

General Cytology

An introduction to functional
morphology of the cell

EKKEHARD GRUNDMANN

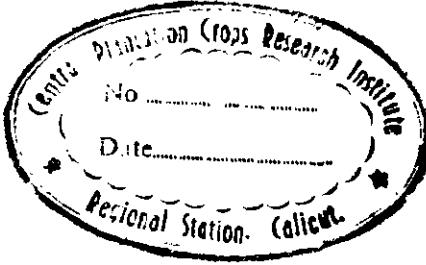
2 ISR
1675

576.3(62
3)

K6

IISR CALICUT





General Cytology

An Introduction to
Functional Morphology of the Cell

ISSR 1675

15

EKKEHARD GRUNDMANN, M.D.

Formerly Head Assistant at the Institute of Pathology, Freiburg University;
Professor of Pathology and Director of the Bayer Institute of Experimental
Pathology, Wuppertal-Elberfeld

English translation by
DIETER M. KRAMSCH, M.D.

Assistant in Medicine, Research Associate and Physician-in-Charge of the Laboratory for
Cellular Biology, Department of Atherosclerosis and Hypertension, Boston University
Medical Center, Boston, Massachusetts

EDWARD ARNOLD (PUBLISHERS) LTD. LONDON



© Georg Thieme 1966

First English edition 1966

English translation of
ALLGEMEINE CYTOLOGIE
Published by Georg Thieme Verlag, Stuttgart

German edition published 1964

Names that are registered trade marks at the same time are not especially specified as such. Hence, from the use of the trade mark as the name of a product it cannot be concluded that it is a free brand name, nor does it imply that these products are not patented or registered.

Central Pat. Office Cross
Registration No. 1675
Date 19.3.81

576.3 (0-)

K4

Printed in Great Britain by
William Clowes and Sons, Limited, London and Beccles

Preface

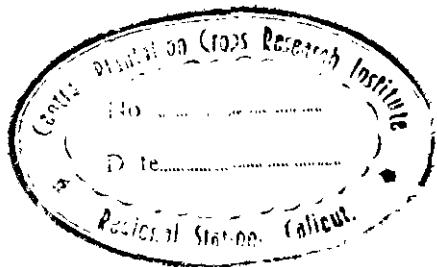
This book was originally conceived as an answer to questions. These questions in part resulted from my own efforts to solve limited problems and classify them within General Cytology; in part, they were brought up by attendants to a lecture on problems of general cytology. The field covered by these questions became gradually so extensive that in a few years the outline was drawn for a "General Cytology".

It is necessary to mention this evolution of the subject, for it may excuse my venturing to deal alone with the whole research in general cytology. This was not possible without many a restriction in this or that chapter, in which one may therefore miss essential points, nor was it possible without laying the stresses and accents according to my personal opinion. On principle, an attempt has been made to arrange and coordinate the chapters and their contents in a clear and logical way, without differing too much from the traditional order. This should enable the reader, as much as possible, to read each section separately and to understand it independently from the others; this I tried to facilitate by introducing many cross-references.

The book does not pretend to compete with those large surveys in many volumes, which were written by many competent specialists in their particular field. On the contrary, constant references are made to those exhaustive studies, for they give the details which are missing here. Furthermore, the book does not want to cover any problems of special cytology; they are mentioned only when necessary for the general understanding. "General Cytology" is meant to be a common preliminary for those who wish to devote themselves to special questions of cytological research. It is therefore intended as an introduction, where, in conception as well as in description, major importance is given to functional morphology, yet without overlooking, let alone minimizing the connections with biochemistry and cell physiology, which are in many respects the leading sciences today. Each of these disciplines will contribute to elucidate the other.

Thus it is apparent which types of persons this book intends to address. These are, first of all, other investigators, whose interest reaches beyond the limits of their special field in cytological research. The book is also meant to guide the beginner who is concerned with cytology or with any of its problems, at medical school or later. To meet his needs, an ample amount of literature has been cited; for reading a comprehensive survey is no substitute for studying the original report. This should not disconcert the third type of reader for whom this book is meant, that is, all those—and they are becoming rare—who are generally interested in this field. For those in particular I designed the plan of this book and carried it through wherever possible; for, after all, it was on their request that the book was written.

Such an extensive framework, where emphasis is laid on functional morphology, needs manifold support, particularly by illustrations which explain and exemplify the text. I made a generous use of them and I wish to thank all those colleagues, as well as the editors, who



kindly placed original photographs at my disposal and permitted their reproduction in this book.

Yet my most special gratitude goes to my teachers: Professor Dr. Franz Büchner, director of the Ludwig-Aschoff Institute, who with constant care guided my whole scientific work; Professor Dr. Hans-Werner Altmann, who stimulated my interest in the subject; and Professor Dr. Hans Marquardt, who supervised my studies and enabled me to gather first-hand experience from his botanic collection. Additional acknowledgements are due to all associates and students for the impetus they gave me with their questions and ideas. Yet, most of all, I want to express my thanks to the group in which my work could grow, all my associates at the Ludwig-Aschoff Institute, my scientific home. They contributed in various ways to the production of this book, passing to me their experience in our daily discussions, or sparing me their time and energy. May I name here first of all Miss Margarete Barner, photographer, Mrs. Irmgard Bühler, secretary, and Miss Inge Motsch, technician. I am particularly grateful to the editor, Mr. G. Hauff, for the broad-mindedness he showed concerning the problems arising from the composition of the book and his understanding of its requirements.

EKKEHARD GRUNDMANN

Kirchzarten bei Freiburg i. Br., 22nd of April 1963

Contents

Preface	v
-------------------	---

1. INTRODUCTION

The problem of the least unit of life	2
Morphology and definition of the cell	6
Origin of the first cell	17
Origin of cytology	21
Cytology today	25

2. THE NUCLEUS

Functions of the nucleus

The nucleus as repository of the genes and general centre of metabolism	28
Interdependence of nucleus and cytoplasm	30
Non-nucleate cells and <i>merotomy experiments</i>	32
Oxidative phosphorylations	33
Glycolysis	34
NAD (DPN) synthesis	35
Phosphate metabolism	35
Metabolism of RNA and protein	36
Review	41

The nucleolus

Shape and structure	42
Relation to the chromosomes	44
Composition	47
Significance and function	49
RNA metabolism. Relation to the cytoplasm	49–52
Review	56

The nuclear envelope

Composition and structure	57
Role of the nuclear envelope in nucleocytoplasmic transfers	61
Origin of the nuclear envelope	64
Review	66

The karyoplasm	
Vital structure and its variability	66
Representation after fixation	68
Persistance of chromosomal individuality	72
Tissue specific structures of the karyoplasm	73
Sex chromatin	77
Nuclear inclusion bodies and nuclear viruses	80
Death of the nucleus	87
Review	88
Size, mass and composition of the nucleus	
Volume	89
Water content and dry mass	92
Proteins	93
Desoxyribonucleic acid	96
Ribonucleic acid and other components	100
Review	100
The chromosome	
Number and size of the chromosomes	101
Constrictions	104
Euchromatin and heterochromatin	105
Fine structure	108
DNA and gene	114
Mechanism of the gene activity	115
Giant chromosomes	118
Review	123

3. REPRODUCTION OF NUCLEUS AND CELL

Mitosis

Cyclic changes in the morphology of the chromosomes

Changes in size and mass. Changes in spiralization. Chemical changes.

Behaviour of the nucleolus. The problem of the chromosomal matrix.

Longitudinal splitting. Review 127-131

Kinetocentres

Shapes and fine structure. Interkaryokinetic constancy. Polar Rays. Review 132-138

The spindle

The central spindle. The kinetochores. The chromosomal spindle. Fine

structure and composition. Review 139-149

Movements of the chromosomes

Prophase. Prometaphase. Metaphase. Anaphase. Telophase. Review 149-165

Cytokinesis	
Interzone structures and phragmoplast. Significance of spindle and kineto-centres. Changes in the cell surface. Review	166-174
Interphase	
DNA synthesis. Protein and RNA synthesis, changes in the nuclear volume. Behaviour of the cytoplasm. Respiration and glycolysis. The mitotic cycle and its stimulation. Review	174-188
Meiosis	
Premeiotic processes	189
The process of meiosis	191
Syndesis	197
Nature of the chiasmata	198
Review	200
Disturbances of mitosis and meiosis	
Interference with interphase	202
Chromosome injury	205
Spindle disturbances	209
Mitotic and meiotic disturbances as the origin of human diseases	211
Review	218
Polyploidy and endomitosis	
Occurrence of polyploidy	219
Polyploidy as a result of abnormal karyokineses	221
Endomitosis	221
Polyteny	225
Conditions in which endomitosis occurs and its significance	226
Review	227
Amitotic division of the nucleus	
Definition	228
Evidence of an amitotic nuclear division	228
The process of amitosis	230
Assumptions concerning the mechanism of amitosis	233
Result of an amitotic nuclear division	235
Conditions of amitosis and their assumed significance	237
Abnormal amitoses—nuclear polymorphism	239
Review	240
4. THE CYTOPLASM	
The endoplasmic reticulum	
Normal representation in the electron microscope	242
Ergastoplasm and its functional and pathological changes	247
Protein synthesis	249
Other functions	253
Review	255

The Golgi apparatus	
Evidence from light microscopy	256
Evidence from electron microscopy	258
Relationship to secretion	260
Other possible functions	263
Review	264
Cytoplasmic ground substance	
Normal representation in the electron microscope	266
Intracellular water transport	272
Coagulative necrosis. Reversible increase in denseness of structures. Cytoplasmic streaming. Amoeboid movement.	273-275
Intercellular water transport	276
Vacuolar degeneration. Vesicular degeneration. Pinocytosis.	277-281
Phagocytosis	286
The lysosomes	290
Deposits	291
Pigments rich in iron. Cytosomes. Pigments devoid of iron. Hyaline drops.	
Glycogen and lipids	291-295
Virus replication in the cytoplasm	296
Review	300
Mitochondria	
Representation in the light microscope	302
Normal representation in the electron microscope	304
Biochemistry and function	306
Isolation of enzymes by electron microscopy. Biochemical multi-enzyme systems. Morphological evidence of the mitochondrial function	306-310
Pathological changes	
Swelling. Biochemical alterations. Other structural alterations and destructions. Problem of regeneration. Review	312-321
Plastids	
Morphology	321
Structure of chloroplasts	323
Composition and function of chloroplasts	326
Chemical structure. Enzymes and photosynthesis. Function and structure	326-328
Formation of plastids	331
The plastidome	333
Review	334
Conclusion	334
References	336
Subject Index	415

I. Introduction

"In order to understand the invisible,
we must reach as far as possible into
the visible" (MAX BECKMAN)

The greatest enigmas lie at the border line which separates living from lifeless matter, life from death.

It was more than a milliard years ago, probably still in archaeozoic times, that life appeared on earth for the first time. The earth was then already very old. Many milliard years before, prodigious energies had concentrated, creating fixed orderly forms: the elements of the periodic system. At the same time, their creation was the origin of the diversity of the matter: gases, liquids, solids, subject to constant dynamic changes, and yet ruled by rigid laws. With the first manifestations of life, this order received a new principle which prevailed temporarily over the old laws. Creation gave birth to its first creature. A new mode of existence was created on earth, that of the living being. And death was created at the same time. Death became the order of the inorganic matter, life its temporary transgression.

We may assume that this new era had been prepared by many events. New molecules had appeared, arranging themselves into large aggregates, which acquired new properties such as increase of energy or self-reproduction. Then, these systems of molecules must have reached a certain equilibrium, composing themselves into structures which supported one another and set up outward bounds against any disturbance of this state of balance. A new, superior organic unit was born: the living cell.

There is much evidence that not just one, but many cells appeared at the same time. However, this is of no particular importance. Life started with the beginning of the cellular order. Thus a limit had been trespassed—the limit between life and death—which, from then on, was always apt to be trespassed. For the living cell not only maintained the new properties of its molecular aggregates—for example transformation of energy and self-reproduction—but blended them into a superior form of dynamism, in which, by virtue of metabolism, growth and excitability, the individual not only could subsist, but also could, and indeed, had to multiply itself. Life extended itself, acquired new forms, which became more and more differentiated, until it brought forth man. Yet through all these stages, life maintained the ground plan with which it started, i.e., the cellular organization of large molecules. This, indeed, is the earthly characteristic of all living matter.

The cell was the first manifestation of life, in the universe as well as in each individual, and its constant reproduction is a constantly recurring transgression of the boundary between the two great arbitrators of all that exists, life and death. This transgression is shrouded in mystery. When we study the living cell, when we recognize the wonderful harmony of its inner structure, we penetrate a part of this mystery. Yet we must not forget that we see only with human eyes and measure by human standards, even if we have managed to unveil many of nature's secrets. The picture we form is a human picture of living nature and, moreover, a surface one, since the methods of investigation can only be those of natural science. The very subject of this book is enclosed within these limitations.

However, as it is the case with many things that disclose part of their concealed aspects the more we get familiarized with them, so it is with the cell: it may also reveal more than the mere system of its mechanisms. At least it can arouse our admiration and fill us with respect for the daily cycle of its birth, life and death.

THE PROBLEM OF THE LEAST UNIT OF LIFE

What entitles us to place the cell, as we do, right in the centre of the process of life? Is the cell really the most important and the least unit of living matter?

We consider today the discovery of the cellular structure of all plant and animal organisms as the beginning of modern biology. This discovery is associated mainly with the names of Schleiden (1838) and Schwann (1839). All the organisms we know consist of at least one cell, and any larger one "appears as a sum of vital units, each of which bears in itself the complete characteristics of life" (Virchow, 1858a). As is the case with the atomic theory in physics and chemistry, this discovery of the past century has given the multiplicity of organisms a common inner bond. All living matter is made of structures which are basically the same. The differences arise on the one hand, from the relationship of the "living particles" to each other, and on the other hand, from the nearly unlimited variety of intracellular architecture.

The second major discovery of the past century in this field was the recognition of the fact that cells can only come from cells. We find this acknowledged with great emphasis by Kölliker (1844), and later by Virchow: "Wherever a cell appears, it must have been preceded by another cell" (Virchow, 1858a); in other words, as Virchow expressed it in 1855 in a remarkable concise formula three years before publication of his *Cellularpathologie*, "omnis cellula e cellula".

Both these assertions, the cellular organization and the cellular origin of all life, were bold, for we must admit today that the evidence on which they were based was most defective. By no means did it justify the broad generalizations made by their authors. We are all the more impressed by the genial intuition that led Virchow, then just 34 years old, to write his "omnis . . .", thus replacing the incomplete specific knowledge of the time by his insight into the interrelations of all living beings. He thereby proved to be a true disciple of the preceding natural philosophy, which his generation claimed to have just overcome. But he was also a typical child of his century, which laid the foundations of our scientific work. Today, we find it much more difficult to discover new relationships and great, clearly outlined theories have become rare among the innumerable and, as yet, unco-ordinated findings.

This theory that the cell is the most essential and, also, the smallest unit of life has been fought with great vehemence up to these last years. It has had to defend itself on two opposite fronts; on the one hand, against the concept that superior structural tissue units are of a greater importance than the order of the cell; on the other hand, against the postulation that there exist incomparably smaller units as least living particles.

Let us start by considering the main argument of the first school of thought. At first sight, it does seem incongruous that the structural elements of a leaf, for example, should be in any way homologous to those of the human brain. Botany knows at least 4000, zoology far

more than a million different species of multicellular organisms. Each of these organisms consists of a great number of specifically differentiated tissues, each playing a different part and having a distinctive function. How can such a multiplicity spring from one single principle?

Consequently, the pre-eminence of the cell as the "anatomical manifestation of life" (Virchow, 1858a) was vehemently contested in favour of multicellular organization units, on the grounds of histology, the science of the tissues. The model for these units appeared to be the central nervous system, where, between the cells, innumerable fine connection fibres could be demonstrated, which grow denser, the greater the central nervous system is developed (e.g., K. Fr. Bauer, 1953; Haugh, 1959). This discovery is to be attributed to Nissl, one of the great neurohistologists and neurocytologists of the past. At first supporting the cell theory, he explored essential details of the nerve cell (Nissl, 1894), but later, influenced by other findings, he abandoned as "obsolete" the idea of the nerve cell performing primarily the central nervous function. Rather, according to him, the substrate of brain activity was the substance which lay between the cell ganglia in the "nervous grey matter" (Nissl, 1903). Indeed, it was there that, later on, it was possible to stain the remarkable network of fine fibrils, so seeming to abolish the cell's individuality in favour of superior functional units (see, e.g., Stöhr, 1941, 1951; K. Fr. Bauer and Müller, 1959). Therefore Nissl and Bethe (1903) both fought vehemently until their death against the cell theory, the first, as an anatomically orientated investigator, the second, as a physiologist. The criticism based on their position subsisted up to quite recently (K. Fr. Bauer and Müller, 1959). It served also as a basis for the "Relationspathologie" and the "Neuralpathologie" by Ricker (1924), Speransky (1950) and Feyrter (1951), where superiority is also given to the nervous system over the cells.

Further: almost in all the other organs, tissue systems could be found, which were morphologically and functionally connected. In the kidneys, each glomerulus constitutes, with its multicellular, differentiated tubule and its collecting tube, a function unit, the nephron. The liver is organized not only morphologically into lobules, but also functionally into multicellular units, the hepatons, which have a synchronous activity. In the lungs, the histological function unit, which serves for respiration, is not the single epithelial cell but the alveolus, bounded by a subepithelial membrane. The protective function of the epidermis is only made possible by the intercellular substance. How, actually, do the extracellular tissue components fit into the cellular theory? What about the fibres, the various basement membranes of endothelia and epithelia, the cartilaginous and osseous structures, are they all dead if the cell is the only one that conveys life (see Cameron, 1952)?

From embryology came another important objection against the primacy of the cell theory. The development of the fertilized egg cell begins in almost all animals and in humans with a division of the egg cell into two halves, followed by a division of those egg cell halves into egg cell quarters etc.: these are the so-called cleavage divisions. In most insects, this cleavage is, however, merely superficial; in the centre of the yolk, only the nuclei divide themselves, and not the cytoplasm. Therefore, these cells end by having many nuclei within a uniform cytoplasm. A similar process occurs in the embryo sac of many seed plants, where a system develops of many hundred nuclei within a single undivided cytoplasmic area, i.e., within a single cell. Only later does each nucleus get enclosed in its cell. In this specific case,

does the individualization of each particular cell not seem to be a mere supplement to a process of tissue growth, which was already completed before?

Yet all these arguments did not succeed in destroying the cell theory. For this theory does not exclude that many cells may associate to form superior functional units. True, it is only through histological organization that the different tissues of a metazoan are more than just the sum of their components, the different cells; such is also the case with the whole organism, which is certainly more than the sum of its organs. The essential point, however, is that the functions of these superior units, the so-called histions (tissue units), are exclusively performed by the cells. For example, it is the liver cell that fulfils the function of the liver in metabolism and bile secretion. The secretion of excretory or incretory glands occurs exclusively in each particular epithelial cell. The histion is a functional community and is, as such, dependent on the function of every single cell. If one transplants a histion onto a culture medium detached from the organism, what will grow is not the tissue unit but the different cells as independent individuals; the histion generally disappears after a short while. Even in those cases where the main function of the particular organ was attributed, up to now, to extracellular membranes—as for example in the lungs or in the kidneys' glomeruli—the importance of the cells which adhere to these membranes has been increasingly recognized with the aid of electron microscopy.

The incomplete cleavage division of insect eggs and the polynuclear appearance of the wall that lines the embryo sacs of many seed plants turn out to represent, after more serious consideration, a mere dissociation of processes normally closely connected with each other: nuclear division and cell cleavage. In both cases, the former precedes the latter, and only after a stage of multicellularity does the division of the cell proceed and come to completion. We shall return to this problem when we explain the reproduction of the cell (see p. 166). At any rate, none of these facts contradicts the cell theory.

Another argument has now lost its weight. Modern electron microscopy has broken up the syncytial groups of cells, almost without exception, into single cells. Heart muscle, which was considered for a long time as an example of the special part played by a syncytium, is constituted of single cells joined together at the intercalated discs. The network of connective tissue fibres or the reticular structures of bone marrow, spleen and lymph nodes are formed by the cells. The intercellular substances of the squamous cell epithelium or the basement membranes of epithelia and endothelia are also, ultimately, condensed material from cells. However, each cell is strictly separated from the other, and no fibre runs through several cells or ends in them. The syncytium of the human trophoblast, which does not seem to show any cell boundaries, is less a syncytium than a plasmodium, resulting from nuclear divisions without cell divisions (Bargmann and Knopp, 1959a).

Electron microscopy, whose present fixation technique is particularly suited to demonstrate membranes, has thereby succeeded in demonstrating the individuality of the single cell, mainly in the central nervous system; the innumerable connections in Nissl's "nervous grey matter" have turned out to be fine cytoplasmic processes, which adhere to the neighbouring cells by small terminal knobs, but do not extend into them (Luse, 1956). In all of these so-called synapses, the neighbouring cells are strictly separated, albeit they constitute special functional junctions (Palay, 1956). The neuron theory, according to which the nervous tissue consists of many separate units, which each have nucleus, cytoplasm and processes (Cajal,

1935), is "but a particular case of the cell theory" (Spatz, 1952). The cell theory itself is more than a classification theory or a mere working hypothesis. It is the very structural principle which unites all tissues. This had been postulated by Leydig (1857) already one year before Virchow published his *Cellularpathologie*, and it is today more valid than ever.

Now what about the lower limit, leading down to the subcellular dimension? Are there really no other smaller units of life than the cell, and can a cell really only derive from another cell? This question did not cease to preoccupy Virchow. Twenty-two years after the publication of the *Cellularpathologie*, he wrote: "The last word has still to be said concerning life units and we must always be prepared to replace the cell by simpler units, as soon as we are convinced of their existence" (Virchow, 1880). Is this the case today?

The triumphant theory of the atomic structure of all matter, which places its units far below the limits of visibility, had such an influence that, already at the end of the 19th century, the cell seemed simply not small enough to be that last unit of life. Great is the number of authors who believed they had found the subcellular life units (ref. see Cameron, 1952). Among them, Richard Altman was the most authoritative, with his theory of the "elementary corpuscles" published in 1894 (essentially identical with his "granule theory"). Less well-known was Wiesner (1892), who gave his mysterious, smallest subcellular units the name of "protomeres". This expression was taken over later by Heidenhain (1907, 1923). He needed such a term, for the cell was, in his mind, "only a certain form of the life substance, consisting of living matter". Nor did Heidenhain believe that "the body of superior organisms represents a mere society of cells"; rather, he saw in them an "association of diversified structural elements (cells, muscle fibres, bundles of connective tissue, intercellular substances)", which, ultimately, are all made of protomeres. The "protomere theory" had many disciples over several decades (e.g., Hueck, 1926).

Today, such conceptions are still far from obsolete. They are frequently based on the fact that the cell contains self-reproducing elements, such as the chromosomes or the plastids—for example in the chromosomes certain molecules, the deoxyribonucleic acid (DNA), are necessary for the self-reproduction of the cell. These, with some restrictions, are representative of the gene substance which, according to the results of genetics, is transmitted unchanged, from cell to cell (see p. 114). Would it not be logical to see in those molecules the least unit of life?

This is confirmed by the findings of microbiology. Pure bacterial DNA is effective as a transforming factor, which changes the cell type; such is the case, for example, with pneumococcus, the typical bacterial agent of pneumonia (Avery *et al.*, 1944). Another fact leads to the same conclusion: many viruses, for example bacteriophages, contain DNA as the essential infectious component, with which they transform the metabolism of the host cell and utilize it for their own purposes (see p. 82). Indeed, have not the viruses already been discovered as being an infinitely smaller vital unit than the cell?

Today the answer to all these questions is clearly no. True, the homology of the cell's own DNA and the virus DNA exists beyond doubt. True, the identity of gene and DNA has been proved to a large extent. Yet a gene only "lives" in co-operation with other structures within a whole cell. All structures need, for their "living", the intact cell. This is also true of the viruses. A virus alone is deprived of life, i.e., it has no metabolism of its own, no transformation of energy, no excitability. The virus needs a living cell, to enter into its

metabolism and thereby multiply itself. Depending on itself, it is incapable of life. In the world as we know it today, the boundary between lifeless matter and living beings is well-defined. It runs between the large viruses and the small bacteria. Lifeless matter can only become a part of living beings through the direct mediation of the cells, or rather, inside the cells.

This occurs for example in every process of growth. Whenever new cellular structures are formed, or cellular substances are synthesized, for instance prior to a replication of the cell, the incorporated matter assumes the structural order of the living cell, which is passed over to the two daughter cells through the cell division. Here we have more than simply a new chemical orientation of the molecules; it is a biological structure formation. "Omnis cellula e cellula"—there seems to be, under the present circumstances, no other possible origin of the cell. This remains true, in spite of the reiterated assertions that protein aggregates, i.e., lifeless matter, are liable to produce living cells within a few minutes (see p. 18). This argument does not stand up to critical examination.

Thus we have today, more than one hundred years after publication of Virchow's *Cellularpathologie*, an unquestionable foundation for the science of the cell. The cell is the least unit of life we can apprehend. We shall now try and analyse it.

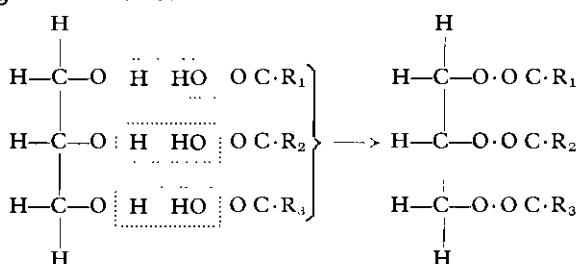
MORPHOLOGY AND DEFINITION OF THE CELL

Here we must simplify in the extreme, for no two cell species are alike. We shall consider the features common to all cells, in other words, the cell in general. What does it look like, what does it contain, how can we define it in its different parts and, eventually, in its whole structural complexity?

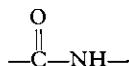
At this point, we discover something quite surprising. There is no difficulty whatsoever in finding features common to all types of cells. All cells have the same structure, whether we investigate plants or animals, whether we study unicellular or multicellular organisms. Indeed, even bacteria more and more fall into this category. Up to quite recently (see, e.g., also Milovidov, 1960), the smallest bacteria and certain blue algae were thought to be non-nucleate cells, which, in the most obvious cases, have certain equivalents to a nucleus. Today we are well informed about the cellular structure of these smallest unicellular organisms, and we know that they, too, have basically the same structure as all other cells, including a nucleus which can be very easily demonstrated (Piekarski, 1939a, 1949; Wilkinson and Duguid, 1960; and others).

Investigation of the chemical composition of the different parts of the cell led to a second surprise. The organic substance consists only of a relatively small number of the elements of the periodic system—mainly of C, H, O, N, P and, to a much lesser degree, of S, Fe and Mg, K, Na, Ca, as well as of a few rare elements. Viewed from the angle of organic chemistry, this preference for the first five elements mentioned here seems almost imperative; yet it may not be so, and there could perfectly well be other combinations. The same can be said of the single molecules. Organic systems consist predominantly of carbohydrates, lipids and proteins, each having their own characteristic properties and functions. Among the carbohydrates, D-glucose, a hexose (i.e., with 6 C-atoms), occurs most frequently and secondarily, a few pentoses. Precisely why D-glucose is preferred biologically is not

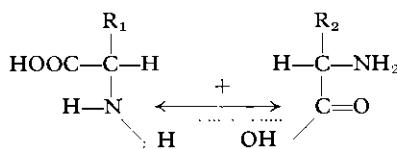
completely understood. The same is true of neutral fats, which are all triglyceride esters of fatty acids with long carbon chains:



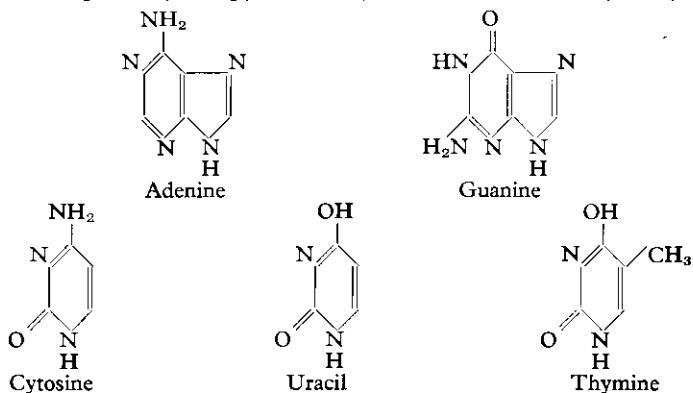
Moreover, of all fatty acids, only a few participate in fat formation. Similarly, proteins, which are particularly important for the variations in living organisms, are all made of amino-acids, of which nature, from the innumerable possibilities, uses by and large only 18, not counting a few rare forms. Those 18 amino-acids are always bonded together in the same fashion by peptide bonds:



i.e., by conjugating the amino group of one amino-acid with the carboxyl group of another amino-acid:



Furthermore, nucleic acids, discovered by Miescher (see, e.g., Miescher, 1871), which play a very special part in the cell's activities, all display a common structure, no matter where they occur in nature. They appear only under two forms: the desoxyribonucleic acid (DNA) and the ribonucleic acid (RNA). Both are uniformly composed of heterocyclic organic bases, pentoses and phosphoric acid and tightly bonded to proteins. The bases are derivatives of purine (adenine and guanine) and pyrimidine (cytosine, uracil and thymine).



As the sugar component, we find the pentoses ribose and desoxyribose, again solely in the d-form. Bases and sugar are linked by a C—N bond (N-glycoside bond) to form nucleosides, to which phosphoric acid is added, thus creating nucleotides, which are the basic components of the highly polymerized nucleic acids. It is interesting to note that the ester-like adjunction of the phosphoric acid to the biologically important nucleic acids takes place at a very definite point, viz., at the CH₂—OH group of the ribose. We will have to discuss the details of structure and composition of the highly polymerized nucleic acids in connection with the chromosomes.

Carbohydrates and lipids serve mainly for the production of energy. The structures of the cell, on the other hand, consist mainly of proteins, partly of lipoproteins and secondarily of nucleoproteins. The latter appear both in the nucleus *and* in the cytoplasm; actually, the term *nucleoprotein* is obsolete.

A first classification of the *protoplasm* (i.e., the whole of the cell substance) is given by the dualism of nucleus and cytoplasm (Fig. 1). The *nucleus* is a viscous vesicle in the interior of the cell, containing, beside acid and basic proteins, both nucleic acids, but mainly DNA; the latter appears only exceptionally outside the nucleus (see p. 97). By *cytoplasm* we mean the rest of the protoplasm excluding the nucleus. It is surrounded by the cell membrane, composed of proteins and lipids, but mainly of carbohydrates.

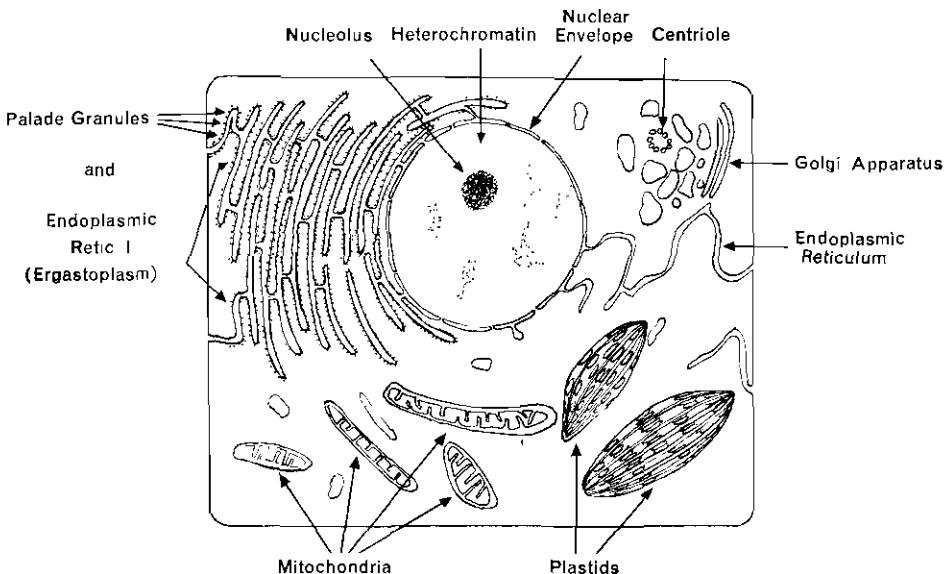


Fig. 1. Schematic representation of a cell with its organelles.

The *nucleus* is a spheroidal structure and lies frequently almost in the middle of the cell. Its shape and position depend, however, on the cytoplasmic components; for example, in an epithelial liver cell, congested with fat, the nucleus is pressed flat against the inner layer of the cell membrane by the fat deposits. Its noticeably constant tendency to round itself

indicates an inner turgor, detectable indeed in isolated nuclei or in living cells with the micro-needle. It can be pushed about freely within the cytoplasm. It is difficult to observe the nucleus in a completely untreated cell. For, even with the phase contrast microscope, which makes visible the phase displacements of the light waves, otherwise invisible to the eye, most of the details of the nucleus cannot be recognized. It is only after staining with basic dyes, that we can discover dense masses, different in size, the largest of which are called *chromocentres* (Fig. 2). Today we know for certain that they are parts of the chromosomes, which have retained the condensation of their previous mitosis.

Smaller lumps of the *karyoplasm* or *nucleoplasm* (term used for the entire nuclear content except the nucleoli) are of chromosomal nature. The very fact that, at the beginning of a mitotic division, the greater part of the nuclear substance goes into the chromosomes and

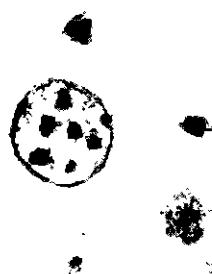
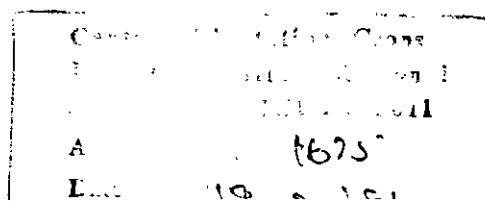


Fig. 2. Nucleus of the rat liver with typical chromocentres.
Feulgen nuclear stain.

reconstitutes itself again from those chromosomes at the end of mitosis, shows that the nucleus consists predominantly of chromosomal matter. Very likely, in the "resting nucleus"—as the nucleus is often called when it is not in the process of a mitotic division—the chromosomes remain as separate entities.

The functions of the chromosomes are very closely dependent on their chemical and morphological structure. For they consist largely of DNA and are the typical and, usually, only structures containing this acid. DNA is, as a rule, complexed with the basic protein histone to form nucleohistones. In accordance with their molecular structure, these are arranged in long threads, generally paired. Microscopy, too, shows that the chromosomes are ordered into bundles of numerous fibrils, which are visible, in many nuclei, in the electron microscope and even, though rarely, in the light microscope. The nucleohistones of the chromosomes are the material substrate of the genes, the heredity units of the nucleus. They control the main metabolic processes of the cytoplasm, more precisely the synthesis of proteins and thus determine the specific character of the cell functions. The mediator material is RNA, which transfers the genetic information into the cytoplasm and delivers it directly or indirectly to the executive organelles located there. In these processes, which are of major importance for the cell, the *nucleoli* play a significant part. They consist largely of acid proteins and RNA. As seen in the electron microscope, they contain a finely granulated matrix and a vermicular *nucleolonema* (Fig. 3).

Electron microscopy shows that the nucleus is surrounded exteriorly by two membranes. In favourable specimens and with optimal fixation, one can see small pores, which punctuate the envelope and establish an access, at least potentially, between nucleus and cytoplasm.



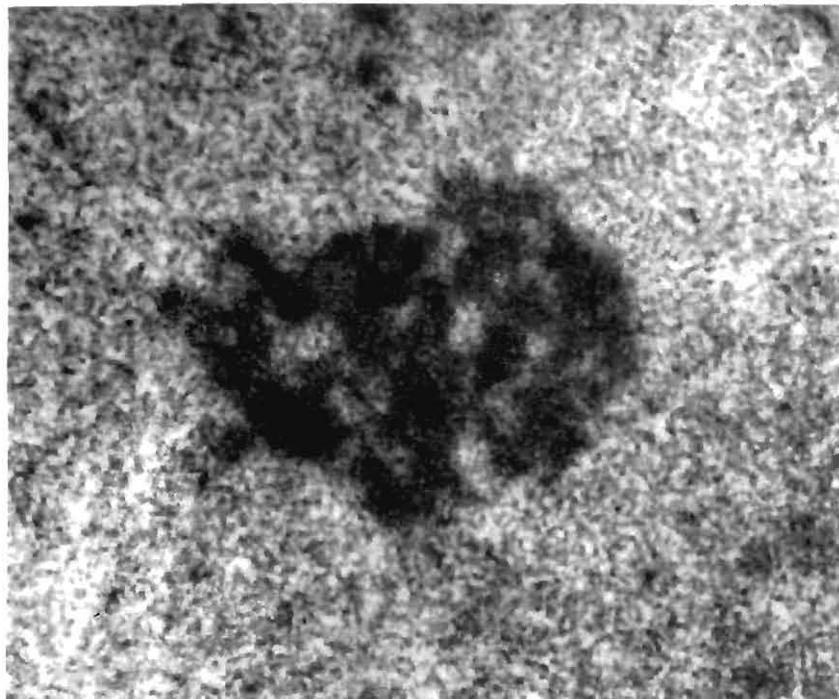


Fig. 3. Electron micrograph of a renal epithelium cell showing nucleolus with typical nucleolonema. $\times 38,100$. (Courtesy G. Thoenes.)

Cytoplasm consists of the most varied structures the most important of which are only mentioned here (see Fig. 1).

Those two membranes, which surround the nucleus, enclose a narrow perinuclear, fluid-containing cistern; they represent parts of a complicated system of tubules, extending throughout the whole cytoplasm as an "*endoplasmic reticulum*" (Fig. 4).

This endoplasmic reticulum is a finely structured and, in its function, doubtless very variable system of tubules, each $50-150\text{ }\mu$ in width, which in some places build small cisternae and are presumably connected with the extracellular space (Fig. 1). They have the function of continuously transporting liquid, in a constant exchange of material between the cell and its environment. Small slender protuberances (microvilli) and invaginations of the membrane enveloping the system of canaliculari (the α -cytomembrane) provide a considerable enlargement of its surface area. Ruska (1959) compared this to moles, lagoons or locks, which facilitate the exchange between the liquid content of the canaliculari and the cytoplasmic substance. The exterior aspect of those α -cytomembranes often shows osmiophilic and therefore in the electron microscope clearly visible granules, the *ribosomes*; these are also called, in reference to their discoverer, *Palade granules* (Fig. 4). They consist mainly of RNA, i.e., to be exact, of a relatively high molecular form of RNA with a molecular weight of 2 millions.

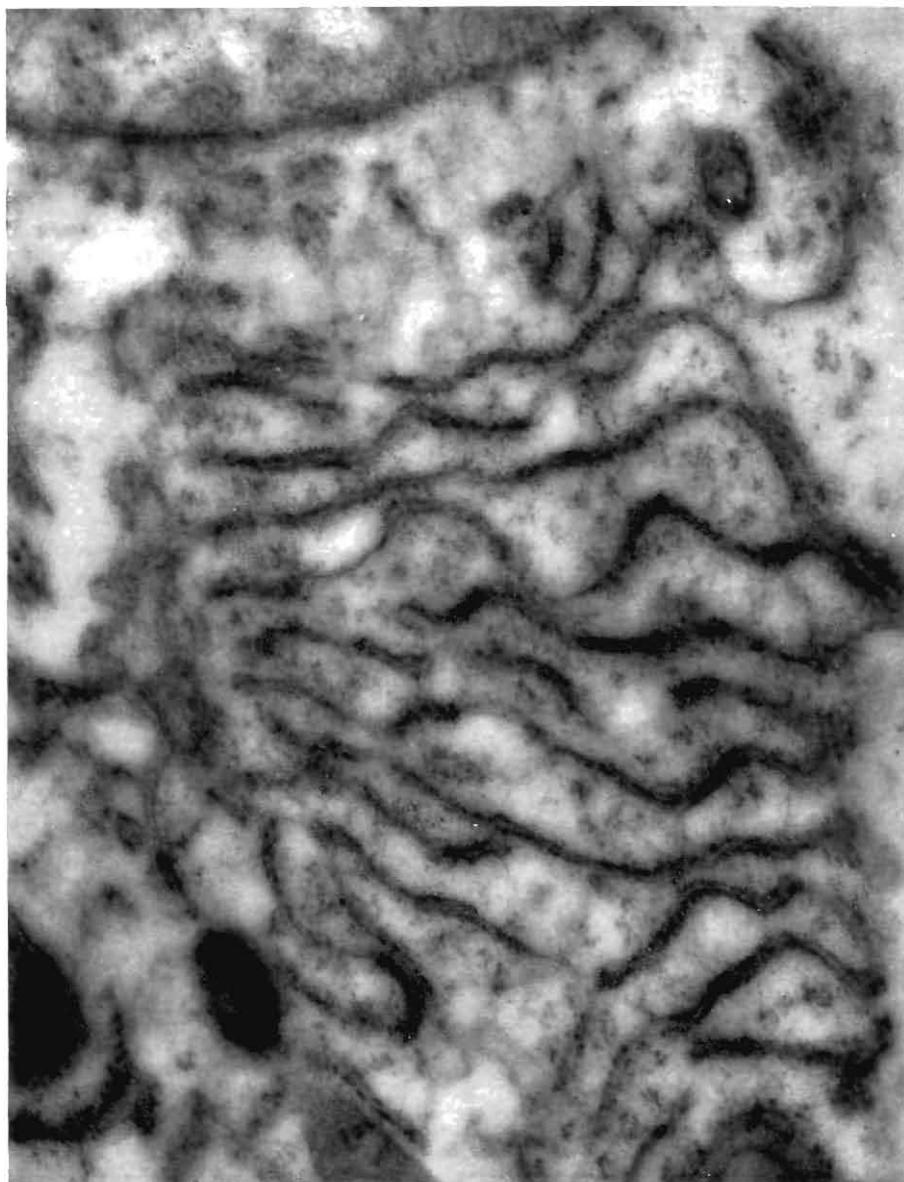


Fig. 4. Electron micrograph of a rabbit liver cell showing part of the lamellar endoplasmic reticulum with ribosomes at the outer aspect of the double lamellae. Note slight translucency of the cytoplasmic ground substance caused by acute hypoxia. *Top:* a part of the nucleus; *bottom left:* a microbody. $\times 40,000$. (Courtesy E. Mölbert.)

They participate in protein synthesis and because of their characteristic staining properties account for the cytoplasmic basophilia. The α -cytomembranes are particularly well developed in secretory cells and thickly settled with ribosomes, thus displaying particularly clearly the basophilic structures of these cells. This explains the term "ergastoplasm" which was used to describe them at the end of the past century. The ribosomes can be isolated for biochemical analyses by centrifugation at an acceleration of 50,000–100,000 g, yet they are always associated with fragments of the cytoplasmic membranes. Together they form the *microsomal fraction*, a term used today only in biochemistry. It contains 50% protein and approximately 40% RNA.

The space between the canalliculi is called *cytoplasmic ground substance*. Its composition is the least well-known of all. Yet it constitutes, in volume, the major part of all cytoplasmic components and is probably their matrix. The electron microscope reveals its details only with difficulty. It contains a variable quantity of free ribosomes, as well as all those deposits which may appear in the cytoplasm, particularly under pathological conditions, such as pigments, fat deposits, protein material, and also crystals or other corpuscles. The cytoplasmic ground substance is often called *hyaloplasm* because of its hyaline aspect. It contains also the cytoplasmic organelles (mitochondria, plastids etc.). Besides, it must probably be the site of essential metabolic processes. Unfortunately, it is difficult to isolate this ground substance for biochemical analyses. True, by fractionated centrifugation we obtain, after isolation of the corpuscular fractions, a supernatant, which was often thought to be identical with the ground substance of the cytoplasm. However, we do not know whether, by this method, part of the ground substance, i.e., the part with the highest specific gravity, does not deposit with the particle fractions, so that we should have to attribute many more enzymes to the ground plasma than can be found in the supernatant fraction. For this ground plasma probably contains protein molecules of different sizes, lipids and water, components which have very different specific gravities. Presumably, the whole glycolytic degradation occurs in the ground substance of the cytoplasm according to the scheme of Embden–Meyerhof. It is also likely that the precursors of the metabolism of both nucleoproteins and proteins originate there. To date it is not yet possible for us to localize exactly all these cytoplasmic processes.

On the other hand, another essential metabolic process of the cell can now be localized with certainty, namely, that of respiratory chain phosphorylation. This, at least in the cells of metazoans, is almost exclusively situated in small, threadlike or rodlike structures, embedded in the ground substance of the cytoplasm, the *mitochondria*. These can be isolated by centrifugation as a well-defined fraction, in which all the substantial components of the cellular respiration can be demonstrated (oxidative phosphorylation as well as transport of electrons to oxygen via the cytochromes). If the mitochondria are destroyed, the oxidative phosphorylation is at first reduced, then completely discontinued. Many enzymes can be extracted; others adhere rather firmly to the mitochondria, i.e., they adhere more specifically, as can be seen in the electron microscope, to a system of fine membranes, which partly enclose the mitochondria, partly traverse them as a series of plates (Fig. 5). These plates, described as "*cristae mitochondriales*" or microvilli (small processes) or tubuli (small tubes), form the inner structure of those corpuscles and are, according to most observations, the site of oxidative phosphorylations.

