to present information by calling upon the memory of past information. This recalls the experiment in 1930, by Makievik at the Institut Pasteur, in Paris: Makievik simultaneously injected a carcinogenic substance in mice and triggered a musical stimulus (trumpet). Evolution of the cancer was then arrested. Several months later, simply in response to the sound of the trumpet, the cancer cells developed anew. In 1973, MacLean (Toronto University), quoted by Vincent (32) constructed an ingenious classification of our three brains:

- the first, *reptilian*, the deepest, not only that of instincts and impulses, but also that of motor function;
- the second, *paleomammalian* or "limbic system", which is of interest in psychotherapy (including the analysis of dreams);
- the third, *neomammalian* or *neocortex*, that of intelligence, the most evolved, which is also of interest in psychotherapy. The second and third brains are, in fact, constantly connected.

A depressive state with no serious neurosis (the affective and sexual states are in balance) is due to stress of some kind. The dermatologist can prescribe a simple anti-depressant and medication for anxiety and also provide supportive psychotherapy. An antidepressant may help the patient to talk but can also reduce hair pulling, perhaps via a serotonin mechanism (33): habit reversal (34) may help repetitive and compulsive behaviors such as skin scratching and hair pulling. Along with this, there should be exploration of underlying tensions. Cognitive-behavioral principles of challenging negative or distorted thoughts may contribute to stress reduction.

Sometimes a deeper, psychoanalytic exploration is required, reviewing childhood experiences and understanding them in a different way: there can be *verbal release* of what had been previously discharged through dermatological symptoms. Why make this comparison?

Before a child acquires the ability to express himself verbally, he can express himself with his body [what Kreisler (35) called "the underground channel of the organs"] and, at certain specific stages of his development, through the direct experience of anxiety (persecution stage at the age of 9–10 months) and depression, as Klein (36) described it.

This has been confirmed by a number of studies in pediatrics and child psychiatry (37) with a major contribution in France by Lebovici and coauthors (38–40), Diatkine and coauthors (41,42), Soulé (43,44), Kreisler (45,46), and de Ajuriaguerra (47–49); in the United States by Mahler (50,51) and Brazelton (52,53).

This channeling through the organs disappears progressively if children receive their share of care and affection and thus reach the stage of verbal exchange. Otherwise, these patients will have to see a specialist in psychosomatic disorders at a later stage of their life. Thus, a predisposition for some kind of dermatitis goes back to early childhood. There were defects, missing steps in the emotional development, before or sometimes after the acquisition of speech.

In cases of severe hair loss (Fig. 2), one of three factors can usually be pinpointed as being the cause: all three are essential for reaching the stage of verbal communication:

1. “Chronic insufficiency of attachment” (54–56), causing an emotional void, evolving toward essential depression.



Figure 2 Alopecia areata in an 18-year-old existing since 3 years of age.
Likely reason: abandoned child neurosis.

2. Excessive stimulation (the exact opposite), “constrained personality,” often detected in the Rorschach and Szondi tests (see below).
3. Irregularity in child care (neglected children), child-minding arrangements (dumped children) and/or inconsistency in parental behavior (this is called a “double bind,” i.e., order and counter-order) (57,58).

In the past, people with this kind of deficiency, with very limited defense potential and lacking the will to live, did not live to reach adulthood. Today, antibiotics and corticoids can prolong their lives despite basic reluctance; this explains why there are increasing numbers of pathological cases of psychosomatic origin.

2. PSYCHOLOGICAL TESTS

If the depressive state is deemed to be long standing, or acute and current, it may help to refer the patient to a psychoanalyst, who will judge if it is necessary to do personality projection tests (59), which might help the patient to be aware of the necessity of undertaking analysis, often lengthy.

Hair loss is really a call for help in these patients, who rapidly become attached to their doctors; these patients do not always realize that they are subject to severe neurosis. Care must be taken not to disturb them further by revealing the truth. What is crucial is gaining the patient's confidence by means of these tests in order to ensure the success of the long-term process.

At the Hôpital Saint-Louis (Paris), more than 100 affected patients undertook the Rorschach test, Szondi test, and the Koch-Stora tree test (to be published). As a result, immature personalities were revealed in these patients, and they were found to have poor identification with their parents (father or mother, usually both). For instance, the only possible identification for one 20-year-old girl was masculine; this young homosexual lost her hair, taking on the appearance of a balding male with hair still on the sides of the head.

2.1. The Rorschach Test (60)

The principle is familiar: the subject is presented with inkblots—grey, black, or colored—forming various nonstructured shapes. He or she is asked to interpret the shapes spontaneously.

Precise modalities govern the coding of these answers, which are used to establish the psychogram: from this, the mode of apprehending reality, the mode of thought, the defense mechanisms, the personality type, adaptable nature, and ability to succeed can be deduced.

The inner personality can be probed thoroughly if this interpretation is done using psychoanalytic data. The Rorschach test explores the identifications, the relationship with the father and the mother, affectivity, sexuality, attitudes toward work, occupational life, and the environment. It also makes it possible to pinpoint the impact of traumas marking the psychic existence.

Therefore, it may be seen as a very complete exploration, although its accuracy can be restricted by reticence or inhibition on the part of the subject. This is why it is recommended to gain the confidence of the subject prior to undertaking the test.

2.1.1. *What Does the Rorschach Test Reveal About Our Patients?*

It can be observed that these subjects do not like themselves. This results in inadequate narcissism, which might be called makeshift, very easily damaged.

The inkblot for reality is always perceived, which is not the case with other dermatological disorders such as constitutional eczema. But this perceived reality is poor, frequently extremely meager. One patient answered: "impression of being in the dark."

When it comes to the inkblot representing sexuality, the answers are invariably very disturbed. They range from “I don’t feel very inspired” (!), to “something which is broken,” to no answer at all. Here, the sexuality is almost nonexistent.

The eighth inkblot, representing affectivity, is the one that patients are most attracted to: this can be viewed as a desire to be loved similar to that felt by children. This childish affectivity is always colored by strong, unresolved aggressiveness.

2.2. The Szondi Test (61)

This nonverbal, nontraumatic test is appropriate for all ages and all socio-cultural environments. This includes children from age 7 onwards, illiterates, and uncouth, highly unsophisticated people. It is generally well accepted even by uncooperative or hostile subjects because it simply involves making choices from a series of eight photographs of men and women.

The subject is asked to designate the two most appealing out of the eight, and then the two least attractive to him. This operation is repeated six times, each time using a different series. The choices are registered on a grid to obtain a “profile.”

This test explores the subject’s impulse dynamics, according to four vectors covering all possible relationships, namely the object relationship, sexuality, emotional reactions, and that of the building up and defense of the ego.

2.2.1. *What Does the Szondi Test Reveal?*

In all cases studied we found the following:

1. An ego, feeling obliged to adapt, but with some claims to independence, and also an ego that does not know how to make a stand, letting itself be torn between the following very strong needs:
 - (a) need for security,
 - (b) need for protection,
 - (c) need for support,
 - (d) need for narcissistic withdrawal,
 - (e) need to bolster up its feelings of self-worth, or else an ego that has been subjected to total neglect, and is therefore weak.
2. An object relationship based on attachment.
3. Sexuality still at a childish stage, characterized by panic and an oedipal attachment, with intense demand remaining unsatisfied.
4. Emotional reactions of depression, with panicky, inhibited subjects or subjects feeling the need to build themselves up.