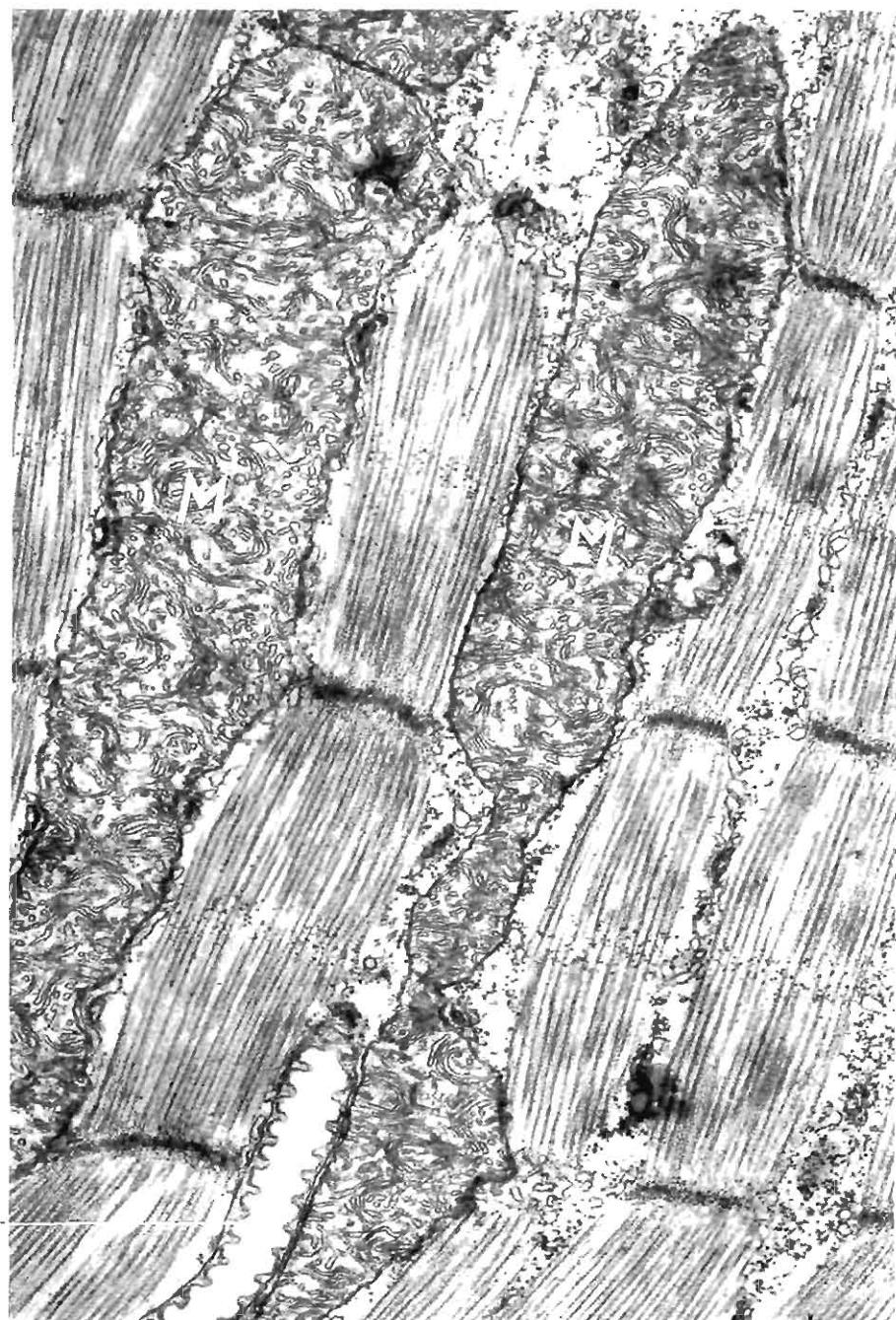
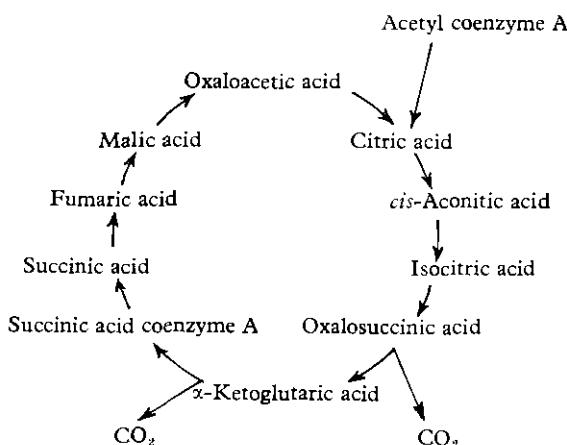


Fig. 5. Electron micrograph of part of a muscle cell from the wing muscle of the African migrant locust, showing a multitude of inner structures in the large mitochondria (M). Bottom left: a tracheole (Tr). $\times 30,000$. (Courtesy W. Vogell.)

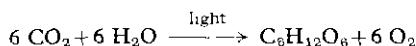
Apart from these phosphorylations and the electron transport in respiration, mitochondria are also the centres of the Krebs citric acid cycle:



This cycle plays a major part in the metabolism of carbohydrates, fats and proteins, for it controls the degradation of all three nutritional substances. This multi-enzyme system is evidently located near the enzymes of the respiratory chain, which perform whatever oxidations are necessary to the Krebs cycle, mainly the oxidation of DPNH. Whereas phosphorylations presumably take place on the membranes, it has been suggested that the components of the Krebs cycle are located in the matrix, i.e., in the fine granulated substance seen in the electron microscope between the cristae in the interior of the mitochondria. The mitochondria are at the centre of the entire energy metabolism; they are "the cell's power station".

The same could be said of other, larger organelles in plant cells, the *plastids*. When they contain the green pigment of leaves, chlorophyll, they are called *chloroplasts*. When they enclose predominantly other colour compounds, such as carotenes or xanthophyll, they determine, as *chromoplasts*, the colour of most flowers and fruits. The great variety of plastids is mainly responsible for the magnificent colouring of nature around us. But there are also colourless types of plastids, the *leucoplasts*, that as storage organelles manufacture and store starch or oil.

The green plastids, the chloroplasts, in the process of photosynthesis transform, by means of their green pigment, the energy of sunlight into chemical energy. Thereby, they absorb CO₂ from the air, bind it organically and produce glucose and oxygen from carbon dioxide and water, according to the following, though very much simplified formula:



The so-formed plant glucose and all substances which derive from it are the basic nutritional elements for animal and man. There is good reason for calling the activity of the green plastids the fundamental reaction of all living beings, for alone this constant absorption of sunlight energy makes life in its present form possible.

As was the case with mitochondria, the metabolic activity of the plastids relies also on a complicated structural system (Fig. 6). We find here disc-shaped corpuscles, the *grana*, composed of lamellae lying parallel to each other, and as with mitochondria, the integrity of those fine structures is the condition for a normal metabolic activity. The chlorophyll molecules can perform photosynthesis only when they are arranged as such lamellae. Despite the similarity of their chemical composition, we must, however, strictly separate the plastids from the mitochondria, for they are completely independent structures.

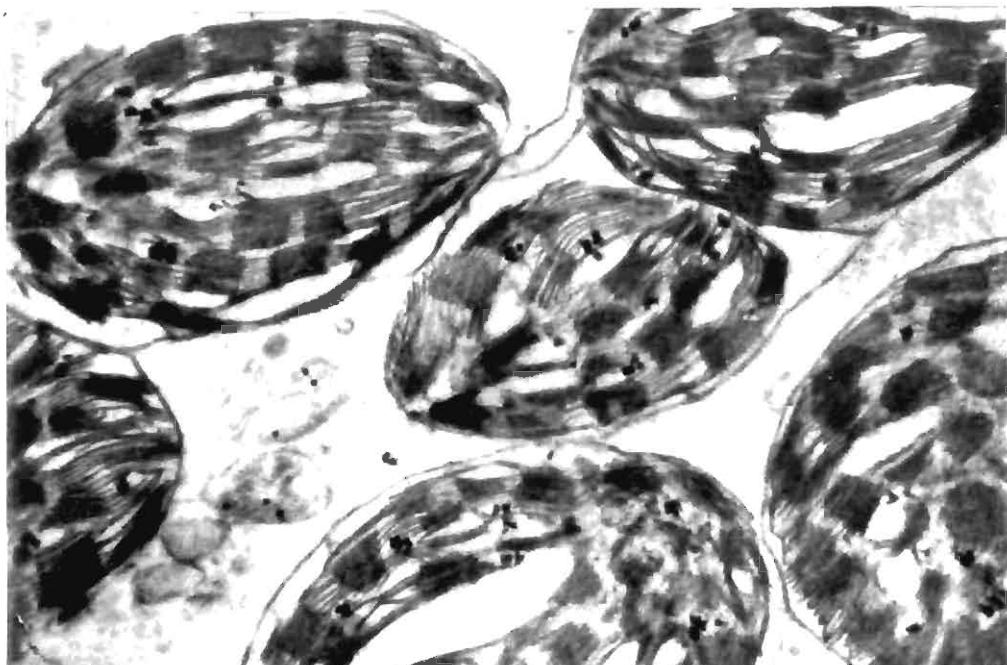


Fig. 6. Electron micrograph of chloroplasts showing typical grana and stroma structure. $\times 20,400$. (From Mühlthaler, K. (1960), *Dtsch. med. Wschr.* **85**, 1065-1067.)

Another independent structure is the *Golgi apparatus*, a region of the cell described by its discoverer Golgi in 1898 as "apparato reticulare interno". It was first detected, by impregnation with silver nitrate, as a network in ganglion cells, and was later found in many other cells by the same method. Electron microscopy has solved the long lasting argument of whether it represents a vital structural complex or only a staining artifact. For, in the electron microscope, the Golgi apparatus appears to be a system of membranes which, like flattened sacs, bound irregularly shaped spaces. Several of these sacs lie next to one another, surrounded by small vesicles. We will deal later with the problem of whether all these spaces are connected with each other and with the canaliculi of the endoplasmic reticulum (see p. 260). There is

still much to be discovered concerning the function of the Golgi apparatus. Suffice it to say here that the striking development of the Golgi region in secretion epithelia suggests its participation in the secretion process.

Lastly, the *centrosome* is a special type of organelle. It can only be demonstrated in a limited number of cell types, but probably it has functional equivalents in all cells capable of a mitotic division; for its function is closely related to mitosis (p. 132) and, further, to the movements of cilia and flagella. Thus it appears in both cases as a centre of well-regulated movements; yet how as such, is still full of enigmas.

All these complicated structures, comprising the cytoplasm with its various organelles and the nucleus, are bounded by the *cell membrane*. Its first function is, of course, to bound and protect the cell contents, but it also has the function of maintaining relations with the neighbouring cells for the formation of cell aggregates or tissues. The third essential function of the cell membrane is to select and control the substances permeating into and out of the cell.

In plants, it has still another function, i.e., the formation of a firm framework for the whole organism. For this particular purpose the envelope of plant cells is usually thicker, i.e., it forms a *cell wall* of many layers, which affords the protoplasm a special protection, as for example against loss of water. It consists basically of three layers: an intercellular middle lamella composed of calcium or magnesium pectates (in wood tissues it contains great quantities of lignin), a primary wall made of pectins and hemicelluloses and frequently a quite large secondary wall composed of polysaccharides, celluloses and hemicelluloses. All these layers of the cell membrane are products secreted by the cytoplasm, and they can outlive the cells for hundreds or thousands of years.

In animals we find similar firm cell walls only in protozoans; some types surround themselves with specific envelopes or shells. Generally, protozoans have a most diversiform covering, the *pellicula*, which can even have the hard consistency of a carapace; as the cell wall in the case of the plant cells, the pellicula here determines the final shape of the animal, and makes any change in shape impossible. Contrarily, the cell membrane of metazoans is very fine and is often not visible in the light microscope. It is, as in plants, also a product of the cytoplasm. As seen in the electron microscope, it is generally only 8 to 10 m μ in width and can have a smooth surface; yet, in certain cases, it may be very irregular because of brushborders or finger-like evaginations, which enlarge its surface area considerably. Special mechanisms serve for the absorption of substances into the cell. One of them is pinocytosis, which has led to a complete revision of the old conception, according to which the cell membrane would be more or less rigid. Most likely, the cell membrane of many metazoan cells is constantly undergoing rapid changes in its inner structure. Yet it remains as the outward bound of each particular cell and, wherever two cells are situated in close contact to each other, the electron microscope can always demonstrate the presence of two separate membranes.

This is the general picture of the cell, these are its elements. This is the architectural pattern of the smallest living elements in nature; and although we find the same design everywhere, it is capable of the utmost differentiation by virtue of its nearly unlimited variations. Evidently, each of the above-mentioned structures is necessary for the life of the cell, except for the Golgi apparatus, which perhaps plays a role in specific cell activities.

Even plastids are indispensable, although they work in plant cells indirectly, so to speak, for animal cells.

Let us now, in conclusion, try and formulate a *definition of the cell*. Today, we can still refer to it as being basically "a lump of protoplasm with a nucleus within it". However, this old definition by Schultze (1861) is no longer sufficient (see Milovidov, 1960). What we see in the cell today is a specific, well-ordered, living system, constituting in itself a harmonious organic unity, capable of storing energy and reproducing molecules and structures as well as producing new ones. There is, to our knowledge, not a single superfluous element in the whole system. Chromosomes, with their specific structure, are absolutely necessary; so are the nucleolus and nuclear membrane; so are the mitochondria, which perform oxidation; so are the centrosomes, which control the division of the cell, and even the cytoplasmic ground substance appears to be the matrix of all other structures and to execute vital metabolic functions.

The cell is the smallest functional unit, composed of elements which counterbalance one another in an endogenous equilibrium and complement one another to carry on the activities of life: metabolism, autochthonous reproduction and specific responses to stimuli. This is the definition of the cell valid today.

ORIGIN OF THE FIRST CELL

The above definition must be our starting point if we now want to examine a major problem of modern biology, namely, the presumptive origin of the first cell. Since we are familiar with life only as a cellular organization, we must consider the origin of the first cell as corresponding, in time and substance, to the origin of life itself. Let us anticipate and say that we are faced here with a process that we do not understand, or at least that we do not yet understand.

But we dispose of many good hypotheses, and, most important of all, of many findings which make these hypotheses acceptable and even partly probable. We will be mainly concerned with these findings in the course of this chapter. We have two reasons for according them such paramount importance.

1. Ever since Virchow's "omnis cellula e cellula" (1855) was recognized and accepted, ever since Pasteur's experiments showed that life can only originate from life, the question has always been raised again and again as to where the first cell came from. An attempt to reduce the problem to the smallest intracellular units, such as the genes or the viruses, which have much in common with the genes, led to a dead end. First, these smallest units again originate from nowhere else but from themselves. Secondly, they all need for this purpose, without exception, intact living cells, most of which require an intense metabolism of their own. We have brought up these problems already once, when we were dealing with the question of the least living unit.

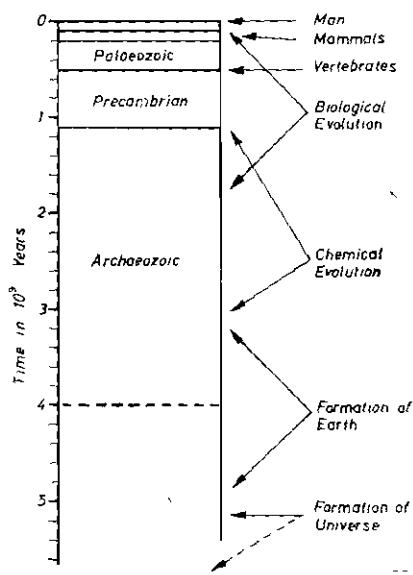
True, there have been, every now and then, descriptions of a more or less sudden generation of living cells from a lifeless substance. This type of "*generatio spontanea*" bears the same characteristics as the theories of the past centuries, according to which living organisms such as worms, crabs or even crocodiles could be born from mud (see Shakespeare's "Anthony and Cleopatra"). Goethe himself, answering an official request from his duke,

stated in 1826 that one could produce fleas from a mixture of sawdust and urine (related by Höhn, 1961).

The modern "generatio spontanea" is applied, however, to smaller subcellular elements, supposedly already capable of living, which are assumed to originate either intra- or extracellularly. In German language publications Busse-Grawitz (1946), for instance, attempted to demonstrate this and in Russia, among others, Lepeschinskaja (1951). All these experiments start by denying that the cell is the smallest elementary organism, and in conclusion that life is also possible outside the cell. In Russia, this theory was strongly contradicted by Zhinkin and Mikhailov (1958). In Germany, however, this doctrine of the cell's generation from non-cellular elements always finds isolated advocates (e.g., Elkeles, 1961), even though all such findings have been proved by critical examination to result from impurities or misinterpretations.

2. All living organisms are built, as we pointed out earlier, according to the same cellular pattern. Further, all cells contain basically the same organelles, which have the same functional relation and all these organelles consist essentially of the same elements (p. 6). This can only have one plausible explanation: all organisms are related to each other and have common ancestors, even maybe one single ancestor or a couple of ancestors. These must have been single cells, a few authentic mother cells. They generated, in a grandiose evolution, the whole of the universe of organisms. We call this process the "biological evolution of organisms".

This conception is based on the assumption that every superior species originated from a more primitive one, and that throughout the whole world of living beings, an unbroken line of evolution can be traced, at the end of which we find man. Let us not mention here the arguments for and against this theory. The important fact is that this evolution theory has, at least since Darwin (1859), ruled and animated the entire modern biology.



The same conception was applied also to the generation of life itself, called "chemical evolution". This evolution fills the two milliard years between the formation of the earth and the beginning of biological evolution (see Fig. 7), i.e., before the first cell appeared. During that time, according to the "hypothesis of probionts", many intermediate stages developed before the coming of life. Previous attempts to locate the origin of the first earthly cell in germs, which have fallen from the universe onto earth (the cosmozoan hypothesis), did nothing else but

Fig. 7. Diagram of the evolution phases of the earth. (After K. Höhn.)

place the site of the first cell formation on to another celestial body; it was, therefore, no satisfactory explanation (see, e.g., Zimmermann, 1960).

Let us now examine cautiously the stages of this chemical evolution, which might possibly have led to the first cell.

During the estimated 2-3 milliard years that the earth had existed before the first cell appeared, surface molecules had condensed and cooled down to such an extent, that they formed the relatively thin earth crust which still supports us today. Simultaneously, it had become possible for small molecules to fuse into larger ones, that had more than one C atom. This could have happened in many different ways (Calvin, 1956): (1) Heterocyclic compounds or amino-acids could emerge, under the influence of ultraviolet rays, from a combination of simple carbon compounds with N-containing molecules such as ammonia or nitrates. (2) Oparin's investigations (1938) lay the emphasis on the generation of large chains of molecules from acetylene, which might have formed itself from water and metallic carbide during the cooling of the surface of the earth. (3) The energy-rich cosmic rays, too, can generate simple organic substances. Thus it is possible to obtain experimentally, in the cyclotron, formic acid and oxalic acid from $\text{CO}_2 + \text{H}_2\text{O}$ and, by irradiating substances with 2 C-atoms, to produce some with 4 C-atoms, such as succinic acid, i.e., substances which play a great part in the entire intermediary metabolism. Glycine too, a simple amino-acid, could be produced by a similar method (Calvin, 1956). (4) It is probable that the original atmosphere was composed mainly of hydrogen, ammonia, methane and steam, and, therefore, did not have oxidizing—as today—but reducing properties, as is the case now with the atmosphere of the big planets Uranus, Jupiter and Saturn. In such a gas combination, Miller (1955) succeeded in producing, by means of electric discharges, a whole series of amino-acids, and, moreover, precisely those kinds of amino-acids which build the natural proteins. By adding sulphur and iron to the experimental combination, he furthered the results considerably.

This meant more than the mere experimental production of an organic substance. It had been demonstrated that the amino acids which are generated under these conditions are also those of the living cell, i.e., that a selection is possible. Now it became relatively clear why living matter is restricted to a fairly small number of chemical compounds, why a selection occurs which is by no means accidental. Probability calculations have led to the conclusion that, if such a selection did not take place, even the whole presumed time of the earth's existence—at least 5 milliard years—would not be enough to produce one single molecule capable of a biological function, that is, not under the conditions assumed to be present on earth at its origins (see Höhn, 1961).

This selection is facilitated by the presence of a catalyst, which gives the reaction a certain direction. In the above-mentioned system it was iron, which, like other metals (Co, Mg) can develop catalytic properties. Development and differentiation of those catalysts may have been the next step in chemical evolution; for example, the catalyzing effect of iron increases by a factor of 1000 when it is a component of porphyrin. Calvin (1956) was able to show how the very stable porphyrin can be produced from pyruvic acid and glycine, i.e., from compounds which themselves can be derived from simple molecules. Porphyrins are essential elements of our blood pigment and of the chlorophyll in plants.

Porphyrins still have another, most fundamental property: they can form more of them—

selves, i.e., they are prone to autocatalysis. Once they are formed, they soon generate other molecules of the same kind under certain experimental conditions, thus giving evidence for one property of living matter, the autoreproduction of large molecules.

Yet the main problem is the production of proteins. These macromolecular structures have, by virtue of the sequence of their amino-acids as well as their three-dimensional arrangement, an extremely high specificity and marked individuality; they represent the ground substance in cells. However, they are most unstable, and, in a chemical system of amino-acids and polypeptides, the production and maintenance of these macromolecular structures demands energy, even in the living cell. *In vitro* this problem has also been successfully tackled during the past years (see Boschke, 1960). One of the experiments consisted, for instance, in letting methane bubble through an aqueous solution of ammonium chloride, then adding iron sulphide and irradiating the system with ultraviolet light; after processing this mixture, peptide-like substances were found (Miller and Urey, 1959). Other experiments led even further. A mixture of amino-acids, in the presence of glutamic acid or aspartic acid, generated under high temperatures (170°C) a so-called proteinoid, i.e., a protein-like structure, which could be given to bacteria as a substitute for peptone; the bacillus then grew slower, but it grew (Fox *et al.*, 1959). By adding polyphosphoric acid to such a system, it was possible to produce, at a temperature as low as $70\text{--}100^{\circ}\text{C}$, proteinoids which largely corresponded to the natural proteins; they contained the 18 most important amino-acids and infra-red analysis showed that they had peptide bonds.

From this we can see that the peptide bond is, at least under those conditions, a most probable and by no means merely accidental reaction. We find here once more an almost imperative regularity; whenever such conditions are present, there appears a sort of protein. However, this protein is still rather "non-specific"; it does not have, for example, any antigen quality, i.e., it is incapable of stimulating antibodies in animal organisms.

Further experiments have shown that this same method can even produce elements of nucleic acids. By heating an aqueous solution of ammonia and hydrocyanic acid to a temperature of 90°C , one can obtain a dark mush containing, among other compounds, adenine (Oro, 1961a). In this reaction, one molecule of adenine emerges from five molecules HCN—the molecular formula for adenine is $\text{C}_5\text{N}_5\text{H}_5$ —and ammonia is not used up (Oro, 1961b). In another reaction system urea and malic acid can in the presence of polyphosphoric acid and likewise under high temperatures of $100\text{--}140^{\circ}\text{C}$, produce another element of nucleic acids: the pyrimidine derivative uracil (Fox *et al.*, 1961).

Thus we can imagine a phase in the evolution of the earth, during which an abundance of "probionts" were generated, owing to an accumulation of macro-molecular compounds, electric discharges and, in limited areas, extreme temperatures within a reducing atmosphere. There may possibly have been enormous quantities of such "precursors of life" with an even greater quantity of "useless" organic substances, and we have good reasons to assume that our petroleum and natural gas are, for a great part, "probionts" waste products (Kropotkin, 1958).

If we pursue this evolution theory further, we should find as the next stage a stabilization of the macromolecules, for example in the form of the coacervates, to use the term given by Bungenberg de Jong (1932) to colloidal droplets of large molecules. Oparin (1957, 1963) believes he has found here the clue to the subsequent evolution. For, indeed, such a coacervate

can originate from proteins, absorb other proteins, fall apart into small particles and, by accumulating proteins over again, grow to become a new coacervate. Yet the resemblance with biological elements is only very superficial, for, as was demonstrated mainly by modern electron microscopy, it is characteristic of living structures to have an utmost differentiated and complicated submicroscopic architecture and composition, which coacervates do not have. This characteristic cannot be found either in experimental compounds made of proteins and oils, although they look like cells in the light microscope (e.g., Moriyama, 1959). All these complexes have nothing in common with living cells.

On the other hand, much emphasis is laid on the following experiment. If one adds an amino-acid to a cell-free homogenate of rat livers, for instance, new protein molecules can be synthesized; but two conditions are necessary for this reaction. (1) There must be a substance present that produces energy in sufficient quantity, as, for example, adenosine triphosphate. The necessity of this source of energy is plausible, for as we remember, energy is necessary for the generation and maintenance of biological, macro-molecular compounds. (2) The protein in process of formation needs a template, according to which the amino-acids can arrange themselves. Incidentally, this is the way all protein and nucleo-protein syntheses occur in the intact cell, including the identical replications of genes or of whole chromosomes.

Therein lie the limitations of the chemical evolution theory. True, some macromolecules tend to have an organized structure (Calvin, 1956); true, we can find examples to show that certain macromolecules, with an almost imperative regularity, always form the same systems, which partly resemble living structures. Yet it is to be doubted whether a mere tendency of relatively simple groups of molecules to associate can generate the highly complicated structure even of the most primitive cell, let alone of all that we call "life". Any technical instrument, be it the most complicated we know, even in this era of conquest of space, is simple as compared to the most primitive form of independent life. From this, it is evident that, despite promising findings concerning the origins of a chemical evolution in which the formation of larger molecules would lead to the generation of unspecific proteins, we are still very far from understanding the evolution that led from the inorganic to the organic, from the lifeless to the living.

The living cell is more than the sum of the groups of molecules it contains, it is more than the sum of the reactions which take place in it. The mystery of its origin is at the same time the mystery of the first generation of life, the first chemical reactions to produce organized structures. We know, especially from findings of pathological cytology, that the proper organization of chemical processes depends on intact structures. Maybe a "structural evolution theory" in the submicroscopic dimension could begin at the point where the methods of the "chemical evolution theory" have reached their limits.

ORIGIN OF CYTOLOGY

Cytology will soon celebrate its 300th anniversary. In 1665, the *Micrographia* of the physicist Robert Hooke, at the time First Secretary of the Royal Society in London, was published. His "Physiological Descriptions of Minute Bodies" contain, among other things, two drawings of cork as it appears under the microscope. They show a structure similar to

that of honeycombs (Fig. 8). Hooke had found such "pores" or "cells" also in many other plants, such as in elder marrow, in fennel, carrots, ferns, etc. Further, he had observed that these "little boxes" contain a liquid, which might possibly go from one cell into the other and thus circulate. A contemporary of Hooke, Nehemiah Grew, also secretary of the Royal Society, demonstrated, in his grandiose illustrated publication *Anatomy of Plants* (1682),

this microscopic organization of plants in nearly all botanic objects he could find. When Grew submitted, in 1671, the first part of his publication to the Royal Society, a manuscript was submitted from Bologna, written by Marcello Malpighi, a professor of medicine already famous at that time. It contained the "Idea" of an "Anatome plantarum", a detailed microscopic description of plant organisms; the latter appeared to be constituted of a series of small

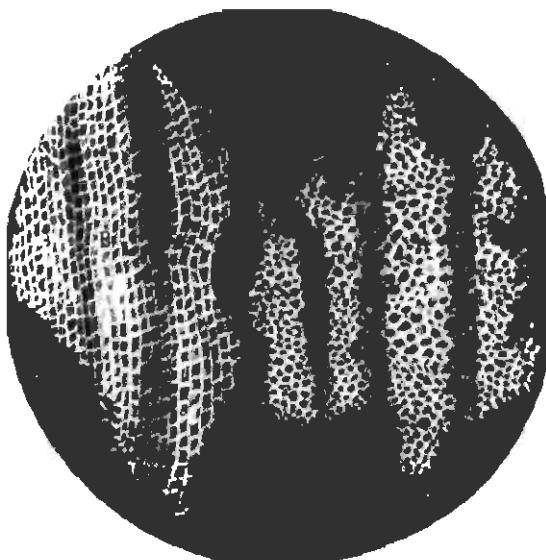


Fig. 8. First drawing of cells. (From Hooke, R. (1665), *Micrographia*.)

"utriculi" or small tubes (Malpighi, 1675), similar to the cells of Hooke and Grew. However, in Malpighi's far more renowned studies on the microscopic structure of animal organisms (Malpighi, 1687), no mention is made of these smallest structure elements.

During the same period of time, in the 1680's, a Dutch merchant, Anton van Leeuwenhoek, published his observations on minute fresh-water animals, which were considered lifeless until then; he had discovered the infusoria. In 1674, he saw for the first time red blood cells and, in 1679, spermatozoa, which he thought to be the smallest animals. In 1702, he drew for the first time nuclei in the erythrocytes of fishes, and this 130 years before the nucleus of cells was actually discovered by Robert Brown (1833). Thus, in the last 30 years of the 17th century, cytology was born in a great breakthrough at three different places: London, Bologna and Delft—even though this is clear to us only retrospectively. For, at that time, neither Hooke nor Grew knew anything of the real nature of the cell. Their "little boxes" were only the walls of those spaces which contained the living substances. Leeuwenhoek did not know that those smallest living beings he had observed were single cells and nobody suspected any inner relationship between the findings of Hooke and those of Leeuwenhoek.

Therefore, this great breakthrough found at first no echo. The 18th century produced but few new discoveries in the field. Among the scanty publications, we find the descriptions of Felix Fontana (1781) who described a nucleus in the epidermis of eel cells, characteristically

with no reference to previous investigations. Ten years before, in 1777, experiments with cells had been posthumously published for the first time; William Hewson had observed that human erythrocytes have the shape of discs which turn into spheres when immersed in water, and that those spheres transform themselves into discs again when immersed in a saline solution. Yet the times were not ready for further discoveries.

Many attempts were made, however, to find a comprehensive classification of the living matter. The great physiologist Albrecht von Haller, in his *Elementen der Physiologie* (1757), even used the expression "cellular tissue"—yet he meant by this a system of fibres, manifoldly interlaced, for it was his opinion that animal tissues are, on the whole, made of fibres and unorganized mass. C. F. Wolff (1759) believed that animal and plant tissues were constituted of uniform "spheres". Wolff mentioned also a sort of "cellular" structure of embryonic tissues, analogous to the "cellulae" in plants, and from this he tried for the first time to conceive animal and plant tissues as having a uniform architectural pattern (see review in *Studnička*, 1926); however, he did not go any further. Incidentally, later on, Milne-Edwards (1823) and Dutrochet (1837) considered similar small spheres as being smallest vital units.

It is noteworthy that, even before natural science had given it an exact basis, the theory of the cell state had been formulated by natural philosophy. Lorenz Oken described in his *Naturphilosophie* (1809) not only how plant and animal organisms are composed of "cells", he added also that these organisms emerge from a union of cells and that they disintegrate into separate cells again when they decay. In his opinion, these disintegration products are the infusoria. Thus, we see accomplished this synthesis of thought which was not possible in the 17th century in spite of Hooke's and Leeuwenhoek's observations. But this was still a purely intellectual concept. Actually, the same idea was the basis of Gruithuisen's theory. In his book *Organozoönomicie* (1811), he mentions a "cell substance" which would be the ground matter of living tissue. According to him, this "cell substance" is composed of small units like the infusoria and provides elements for all animal and plant organisms (for other precursors of the cell theory, see Cameron, 1952).

One should not underestimate the influence of all these books, especially that of Oken, since the findings on which soon afterwards the cell theory could rely, provided an extremely narrow and insufficient base. If we want to understand how modern cytology was born, in the fourth decade of the past century, we must not forget that the way had been prepared by natural philosophy.

However, the new theories were thought to rest exclusively on observations and experiments and, indeed, Treviranus (1806) and Link (1807) had succeeded in isolating single cells from groups of plant cells and Robert Brown had discovered in 1833 the nucleus as an organelle present in all cells. Those were authentic natural science discoveries, which were in direct relation to those of Hooke, Grew, Malpighi and Leeuwenhoek, and scientists largely restrained themselves from forming too hasty conclusions.

At the same time, Purkinje and his associates accomplished strict natural science work. Purkinje, professor of physiology and pathology in Breslau, established in 1825 a structural homology between the "germinal vesicle" of a bird's egg and spheric bodies (cell nuclei) in cerebrum and cerebellum (see Valentin, 1836). In the cells of the cerebellum, which later were named after him, he described the nuclei as "central discs" and mentioned also the

nucleolus, calling it "central granule". At the 15th session of the *Deutsche Gesellschaft für Naturforscher und Ärzte* in Prague in 1837, Purkinje presented his theory. In the mucous membrane of the stomach, in the excretory glands, in the kidneys, in the epidermis, everywhere he had found granules of the same nature. "The granular basic structure imposes once more the analogy with the plant, which, as we know, is almost entirely composed of granules or cells." This statement was made in 1837, i.e., one year before Schleiden published his *Beiträge zur Phytogenesis* (see also Frankenberger, 1961).

Schleiden's fields of interest were, even for his time, almost too widespread. He started as a law student, switched over to the study of philosophy and medicine and finally became a botanist and was renowned as such during his life and after. However, his main interest was philosophy; so it is no wonder that his famous *Beiträge zur Phytogenesis*, published in 1838, abounds in speculations. The existence of a cell state in plants he took more or less for an established fact. The object of his research was rather the origin of plant cells. He assumed that a granular thickening could occur at a certain site in the inner surface of the pre-existing cell wall, and that the nucleus gradually formed itself around this thickening, thus becoming a "cytoblastema". It would then grow and build a fine, transparent vesicle; thus, the young cell would be born, which would continue to grow and eventually be able to surround itself with an individual cell wall. According to Schleiden's theory, the mature cell has no nucleus any more; the latter only appears again with the formation of a new cell.

The interpretation which Schleiden deduced from his preparations is the same as that of the followers of the "endocytogenesis" theory, which postulates the formation of a cell within another. This theory very soon met a strong opposition, especially from Kölliker who, in 1844, recognized the cleavage divisions of the animal egg as being a continuous division of the cells. It was, however, to be fatal for the further development of cytology that Schleiden's conception was enthusiastically adopted and carried on by his contemporaries and followers, also for instance, by Virchow (1851). Schwann too adopted this theory from Schleiden, who was at the time 6 years older than he, and he applied it to animal organisms. In Schwann we find the clear method of thinking of his great master Johannes Müller. Schwann's *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und im Wachstum der Tiere und Pflanzen** (1839) have rightly become the "basis of the cell state theory" (Heidenhain, 1907). In this book we read: "These cell individuals, however, are not juxtaposed as a mere aggregate; they cooperate, we do not know how, in such a way that they build a harmonious organic unity." In another passage he mentions the cells as being elementary particles: "... each elementary particle has an independent energy, an independent life, and the whole organism exists only through the interaction of each of its elementary particles." The following was new too: "The eggs of superior animals are also such independent cells, growing separately from the organism. Since all cells grow according to the same laws . . . and since it has been proved that certain cells, albeit they do not differ from the others in their pattern of growth, follow, however, an independent evolution, we must conclude that cells in general lead an independent life." We have here the first clear-cut formulation of the cell theory and of the concept that the cell is the smallest vital unit. Schwann's writing found the greatest response and thus, the cell theory was founded in a form which is valid up to this day.

* "Microscopic investigations on the correspondence in structure and growth of animals and plants."

There followed many decades of intensive research, during which cytology was built stone by stone. The better optics of the apochromatic instruments (Abbé, 1886) made possible the beginning of "classical cytology", which revealed to us the details of nuclear and cellular division as well as the light microscopical structure of the cytoplasm. Let us mention, among many others, Flemming (1843-1915), Strasburger (1844-1912), Boveri (1862-1915) and Heidenhain (1864-1949).

During the same period, Miescher (e.g., 1871) and Kossel (1882) had discovered the essential components of the nuclei, the nucleic acids. Thus, chemical cytology, today in full bloom, was born parallel to morphological cytology, though limited by the modest technical possibilities of that time. Owing to an abundance of special methods such as ultracentrifugation-sedimentation, isotope research, micro-electrophoretic techniques, cytophotometry and many others, chemical cytology is responsible for the modern "renaissance" of cytological research. Phase contrast microscopy extended the possibilities of investigating the living cell, an object that was more widely studied in the past; and since the use of thin-section methods made the electron microscope a cytological instrument, an entirely new morphological dimension has been made available, which extends down to the level of macromolecules. At this point, morphology and chemistry meet in a most remarkable way (Büchner, 1959a, 1960).

CYTOTOLOGY TODAY

In both its previous periods of bloom, from 1665 to 1675 and from 1830 to 1840, cytology was chiefly a field of botany. Plants displayed those "little boxes" indeed much more clearly than animal organisms. Purkinje and Schwann were the first to join both realms, and ever since, cytology concerns the whole of biology. Even though each of the investigators has had to restrict himself by necessity to few methods and objects of investigation, thus limiting now and then his field of interest, never again did they cease to be conscious of the common points of concern.

The foregoing remained true, when, at the beginning of the 20th century, a new branch of biology appeared, namely, genetics. It was Correns (1900) who, in relation to his own results, called attention to the fundamental observations made by the Augustinian monk from Brünn, Father Mendel (1865). They were the foundation for the newly born science of heredity. It started as a physiological study, in which interest was directed mainly to the laws by which genetic factors are transmitted. Yet the question soon arose as to where the genetic factors are located within the gamete; thus, cytogenetics were born. The question of how the factors of heredity contributed to the formation of each character, also belonged at first to the physiology of heredity. However, the transport of genetic information from the genes of the nucleus to the executive organelles of the cytoplasm has become the specific concern of cytology; thereby, investigation of the gene activity is primarily a cytological problem.

In modern genetics, another field has become increasingly important, viz., bacteriology. It was originally a medical science; today, however, it has become a most modern branch of biology, since its objects, bacteria, are particularly suited to genetic experiments because of their relatively simple metabolic systems and their high rate of division. It is bacteriology

which is responsible for important discoveries concerning the action of the genes and many other cytological problems.

There are equally close relations between cytology and virology. Investigation of virus diseases is getting more and more involved with problems of cytology. For, as the virus develops its qualities only in the cell, so it is also only in the cell that it releases its pathogenetic reactions. Thus, the experience gained in virology contributes a great deal to extend the knowledge of the cell's reactions under pathological conditions. We can even say that the viruses' extremely simplified and, therefore, especially clear structural elements have taught us a great deal during the past decade in regard to the role of nucleoproteins and their relationship to protein synthesis.

Assuredly, cytology today is quite different from what it was 120 years ago. Many of its most widely used methods come from other sciences, mainly from biochemistry, whose methods of investigation become more and more refined as they need smaller and smaller quantities of material. Today, biochemistry can almost be considered a branch of general cytology. This may surprise many biochemists. Yet quite a few biochemical problems, such as the transport of electrons, the action of enzymes or the synthesis of proteins or nucleoproteins are also cytological problems, and questions of a primarily cytological interest, as the function of the nucleus or the role of the mitochondria, concern today also the biochemist. Contrarily to the much deplored splitting up of research, many branches of natural science have recently gathered around the smallest living unit, the cell.

This is also particularly true of medicine, which, owing to Virchow, has been inseparably related to cytology for more than a hundred years. "Cellular pathology" is not, and Virchow repeatedly emphasized this, a new solidistic pathology. Nor is it opposed to humoral pathology under its old or new form. It was founded "to unite both humoral and solidistic pathology into one, empirically based cellular pathology" (Virchow, 1858a). It is, therefore, a superior factor of unity, and has become even more so since new fields have been opened to research.

It was in a polemic writing against a doctor Spiess from Frankfurt that Virchow (1858b) gave the most adequate definition of cellular pathology: "Each disease originates from the alterations that affect a smaller or larger number of cellular units within the living organism; every pathological disturbance, every therapeutic effect can only then be ultimately interpreted, when it is possible to tell which particular group of living cellular elements is concerned, and which kind of alterations each element of such a group has undergone. The long searched for essence (*ens*) of disease is the altered cell."

For cell research, pathology and orthology are merely two aspects of the same principle. The former requires the knowledge of the latter, and the latter itself can often be understood from the very phenomena of pathology.

Constantly bearing in mind this unity of object, methods and goal, let us now attempt to elucidate the different parts of the cell.

2. The Nucleus

In our introduction, following the general custom, we divided the cell into two components: the nucleus and the cytoplasm. This has the advantage of providing a clear-cut classification, but it should not disrupt their correlation. For, the nucleus being entirely surrounded by cytoplasm, its nature can only be understood in its close relation to the cytoplasm. However, the many peculiarities of its structure and functions place it in a singular position. Thus, the science of the nucleus, or *karyology*, has long been regarded as a separate branch of cytology. This is not so much due to the use of special methods of investigation; in fact, it reflects an independent way of thinking of this discipline, as will be shown clearly in the following paragraphs. Karyology still has to solve a large number of problems; these will be the main subject of this chapter.

They appear already at the level of macroscopic morphology. In the untreated living cell—insofar as such a cell is accessible at all to observation—the nucleus is practically not visible. Even with the aid of phase-contrast microscopy, we see, for instance in living human granulocytes obtained from the blood stream, only a negative print of the nucleus, that is, one detects the region it occupies as an empty space between the cytoplasmic granules (Fig. 9, left); the nucleus itself is almost invisible. Only when the cell is damaged or even has died as a result of the abnormal conditions of histological processing, does the nucleus reveal its typical shape, which corresponds largely to its appearance after fixation and dyeing

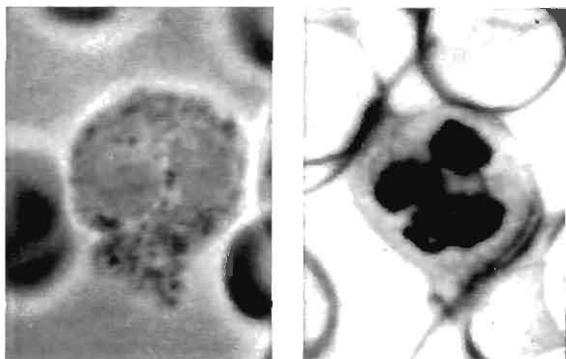


Fig. 9. Human neutrophilic granulocytes. Left: intravital phase contrast micrograph; right: fixed and stained with Giemsa.

(Fig. 9, right). We shall return later to the explanation of this phenomenon (p. 68). Suffice it to say here that the striking appearance of the nucleus within the cell, as can be seen on cytological sections, is due to the methods of preparation.

Most of the routine staining methods today are based, in a purely empirical way, on a difference in colour between nucleus and cytoplasm. Each combination of dyes contains one component for the nucleus and one or more for the cytoplasm, the former being more basic, the latter more acid in character. In short, one could say that the difference in staining

indicates a difference in the pH, i.e., that the constituents of the nucleus are more acid than those of the cytoplasm. This is only grossly correct. Yet, this simple classification of the constituents of the cell in "basophilic" and "acidophilic" structures has dominated the entire "classical" cytology for almost 50 years (Hertwig, 1929).

Three discoveries directed the emphasis to the nucleus. They were all made in the past century, but only today is their importance fully appreciated. Historically, the first one was of a chemical nature. Miescher (e.g., 1871) discovered in the nuclei of spermatozoa, thymus cells and nucleated red blood cells, a specific nucleic acid. Kossel (1911) isolated it and called it "thymonucleic acid"; Feulgen and Rossenbeck (1924), by a cytochemical reaction, found it to be a specific component of the nucleus: the deoxyribonucleic acid (DNA). Since then, we know that there is no nucleus without DNA, and that polymerized DNA occurs, with a few exceptions, only in nuclei. Hence the nucleus is the only cell component to be characterized by a special chemical substance. Its function is closely related to this substance.

The second discovery was morphological. Virchow postulated the continuity of the cell generations and extended his doctrine "omnis cellula e cellula" (see p. 2) to "omnis nucleus e nucleo" (1858a). He assumed, however, that the mode of division was that of a simple cleavage of the nuclei without change in their vesicular structure. True, this observation was due to artifacts; nevertheless, the principle discovered was correct. Then, in the last three decades of the 19th century, Flemming (1882) discovered what he himself termed the "thread metamorphosis" of the nucleus during its division, that is, its rearrangement in threadlike loops, named chromosomes, which split and move to two opposite poles in the mitosis. These findings provided a new definition of the nucleus as that part of the cell "during the division of which chromosomes appear" (Belar, 1926). As we now know, this also was only partly true. Yet we have today unquestionable proof of the fact that the nucleus consists mainly of a material which merges into the chromosomes during the mitotic division.

Thus the path was paved for the third discovery. It was found that the main genetic factors of the individual, the genes, are closely associated with the specific DNA mentioned above. This DNA, therefore, can rightly be considered as being the gene substance *per se* (p. 114). Thus, according to modern definition, the nucleus is that part of the cell which contains the mass of the genes by virtue of its DNA.

FUNCTIONS OF THE NUCLEUS

The nucleus as repository of the genes and general centre of metabolism

As a key repository of the genes, the nucleus is, functionally, at least equal to the sum of its genes. We will have to deal in the following chapters with the gene and its possible definitions (p. 115). Suffice it to say here that the functions of the nucleus correspond mainly to the action of its genetic units.

What then, one may ask, do genetic units, carrying characteristics specific of a given genus or species, have in common with the action of the nucleus in the metabolism of the cell? Are these not two fundamentally different functions, one genetic, the other metabolic?