These various constants emerge first in the Rorschach test. They prove the psychic relationship in those suffering from alopecia areata.

2.3. The Koch-Stora Tree Test (62)

The Koch-Stora tree test is also a good test of personality projection. It was conceived by Junker, revised by Koch and developed further by Stora (63). The subject is asked to draw a tree on a sheet of paper in a given format. Then the subject is given another piece of paper and asked to do another tree. The third time, the subject is asked to draw his or her dream tree, real or imaginary.

A drawing of a tree is a good medium for projection. More expressive than writing and less restricted by formal requirements, it allows tendencies to be dramatized. Each of the three trees fulfills one aspect of the personality, showing the inner self, the social self, and desires and hopes.

The constants for trees drawn by subjects experiencing hair loss were as follows:

1. The first shows a desire to keep up appearances.
2. The second shows impulsiveness, and a great deal of anxiety.
3. The third, being the dream tree or tree of the imagination, always bears leaves, and in fact signifies a head with hair.

In addition, they add comments such as "I just love leaves!" or "I love trees with lots of leaves." The transposition in cases of alopecia areata is fairly straightforward; the leaves, of course, signify hair. "It would be fantastic to have a lot of hair, even too much!" said one 18-year-old boy with alopecia totalis.

The third tree is often phallic as well. It shows a desire for power, which corresponds to the childish stage in which these patients have remained trapped.

3. WHAT KIND OF THERAPY CAN BE PROPOSED FOR THIS KIND OF PATIENT?

The most appropriate therapy is carried out face to face and makes use of dream analysis. It is out of the question to suggest psychoanalysis on the couch to patients like this. Instead of destroying and then rebuilding, what must be undertaken is a process of building up, of strengthening the ego (The doctor's presence is necessary to afford "support"). This therapy should also repair or produce the identifications that had not been possible with the parents. Lagache (64) put it nicely when he called these the "heroic identifications."

The analysis of dreams and their content allows the subject to master progressively his childlike, violent impulses of love and hate. (The dreams of these patients contain numerous fantasies of death, devouring, and persecution.)

A 30-year-old woman with severe diffuse alopecia hated her mother. In her dreams, she saw her excrement come out of her mouth in the shape of a snake. This is a very precise fantasy, that of ridding herself through speech of the anal, omnipotent, and death-dealing mother. Months of analysis were required to put an end to this utterly ambivalent dependency. Her hair would stop falling out, and then would begin to fall out again. After 3 years of analysis, the young woman went to live abroad for professional reasons. She was then able to be cured of the symptom and find emotional fulfillment, thus completing the severing of her dependency on the "wrong object."

It seems as if the patient losing hair cannot break out of a circle of self-excitation, the end results of which is a physicochemical sort of intoxication.

Given this background disorder, tolerated to varying degrees in everyday life, hair loss might be triggered by the occurrence of an accident or disaster or fear. Many authors have stressed the fact that the triggering factor is frequently related to "death": the loss of a loved one, an abortion, a drowning, or the arrival of a second child for an overprotected first child.

With confidence on the part of the patient, and neutrality and steady encouragement from the psychoanalyst, the patient wishing above all to recover his or her hair will succeed in getting rid of the death instincts directed toward the parents, and of the self-directed castration instincts. Hair, symbol of life, stops falling and can start growing again, since the hair bulbs themselves are not destroyed.

We followed eight cases of alopecia totalis (Fig. 3), two of which were of universalis type. Through this method, all eight experienced new hair growth (Fig. 4) after a period of 1–5 years (Figs. 4 and 5). One of the eight cases, that of a Tunisian woman, is a beautiful story. Her marriage had been arranged by her family with a young man whom she had never met. She loved another with all her heart and plunged into a severe depressive state causing her to lose all her hair. At this point, the future mother-in-law decided that her son could not marry a girl who was bald, and the wedding was called off. Several months later, the young girl left her country with the young man she loved and they settled in Paris, where she began to study in the computer field. But her hair did not grow back. Finally, she went to the Hôpital Saint Louis, for treatment.

Psychotherapy including dream analysis over an 8-month period was necessary to help her release herself from all the guilt that she still felt because she had violated her parents authority, despite her happiness with her lover. Her hair began to grow after the sixth month of consultations, all over the scalp. Toward the end of the therapy she became pregnant (she had wanted to have a baby for 4 years). A number of



Figure 3 Alopecia totalis in a 35-year-old woman following intense fright when she rescued her small son from falling from a sixth floor window.



Figure 4 Same woman as in Fig. 3 showing total regrowth following 10 month weekly analytical psychotherapy.

gynecological treatments had not succeeded in curing her infertility, no doubt also due to psychologic factors.

Another symptom within the pathological domain concerned with the interaction between psyche and hair is that of trichotillomania (65) (Fig. 5). These are patients with graver illnesses; they are psychotic or borderline cases. The dermatologist should immediately recommend consultation with a psychoanalyst. In cases of trichotillomania, or the habit of pulling out hair, an impulse of self-destruction is acted out; whereas in cases of hair loss, the self-destructive instinct is expressed more or less unconsciously.

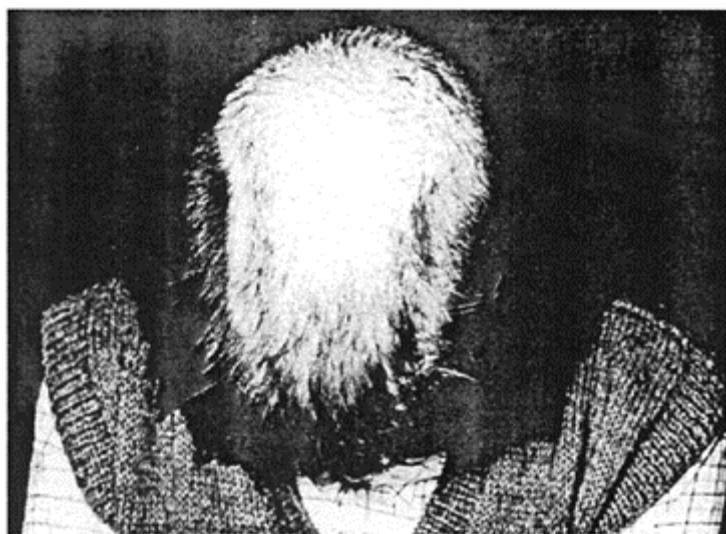


Figure 5 Trichotillomania limited to the apex in a female teenager.

Trichotillomania occurs most frequently in girls in conflict with their mother. In two of 10 girls examined, it was associated with nail biting. The triggering factor in the case of a 15-year-old was the removal of her teddy bear, her mother saying that she was too old to sleep with a teddy bear. Here, it is a case of frustration of the transitional object, the "right" love object. In another case, a girl was whipped at the slightest hint of opposition. In all cases the father was absent (seven cases were children of divorced parents) or was a weak figure upon whose support the girl could not count. During a fifth talk with one patient, I noted down the account she gave of a dream: "I was at my mother's burial and I laughed." She rid herself of her sadistic mother in fantasy, which she could not do in reality; she turned the impulse of destruction against herself by pulling out her own hair.

Do not people frequently use the expression that something is enough to make you tear your hair out? In Ancient Greece, the mourners tore out their hair when a loved one died.

Women are not alone in being disturbed by hair loss. Men, too, may experience similar difficulty in identifying with their parents. They are also predisposed to hair loss, independently of all heredity-related factors. Frequently, men suffer from even more severe neurosis than women. These patients always have authoritarian, castrating, or even psychotic mothers. Some opt for hair transplants and become attached to their surgeons as they would to a therapist. If the dreams of a narcissism restored at least from an esthetic standpoint are disappointed by the results, then the subject may well sink into a depressive state requiring the assistance of a psychiatrist.

A 36-year-old man, dissatisfied with his hair transplant, which was actually very successful, requested help in the midst of an anxiety crisis because he had begun to lose hair on the sides of his head. It was very difficult to persuade him to undertake

psychotherapy. He would only agree if it were free of charge, because he had already spent so much on surgery.

In conclusion, it seems useless to treat the affected areas of hair loss exclusively using lotions, dry ice, cortisone, PUVA therapy, DNCB, etc. when the cause of the disorder lies elsewhere.

It seems preferable to try to break the psychopathological chain set up between emotions and the target organ with the help of antidepressants, sedatives, and even hypnotics, and with supportive psychotherapy and dream analysis if possible. In cases of acute depression, drugs are necessary, especially in the beginning. The skin lesion or the hair loss are eloquent even if the patient, hiding behind the barrier set up by the physical disorder, is silent; the doctor must seek out the true source of illness. Getting patients to talk freely is therefore essential in helping them.

Today, the association between dermatologists, psychologists, psychiatrists, and psychoanalysts is becoming a reality. In France, this kind of versatile team work exists in Paris, Lille, Toulouse, Brest, Marseille. In the United States there is a foundation called the "National Alopecia Areata Foundation," dedicated to helping this kind of patient.

It is to be hoped that this kind of exchange will spread and that one day, each specialized hospital service and each dermatologist will find a corresponding practitioner to help cure this kind of distressing hair loss. Psychosomatic medicine, which "sounds the psyche," should receive full recognition in dermatology and all other medical fields. This would require that medical students be taught to reconcile psyche and soma. With this in view, a university degree has been created in 2002 at Cochin-Tarnier University, Paris which is awarded after a 6-month course in "Psychoanalysis and Dermatology."

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Index

- 13-cis Retinoic acid, 133, 163
 - 18-MEA, 5, 145, 397
 - 1,2-Diaminobenzene, nitro derivatives
 - (*See also* o-phenylene diamines, nitro), 264
 - 1,3-Diaminobenzenes, 278
 - 1,4-Diaminobenzenes
 - (*See also* p-phenylene diamines), 263, 277, 278
 - nitro derivatives, 263
 - 2,4-DPO, 138
 - 2,4-Diaminoanisole, 329, 330, 483
 - 2,4-Diaminophenoxyethanol, 278, 287
 - 2,4-Diaminotoluene (*See* m-toluylenediamine)
 - 2,5-Diaminotoluene (*See* p-toluylenediamine)
 - 5,6-Dihydroxyindole, 27, 549
 - 5,6-Dihydroxyindoline, 270
 - 17 β -Estradiol, 567
 - 5 α -Reductase, 76, 567, 599
 - α -Helix, 11, 34
 - α -Keratins, 3, 71
 - heterodimer, 12
 - α -Hydroxyacids, 144, 416
 - in hair care, 144
 - β -Layer, 15, 17, 21
 - β -Keratin, 33
 - δ -Layer, 17
 - Δ^4 Androstenedione, and sebum production, 587, 590
-
- A layer, 5, 17, 545
 - Acne necrotica, 651, 652
 - Acrylic/acrylates copolymers, 157, 177, 189
 - cationics, 258
 - Active ingredients, in shampoos, 105, 107, 110
 - Adhesive tapes, 616, 619
 - for patch testing, 498
 - for scale sampling, 619
 - Adsorption properties of hair, 45, 86, 150
 - of bleached hair, 242, 244
 - Adverse reactions, 318, 498, 511
 - to bleaches, 356, 526
 - hairdressing shampoos, 520
 - hair dyes, 249, 512, 529
 - hair products, 352, 358, 440
 - metallic dyes, 267, 525
 - permanent waving, 242, 420, 543
 - permanent dyes, 258, 524

- semipermanent dyes, 257, 259, 273
- straightening products, 221
- vegetable dyes, 253, 255, 525
- Aerosol sprays, 199, 259
 - technology, 181
- African American hair, 146, 166, 451
- African hair, 2, 23, 40
- Alkali, effects on hair, 144, 203, 223
- Alkali solubility, 244, 249, 418
 - and bleaching level, 46, 244, 418
 - and friction coefficient, 46, 144, 396
- Alkaline technology, to straighten hair, 221, 222, 224
- Alkanolamides, 92, 108
 - sulfates, 85, 86
- Alkylethersulfates, 86, 88, 106
- Alkyl glycosides, 91
- Alkyl sulfates, 86, 106, 111
- Allergens, 493, 495, 500
 - patch testing, 494, 496, 535
 - predictive tests, 493, 494, 495
 - reactions to, 374, 538, 655
 - standard series, 498, 533, 539
- Allergic contact dermatitis, 495, 520, 531
- Allergic reactions, 318, 513, 522
 - delayed-type, 496, 502, 536
 - immediate, 495, 509, 638
 - types of, 501, 513, 638
- Alopecia, 77, 559, 570
 - androgenetica, 77, 163, 543
 - and lymphocytic infiltrate, 77
 - in female, 165, 565, 688
 - in male, 142, 163, 573
 - areata, 77, 559, 574
 - and immune system, 644, 702
 - and neuropeptides, 703
 - and psyche, 701
 - psychotherapy, 701, 704, 711
 - cicatricial, 576, 577, 578
 - diffuse, 559, 569, 570
 - drug-induced, 570
 - localized, 559, 561, 574
 - scarring, 511, 522, 541
 - totalis, 702, 708, 709
 - toxic, 570
 - traumatic, 579, 662
 - universalis, 77, 702, 709
- Alpha olefin sulfonates, 123
- Aminophenols, in oxidation coloring, 261, 269, 285
- Ammonia, 188
 - in bleaching products, 237
 - in oxidation dyes, 295
 - in waving products, 205, 210, 211

- Ammonium carbonate, in waving lotions, 205
- chloride, in shampoos, 108
- Amine oxides, 92
- Aminoacid analysis, 16, 55, 248
- Amphiphilics, 91, 92
- Amphoteric polymers, 107, 177, 191
- Amphoteric surfactants, 88, 89, 300
- Anagen hair, 69, 76, 553
- Anagen to catagen transition, 73, 76
- Anagen effluvium, 559, 569, 570
- Andean Community regulations, 461, 464, 471
- Androgenetica, 77, 163, 543, 676
- Androgens, 76, 80
 - and hair growth, 76
 - and sebocyte, 586, 598
 - and sebum secretion, 600
- Angiosarcoma (scalp), 670
- Anion-cation complex, 152, 267, 268
- Anionic polymers, 107, 188, 257
 - in hairstyling products, 136, 150, 177
- Anionic surfactants, 85, 102, 106
 - in shampoos, 88, 89, 90
- Anthraquinone dyes, 266, 274
- Antidandruff active ingredients, 466, 470, 480
- Antigrease agents, 133
- Antioxidants, reactions to, 537
- Aromatase, 567, 572, 581
- Aromatic diamines, 279
 - in hair coloring, 279, 316
 - in oxidation coloring, 279
 - toxicology, 314, 316, 317
- Arrector pili muscle, 72, 74, 653
- Asean countries regulations, 461, 464, 472
- Asian hair, 2, 49, 695
- Assessment of product efficacy, 377
 - in vitro, 377, 378
 - in vivo, 399, 416
- Atomic force microscopy, 6, 47, 389
- Atopic dermatitis of the scalp, 637
- Auber line, 69, 70
- Autografts, 675, 697, 698
- Autoimmune diseases, 641, 644
- Azine dyes, 268
- Azo dyes, 260, 271, 273
- Bandrowski's base, 310, 320, 321
- Baryum binoxide, 237
- Basal cell carniroma (scalp), 656, 668, 669
- Basic dyes, 257, 258, 270
- Bcl2 protein, 69
- Bending, 5, 38, 193