Let us disregard the genetic factors of the cytoplasm, since they are subject to special conditions. A glance at fertilization and germinal development shows that the male gamete

consists predominantly of nuclear material. Once it has entered the egg, it combines itself with the nucleus of the egg cell, and from this merger, by means of repeated divisions, a whole organism is eventually formed. The structures of the living system, which are now taking shape, are equally dependent on the paternal and maternal information, i.e., they are primarily determined by the nuclear material, which, alone, comes in equal parts from both parents. These inherited units, which are enclosed in the nuclei and determine the future organism, begin their activity during the very development of the embryo. They induce, by means of complicated, only partly known mechanisms, the formation of the different cell species and direct the development of tissues, which each have a specific role and function, different from that of the others. It is still not clear how these determining units, which are identical with the genes, influence the organization of the embryo.

The nucleus of the zygote after fertilization can, by no means, be held alone responsible for the differentiation. If, in an embryological experiment, parts of the cytoplasm of unfertilized egg cells are resected, such as in the roundworm *Ascaris* of the horse (Boveri, 1910) or in certain snails, the result is that of defective embryos (Wilson, 1904). Thus, in these "mosaic eggs", the cytoplasm of the unfertilized egg cell contains already differentiated areas, which are the prerequisite of a regular development. Yet the formation of such areas needs to be induced and directed by the nucleus.

In the mature organism, the different cellular functions are performed mainly by the organelles of the cytoplasm: the muscle contraction by the contractile substance of the muscle cells, the transmission of the excitation by the nerve fibres, the secretion by the ergastoplasm, etc. Yet, the formation of such organelles is directed by the nuclei; indeed, they may even, as for example the neurofibrils, originate from material released directly by the nucleus (Geiger, 1958).

So far, we can conclude by saying that the different cellular functions are the sign of a cytoplasmic activity, the mode of which, however, is determined by the nuclei. The determining factors in the nuclei are, obviously, the genes, which have come from the parental gametes, and carry on the same functions. This solves the contradiction of the gene being at the same time the carrier of individual characteristics and an element of the cell function; the differentiated action of the cell is a manifestation of the gene activity; the genes preside over the cellular function.

What is true of the genes, is also necessarily true of the sum of genes, the nucleus. The nucleus acts practically as "chief of the cell" (Felix, 1959); it directs and supervises the activities of the cytoplasmic organelles. Non-nucleate cytoplasmic particles are bound to perish. Their death can come about rapidly, or may be delayed for weeks or even months, as it occurs normally in the mammalian erythrocytes, which lose their nucleus during the process of maturation. Experimentally, this fact can be neatly demonstrated on the ciliated protozoan *Stentor*. With the aid of a micromanipulator, this unicellular organism can be easily cut into several parts. Almost each of these is able to regenerate to a complete protozoan, yet only if it contains a portion of the worm- or rodlike nucleus. Pieces without nuclear material assume a round shape and then disintegrate (see, e.g., Belar, 1924). Inability to take up food supply seems to be the decisive factor leading to this fate, since, when nutrition is withheld, both fragments, those without nucleus and those which contain nuclear particles and regenerate, die after the same period of time (Tartar, 1956).

So far, we can summarize the role of the nucleus in three points:

1. It contains the main genetic factors.
2. Its genes control the formation of the differentiated structures.
3. It initiates, directs and supervises the activities of these structures.

Let us, at this stage, consider all three points as being hypothetical; we will prove them step by step in the following chapters.

Interdependence of nucleus and cytoplasm

The nucleus, initiator of cell metabolism—as we may rightly call it now—is by no means an independent ruler. Rather, it depends in all its functions on the cytoplasmic organelles. We already indicated how important primary cytoplasmic differentiation is for the embryogenesis of mosaic eggs (p. 29). Furthermore, biology and pathology have recognized innumerable other examples where the nucleus is influenced by the cytoplasm. By this we mean less the participation of the nucleus in degenerative processes of the whole cell, than its normal involvement when functional stimuli are exerted on the cell. These, it is true, affect primarily the cytoplasmic organelles, but secondarily also the nucleus.

We can see this particularly well in the large ganglion cells of the nervous system. In the anterior grey column of the spinal cord, the mass of basophilic granules disappears from the cytoplasm of the ganglion cells after intensive muscle contraction; the cytoplasm is depleted of protein and nucleoproteins (Hyden, 1943). Those cells are exclusively affected, which transmit the motor impulses to the muscles. Analogous reactions in sensory cells are caused by stimulation of sensory nerves (Hamberger and Hyden, 1945). On the other hand, these alterations are accompanied by an enlargement of the nucleus and nucleolus, and soon new basophilic granules are formed again. Benninghoff (1950) called this increase in nuclear volume during or after functional stress on the cytoplasm “functional oedema of the nucleus”. He demonstrated it chiefly in the spinal cord of mice, which after amputation of one leg, acquired a muscular hypertrophy in the opposite extremity (Benninghoff, 1951). Meanwhile, innumerable similar observations have been reported (summarized by Räther, 1958), which all lead to the same conclusion, viz., the response to each functional stimulation of the cell is, after a certain latency, an enlargement of the nucleus. For instance, after resection of two-thirds of the liver in rats, the volumes of the liver cell nuclei double in the remaining tissue (Grundmann and Bach, 1960); concomitantly, the nucleoli increase in size (Fig. 10). The latter occurs so inevitably that it is considered as a means of measuring cellular activity and of distinguishing between a functionally active and a resting cell (for example, Dolley, 1925; Caspersson and Holmgren, 1934; Puff, 1951; Kulenkampff and Wüstenfeld, 1954; Bucher, 1959; and others).

Intensification of cytoplasmic activity does not only cause quantitative, but also qualitative reactions of the nucleus. As Altmann (1952) has shown, for example in endocrine tissue of the mouse pancreas, the nucleus releases, after extreme hunger or stimulation by pilocarpine, substances into the cytoplasm throughout the course of cytoplasmic protein synthesis. During that phase, its shape undergoes cyclic alterations which Altmann (1952) termed “functional cycle of nuclear shape” (see also Altmann and Meny, 1952). These observations

have been confirmed recently by radioautographic findings with ^3H -cytidine (Stoecker *et al.*, 1961a; Stoecker, 1962b).

The same object has been used for studying protein synthesis biochemically, with ^{15}N labelled amino-acids (Allfrey and Mirsky, 1955). True, hunger impairs their incorporation into the cell; however, only 30 minutes after refeeding, an intensified protein synthesis occurs simultaneously in nucleus and cytoplasm.

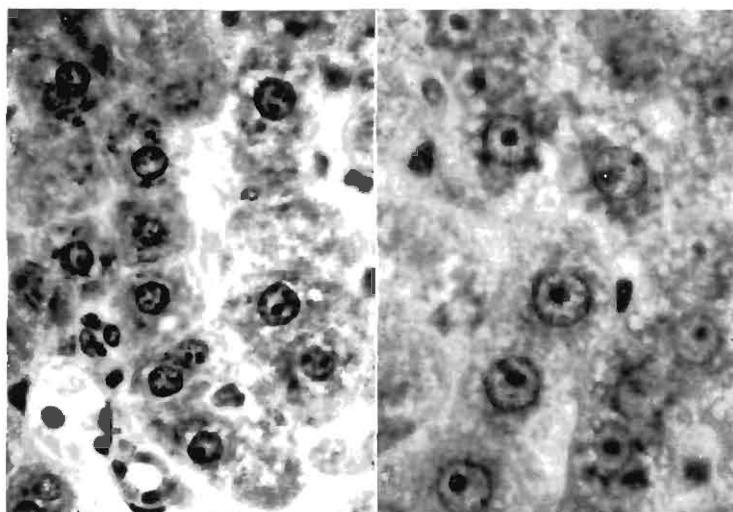


Fig. 10. Enlargement of nuclei and nucleoli in rat liver cells following partial hepatectomy. *Left*: normal liver. *Right*: 30 hours after partial hepatectomy. In both cases, $\times 800$. Haematoxylin eosin.

How much the nucleus relies on the functional conditions present in the cytoplasm has been documented very elegantly in *Acetabularia mediterranea* (Hämmerling, 1958). The nucleus of this large unicellular alga, is located in one of its rhizoid branches (Hämmerling, 1931). If this branch is clipped from the cell, the nucleus shrinks to one-fifth of its original size; at the same time, the previously relatively large nucleoli deteriorate. During the following regenerative growth of the cell, nucleus and nucleoli expand again. A similar shrinking and re-expansion of the nucleoli occurs when the plant is kept in the dark for 10 days and then exposed to light again (Stich, 1951b, 1956). The relation to sunlight of the nucleolar size, especially in cells that contain chlorophyll, was already shown in earlier investigations on cells of leaves. In the afternoon of a sunny day their nucleoli are almost double the size they are the next morning before sunrise (Fischer, 1934). It indicates that the nuclei are dependent on the energy production of the cytoplasm, which controls the functioning of the whole cell, as well as that of the nucleus (Hämmerling, 1959).

On the other hand, as we will see later, the cytoplasmic production of energy relies in turn heavily on the nucleus. Nucleus and cytoplasm are interdependent; nuclear syntheses require substances from the cytoplasm, yet the metabolic functions of the latter need to be initiated and directed by the nuclear genes.

Non-nucleate cells and merotomy experiments

Great efforts have been made to identify with precision the single functions of the nucleus in the metabolism of the cell. Our knowledge of this matter is still incomplete and is based on a series of hypotheses. In the following, we will attempt to understand the main findings regarding this problem.

First of all, our interest is directed towards those cells that entirely lack a nucleus, as, for instance, the normal mammalian erythrocytes. These red blood cells lose their nucleus at a certain stage of maturation. They are then called reticulocytes, for they contain a basophilic net-like structure composed largely of RNA. There appears to be no immediate radical change in the cell metabolism after loss of the nucleus. At least, all studies have shown that the non-nucleate reticulocytes carry on the synthesis of haemoglobin as before (Rabinovitz and Olson, 1959; Kruh *et al.*, 1960; Allen and Schweet, 1960). Accordingly, they are equally able to take up and incorporate new amino-acids (Borsook *et al.*, 1952). Later, however, in the process of further maturation, the basophilic network gradually disappears, the cells become depleted of RNA and, concomitantly, the incorporation of labelled amino-acids ceases (Gavosta and Rechenmann, 1954). The fully matured erythrocytes are now completely filled with haemoglobin. As a rule, they are free of RNA and unable to synthesize protein. Their life span is restricted to 120 days; their oxygen requirement is lowered by a factor of 200 as compared to their nucleate precursors (Frick, 1961).

At this point, we encounter for the first time a correlation which is of paramount importance for all of our further discussions. There is a close interrelationship between RNA content and protein synthesis (Caspersson, 1941; Brachet, 1941), and this production of protein, for which the presence of RNA is necessary, is even possible in a cytoplasm without a nucleus.

Considerable importance has been attributed to experiments in which artificially enucleated cell fragments were produced. We mentioned already the evidence brought by merotomy experiments on the protozoan *Stentor* (p. 29). Analogous experiments were performed in amoebae by Brachet (1959b, 1961). The formation of pseudopodia ceases a few minutes after resection only in the half left without a nucleus, and this cell fragment takes on the round shape of a pellet. This impairment of the cell's motility is caused by a rapid change in the viscosity of the cytoplasm (Hirshfield, 1959). As was the case with the enucleated cell fragments of the protozoan *Stentor* in the experiments of Belar (1924) and Tartar (1956) (p. 29), these enucleated cell portions are also doomed to die of starvation. If one succeeds in implanting another nucleus into this enucleated cytoplasm fragment, pseudopodia rapidly form again and the cell survives (Comandon and de Fonbrune, 1939). These studies on protozoans demonstrated that amoeboid movements and, therefore, intake of food supply, both resulting from alterations in the cytoplasmic viscosity, are dependent on the nucleus.

Further merotomy experiments brought the following results. If fragments of amoebae are kept without nutrition for one week, the nucleate portions will have utilized almost all their carbohydrates and fats, whereas enucleated fragments are still filled with these reserve materials (Fig. 11). This fact could indicate that nuclei have a particularly high requirement for energy, but it can also be interpreted as the inability of the cytoplasm to oxidize normally without the presence of a nucleus. Brachet (1955) has demonstrated that after 3 days enucleated

Non-nucleate cells and merotomy experiments

Great efforts have been made to identify with precision the single functions of the nucleus in the metabolism of the cell. Our knowledge of this matter is still incomplete and is based on a series of hypotheses. In the following, we will attempt to understand the main findings regarding this problem.

First of all, our interest is directed towards those cells that entirely lack a nucleus, as, for instance, the normal mammalian erythrocytes. These red blood cells lose their nucleus at a certain stage of maturation. They are then called reticulocytes, for they contain a basophilic net-like structure composed largely of RNA. There appears to be no immediate radical change in the cell metabolism after loss of the nucleus. At least, all studies have shown that the non-nucleate reticulocytes carry on the synthesis of haemoglobin as before (Rabinovitz and Olson, 1959; Kruh *et al.*, 1960; Allen and Schweet, 1960). Accordingly, they are equally able to take up and incorporate new amino-acids (Borsook *et al.*, 1952). Later, however, in the process of further maturation, the basophilic network gradually disappears, the cells become depleted of RNA and, concomitantly, the incorporation of labelled amino-acids ceases (Gavosta and Rechenmann, 1954). The fully matured erythrocytes are now completely filled with haemoglobin. As a rule, they are free of RNA and unable to synthesize protein. Their life span is restricted to 120 days; their oxygen requirement is lowered by a factor of 200 as compared to their nucleate precursors (Frick, 1961).

At this point, we encounter for the first time a correlation which is of paramount importance for all of our further discussions. There is a close interrelationship between RNA content and protein synthesis (Caspersson, 1941; Brachet, 1941), and this production of protein, for which the presence of RNA is necessary, is even possible in a cytoplasm without a nucleus.

Considerable importance has been attributed to experiments in which artificially enucleated cell fragments were produced. We mentioned already the evidence brought by merotomy experiments on the protozoan *Stentor* (p. 29). Analogous experiments were performed in amoebae by Brachet (1959b, 1961). The formation of pseudopodia ceases a few minutes after resection only in the half left without a nucleus, and this cell fragment takes on the round shape of a pellet. This impairment of the cell's motility is caused by a rapid change in the viscosity of the cytoplasm (Hirshfield, 1959). As was the case with the enucleated cell fragments of the protozoan *Stentor* in the experiments of Belar (1924) and Tartar (1956) (p. 29), these enucleated cell portions are also doomed to die of starvation. If one succeeds in implanting another nucleus into this enucleated cytoplasm fragment, pseudopodia rapidly form again and the cell survives (Comandon and de Fonbrune, 1939). These studies on protozoans demonstrated that amoeboid movements and, therefore, intake of food supply, both resulting from alterations in the cytoplasmic viscosity, are dependent on the nucleus.

Further merotomy experiments brought the following results. If fragments of amoebae are kept without nutrition for one week, the nucleate portions will have utilized almost all their carbohydrates and fats, whereas enucleated fragments are still filled with these reserve materials (Fig. 11). This fact could indicate that nuclei have a particularly high requirement for energy, but it can also be interpreted as the inability of the cytoplasm to oxidize normally without the presence of a nucleus. Brachet (1955) has demonstrated that after 3 days enucleated

cytoplasmic fragments no longer degrade glycogen. This leads us, on the long run, to favour the second of the above-mentioned possibilities. Apparently, neither fats nor carbohydrates can be degraded in the absence of a nucleus, though, seemingly, a certain impulse still persists for a short period of time in enucleated cytoplasmic fragments, that soon abates.

Conditions are somewhat different in the alga *Acetabularia*. Here, the cell fragment survives enucleation for several months; moreover, not only is it still able to grow, but it can also produce differentiated structures, as, for example, a second umbrella-like cap at the site of the

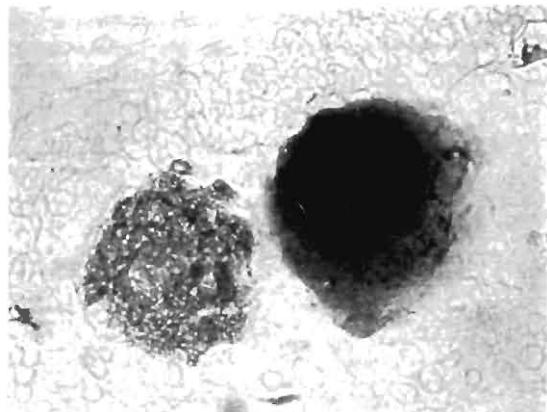


Fig. 11. Merotomy experiment in amoebae. Left: nucleate, right: non-nucleate portion of an amoeba one week after merotomy. In the nutrition free medium, the nucleate portion has used up most of its glycogen, the non-nucleate portion is still filled with glycogen. (From Brachet J. (1955), *Biochim. Biophys. Acta*, 18, 247-268.)

resection (Hämmerling *et al.*, 1958). The energy-providing breakdown of carbohydrates goes on normally for a long time, even in the absence of a nucleus.

The difference between these experiments and those with amoebae is readily explained. The *Acetabularia* possesses chloroplasts, not so the amoeba. Thus we could say that the faster disintegration of enucleated amoeba fragments is simply a result of an energy insufficiency within the cell. The question here is: does the nucleus play a special part in the production of energy?

Oxidative phosphorylations

There are two pathways for the production of energy. One is aerobic, and runs, in the presence and with the co-action of available free oxygen, via the respiratory chain; the other is anaerobic and goes via the substrate chains. The energy gained is only partly released for immediate use. The main part is stored as chemical energy, especially in the form of "high energy phosphates", among which adenosine triphosphate (ATP) is of paramount importance. The normal tissue cell obtains its share of ATP predominantly from the respiratory chain by oxidative processes. Therefore, in the light of the merotomy experiments described earlier, the possibility must be examined, whether or not the nucleus is the centre of the oxidative metabolism (see Loeb, 1899).

Let us anticipate the result and say this is not the case. Oxidative metabolism is the function of the mitochondria, as we have indicated earlier in our introductory survey of the cell components (p. 12). Yet, as it seems, the nucleus is not entirely inactive in this

process. Mirsky *et al.* (1956) reported an oxidative phosphorylation in isolated nuclei of thymus cells, which could be entirely blocked by cyanogen compounds or dinitrophenol. Obviously, it is a different kind of phosphorylation than that of the mitochondria, since substances like Dicumerol, Janus green B, methylene blue or Ca ions, which inhibit mitochondrial phosphorylations, have no influence on the phosphorylations in nuclei. Furthermore, this phosphorylation is of necessity dependent on the presence of nuclear DNA (Allfrey and Mirsky, 1958); the formation of ATP is immediately blocked, if the DNA is removed by desoxyribonuclease. As indicated by these investigations, DNA appears to play the part of a co-factor in nuclear oxidative phosphorylations. In this context, apparently, DNA acts primarily by virtue of its polyphosphate character, since its action can be replaced by other polyanions, such as polyacrylic acid, but not by polycations (Mirsky and Osawa, 1961). The energy gained this way intranuclearly is stored chiefly as ATP, for nuclear ATP synthesis is dependent on the presence of DNA (Allfrey and Mirsky, 1957).

These data did not remain unopposed (see, e.g., Siebert, 1958a); yet, so far, they cannot definitely be proved false. The existence of nuclear ATP synthesis seems to be confirmed, but its pathways are still not clear. There is also, as yet, no general agreement on the extent of nuclear energy production. As for now, it is to be assumed that this energy serves mainly the specific needs of the nucleus. Whether or not the supply is adequate, is another question. Nuclei of animal cells that need further oxidatively-produced energy, have to draw it from the mitochondria. In this regard, the observation of Frederic (1953) is, possibly, of some significance. It is a striking feature of mitochondria, when observed in the living state, that one or the other frequently nestles against the nuclear membrane, stays there for a while and then recedes. By this means, a transmission of energy—conceivably in form of ATP—could take place. But these are mere speculations.

Glycolysis

We have a well-grounded knowledge of the other possible route by which intracellular ATP is formed. This ATP synthesis occurs anaerobically via the phosphorylation of the substrate chains in the process of glycolysis. The typical oxidative enzymes are missing in the nucleus, e.g., succinic dehydrogenase or cytochrome-C-oxidase (Dounce, 1943; Hogeboom *et al.*, 1952). However, important glycolytic enzymes, such as aldolase, glyceraldehyde phosphate dehydrogenase or enolase and their respective substrates, have been found, for example, in nuclei of wheat germs or isolated nuclei of rat liver cells (Stern and Mirsky, 1952; Siebert, 1958b). There are considerable differences between the enzyme content of the total tissue and that of the nuclear fraction. The data suggest that high energy phosphates are produced in the nucleus via anaerobic glycolysis (see Siebert and Smellie, 1957).

These transformations, however, play a comparatively minor part within the total cellular energy metabolism. For example, if, after the method described by Harvey (1934), unfertilized sea urchin eggs are centrifuged in such a way that they are separated into a lighter, non-nucleate, and a heavier, nucleate fraction, the O₂ uptake of the non-nucleate portion exceeds by far that of the nucleate fraction (Shapiro, 1935). Accordingly, the dehydrogenase activity, which is closely related to the respiratory chain, is 70% higher in non-nucleate than in nucleate fractions of sea urchin eggs (Ballentine, 1939).

In this context, let us consider once more those merotomy experiments with amoebae, which we had placed at the beginning of our discussion on nuclear energy production. Though the fact of energy production in the nucleus has been established, it is difficult to conceive that the lack of energy from the nucleus should be solely responsible for the inhibition of fat and glycogen degradation in enucleated fragments (Fig. 11). Let it be said again: the energetic activities of the nucleus are minor as compared to those of the cytoplasm. In fact, the nuclear energy balance is most likely negative.

NAD (DPN) synthesis

However, another observation could provide an explanation for these merotomy data. Nuclei contain much diphosphopyridine nucleotide (DPN) (Stern *et al.*, 1952), which, according to modern nomenclature, should rather be termed nicotinamide adenine dinucleotide (NAD). According to Hogeboom and Schneider (1952), NAD is formed exclusively in the nuclei. At least, such is the case in the liver. NAD can be found in the nucleate erythrocytes of birds, but not in the non-nucleate red blood cells of mammals (Malkin and Denstedt, 1956). It is one of the most important co-enzymes of the intermediary metabolism; it is, in particular, a co-enzyme of many dehydrogenases, which are of great importance in energy metabolism. Since an NAD-synthesizing enzyme apparently exists exclusively in nuclei (Hogeboom and Schneider, 1952)—in certain oocytes it is located predominantly in the nucleoli (Baltus, 1954)—the nucleus must be considered as playing an important role in NAD synthesis. Thus, the absence of a nucleus is necessarily followed by a gradual decrease in the NAD content of the cytoplasm; conceivably, NAD is the material basis for this nuclear “impulse”, which, as we noted earlier (p. 33), is necessary for the metabolic processes in the cytoplasm, for example for the combustion of fat and carbohydrates. Unfortunately, NAD determinations in merotomy experiments on amoebae have not yet given any useful results (see Brachet, 1961).

Phosphate metabolism

All these metabolic activities inside the nucleus still do not explain the first and most simple merotomy observation, namely, the rapid cessation of pseudopodia formation, which leads ultimately to the starvation of the enucleated cell fragments; nor does it explain the fast recovery of motility after implantation of a new nucleus (see p. 32). There also occurs concurrently an entirely different metabolic process in the enucleated cell. Only a few hours after enucleation of an amoeba, the cytoplasmic uptake of ^{32}P is reduced to 25–30% of the norm (Mazia and Hirshfield, 1950). After 3 days, the ^{32}P uptake of enucleated fragments has decreased to one-sixth, after 6 days to one-thirtieth of that in nucleate portions (Brachet, 1961). The uptake of ^{32}P into the nucleus itself plays only a minor part in these processes. Variations in the degree of permeability of the cell membranes are of no significance either.

It is hitherto impossible to decide which of the metabolic pathways are traced down by the radioactive phosphorus, for phosphorylations are ubiquitous in the metabolism of the cell. In all likelihood, these differences are partly due to the synthesis of NAD, which is missing in

enucleate cell fragments. The inhibition of ATP formation, caused by the decrease in NAD content, might be also of some importance.

Entirely different findings have been demonstrated in the alga *Acetabularia*. Here, ^{32}P is incorporated chiefly into the nucleus, especially into the nucleolus, with the latter taking up 80% of the total activity (Stich and Hämmerling, 1953). This incorporation is dependent on the metabolic activity of the whole cell; lack of light, i.e., inhibition of the formation of energy by the chloroplasts or intoxication with dinitrophenol lower the nuclear incorporation. Trypaflavine has no effect (Hämmerling and Stich, 1956).

Metabolism of RNA and protein

In addition to these results, experiments with *Acetabulariae* have helped to identify what is perhaps the most important component of phosphorylations, viz., the metabolism of RNA and protein. For, if the incorporation of ^{32}P into the nucleus is reduced by means of inhibition of energetic cell activities, the ^{32}P uptake into the cytoplasm is also lowered, particularly that into the RNA-containing granules (Hämmerling and Stich, 1956). Considering the close relations of RNA and protein metabolism, which we already emphasized above, this branch of the cell metabolism may be made responsible for a large part of the mentioned phosphorylations.

Let us turn once more to the regeneration studies of Hämmerling (1934a) mentioned above (see p. 33). An enucleated alga, consisting of pedicle and umbrella-like cap, is capable of surviving for several months; it still can grow and even form one or more new caps. It possesses indeed an impressive morphogenetic capacity. Actually, this proliferative potency is greater in the anterior, than in the posterior parts of the alga. From this evidence, Hämmerling (1934b) inferred the existence of a "morphogenetic substance", probably produced by the nucleus and distributed throughout the cytoplasm in an anterior-posterior gradient. The regenerative power of enucleated fragments would then depend on the amount of "morphogenetic substance" stored in the cytoplasm. In nucleate fragments, however, this substance can be produced continuously, either in the nucleus itself or at least under its direct influence (Hämmerling, 1943, 1953).

These data have been confirmed by the following transplantation experiments. Hämmerling (1943) and his co-workers (Beth, 1943; Maschlanka, 1946) used two kinds of *Acetabulariae*: *Acetabularia mediterranea* and *Acetabularia crenulata*. They are distinguished from each other by differently shaped caps. If, by transplantation, one produces binuclear algae which each contain one nucleus from either type, an "intermediary" cap shape will be formed (Fig. 12). Trinuclear *Acetabulariae*, which contain two nuclei from *A. crenulata* and one from *A. mediterranea*, grow caps that resemble more those of *A. crenulata*. If an enucleated pedicle of *A. crenulata* is grafted onto a nucleate rhizoid of *A. mediterranea*, again there will develop at first an intermediate form of cap. But, if this cap is clipped, then the typical cap of *A. mediterranea* will appear. The interpretation of this fact is obvious; there was still sufficient "morphogenetic substance" present in the enucleated pedicle of *A. crenulata* to induce the old cap form. But the newly produced "morphogenetic substance" is derived from the nucleus of *A. mediterranea*.

The behaviour and nature of this "morphogenetic substance" were studied in many other

experiments. It was demonstrated, for example, that it was also produced in the dark, i.e., in the absence of photosynthesis (Hämmerling and Hämmerling, 1959a), and that their "potential gradient" from apex to basis is also independent of photosynthesis (Hämmerling and Hämmerling, 1959b). The question as to what material composes the "morphogenetic substance" has also been widely discussed. It is an established fact that it is consumed gradually in enucleated fragments. No evidence could be found of the fact that it is a protein, as was believed initially. For, in contrast to the "morphogenetic substance", the protein

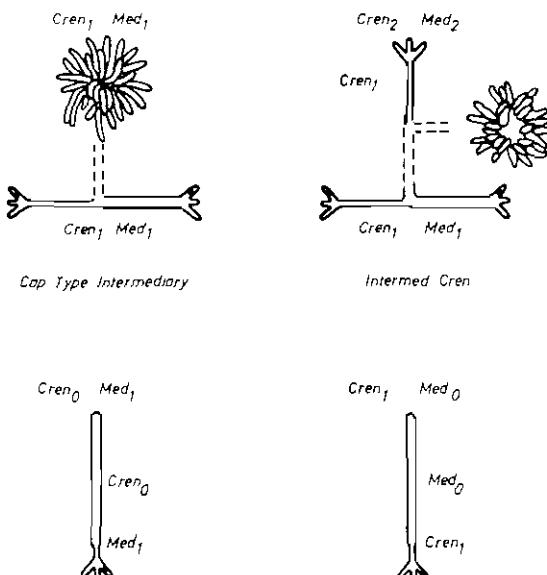


Fig. 12. Schematic representation of the transplantation experiments of Hämmerling. *Top left*: one pedicle of a nucleate rhizoid of *Acetabularia cren.*, combined with one pedicle of a nucleate rhizoid of *A. med.*, gives rise to an intermediary type of umbrella-like cap. *Top right*: two pedicles of *A. cren.* and one pedicle of *A. med.*, all three nucleate, produce at first, when combined, an intermediary type of cap, later, the *Crenulata* type of cap. *Bottom Left*: transplantation of a *Crenulata* pedicle onto a nucleate *Mediterranea* rhizoid gives rise first to an intermediary type, but changes then into the *Mediterranea* type of cap. *Bottom right*: the reverse happens when a *Mediterranea* pedicle is transplanted onto a nucleate *Crenulata* rhizoid. (From Hämmerling J. (1953), *Int. Rev. Cytol.* 2, 475-498).

content in enucleated fragments goes on increasing immediately after clipping, albeit less markedly than in the nucleate fragment (Clauss and Werz, 1961). One particular enzymatic protein, a phosphorylase, rises to more than 600% after enucleation (Clauss, 1959).

Of course, protein synthesis without a nucleus is limited, also in the case of the *Acetabularia*. The incorporation of ^{14}C remains equal in nucleate and enucleated fragments for two weeks (Clauss and Werz, 1961), then, however, it decreases progressively in the enucleated fragment. After seven weeks, it is reduced by a factor of 2·4. Here, evidently, the nucleus is

not absolutely necessary for the actual protein synthesis—a fact that is confirmed by experiments on non-nucleate reticulocytes (p. 32). Nevertheless, it is indispensable for the long-term production of protein. Again we must infer the presence of a factor which is produced under the decisive influence of the nucleus, maintains protein synthesis in the cytoplasm, yet is consumed after a certain period of time—exactly like the “morphogenetic substance”. Both factors may possibly be identical.

From these findings, we cannot help thinking primarily of the RNA. Is it not, like the “morphogenetic substance”, especially concentrated in the zone of growth, i.e., in the pedicle tip of *Acetabularia* (Werz, 1960)—and clearly dependent on the nucleus? Moreover, in enucleated posterior pieces of *Acetabulariae*, which are incapable of growth, there is no accumulation either of RNA or of “morphogenetic substance”. Thus, in all instances so far, there has been an evident parallelism between RNA and “morphogenetic substance” (Werz, 1961).

We can also understand now why, in the merotomy experiment, the syntheses of RNA and protein appeared to be dissociated. Whereas, in enucleated fragments, the protein synthesis remains at first unaltered, the net production of RNA decreases considerably at once (G. Richter, 1959). Enucleated *Acetabularia* fragments are perhaps still capable of a limited RNA synthesis (Schweiger and Bremer, 1960, 1961). Yet, the amount of synthesized cytoplasmic RNA is small and its formation, which is evidently independent from the nucleus, is, as attested by all observations made until now, limited to plants and bacteria; it is, according to Naora *et al.* (1960), localized in the chloroplasts, at least in plant cells.

Undeniably, the alga *Acetabularia* represents an exceptional case. On the other hand, there is hardly a more favourable model for the investigation of nuclear function, and for this reason we cannot but grant these data a special place and status. In the light of these experiments, we are now in a position to answer indisputably the very question of the part played by the nucleus in the synthesis of protein and RNA. The nucleus is not necessary for that part of the cytoplasmic protein synthesis which is actually in the process at a given time. Yet, a long-term protein synthesis is impossible without the presence of a nucleus. The RNA, which is predominantly of nuclear origin, seems to act as a mediator between nucleus and cytoplasm.

These data have been demonstrated in an equally impressive way by merotomy experiments on amoebae. Only a few days after resection, the basophilia of enucleated fragments is markedly reduced and 10 days later, the RNA content has decreased to 60% (Brachet, 1955). From these results, it was to be assumed that the nucleus is the main area of RNA synthesis, and Goldstein and Plaut (1955) brought further evidence of this fact. They fed amoebae a ³²P-containing food, resected the markedly radioactive nucleus and implanted it into other, non-labelled amoebae. As early as 12 hours after transplantation, the cytoplasm of these cells was radioactive, whereas the nucleus of the recipient amoeba stayed free of radioactive phosphorus. From this it was to be concluded that the phosphorus-carrying substance could indeed be transferred from the nucleus to the cytoplasm, but not from the cytoplasm to the nucleus.

Here again the question was raised whether or not the RNA of amoebae cytoplasm was produced in the nucleus *and* the cytoplasm or in the nucleus *alone*. Studies by Brachet (1959b) with tagged adenine led initially to the assumption that, in analogy to the findings in *Acetabu-*

lariae, amoebae also are capable of an at least limited RNA synthesis in their cytoplasm. However, experimental pollutions evidently played a great part in these investigations. For, when the nutrient medium was kept under sterile conditions, it was no longer possible to demonstrate any RNA synthesis in the enucleated fragments (Prescott, 1959a). Recent experiments with the ciliate infusorian *Tetrahymena* have confirmed these results (Prescott, 1961a, 1961b) (Fig. 13).

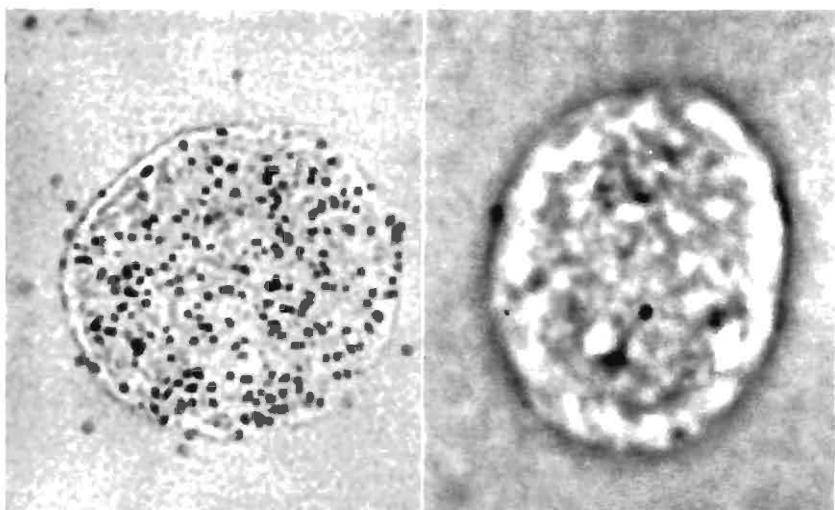


Fig. 13. Nucleate (left) and non-nucleate (right) fragment of a protozoan following 2 hours incubation with ^3H -cytidine. Extensive labelling of RNA in the nucleate, no labelling in the non-nucleate fragment. (From Prescott, D. M. (1961), *Proc. I. IUB/IUBS Symp.* Vol. II Acad. Press.)

Later, the particular importance of nuclear RNA synthesis was noted in many metazoans. Woods and Taylor (1959) found for example in the root tips of the bean *Vicia faba*, that the RNA precursor ^3H -cytidine, after a short time of incubation, was to be encountered exclusively in the nucleus, and only 8 hours later in the cytoplasm. In liver cells of mice the incorporation of ^{14}C -adenine was four times more pronounced in the nucleus than in the cytoplasm (Ficq and Errera, 1959). Equally, the uptake of ^3H -cytidine in the rat pancreas was, during the first hours, limited to the nuclei (Fitzgerald and Vinijchaikul, 1959). Further data concerning this problem were brought by Oehlert (1961) after extensive studies of various rat tissues. He too noted in his experiments that in all cells the incorporation of ^3H -cytidine occurred at first in the nucleus, especially in the nucleolus. It was only later that this labelled RNA precursor could be found in the cytoplasm in cells of the small intestine after 30 minutes, in the ganglion cells of the central nervous system after 180 minutes.

As we can see, these findings in the cells of metazoans led to the very same concept as the merotomy experiments on amoebae and *Acetabulariae*. They all suggest that the bulk of macromolecular RNA is produced in the nucleus, or, at least, under its direct control. This

statement is assumed to be valid for at least 99% of the total RNA (Zalokar, 1960). Up to now, there has been no definite proof of a cytoplasmic synthesis of high-molecular weight RNA occurring independently from the nucleus—at least, not in animal cells. The relatively low-molecular weight “soluble” RNA possibly follows other rules (Canellakis and Herbert, 1960).

Protein synthesis, however, is possible in the cytoplasm even without a nucleus, at least for a certain period of time, as indicated by the experiments on *Acetabulariae*, which we discussed previously. True, enucleation of an amoeba leads to a decrease in protein synthesis, but this reduction only goes down to 40% and then stays unaltered for a long time at this level (Brachet, 1959b). If a nucleus, labelled with radioactive methionine, is implanted into another amoeba, the radioactivity is transferred not only into the cytoplasm, but also into the nucleus of the recipient cell (Goldstein, 1958). In other words, protein and RNA forerunners show a different behaviour and we must conclude that protein can pass from the nucleus to the cytoplasm as well as from the cytoplasm to the nucleus.

According to radioautographic findings by Ficq and Errera (1959), mouse liver nuclei *in situ* take up considerably more phenylalanine than the cytoplasm. Investigations carried out with ^{35}S -thioamino-acids in rabbits (Schulze *et al.*, 1959) and with ^3H -leucine as well as ^{14}C labelled amino-acids in rats and mice (Oehlert *et al.*, 1960) have proved that the nucleus synthesizes its proteins primarily at sites where chromatin structures can be stained. Isolated nuclei, too, incorporate free amino-acids into their proteins (Allfrey, 1954) and, moreover, in close connection with the nuclear ATP synthesis. All substances that inhibit ATP formation, also block the incorporation of labelled amino-acids (Mirsky and Osawa, 1961). Thence, we understand that oxygen restriction too can influence nuclear protein synthesis. A further prerequisite for nuclear protein synthesis is the presence of Na^+ ions (Allfrey *et al.*, 1961), which possibly perform the transport of amino-acids into the nucleus. Most likely, the different steps of nuclear protein synthesis correspond largely to those of cytoplasmic protein synthesis (see p. 249).

If both, nucleus as well as cytoplasm, produce protein and if both syntheses follow the same principles, then the question is: Is there any difference between these two, i.e., has nuclear protein synthesis a special significance in the metabolism of the cell?

This question was answered affirmatively by the findings of Danielli *et al.* (1955) (see also Danielli, 1958). If the nuclei of two closely related species of amoebae, *A. proteus* and *A. discoides*, are exchanged, sterile hybrids appear. The morphology of each cell is determined by the respective cytoplasm, but the mode of motion is of an intermediary type. However, if one produces antibodies against both amoebae and tests their action on the hybrids, it appears that the antigen properties of these cells are exclusively determined by the nucleus. Danielli (1960) concluded from these and other data that the nucleus or more precisely the chromosomal genes determine the formation of macromolecules, which are later organized into structures under the control of the cytoplasm. He assumed that there are “messenger” or “signal” molecules, capable of travelling to and fro between nucleus and cytoplasm and between the cytoplasmic organelles, and controlling the specific syntheses (see also Danielli, 1958). Apart from this messenger RNA, which we will discuss in detail elsewhere (p. 251), other macromolecules have been thought responsible for that action, especially proteins, which might be partially also of nuclear origin. These substances would

be capable of serving two purposes. They would transmit information from the genes to the cytoplasm and, vice versa, inform the genes of alterations in the cytoplasm. Such a function would again provide an explanation for the interdependence of nucleus and cytoplasm. The assumption that some of the protein macromolecules are of nuclear origin is supported by an observation made by Dutcher and Fahey (1960), who demonstrated, in the case of macroglobulinaemia Waldenstrom, that 18-S-gamma globulins are found chiefly in the nuclei of lymphoid cells, where they are produced.

Review

With the discussion of the formation of messenger RNA and certain nuclear protein molecules, we are back again at the starting point of our analysis, and we are in a position to make the following statements. The nucleus exerts control over the cytoplasmic organelles and utilizes for this purpose mainly the synthesis of RNA and protein. In contrast to this primary function, its energy production is probably comparatively insignificant. It is still not clear, whether or not the nucleus is able to meet its own energy demands. Energy is chiefly produced via glycolysis; but, additionally, an oxidative formation of ATP is also possible. The nuclear formation of NAD is certain. Furthermore, all findings suggest that the entire macromolecular RNA of the cytoplasm is of nuclear origin, except, possibly, the RNA of the chloroplasts. The specific role of RNA will be discussed later. As we have seen above, it is closely related to protein synthesis.

The processes involved are decisive for the entire metabolism of the cell; however, they can only be understood if we know the composition and fine structure of the nucleus and its components. This we will discuss in the following chapter.

THE NUCLEOLUS

In our search for a morphological substrate for the nuclear functions discussed in the previous chapter, our attention is drawn at first to the nucleolus, the nuclear corpuscle, as this structure has been called since Schwann (1839). Indeed, *in vivo* the nucleolus is the most striking, even the only clearly discernible, of the nucleus' inner structures (Fig. 14); the main components of the nucleus, the chromosomes, are hardly visible (p. 27). For this reason it is understandable that in Schleiden's cytoplasm theory, which

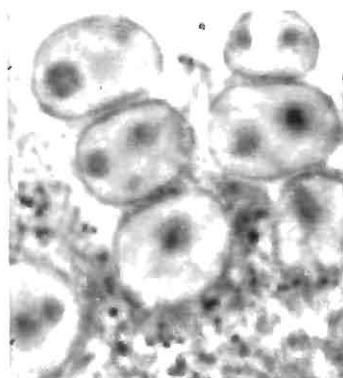


Fig. 14. Supravital phase contrast micrograph of nuclei of mouse pancreas epithelium following storage in a sugar solution. Only the nucleoli and the nuclear envelope are visible; the nucleoli have no membrane. (From Altmann, H.-W., Grundmann, E. (1955), *Beitr. path. Anat.* 115, 313-347.)

we mentioned earlier (p. 24), the nuclear corpuscle was taken for the first and really genuine cell structure, around which the nuclear material and, later, the cytoplasm was thought to deposit during the process of cell formation (see also Schwann, 1839). Today, this theory is a mere historical reminiscence, understandable on account of the particularly striking appearance of the nucleolus, which led Fontana, as early as half a century before Schwann, in 1781, to give the first description of a nuclear corpuscle as "... une tache dans son (the nucleus) milieu".

Shape and Structure

The question of nucleolar function, that interests us most after the foregoing, will be discussed at the end of this chapter. For, in order to answer this question, we must at first describe the shape of the nucleolus. Usually a spheroidal structure it is situated in the centre or at the periphery of the nucleus. Generally, each nucleus has at least one of these structures. The fixed and stained section as prepared for light microscopy shows that it is surrounded by a sharply defined membrane, similar in quality to the nuclear membrane (see e.g., Fig. 10).

This is, in general, the typical appearance of the nucleolus, but there are many variations. For example, the shape can be quite irregular in cells of tissue cultures as a result of many evaginations (e.g., Stockinger, 1953; Bierling, 1960). In the large alga *Acetabularia mediterranea* the nucleolus expands during the period of cell growth by means of lateral evaginations and ramifications until it finally has the appearance of a large sausage-like structure (Hämmerling, 1931). On the other hand, the nucleolus of the closely related *A. Wettsteinii* is of a very irregular shape and has, particularly *in vivo*, very ill-defined contours (Schulze, 1939). Furthermore, undifferentiated embryonic cells usually have no nucleolus, ganglion cells, a particularly large one, whereas other cells, such as fibroblasts, endothelial or neuroglia cells, contain a very small and in many cases unostentatious nucleolus. From these examples we may deduce that shape and size of the nucleolus are dependent, within certain limits, on the species and the specific differentiation of the cell.

The same rule can be applied, with similar restrictions, to the number of nucleoli. Whereas most ganglionic cells have only one large nucleolus, many liver cells and a certain type of lymphocytes, possess two or more. Polyploid nuclei contain more nucleoli than diploid ones. The amphibian oöcytes have, under certain conditions, even several thousands of nucleoli, which, according to observations by Gall (1955), are equivalent to the nucleoli of somatic cells. Older data should be evaluated with reservation, since other dense particles, that have—as we know today—nothing in common with them, were frequently mistaken for nucleoli. Also, there are undoubtedly differences between various types of nucleoli, as for example the "special nucleoli" of the chironomids, which are very similar to normal nucleoli in appearance, but are functionally not equivalent (Beermann, 1960). This homology of all nucleoli and the fact that light microscopists have not yet found an adequate definition for the nucleolus, have led to the conception that the number of nucleoli in a nucleus is almost arbitrary, or at least entirely unspecific (e.g., Stockinger, 1953); even the coalescence of nucleoli into a "collective nucleolus" is no rarity.

The frequent round shape of nucleoli and the observation that several of them may coalesce suggest that the nucleolar content is liquid. Most likely its consistency is that of a

fat droplet. In the large salivary gland cells of *Chironomus* it is possible, by applying pressure cautiously, to separate the nucleolus into two parts, that immediately take on a round shape (Kolmer and Fleischmann, 1928). Owing to their greater light refraction, nucleoli appear to be more compact than the surrounding karyoplasm and their relative dry mass is higher (Sandritter *et al.*, 1960). In frozen-dried rat liver cells, their mass is more than 30 vol.^o as compared to the 12–20 vol.^o of the karyoplasm (Sternram, 1961).