- modulus, 413, 448,
- properties, 5, 61, 383, 412
- Benzene trihydroxy, 256, 269, 278
- Betaine, 89, 111
 - sulfo, 89
- Bird's nest hair, 513, 527
- Bjornstadt's syndrome, 541
- Bleached hair, 89, 144, 150
 - characteristics, 211, 242
 - dye uptake, 245
 - friction properties, 243
 - shampoo for, 89
 - surface properties, 236
 - tensile properties, 243, 408
 - water uptake, 244
- Bleaches (*See* Bleaching products)
- Bleaching, 107, 114, 214, 229
 - chemistry of, 231
 - creams, 238
 - effects on hair, 237
 - gels, 241
 - importance of, 245
 - light-induced, 250
 - oils, 238
 - pastes, 241
 - powders, 237, 239
 - products, 238, 241, 353, 525
 - adverse reactions to, 318, 512
 - and occupational dermatitis, 531, 534
 - shampoos, 114
 - technique, 241
- Biodegradability, 121, 365
 - intrinsic, 122, 371
 - ready, 122, 365, 370
- Block polymers, 108, 178, 180
- Blond, Venetian, 229
- Blow-drying, and hair breakage, 427, 430
- Body properties, 30, 116
- Borderline products, 464, 467, 491
- Boric acid, 476, 570
- Bounce test, 382, 383
- Bovine cornea test, 120, 126, 357
- Brocq pseudopelade, 542, 582, 642
- Bromates, 214, 221
- Bulge, 72, 74, 553
- Burns, from hair products, 256, 511, 517

- Capsaicin test, 494, 515, 527
- Carcinogenicity testing, 329, 335, 340
- Carcinomas (scalp), 657, 667, 668
- Catagen hair, 73, 139, 575,

- Cationic dyes, 245, 272
- Cationic polymers, 246, 258, 426
- Cationic surfactants, 92, 102, 115
 - for hair care, 153
 - in semipermanent coloring, 252, 254, 268
 - for shampoos, 92
- Caucasian hair, 2, 21, 34
- Cell membrane complex, 14, 17, 21
- Ceramides, 21, 144, 145
- Chamber test, 505, 506
- Chamomile, 254, 255, 519
- China regulations, 466,
- Chlorite, sodium, 5
- Cholesterol, in sebum, 583, 587, 588
- Cholesterol esters, in sebum, 583, 587, 588
- Chroma, 431, 432
- Chromosomal aberrations data, 322, 325, 327
- CIE-LAB system, 431, 432
- Citric acid, in shampoos, 107, 108, 144
- Cleansing base, 90, 105, 106
- Cleansing of hair, 83, 94, 114
 - basic mechanisms, 93
 - and calcium ions, 101
 - and redeposition, 92, 101, 109
- Clinical testing, 358, 359, 361
- Coal tar, 466, 474, 655
 - hair dyes, 466, 474, 482
- Cocamidopropyl, 520, 528, 537
- Color, 4, 24, 25
 - accidents, 513
 - fastness, 257
 - to sunlight, 435
 - to shampoo, 289
 - measurement, 430
 - and pH, 110
 - and surfactants, 298, 299
 - uptake by hair, 290
- Colored hair sprays, 257, 258, 259
- Colorimeters, 245, 433
- Colorimetric coordinates, 432
- Coloring, 107, 112, 178
 - conditioners, 258
 - mixture, 293
 - models, 294
- Coloring (hair), 251, 252
 - direct, 259
 - with lead salts, 255, 256
 - lightening, 294
 - nonoxidation, 253
 - oxidation, 257, 277, 294
 - semi-permanent, 296
 - with silver salts, 255

- temporary, 256
- tone on tone, 272, 296
- Combability, 150, 238, 400
- Combing, 45, 46, 143
 - ease, evaluation of, 116
 - machine, 78
 - noise at, 399
 - stress, 402
 - work, 401, 430
- Companion layer, 70
- Comprehensive license standard, 460, 485, 489
- Conditioners, 84, 109, 159
 - deep, 158
 - free from cationic surfactants, 159
 - leave on, 161
- Conditioning, 34, 46, 84, 106
 - agents, 105
 - after coloring, 300, 434
 - creams, 117
 - emulsions, 158
 - liquid gels, 158
 - lotions, 158
 - nano-emulsions, 159
 - shampoos, 112
- Confocal laser scanning microscopy, 389
- Consumer tests, 355, 440
- Cortex, 3, 5, 7, 17
- Cortical cell, 8, 21, 49
- Cosmetic product, 119, 120
 - definition, 462
 - labeling, 472, 473
- Cosmetic Ingredient Review, 316, 354, 470
- Cosmetovigilance, 119, 362, 363
- Coupler, 280, 283, 286
- Coupling reaction, oxidative, 283, 292, 317
- Critical packing parameter, 99
- Cross-reactivity, 500, 519, 539
- Curl bounce test, 382
- Curl retention test, 379, 381, 385
- Cuticle, 3, 4, 6
 - cohesion, 5, 14
 - cracks, 427
 - mechanical properties, 5, 8
- Cylindroma, 666, 668
- Cysteamine, 208, 212, 448
- Cysteic acid, 19, 234, 244
- Cysteinyl dopa (*See Dopa*)
- Cystine linkage, 143, 150, 210
 - action of alkali, 232
 - action of mercaptans/thiols, 213
 - action of sulfites, 232
 - breakage/disruption, 150

- Cystine oxidation, 421, 450
Cysts, 666, 667
Cytokines and hair growth, 74, 76
- Dandruff, 84, 137, 465, 609, 663
 evaluation, 628
 and hair loss, 138
 histology, 613
 inflammation, 620
 microbiology, 629
 scaling, 165
 treatment, 616, 630
- DDT, 666
- Definition of cosmetic product, 461, 462, 466
- Delaney clause, 483
- Dermal papilla, 67, 69, 73, 76
- Dermal sheath, 67, 68, 69
- Dermatitis, 130, 318, 503
 allergic, 497
 atopic, 637
 contact, 633
 eczematous, 514
 from hair dyes, 369, 526
 from hair products, 498
 in hairdressers, 520, 528, 535
 and pityriasis capitis, 609, 610
 rubber gloves and occupational, 531, 534, 536
 seborrheic, 130, 635
- Dermatomyositis, 571, 610, 641, 643, 657
- Dermatophytes, 583, 657, 663
 anthropophilic species, 621, 657, 659, 662
 ectothrix type, 657, 658, 661
 endothrix type, 657, 658, 661
 favus type, 657, 658, 661, 662
 geophilic species, 657
 zoophilic species, 657, 660, 664
- Detergency, 99, 115
 and calcium ions, 101
 evaluation of, 115
- Diagnostic patch testing, 496
- Diameter (hair) and physical properties, 35, 40, 45
- Dielectrical properties of hair, 85
- Diffuse alopecia, 559, 560, 569
- Diffuse reflection, 403, 405, 406
- Dihydrotestosterone, 76, 567, 598
- Dimethyl ether, 186, 187, 195
- Dipolar surfactants, 89
- Disentangling assessment, 400, 437
- Disruptive load, 34, 220
- Disperse dyes, 266
- Dispersion process in shampooing, 101, 109, 258