The nucleolar content is seldom homogeneous. In fact, it contains frequently a few or many vacuoles, as for example in *Acetabulariae* (Hämmerling, 1931), in the salivary glands of chironomids, in human or animal egg cells (Shettles, 1959) or in ganglion cells. In oöcytes of *Copepoda* the vacuoles increase from 15^o to approximately 75^o of the nucleolar volume during the period of growth (Stich, 1956). In the light microscope as well as in the electron microscope, they seem to be empty, i.e., they probably consist mainly of water. Sometimes, however, we find isolated, strongly stained granules, the nucleolini, whose refractive index is even greater than that of the entire nucleolus.

By employing a special silver impregnation technique, Estable and Sotelo (1951) were able to demonstrate an infinitely fine vermiform mass, which they termed "nucleolonema". It is embedded in a homogeneous "pars amorpha" and its fine threads seem to extend throughout the whole nucleolus.

This light microscope evidence has been confirmed and defined in greater detail by electron microscopy. In 1951, Borysko and Gang saw for the first time, in the electron microscope, such fine threads within a homogeneous nucleolar substance and since then, this finding has often been confirmed (Fig. 3) (Bernhard *et al.*, 1952, 1955. For ref. see Vincent, 1955; Wischnitzer, 1960). Georgiev and Chentsov (1962) then discovered that each thread consists of granula, arranged in chains of a diameter of 100 to 200 Å and that these granula are held in this particular array by a still finer thread, 80 Å in diameter. Occasionally this chain-like arrangement of the granula brings about a slightly undulated striation of the nucleolonemata (Horstmann and Knoop, 1957). Kurosumi (1961) was able to observe in human sweat glands this arrangement of granula with even greater precision. The granula, averaging 150 Å, are lined up like beads along the axis of the large nucleolonemata; they represent "primary nucleolonemata". When these threads are twisted into spirals of many layers, they become the "secondary nucleolonema" which has a diameter of 1000 to 2000 Å. The latter then corresponds to the original nucleolonema as described by Estable and Sotelo (1951) with the light microscope.

Apart from these nucleoli, there are others that have no "secondary" nucleolonemata. Nucleolonema-containing and nucleolonema-free nucleoli may occur together (Bolognari, 1959, 1960, 1961). Therefore, the "secondary" nucleolonema is not, as for example Estable and Sotelo (1954) had assumed, a permanent structure. It is transient like the vacuoles of the nucleoli (Serra 1958) and from the presence or the absence of "secondary" nucleolonemata one can distinguish two types of nucleoli, which may even represent two different functional stages of the nucleolus (Bolognari, 1961). Moreover, there are many transitions between the two forms (Davis, 1960).

Relation to the Chromosomes

Whereas electron microscopy on one hand was able to extend considerably our knowledge of the morphology of the nucleolus, it furnished on the other hand one particular fact that was, at first, difficult to interpret, viz., the fact that the nucleolus invariably lacks a membrane (Fig. 3), except for the special case found in a mouse liver (David, 1960a). The above-described fine granular threads of the nucleoli seem to extend into the karyoplasmic ground structure, made of equally fine threads (Bopp-Hassenkamp, 1959a; Peveling, 1961). This observation is in extreme contrast to the light microscopic findings in fixed and stained sections, where a distinct fringe of chromatin can frequently be seen surrounding the nucleoli. From this we must conclude that the membrane we see in the light microscope is an artifact. This is confirmed by a comparison with the supravital picture. Here too, the nucleoli are as "naked" as we see them in the electron microscope (Fig. 14).

How does the nucleolus acquire this artificial membrane?

In the Feulgen stain for DNA, the nucleolar membrane is always positive, yet the interior portion of the nucleoli is usually negative. Hence, if nucleoli, after fixation and staining, are surrounded by a Feulgen-positive membrane, that we recognized as an artifact, this membrane is clearly the result of a deposit of chromosomal material (see Altmann *et al.*, 1963). Further and more detailed explanation of this fact will be given in the chapter on the structure of the karyoplasm (p. 68).

The occurrence of such an artifact requires that nucleolus and chromosomes are located at adjacent sites. This close topographical relation, still rejected in 1929 by Wassermann, has now been proved. Actually, the nucleolus is a product, strictly speaking, even a part of the chromosomes of the interphase nucleus. This fact determines today our conception not only of the morphology, but also of the function of the nucleus.

Investigations of this matter were initiated by the long-known fact that nucleoli disappear during mitosis and reappear, after completed division, in the reconstruction phase at very precise sites in the chromosomes. In plants with particularly large chromosomes, these chromosome sections are free of stainable DNA. For this reason, they were termed by Heitz (1931a) "SAT segments", i.e., "sine acido thymo-nucleinico". The diploid nuclei of the bean *Vicia faba* have, for example, two chromosomes with such SAT segments, on which, in the interphase nucleus, two nucleoli develop, exactly at the same site where these chromosome sections are located after the post-telophase reconstruction. Nuclei of other species behave the same way (Fig. 15). The universal validity of this principle was soon recognized and the sites where nucleoli are formed were termed "nucleolar organizers". Frequently, at these sites no SAT segments are discernible but "heterochromatic" portions of the chromosomes that stain particularly strongly (McClintock, 1934). At least, such heterochromatic areas are often found adjacent to the nucleoli in interphase nuclei of animal cells (Caspersson, 1941, 1950; Hyden, 1943; Vogt and Vogt, 1946; Barigozzi and Feletig, 1950) and in egg cells as well (e.g., Arndt, 1960). Sometimes, these chromatin densities at which nucleoli are formed are located near the nuclear membrane (Ludford, 1954; Iversen, 1960a); frequently, however, they lie in the middle of the karyoplasm (Grundmann, 1961a). There, they can be well demonstrated with the electron microscope (Peveling, 1961). They consist of a conglomerate of helically coiled threads having a mean width of 130

to 150 Å, i.e., a diameter quite similar to that of the chromonemata of chromosomes (see p. 108).

Recently, this relation between nucleoli and chromosomes has been demonstrated in even more precise terms. There is much evidence to suggest that the nucleolus is generated around the chromosome in a circular fashion. In the final stage, the chromo-

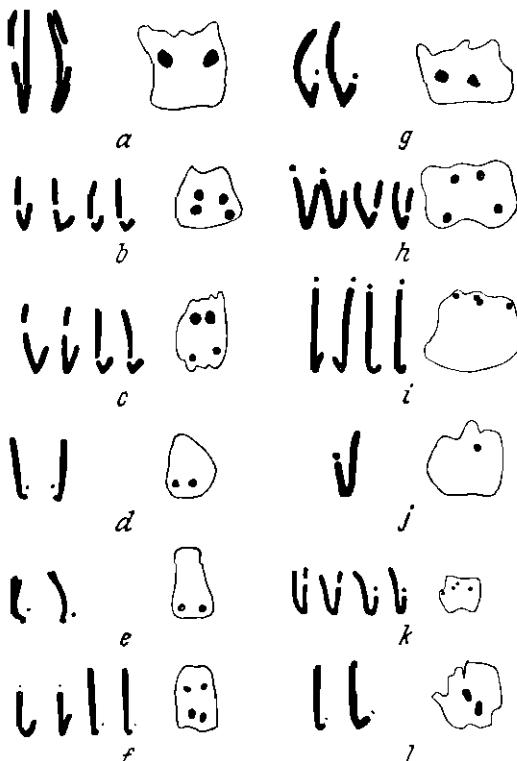


Fig. 15. Scheme showing relation between SAT chromosomes and sites of nucleoli formation. Number, position and size of nucleoli correspond to the SAT segments of the chromosomes. (a) *Vicia faba*, (b) *Vicia pannonica*, (c) *Vicia hybrida*, (d) *Crepis sibirica*, (e) *Crepis virens*, (f) *Crepis pulchra*, (g) *Allium cepa*, (h) *Drosophyllum lusitanicum*, (i) *Aloe arborescens*, (j) *Makinoa crispata*, (k) *Hordeum vulgare*, (l) *Aneura pinguis*. (From Heitz, E. (1931), *Planta*, 15, 495-505.)

some then runs through the nucleolus and does not just adjoin its peripheral aspect. Fig. 16 shows the chromosomal substance in the centre of the nucleolus as it appears in the electron microscope. True, the final proof that this is indeed the nucleoli-generating segment of a chromosome is still lacking. Also, the Feulgen-positive, i.e., DNA particles which many investigators assume the nucleolus to contain (Mulnard, 1956; O'Donnell, 1961; Bhattacharyya and Ghosh, 1961; and others) may be partially explained by supposition of heterochromatic chromosome centres, just as the assumption that the "nucleolonema" contains DNA (Davis, 1960; Lettré and Siebs, 1961a) could never be demonstrated with certainty. Yet, today we are almost certain that the nucleolus too contains DNA and that, by radioautography, DNA synthesis can be demonstrated also in the nucleolus, probably indicating replication of that chromosomal segment which carries the nucleolus (Altmann et al., 1963).

The findings on the giant chromosomes of *Diptera* are quite impressive. Here the nucleolar substance always surrounds a distinct chromosome segment as an irregularly shaped structure (Fig. 17). These areas are sharply defined against the adjoining transverse bands (Beermann, 1952a). They are so to speak, inserted, as additional structure elements, in the pattern of transverse bands of the giant chromosomes. The insertion occurs at sites which are specific for each tissue (Beermann, 1960). When nucleolar substance is being generated,

these well-defined chromosome segments swell and their fibrillar elements spread out into many ramifications (Fig. 18). The nucleoli are here definitely the products of distinct chromosomal loci with which they stay in close topographic relation. Indeed, they are themselves parts of the chromosomes.

The chromosome that carries the nucleolar organizer apparently also determines largely the composition and size of nucleoli. For instance, the microsporocytes of *Zea mays* have, as diploid cells, two chromosomes carrying nucleolar organizers; if the number of chromosomes is increased by adding another "organizer" chromosome, the nucleolar RNA- and

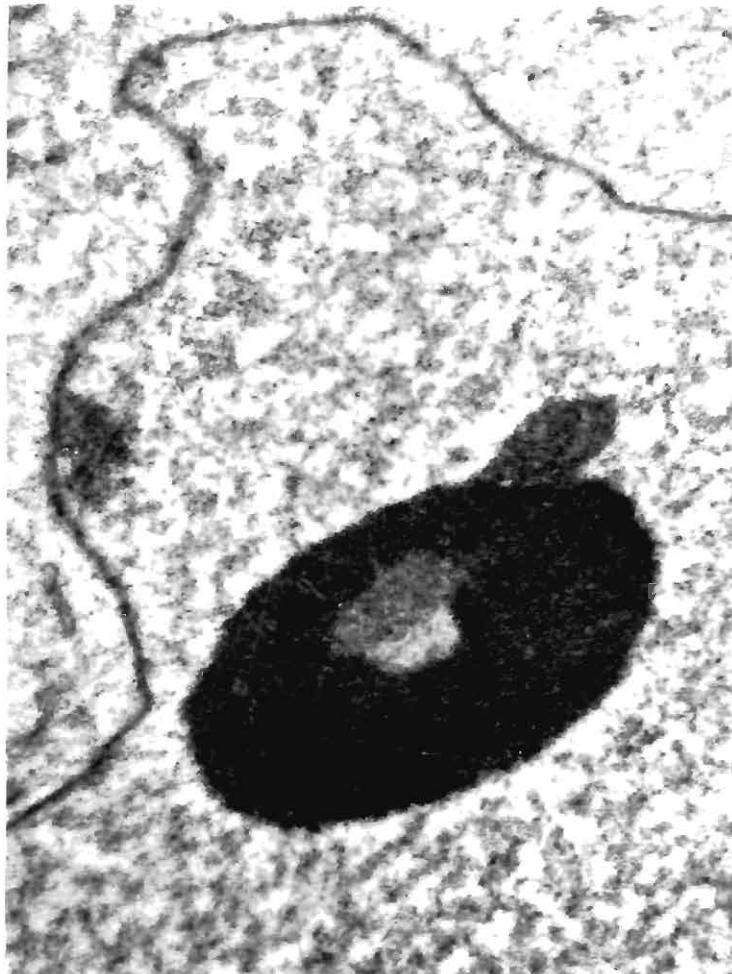


Fig. 16. Compact nucleolus with a central vacuole and a rodlike chromocentre which is located adjacent to and inside the nucleolus. The chromocentre is less blackened than the nucleolar content. Interphase nucleus of *Cucumis sativus*. (From Peveling, E. (1961), *Planta*, 56, 530-554.)

protein content will increase to exactly one and a half times its normal value (Lin, 1955). Addition of a "non-organizer" chromosome has no effect.

We must, however, point out the fact that nucleoli can also occur at other points on the chromosome. As early as 1931, Heitz had made the observation that micronuclei which are the product of a mitotic disturbance and which do not contain any organizer chromosome,

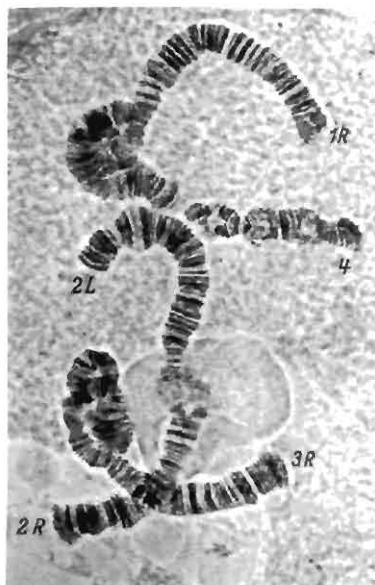


Fig. 17. Phase contrast micrograph of salivary gland chromosomes of *Chironomus pallidivittatus* with adjoining nucleolus. Note lesser density of the chromosome in the area where it is surrounded by the nucleolus. Acetocarmine preparation. (From Beerman, W. (1960), *Chromosoma*, 11, 262-296.)

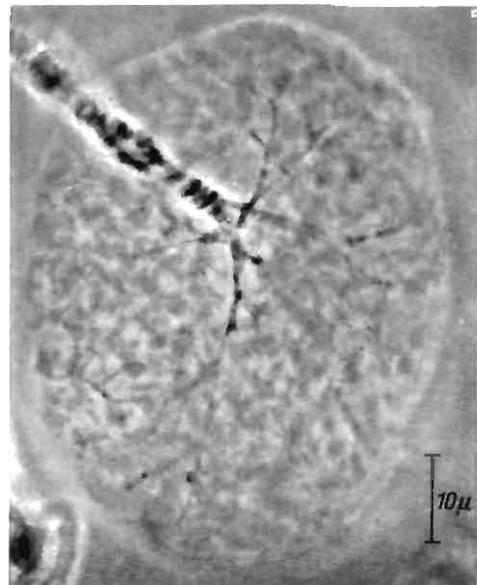


Fig. 18. Phase contrast micrograph of the special nucleolus at chromosome 4 in *Chironomus pallidivittatus*, clearly showing the splitting of the chromosome's longitudinal fibrils. Acetocarmine preparation. (From Beermann, W. (1960), *Chromosoma*, 11, 262-296.)

generate small nucleoli; apparently, other chromosomes are able to take over the function of forming nucleoli. This fact has been demonstrated also in wheat (Longwell and Svhla 1960). In these cases there are, apart from the main organizers, other "latent" organizer chromosomes present, which start functioning when the main organizers are absent.

Composition

When the nucleoli-generating transverse bands swell, as for instance in the giant chromosomes of chironomids, the DNA-containing chromosomal substance is increasingly diluted in this region. The fact that in most nucleoli DNA cannot easily be demonstrated, may,

therefore, be regarded as a dilution effect. On the other hand, there is heterochromatic material, i.e., material rich in DNA, directly adjacent to the nucleolus and serving as "organizer". This is probably the explanation for the frequently contradictory results of isolation experiments. Isolated nucleoli of liver cells have been demonstrated to contain up to more than 17% DNA per unit of dry mass (Monty *et al.*, 1956), whereas nucleoli of fish oocytes (Vincent, 1952, 1955) and of peas (Stern *et al.*, 1959) were found to be free of DNA. The difference may be due merely to the isolation technique; in one case, larger portions of the carrier chromosomes were contained in the isolation product, in the other, only the nucleolar substance was isolated. Probably, the actual nucleolar material, i.e., that substance which accumulates on or in specific segments of the chromosome, is free of DNA.

The nucleolar substance of all cells, however, contains RNA. Caspersson and Schultz (1939) and Brachet (1940) were the first to demonstrate this fact with cytochemical methods. The strong basophilia had for a long time suggested a particularly high RNA content. Exact studies have proved this assumption false; 10% of the total nucleolar mass, at the most, is RNA; in most cases, the figure is 5% or less (Vincent, 1952, 1955; Baltus, 1954). Even if we take into account the loss of RNA resulting from the isolation procedure, the amount of RNA concentrated in the nucleolus is hardly, if at all, greater than in the karyoplasm (Vincent, 1955; Stern *et al.*, 1959). Incidentally, Caspersson and Schultz (1940) had already measured an RNA concentration of only 3.5% in the nucleoli of *Drosophila* (see also Vogt-Köhne, 1961).

Therefore, the strong basophilia of nucleoli is not due to RNA, as was assumed earlier (e.g., by Serra, 1947). Rather the proteins play a considerable role and all investigations demonstrated that nucleoli contain much protein. Vincent and Huxley (1954) had measured the protein content of isolated fish nucleoli and found a maximal value of 85%. What kind of proteins these may be, is, as yet, largely obscure. The assumption, made originally by Caspersson (1941), that they are predominantly basic proteins of the histone type, or, to use a better expression, proteins with many di-amino-acids (Caspersson, 1950), has now been refuted (for ref. see Vincent, 1955; Brachet, 1957; Mirsky and Osawa, 1961). Probably in the nucleolus mainly phosphoproteins are present which, as revealed by electrophoresis, prove to be, significantly different from proteins of the karyoplasm (Poort, 1961). In fish oocytes, paper chromatography has shown only a single type of protein (Vincent, 1960), which was interpreted as being the product of a single gene site. It is also noteworthy that nucleoli can be stained with a non-specific basic dye, methylene blue, at pH 4.9 (Haenel, 1950; Stockinger and Kellner, 1952); this may be also due to a protein that is typical of both the nucleoli and certain portions of the cytoplasm.

On the other hand, it has been frequently confirmed that nucleoli contain several enzyme proteins as, for instance, the NAD synthesizing enzyme, nucleotide phosphorylase and especially acid phosphatase (Vincent, 1952, 1955). Cytochemically positive reactions for alkaline phosphatase proved to be diffusion artifacts (Mirsky and Osawa, 1961). Whether this also holds true for the succinic dehydrogenase, whose presence De *et al.* (1962) were able to demonstrate cytochemically in the nucleoli of liver and tumour cells, is not decided as yet. Since succinic dehydrogenase is a flavoprotein especially closely connected with the respiratory chain, its demonstration would be of considerable importance for the above-discussed question as to whether or not the nucleus has an energy metabolism of its own (see

p. 33). Tests for RNase, previously mostly negative, were more recently found, in the nucleoli of oocytes of *Asterias rubens*, to be definitely positive (Baltus 1960).

Significance and function

RNA metabolism

Whereas the enzymatic composition of the nucleolus does not allow us to make any definite statements about the nucleolar function, the fact that all nucleoli contain RNA has proved to be important. However, nucleolar RNA does not seem to be of a uniform nature. This is already suggested by certain metachromatic effects obtained by employing Toluidine blue molybdate (Love and Bharadwaj, 1959; Stolk, 1961). The heterogeneous composition of RNA has been more precisely demonstrated in fish oocytes (Vincent and Baltus, 1960); on the one hand, incorporation of ^{32}P into RNA is an indication that RNA is synthesized anew, however on the other hand ^{32}P is bound to the end groups of another RNA, just as free nucleotides are bound to the end groups of the cytoplasmic "soluble" RNA.

This heterogeneity of the RNA must be kept in mind when we now attempt to answer the question of the location of RNA synthesis. In the foregoing chapter, we have brought evidence that the nucleus, at least in most cells, is the main, if not the only site of RNA formation (see p. 38). It is equally certain that the nucleoli play a part in this process. The principal question in a simplified form is whether or not the nucleolus generates its RNA by itself or whether it merely collects RNA from the chromosomes. If the nucleolus itself synthesizes RNA, then the question arises as to whether this is a function restricted solely to the nucleolus or whether the chromosomes too, independently, take part in RNA metabolism. Finally, we must find out to what extent the above-postulated transfer of nuclear RNA to the cytoplasm is mediated by the nucleolus. In this context we must also be aware of the close relationship between RNA and protein synthesis (p. 32).

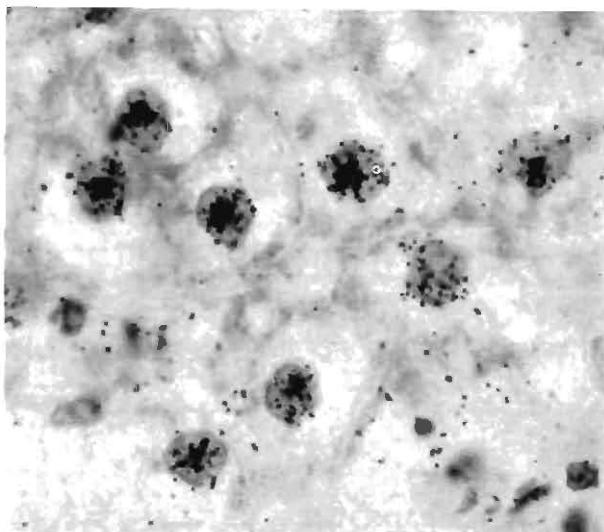


Fig. 19. Radioautograph of nuclei of rat liver cells after injection of ^3H -cytidine. Marked labelling of RNA, with preference for the nucleoli, and exaggerated here because of previous administration of diethylnitrosamine, a carcinogen. (From Oehlert, W. and Hartje, J. (1963), *Beitr. path. Anat.*, **128**, 376-415.)

Does the nucleolus synthesize its RNA on its own?

Radioautography has been the best method for tackling this problem, since the time it takes for an isotope to be incorporated can be held, to a certain extent, as a measure for the speed of the syntheses. In ^{32}P studies on fish oöcytes (Ficq, 1955) on the giant nuclei of *Drosophila* salivary glands (Taylor, 1953) or in isolated nucleoli of *Acetabularia* (Hämmerling and Stich, 1956), the labelled material showed unequivocally, in time and quantity, a preference for the nucleolus, where ^{32}P incorporation was in certain cases a hundred to a thousand times more intensive than in the cytoplasm (Vincent, 1955); it was also considerably greater than in the karyoplasm. When the much more specific labelling of RNA precursors with tritium became available, many studies were performed with ^3H -cytidine and ^3H -uridine. Again, the result was, in many different cell types, an especially intensive labelling of the nucleolus (Harris, 1959; Sirlin, 1960a; Sisken and Kinosita, 1961; Oehlert, 1961; for further references see there; Lauf *et al.*, 1962; Sirlin and Schor, 1962a and b). All these observations demonstrate clearly a high rate of RNA turnover in the nucleolus (see Fig. 19); findings in living cells of tissue cultures (Chèvremont and Baeckeland, 1961; Bogoroch and Siegel, 1961) and in the giant nuclei of insect salivary glands (McMaster-Kaye, 1962), obtained by using ^3H -uridine, ^3H -cytidine or ^{14}C -adenine, were along the same line.

The other alternative was that RNA is not synthesized in nucleoli at all but on the chromosomes, the nucleolus serving merely as a collection site. This concept has found since McClintock (1934) many followers (ref. see Altmann, 1957). It was reported, for instance, that labelling of the chromosomal substance occurred in the giant chromosomes of chironomids soon after their exposure to ^3H -uridine and ^3H -cytidine (Sirlin, 1960b; Sirlin and Schor, 1962a, 1962b). In these objects one could even localize with precision the chromosomal sites at which primary RNA synthesis started (Pelling, 1959); maximal ^3H -uridine incorporation was found first in the immediate vicinity of the nucleoli, i.e., in the region of the nucleolar organizer, secondly—and mainly—in the so-called Balbiani rings and in the “puffs”, which, as we will see later on (see p. 119), are the morphologically demonstrable sites of marked gene activity. The logical interpretation of these findings is that at least parts of the nuclear RNA are a product of active chromosomal genes (see, e.g., Ficq, 1961). This interpretation has been confirmed by more recent observations in nuclear fractions (Rho and Bonner, 1961).

Whether chromosomal RNA secondarily gets into the nucleolus is another question. Most observations contradict this possibility. For instance, in a nucleolus that had been damaged by selective ultraviolet-irradiation, only very little newly synthesized RNA can be demonstrated radioautographically (Perry, 1960a, 1960b; Perry and Errera, 1960). This indicates, if the concept is correct, that at least under these conditions the nucleolus does not accept chromosomal RNA any longer. Other radioautographic findings clearly demonstrated a strict quantitative difference between the RNA synthesis of the nucleoli and that of the “chromatin”. For example, the incorporation of ^{14}C -labelled RNA precursors into nucleoli of insect larvae salivary glands is, during the first four hours of incubation, up to seven times as high as the incorporation into the rest of the chromatin (McMaster-Kaye, 1962). Quantitative studies also brought evidence of the fact that nucleolar RNA, soon after its synthesis, is transmitted into the cytoplasm whereas the fate of chromosomal RNA could not be determined with certainty (Leblond and Amano, 1962).

As we have seen, RNA is generated at the nucleolus as well as at other loci of the chromosomes. The "nucleolar organizer" probably, is nothing but a specially designed gene area (Beerman, 1960) in which largely its own products are being accumulated. All these findings point to the fact that there are at least two kinds of nuclear RNA, of which one is synthesized under the influence of the nucleolar organizer, the second at other chromosomal loci. Differentiation of nucleolar and chromosomal RNA has also been, in many studies, achieved by fractionation of nuclei. In these experiments, tracing of ^{32}P incorporation showed that nucleolar RNA was more active than chromosomal RNA (Georgiev *et al.*, 1960).

A qualitative difference was suggested by the observation that larvae of chironomids at late stages hardly incorporate ^3H -labelled pseudo-uridine any longer into nucleoli but extensively into chromosomal RNA (Sirlin *et al.*, 1961). Both kinds of RNA differ in their base composition. Recent, particularly elegant microanalyses in egg cells (Edström, 1960; Edström *et al.*, 1961) demonstrated that chromosomal RNA contained adenine and uracil in higher concentrations than guanine and cytosine, whereas in nucleolar RNA the concentration of guanine and cytosine is higher. Hence, the base composition of chromosomal RNA is similar to that of DNA; nucleolar RNA, however, is but little different from cytoplasmic RNA. This is not only true of egg cells but it is also basically true of those chironomid nuclei that contain giant chromosomes. Furthermore, in the latter, it was possible to show that different portions of chromosomes generate chromosomal RNA of different composition and that chromosomal regions with particularly active gene sites have a higher RNA/DNA ratio (Edström and Beermann, 1962).

We can today only roughly outline the significance of these two kinds of RNA. If it is true, as the observations on giant chromosomes of *Chironomus* suggest, that chromosomal RNA is directly related to the activities of the gene sites, then we are perhaps dealing here with the messenger RNA which transmits genetic information to the cytoplasm. This messenger RNA has been found, for instance, in rat liver nuclei (Scholtissek and Potter, 1960) but foremost in bacterial systems. It apparently determines the specificity of the ergastoplasmic ribosomes (Brenner *et al.*, 1961; Gros *et al.*, 1961); its composition in the nuclei varies from organ to organ (Scholtissek, 1962) and is, therefore, at least roughly specific for a given tissue. Whenever, in the course of our foregoing discussions, we have been talking of a genetic control of the cell function, we were referring to the co-operation of this messenger RNA.

Chemically it is comparatively unstable in contrast to the RNA of ribosomes, which, in turn, is similar to nucleolar RNA. Indeed, all quantitative and qualitative relations between nucleolus and cytoplasm, which are to be discussed later, concern mainly this stable, macromolecular ribosomal RNA. The 100–150 Å granules of the nucleolonemata and of the ergastoplasm are predominantly composed of this RNA. Ribosomal RNA is indeed responsible for the early and extensive incorporation of RNA precursors into the nucleoli (see p. 50). It is quantitatively, not necessarily qualitatively, by far the most important; moreover, nucleolar and ribosomal RNA constitute perhaps a comparatively "crude" form of RNA which may still be re-shaped under the influence of messenger RNA.

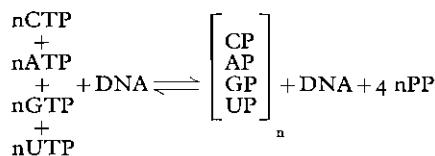
We have not, as yet, taken into account a third form of RNA, the "soluble" RNA. This type is of a low-molecular weight. Its function in protein metabolism is to bind specifically the amino-acids which are derived from amino-acid activating enzymes, and to transfer

them to the ribosomes. We will discuss this in greater detail in the chapter on cytoplasmic protein metabolism (see p. 251). According to findings in fish oöcytes (Vincent and Baltus, 1960) this "soluble" RNA, too, is synthesized in the nucleolus (see p. 49).

Nuclear protein synthesis is apparently dependent on chromosomal RNA (Amano and Leblond, 1960). The question as to which part the nucleoli play in this process has found different answers. There is evidence clearly supporting an involvement of the nucleoli (Stich, 1956; Ficq, 1959; Ficq and Errera, 1959; Bogoroch and Siegel, 1961; Olszewska and Brachet, 1961) but there is also evidence denying the fact (Carneiro and Leblond, 1959; Oehlert *et al.*, 1960); all of these findings were obtained by radioautography. Probably, variations specific of different types of tissues and of the functional stage of the nucleus must be taken into account. Perhaps the nucleolus even synthesizes only one very specific kind of protein (Vincent, 1960) which the cytoplasm does not always need and certainly not in each of its functional phases.

If the nucleolus generates any protein, its synthesis must certainly be related to the presence of an organizer region. For the synthesizing activity of nuclear RNA is generally dependent on the presence of DNA histones in the immediate vicinity (Frenster *et al.*, 1961). *In vitro*, too, RNA synthesis needs the contact with DNA (Huang and Maheshwari, 1961; Prescott, 1961b) which give it, no doubt, its specific character (see, e.g., Zubay, 1958).

This process of DNA-dependent RNA synthesis has been of particular interest to biochemists. It is probably very similar to the identical replication of DNA molecules (see p. 116), i.e., the bases are bonded to one another in the presence of DNA by an RNA polymerase. The latter was discovered first in preparations of mammalian tissue (Weiss and Gladstone, 1959; Weiss, 1960). The reaction is represented by the formula (Weiss, 1962)



The newly formed RNA has a composition similar to that of the "primer" DNA (Weiss and Nakamoto, 1961). Furthermore the synthesis of messenger RNA may be affected by cytoplasmic factors (Scholtissek, 1962), which are able to derepress certain specific parts of the DNA genome. If we take all these interactions into account, we realize the close interplay of DNA and RNA. This interplay would explain many problems of tissue differentiation and of the loss of differentiation in carcinogenesis as well (Büchner, 1961a and b; Büchner *et al.*, 1962).

Relation to the cytoplasm

The organelles affected by the action of nuclear RNA are located in the cytoplasm. If the nucleolus represents the main site of RNA synthesis for the cytoplasm we should be able to find evidence for such a relation.

The most simple evidence suggesting this relation is brought by differences in the nucleolar volume. The more the cytoplasmic organelles are stimulated, the larger are the nucleoli. This fact has been observed in an endless number of studies (for references, see Stockinger,

1953; Vincent, 1955; Stich, 1956; Mirsky and Osawa, 1961). Since RNA and protein metabolism are so closely interrelated, stresses on the latter have particularly been followed. A reaction has been found to occur in the liver of rodents after parenteral protein administration (Schlager, 1960) and also after a diet deficient in protein (Stenram, 1958b, 1962a); it appeared to be the typical reaction to an overstraining of the metabolism following partial hepatectomy (Fig. 10) (Grundmann and Bach, 1960; Oehlert *et al.*, 1962) or feeding with toxic agents (e.g., Bernhard and Bauer, 1954; Grundmann, 1954; Grundmann and Sieburg, 1962). Particularly large nucleoli result in the liver of rodents after the administration of thioacetamide (for references, see Rather, 1958; Georgii and Mehnert, 1961; Altmann and Osterland, 1961), in which case both RNA (Laird, 1953; Rüttner *et al.*, 1959) and protein content are greatly increased (Judah and Rees, 1959). Large nucleoli are usually also characteristic of cells infected by viruses (Caspersson and Thorsson, 1953; see also p. 82), of carcinoma cells (for references, see Ludford, 1954; Hamperl, 1956; Oberling and Bernhard, 1961) and of growing plant galls (e.g., Woll, 1954); indeed, they are characteristic in general of growing or secreting cells (e.g., Sandritter and Hübotter, 1954; Kracht, 1958; Long *et al.*, 1958; for further ref. see Gabe and Arvy, 1961) and also as the manifestation of an increased stimulus of protein synthesis. In *Acetabularia* the inhibition of energy-providing syntheses in the cytoplasm leads to a shrinking of the nucleoli (Stich, 1956); in *Chironomus*, the incubation in media deficient in nutriments has the same result (Vogt-Köhne, 1961).

The RNA/protein ratio may, to a large extent, remain constant through these metabolic changes in volume (Vogt-Köhne, 1961) even though an absolute increase in nucleolar RNA has been observed in cases of protein deficiency (Stenram, 1958b) and, for instance, in the maturation process of normal blood cells (Thorell, 1947) as well as the already mentioned thioacetamide studies. Frequently, in these cases, the RNA turnover has also increased (Stenram, 1962b). In the electron microscope these cells often show a particularly marked accumulation of those granules which are identical with the ribosomes; fully developed nucleolonemata are often missing (Bolognari, 1961).

Yet an increase in the size of the nucleolus need not necessarily reflect a genuine increase in substance. It may rather be the effect of hydration and thus, represent a stage of functional stimulation (Beermann, 1960). In the light microscope an increased number of vesicles are to be seen in the nucleoli and these vacuoles can be distributed throughout the nucleolus or make it swell like a mulberry (Höpker, 1953). Other cases show large pellets developing in the nucleus (e.g., Hett, 1937; Bargmann, 1942; Schiller, 1944; Ortmann, 1949; Seifert, 1952; Altmann, 1955b; Johannsen and Flint, 1959), yet, that they originated from nucleoli is now often impossible to prove. Sometimes, they certainly are nothing but invaginations of cytoplasmic substance into a nuclear indentation (Wessel, 1958; Fig. 20, right), in other instances they represent depositions of material or genuine nucleolar vesicles (Fig. 20, left).

The close relation between nucleolus and cytoplasm is particularly apparent when the content of these nucleolar vesicles is ejected into the cytoplasm. Though artifacts may occur, owing to sectioning and fixation techniques, the possibility of such a process has been demonstrated in a series of cases (Bargmann, 1942; Schiller, 1944; Altmann, 1955), especially since it could be observed in *in vivo* preparations (Altmann and Grundmann, 1955) and in electron micrographs (Clark, 1960). How the nuclear membrane acts in this case will be one of the topics of the next chapter. Probably a broad contact between nucleolar

substance and cytoplasm is possible even without any defect in the nuclear membrane, yet only under the condition of extreme metabolic strain (e.g., Dittus, 1940; Huber, 1945; Altmann and Meny, 1952; Grundmann, 1958a; Weber and Kriesten, 1960; Müller, 1961a).

This problem is still highly controversial. How do nucleolar RNA molecules get into the cytoplasm?

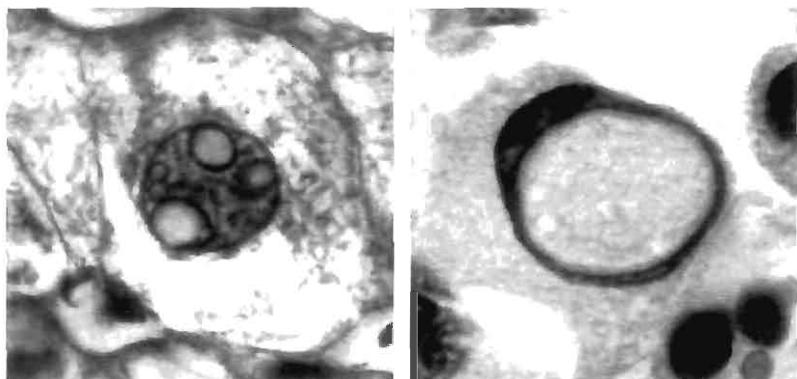


Fig. 20. Biopsy of human liver in toxic liver degeneration showing different forms of nuclear vesicles. To the left are nuclear or nucleolar vesicles which are probably genuine. The large vesicle to the right, whose content is of the same quality as the surrounding cytoplasm, is probably the result of an invagination or engulfing of cytoplasmic material. Haematoxylin eosin.

The fact that nuclear or rather nucleolar ribosomes are morphologically identical with those of the ergastoplasm (see p. 242) suggests that these ribosomes pass *in toto* through the nuclear envelope. This passage may be aided by an intensified locomotion of the nucleoli inside the nucleus, as can often be observed in active cells (Altmann and Grundmann, 1955; Sirlin, 1961) and by the fact that they migrate to the periphery of the nucleus and nestle against the inner surface of the nuclear membrane (e.g., Chèvremont and Frederic, 1954; Chèvremont, 1961a; Lettré, 1954; Lettré and Siebs, 1961b; Mölbert, 1951a; Sirlin, 1961). Nucleoli which stay in the inner portions of the nucleus are apparently able, with the aid of chromosomal structures, to get into contact with the nuclear membrane by formation of nucleolar processes (e.g., Bachmann, 1948). Altmann (1952) could discern, in the exocrine tissue of the mouse pancreas, a regular "functional cycle" (Altmann and Meny, 1952) whereby in several phases nucleolar material was being ejected along chromosomal "*Leitbahnen*" or channels (see also Beck, 1956; Beck and Michler, 1960; Hertl, 1957; Müller, 1961a; Stöcker, 1962b; and others). Perhaps the nuclear envelope can form invaginations at such sites (Pomerat, 1961) which would explain the old observation of deep folds in the nuclei of intensely active cells. In *Lophius piscatorius* (Holmgren, 1899) as well as in tumour cells (Hoshino, 1961), particularly when the latter have been treated with cytostatics (Fig. 21), a large surface area for exchange is created by finger-like nuclear processes, which protrude into the cytoplasm, and by many deep invaginations of the cytoplasm into the nucleus; it is at these sites that the cytoplasmic RNA and protein concentration is by far the densest.

This is the accumulation of "membrane nucleotides of the nucleus" which led Caspersson (1941, 1950) to the conclusion that RNA is generated here.



Fig. 21. Jensen's sarcoma cells of the rat under cytostatic treatment showing deep invaginations in the nucleus and finger-like nuclear processes projecting into the cytoplasm. Marked increase in surface of the exchange area between nucleus and cytoplasm. Haematoxylin eosin.

The observations on which these postulations of RNA passage through the nuclear membrane were based, have been recently confirmed with modern methods. Electron microscopy has revealed in several cells that nucleolar ribosomes leave the nucleolus and migrate to the nuclear membrane (Hortsmann and Knoop, 1957; Cotte, 1959); also in trophoblast cells of young rat embryos the passage of nuclear material through pores of the nuclear wall into the cisternae of the endoplasmic reticulum has been observed with certainty (Hadek and Swift, 1962). In the exocrine tissue of the mouse pancreas where Altmann (1952) had found the "functional cycle", electron microscopy also succeeded in demonstrating nuclear vesicles which are expelled through the nuclear membrane into the cytoplasm and contain ribosomes (Clark, 1960). Radioautographically, the expulsion of RNA-containing material into the cytoplasm has been observed in the form of a radioactive "cloud" (Stöcker *et al.*, 1961a; Stöcker, 1962b).

The particularly large nuclei of *Acetabularia* have not, as yet, revealed any of these processes (Stich, 1956). In *Chironomus* we have at least some evidence suggesting the passage of small pellets through the envelope of the nucleus (Kimoto, 1958a). In growing oocytes of amphibia the situation is clearer. Here, hundreds of thousands of nucleoli are generated on the lampbrush chromosomes (see p. 119), detaching themselves from the latter and migrating to the inner surface of the nuclear membrane where they are deposited in saccular evaginations before being turned over to the cytoplasm (Duryee, 1950). In the oocytes of ophidians, small nucleolar granules separate from the main nucleolus and migrate into the cytoplasm (Kaushiva, 1961).

This morphologically detectable passage of nucleolar material into the cytoplasm certainly represents the intensification of a process which, as Caspersson (1950) had assumed, goes on in most cells below the level of visibility. The threshold of visibility for this process is in most cases even below the dimension of the electron microscope. This fact only increases the value of confirmed positive evidence for our understanding of the exchange processes between nucleus and cytoplasm, in which the nucleolus certainly plays a central part. The

previously mentioned argument against this close relation, namely, that the base composition of nucleolar RNA and that of cytoplasmic RNA are not equivalent (for ref. see Vincent, 1955), has been refuted by a more modern method (Edström, 1960; Edström *et al.*, 1961).

Lately, it has been possible, in the oöcytes of sea urchins (Vincent and Baltus, 1960) and in the salivary glands of chironomids (Sirlin, 1961) to isolate soluble RNA, which in the course of protein synthesis binds the amino-acids, thus bringing them together on the surface of messenger RNA (p. 251) for the synthesis of proteins. If we interpret the synthesis of this soluble RNA and of ribosomal RNA as the main function of the nucleolus, we understand its central position as the functional link between nucleus and cytoplasm. Disturbances of the nucleolar function, caused for instance by selective irradiation of the nucleolar area, lead consequently to the inhibition of nucleic acid synthesis in the cell (Seed, 1960), i.e., predominantly to the inhibition of RNA synthesis (Errera *et al.*, 1961a). To a lesser extent the synthesis of nuclear proteins, too, is inhibited (Errera *et al.*, 1961b) and also the synthesis of proteins in the cytoplasm, a fact which is of particular importance in this context (Perry *et al.*, 1961).

Review

All this is sufficient proof why the nucleolus is a necessary element of a differentiated cell and why zygotes of *Chironomus* experimentally deprived of nucleoli cannot develop as embryos (Beermann, 1960). The nucleolus, as a chromosomal element, is a decisive factor in this feedback mechanism of the cell (Vogt and Vogt, 1947; Glinos, 1960; Büchner, 1961a) that stimulates the loci of the chromosomes and particularly the "nucleolar organizer", according to cytoplasmic demands, to produce messenger RNA, macromolecular ribosome RNA and "soluble" RNA (Stich, 1956; Häammerling, 1958.) Probably, the RNA stays only for a very short time in the nucleolus or perhaps for one or several hours (Woods, 1960); it then migrates to the cytoplasm where it becomes available for the specific protein metabolism of tissue. Messenger RNA perhaps takes another route. Whether or not the nucleolus has still other functions has not been determined. The known functions, however, sufficiently explain the alterations of its volume and of its composition as they are dictated by the cell's metabolism. We also understand from the function of the nucleoli why they develop at definite points on the chromosomes, at the nucleolar organizers, and why they are ultimately a part of the chromosome that bears the organizer. Their number is, therefore, basically determined by the number of chromosomal sets, i.e., by the degree of ploidy; formation of accessory nucleoli, however, can increase and fusion into collective nucleoli can decrease their number. Their fine structure of granules, 100–150 Å in size, often ordered to filamentous structures, the nucleolonemata, confirms morphologically their relation to the elements of protein metabolism in the cytoplasm; for, indeed, we will meet again these same granules as ribosomes in the ergastoplasm. Finally, since nucleolar RNA, at least in cells where it is intensely metabolized, resembles cytoplasmic RNA in its composition, morphological and biochemical evidence merges here and we can call the nucleolus a mediator between nuclear and cytoplasmic RNA.

THE NUCLEAR ENVELOPE

Our discussion of the nuclear function and of the nucleolus has brought forth evidence, in several instances, of an exchange of material between nucleus and cytoplasm. Emphasis has been laid on substances delivered by the nucleus, yet, an equally intensive absorption of cytoplasmic particles is necessary for the nuclear syntheses. This exchange of material must, in every instance, penetrate the interfaces that separate nucleus and cytoplasm; these interfaces will be the topic of this chapter. In summary, they are generally termed "nuclear membrane", a term whose validity is the object of much controversy today. For we are probably dealing here with several membranes which are possibly of different origin. Therefore, the term "nuclear interface layers" (Peveling, 1961) seems more suitable. Yet, since this term is liable to be misinterpreted we will use the simple term "nuclear envelope".

Composition and structure

In the fixed and stained preparation under the light microscope (Fig. 10, 20) the nuclear envelope appears as *one* membrane which, as a rule, has sharp contours and stains with Feulgen dye, i.e., it appears to contain DNA. This impression is enhanced by the chromocentres which are frequently situated on the inner surface of the nuclear envelope. However, the appearance changes greatly if we investigate the nucleus of a living or surviving cell. In this case, the nuclear envelope looks like a fine pellicle whose refractive index is somewhat higher than that of the interior of the nucleus (Fig. 14). In well preserved cells this pellicle is so delicate that it can hardly be seen (Fig. 9, left). When the cell dies, the pellicle becomes gradually thicker and thus more visible. This leads to the conclusion that the compact appearance of the nuclear envelope is the result of cell death and therefore an artifact. As revealed by a positive Feulgen reaction (Fig. 2), this artifact is due to DNA-containing structures from the chromosomes and can be explained by the deposition, during agonal denaturation, of chromosomal particles at the inner aspect of the nuclear envelope where they coagulated. As we recall, the Feulgen positive membranes of nucleoli must be likewise interpreted as a precipitation of chromosomal material (p. 44).

Hence, the nuclear envelope, too, has been thought to be a mere adsorption layer between nuclear and cytoplasmic colloids (Lepeschkin, 1924) similar to the fine pellicles which can be found at the surface of protein corpuscles. This view has been supported by observations showing that the nuclear envelope can easily be dissolved by mechanical pressure, high temperatures and certain saline solutions (for ref. see Milovidov, 1949). All mechanical alterations have demonstrated a plasticity of the nucleus, as observed for instance in blood smears, where we may see leucocytes or lymphocytes forcing their way through narrow gaps between two erythrocytes. Hence, the nuclear envelope is neither rigid nor does it determine the shape of the nucleus.

The existence of a nuclear envelope has been proved in early experiments. In 1893, O. Hertwig described on eggs of amphibia how it could be detached micro-surgically from the content of the nucleus as a fine membrane (Hertwig, 1893). Investigations using polarized light also suggested its existence. Further, the birefringence of the nuclear surface, which can be demonstrated under certain conditions, have led to the conclusion that it is

composed of a protein-lipid system (Schmidt, 1939, 1942), whereby the protein molecules, lying parallel to the nuclear surface, appeared to enclose the lipids, thus forming a film. Antimony sulphide was found to enhance considerably the birefringence effect (Baud, 1949) and so it was possible, in many types of nuclei, to distinguish an inner and an outer nuclear membrane; the inner one consisted apparently only of proteins, the outer one of proteins and lipids (Baud, 1953).

This subdivision of the nuclear envelope into two layers has been demonstrated even before these observations, in epidermis cells of plants (Cohen, 1937) and, by dark field microscopy, in testes of insects (Klingstedt, 1928). This was confirmed by the very first investigations with the electron microscope (Callan and Tomlin, 1950; Bairati and Lehmann, 1952; Harris and James, 1952). Interest has now been focused on a new finding, viz., that these membranes display small apertures, the "nuclear pores". At first, it was not possible to determine with certainty whether these pores belonged to the outer (Baud, 1953) or to the inner film (Harris and James, 1952); one of these two layers seemed to be continuous.

The matter was clarified only with the improvement of thin-sectioning technique, and it was surprising to see how uniform a picture emerged in the most different nuclei of humans, animals and plants, including algae and yeast (for ref. see Watson, 1955; Denues, 1958; Miller, 1958; Bernhard, 1958; Barnes and Davis, 1959; Marinos, 1960; Wischnitzer, 1960; Kurosumi, 1961; Mirsky and Osawa, 1961; Marquardt, 1962). Almost all nuclei are bounded by two osmophilic membranes (Fig. 1, 16), each of which has a thickness of 60–100 Å. Between them there is an osmophobic layer with a mean width of 100–150 Å. Sometimes each of the osmophilic films is again composed of two half films (André and Rouiller, 1957; Peveling, 1961). Thence, the whole system of membranes has a width of approximately 300–350 Å. It often constitutes the only nuclear boundary discernible in the electron microscope. Larger depositions of karyoplasm at its inner aspect are, as in the light microscope, mostly artifacts.

This system of membranes is punctuated by the nuclear pores, which are located at the same site in both membranes; the osmophilic membranes of the inner and outer film are joined at these points, forming true gaps in histological sections. The three-dimensional reconstruction shows a curved bilamellar plate with small openings that all have approximately the same size (Fig. 22). The pores have a diameter between 300–1000 Å (Gall, 1959a; Whaley *et al.*, 1960; Marquardt, 1962; and others). In egg cells (e.g., Afzelius, 1955), the pores lie relatively close to one another, in other cells they are more dispersed and sometimes along the circumference of a nucleus there are found only a few or none at all (e.g., Kurosumi,

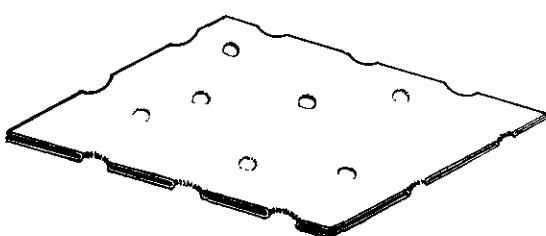


Fig. 22. Schematic representation of the nuclear envelope with pores. (From Wischnitzer, S. (1960), *Int. Rev. Cytol.* **10**, 137–162.)

1961). Thus, the portion of the total nuclear surface area covered by the pores varies considerably. The acidophilic cells, for instance, in the posterior lobe of the mouse pituitary gland contain 800 pores per nucleus; hence, they cover 3% of the nuclear surface area (Barnes and Davis, 1959). In other animal cells, the share of the pores is 5-15% (Watson, 1955). Sometimes the pores appear to be dammed by a very fine diaphragm (e.g., Watson, 1955; Marinos, 1960); thus, they do not constitute real interruptions of the membrane but appear rather as thinned out regions. However, these are probably nothing but an optical illusion due to the fact that the pores at this level of sectioning were cut tangentially (Barnes and Davis, 1959). Apart from these pores, still wider gaps have been discovered (Fig. 23), which contain in certain cases cytoplasmic organelles like mitochondria or proplastids apparently in direct contact with the karyoplasm (Whaley *et al.*, 1960).

In the vicinity of nuclear pores have been found, first in oöcytes (Gall, 1954a; Afzelius, 1955), later also in many other cells (Meriam, 1961, 1962) ring-shaped osmiophilic structures, the so-called "annuli". In some objects they are in turn composed of smaller rings, the "subannuli" (Gall, 1956; Rebhun, 1956; Wischnitzer, 1958, 1959). They represent perhaps small hollow cylinders perpendicular to the surface of the nucleus (Watson, 1959; Wischnitzer, 1959, 1960). In amoebae there are quite similar structures, but there they are fortified by a layer, 2800 Å thick, of tightly packed hexagonal prisms, the centres of which contain the "nuclear pores" (Pappas, 1956; Bernhard, 1958).

In extending our knowledge of the fine structure of the nuclear wall, we have gone far beyond the original concept of a simple, more or less flexible envelope and we have encountered a most complicated system of membranes which perhaps plays an active role of its own in the nucleocytoplasmic transfer of material.

This system of membranes is but a part of that network of fine canaliculi, the endoplasmic reticulum, which extends throughout the entire cytoplasm (Figs. 1 and 23) (see p. 241). The interspace between the osmiophilic lamellae or "perinuclear space" communicates directly with the cytoplasmic cisterns (Policard and Bessis, 1956; Epstein, 1957) and the external membrane of the nuclear envelope extends directly into the system of lamellae of the endoplasmic reticulum (e.g., Watson, 1955; Wellensiek, 1957; Whaley *et al.*, 1960). The nucleus has, so to speak, an extracellular position (Miller, 1958), yet it is only seemingly so, for, in the areas of "nuclear pores", cytoplasm and nucleus communicate with each other. On the other hand, its location within the system of canaliculi of the endoplasmic reticulum puts the nucleus into direct contact with the substances from the extracellular space. Evidence supporting the fact that such a direct contact exists is brought by the presence of resorbed fat droplets in the "perinuclear space" of intestinal epithelia (Palay, 1960a).

All these structures are by no means permanently fixed elements but are, as all the other particles of the cell, undergoing constant changes. Probably, the above-mentioned potential communications between the interior of the nucleus and the cytoplasm can be rapidly established and as rapidly interrupted again according to functional requirements. This is already suggested by the fact that the nucleus has, within the cytoplasm, a relatively high degree of mobility. It can be translocated to the sides or rotate within the cell as has been demonstrated by filming tissue cultures. One complete rotation, in certain cases, does not take much longer than 1 minute (e.g., Pomerat, 1953, 1961).

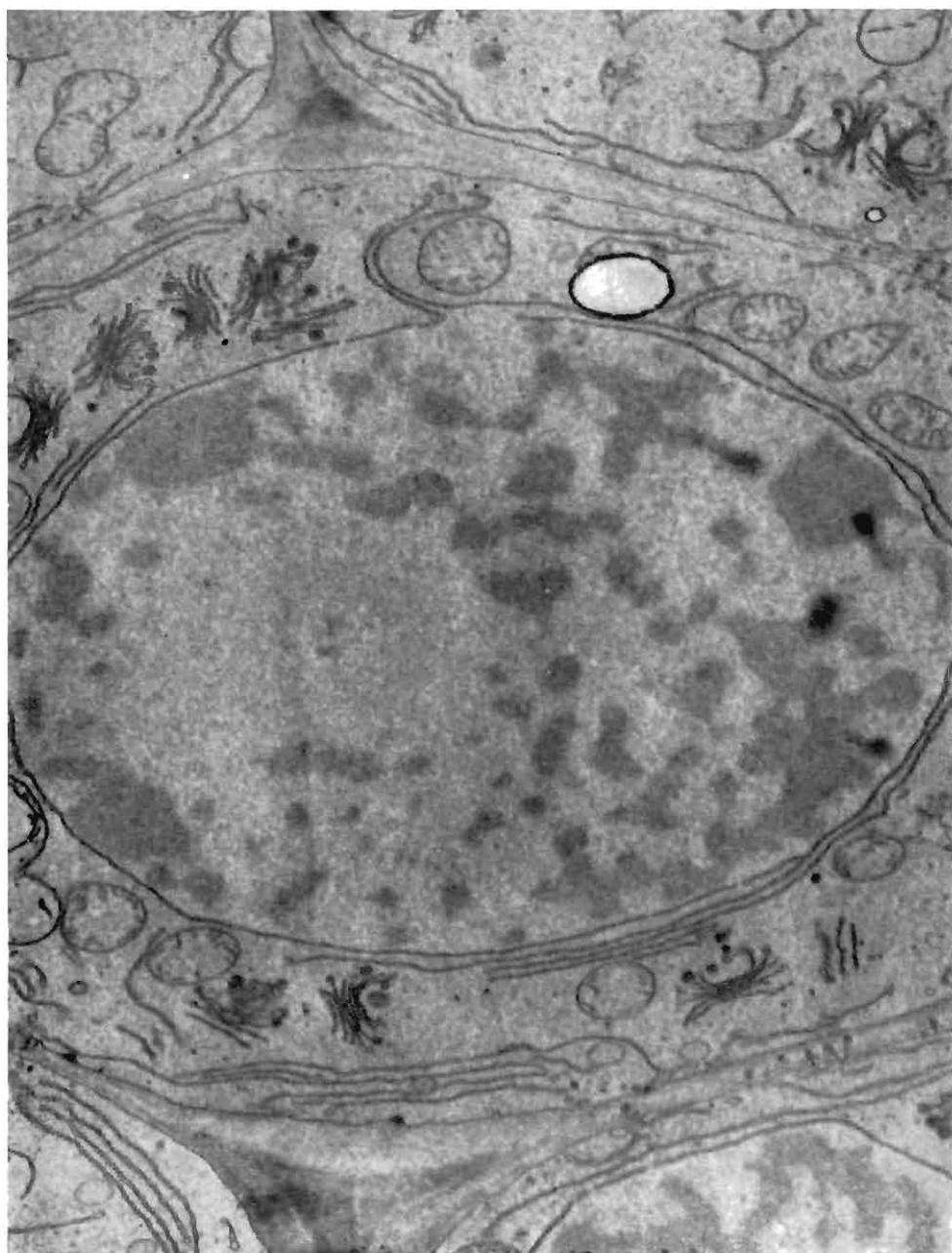


Fig. 23. Meristem cell of corn root, fixed in KMnO_4 . Electron micrograph shows nuclear envelope as part of the endoplasmic reticulum. Note small pores and wider gaps (*top*) in the nuclear envelope. $\times 12,600$. (From Whaley, W. A., et al. (1960), *J. biophys. biochem. Cytol.*, 8, 233-245.)

Role of the nuclear envelope in nucleocytoplasmic transfers

The structural relations between nuclear surface area and cytoplasm may be manifold. The "pores" represent so to speak the most simple type of communication. After fixation with osmic acid we may sometimes discern within the pores granules of the size and density of ribosomes, likely to be RNA granules (e.g., Pollister *et al.*, 1954; Kautz and Marsh, 1955; Bernhard, 1958; and others). The same material may be found deposited on both aspects of the nuclear envelope (Anderson and Beams, 1956) and in Fig. 24 such a passage of substance may perhaps just be taking place.

If we include the above described annuli in the "pore complex" (Watson, 1959), the latter may possibly be another specific manifestation of this exchange process. This "complex" consists of one nuclear pore and of one canaliculus for each pore, extending into the interior of the nucleus where it is assumed to anastomose with other canaliculi. The content of these canaliculi appears more translucent than that of the rest of the karyoplasm and a similar translucency may be observed in the cytoplasm in the vicinity of a nuclear pore (Watson, 1959; Marquardt, 1962; and others). We may conceive these canaliculi as being channels or "*Leitbahnen*" (Altmann, 1952), i.e., a chromosomal system of canaliculi which serve for the extrusion of nucleolar substance. However, this is as yet a hypothesis.

The extrusion of nuclear material seems to be also possible via another mechanism, that is, by evaginations of the nuclear double membrane and the pinching off of small portions of the karyoplasm which finally dissolve in the cytoplasm (e.g., Gay, 1956; Bernhard, 1958; Peveling, 1961). This is how many nuclear viruses are transferred into the cytoplasm (p. 82). This process has its counterpart in deep invaginations of the cytoplasm into the nuclei (Fig. 21) (Bernhard, 1958; De Groot *et al.*, 1958; Pomerat, 1961; and others).

A further possibility of transfer of material is implied in the close relationship of nuclear envelope and ergastoplasm. Frequently, the nuclear envelope consists of several double folia (e.g., Swift, 1956; Merriam, 1959; Meyer, 1960; Kurosumi, 1961; and others) or there are single processes of the nuclear double membrane protruding into the cytoplasm (Fig. 23) (Whaley *et al.*, 1960). It is assumed that the endoplasmic system of canaliculi can grow from the nuclear envelope; this again would make a transfer of nuclear material into the cytoplasm possible (e.g., Wischnitzer, 1960; Horstmann, 1961).

Though the details of this process have not as yet been clarified nor completely confirmed, we still have the impression that the nuclear envelope is neither a simple separating membrane nor is it necessarily always a connecting structure. It seems to have an alternating function in the total metabolism of the cell, separating and connecting at the same time. For, even when a direct passage of substances via nuclear pores or pore complexes appears possible, it has by no means been proved that at these points nucleus and cytoplasm communicate directly. On the contrary, each experimental perforation of the nuclear membrane brings about the death of the cell (Chambers and Fell, 1931). Studies on the relative concentration of particles in both karyoplasm and cytoplasm have shown that, at least in the developing chick nerve cell, the general concentration of substances is considerably higher in the cytoplasm than in the karyoplasm (Merriam and Koch, 1960).

Furthermore, it has often been confirmed that substances of high-molecular weight are incapable of passing through the nuclear envelope. In experiments with isolated nuclei,

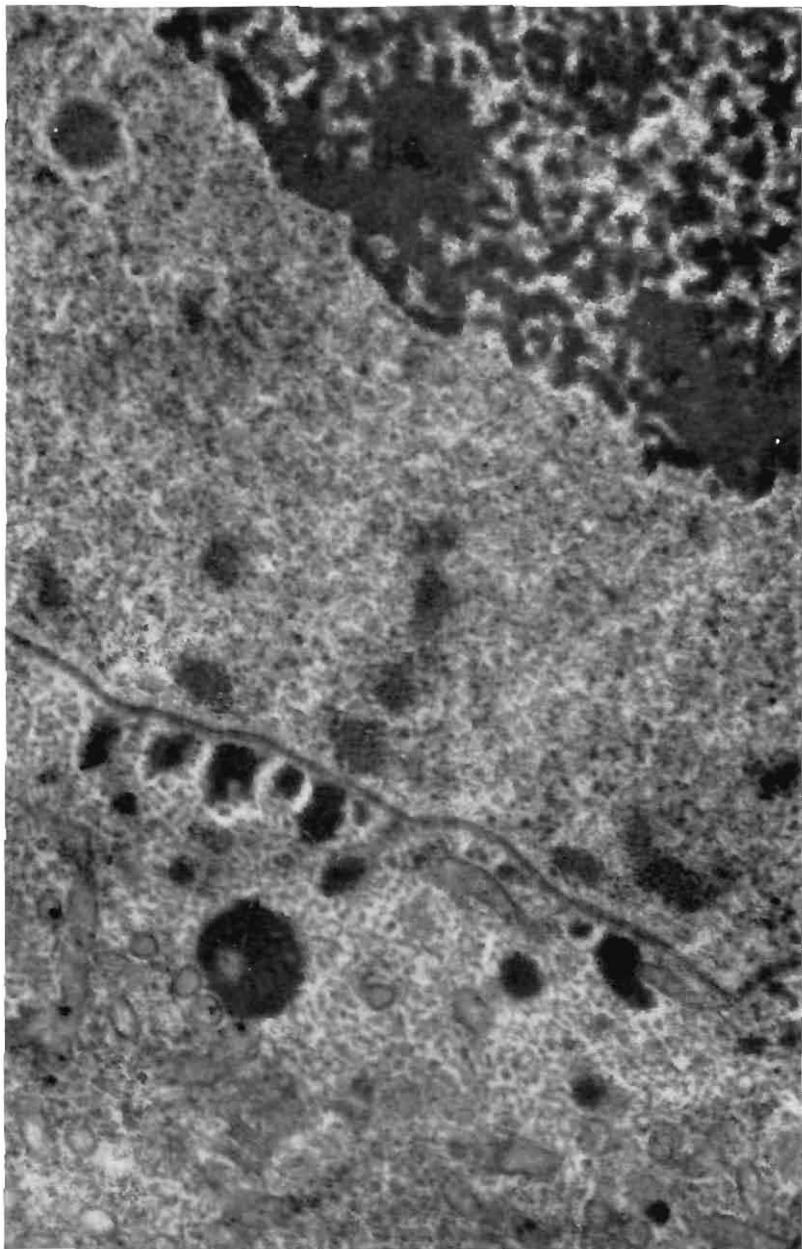


Fig. 24. Nutritional cell of *Rhodnius* egg displaying accumulation of electron dense material in direct vicinity of the nuclear pores and on both sides of the nuclear envelope, suggesting a possible transfer of RNA granules. (Courtesy E. Anderson.)

substances of low-molecular weight, like sugars and amino-acids up to the low-molecular polypeptides, do penetrate into the nuclei, but not so the higher molecular proteins like egg albumin or serum albumin (e.g., Callan, 1952). In similar experiments, RNase, protamines or histones have been shown to pass through the nuclear envelope from the outside (Fischer and Wagner, 1954; Brachet, 1957), yet it cannot be excluded that the process of isolation or the lytic properties of some substances may have influenced the permeability of the nuclear envelope (Holtfreter, 1954; Becker and Green, 1960; Mirsky and Osawa, 1961).

The final answer to the question under what conditions the nuclear envelope is permeable can only be obtained by observations in intact cells. These observations have demonstrated unequivocally that the nuclear envelope constitutes an impermeable barrier for many high-molecular weight substances coming from outside. Fluorescent albumins and globulins can permeate into the cytoplasm but not into the nucleus (Schiller *et al.*, 1952; Ortega and Mellors, 1957). This fact has been most clearly demonstrated by the microinjection of fluorescent γ -globulins (molecular weight 16,500) into isolated egg cells (Feldherr and Feldherr, 1960). As a consequence of the semipermeability of the nuclear envelope, the size of the nucleus is dependent, to a large extent, on the colloid osmotic pressure of the surrounding cytoplasm; intracytoplasmic injections of polyvinyl pyrrolidone, a plasma expander with a molecular weight of 40,000, provoked, without passing into the nucleus, in low concentrations (0.5%) an increase and in higher concentrations (2% and more) a decrease in nuclear volume (Harding and Feldherr, 1958). If the nuclear pores were a constant access between nucleus and cytoplasm, such an effect would be impossible. It is of interest that colloidal gold particles can migrate from the cytoplasm into the nucleus (Moore *et al.*, 1961) and their migration via the system of pores can be made visible (Feldherr, 1962).

The nuclear envelope seems to protect the karyoplasm from foreign high-molecular weight substances, on the other hand, however, it appears to be permeable to certain high-molecular weight products of nuclear metabolism; that is, for substances that come from inside the nucleus. For, indeed, by this means, the cytoplasmic organelles are controlled by the RNA produced in the chromosomes (p. 39). High-molecular weight particles of viruses, too, can leave the nucleus directly (Tournier *et al.*, 1957; Morgan *et al.*, 1957) and be transferred to the cytoplasm (p. 82). Conceivably, the special structural devices of the nuclear envelope may be responsible for this one-way transport, whereas low-molecular weight substances, which the nucleus needs for its synthesis, are apparently able to pass directly through the nuclear envelope.

A further statement may perhaps be of some significance; all of today's electron micrographs are limited to the representation of the double contour of the nuclear membrane as the analogue or as part of the endoplasmic system of canaliculi. The boundaries of the chromosomes in the karyoplasm are not represented; however, they exist, and possibly also in the areas of the nuclear pores. Why should not these, too, have some influence on the permeability of the nuclear envelope?

Origin of the nuclear envelope

The fact that the nuclear envelope is ultimately composed of two entirely different elements, i.e., of parts of the endoplasmic reticulum and of the boundaries of the chromosomes, constitutes a problem which has not yet been solved by electron microscopy. For the double lamellae which we see in the electron microscope are part of the cytoplasm; the actual nucleus appears to have no membranes.

And yet, the condition of the chromosomes is important for the existence of the nuclear envelope. This is demonstrated by their action during mitosis; the nuclear envelope begins to dissolve at the same time as the chromosomes contract at prophase. This breakdown has two phases. At first, the membrane lamellae break up into pieces of different sizes without losing their double structure; thus, they become indistinguishable from the lamellae of the endoplasmic reticulum (Porter and Machado, 1960). Then they migrate away from the prophase bundle of chromosomes and are dissolved in the periphery of the cell (Jones, 1960), perhaps with the aid of the mitochondria (Yasuzumi, 1959). During the time elapsing between the end of prophase and the beginning of reconstruction at post-telophase, the nucleus is without an envelope except for some special cases, as in *Amoeba proteus*, in which only the inner prisms (p. 59) are dissolved (Cohen, 1957).

There are basically two theories as to how the nuclear envelope reconstitutes after telophase; either the pair of lamellae is synthesized *de novo* at the sites where chromosomes and cytoplasm are in contact with each other (Jones, 1960), or parts of the already present endoplasmic reticulum adjoin the telophase nucleus and merge to form the nuclear envelope (Amano and Tanaka, 1957; Kurozumi, 1958, 1961; Yasuzumi, 1959; Porter and Machado, 1960). In many instances it is difficult to decide whether the double lamella is due to a mere apposition or to a genuine local *de novo* synthesis; at least this is not easily disproved. In locust spermatocytes, for instance, the 0.5 μ vesicles which are formed at the surface of the chromosomes, are indistinguishable from the vesicles of the endoplasmic reticulum (Bahr *et al.*, 1959, 1961). They gradually arrange themselves so as to lie parallel to the surface of the nucleus and then merge to form a continuous nuclear envelope (Fig. 25).

What we can conclude from these and many other findings is, at least, that the formation of the nuclear envelope is concomitant with a rearrangement of the endoplasmic reticulum and that this rearrangement only takes place when the chromosomes unfold at the end of karyokinesis and come into broad contact with the cytoplasm (Claude, 1961; and others). We can conclude that the stimulation to the formation of the membranes comes from the chromosomes; for, all those chromosomes or portions of chromosomes that become independent by breakage in pathological mitoses, surround themselves with a genuine nuclear envelope and become so-called micronuclei (p. 205).

Fig. 25. Electron micrograph showing new formation of a nuclear envelope after nuclear division (meiosis in spermatogenesis of insects). (a) Beginning of the deposition of endoplasmic reticulum (ER) on the telophase chromosomes (C). (b) Advanced stage of apposition of endoplasmic reticulum (ER); the single chromosomes (C) are often separated from one another by endoplasmic canaliculi and mitochondria. Permanganate araldite fixation. $\times 17,000$. (From Barer, R., *et al.* (1960), *Proc. Roy. Soc. London, Ser. B* **152**, 353-366.)

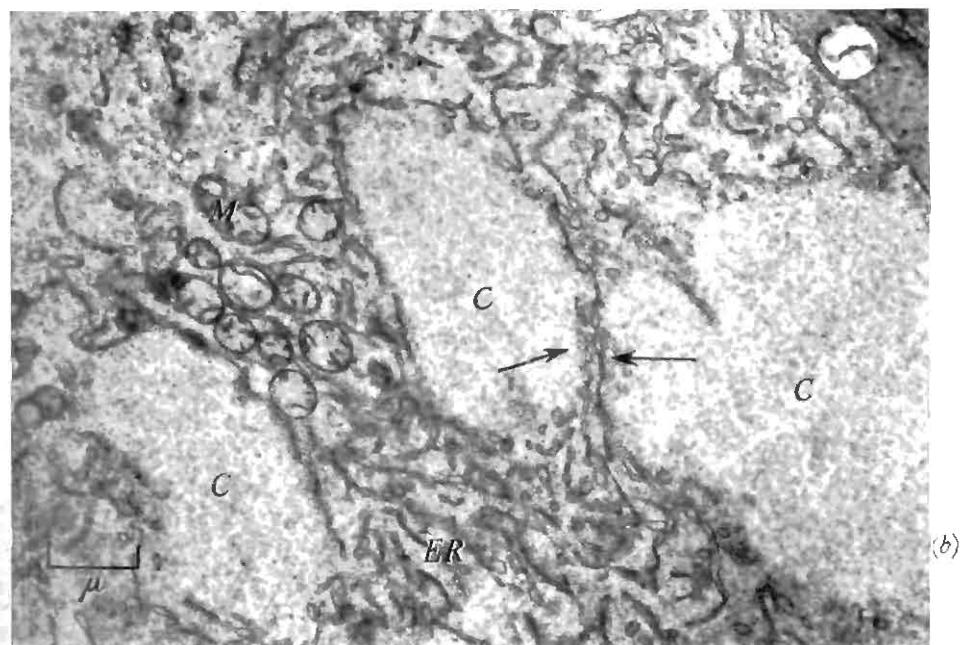
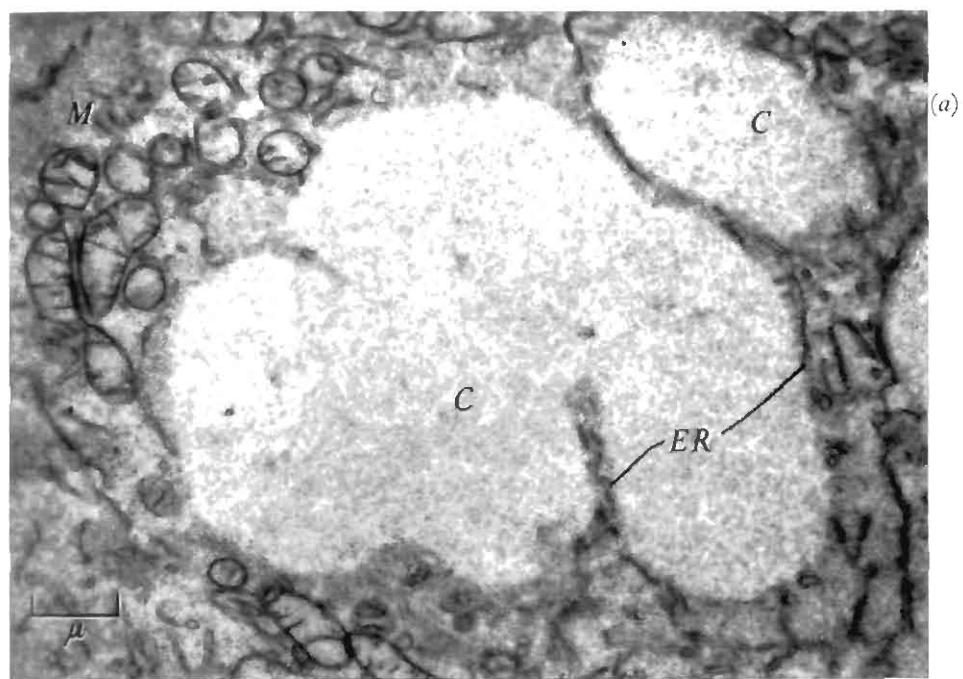


Fig. 25

Review

The nuclear envelope is ultimately a structure of both the nucleus *and* the cytoplasm; it is generated from and also most likely composed of both elements. It is more difficult to understand the one-way semipermeability of the nuclear envelope. Substances of low-molecular weight can pass it in both directions, high-molecular weight proteins can only go from the nucleus into the cytoplasm. This passage of high-molecular weight products of the nucleus probably occurs via certain special devices such as the nuclear pores. The nuclear envelope is one of the active structures of the nucleus. It is more than just a membrane separating two portions of the cell. Indeed, it separates nucleus and cytoplasm, but at the same time it combines them into a functional unit.

THE KARYOPLASM

The preliminary definition of what we call "karyoplasm" ensues directly from our discussions on the nuclear envelope. The karyoplasm is the substance which is situated inside the nuclear envelope and outside the nucleolus; therefore, the karyoplasm is that essential nuclear substance which contains the genetic material.

Vital structure and its variability

To understand the composition of the karyoplasm we must consider two facts. First we must keep in mind that this substance contains the material of the mitosis chromosomes. Secondly, we must remember that we generally cannot discern in a living nucleus any structures at all except for the nucleolus and the nuclear envelope, not even with the phase contrast microscope (Altmann and Grundmann, 1955). The optical appearance of the karyoplasmic area is homogeneous and frequently seems to be empty. In what form is the chromosome material then present in the karyoplasm?

The problem becomes even more complicated if we consider the picture revealed in the light microscope by fixed and stained preparations. Dispersed throughout the karyoplasm there are a multitude of corpuscles and filaments which frequently seem to form a stable framework; thus investigators have often talked about a "nuclear framework". What is the meaning of these corpuscles and filaments? Can they help us gain a better understanding of the karyoplasm?

The answer to this question, from many investigators, is absolutely negative, particularly in the light of electron microscopical findings, which revealed no equivalents to these structures. However, the doubt cast upon the reality of the nuclear structures seen in the light microscope is nothing new (e.g., Tellyesniczky, 1902). Criticisms were particularly vehement at the time when colloid chemistry was in the lead; the nucleus was then regarded as a solution, a sol in which little droplets, the so-called karyotine droplets, representing a somewhat more solid "phase", were dispersed, that is, they were thought to float more or less freely in the nucleus (e.g., Schaede, 1927, 1929). According to this concept, a solid structure would then be due to fixation, which produces a gel, and thus precipitates the "karyotine droplets" at the site where they just happened to be.

Microsurgical experiments on living nuclei seemed to confirm this concept. A fine needle can be moved about freely in living nuclei (Chambers, 1924; Pèterfi and Kojima, 1936). When perforated by a needle, the nucleus yields only small amounts of liquid material; other parts of it adhere to the needle and can be withdrawn as filaments (Bancher, 1938). These findings supported the view that the karyoplasm was composed of two "phases", a "sol" portion which can flow out of the nucleus, and a "gel" portion that congeals. The first had been called "karyolymph" and the latter "chromatin", with regard to older experience with stained preparations (e.g., Strugger, 1930). This concept has remained essentially unchanged. We still speak of a liquid "nuclear sap" and of chromatin to designate the stainable chromosomal substance. It is quite certain that the karyoplasm is of a viscous consistency which can be compared best with that of Canada balsam, used for mounting fixed preparations (Pèterfi and Kojima, 1936).

But these observations do not yet clarify the nuclear fine structure. Further perforation experiments brought forth surprising findings that have not yet been fully explained. If one only touches the hairlets of *Tradescantia stamens*, small granules appear in the previously homogeneous nuclear space (Pèterfi and Kojima, 1936). When these experiments are carried out in different media, for instance in saline solutions of different concentrations, these granules can be made visible with variable degrees of intensity (Bancher, 1938) up to the demonstration of the filamentous "nuclear framework" of the fixed and stained preparations. To achieve this, it is not even necessary to injure the cell; all it takes to produce these different representations of the nucleus, is to immerse the cells into saline solutions of different concentrations (Zeiger, 1935; Bank, 1939; Anderson and Wilbur, 1952; Schneider, 1955; Altmann and Grundmann, 1955; and others). Distilled water and sugar solutions, however, produce a rather homogeneous nucleus in which we frequently cannot even discern the nucleoli any more. Fig. 26 shows two representations of pancreas nuclei, one in a 0·9% NaCl solution, the other in a 10% sugar solution. In saline solutions, the nuclei shrink, in sugar solutions, they expand.

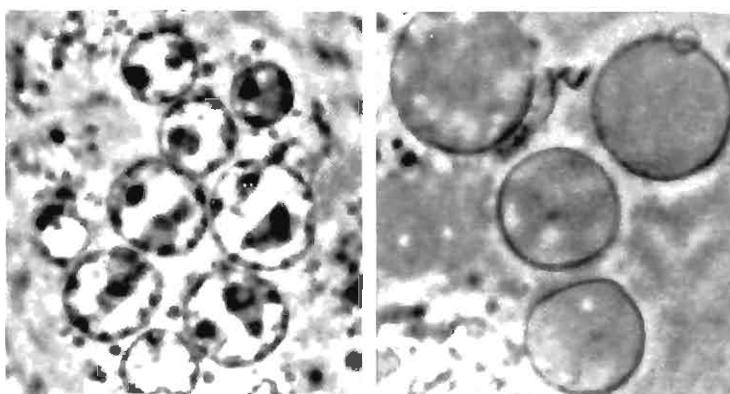


Fig. 26. Epithelium nuclei from mouse pancreas immersed in the living state into a 0·9% NaCl solution (left) and into a 10% sugar solution (right). (From Altmann, H.-W. and Grundmann, E. (1955), *Beitr. path. Anat.*, 115, 313-347.)

These experiments have often been varied (e.g., Kuwada and Sakamura, 1927; Sakamura, 1927; Shinke, 1937; Bancher, 1938; Zollinger, 1948a; Anderson and Wilbur, 1952; Enderlin, 1953; Altmann and Grundmann, 1955; and others). Two conclusions were drawn from the results of all these studies. The first is that the representation of the nucleus at a given time is due to the kind of experiment and depends on the media in which the nucleus is immersed. The structures which are made visible are reversible at least as long as the nucleus is still living (Zeiger, 1935). Filamentous and granular structures, however, that appear for instance in acid media, are irreversible (Kuwada and Sakamura, 1927; Zeiger, 1938). W. Flemming, one of the pioneers of cytology, arrived in 1892 at the same conclusions without having at hand the wealth of observations that we have now.

The second conclusion is based mainly on the mentioned alterations in volume; salt solutions cause a release of water from the nucleus, sugar solutions an uptake of water from the extranuclear space. This transfer of water, however, is not a uniform process in all areas of the nucleus, on the contrary, the visualization of nuclear structures in saline solutions is due to a dehydration of very definite components of the karyoplasm (Kuwada and Sakamura, 1927; Sakamura, 1927; Shinke, 1937; and others). It is precisely this manifold variability and also the reversibility of nuclear structures which is the manifestation of the variable and reversible hydration and dehydration processes in the karyoplasm; the nuclear framework, too, as we see it after fixation, is a result of such a dehydration of karyoplasmic components (Zeiger, 1938). The optically homogeneous appearance of the living nucleus must be due to the hydrated and oedematous condition of the karyoplasm (Ris and Mirsky, 1949). This condition is very labile and may be altered already by minor exterior forces to the effect that the karyoplasm enters a state of condensation of different density (Ris and Mirsky, 1949; Altmann and Grundmann, 1955; Grundmann and Stein, 1961a). A pre-requisite for this water transfer is, apparently, the presence of nucleoproteins. The dehydrating capacity is lost when the nucleoproteins are extracted by molar NaCl (Zollinger, 1948; Ris and Mirsky, 1949; Enderlin, 1953; Altmann and Grundmann, 1955; and others).

These perforation experiments have already shown how sensitive is the hydrated karyoplasm. Thus we well understand why it is so difficult to visualize the actual living state of a nucleus, as some sort of pre-treatment, and be it the most cautious one, is unavoidable. Slight pressure with the cover glass causes already an alteration of its phase contrast representation (e.g., Bessis, 1949) and results in dehydration but frequently also in hydration and homogenization of the nucleus. The cell's capacity to maintain life need not be disturbed by these manipulations and we speak, therefore, of "*in vivo* artifacts" (Belar, 1930a). And these "artifacts" provide us with an explanation why most living cells do not display clearly visible structures in their nuclei, whereas some cells do, as, for instance, the macronuclei of certain protozoans (Schwartz, 1958), or plant cells (e.g., Shinke, 1937).

Representation after fixation

The reversibility of nuclear structures has certain implications for the interpretation of the fixed and stained preparation. The condensation products, for example after treatment with a 0·9% NaCl solution, correspond largely to the densities which we see in a fixed nucleus; these densities are still reversible if acetic acid is employed in low concentrations and they

occur always at the same site (e.g., Zeiger, 1935). Therefore, random condensations cannot be held responsible for these densities, they must have been present in some form before dehydration. Thus, however, the fixed and stained preparation can no longer be considered to be a meaningless artifact but gains the character of a genuine though limited representation, namely, it reveals structures that were previously indiscernible because of the homogeneous hydration of the karyoplasm, but were nevertheless present. Yet this does not necessarily mean that the components which are visualized in the fixed nucleus correspond exactly to those of the living nucleus. It is a well-known fact (e.g., Zeiger, 1938) that fixatives alter especially the proteins; acetic acid, which is a component of a great many fixative blends, precipitates protein (Lassek, 1955). The molecules during this process lose their water hull and, at the same time, their electrostatic charge; they then rearrange themselves closer to one another by means of new direct bonds (Wolman, 1955). In essentially the same way the nucleoproteins are precipitated (Baker, 1945); the reaction is pH dependent (Lassek, 1950).

The representation of the nucleus as it results for example from treatment with protein precipitating solutions is determined by the media and, also, by the karyoplasm. Thus, if we know the action of the media, we can draw from the visualized structures certain conclusions on the nature of the pre-existing vital structures. If we compare the fixed and stained representations for instance of a human egg cell, a liver cell, a tissue plasma cell and a cancer cell (Fig. 27), we can easily deduce that the structures of the egg cell, for example, represent a different state of the karyoplasm than those of the liver cell; the former are precipitated by fixation as filaments, whereas the latter give rise to granular corpuscular densities with fine filamentous bridges. The karyoplasm of plasma cells is very compact and contains larger corpuscles at the nuclear envelope. Cancer cells may display denser filaments than egg cells and in certain cases several small nucleoli beside the large main nucleolus. These observations lead to the conclusion that the fixed and stained representation must be the manifestation of different vital structures since the influence exerted by the media was the same in all cases (see also Barigozzi, 1952, 1954).

In view of the considerable differences between the elements of the karyoplasm as revealed in the light microscope, great hopes were set on electron microscopy as a means of obtaining basic information about the nuclear fine structure. Yet the first results were a bitter disappointment; in contrast to the rich structuration of the cytoplasm, the karyoplasm appeared to be in most instances homogeneous and almost structureless. At first, the widely used fixation with osmium tetroxide was held responsible for the failure, for it was thought that, by this method of fixation, the nuclear components were destroyed (Sjöstrand, 1956); yet this was in apparent contrast to the experiences gained from the light microscope where fixation with OsO_4 brought forth the best representations of the nucleus (e.g., Brun and Chevassu, 1958). This discrepancy can be explained only by confronting the electron micrograph with the equally structureless appearance of the nucleus *in vivo*; the electron microscope representation after treatment with osmium corresponds largely to the *intra vitam* state of the karyoplasm (Moses, 1960) and the seeming lack of structures in the nuclear space can be taken as a criterion for the cell's state of preservation. Large densities turned out in most cases to be undesirable artifacts.

With the increasing optical resolving power of electron microscopes in recent years, more

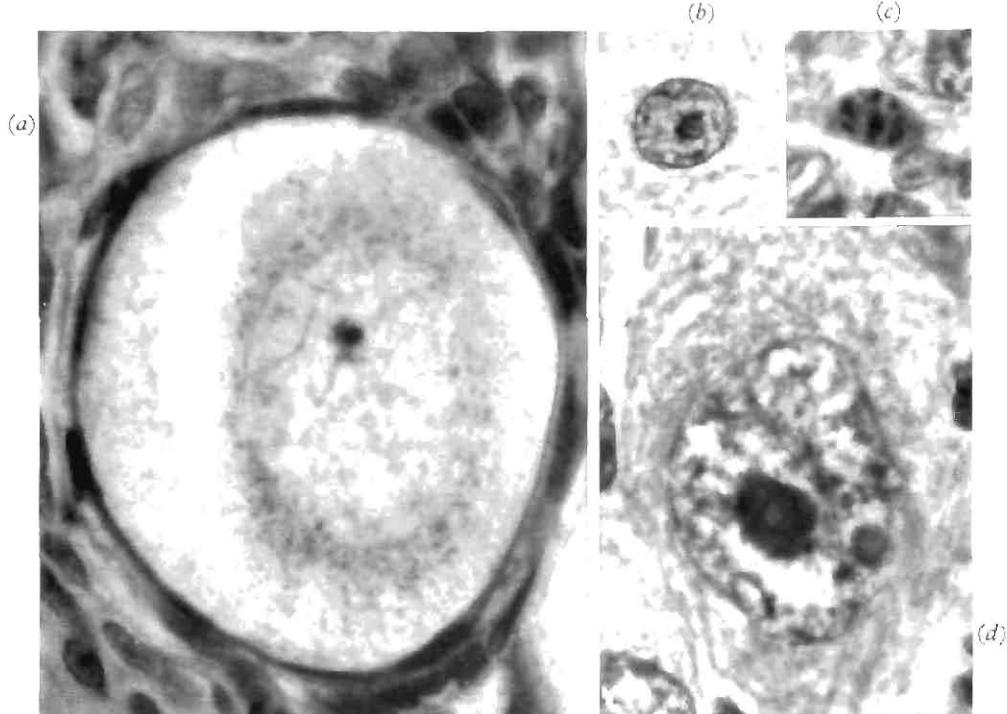


Fig. 27. Different types of nuclei as they appear after fixation and staining (formalin, haematoxylin eosin); all $\times 2000$. (a) Human egg cell showing fine filamentous chromosome-like structures in a nucleus with extremely loose content. (b) Nucleus of human liver cell with granular filamentous densities which are predominantly situated at the periphery. (c) Human tissue plasma cell showing characteristic peripheral densities at the inner aspect of the nuclear envelope, and a central nucleolus. (d) Human carcinoma cell with a very large vacuolized nucleolus and a ramified system of canaliculi in the loose karyoplasm.

and more observations are being compiled, which allow at least preliminarily a certain sub-classification of this homogeneous nuclear content. Thus it was found that the basic structure is always of the same nature; homogeneity is simulated by a large number of small granules, which are by no means dispersed at random but ordered into filaments like beads on a chain. The single filaments are coiled around each other in spirals, thus forming fibrils of a higher order, which in turn may be coiled around each other to form an even bigger spiral. Some representations allow even approximate measurements of the thickness of such fibres. The values thus obtained differ considerably from species to species but they are all within a certain order of magnitude. It was possible to demonstrate most delicate filaments of 30–60 Å in thickness, e.g., in mouse monocytes (Amano *et al.*, 1956), in algae (Ueda, 1960), in lymphocytes of calf thymus (Ris, 1958) and in the nuclei of human sweat glands (Kurosumi, 1961). These structures have been termed primary chromonemata and pairs of these frequently form double helices, thus creating bigger fibrils (secondary chromonemata) of 300 to 500 Å in diameter (Fig. 28). Elements of this dimension have been found by many

investigators, e.g., in plasma cells of mouse lymph nodes (Amano *et al.*, 1956) and also in the macronuclei of a certain type of ciliate (Kaneda, 1961). In other cells, these fibrils apparently form finer helices of little more than 100 Å in diameter as, for instance, in a certain type of *Cyanophyceae* (Shinke and Ueda, 1956), in lymphocytes of calf thymus or in spermatocytes (Ris, 1958; Kaye, 1958), in frog erythrocytes (Yasuzumi, 1960) and in the root tips of plants (Peveling, 1961). Whether there are still bigger bundles of fibrils up to 1000 or 2000 Å, we do not know. Also, we can by no means attribute the same significance to all filamentous structures of the karyoplasm. Some are apparently related to the function of the nucleus, as for example the 300 to 400 Å tubuli of *Drosophila* spermatocytes (Meyer, 1960; Meyer *et al.*, 1961). Finally, there are also in the nucleus a large number of those nucleolar granula, 150 Å in diameter, which are probably equivalents of the cytoplasmic ribosomes.

The range of these observations is expanding constantly; details are of minor importance. It is decisive that in the electron microscope too the karyoplasm is not structureless but ordered in fine fibrils. In the discussion of the fine structure of the mitotic chromosomes we will meet again such fibrils, composed of a helix of still finer fibrils; and when we were talking previously of primary and secondary chromonemata, the parallelism became obvious. These fibrillar components are identical with the components of the chromosomes. Since they fill, in the electron micrograph, almost evenly the entire nucleus, we can conclude that the karyoplasm probably consists mainly of chromosomes. The free nuclear sap possibly exists only under special conditions as for instance before the onset of mitosis. It is released from the chromosomal material under treatment with dehydrating solutions, e.g., acid fixatives. The dehydrated chromosomes are then, indeed, well demonstrable in the electron microscope, and the optically empty space between them corresponds to the nuclear sap (Resch and Peveling, 1962). It is evident that the components of the chromosomes still subsist even after completion of mitosis and that the nuclear space is not filled with an irregular sol.