- Disulfide, in waving lotions, 207
- Dithiodiglycolic acid, 210, 217
- Dopa, 28, 59
- Dopachrome, 27
- Dopaquinone, 27, 28
- D'Orbigny papyrus, 699
- Drug-induced alopecia, 570
- Dry hair, 142, 170, 399
- Dry combing work, 401
- Dyes (hair), 241, 251, 253, 257, 314
 - acid, 267, 268
 - adverse reactions to, 318
 - anion-cation, 268
 - Arianor, 271, 272
 - assessment of, 315
 - basic, 257, 258
 - carcinogenicity, 328
 - contact allergy, 318, 367
 - coal tar, 482
 - disperse, 266
 - endocrine effects, 336
 - epidemiology, 328
 - exemption, 481
 - genotoxicity, 315, 319
 - human exposure, 329
 - ingredients, 317, 318
 - metallic, 266, 525
 - metallized, 255
 - nitro, 261
 - nonoxidation, 296
 - oxidation, 293
 - percutaneous penetration, 347, 506
 - permanent, 256, 277
 - reactive, 271
 - removal from hair, 241
 - reproductive toxicity, 313, 315
 - risk assessment, 314, 328
 - safety requirements, 315
 - selectivity, 257
 - self-oxidizing, 269, 272
 - semipermanent, 257
 - systemic exposure dose, 315, 334
 - systemic toxicity, 313, 329
 - toxicity studies, 318
 - relevance to human health, 326
 - vegetable, 253, 525
- Dynamic hold test, 382
- Dynamic measurements, 37, 40, 61
- Ecotoxicity, 121, 122, 365
- Ecdysplasin, 77

- Eczema, 318, 368, 501
- Efficacy of hair products, 488, 624, 625
- Elastic properties of hair, 37, 391, 398
- Elasticity modulus, 6, 40
- Electrical properties of hair, 50, 52, 64
- Ellipsicity, 2
- Embryogenesis (hair), 67, 550
- Endocuticle, 5, 7, 17
- Environmental safety of hair products, 121, 364
- Epicuticle, 5, 7, 94
- Esterquats, 151
- Estrogens, and sebum production, 599, 600, 606
- Ethanolamine, 86, 290, 521
 - in oxidation coloring, 294
 - in waving lotions, 205
- EU Directive, 7th amendment to, 458, 487, 488
- EU regulation of cosmetic products, 195
 - definition, 336, 464
 - illustrative list, 364, 367, 463, 467
 - labeling, 464
 - marketing requirements, 351
- Exocuticle, 5, 17, 38
- Exogen phase, 72, 139
- Expert evaluation (*See* Sensory evaluation)
- Exposure to hair products, 527
- Eye irritation potential, 126
 - alternative test methods, 119, 120
- Fading, index of, 435, 436
- Fatty acids,
 - in hair care, 85, 588
 - in sebum, 589
- Fatty alcohols, in hair care, 157, 297, 298
- Favus, 657, 658, 662
- Fibronectine, 68, 69, 552
- Finasteride®, 134, 137, 138
- Fine hair, 143, 159, 569,
- Finished product data, 355
- Flexabrasion, 144, 414, 415
- Flexion properties, 382
- Fly away, 388, 400, 402
- Fixation, in permanent waving, 209
- Foam (*See* Lather)
 - boosters, 91, 99, 176
 - characteristics, 83, 84, 91
 - softeners, 238, 297, 300
 - stabilizers, 92, 99, 238
- Follicle (*See* Hair follicle)
- Follicular automaton, 73, 80
- Follicular unit, 677, 680, 681
- Folliculitis, 579, 662, 663

- Frame formulae, 469
- Friction, 46, 47, 243
 - fiber to fiber, 378, 395
 - macroscopic, 393, 396
 - nano, 396, 397
 - noise generated by, 398
- Formaldehyde sulfoxylate, 218, 241
- Frictional properties of hair, 393
- FT-IR microscopy, 21
- Full ingredient labeling, 458, 460, 474
- Fungal infections, 622, 657
 - inflammatory, 661
 - noninflammatory, 658, 660
- Fungi (*See* Dermatophytes)

- Gammexane, 666
- Gray hair, 251, 269, 299
- Genetics, 77, 78
 - of hair growth, 77
 - of hair pigmentation, 78
- Gonioreflectometer, 403, 405, 406
- Glucamides N-acyl, 91
- Graft (hair), 675, 680, 697
 - African hair, 2, 23, 40
 - Asian hair, 2, 23, 34
 - evolution of, 684
 - eyebrow, 675, 686, 690
 - and Minoxidil®, 137
- Grafts, 677, 681, 695
 - curly hair, 697
 - implantation, 691, 692
 - indications for, 685
 - in female, 688
 - in male, 691
 - long hair, 685, 691
 - micro, 677, 680
 - mini, 677, 680
 - preparation, 680, 695
 - placing, 687
 - pubic hair, 690
 - regrowth on, 685, 690
- Grafting instruments, 686,
- Growth factors, 69, 74, 76

- Hair and psyche, 701, 710, 711
- Hair as a symbol, 709
- Hair bulb, 27, 30, 549
 - bleaching, 230, 245, 420
- Hair body, 378, 387, 412
 - breakage, 34, 430, 525
- Hair care, 106, 129, 463

- chemistry, 84, 400
- color, 24, 29, 251
- cleansing, 94, 353
- coloring, 149, 252, 258
- condition, 30, 113, 129
- cross-section, 2, 3, 40
- cycle, 73, 74, 552
- ingredients, 144, 147
- product evaluation, 377
- Hair damage, 146, 656, 512
 - density, 25, 141, 574
 - diameter, 2, 143, 335
 - dyes (*See Dyes*)
- Hair ellipsity, 2, 25, 35
 - elongation/extension, 34, 223, 242
 - embryogenesis, 67, 550
- Hair fibrillation, 4, 411, 429
 - follicle structure, 67
 - functions, 84, 104, 584
- Hair follicle, 1, 11, 67
- Hair germ, 71, 72
 - and androgens, 76, 567, 599
 - grafts (*See Grafts*)
 - graying, 549, 555, 556
 - growth, 74, 76, 77
 - and substance P, 519, 703, 712
- Hair lipids, 21, 94, 144
- Hair loss, 142, 621, 688,
 - evaluation of, 164
 - and neurosis, 706, 711
 - and psyche, 701, 710, 711
 - and stress, 569, 574, 703
 - and treatments, 624, 666, 676
- Hair mascaras, 257, 259
 - matrix, 67, 69, 558
 - morphogenesis, 79, 552
 - morphology, 54, 443, 391
- Hair peg, 71, 72
 - permeability, 120, 425, 638
 - pigmentation, 24, 77
 - regulation of, 554, 555
 - unit, 74
 - pigments, 8, 25, 26
 - plugs, 577, 579, 644, 675
 - products, 351, 462, 498
 - efficacy assessment, 377
 - regulations, 194, 455, 486
 - safety assessment, 120, 315, 344
 - sensory assessment, 184, 399, 439
- Hair pull test, 141
- Hair sprays, 180, 181, 190
 - straightening, 174, 220, 221