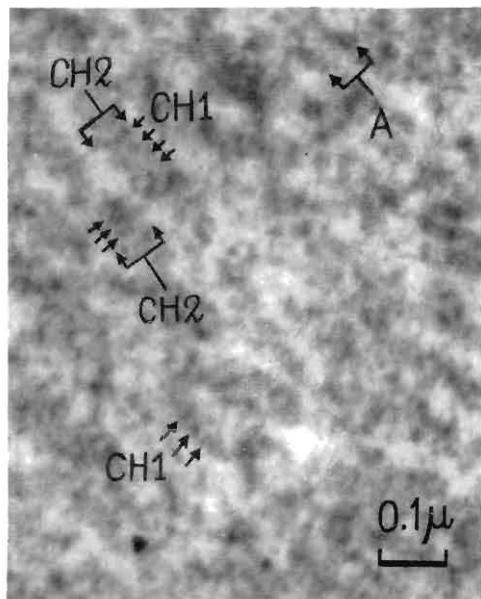


Fig. 28. Section of electron micrograph of human sweat gland nucleus. $\times 90,000$. Many fibrillar structures are discernible (secondary chromonemata = CH^2), that appear to be composed of helices of finer filaments (primary chromonemata = CH^1). The latter reveal superimposed granular densities. The letter A represents a cross section of a secondary chromonema showing filaments arranged in pairs. (From Kurosumi, K. (1961), *Int. Rev. Cytol.* 11, 1-124.)

Persistence of chromosomal individuality

The bulk of the available evidence suggests that chromosomes even subsist as definite karyoplasmic entities, i.e., each chromosomal component has its definite location in the nucleus. The observation that, for example, the giant chromosomes of *Chironomus* are adherent to the nuclear envelope fits well into this concept (Kimoto, 1958a); this arrangement is probably also present in many other nuclei, for, in early prophase, the chromosomes appear as bridges between nucleolus and nuclear envelope (Fig. 29). In suitable objects, as for instance in the rat mesothelium nucleus, it was possible to demonstrate even *in vivo*, by phase contrast microscopy (i.e., still in the light microscopical dimension), the presence of fine granular chains which essentially follow the same course (Ohno and Kinoshita, 1956). In the macronuclei of ciliates, too, similar structures have been found in the light microscope (Schwartz, 1958).

We know, however, of still earlier evidence supporting the fact that in the nucleus, chromosomal material does not dissolve in a diffuse solution but persists in certain areas and becomes visible again at the same site in the next prophase. For example, the single chromosome areas persist, in certain cleavage divisions, even after completion of telophase, as separate vesicles, i.e., the karyomeres. Each karyomere has its own nuclear envelope and during preparation for the next division, a chromosome appears in each karyomere (Heberer, 1927). There are many transitional stages between these karyomere nuclei and those nuclei in which no boundaries of the chromosomes are visible, as for instance in egg cell nuclei where boundaries between chromosomal territories are visible for certain periods and invisible in others, e.g., in *Cyclopes* eggs in cold water (Hertwig, 1929).

In fact, even when the chromosomes disappear in the karyoplasm "without leaving a trace", there is in certain cases evidence of their persistence. The best known examples are the nuclei of equine *Ascaris* blastomeres (Boveri, 1904). These contain large collective chromosomes with clubbed ends. When telophase reconstruction begins, the chromosome ends protrude from the reconstituting nuclei, thus producing eight invaginations in each completed nucleus (Fig. 30). In the next prophase the same chromosome ends appear again in each evagination; their material, therefore, had persisted at the same site. In single instances doubt has been voiced about the continuity of chromosomal individuality (Makarov, 1957); however, this is probably due to a lack of knowledge of the old observations.



Fig. 29. Early prophase in root tip meristem of *Vicia faba* showing radial arrangement of chromosomes extending between nucleolus and nuclear envelope. Feulgen nuclear stain. Approx. $\times 1000$.

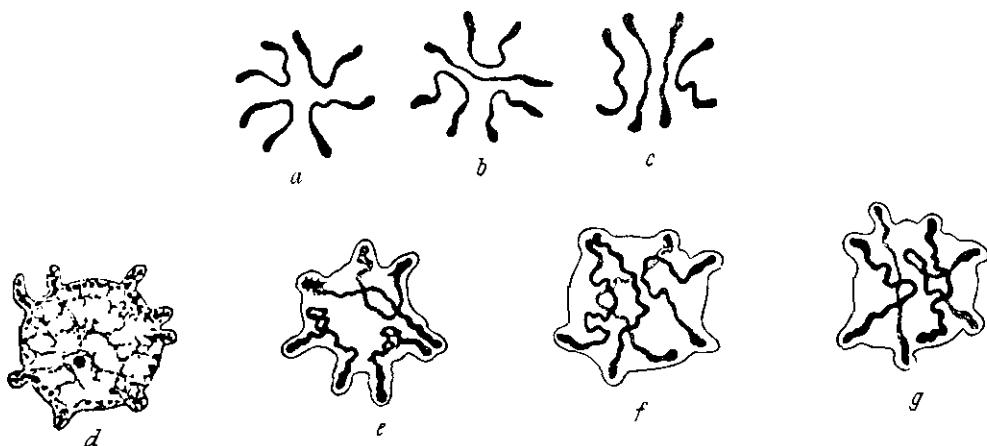


Fig. 30. Nuclei and chromosomes from the first cleavage divisions of *Ascaris* of the horse. (a-c) Metaphase figures with four chromosomes each. (d) Nucleus showing evaginations due to chromosomes. (e-g) Nuclei with the chromosomes becoming visible at early prophase. (From Boveri, Th., *Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns* (1904), Fischer, Jena.)

Tissue specific structures of the karyoplasm

On the basis of the above discussion, we are in a position to understand another phenomenon. We have noticed already when we compared different types of nuclei in Fig. 27 gross differences in the appearance of the karyoplasm. More subtle differences can be demonstrated with the acetocarmine squash technique. Here we find, e.g., in a liver cell nucleus (Fig. 31), single large chromatin corpuscles, some of which are located at the nuclear envelope, some at the nucleolus and others in the middle of the karyoplasm. In addition, we see small granular densities and a cloudy ground texture with ill-defined contours. Nuclei of renal cortex (Fig. 31, top right) contain, by contrast, a multitude of small granules and, rarely, large corpuscles, whereas epithelial nuclei from the upper small intestine (Fig. 31, middle left) display particularly large corpuscles which are connected with one another by ill-defined cloudy densities. In lymphnode preparations (Fig. 31, bottom), we are able to differentiate very well the finely granulated endothelial cells from the different lymphocytes. Such differences exist in many other tissues (Grundmann and Stein, 1961b). Thus the older observations that nuclear structures are specific of a given organ or tissue (Heidenhain, 1907; Olszewski, 1947; Barigozzi, 1952, 1954; Oexle, 1954) were confirmed in greater detail.

What are these corpuscles and granules? Let us recall our previous conclusions that acetic acid causes precipitation of proteins and particularly of nucleoproteins (p. 69). Thus, we are dealing here with precipitations of chromosomal material. If we compare the results with Feulgen-stained nuclei, we realize that it is mainly DNA which is demonstrated by the acetocarmine squash technique (Grundmann and Stein, 1961b). Since the precipitating forces of acetic acid act equally upon all portions of the karyoplasm, the different condensations can only be due to differences in the karyoplasm itself, i.e., in the chromosomes.

Such differences in the density of chromosomes are known since the studies of Heitz (1929) as the subdivision of the chromosomal substance into heterochromatin and euchromatin or, rather, into heterochromatic and euchromatic regions of the chromosomes, whereby the heterochromatic regions constitute the chromocentres. It has become increasingly difficult, in the course of the past twenty years, to give a definition of this dualism of chromatin, which we will discuss in greater detail in our chapter on the chromosomes (p. 105). It was originally agreed to apply the term heterochromatic to those chromosome components that underwent uncoiling and dispersion not during the post-telophatic reconstruction phase of the nucleus, but only just before the beginning of the next prophase. This feature has been called "allocycle" by Darlington and LaCour (1940). In principle,

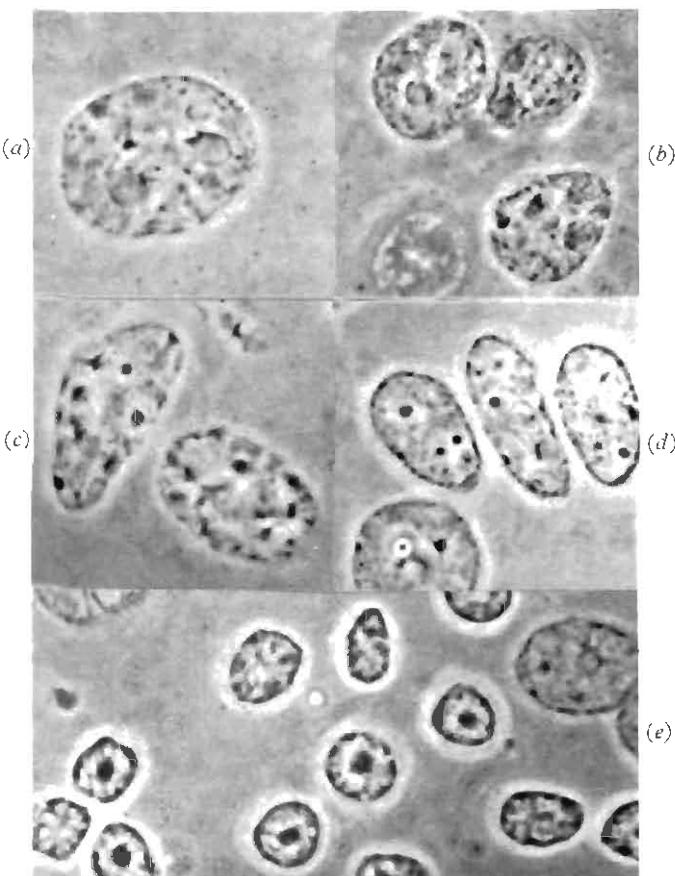


Fig. 31. Tissue specific nuclear structures in the acetocarmine squash preparation (phase contrast) of different rat organs. (a) Liver cell nucleus; (b) nuclei of renal epithelia; (c) nuclei from the upper small intestine; (d) nuclei of gastric epithelium; (e) macro- and micronuclear lymphocytes from lymphnode. Number and arrangement of the chromatin densities differ from tissue to tissue.

each heterochromatin is of transient nature and may become euchromatic just as, on the other hand, euchromatin is, during mitosis, temporarily heterochromatized (Kaufmann *et al.*, 1960). Hence, euchromatin and heterochromatin are but two different aspects of the chromosomal material.

The chromocentres are electron microscopically composed of the same delicate structures as the rest of the karyoplasm (Fig. 32). They too consist of coiled fibrils (e.g., Peveling, 1961; Rossner, 1961); however, the fibrils are here, in contrast to the euchromatin, more tightly coiled and lie, therefore, closer to each other. Thence, heterochromasia is the manifestation of a different degree of coiling (Ris, 1957) and, at the same time, of a different hydration (Altmann and Grundmann, 1955); the heterochromatic areas are more tightly coiled and less hydrated than the euchromatic portions, at least following treatment with dehydrating solutions. Yet, this does not explain what these differences are *in vivo*. Nuclei, at least in animal cells, are homogeneous *in vivo*, i.e., they do not contain markedly dehydrated components and perhaps the heterochromatic areas start to condensate only when exposed to dehydrating media (Grundmann, 1958a). It is possible but by no means certain that they were already previously more dehydrated. Now we also understand why there are several degrees of heterochromatic intensity (e.g., Hannah, 1951; Tschermak-Woess and Hasitschka, 1953) according to the ability of each chromosome component to condense and coil.

Thus we may interpret the corpuscles and grains in Fig. 31 as representing different degrees of heterochromasia. The large, strongly stained corpuscles correspond to the chromocentres (Baccarini, 1908), the smaller grains most likely to the β -heterochromatin of Heitz (1934). The smallest granules, which frequently merge to form cloudy structures, represent already intermediary stages between heterochromatin and euchromatin; the fully developed euchromatin is assumed to occupy the hardly stained spaces between the mentioned heterochromatic particles. The hypothesis put forward by Ludford (1954) and more recently by Iversen (1960b) that the chromosomes line as a narrow fringe the inner aspect of the nuclear envelope and only a few of them extend to the nucleolus, is highly unlikely (Grundmann, 1961a). For we find as a rule chromatin granula in the middle of the karyoplasm (Fig. 31) and one would not even have to use the same technique as we did to visualize these structures. Osmium fixation is equally adequate to demonstrate, in animal cells, those dense structures which differ from tissue to tissue (Pasteels, 1954).

We shall reconsider such tissue specific differences in structure when we discuss the fine structure of the chromosomes (p. 119). In the giant chromosomes of insect larvae there are localized expansions of the chromosomes, especially the "Balbiani rings" which are interpreted as being particularly marked functional uncoilings of chromosomal substance (Beerman, 1952a, 1956). This is in the chromosome itself the analogue to the above-described tissue specific differences in structure, i.e., a tissue specific hydration and uncoiling as the expression of a local function. Heitz (1929, 1934) had already, along similar lines, thought the euchromatic parts of chromosomes to be genetically active, the heterochromatic, however, to be comparatively inactive. Despite some restrictions (Cooper, 1959), this concept is still valid (Heitz, 1956).

Radioautographic findings by Hsu (1962) have provided compelling data to support this concept. Tritium labelled precursors of RNA are, in the interphase nucleus, selectively incorporated into euchromatic portions of the chromosomes; the heterochromatic



Fig. 32. Electron micrograph of a nucleus in a mouse liver cell, with three nucleoli and adjoining chromocentre (1% OsO_4 , pH 7.3). The filamentous granular fine structure of the chromocentres is visualized at many points. $\times 26,000$. (From Altmann, H. W. *et al.* (1963), *Z. Zellforsch.*, 59, 116.)

chromocentres are almost free of labelled material, i.e., they are not able to synthesize RNA, or, at least to a considerably lesser extent.

Finally, comparison of nuclei during embryogenesis, too, reveals a direct relationship between function and differentiation on the one hand and structure of the nucleus on the other. According to Barigozzi (1950), the nuclei of liver cells and of striated muscles in young insect larvae are entirely free of chromocentres. In the early cleavage stages of amphibian embryos, too, Olszewski (1945) and Oechsle (1954) were unable to find any chromocentres. It is only at the gastrula stage that the characteristic nuclear structures start to appear gradually as the cell becomes more differentiated. Serial transplantations of embryonal nuclei demonstrated that precisely at the gastrulation stage the possibility of exchanging nuclei, i.e., their functional omnipotence comes to an end (King and Briggs, 1956).

All these data complete the picture; different stages of uncoiling (or perhaps only a variable tendency to uncoil) in connection with the different functions are responsible for the tissue specific structures of the nuclei. Nuclei that are ontogenetically related have a similar nuclear structuration. In undifferentiated nuclei, e.g., in nuclei of cancer cells (Fig. 33), such characteristics are missing (Grundmann and Stein, 1961a and b).

Sex chromatin

Among all these chromocentres, one has proved to be of a special significance. In 1949, Barr and Bertram found in the nuclei of ganglion cells of female cats one large chromocentre which was missing in male animals, the so-called sex chromatin. It is located, in nuclei of feline hypoglossus nerve cells (Barr and Bertram, 1949), lying directly by the nucleolus and for this reason it was at first termed "nucleolar satellite" (Barr *et al.*, 1950). Numerous studies by many investigators of almost all available mammals (ref. see Hienz, 1959; Hamerton, 1961) including man (e.g., Moore and Barr, 1954) revealed that this sex chromatin is not, in most cases, associated with the nucleolus but closely applied to the inner aspect of the nuclear envelope (Fig. 33). It is a corpuscle of approximately 1μ in diameter (Barr, 1960); it sometimes displays three tassels but is usually almost round and seems in some cases to consist of two parts (Graham and Barr, 1952; Klinger, 1958; Miles, 1959). Like all other chromocentres it takes up basic dyes such as galloxyanine chrome alum or the Feulgen dye (Barr, 1960) and, therefore, contains DNA; but considerable amounts of



Fig. 33. Phase contrast micrograph of two cancer cells of a human portiocarcinoma showing the characteristic finely granulated nuclear structure and clearly visible sex chromatin (left in both nuclei). Acetocarmine squash preparation.

RNA are also present (James, 1960a). At the electron microscope level, we see that its fine structure resembles exactly that of the other chromocentres (James, 1960b; Thoenes, 1961).

Each diploid nucleus of female individuals contains one of these large chromatin corpuscles. Polyploid nuclei may have two or three of them (Ohno *et al.*, 1959); thus we are able to determine to a certain extent the degree of ploidy from the number of these nuclear densities (Bürger, 1961). Frequently, however, two or more adjoining chromocentres merge (Klinger and Schwarzacher, 1960). In many birds or in the fly *Drosophila* (Lüers, 1955; Brum *et al.*, 1959) the presence of sex chromatin could not be demonstrated with certainty; the difference with other chromocentres is too slight and this can make it difficult, in rodents too, to establish a diagnosis (Hinrichsen and Gothe, 1958). In histological sections from parenchymal organs of female individuals we can observe the presence of a sex chromatin in approximately 60–80% of all nuclei; in the intact preparation, e.g., of rabbit mesentery, it appears in almost 100% of the cases (James, 1960b). The same result is obtained when we employ the aceto carmine squash technique (Fig. 33), which is particularly useful in bringing out the chromocentres (Sanderson and Stewart, 1961).

Our definition of the sex chromatin as a particularly large chromocentre gives us at the same time an indication about its nature; it is a portion of a chromosome. Furthermore, there are many observations suggesting that the sex chromatin is due to the presence of an X chromosome and that the single X chromosome which is present in male individuals is either alone too small to be visualized as such or is inhibited in its heteropycnotic condensation by the Y chromosome. Another possibility is that one X chromosome suffices for the metabolic function of diploid nuclei and that in female individuals the second one condenses without functioning and thus becomes visible (Stewart, 1960). In the nuclei of male individuals there has frequently been found at the same site a smaller chromocentre (Graham and Barr, 1952, 1959) which may be the X chromosome of an XY nucleus. All these speculations, however, are valid only if there is, indeed, a regular pairing of the sex chromosomes in the nuclei of all somatic cells. This has been demonstrated, as yet, only in tadpoles (Boss, 1955a). At present, most investigators believe that only one X chromosome generates the sex chromatin (for ref. see Hamerton, 1961; Schwarzacher, 1962), particularly since a heteropycnotic cycle of the X chromosome has often been found (Ohno and Hauschka, 1960; Ohno and Makino, 1961).

Though many features of the sex chromatin are still unknown, it has gained much practical value in medicine. It can be well demonstrated especially in smears of the easily accessible mucous membranes, e.g., of the mouth (Moore and Barr, 1955; Marberger and Nelson, 1955) and this is why the use of the "mouth epithelium test" for nucleomorphological determination of the sex is so widespread in present-day medicine. It can equally be found in the mucous membranes of vagina and urethra (Carpentier *et al.*, 1955) as well as in all body fluids that contain cells such as the amniotic fluid, where desquamated foetal cells allow prenatal sex determinations (Serr *et al.*, 1955; Haempel, 1956).

Furthermore, sex determination from the nuclear morphology has proved to be of great value in all disturbances of the development of gonads. With the aid of the mouth epithelium test it is easily possible to determine the chromosomal sex of genuine intersexes (Assis *et al.*, 1960) as well as of male or female pseudohermaphrodites (Hienz, 1959; Shah *et al.*, 1961).

Some disturbances of male fertility can be explained by the presence of the female sex chromatin (Kosenow and Niermann, 1960). Recently, statistics showed that 2·65% of newborn boys are of "female" sex according to their nuclear chromatin and 1·33% of newborn girls are of "male" sex (Maclean *et al.*, 1961). This fact is probably due to supernumerary or absent sex chromosomes (XXY or XO constellations, see also p. 216); there are often exterior malformations present at the same time. In the Turner's syndrome of the female (Turner, 1938), a hypogonadism associated with inhibition of growth, infantilism, primary amenorrhoea and XO constellation (see p. 215), the female sex chromatin, in most cases, is absent; in the Klinefelter's syndrome (Klinefelter *et al.*, 1942), a eunuchoid phenotype associated with gynecomastia, hypoplasia of the testes and XXY constellation, the males are females according to their sex chromatin (for ref. see Hienz, 1959; Hamerton, 1961; see also Barr, 1959). In benign or malignant tumours, the nuclear sex corresponds usually to that of the individual afflicted with the tumour (Hanschke and Hoffmeister, 1960; Schwarzacher, 1962). The hope to counteract tumours of the mamma which are seemingly chromatin negative (i.e., tumours without the female sex chromatin) by therapy with female hormones (Hienz, 1959; Spechter *et al.*, 1962) has not generally materialized (Zanella *et al.*, 1961; Schwarzacher, 1962).

Nuclei of granulocytes also display a special feature pertaining to the female sex (Fig. 34). It is an accessory nuclear structure which has been called drumstick because of its shape (Davidson and Smith, 1954). It occurs in women in roughly one out of every 38 granulocytes; men have it much more seldom. Other types of nuclear processes as the sessile nodules, rackets, small lobes or minor lobes (Davidson and Smith, 1954) are also characteristic of the female sex but less easy to interpret (for ref. see Kosenow, 1959). For the detection of all disturbances in the development of sex, one can employ the leucocyte test or the mouth epithelium test with equally good results (Kosenow, 1959; MacLean, 1962; and others). Old age (Brüschke and Hermann, 1962) or castration (Krueger and Dihlmann, 1957) are of no consequence for these structures. The fact that these processes of the nuclear chromatin are always Feulgen positive and hence contain DNA (Schaumkel *et al.*, 1957) suggests that we can consider them to be analogous to the sex chromatin, especially since they often contain heterochromatic chromocentres (Gothe and Hinrichsen, 1959). The formation of these processes may be explained by a retraction of euchromatic nuclear material from the denser heterochromatic material (Dihlmann, 1959; Gothe and Hinrichsen, 1959; Felsch, 1961), particularly since living leucocytes are in constant amoeboid movement. And precisely this amoeboid movement provides a clue as to why chromocentres that resemble the sex chromatin may be found also independently from the drumstick processes



Fig. 34. Human neutrophilic granulocyte displaying the "drumstick" process (*top left*), characteristic of female individuals. Blood smear, Pappenheim stain.

(Burghold and Spreer, 1960). The reason is that the nuclear shape changes rapidly and the nuclear segments vary.

Nuclear inclusion bodies and nuclear viruses

We must strictly distinguish from the chromocentres those structures which are called nuclear inclusion bodies. This term should really be used only to describe foreign bodies or genuine cellular particles, which were situated previously outside the nucleus and are now found inside. However, the expression is used today in a much wider sense. It generally embraces all substances which are surrounded by nuclear material but are themselves no structures of the nucleus. This definition leads us automatically to a rough classification of the structures in question into those that came from the outside, viz. the cytoplasm, and those that were primarily formed inside the nucleus.

Most eosinophilic inclusion bodies in mammalian liver cell nuclei (Schiller, 1949; and others) seem to be cytoplasmic particles that were secondarily engulfed by the karyoplasm. They can assume the most different shapes and sizes and may often contain small vacuoles or ill-defined densities. Some of them are spheroid and apparently rather compact; these are usually surrounded by a brighter halo (Fig. 35). Others have ill-defined boundaries. Quite



Fig. 35. Human liver biopsy showing eosinophilic inclusion body in the liver as a remnant of cytoplasmic inclusions of the nucleus (see Fig. 20, right) after toxic insult to the liver parenchyma.
Haematoxylin eosin.

frequently they are also surrounded by a Feulgen positive fringe similar to the nucleolar membrane as we see it under the light microscope. Electron microscopy, however, reveals in many of these inclusion bodies mitochondria or components of the endoplasmic reticulum (Wessel, 1958; Leduc and Wilson, 1959a); histochemistry brings evidence of lipids, glycogen, iron pigment, etc. (Leduc and Wilson, 1959b). Most likely the fat droplets which we frequently find in the nucleus in severe toxic liver cell degeneration (e.g., Wotton and Levin, 1957) are also such engulfed cytoplasmic fragments (Altmann, 1961b); they give rise, after treatment of the sections with alcohol, to vacuolised nuclei ("Lochkerne") (e.g., Winckler, 1960). The frequent glycogen nuclei of liver cells (i.e., expanded nuclei whose chromatin is pushed to the periphery and whose central portions seem to be empty under the microscope) are the result of a transfer of glycogen into the nucleus where it can be well demonstrated at the electron microscope level (Fig. 36) (Sheldon *et al.*, 1962).

Other inclusion bodies as for example the nuclear pellets of the epiphysis (see, e.g., Bargmann, 1942) or of the rodent hypophysis are generally thought to be related to the

secretion mechanism of these cells, insofar as they are not large nucleoli or simply artifacts due to fixation (Kevorkian and Wessel, 1959) or invaginations of the cytoplasm (Serber, 1961). It seems possible to interpret in a similar manner the small vacuolar inclusions which are frequently observed in lymphocytes and plasma cells, even though they tend to occur more frequently in certain metabolic diseases (e.g., Derwort and Detering, 1959; Maurer, 1962). The significance of those very dense nuclear inclusion bodies in the rat kidney that are to be found in the electron microscope after lead poisoning has not yet been clarified (Beaver, 1961).

It is essential for the interpretation of nuclear alterations in *virus diseases* that we should be aware of all these nuclear inclusion bodies. For these inclusion bodies are quite frequently mistaken for virus aggregates or, even, virus diseases are diagnosed from their presence. The above-mentioned examples have demonstrated how non-specific insults to cells may

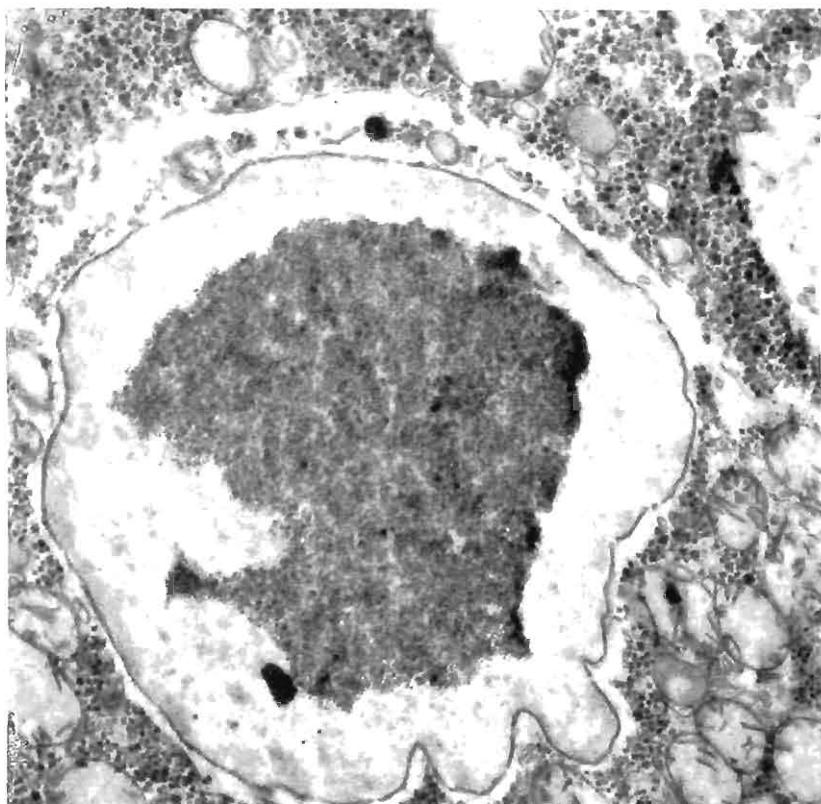


Fig. 36. Electron micrograph of a liver cell in Gierke's disease. $\times 8800$. Both nucleus and cytoplasm show glycogen granules which are clearly smaller in the nucleus than in the cytoplasm. The pale grey area outlining the nuclear periphery corresponds to the chromatin. (From Sheldon, H., et al. (1962), *J. Cell Biol.*, 13, 468-473.)

lead to nuclear inclusion bodies which are in no way related to a virus (see also Reuter, 1959).

On the other hand, it has been demonstrated that certain virus diseases can produce in the nucleus inclusion bodies of a certain diagnostic significance. This is the case for example with the inclusion body encephalites (e.g., Weisse and Krücke, 1959) and there are other viruses which multiply exclusively in the nucleus and which cause characteristic alterations there.

A virus does not have a metabolism of its own; it needs a living cell as a media for its multiplication. There, the nucleic acid component of the virus is incorporated into the nucleoproteins of the host cell or it takes their place. The host cell then synthesizes both the genuine nucleoprotein form and that of the virus. Which nucleoprotein form is reduplicated in preference to the other depends on the "virulence" of the virus. The protein component of the virus is also synthesized and nucleoprotein and protein are then bound together thus forming the complete virus. Now the mature viruses leave the host cell and penetrate into other cells where they start the same cycle all over again. There are countless variations of this mechanism described here in a simplified way. These variations depend on whether the virus consists only of nucleic acid and protein or whether it is a larger virus of a more complicated composition. However, all virus diseases follow the same basic rules. It is important for cytomorphology that the small simple viruses, as well as the intracellularly generated precursors of more complex viruses are particles of uniform size and uniform structure which, therefore, may form a regular crystal lattice. This we then find in the cell, especially in the nucleus, and the presence of such crystal lattices is cytomorphologically a particularly sure sign of a virus infection.

A few examples may illustrate this fact. Typical nuclear viruses are those of the herpes group. They all lead, with certain variations, to reactions which are at least similar. After they have penetrated the host cell, the nucleus of the latter swells and sometimes crystalline structures develop in the nucleus arranged in the pattern of a spatial lattice (Fig. 37); this lattice consists of particles that are 70–80 m μ in diameter in the central portions of the lattice, and 120–130 m μ at its periphery (Morgan *et al.*, 1959). The nucleoli are gradually dissolved (Barski and Robineaux, 1959) and the karyoplasmic chromatin is pushed to the periphery of the nucleus. We also find then many mature viral particles applied to the inner aspect of the nuclear envelope. At times, single viral particles may be seen bulging the nuclear envelope and finally pinching off a part of it; the viruses then appear in the cytoplasm surrounded by portions of the nuclear envelope. The DNA content of the nuclei is often considerably increased during the virus replication. However, gradually the basophilia and the DNA content of the nuclei diminish until finally the nucleus represents only a compact eosinophilic inclusion body in the dying cell. The viruses have by that time already left the cell and infested other cells.

The viruses of the adeno-virus group provoke similar reactions. Here also the nuclei swell and under the light microscope we find an acidophilic network with ill-defined boundaries, that soon extends throughout the entire nuclear space (Godman *et al.*, 1960). Again we see, under the electron microscope, crystalline spatial lattices (Barski and Cornefert, 1958; Armstrong and Pereira, 1960) which may grow into large nuclear crystals (Leuchtenberger and Boyer, 1957; Godman *et al.*, 1960); the nuclear envelope is at that time still

The development of these large nuclear crystals and their relation to the viral particles is particularly interesting (Boch *et al.*, 1957; Morgan *et al.*, 1957, 1960). They are pure protein crystals which stretch the nucleus along its longitudinal axis (Fig. 38). In cross-sections they are hexagonal (Fig. 39) and the small dots and rings which are seen inside the crystals represent the replicated protein component of the virus. The viral component, which contains

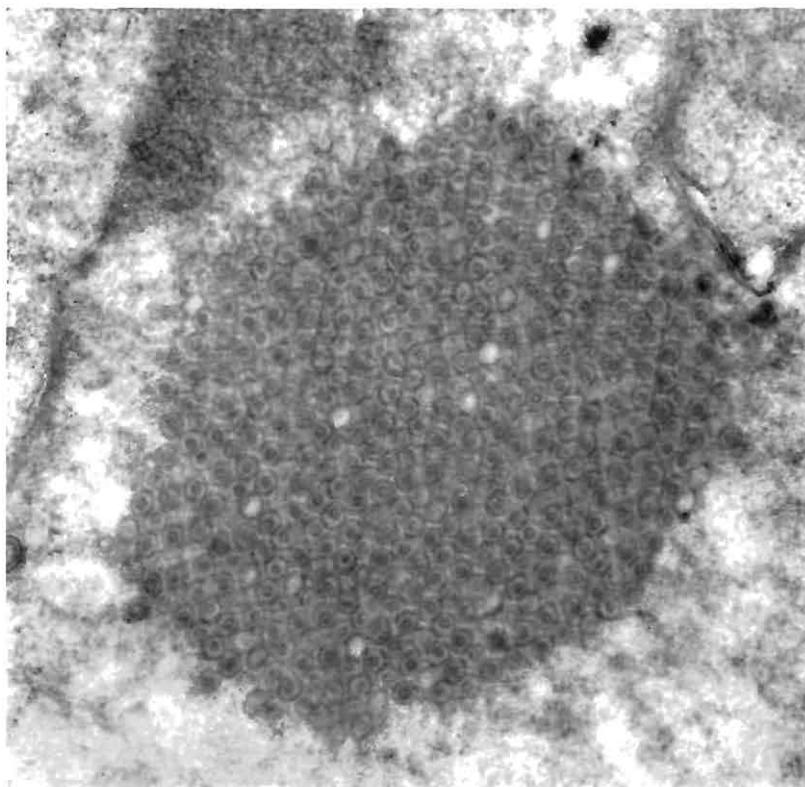


Fig. 37. Electron micrograph of a particle of the herpes virus in crystalline array. $\times 40,500$.
(From Morgan, C., *et al.* (1959), *J. exp. Med.*, **110**, 643-656.)

the DNA, is situated beside the protein crystals. It is either of crystalline structure (Fig. 38, right) or more diffusely ordered, but is also always located inside the nucleus. In the polyhedron disease of insects, too, large intranuclear bodies are formed (Smith, 1958b) which have a crystalline structure (Day *et al.*, 1958). They also contain within the crystal, in contrast to the adeno-virus group, the DNA-containing virus components. Smaller nuclear inclusion bodies are also found in the human skin papilloma (ref. see Gaylord, 1958), in viral diseases of human salivary glands (Luse and Smith, 1958) or in poliomyelitis (Reuter, 1959); in the latter, the virus antigen can be demonstrated in the nucleus 3 to $3\frac{1}{2}$ hours after inoculation

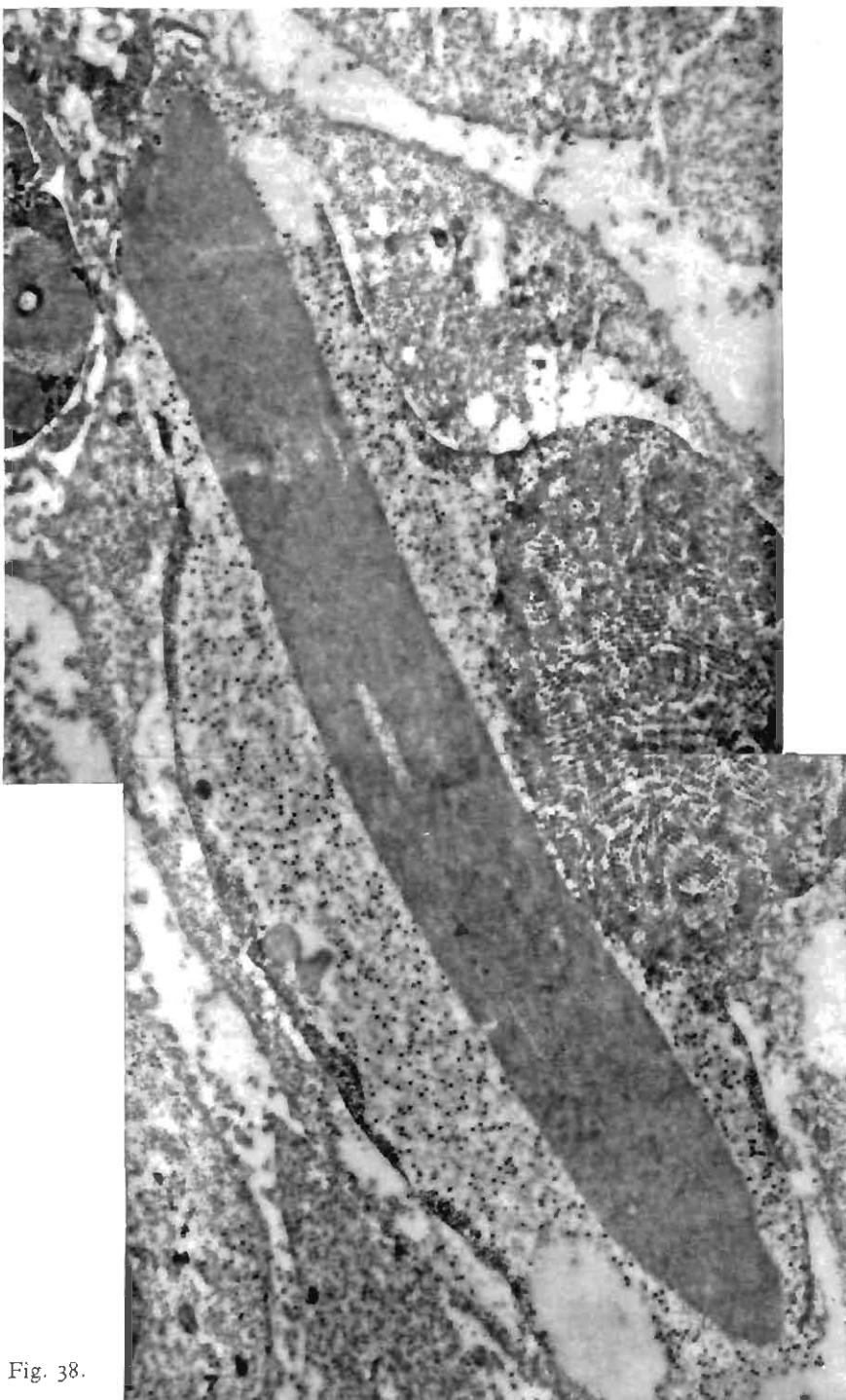


Fig. 38.

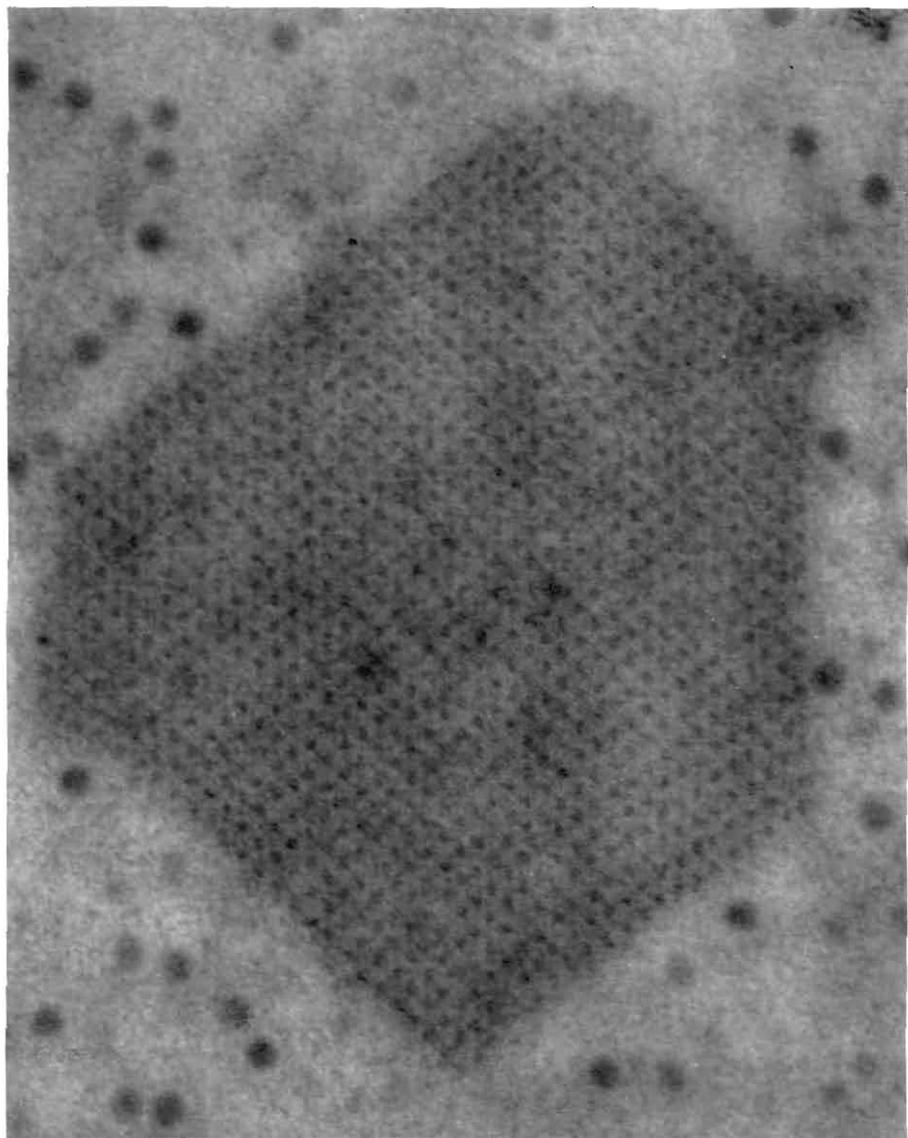


Fig. 39. Electron micrograph of tissue culture cells infected with adenovirus, showing hexagonal protein crystal in cross section. $\times 80,000$. (From Morgan, C., et al. (1960), *J. exp. Med.*, **112**, 373-382.)

Fig. 38. Electron micrograph of large intranuclear virus crystals of the adenovirus type 5. At left, extending longitudinally, a protein crystal; to the right of the latter, nucleoprotein particles are seen in a crystalloid array. $\times 7360$. (From Morgan, C., et al. (1960), *J. exp. Med.*, **112**, 373-382.)

(Levy, 1961). One component of the poliomyelitis virus is perhaps replicated in the immediate vicinity of the nucleolus (Ruska *et al.*, 1955; Braunsteiner *et al.*, 1958) as is, similarly, the varicella virus (Tournier *et al.*, 1957) or the virus of the rabbit papilloma (Stone *et al.*, 1959). In other cases, the DNA content of the virus and the presence of large nuclear inclusion bodies suggest a nuclear replication as for instance in the polyoma virus (Love and Rabson, 1961) which is an important virus for cancer research or in a certain "rat virus" (Dawe *et al.*, 1961; Rabson *et al.*, 1961) which is very similar to the polyoma virus. For the inclusion bodies in cytomegaly (Fig. 40) which also contain DNA (Sandritter *et al.*, 1960c),

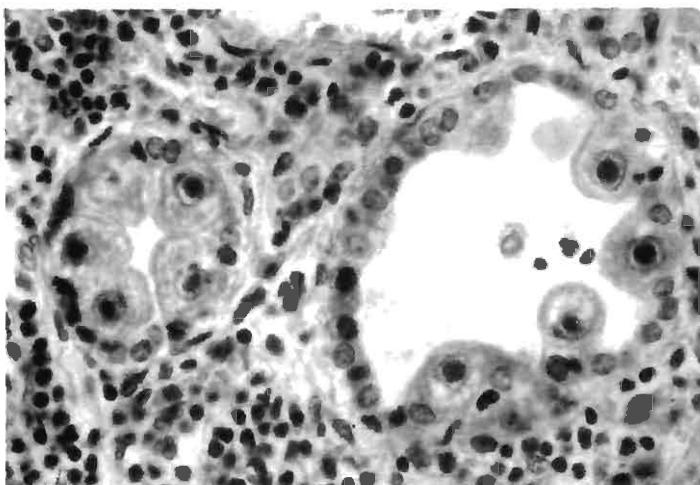


Fig. 40. Intranuclear inclusion bodies in cytomegalic kidney epithelium cells. Haematoxylin eosin.

the nuclear replication has been clearly demonstrated by electron microscopy (Stern and Friedmann, 1960). The details of the process resemble very closely those of the replication of Herpes viruses (see above).

In other cases, however, the nuclear inclusion bodies do not contain any viral particles at all. They are merely the symptoms of a nuclear reaction or, in most cases, of nuclear degeneration. For, in all virus diseases, non-specific alterations of the nucleus play a major role; they are associated with enlargement of the nucleoli, hyperchromatosis of the nuclear envelope and degeneration of the nucleus, as for instance in the human virus hepatitis (e.g., Büchner, 1957b; Cossel, 1961); the inclusion bodies which we then see are the remnants of dead nuclear particles. Such inclusion bodies are usually free of DNA for, either it has been used up by the virus or it has been depolymerized or destroyed. The Torres' inclusion bodies in yellow fever, for example, consist only of histones, lipoproteins and isolated amino-acids (Bearcroft, 1960), in other words, they do not contain nucleic acids. It is only from their histone content that we are able to deduce their origin from chromosomal material. Furthermore, we understand now that virus diseases are typical cellular diseases, which lead in

many cases to the death of the cell, the virus disturbing or completely taking over the cell's metabolism or poisoning the cell with "viral toxins" (ref. see Walker, 1960).

Death of the nucleus

In the case of nuclear viruses the destruction of the nucleus is, in the light microscope, the most striking feature. In most cases, we observe a dissolution of the nucleus, a "karyolysis", that is, the DNA-containing chromatin diminishes or is deposited against the nuclear envelope. This "hyperchromatosis of the nuclear envelope" is absolutely non-specific (see e.g., Wendt, 1959) and has been known for a long time, in ganglion cells or liver cells, to result from a massive insult to the cell (ref., see Müller 1955). If the process goes on any further, the nuclear substance dissolves or forms an eosinophilic body that still contains remnants of basophilic nucleic acids. Such dying cells may, in the liver, be phagocytized by other cells (Fig. 41); frequently, however, we cannot distinguish this condition from others in which one nucleus of a binuclear liver cell has died together with its respective cytoplasmic area.

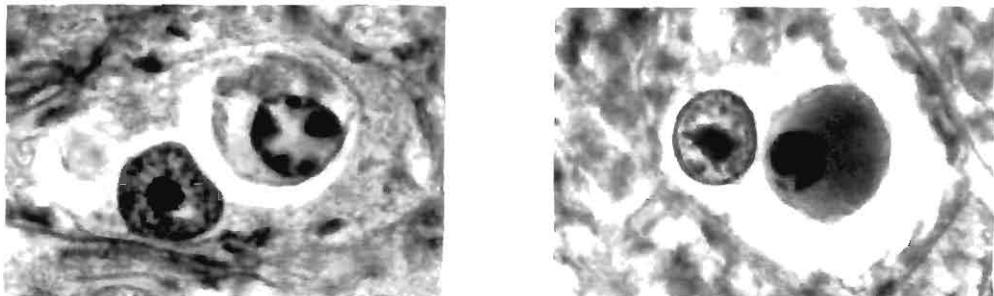


Fig. 41. Representations of dead epithelial cells of rat liver, which are phagocytized by intact liver epithelial cells, following toxic liver damage by the carcinogenic substance diethylnitrosamine. Haematoxylin eosin.

In other instances, following the above-described hyperchromatosis of the nuclear envelope, the nucleoproteins stay condensed even after the nucleus is destroyed and the interior parts of the nucleus show also such droplet-like precipitations; this situation is called "karyorrhexis" (see Müller, 1955) (Fig. 42, left). From what we have learned of the nuclear fine structure (see p. 68), we can easily deduce that these precipitations are irreversible coagulations of nucleoproteins due to the loss of almost the entire binding water.

In most cases, however, the stage at which these coagulation granules can be seen is never reached. The nucleus shrinks instead and forms a compact structure which is either round or has an irregular outline (Fig. 42, right). This "pyknosis" entails an intensive basophilia of the nuclear material and is represented in the phase contrast microscope by a brilliant contrast (Zollinger, 1948b). Under the electron microscope (see, e.g., Miller, 1958; Dalgaard and Pedersen, 1959; Ule and Rossner, 1960; and others), we see already at early stages within the nucleus osmiophilic condensations which develop into increasingly larger

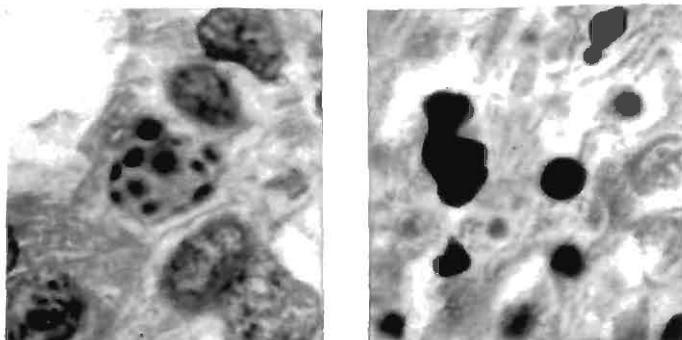


Fig. 42. Types of nuclear death in human carcinomas. *Left:* karyorrhexis. *Right:* several pycnoses. Haematoxylin eosin.

bodies, leaving empty interspaces between them. The degradation of DNA (Leuchtenberger, 1950) is preceded by the loss of histone protein (Alfert, 1958a). The course of these nuclear alterations that lead to pycnosis does not depend on the type of insult to the cell; it always passes the same stages, whether it occurs after irradiation with ionizing rays (e.g., Schümmelfeder, 1959; Wendt, 1959; Schrek, 1960), after administration of nitrogen mustard (e.g., Grundmann, 1952) or after other types of intoxications (ref. see Müller, 1955). In the "nucleomalaenia" of white blood cells (e.g., Undritz, 1942), we have basically the same process. Antibodies to nuclei, as for example in lupus erythematosus, bring about the same type of cell death. The pycnotic remnants of the nucleus are here phagocytized by other granulocytes and lie then in their cytoplasm as bodies of various size; this fact is the basis of the "LE cell phenomenon" (Fig. 43) (ref. see Miescher, 1959).

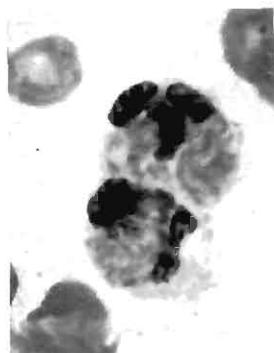


Fig. 43. "LE-cells" in lupus erythematosus, human blood smear. They represent neutrophilic granulocytes which have phagocytized the remnants of dead nuclei. Pappenheim stain.

Review

These processes which are connected with the cell's death illustrate clearly that in a living nucleus the equilibrium of the structure is very labile. Every disturbance in the extranuclear or intranuclear conditions results in condensations of the nuclear components which, at first,

are reversible but soon lead to irreversible condensations and coagulations. Thus, the previous order is destroyed. The karyoplasm is not a solution of proteins and nucleoproteins suspended with no specific order within a nuclear sap; it consists of chromosomes which most likely lie in a definite spatial arrangement to one another. The regular occurrence of such a morphology can be inferred from the persistence of the reversible nuclear constellations as we see them after treatment with dehydrating agents; it is further indicated by the evident permanence of the chromosomes' individuality and, last but not least, by the fixed localization and the regular occurrence of the sex chromatin, a particularly characteristic chromocentre of chromosomal nature. In addition, there exists an inner structure of the nucleus, which is to a certain extent tissue specific. It is made of two different components, euchromatin and heterochromatin. This differentiation is the result of different stages of hydration of the chromosomal components or, to be more exact, probably of a different susceptibility to dehydration. With the electron microscope, the delicate elements of the chromosome can be recognized as fibrils of different diameter and different order, coiled around one another.

SIZE, MASS AND COMPOSITION OF THE NUCLEUS

Now that we have a fair knowledge of the structures of the nucleus and of their functional interrelations, we can proceed and examine what substances these structures are composed of and what significance these substances have for the nuclear function. The karyoplasm represents by far the largest portion of the nucleus and, therefore, all quantitative data available for the nucleus are mainly concerned with the karyoplasm and less with the nucleolus or the nuclear envelope; besides, we have already discussed the chemical composition of the latter (see pp. 48 and 57).

Volume

The size of the nucleus is mainly determined by the amount of substances present in the karyoplasm. Important, however, is not only their amount but also the form under which they occur, in other words, their water content. In the course of our discussion on the difference between euchromatin and heterochromatin we mentioned already that the karyoplasmic material can bind water in different amounts.

As we have shown in the previous chapter, for instance in Fig. 27, the nuclei of different tissues differ in size. The largest nuclei in mammalians are those of ganglion cells and egg cells, the smallest are those of lymphocytes, glia cells and erythroblasts. In many cases, there is a direct relation between nuclear size and the size of the whole cell. The size of the nucleus is relatively specific for a given species; seed plants, for instance, have fairly large, fungi fairly small nuclei. In one and the same organism, the larger nuclei have a more dispersed inner structure, the smaller nuclei a more compact one (see Figs. 27 and 31).

The three components of the nucleus are classified as follows:

1. The essential nuclear substance; this is the chromosomal mass which is, in an individual, equal in all nuclei that have equal ploidy;
2. RNA and certain proteins, present in variable amounts;
3. The binding water of proteins and nucleoproteins which varies from nucleus to nucleus.

An increase in volume for instance to twice the original volume, can be brought about by (a) an increase of all three components, (b) an increase of component 2 and 3, whereas the first component of our classification remains constant. The first process occurs prior to mitotic division (p. 175) or in endomitosis (p. 223), when the entire chromosomal material doubles. The second of these processes is responsible for the above-mentioned tissue specific differences of nuclear volumes. The first can be compensated only by the division of the nucleus; it is part of the cellular growth. The second is an increase in substance which can easily be reversed. We must always clearly distinguish between these two processes.

Boveri (1905) has termed this principle of nuclear growth the "rule of proportional growth of the nucleus"; each nucleus grows by doubling its previous substance. The "particle theory" or "protomere theory" of Heidenhain (1907) was based on the same concept; he postulated that the living material is composed of elementary particles ("protomeres"), which double in number during growth. This led Jacobj (1935, 1942) to formulate his "Gesetz des rythmischen Kernwachstums in Verdoppelungsschritten".* When we measure the nuclear volumes, for example, of the rat liver, we obtain at least three classes of nuclei; their mean values are exactly in a ratio of 1:2:4. Such curves are typical of all tissues with different stages of ploidy, i.e., tissues which contain nuclei with different numbers of chromosome sets. In the rat liver (Fig. 113), the small nuclei which form the first class are diploid (two sets of chromosomes), the medium-sized ones of the second class are tetraploid (four sets of chromosomes) and the large nuclei of the third class are octoploid (eight sets of chromosomes). Two methods were used to determine these values: chromosome count and measuring of nuclear DNA content (p. 219), and the data from both methods are consistent. The rhythmic reduplication has been demonstrated in many tissues and its causality has been essentially clarified (for summarized ref. see Helweg-Larsen, 1952; Rather, 1958). We will return to this subject later in our chapter on polyploidy.

However, nuclei of high ploidy are not in every instance larger than diploid ones (e.g., Nemec, 1910) and the rhythmical doubling of nuclear volume is by no means always based on polyploidy. In this regard, the case of the spermatocytes and spermatids of the insect *Arvelius albopunctatus* became well-known; all three classes of their nuclei (each higher class having double the nuclear volume of the former) have the same DNA content, but the protein content increases proportionally from one class to the other (Schrader and Leuchtenberger, 1950). This situation can best be explained by the special conditions existing in the maturation process of spermatocytes. It also is of interest that nuclei of female individuals are frequently larger than those of male individuals (Sauser, 1936; Kulenkampff and Köhler, 1961).

These ratios in nuclear volume can easily be explained by differences in the number or the mass of the chromosomes; this kind of relation, however, is lacking in all these alterations in nuclear volume which we generally call "nuclear swelling" or "nuclear shrinking". These are mainly due to exogenous factors. An elevation of environmental temperature, for example, can significantly diminish the size of the nuclei in plants or animals, lower temperatures can increase the volumes of the nuclei (Hertwig, 1903b; Hartmann, 1918a, 1918b, 1919). In the mouse liver (Caspersson and Holmgren, 1934), in the Langerhans islet cells of the pancreas (Helman and Hellerström, 1959) and, similarly, in the ependyma cells of

* "Rule that nuclei grow rhythmically by stepwise doubling of their nuclear substance."

the spinal cord, too (Kulenkampff and Kolb, 1960) a diurnal rhythm of alterations of nuclear volume has been found; it appears to be associated, in the liver, with the glycogen content. These rhythmic alterations are probably directly related to periodic functions of the cell. In other organs too, as for instance in the epithelial cells of the small intestine (Hinztsche and Tanner, 1937), the nuclear volume rises as a rule when the tissue is under functional strain.

This relation between nuclear volume and function of the nucleus has been known for a long time (see, e.g., Dolley, 1925); Benninghoff (1950) termed it "functional nuclear oedema" and demonstrated it in his studies on experimental hypertrophy of the small intestine of the rat (Benninghoff, 1951). Many other investigations followed. Complete relaxation of the muscles by ether anaesthesia resulted, in the frog, in a decrease in size of the ganglion cell nuclei of the anterior horn of the spinal cord; when a stimulus was applied before or after anaesthesia they became larger (Krantz, 1947). If, after hypophysectomy in rats, the function of the thyroid gland is diminished, the nuclei of this gland become smaller, whereas their volume rises after stimulation of the gland by propylthiouracil (Alfert *et al.*, 1955). The nuclei of rat liver cells grow larger with experimental protein feeding (Mayersbach and Schlager, 1960) or, after partial hepatectomy, even before the onset of the interphase replications (Grundmann and Bach, 1960). In organs with cells of a different function it is possible to test by means of this "nuclear volume reaction" the specific function of each cell type; for instance, in excretory glands (hypophysis, pancreas islets, adrenals, etc., ref. see, e.g., Rather, 1958), or in the retina, where the theory of the two cell types (rods and cones) can be confirmed by determinations of the nuclear volumes (Puff, 1951). In the kidney it is possible experimentally to apply stresses to single portions of the nephron, thus producing characteristic swellings of the nuclei, for instance with trypan blue in the cells of the proximal convoluted tubules where this dye is mainly stored (Bucher and Gailloud, 1958). Also in the proximal convoluted tubule we find, in experimental protein nephrosis, the most extensive storage of protein and, therefore, the most intensive swelling of the nuclei (Polster, 1959); in the nuclei of the collecting ducts swelling can be induced by prolonged stimulation of water reabsorption (Eichner, 1959).

The list of examples could be continued almost indefinitely. They all show a direct correlation between functional strain and nuclear volume, a fact that could also be demonstrated by radioautographic determinations of amino-acid incorporation (Oehlert and Schultze, 1960; Gerbaulet *et al.*, 1961). In the excretory pancreas tissue of white mice for example, protein and RNA syntheses remain parallel to the volume of the nucleus during the entire functional cycle (Stöcker *et al.*, 1961b; Stöcker, 1962a).

It is obvious that increases in nuclear volume can be brought about not only by the stimulations of normal metabolism but also by insults to the cell which provoke an enhanced metabolic compensation. The resulting "pathological nuclear oedema" (Grundmann, 1952) can be found in the liver after exposure to a variety of toxic substances (e.g., Wermel and Ignatjewa, 1933, 1934; Langer, 1942; Grundmann and Sieburg, 1962) but also in endogenous liver diseases (Bock *et al.*, 1953). This pathological oedema of the nucleus has also been demonstrated in the glomerular cover cells in the early stages of extracapillary glomerulonephritis (Noltenius, 1960), in renal epithelium cells eight hours after ischaemia (Cain, 1961) and in ganglion cells after severing the axon (Altschul, 1958; La Velle and La Velle, 1958).

The nuclear swelling in all these cases is due to an increased hydration of the karyoplasm. Stimulation of the nuclear metabolism is usually associated with incorporation of water either diffusely into the nucleus or into specific portions of chromosome. We will come across further evidence of this fact when we discuss the structure of chromosomes.

Water content and dry mass

Determinations of the nuclear dry mass and its correlation to the respective nuclear volume provide an even more exact measurement of this hydration than determinations of nuclear volume alone, especially since it is possible, with the aid of interference microscopy, to measure single nuclei or even single portions of a nucleus (Dyson, 1949; Davies *et al.*, 1957; Schiemer *et al.*, 1957; Hale, 1958; Hofmeier and Grundmann, 1962; and others). Considering the difficulties of this technique and the even more difficult preparation of the cells for such measurements, it is no wonder that the reported data differ widely. Indeed, 50% and more of the dry mass is sometimes lost by preparing cell suspensions or by isolating nuclei in aqueous media such as isotonic tyrode or saline solutions (Hale, 1960; Grundmann, 1963a). We understand now why one group of investigators (Mellors *et al.*, 1954) has reported a dry mass of 16×10^{-12} g for head pieces of mouse spermatozoans and 11.8×10^{-12} g for head pieces of rat spermatozoans, whereas another group (Sandritter *et al.*, 1960a), after suspending the same cells in tyrode solution, found only 8.6 and 6×10^{-12} g respectively. Rat liver cell nuclei that were isolated waterfree contain 66 to 86×10^{-12} g dry mass (Hofmeier and Grundmann, 1962) depending on what specific weight was used, 1.0 (Nurnberger *et al.*, 1952) or 1.3 (Barer and Dick, 1957; Davies *et al.*, 1957). Earlier values of nuclear dry weight that were taken from large pools of nuclei (see Lang and Siebert, 1954) are consistent with these data. Nuclei of tumour cells usually have a greater dry mass (McIndoe and Davidson, 1952; Sandritter and Schiemer, 1958; Broghamer and Christopherson, 1961).

According to these data, the mean water content of nuclei varies between 50% (Mellors, 1953) and 88% (Schiemer, 1961). In the rat liver, for instance, the water content is 80% (Stenram, 1961; Hofmeier and Grundmann, 1962) and it rises following a diet deficient in protein (Stenram, 1961). On the other hand, in certain diseases the nuclear dry mass may increase significantly, as for instance in the squamous cell epithelium of the mucous membranes of the mouth or in the human liver (Schiemer, 1961); this increase in dry mass may well be related to the above-mentioned "pathological nuclear oedema" (see p. 91), for the "functional nuclear oedema", too, is associated with a significant increase in the nuclear dry mass (Grundmann and Hofmeier, 1962). True, all these data that are available at present give only a crude idea of the actual extent of water transfer in the nucleus and the variability of its dry mass; yet they add to our knowledge about functional and pathological increases in nuclear volume.

The growth of nuclei by an increase in the chromosomal substance, the "rhythmic nuclear growth" (Jacob, 1925, 1942) is also reflected by alterations in the dry mass. The diploid thymus lymphocytes, for example, have generally twice the dry weight of the haploid spermatozoans (Sandritter, *et al.*, 1960a); in polyploid tissues the dry mass of the nuclei corresponds to the number of chromosomal sets (e.g., Watkins, 1961).

Proteins

What has been said about the preparation of nuclei for the determination of their dry mass applies also to the preparation for the determination of their chemical composition; absolutely waterfree media are essential, for only by this means can we almost completely prevent a loss of substance. Table I gives some of the more important results of such an assay in a waterfree medium (Allfrey *et al.*, 1955). According to this study, the dry mass of a nucleus consists of 79% protein (mean values), 19% DNA, 2% RNA and other substances. The above-mentioned changes in the nuclear dry mass are, therefore, predominantly changes in the protein content.

TABLE I. DNA-P, RNA-P and protein concentrations in nuclei after isolation in non-aqueous medium.

		DNA-P	RNA-P	Ratio DNA-P RNA-P	DNA	Protein	Ratio Protein DNA
		(%)	(%)		(%)	(%)	
Calf	Thymus	2.56	0.17	15	26.5	71.8	2.7
	Heart	1.90	0.11	17	19.7	79.2	4.0
	Kidney	1.71	0.16	11	17.7	80.7	4.6
	Liver	1.50	0.15	9.7	15.6	82.9	5.3
	Small intestine	1.96	0.14	14	20.3	78.3	3.9
	Bone marrow	2.34	0.09	26	24.3	74.8	3.1
	Pancreas	1.70	0.11	15	17.6	81.3	4.6
Cow	Heart	1.65	0.22	7.6	17.1	80.7	4.8
	Pancreas	1.71	0.13	13	17.7	81.0	4.6
Chick	Kidney	1.45	0.20	7.3	15.0	83.0	5.5

(After Allfrey, V. G., Daly, M. M., and Mirsky, A. E. (1955), *J. gen. Physiol.* **38**, 415)

We can determine cytophotometrically the protein content of a nucleus, by employing the Millon reaction for tyrosine and tryptophane (Pollister and Ris, 1947; Pollister and Ornstein, 1955; Swift and Rasch, 1956; and others); however, the method is only applicable when similar proteins are compared and even then the margin of error is approximately 20% (Rasch and Swift, 1960). However, it has been possible to demonstrate by this method that a stimulated nuclear function is associated with an increase in protein, for instance in the thyroid gland (Alfert *et al.*, 1955) and in the rat adrenals (Sandritter, 1961). The case is analogous in other organs.

The bulk of hitherto available evidence suggests that these variable protein substances of the nucleus are "acid proteins", i.e., proteins which are characterized mainly by the fact that their isoelectric point lies between pH 5.5 and 6.0. They contain, in contrast to the "basic proteins" of the histone type, tryptophane in measurable amounts (Table 2) (Lang and Siebert, 1954). Since they remain in the nucleus after total extraction of the histones (Mirsky and Pollister, 1946), they are generally referred to as "nonhistone proteins" (Felix,

1949; Mirsky and Osawa, 1961). Their amount is difficult to determine, especially as it apparently depends on the functional state of the nucleus (see above). It is assumed that in some nuclei, the "nonhistone protein" accounts for 40 to 50% of the total protein (Siebert, 1958a). Part of it can be washed out by dilute saline solutions. More than 50% of this part are, in the nuclei of thymus cells, composed of ribonucleoproteins of uniform size (Frenster *et al.*, 1960). As we recall, the manufacturing of RNA is one of the characteristic functions of the nucleus (see p. 36).

TABLE 2. Composition of amino-acids from histones and acid protein (values in percentages of amino-acid in the protein)

	Total histones	Acid protein
Alanine	6·1	
Arginine	13·2	7·5
Aspartic acid	4·7	7·6
Cystine		1·8
Glutamic acid	9·0	14·4
Glycine	4·4	
Histidine	2·0	2·8
Isoleucine	4·0	
Leucine	7·8	
Lysine	10·2	7·5
Methionine	0·8	3·3
Phenylalanine	3·1	
Proline	2·9	
Serine	3·4	
Threonine	5·5	
Tryptophane		2·6
Tyrosine	3·5	5·7
Valine	4·7	
Total nitrogen		14·4

(After Siebert, G. (1958), 9. *Colloq. Ges. physiol. Chem.*)

Even after extraction with dilute saline solutions, some "nonhistone protein" still remains in the nucleus. The nucleus maintains its original shape even when the histones and the DNA are removed. This "remainder protein" (Allfrey *et al.*, 1955) accounts for 28·6% of the proteins in isolated liver cell nuclei (Mirsky and Osawa, 1961). It is possibly bound to DNA; at least, it has been shown that without histone proteins, the chromosomes are still capable of maintaining their shape by means of a DNA-remainder protein complex (Mirsky and Ris, 1951). If, however, this remainder protein is removed by digestion with trypsin, the chromosomes lose their shape and the result is an amorphous mass of DNA. The remainder protein, therefore, appears to be a kind of "structure protein" of the chromosomes. It is of interest, in this context, that according to viscosity determinations by Fisher and his associates (1959) two types of protein-DNA bonds must be postulated. Perhaps, DNA is bound to histones and to the remainder protein at the same time (Dounce, 1959).

On the other hand, this remainder protein seems to be closely related to the nuclear function and also to differentiation. For nuclei of cells with a high protein metabolism, such as the epithelium cells of liver and kidney, contain much remainder protein; lymphocytes, by contrast, contain very little. The amount of "active cytoplasm" appears to be correlated to this remainder protein (Mirsky and Osawa, 1961).

We are better informed about the basic proteins of the nucleus, especially the histones. These—and this seems to be one of their characteristics—can be extracted from the nucleus together with DNA as a complex of nucleohistones, in which many small histone molecules are bonded to each of the long DNA molecules, probably by a salt-like bond. At the electron microscope level, this DNA-histone complex appears to consist of long coiled fibrils; these are 30 Å in diameter (Zubay and Doty, 1959) which is quite similar to the diameter of pure DNA (20 Å). Ever since their discovery by Kossel (1884), the histones have been known to be characteristic components of the nucleus and they have been found, since then, in all tissues including bacteria (Palmade *et al.*, 1958). Their amino-acid composition is very different from that of the acid proteins (Table 2). Most striking is their lack of tryptophane. There are two types of histones present in most of the tissues studied so far. One type contains much lysine (approximately 40% of the total nitrogen, arginine roughly 5%), the other type much arginine (approximately 28% of the total nitrogen, lysine roughly 16%) (Davison and Butler, 1954; Daly and Mirsky, 1955). By determining their end groups, it was possible to subdivide these types even further (e.g., Davison, 1957) and it was even postulated that each type of tissue has its own characteristic histone composition (Stedman and Stedman, 1950); i.e., histones were thought to be the chemical equivalent to differentiation. Such differences were found in some tissues (Fischer *et al.*, 1959), in others they were not demonstrable (Davison, 1957; Vendrely *et al.*, 1960). Thus the question of the significance of histones is still unanswered. The existence of so many types is evidence against the simple explanation that the role of the histones is merely to neutralize the negative charges of the DNA.

It is noteworthy that the ratio of the amounts of histones and DNA is always constant in each nucleus; during polyploidization, the histones are reduplicated along with the DNA. This has been demonstrated with the aid of the fast green stain by cytophotometrical determinations in many tissues (Alfert and Geschwind, 1953; Alfert, 1958b; Alfert and Goldstein, 1955; Rasch and Woodard, 1959; and others). In some carcinomas, however, the stainability with the fast green dye is abnormally enhanced (Sandritter and Schiemer, 1958; Grundmann *et al.*, 1961); the same is true of leukaemias (Perugini *et al.*, 1957) and also of other fast proliferating tissues (Perugini *et al.*, 1956). It appears to be less a characteristic of malignant growth than of fast proliferating tissues in general. In fact, it is still not clear whether this staining effect is indeed due to a genuine elevation of histone content. For in this kind of reaction masking and demasking effects are to be expected (Alfert, 1958b). Sakaguchi's (1925) cytochemical reaction (Deitch, 1961), whose intensity is also dependent on the degree of ploidization (McLeish, 1960), gives an equally limited indication of the amount of the arginine-rich histone component. This reaction, however, is abnormally intensified in spermiogenesis (Alfert, 1958b) and in tissue differentiations of plants (McLeish, 1960).

In the case of spermatogenesis this increase in arginine probably indicates a real rearrangement of the proteins. The histones are here replaced by proteins of a much simpler structure,

by protamine or protamine-like proteins. The protamines have a molecular weight of 3000 to 4000, which is many times smaller than that of histones. The protamines which have been discovered in fish spermatozoa by Miescher in 1897 contain only few amino-acids (Felix, 1959), among which arginine prevails (Fig. 44). In histones of erythrocytes for example, the ratio of arginine molecules to lysine molecules is 3:7; in protamine of spermatozoa it is exactly the reverse (Vendrelly *et al.*, 1960). The simple structure of protamine makes it seem probable that these proteins are used mainly if not exclusively to neutralize the acid groups of DNA which, in spermatozoa, have indeed no function (Felix, 1959).

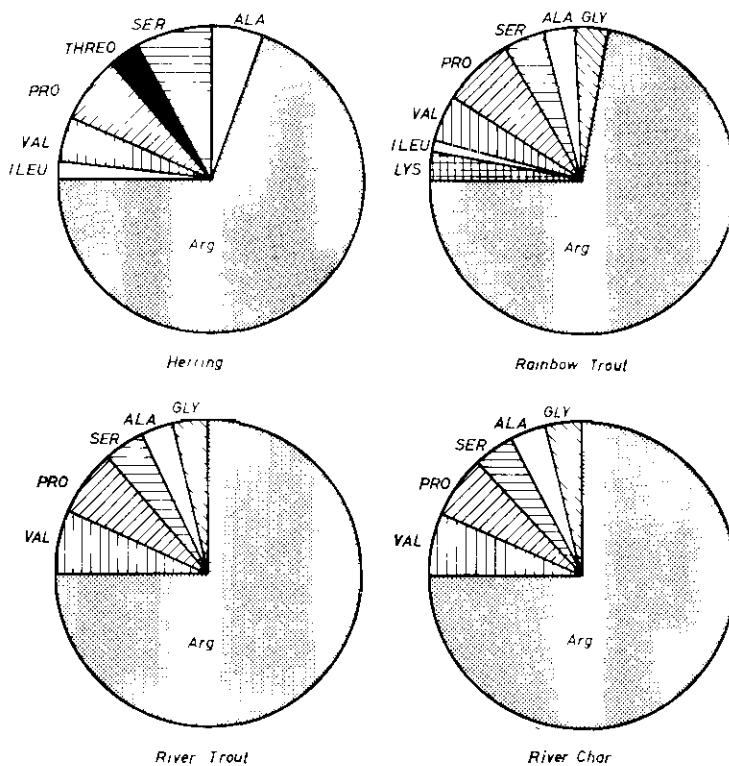


Fig. 44. Amino-acid patterns of several protamines. (From Felix, K. (1959), *Naturwiss.*, **46**, 29-35.)

Desoxyribonucleic acid

The nucleic acids have been given their name because they occur predominantly in the nucleus. They too were discovered by Miescher (ref. 1871). One of these, desoxyribonucleic acid (DNA), is closely related to the genetic substance of all individuals; hence, scientific interest has been focused on it for the past 30 years. The problems involved are inseparable

from the concept of the gene and its function and, therefore, we will devote a special section to this gene-DNA correlation after our discussion of the chromosomal structure (see p. 114). As for now, suffice it to say that DNA is a substance of high-molecular weight in which many nucleosides are bonded to one another by phosphoric acid, thus forming long chains of a molecular weight of 10 millions or more. These chains are a basic structural element of the chromosomes and, therefore, of the entire nucleus. Today we can, without hesitation, make the generalizing statement that there is no nucleus without DNA.

This has been confirmed by all biochemical and cytochemical studies. In the Feulgen dye (Feulgen and Rosenbeck, 1924) we have a highly specific staining method for DNA which is based on an aldehyde reaction (ref. see Milovidov, 1938, 1949; Kasten, 1960). This method can be employed for qualitative as well as quantitative determinations and it allows exact cytophotometric measurings of light absorption, which can be compared; hence we can analyse individually the amount of DNA present in each single nucleus (Pollister and Ris 1947; Pollister and Ornstein, 1955; Swift and Rasch, 1956; Leuchtenberger, 1958; Naora, 1958; McLeish and Sunderland, 1961; and others). Problems caused by a lack of homogeneity (see Iversen, 1960b, 1961) are largely negligible today. The method of direct analysis of the absorption of ultraviolet light (Caspersson, 1941; Sandritter, 1958 and others); makes it necessary to remove the RNA with enzymes. For qualitative morphological determinations it is advantageous to combine the Feulgen stain with a certain fluorescence method (Culling and Vasser, 1961). The specificity of this method is certainly superior to that of the acridine orange-green fluorescence (Schümmelfeder *et al.*, 1958; Bertalanffy, 1961). Other quantitative staining methods as, for example, that with methyl green (Kurnick, 1950; Specht, 1959) have lost popularity despite their clearly demonstrated stoichiometry (Kurnick and Foster, 1950). Two new methods need to be mentioned here; they are still in a developmental stage but may bring forth important contributions. In one of these (Edström and Kawiak, 1961), attempts have been made to measure, microchemically and photometrically, the DNA content of single nuclei after extraction with desoxyribonuclease, an enzyme that specifically dissolves DNA. In the other method (Leduc and Bernhard, 1960, 1961) the action of desoxyribonuclease, pepsin and acid hydrolysis is being followed at the electron microscope level; thus, it is possible to gain further insight into the chemical structure of the nucleus.

DNA is a substance which is characteristic of the nucleus; however, it is present also outside the nucleus. Nuclei of nutritional cells in many types of insect ovaries excrete a substance which contains DNA and which can be demonstrated, often after a long period of time, in the cytoplasm; its presence there is apparently important for the maturation of the egg (e.g., Konopacki, 1936; Fautrez-Firlefyn, 1950; Schrader and Leuchtenberger, 1952; and others). In the tapetum cells of plants a similar transfer of DNA-containing substances from the nucleus into the cytoplasm has been reported (Sparrow and Hammond, 1947; Cooper, 1952); this, however, may be also a mere artifact (Takats, 1959). The so-called ring bodies in the cytoplasm of secondary oögonia of *Dytiscus* (Bauer, 1933) and of certain mosquitoes (Bayreuther, 1952) contain DNA and they seem, by contrast, to be genuine elements. They are spheroid bodies which appear in the prophase of the second mitosis of oögonia and which can be demonstrated later in the egg cell and in one of the nutritional cells. The cytoplasm of frog egg cells contains large amounts of desoxyribonucleosides

(Hoff-Jørgensen and Zeuthen, 1952); possibly, there are large amounts of polymerized DNA stored in the nuclei of certain egg cells (Finamore, 1961). It is a fact, however, that egg cells of mice and locusts have the same DNA content as nuclei of spermatozoa in these species (Alfert, 1950; Swift and Kleinfeld, 1953). More recent observations on a cytoplasmic DNA synthesis in tissue cultures of fibroblasts are still not clarified. These experiments showed that Feulgen positive corpuscles appear in mitochondria following treatment with acid desoxyribonuclease and that these corpuscles are capable of incorporating ^3H -thymidine (Chèvremont *et al.*, 1959, 1960). The same result can be achieved by incubating the cells at 16°C (Chèvremont *et al.*, 1961). If these findings are confirmed we have to revise our theory that DNA synthesis is restricted to the nucleus. It is noteworthy, in this context, that the kinetoplast of trypanosomes, which lies extranuclearly, appears to consist of a mitochondrial and a Feulgen-positive portion (Steinert, 1960). Yet, all these findings do not suffice to justify the assumption that the cytoplasm generally produces DNA (Chayen, 1959) or that mitochondria can facultatively synthesize it. On the contrary, DNA should still be considered to be the characteristic substance of the nucleus, whose DNA content is one of the constant factors in biology. The diploid nuclei of mammals and humans contain uniformly 5 to 7×10^{-12} g DNA (Table 3) (Boivin *et al.*, 1948; Vendrelly and Vendrelly, 1948; see also Lang and Siebert, 1954; Siebert, 1958a; and others). All nuclei of reptiles have a mean DNA content of 5×10^{-12} g (Allfrey *et al.*, 1955) (see Table 3).

TABLE 3. DNA content of mammalian nuclei in 10^{-9} mg

Liver	5.5 – 6.5	Leucocytes	6.7 – 6.9
Kidney	5.2 – 6.5	Prostate	7.7
Pancreas	6.7	Bone marrow	6.2 – 9.7
Thymus	6.3 – 6.9	Spermatozoans	2.8 – 3.4
Spleen	6.5		

(After Siebert, G. (1958), *9. Colloq. Ges. physiol. Chem.*)

TABLE 4. Mean DNA content (in relative values) of nuclei of mice according to cytophotometric DNA determinations

	Nuclear classes	DNA content	Standard deviation
Liver	I	3.34	0.05
	II	6.77	0.07
	III	13.2	0.25
Pancreas	I	3.10	0.06
	II	6.36	0.09
	III	12.4	—
Lymphocytes	I	3.20	0.08
	II	6.00	0.22
Spermatids		1.68	0.02

(After Vendrelly, R. and Vendrelly C. (1956), *Int. Rev. Cytol.* **5**, 171)

This constancy in the DNA values depends, however, on the number of chromosome sets. Haploid heads of spermatozoa contain exactly half the DNA amount of corresponding soma cells (see Table 3) (Boivin *et al.*, 1948; Vendrelly and Vendrelly, 1948); in parenchymal organs of mammals and humans where polyploidy is the rule, there are several classes of DNA content corresponding to the classes of ploidy; the DNA content rises in each class successively, as do the nuclear volumes, by a stepwise doubling of the values found in the preceding class. This has been shown especially by cytophotometrical analyses of DNA by which determinations in single nuclei are possible. For example, liver and pancreas cells have under normal conditions three such classes of nuclei, lymphocytes and Sertoli cells have two and other tissues only one class; but the DNA value of this single class is exactly double that of haploid spermatozoa (Table 4) (compare, e.g., Swift, 1950*a*, 1950*b*, 1953; Leuchtenberger *et al.*, 1952; Vendrelly, 1952; Vendrelly and Vendrelly, 1956; and others). The same constant correlation between DNA content and degree of ploidy occurs also in lower organisms as, for instance, in yeasts (Ogur *et al.*, 1952) or in *Aspergillus comidia* (Heagy and Roper, 1952). Accordingly, in the nuclei of mammals there is a correlation between DNA content and nuclear volume (Fautrez and Laquerrière, 1957; Fautrez, 1960; Grundmann and Bach, 1960; Hobik and Grundmann, 1962). This is another expression of the above-mentioned rhythmic growth of nuclei (Jacobj, 1925, 1942) (see p. 90). Both DNA content and nuclear volume are related to the respective degree of ploidy and we can, to a certain extent, predict the number of chromosome sets from the DNA values.

Accordingly, aneuploidies, i.e., abnormal numbers of chromosomes, which bear no relation to whole chromosome sets, present DNA values that do not correspond to the normal duplication rhythm. This occurs frequently in malignant tumours in humans (Stowell, 1949; Leuchtenberger *et al.*, 1954; Sandritter and Schiemer, 1958; Stich *et al.*, 1960). Abnormal chromosome sets that replicate are termed "stem lines" of that specific tumour (Makino, 1957; Levan and Hsu, 1960; and others). It is of practical importance that in the ejaculate of infertile mammals, the variations of DNA values per spermatozoan cell are much greater than normal (Leuchtenberger *et al.*, 1953; Welch *et al.*, 1961). All these are special cases; however, they only confirm the rule of DNA constancy of each chromosomal set.

If this is the general rule, then the amount of DNA should not be influenced by variations in the cell's metabolism; for, according to present day knowledge, the genetic substance controls the metabolism but does not take part in it. It was possible to demonstrate this metabolic stability of DNA in mice by injecting ^{14}C -adenine into pregnant animals and then measuring the radioactivity in the offsprings. The result was that the DNA of liver and brain in the offsprings still contained after 1 year constant amounts of ^{14}C whereas the RNA which had been labelled at the same time had lost most of its radioactivity (Bennett *et al.*, 1960). Many other and earlier investigations have brought forth the same result. Yet there have been in recent years many reports of variations in the DNA content due to functional changes of the nuclei of parenchyma cells, especially in endocrine organs or tissues exposed to hormones (e.g., Lison and Valery, 1955; Roels, 1956, 1957; Lowe *et al.*, 1959; Kerckhove, 1960; Roels-de-Schrijver, 1961; and many others); these findings seem to contradict the theory of the stability of DNA. Many other authors, however (e.g., Alfert and Bern, 1951; Alfert *et al.*, 1955; Sandritter *et al.*, 1959) could not detect any such variability due to

hormones. Perhaps it is necessary to postulate, apart from the DNA which is demonstrable by the Feulgen stain, the existence of another type of DNA which may be soluble (Taylor and McMaster, 1954; Welsh, 1960); this latter DNA may perhaps participate directly in metabolism (Painter, 1955; and others). According to staining reactions with methyl green, we have to expect inner rearrangements in the nucleoprotein macromolecules (Alfert, 1958a; Barnard and Bell, 1960) and these processes may be responsible for a decrease in the Feulgen intensity in aged individuals (Naora, 1957) and for a depolymerization of DNA in undercooling (La Cour *et al.*, 1956; Evans, 1956). At least, it is too early to draw from these and other observations any conclusions against the law of DNA constancy (Wassilewa-Dranowska, 1961).

Ribonucleic acid and other components

With RNA, the situation is different. We have seen already that this substance is a characteristic component of the nucleolus, i.e., a component of a typical organelle for the cell's metabolism (see p. 48). We have discussed its manifold relations to the nuclear function (see p. 36). The RNA content of nuclei varies therefore a great deal according to functional conditions. The DNA-P:RNA-P ratio varies between 7 and 26 (Table 1); in other words, the entire nucleus, including the nucleolus, contains 15 to 20 times as much DNA as RNA. Furthermore, there are several types of RNA with different activities in the metabolism and a probably different significance for the protein syntheses in nucleus and cytoplasm (see p. 251). The chromosomes themselves apparently contain a structural type of RNA, which is related to the function of the genes (see p. 116). The theory of the different cytological significance of both types of nucleic acids as put forward by Caspersson (1939, 1941, 1950) and Brachet (1941, 1957) can be specified today as follows: DNA is an essential chromosomal substance and as such excluded from the metabolic turnover. RNA is, by contrast, an essential mediator between nucleus and cytoplasm and it is in all likelihood a most important component of the cell's metabolism. RNA is the functional link between DNA and protein synthesis (p. 40).

By means of incineration many other components were found in nuclei; their significance, however, has not yet been clarified. For example, copper and zinc are apparently bound to nucleoproteins (Horning, 1951) and according to isotope studies this probably applies to cobalt and other elements too (Rosenfeld and Tobias, 1951; Poulsen and Bowen, 1952). Bound to nucleoproteins, especially to RNA, are also several lipids (ref. see Denues, 1958); these bonds are sometimes of such a stability that they cannot be broken by the usual lipid extraction media (Chayen *et al.*, 1959).

Review

The nucleus consists, except under pathological conditions, of almost 80% proteins and 15 to 20% DNA. This DNA, which is bound to basic histones and forms the nucleohistone complex, is the most important component of the chromosomes and, therefore, of the entire nucleus. Each set of chromosomes contains a specific amount of DNA, which ranges in all mammals from 2.5 to 3.5×10^{-12} g. Diploid somatic nuclei contain double, tetraploid nuclei four times that amount. A ratio corresponding to the degree of ploidy is also to be

found in determinations of histone content and in measurements of nuclear volume. This ratio is based on the "rhythmical growth of nuclei" and it provides evidence for the constancy of the DNA-histone complex. At present, it is still unclear whether or not metabolic alterations of cellular function have any influence on the DNA content of the nucleus; we do not know either whether we should postulate, apart from the fixed DNA, a second perhaps soluble type of DNA. It has been well demonstrated that DNA is bound, though by an unknown mechanism, to the acid remainder protein which remains alone in the nuclei after extraction of DNA and histones. This remainder protein, together with DNA, forms the actual chromosomal structure. Both acid and basic proteins represent the main portion of nuclear dry mass. The latter, too, is strictly correlated to the degree of ploidy. Both dry mass and nuclear volume are subject to alterations due to the function of the nucleus; however, hydrations of nucleoproteins and histones are by far more variable than alterations of the dry mass. Such differences in hydration are also responsible for the "functional" and the "pathological oedema of the nucleus". The former is the manifestation of an intensified nuclear metabolism, the latter is due to a damage to the karyoplasm.

THE CHROMOSOME

In the previous chapters, we often had to refer to those subunits of the nucleus, which are called chromosomes because of their strong staining capacity. For instance, we had noted that they form the karyoplasm, that the nucleoli develop on the chromosomes and that the chromosomes probably also play a part in the formation of the nuclear wall. Furthermore, in our preliminary exposition of the function of the nucleus, we had mentioned that they are the most important carriers of the nuclear metabolism, since the main factors of heredity, the genes, are located in them or on their surface. The fact that chromosomes are not visible in the active nucleus was found to be due to their high water content, which erases their contours. We were given evidence of the continuity of chromosomal individuality in the nucleus (p. 72) and we can now proceed from the fact that the "resting nucleus", i.e., the non-mitotic nucleus, too, contains chromosomes. However, only during the mitotic division of the nucleus do they appear as well-defined individuals, and then, only at meta- and anaphase, that is, during a fairly short period of time.

What entitles us to give such a great importance to corpuscles which are visible only for so short a time? Would it not be more logical to consider the nucleus as an indivisible whole and the chromosomes as mere temporary structures with no proper individuality?

Number and size of the chromosomes

Their number gives a first idea of their importance. All metaphase plates of one individual contain, apart from a few exceptional cases, always the same number of chromosomes. This is even true of all individuals of one species, and related species usually have similar numbers of chromosomes. A rough examination of shape and size of the chromosomes shows that in tissue cells each chromosome is present in duplicate. Male and female germ cells contain half the number of tissue cell chromosomes and each chromosome is represented only once; thus they each contain only one set of chromosomes. They are called haploid

and the somatic cells, which contain two sets of chromosomes, diploid. In each of these diploid cells, one chromosome set comes from the father and one from the mother. Both sets are identical insofar as those of the parents were identical. Species closely related to one another are liable to have chromosome sets with such small differences that a meiotic pairing of the chromosomes is still possible. This may produce hybrids with a diploid nucleus showing certain differences between the homologous chromosomes, but these differences can disappear in certain cases quite rapidly (Brown, 1954).

In one of the two sexes, generally in the male sex, one chromosome pair is not homologous. These are the sex chromosomes X and Y; the first comes from the mother, the second from the father (XY type). Female individuals thus contain two X chromosomes, which are homologous (XX type). Some species have no Y chromosome. In this case, the diploid number of chromosomes of the one sex is odd, since one of the X chromosomes has no partner (XO type). Sex chromosomes are called heterosomes in opposition to the autosomes which form homologous pairs. If the autosomes are represented three times, the nucleus is triploid, if they are represented four times, it is tetraploid etc. In general, nuclei which have more than two autosomes of a kind are called polyploid.

The number of chromosomes in the haploid set of most plants and animals lies between 6 and 25. Man has 22 autosomes and one heterosome in a haploid set, that is, in a diploid set, 46 chromosomes (Fig. 45). Haploid chromosome sets with only one chromosome can be found in *Ascaris megalocephala* of the horse. However, this is a collective chromosome which divides itself, in somatic cells, into several chromosomes (up to 190); polyploid nuclei can contain several thousands of chromosomes.

The chromosomes are ordered according to their size. Thus it is possible to serialize them and give them numbers (Fig. 45, bottom). Two chromosomes of a diploid set always have exactly the same size. Only the sex chromosomes are of different sizes; in man, the X chromosome is fairly large and the Y chromosome small. The large chromosomes in man have, at metaphase, a length of 5 μ and more. However, much depends on the preparation, so that as a rule, only relative measurements are given, generally applied to the total length of all the chromosomes. Table 5 shows that in man, the chromosomes 8 and 9 (according to the nomenclature proposed at the Denver meeting) have approximately the same size (Chu, 1960). All the others have different sizes and the size of the chromosomes is the first proof of their individuality.