- style evaluation, 171, 181, 437
- Hair volume, 175, 378, 387
 - waving, 378
 - wetting, 387
- Hairdressers, 237, 437, 531
 - nickel allergy to, 533, 535, 538
 - occupational disorders of, 531, 533, 535
 - protection, 455, 504, 532
- Hard water, and cleansing of hair, 89, 100, 144
- Harris papyrus, 699
- Hairless gene, 77, 81, 569
- Hairstyling
 - foams, 179
 - gels, 175, 180, 196
 - lotions, 136, 158, 175
 - waxes, 175, 179
- Hamartoma (scalp), 667, 668
 - sebaceous, 667, 669, 670
- Heating sachets, for hair waving, 201, 205
- Henle layer, 69, 71
- Henna, 252, 253, 254
 - compounded, 256
- HET-CAM test, 120, 126, 357
- HGT proteins, 14, 21
- hHa/hHb proteins, 71
- Hamilton classification, 687, 691
- Hold, hairstyle, 116, 136, 169
- Hookean region, 33, 60, 62
- Hot iron, 427, 430
- Hue, 252, 255, 292
- Huxley layer, 69, 71
- Hydrocarbon propellents, in hair sprays, 189, 191
- Hydrogen bonds, 11, 24, 232
- Hydrogen peroxide, 236, 238, 239
 - action on hair
 - (*See also* Bleaching effects on hair), 237
 - action on keratin, 205
 - in bleaching products, 236
 - in oxidation coloring, 296
- Hydrosulfite, sodium, 241
- IF keratins, 17, 20, 21
- Immunological contact urticaria, 505, 507
- Immune mediated diseases, 505, 516, 537
- Indamine (*See* Indoamines)
- Indigo, 252, 255
- Indophenol, 257, 285, 291
- Indoamines, 257, 284, 291, 317
- Indoaniline, 285
- Inflammatory diseases (scalp), 641
- Inflammatory ringworm, 661, 663

- In market control system, 456, 460, 467
- Inner root sheath, 69, 70, 552
- Ingredient data, 354
- Ingredient lists, 486
- Ingredient regulation, 478, 481, 484
- Integrity, hair fiber, 408, 425, 430
- Intermediate filaments, 8, 42, 419
- Intermediates, primary, in oxidation coloring, 279, 280, 292
- International Nomenclature of Cosmetic Ingredients (INCI), 473, 474, 479
- Inventory of cosmetic ingredients, 458
- Ionenes, 156
- Ionic bonds (salt links), 36, 206, 213
- Irritant contact dermatitis, 517, 520
- Irritant vs. allergic contact dermatitis, 513, 514
- Irritants, predictive tests, 493, 494, 495
- Iron deficiency, 567, 573, 574
- Isethionates, 88, 106
- ISO standards, 462
- Isthmus, 67, 71, 621
- Japan regulations, 484, 485
- Keratin,
 - $\alpha \rightarrow \beta$ transition, 33
 - action of mercaptans/thiols, 207
 - action of sulfites, 218
 - oxidation, 244,
- Keratin associated proteins (KAP), 5
- Ketoconazole, 107, 625, 636
- Kerion, 661, 664
- Knot adhesion test method, 384
- Knitting of hair, 544
- Köbner phenomenon, 649
- Koch-Stora tree test, 706, 708
- Korean hair, 72
- Labeling regulations, 473, 474, 475
- Lactic acid test, 494
- Lamellar ichthiosis, 654, 673
- Laminin, 68, 69, 552
- Lanthionine, 203, 207, 222
- Lanugo hair, 71, 141
- Latency phase, 72, 73, 139
- Lather, 85, 86, 96, 162
- Lathering, 97
 - potential, 97
 - process, 98
- Lawsone, 254, 272, 324
- Lead acetate, 317, 482, 525
- Lef-1 factor, 72
- Lichen planopilaris, 577, 650, 653

- Lichen planus, 542, 577, 650
Lichen simplex nuchae, 654, 655
Lightness, 157, 158, 432
Lightening
 (See also Bleaching), 231
 degree, 231
 setting lotions, 231
 tones, 231
Limp hair, 143, 144, 158
Lipoma (scalp), 670
Lipids (hair), 15, 144, 145
Liquid crystals, 95, 98, 100
Liquid membrane method, 409
Lists of substances/ingredients, 477
 positive, 477
 negative, 477
 restrictive, 477
Load/elongation curve, 409, 410
Loose anagen syndrome, 576
Louse, 664, 665,
Ludwig classification, 564, 688
Lupus erythematosus, 641
 chronic, 644
 discoid, 542
 systemic, 644
Lupushair, 644
Lysinoalanine, 203, 204, 420
- m-Aminophenols, 280, 285, 317
 co-oxidation with PPD, 284
m-Phenylenediamines, 317
m-Toluylenediamine, 322, 349, 355
Macrofibril, 8, 9, 54
Magnesium dioxide, 237
Malassezia sp., 621
 furfur, 621
 ovalis, 620, 622
Malathion, 666
Male pattern baldness (See Androgenetic alopecia)
Maleic hemiesters/vinyl ethers copolymers, 189, 194
Margin of safety approach, 217, 315, 338
Matrix, 14, 42, 69
Matting of hair, 513, 522, 525
Maximization test, 315, 494
Mechanical properties of hair, 32, 383, 408
 simulation models (See Models)
 and temperature, 40
 and water, 38
Melamine peroxide, 237, 296
Melanin, 24, 25
 biosynthesis, 28, 31, 550

- bleaching, 231
- oxidation, 231
- Melanoblast, 552, 557
- Melanogenesis, 549
 - and hair cycle, 552
- Melanoma (scalp), 671
- Menke's syndrome, 541
- Mercaptans (*See* Thiols)
- Mercosur, 461, 471, 487
- Merkel cells, 70, 71
- Metallic dyes (*See* Dyes)
- Methyl eicosanoic acid (*See* 18-MEA)
- Micellar dispersion, 100
- Micellar solubilization, 95, 100
- Micelles, 94, 100, 114
- Microfibril, 8, 24, 34
- Micrografts, 675, 680, 681
- Micronucleus test, 326, 342, 346
- Microsporum* genus, 659
 - M. audouinii*, 659
 - M. canis*, 659, 661
 - M. ferrugineum*, 659
 - M. gypseum*, 661
 - M. langeronii*, 659, 660
 - M. rivalieri*, 659
- Minigrafts, 677, 680, 681
- Minoxidil®, 137, 575, 685
- Models, 42
 - mechanical, 42
 - rheological, 45
- Monilethrix, 545, 546, 547
- Monographs, FDA, 469
- Mutagenicity testing, 319, 345
- Mutual recognition, 457, 461, 471
- Myelinic figures, 100
- Necking, 33, 34
- Nessler's waving system, 201
- Netherton's syndrome, 547
- Neurosensory irritation, 494, 511, 515
- Neutralization
 - by atmospheric oxygen, 209
 - in permanent waving, 216
- Neutralizers, for perming, 209
- Nickel, allergy in hairdressers, 433, 435, 537
- NICU reaction, 505
- Nitro derivatives of 1,2-diaminobenzen, 264
- Nitro derivatives of 1,4-diaminobenzene, 263
- Nitro dyes, 261, 269, 272
- Nitro phenylenediamine (*See* Phenylenediamine, nitro)
- Nitroaminophenols, 317

- Nitroaminophenylethers, 265
- Nits, 638, 665, 666
- NOAEL, 315, 338, 339
- Noise, at combing, 399
- Nominal content, 473
- Nonionic polymers, 137, 177, 214
- Nonionic surfactants, 451
- Notes of Guidance for safety testing (*See* SCCNFP)
-
- o-Phenylenediamines, 317
 - nitro derivatives, 317
- Occupational dermatitis (*See* Dermatitis)
- Occupational disorders of hairdressers, 531, 533, 535
- Ocular adverse effects, with hair products, 526
- Oils, for hair care, 106, 162, 191
- Olefin sulfonates, 123
- Opacifiers, in shampoos, 112
- Open tests, 500, 505
- OTC drugs, 455, 464, 484
- Outer root sheath, 69, 70, 552
- Oxidation coloring (*See* Coloring, oxidation)
- Oxidation dyes, 277
 - creams, 297
 - gels, 298
 - liquids, 297
 - powders, 299
 - shampoos, 298
- Oxidizers, in permanent waving (*See* Neutralizers)
- Oxidizing products, 236, 242, 246
 - action of, 238
 - for hair bleaching, 230, 246
-
- p-Aminophenol, in oxidation coloring, 278, 286, 323
- p-Toluylenediamine, 277, 320, 331, 536
- p-Phenylenediamines
 - (*See also* PPD), 284, 332
 - nitro derivatives, 317
 - oxidation and coupling, 283
 - tertiary derivatives, 287
- Papilla (*See* Dermal papilla)
- Paraffin sulfonates, 87
- Parakeratotic index, 617, 619, 620
- Patch testing, 496, 498
 - complications, 501
 - false negative reactions, 501
 - open, 503
- Pediculosis, 638, 652, 663
- Pediculus humanus, 664, 665
- Perborate, sodium, 209, 237, 296
- Perborates, 209, 237
- Percarbonatess, 240

- Peripilar signs, 580, 581
cupules, 561, 568
halos, 561
- Permanent waving, 201, 385, 521
adverse reactions to, 521
assessment, 218
lotions, 206
occupational dermatitis and, 531
yield, 136
- Persistence, 118, 364, 365
- Personality projection tests, 706
- Persulfates, 236, 246, 519
adverse reactions to, 519
associated occupational disorders, 536, 537
- PPD (p-phenylenediamine)
allergy to, 367, 536
epidemiological data, 367
co-oxidation, 284
genotoxicity data, 327
occupational dermatitis and, 534
oxidation, 283, 290
penetration studies, 514
- PPD/PAP combinations, 286
- Pheomelanin, 25, 28, 549
- Phenylenediamine, nitro, 317, 348
- Photogoniometer, 597
- Photopatch test, 508
- Photographs, hair global, 141, 142
- Photometric evaluation, sebum, 593
- Phototrichogram, 139, 140, 141
- Physical properties of hair, 30, 419
- Pigment cell, 549, 554, 558
embryogenesis, 550
- Pili annulati, 542, 547
bigemini, 677
torti, 541
trianguli and canaliculi (*See* Uncombable hair)
trigemini, 677
- Piperazine cationics, 156
- Piroctone olamine, 107, 625, 626
- Pityriasis amiantacea (*See* Tinea amiantacea)
- Pityriasis capitis (*See* Dandruff)
steatoides, 613, 633
- Pityriasis rubra pilaris, 652, 653, 654
- Pityrosporon, 635, 649
orbiculare, 620
ovale, 610
- Poison centers, 486
- Polyamides, 156,
- Polyethoxylated derivatives, 90
carboxylates, 88
fatty alcohols, 90

- sulfates, 85
- Polyethyleneimines, 156,
- Polyglycosides, alkyl (APG), 91
- Polyhydroxylated derivatives, 91
- Polymer film evaluation, 192
- Polymethylvinylethers/maleic acid hemiesters, 177
- Polyphenols, in hair coloring, 269, 280, 291
- Polysaccharides, cationic, 153
- Poly vinylacetate (PVA)
- Polyvinylpyrrolidone (PVP), 177
- Post-marketing follow-up, 382
- Post-yield region, 33, 34, 38
- Predictive tests for, 493
 - delayed-type allergy, 496
 - irritants, 494
 - sensitizers in human, 495
 - sensitizing potential, 494
- Pre-market control approval/system, 456, 461, 467
- Pre-rolling lotions, 216
- Preservatives, in shampoos, 110, 119, 178
- Prick test, 505, 506, 522
- Primary intermediates, 279, 282, 292
- Product information, 458, 460, 473
- Propellants, for hair sprays, 183
- Protein analysis, 420, 421,
- Protein derivatives, 105, 146, 192
- Protein loss, 427, 428,
 - after brushing, 427
 - after repeated combing, 427
- Protective colloids, 101
- Protofilament, 10, 12, 14
- Pseudopelade (Brocq), 542, 582, 642
- Psoriasis (scalp), 134, 465, 576
- Pump-spray, 161, 181, 183
- PVA/crotonic acid copolymer, 188
- PVP/VA, 190, 191, 194
- Pyrimidine derivatives, in hair coloring, 271
- Pyrazole derivatives, in hair coloring, 282
- Pyrethroids, 666,
- Pyridinethione, zinc, 107
- Quasi-drugs, 316, 455, 460
- Quillaic acid, 93
- Quinoneimine, 27, 291
- Radiodermatitis, 655, 656, 669
- Raman spectroscopy, 421, 451
- Reconstructed skin model, 357, 358
- Reflectance indicator, 403, 406
- Reflection, 404
 - diffuse, 405

- internal, 405
- specular, 404
- Regreasing after shampoo, 135
 - hair, 134
 - scalp, 131
- Regulation of hair products, 455
- Relaxation process, 38
 - under traction, 410
- Relaxers, 222, 353, 378
- Resistance to fatigue, 414, 415
- Resorcinol, 281, 286, 291
 - co-oxidation with PPD, 284
- Rheological models (*See Models*)
- Ring method, 165, 387, 443
- Ringworm, 583, 649, 655
- Rinse, final (or neutralizing), in oxidationcoloring, 84, 92
- Risk assessment, 314, 328, 335
- ROAT, 494, 495, 500
- Root sheath, 68, 69, 546
- Rorschach test, 706, 707
- Rolling up process, 99, 100, 133
- Rub test, 505

- Safety assessor, 119, 315, 352
- Safety evaluation, 119, 313, 315
- Salmonella assay, 315, 319, 328
- Saponins, 92, 93
- Sarcosinates, N-acyl, 88
- Sarsaparilla, 92, 93
- Scaling, 107, 165, 521
- Scalp condition, 118
 - diseases, 641
 - flora, 613
 - regreasing, 131, 134
 - seborrhea, 131
 - tumors, 666
- Scanning calorimetry, 33, 47, 419
- Scarring alopecia, 511, 522, 644
- SCCNFP and CIR, 315, 479, 484, 488
- Scratch test, 505
- Sebaceous gland, 67, 71, 76
- Sebocyte, 586, 587, 598
- Seborrhea, clinical signs, 137, 585
- Seborrheic dermatitis, scalp, 130, 585, 600
- Sebum, 583, 613
 - casual level, 595
 - composition, 587
 - excretion, 595
 - hormonal regulation, 598
 - removal, 591, 595
 - spreading, 598

- Sebum excretion rate, 595
 - and age, 600
 - and neuropeptides, 704
- photometric evaluation, 593
- tape evaluation method, 592
- Secondary ion mass spectrometry (SIMS), 146, 421, 422, 423
- Selectivity, in hair coloring, 257, 261, 268
- Selenium sulfide 107, 626, 636
- Self-heating permanent waving, 217
- Self-oxidizing dyes, 269, 270, 272
- SEM studies, 43
- Semipermanent coloring, 266, 296, 434
- Sensitivity test, for hair dyes, 364, 474
- Sensitizing potential, predictive tests, 356, 360, 495
- Sensory analysis, 117, 377, 381
- Sensory evaluation, 184, 395, 439
- Series-zone model, 43
- Setting lotions, 169, 178, 238
 - colored, 257
 - evaluation of, 192
 - lightening, 238
 - principles of, 169
- Setting products, 172, 173, 192
- Shampoo
 - adverse reactions to, 512, 513
 - antidandruff, 113
 - baby, 113
 - basic mechanisms, 93
 - biodegradability, 122
 - cationic, 92
 - classic, 111
 - conditioning, 112
 - coloring, 109
 - environmental safety, 121
 - evaluation, 121
 - frequency, 83, 117
 - for greasy hair, 108
 - and occupational dermatitis, 535, 537
 - opacifiers, 109
 - pearls, 109
 - perfume, 109
 - safety, 118
 - silicones in, 106
 - styling, 112
 - thickeners, 108
 - treatment, 113
 - types of, 110
- Shellac, 181, 188, 200
- Shine, 402
 - contrast, 403
 - and hair treatments, 405
 - specular, 403

- Skin reactions to patch tests, 318, 497
Skin tests, 493, 509, 651
Silicones, 146
 glycol, 149
Siloxanes, 147
 cationics, 149
 copolymers, 149
SLIM program, 459
Soap bark, 92, 93
Soaps, 85, 144
Soapwort, 92, 93
Soil
 on hair, 92
 removal from hair, 115
Solvent-assisted dyeing, 266
Specular reflection (*See* Reflection)
Spectroscopic analysis, 421
Split ends, 5, 45, 143
Split-fuse hypothesis, 74, 75
Squalene, 144, 583, 587
Static curl retention test, 380, 381
Staphylococci, in scalp dermatoses, 638, 651
Steam, action on hair, 34, 203, 521
Stem cells, 74, 80
Steric stabilization, 101
Stiffness index, 414
Stinging test, 494, 515
 capsaicin, 515
 lactic acid, 494, 495
Strontium dioxide, 237
Straightening (hair), 201, 220
 alkaline technology, 203, 222
 reducer/oxidant technology, 204, 221
Strip harvesting, 680, 681, 685
Style retention, 46, 377, 378
Styling products, 174
 shampoos, 174
Sulfates, 85
 alkyl (*See* Alkyl sulfates)
 alkylether (*See* Alkylethersulfates)
 fatty alcohols (*See* Alkyl sulfates)
Sulfites, 201, 204, 205, 218
Sulfobetaines, 89
Sulfosuccinates, 87, 88, 106
Sulfoxylate, sodium formaldehyde, 218, 241
Sulfoxylate, zinc formaldehyde, 241
Sun-induced bleaching, 233, 247
 damage, 656
Suntest, 435
Surface (hair), 45
 charges, 425
 condition, 45, 46

- lattice model, 13, 14
- properties, 45, 388
- Surfactant, 84
 - and denaturation, 102
 - and hair, 102, 105
 - and skin, 102, 103
 - and scalp, 104
 - penetration, 100
 - protein interactions, 23
- Swelling properties (hair), 416, 418
- Szondi test, 706, 707, 714

- Tape techniques, 592
- Taurides N-Acyl, 88,
- Telogen (hair), 72, 73, 78
- Telogen effluvium, 139, 141, 559
- Telogen to anagen transition, 73
- Tensile properties, 32, 243
 - effects of hair bleaching, 245, 246
- Terminal hair, 69, 76, 129
- Tests in vitro, 356
 - for efficacy assessment, 357
 - for safety evaluation, 354
 - in safety evaluation process, 361
- Tetrathionate, 209
- Thermal properties (hair), 40, 47
- Thermal analysis, 47, 64, 419
- Thesaurosis, 194, 199, 200
- Thioglycerol, 208
- Thioglycollic acid, 203, 206, 208
 - and derivatives, 208, 210
 - formulations, 210
- Thiolactic acid, 208, 292, 539
- Thiols, 202, 225
 - pK of, 207, 208
 - in waving lotions, 207, 208, 217
- Time and extent application, 470
- Tinea amiantacea, 576, 633, 637, 647
- Tones, 230, 236, 239
- Tone on tone coloring, 259, 272, 296
- Torsion (hair), 32, 35, 36
 - modulus, 36
 - pendulum, 35
- Torsional stress, 40, 143
- Toxic alopecia, 570, 571
- Trace elements in hair, 22
- Traction alopecia, 511, 575, 689
- Tractiotrichogram, 140, 165,
- Transient irritant response, 516
- Transplantation (hair), 675, 685, 696
- Traumatic cicatricial alopecia, 579

- Triboelectric charges/effects, 401, 402, 446
Tribologic properties (hair), 392, 393
Trichanalectia, 142
Trichogram, 139, 141, 164
Trichohyaline, 70
Tristimulus colorimeter, 433
Trichophyton, 578, 583, 657
T. ochraceum, 661
T. rosaceum, 661
T. rubrum, 661
T. schonleinii, 661
T. soudanene, 660, 661
T. tonsurans, 661
T. verrucosum, 661
T. violaceum, 660, 661
Trichorrhesis nodosa, 512, 542, 544
Trichoiodystrophy, 545
Trichotillomania, 575, 662, 689
Triglycerides, 85, 135, 144
 in hair care, 145
 in sebum, 587, 588
Two-phase model, 43
Tyrosinase, 27, 30, 269
Tyrosine, 14, 21, 27
- UHS proteins, 17, 21
Uncombable hair, 544
Urea peroxide, 209, 237, 317
Urticaria type reactions, 516, 525, 526
US regulations, 486, 482
 definition of cosmetic products, 455, 462, 466
 labeling, 364, 455
Use-by date, 473
USAN, 476
- Value, 43, 86, 363
Vellus hair, 71, 564, 575
Viscoelastic properties of hair, 391, 398, 414
Vitamins, 146, 159, 570
Videotrichogram, 139
Vitreous membrane, 68, 73
- Water absorption/desorption by hair, 23, 24, 37
Waving (hair), 50, 83, 201
 acid, 208
 cold, 206
 evaluation of, 218
 hot, 204, 205
 indirect, 206
 lotions, 206, 207, 210
 self-heating, 217

- self-neutralizing, 209, 210
 - self-regulated, 216
 - soft, 212, 215
 - techniques, 215, 521
 - tepid, 206
- Weathering, hair, 30, 105, 143
- Wettability, hair, 47, 407
 - effects of oxidation, 407
 - effects of reduction, 407
- Weight of evidence approach, 333, 340
- Whn factor, 72, 77
- Wnt factor, 72
- Woolly hair, 543
- X-ray diffraction diagram, 8, 13, 42
- X-ray photoelectron spectroscopy, 421, 422
- Xenotest, 436
- Yield region, 33, 38, 42
- Young modulus, 38, 40, 409
- Zinc pyridinethione, 107