Fairly large chromosomes are to be found in seed plants; this explains why our basic knowledge of the structure of the chromosomes has been gained through investigations on botanic objects (for a summary see, e.g., Geitler, 1938d; Tischler, 1934-1951; Straub, 1943). Here we meet chromosomes up to 30 μ in length. The chromosomes of the salivary glands in *Diptera* are even longer. Their total length, in favourable preparations, was found to be 2 mm (Bridges, 1942); yet they should not be compared to other chromosomes, since they are not in a state of metaphase contraction and represent, also in other respects, a special case (p. 118). The smallest chromosomes of all are probably contained in fungi and certain birds; most likely there are even chromosomes which are so small that they lie below the resolving power of the light microscope. At least, it was possible to generate such an "invisible chromosome" in *Drosophila* by Roentgen irradiation (Kodani and Stern, 1946); this chromosome can be demonstrated in the salivary gland only under its macroform.

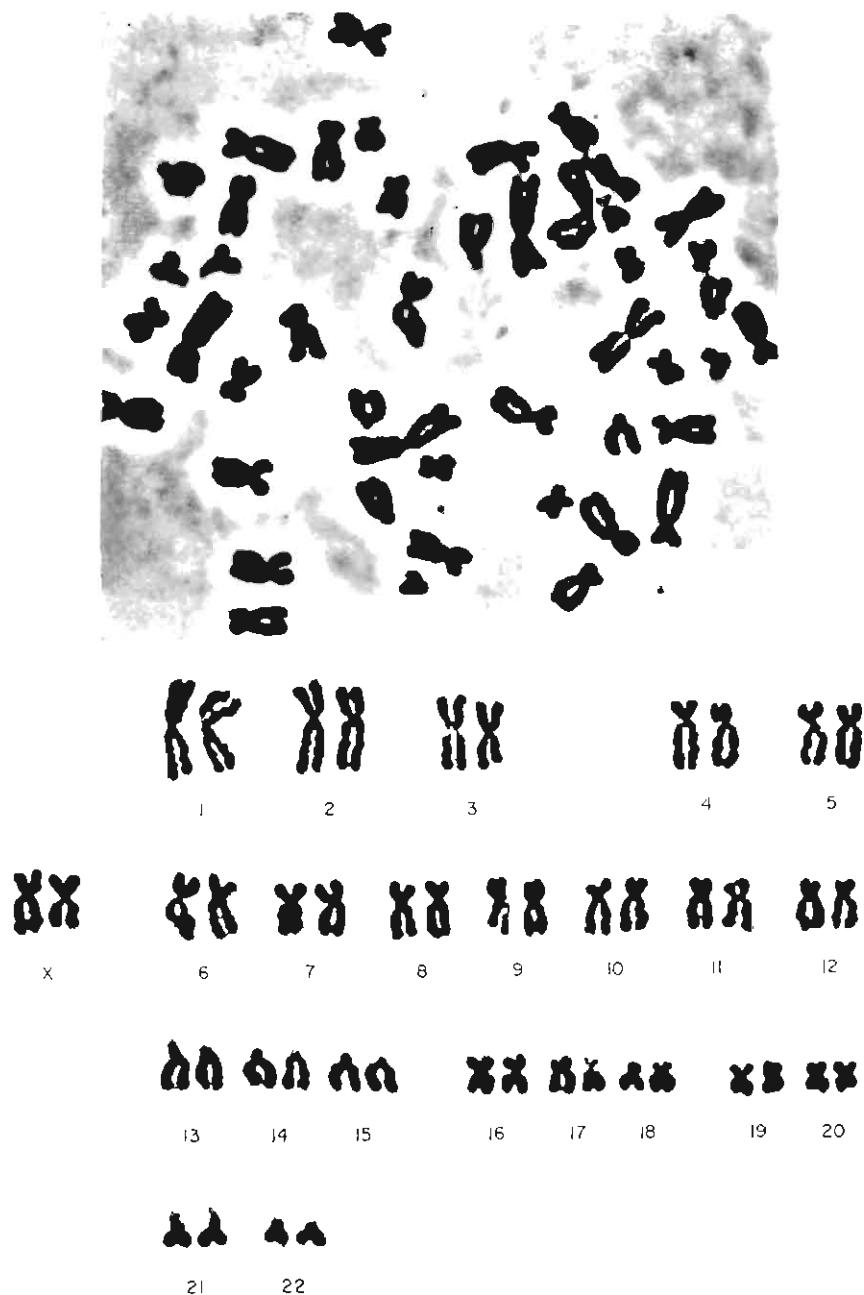


Fig. 45. Human chromosomes. *Top*: Squash preparation of a metaphase plate. *Bottom*: The same chromosomes arranged and numbered according to their size. (From Hamerton, J. L. (1961), *Int. Rev. Cytol.*, 12, 1-68.)

Constrictions

Most chromosomes have the shape of a small rod. This rod varies in length, but its diameter within one set of chromosomes remains generally constant (Fig. 45). Extremely short chromosomes appear as small pellets having the same diameter as the other chromosomes. Here and there, there are also ring chromosomes to be found (McClintock, 1942; Morgan, 1933; Schwartz, 1953; and others).

At the point where the spindle fibres are "inserted" at metaphase (p. 141), chromosomes show a "primary" constriction. This is where the centromere or kinetochore lies. During anaphase migration the bend in the chromosomes, which existed already at metaphase, is

TABLE 5. Description of human chromosomes according to length and arm ratio (ratio of the long to the short arm). The length is given in percentages of the total length of the haploid set of autosomes (after Chu and Giles, modified according to the Denver convention)

Classification of chromosomes	Length in percentages of total length of the haploid set	Arm ratio	Position of kinetochore
1	9.53 ± 0.02	1.07 ± 0.00	M
2	9.15–0.05	1.48 0.01	M
3	7.60 0.14	1.16 0.01	M
4	6.57 0.30	2.89 0.03	S
5	6.10 0.05	3.17 0.22	S
6	5.88 0.10	1.77 0.07	M
7	5.45 0.03	1.89 0.10	M
8	4.90 0.00	1.65 0.07	M
9	4.90 0.00	2.40 0.23	S
10	4.72 0.00	2.31 0.12	S
11	4.55 0.03	2.12 0.10	S
12	4.46 0.05	3.13 0.31	S
13	3.43 0.04	9.67 0.27	A
14	3.34 0.06	11.94 1.80	A
15	3.60 0.03	9.53 0.57	A
16	2.79 0.06	1.60 0.06	M
17	3.17 0.04	2.07 0.04	S
18	2.58 0.04	3.75 0.43	S
19	2.32 0.09	1.95 0.18	M (S)
20	2.02 0.06	1.28 0.03	M
21	1.59 0.08	6.83 0.17	A
22	1.25 0.06	6.00 0.00	A
X		2.05 0.14	S
Y		5.00 0.00	A (M)

i.e.: M = kinetochore located in or near centre

S = kinetochore located subterminally

A = kinetochore located almost terminally (acrocentric chromosomes)

(After Vogel: *Humangenetik*, Springer, Berlin, 1961)

accentuated at that point and each chromosome finally consists of two ends or arms. When the primary constriction is median, both arms have the same length and the chromosome is V-shaped. If the constriction is near one of the tips of the chromosome ("subterminal"), both ends are of a quite different length. The smallest end can be so small, that it remains invisible in the usual preparation and the spindle insertion appears "terminal". This was the case with the chromosome 4 in *Drosophila*, which for a long time was thought to be "telocentric", that is, it was assumed that the centromere lies at one chromosome tip. However, detailed investigations brought evidence of a second, though very small arm. Real terminal insertions are extraordinarily rare (Marks, 1957a) and are often fatal when due to the action of mutagenic agents (Marks, 1957b). At least, such chromosomes are generally eliminated sooner or later (Rhoades, 1936). They can derive, for example, from a transverse division of the centromere and form two "isochromosomes" (Darlington, 1940), the arms of which are not only of the same length but also genetically homologous. A terminal spindle insertion is the rule in certain protozoans (Cleveland, 1949); however, this case is exceptional in other respects too (p. 141).

The relation of the long arm to the short one is given as the "arm ratio" and characterizes, beside its total length, each single chromosome. Five autosomes and the Y heterosome in man have such a high arm ratio that the centromere is nearly terminal (Fig. 45). We speak of "acrocentric chromosomes". Table 5 establishes the arm ratios of the human chromosomes (Chu, 1960; modification after Vogel, 1961). They are the second characteristic of each chromosome.

The "secondary constrictions" are the third characteristic of chromosomal individuality and appear with almost the same constancy. They include in principle all constrictions without a centromere. Heitz (1931a) called the chromosomes which displayed such secondary constrictions "SAT chromosomes" (sine acido thymonucleinico), since no DNA (thymonucleic acid) could be demonstrated at this point by staining methods (Fig. 15). We have shown that it is, as a rule, on this SAT segment that the nucleolus is formed during the postmitotic reconstruction phase (p. 44). Its relative DNA deficiency is possibly only the sign of a special "stretching", that is, of a particularly loose coiling of the chromosome. This fact is confirmed by the observation that the SAT segment is often no real constriction at all, but merely a less compact section, having the same diameter as the rest of the chromosome (Stewart and Bamford, 1942; Therman-Suomalainen, 1949). These "regions of loose coiling" in the chromosomes were often found independently from the formation of nucleoli (Kaufmann, 1934, 1948; Kaufmann *et al.*, 1960; Hinton, 1942). They are in many cases quite stretchable and can extend at prophase through the whole nucleus; however, they always constrict again at metaphase and are, therefore, called "elastic" constrictions.

Euchromatin and heterochromatin

Of a different nature but often very much like the secondary constrictions in their exterior aspect are those weakly staining gaps which can be produced on certain chromosomes by exposure to cold (Darlington and La Cour, 1938, 1940). These reactions to cold have been called "negative heteropycnotosis", for it was assumed that they were due to a special kind of heterochromatin, which gives under exposure to cold the effect opposite to that of normal

heterochromatin. This assumption was based on the theory of the "nucleic acid load" of the chromosomes (Darlington and La Cour, 1940) and the investigators saw in this "negative heteropycnosis" the sign of a "deficient DNA load" of the chromosome. Normal heterochromatin was considered, accordingly, as a region of the chromosomes "overloaded" with DNA.

However, the main difference between euchromatin and heterochromatin is morphological. The terms themselves came from morphological studies (Heitz, 1928, 1929). It was noticed that certain segments in prophase chromosomes regularly stained more strongly than the others (Fig. 46). Since it was assumed that the strongly staining portions were the site of a premature mitotic condensation and spiralization, they were given first the name prochromosomes and were later called heterochromatin in contrast to the more weakly staining chromosome segments of euchromatin. The heterochromatic segments lie frequently in the

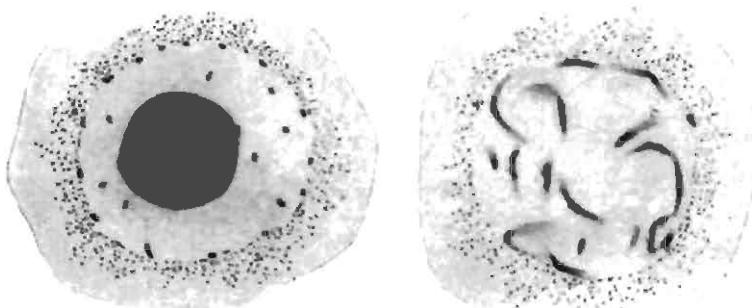


Fig. 46. Heterochromatin in seed plant *Citrullus vulgaris*. Left: Interphase nucleus with large nucleolus and many small chromocentres. Right: early prophase, in which the chromocentres reveal themselves as the heterochromatic elements of the chromosomes. (From Doutreligne, J. (1933), *Cellule*, 42, 29–100.)

vicinity of the constrictions, next to the centromere as well as next to the SAT segment; in this last case they play a special role (p. 44) as nucleolar organizers (McClintock, 1934). Sometimes the chromosome tips or telomeres are heterochromatic, sometimes it is a whole chromosome, as for instance in certain sex chromosomes of insects.

Yet, heterochromatin is no constant structure. Between both extremes, euchromatin and heterochromatin, there are many intermediary stages; moreover, it happens frequently that a chromosome segment is euchromatic under certain conditions, but heterochromatic under others (Schultz, 1941; Dobzhansky, 1944). In certain plants, the phylogenetically superior families and, among these, the phylogenetically superior species have more heterochromatic segments than the primitive ones (Tatuno, 1960a, b and c). Environmental conditions, such as temperature, can also vary the formation of heterochromatin (Rutishauser, 1956). Typical of heterochromatin is its tendency to frequent breaks and a marked "stickiness". These characteristics become noticeable when the chemical environment changes (McLeish, 1953; Gläss, 1956; and others) or under treatment with ionizing rays (e.g., Keyl, 1958).

There are many different genetic definitions of heterochromatin. Some investigators believe that heterochromatin is relatively poor in genes and, consequently, functionless (for ref. see Swanson, 1960). Others think that it contains, on the contrary, a series of complementary genes, the polygenes (Mather, 1944) or that it plays a certain metabolic role via the so-called "position effect" (for ref. see Hannah, 1951; Schultz, 1956). However, if we consider that neither gene deficiency nor position effect are constant or exclusive characteristics of heterochromatin (Cooper, 1959; Swanson, 1960), we must admit that the concept of heterochromatin in cytogenetics has become most vague, so that it is doubtful whether we should use it here (Östergren, 1950a; Baker and Callan, 1950; Kaufmann *et al.*, 1960; and others).

Finally, the difference between euchromatin and the heterochromatin is most likely not a qualitative but merely a quantitative one (p. 74). Heterochromatin is a segment of the chromosome in which the condensation is particularly strong and the coiling tightened (Ris, 1957) and which contains perhaps a particularly large amount of RNA (Kaufmann *et al.*, 1960) or phospholipids (La Cour and Chayen; 1958; La Cour *et al.*, 1958). Thus we do not have two sorts of chromatin, but merely two, or several (Heitz, 1933a, 1934; Geitler, 1938d; Hannah, 1951; Tschermak-Woess and Hasitschka, 1953) different conditions of the chromosomal substance, and the aspect under which we know it at present is perhaps only a preparation artifact (Grundmann and Stein, 1961a).

And yet, the concepts euchromatic and heterochromatic have become indispensable. The best definition of heterochromatin is based on morphology. The expression heterochromatic chromosome segments should be used only when they are allocyclic, i.e., when condensation and coiling of heterochromatin occurs in the mitotic cycle at different times than that of euchromatin. At interphase, only the heterochromatic chromosome segments are condensed, whereas at meta- and anaphase it is the whole chromosome, i.e., euchromatin is then temporarily heterochromatized (Kaufmann *et al.*, 1960).

The subdivision of each chromosome into euchromatic and heterochromatic segments is the fourth and last possible way of characterizing a chromosome morphologically. We mentioned already, as other possible characteristics, the total length of the chromosome, the comparative length of both arms, determined by the primary constriction, and the position of the secondary

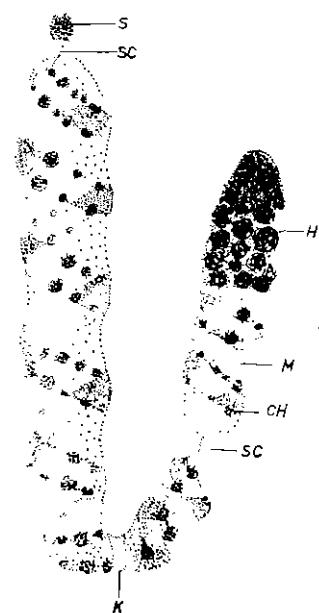


Fig. 47. Schema of a metaphase chromosome with heterochromatin (H), matrix substance (M), chromomeres (CH), two secondary constrictions (SC), the kinetochore (K) and a satellite (S). (From Heitz, E. (1935), Z. indukt. Abstamm.- u. Vererb.lehre, 70, 402-447.)

constrictions. These four characteristics are represented in Fig. 47. They make it possible to attribute to each and every chromosome an individuality of its own, since each chromosome always appears under the same aspect. All the chromosomes of one set form the karyotype specific for each species and with such a chromosomal idiogram it is possible in certain cases to demonstrate relationships between the species. Abnormal idiograms in man play a great part in medicine today (p. 211) and are the only means of solving many a problem of the evolution of organisms.

Fine structure

A chromosome is no indivisible unit. The metaphase chromosome, which we considered until now as a prototype, is always split longitudinally (Fig. 45). It is composed of two chromatids, which are joined during metaphase at the centromere and move separately towards the spindle poles at anaphase. However, in certain cases, another longitudinal split appears at anaphase, and in favourable specimens, even a fourfold split occurs (Nebel, 1939; Marquardt, 1941).

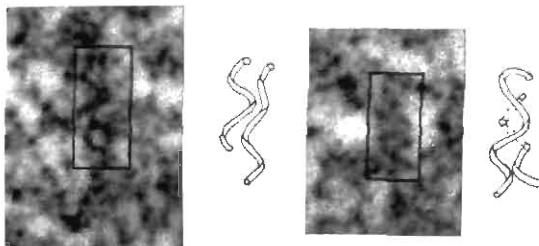
In principle, every linear subunit of the chromosome may be called chromonema. Radiation damage brought evidence of the fact that each chromosome contains at least four sub-units, i.e., two chromatids, with each two chromatid halves (Nebel, 1939; Swanson, 1947). The mosquito prophase chromosomes seem to contain at least 16 if not 32 chromonemata (Berger, 1938; Grell, 1946).

Electron microscopy, with its far greater resolving power, made it possible to measure the single fibrils. In the leptotene chromosomes of *Tradescantia*, each chromatid was found to consist of eight fibrils (16 in the total chromosome), each having a diameter of approximately 200 Å. These fibrils appeared twisted around each other two by two and the whole chromosome after DNA synthesis had a diameter of 0.39 μ. Each chromatid would be, accordingly, 0.2 μ thick; this value is evidently a constant for many objects (Steffensen, 1961).

If one admits that the single chromatids are subdivided, a fact which has been already demonstrated in the light microscope (see above), each chromatid half—provided there is a division into exactly two halves—would be some 1000 Å thick and could be separated into two chromatid quarters, each averaging 500 Å in diameter. Fibres of this dimension were found in several objects, for example in animal and plant pachytene chromosomes (Ris, 1954) or in salivary gland chromosomes in *Diptera* (Ris, 1956). However, these chromonemata, too, consisted of two fibrils coiled around each other, each being 200 to 250 Å thick. Fibrils of this diameter had been demonstrated already earlier after supersonic treatment (Yasuzumi and Kondo, 1951). They were also found forming the middle fibre in the lampbrush chromosomes (p. 119) (Tomlin and Callan, 1951), and were demonstrated too in sea urchin spermatozoa (Bernstein and Mazia, 1953a and b) and in the carp erythrocytes (Yasuzumi and Higashizawa, 1955). Fibres of this diameter can probably be considered as a structural unit common to all chromosomes (Ris, 1957). However, they are not the finest units. The meiotic prophase chromosomes in many plants contain microfibrils ("elementary fibrils") which have a diameter of 100 to 120 Å (Fig. 48) (Marquardt *et al.*, 1956; Bopp-Hassenkamp, 1959b); such is also the case with the chromosomes of the pollen tube of *Tradescantia*.

(Kaufmann and De, 1956) and many spermatid nuclei (see Ris, 1963). These too can in certain cases each be subdivided once more into two fibrils, averaging 20–40 Å in diameter. This is the lowest limit reached up to now and is probably the absolute lowest limit possible. For these 20–40 Å "subfibrils" which have been seen many times in the interphase nucleus (Amano *et al.*, 1956; Ris, 1958, 1963; Ued, 1930; Kurosumi, 1961) or in mitotic chromosomes (e.g., Kaufmann and De, 1956; Nebel, 1957; Bopp-Hassenkamp, 1959b; Dales, 1960;

Fig. 48. Electron micrograph of *Lilium candidum* at pachytene, showing chromosomal microfibrils of 100–120 Å in diameter. OsO₄ fixation and staining with potassium dichromate. $\times 47,520$. (From Bopp-Hassenkamp, G. (1959), *Protoplasma*, **50**, 243–268.)



Kaufmann *et al.*, 1960; and others), belong already to the order of magnitude of the single nucleoprotein molecules, and each of them is probably one double helix DNA molecule (Bopp-Hassenkamp, 1959b). According to the model of Watson and Crick (1953) the helix has a diameter of approximately 20 Å. If we add to it the protein component, left aside in the model, then we must definitely consider even somewhat thicker "subfibrils" (e.g., Ris, 1956; De Robertis, 1956) as having molecular dimensions.

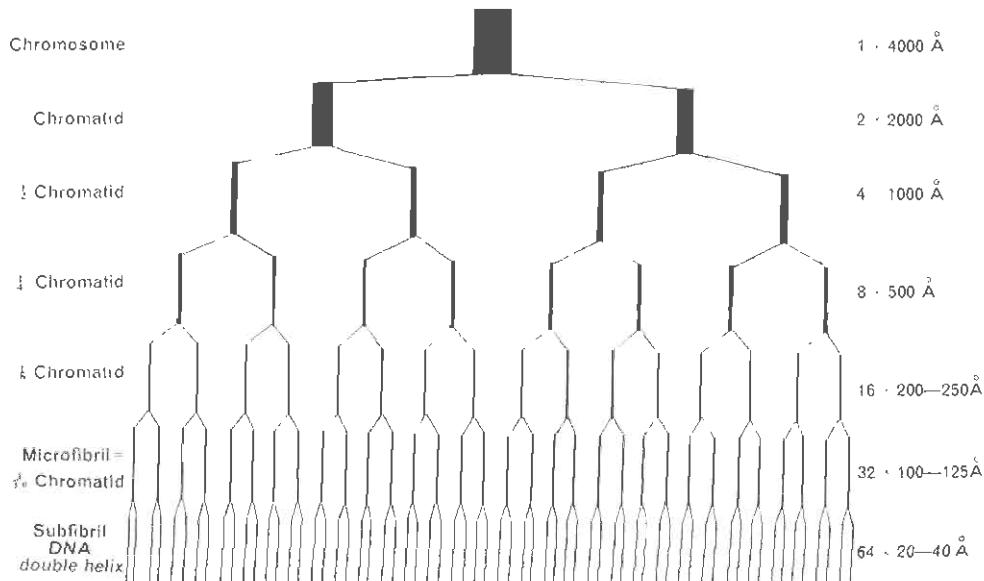


Fig. 49. Schema of a chromosome according to the multiple strand hypothesis.

The above-mentioned findings bring further support to the old theory, that the chromosome is composed of many fibrillar subunits. This conception can be represented schematically (Fig. 49) with very precise diametrical measurements (for ref. see Ris, 1957; Kaufmann, 1960; Kaufmann *et al.*, 1960; Wischnitzer, 1960; Steffensen, 1961).

And yet, it is merely a hypothesis. The reason for this is not so much the relative uncertainty and variability in the single measurements, depending on the object and the methods of investigation, for the principle remains correct. It is really that the "multiple strand" hypothesis is not consistent with all the findings and that other hypotheses also have evidence to support them.



In fact, it is quite possible that all these fibrils of various thickness are mere optical illusions and that there is in reality only one single fine fibril coiled and folded over on itself many times, as represented in the model of Fig. 50. Most of the electron micrographs (see Fig. 48) can perfectly be interpreted in this manner and many recent findings concerning DNA synthesis *in vitro* (Kornberg, 1960) or in bacteriophages (Hall and Spiegelmann, 1961; and others) support this "single strand hypothesis" (p. 116).

Fig. 50. Chromosome model according to the single strand hypothesis after Nebel. (From Klingmüller, W. (1962), *Naturw. Rdsch.*, 15, 363-372.)

For all further discussions of this matter, the following observation is of a great importance (Taylor *et al.*, 1957): in *Vicia* mitotic chromosomes treated with colchicine and injected with ^3H -thymidine, only one chromatid is labelled in the second division, the other is not (Fig. 51). If, according to the old multiple strand hypothesis, DNA synthesis occurs in the same way in all chromosomal subunits, each chromatid half should contain the same number of newly-formed and, hence, isotope-labelled DNA molecules; according to the schema of the multiple strand hypothesis in Fig. 49, one would expect non-labelled chromatids only in the fifth generation, and non-labelled chromosomes only in the sixth generation. Yet, already in the second division following incorporation of the tritium-labelled thymidine, we find a clear-cut separation into labelled and non-labelled chromosome arms—if we disregard the possibility of a multiple chromatid exchange (Fig. 51). From the sum of these findings (Taylor *et al.*, 1957; Taylor, 1958a and b, 1960a, 1962), it must be assumed that every chromosome behaves during DNA synthesis as if it were composed not of 64 strands but of two. The chromatid would be accordingly a kind of replication unit of the chromosome.

The findings shown in Fig. 51 have been interpreted as the result of an incomplete colchicine effect (Neary *et al.*, 1959) or of the technical limitations of radioautography (Steffensen, 1961). However, since other findings, mostly in genetics, are not always consistent with the multiple strand hypothesis, since, on the other hand, the single strand hypothesis is not completely satisfying either, scores of more complicated chromosome models have been designed in an attempt to cover all findings. From the observations we have just mentioned, such a model should be able to explain for example how a chromatid can appear morphologically as a double or multiple fibril when it is seen only as one unit during reproduction. This model should also take into account the separation of the DNA chains after reproduction. Further, it should be able to explain the chromatid exchange (Fig. 51), yet without being in contradiction with the observation, discussed elsewhere, that changes in one single base of the DNA helix can already bring about a mutation (p. 117).

Fig. 52 shows one of these tentative models (Taylor, 1962).

It is a "ladder model", in which each subunit of the chromatids, mainly the chromatid halves, consists of transverse DNA double helices, the ends of which are joined by two different kinds of linkers. The first type of linkers (R) is the most stable, the second (H) breaks more easily. When, for example, the H linkers break, there results a long double spiral in which the DNA segments are arranged

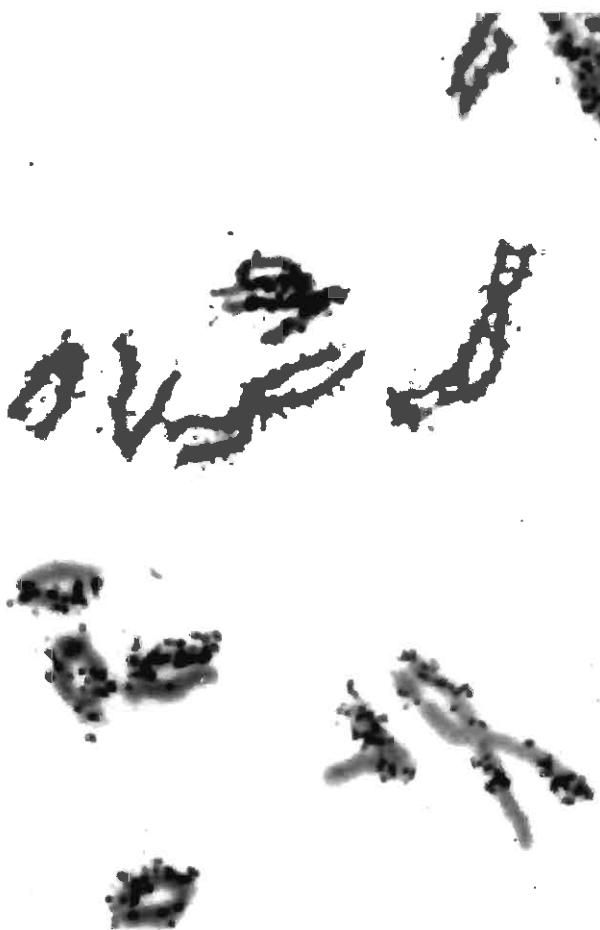


Fig. 51. Mitotic chromosomes from root tip of a bean following incorporation of ^3H -thymidine. *Top*: First division after incorporation of thymidine. All chromatids are labelled. *Bottom*: Second division after incorporation of thymidine. In each chromosome only one chromatid is labelled. Note the chromatid exchange at several sites. (From Taylor, J. H. (1962), *Int. Rev. Cytol.*, 13, 39-73.)

one behind the other. In the case of chromatid halves, there is, according to this model, on one side a chain of R and H linkers, to which the ends of the DNA spirals are attached. Thus the chromosome forms a band which can twist itself like a helix in various ways, a shape confirmed by light microscopical observations. By contrast, in the condensation stage of cell division or during DNA replication, the ladder model emerges from alternating R and H linkers on both sides of the DNA chain and explains at the same time the division into two chromatid halves. Accordingly, the fine structure of the chromosome would be changing constantly, depending on whether the chromosome is in a condensation stage or in the loosened function stage. It is a fact that this model can account for most of the findings (Taylor, 1962). Even the genetic arguments for a single strand hypothesis are included in this changeable chromosome model. However, the systematic coupling of the subunits at the various levels, which we deduced from the bulk of the various findings, is neglected in this model.

A general structural principle is that of the helical coiling of chromosomal subunits around one another. It can be observed in the chromatids mainly during prophase, especially during the meiotic prophase, but also during metaphase. This general helix shape often gives the chromosomes in cross-sections the shape of a ring, a feature which may occur in the finer chromosomes too, maybe even with the microfibrils and the subfibrils, according to the multiple strand hypothesis in Fig. 49. From the Watson-Crick model, this structural principle extends down to the molecular level (Fig. 53) and the helical coiling of both DNA strands in the double molecule is probably responsible for the bigger spirals which may possibly result from an inner tension in the chromosome (for ref. see Swanson, 1960).

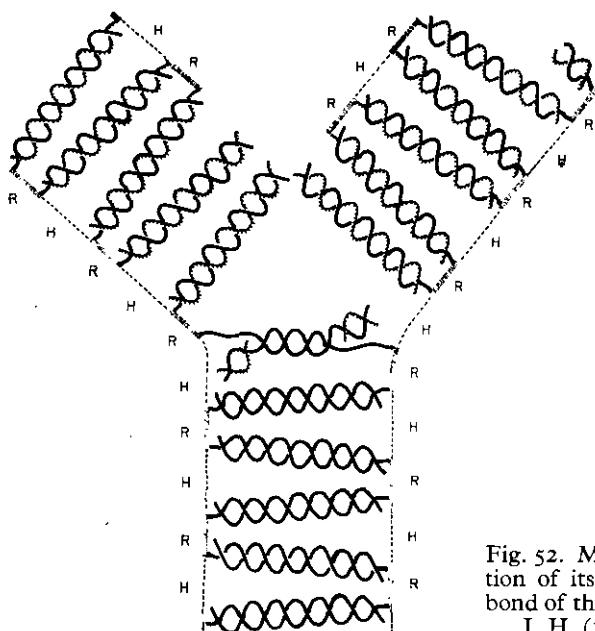


Fig. 52. Model of a chromosome and the replication of its subunits. R = tight bond, H = looser bond of the DNA double helices. (From Taylor, J. H. (1962), *Int. Rev. Cytol.*, 13, 39-73.)

In the DNA model of Fig. 53, both nucleoside chains are coiled around one another into an "orthospiral". In this "plectonematic" type of coiling, the two chains cannot separate without the spirals being uncoiled, at least for a short time. However, since both chains must separate from one another after each duplication, several possibilities have been taken into consideration, one of which has already been represented in Fig. 52. The same problem is raised by the coiling of the half chromatids, which clearly consists, from many observations, in orthospirals (Sparrow, 1942; Swanson, 1943; Mickey, 1946; and others). However, it is also clear that the chromatids separate at prophase with no difficulty, and that even the ring chromosomes in *Drosophila* disjoin themselves at anaphase without getting entangled. This can be explained only by a change in the orientation of the coiling, evidently occurring at interphase at the level of the half

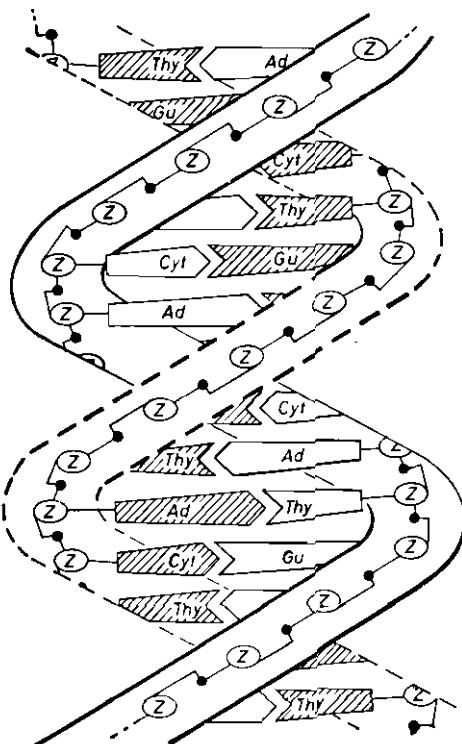


Fig. 53. Model of the structure of DNA according to Watson and Crick (1953). Double helix of two chains composed of sugar and phosphoric acid, held together via the bases cytosine, adenine, thymine and guanine. Hydrogen bonds connect the bases, which lie opposite one another and form pairs (thymine always paired with adenine and cytosine with guanine). (From Karlson, P. (1963), *Kurzes Lehrbuch der Biochemie*, 4. Aufl., Thieme, Stuttgart.)

chromatids or of the chromatids. Since such an uncoiling has indeed been observed (Huskin and Wilson, 1938; Cleveland, 1949), an orthospiral can, by uncoiling, turn into an anorthospiral with a "paranematic" arrangement in which both strands run parallel to each other so that they can separate without getting entangled. However, the details of such rearrangements are still unknown. The coiling phenomenon was frequently thought to be due to exterior traction forces, mainly to the so-called matrix, with which we will deal in the following chapter (p. 130).

Let us mention one last phenomenon, viz., that of those small pellets which, on our chromosome schema of Fig. 47, are lined up on the chromonemata like beads on a thread. They are the chromomeres. They may be seen particularly clearly at meiotic prophase and also in the giant chromosomes of *Diptera*. We will mention them again when we examine

these giant chromosomes (p. 118). They were previously thought to be the morphological substrate of the genes because of their linear arrangement on the chromosomes (Belling, 1928). Today they are interpreted rather as more tightly coiled regions of the chromonemata (Ris, 1954, 1957). The last chromomeres at both tips of a chromosome are called telomeres (Müller, 1938). Their behaviour in meiosis is sometimes different from that of the other chromomeres (Ribbands, 1941) and they have perhaps a special relation to the nuclear wall. In the "bouquet" stage in meiosis (Hughes-Schrader, 1943) as well as in the giant chromosomes of chironomids (Kimoto, 1958a), the telomeres are attached to the nuclear wall, and in the spermatids of other insects the chromosomes radiate similarly from the wall to the inside of the nucleus (Sotelo and Trujillo-Cenoz, 1960). Whether this is the sign of a basic arrangement of the chromosomal structure in interphase nuclei (see Fig. 29), is still open to question (p. 72). Finally, a special structure can be found in the centromere of the spindle insertion; its function can be explained only from the mechanism of mitosis and will be dealt with, together with its fine structure, in the chapter on mitosis (p. 141).

DNA and gene

In principle the chemical composition of the chromosomes is identical with that of the karyoplasm which is formed by them (p. 93).

The interest of cytologists, biochemists and geneticists has been concentrated on the DNA. As we already emphasized in our discussion of the chromosomal fine structure (p. 109), DNA seems to be the typical structural element of the chromosomes, which, by virtue of its double helical arrangement, determines also the double helix of chromonemata, chromatids and chromosomes.

DNA has not only a structural but also a functional significance. There are several lines of evidence implicating that DNA is closely correlated in its substance with the gene, and that it even represents the genetic material itself, together with proteins (Ycas, 1962).

The first evidence is the regular occurrence of DNA in every nucleus, which allows us to define the nucleus by its DNA content (p. 28). In the nucleus, the DNA is located exclusively in the chromosomes. Wherever DNA occurs in the nucleolus or in the inner membrane of the nuclear wall, it is of chromosomal nature (see pp. 45 and 57). DNA can be found also in every nucleus of bacteria. Cytoplasmic DNA is only an exception (p. 97).

The second evidence is the constancy of the amount of DNA in the nucleus. Or rather, every chromosome set has a specific DNA content. Accordingly, the haploid germ cells contain half as much DNA as the diploid tissue cells and these, in turn, contain half as much DNA as the tetraploid cells, which contain half as much as the octoploid cells etc. (p. 99).

A third evidence is the remarkable metabolic constancy of DNA (p. 99). Except for a few rare and still controversial cases, the functional cell activity entails a change in the DNA content only when it provokes an endomitotic polyploidization or nuclear fusion (p. 226). Moreover, an incorporation of ^3H -thymidine, for example, can be obtained autoradiographically only when it coincides with a mitotic or endomitotic period of growth (see Hughes, 1959); only then is there a synthesis of DNA.

Mutation studies have given the fourth evidence. Ultraviolet rays have their strongest mutagenic effect at 2600 Å, the point of maximal light absorption of the nucleic acids. As a

general rule, many mutagens act primarily on DNA. Thus, Roentgen rays can impair DNA synthesis in meristematic tissues already at fairly low doses (Grundmann, 1953).

The fifth evidence comes from virus investigations. Bacteriophages T2 consist of a head and a tail. Both structures are enclosed in a protein envelope, but DNA occurs only in the head structure. When a bacterium cell gets infected, the virus places itself against the cell membrane and injects only the DNA into the bacterium (Hershey, 1955); the protein envelope remains outside and breaks down (Hershey and Chase, 1952; Hershey and Burgi, 1956). Of importance here is that the reproductive capacity of the bacteriophages, which is responsible for their genetic continuity, depends solely on DNA.

The most powerful direct evidence is the sixth. A genetic character may be transferred via DNA from a donor to a recipient cell. This "transformation" was obtained for the first time on non-capsulated R pneumococci, which were transformed into capsulated S pneumococci via the DNA of the capsulated S pneumococci (Avery *et al.*, 1944). Since then, the experiment has been repeated successfully on many other bacteria (see ref. in Wacker, 1958).

These six points of evidence suffice to support the present theory of the importance of DNA for the inheritance of cellular characters. No other substance meets as well as DNA the criteria which are required for such a genetic material. The fact that many viruses do not contain DNA but RNA, is not inconsistent with this theory, since viruses can only multiply within a living cell, i.e., with the help of cellular DNA; they are not able to live on their own.

Mechanism of the gene activity

As compared with the manifold possible arrangements of polypeptides, the molecular structure of DNA appears simple and monotonous. The sugar-phosphoric acid chain does not vary; hence, the genetic specificity must depend solely on the sequential arrangement of the paired bases. How can this fact be explained?

The specificity of the genes is reflected ultimately by the specificity of the enzyme proteins produced under the control of the genes, or more precisely, by the sequence of amino-acids in these proteins. There are approximately 20 naturally occurring amino-acids and four nucleotide bases, and the question arises as to how a sequence of four such bases in the DNA molecule can determine the much more complicated sequence of the 20 amino-acids in the protein molecule. This is the problem of the gene function at molecular level (see review in e.g., Gierer, 1961; Friedrich-Freksa, 1961; Eglhaaf, 1961; Klingmüller, 1962; and others.)

As we mentioned already, it is first of all a purely mathematical problem. The question is how to establish a codified "alphabetic text" representing in this case the various amino-acids, by grouping together a few symbols as in the Morse telegraph code. If we replace the four bases of the nucleic acids by the letters, A, B, C and D and the 20 amino-acids by numbers, a combination of paired bases (AA, AB, AC, etc.) would allow a code of $4^2 = 16$ numbers. Therefore, for 20 amino-acids there must be at least groups of three bases present or triplets, that act in combination (Gamow; Dounce *et al.*, 1956; Quastler, 1959; and others). However, there may be mixed codes, for instance groups of two, three or four that alternate. Such mixtures however are hardly likely. It seems rather that codons (a codon is an ordered

series of bases which code for an amino-acid) consisting of 3 bases, or possibly 3^2 , 3^3 or higher factors of 3, are used to code for the amino-acids in the polypeptide chain which forms on the cytoplasmic ribosomes.

In addition, it is not at all clear whether or not a single amino-acid can be controlled by more than one triplet (a "degenerate code"). Probably, the codes do not "overlap", that is, each letter of a code belongs to only one triplet (Gamow *et al.*, 1956). For with an overlapping code it would be possible through a change in only one base to change two or more neighbouring amino-acids at the same time. As yet this has not been shown.

A great leap forward in sequence analysis was the "Kornberg system" which made it possible for the first time to synthesize an artificial DNA *in vitro* from four nucleotides in the presence of the enzyme "polymerase" (Kornberg, 1960). However the presence of a small amount of naturally occurring DNA ("primer DNA") is necessary for the experiment and the newly manufactured DNA resembles exactly this "primer DNA". If this artificial DNA is broken up by enzymes, it yields mononucleotides which, especially when isotopes are used, allow certain conclusions as to which groups of polynucleotides occur together most frequently. A particularly elegant technique is the method of offering the cells "false" precursors for the synthesis of DNA; these are substances that are the analogues of bases, as for example 5-bromouracil which is an analogue of thymine. If there is a deficiency in thymine, 5-bromouracil will replace it, therefore, pairing errors may occur, which make it possible to draw certain conclusions about the identification of single nucleotides (Strelzoff, 1961). Here, we can touch only briefly on these investigations, for they are still preliminary and subject to frequent alterations; yet probably they will call very soon for a complete reconsideration of our concept of the molecular fundaments of genetics. In addition, it is frequently not taken into consideration that DNA never occurs isolated but always associated with a protein component. This protein component should not be neglected in the attempts to analyse codings. (Ycas, 1962; and others).

However, the DNA does not determine the specificity of enzymes directly; it needs, as a mediator, RNA. There is sufficient evidence to suggest that the latter is generated directly on the DNA and that it obtains from the gene a specific "information"; in other words, it is given by the DNA a specific structural composition which enables it to transfer as "messenger RNA" or "template RNA" the information to the elements of protein synthesis in the cytoplasm. Hence, the function of genes is not at all, apart from certain exceptions (e.g., Pardee, 1959; Jacob, 1959), to determine the sequence of amino-acids, their function is primarily to determine the structure of the messenger RNA onto which the code is transferred. How this happens in detail is not yet known. One possibility is that RNA is synthesized directly on the double helix of the DNA, whereby the paired bases of the DNA determine the location of the corresponding paired bases of the RNA (Felsenfeld and Rich, 1957; and others). For RNA consists essentially of the same components as DNA, the only difference being that RNA contains ribose instead of desoxyribose and uracil instead of thymidine and that it contains, in small amounts, other bases. Best suited as a matrix for the generation of messenger RNA would be a one-stranded DNA (Volkin and Asrachan, 1956; Volkin *et al.* 1958; and others), especially after the discovery, by Weiss (1960, 1962), of an important RNA polymerase which is capable of bonding bases in the presence of a "RNA primer" in a manner similar to that of DNA polymerase (see above). "Soluble" RNA

has to be a specific nucleic acid, it is of a lower molecular weight than messenger RNA and it is bound, during protein synthesis, primarily to the activated amino-acids (p. 251). The problem of the base sequences in soluble RNA has been somewhat easier to tackle. It has been possible, for instance, to determine three nucleotides at the end of the chain which were found to be of the sequence cytidylic acid-cytidylic acid-adenylic acid, the latter being the last member of the chain. The last member at the other end of this RNA chain is guanylic acid. Fig. 54 depicts schematically (Klingmüller, 1962) this relation between the DNA code, the two RNA components and the sequence of amino-acids. Furthermore, in cell free systems, it has been possible to synthesize, by

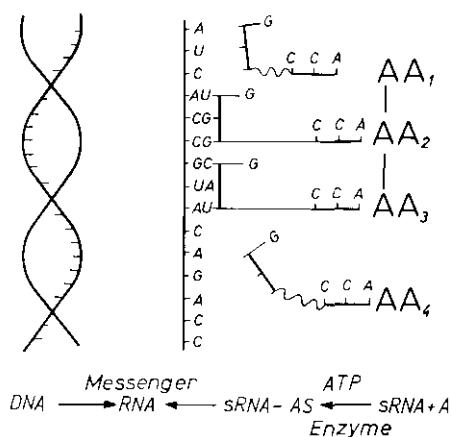


Fig. 54. Schematic representation of the relation between RNA code and amino-acid sequence of a protein enzyme. A = Adenine, U = Uracil, G = Guanine, C = Cytosine, AA = Amino-acid. (From Klingmüller, W. (1962), *Naturw. Rdsch.*, 15, 363-372.)

adding artificial polyribonucleotides, protein-like substances which consisted of only one single amino-acid. If polyuridylic acid was used, polypeptides were obtained that were composed of phenylalanine only (Nirenberg and Matthaei, 1961).

In our discussion, so far, of the probable position of DNA in the cell's metabolism, i.e., chiefly in the metabolism of proteins, the gene appeared to be mainly the location of a specific physiological activity. A gene location, a "locus", would then mean, generally speaking, a location on the chromosome that has a specific and possibly uniform function. This is the one-gene-one-function hypothesis which has been extended and re-formulated as the one-gene-one-enzyme hypothesis (Horowitz and Urs, 1951).

However, many studies have shown that one gene can appear under several variants and that mutations occur at many different sites of the gene. These "sites" (Demerec, 1955) are, therefore, subdivisions of a gene and the gene itself can no longer be considered to be the least genetic unit. There are even "intragenetic" recombinations, in other words, an exchange of genetic characters within the same gene structure. Ishikawa (1961) has been able to demonstrate that some loci, for instance of *Neurospora*, consist of several hundred sites. In the early "gene maps" which were derived mainly from studies of mutation and recombination frequencies, the gene was thought to be predominantly a "recombination unit" (ref., e.g., Swanson, 1960); hence, these maps provided only a first and crude subdivision of the chromosomes. Probably, the specificity of genetic units must be traced down

to the level of the single nucleotides. This is the level of the smallest unit whose alteration may yield a mutation, these smallest units are termed "mutons". In the RNA of the tobacco mosaic virus, the alteration of one of the 6000 nucleotides suffices to bring about a mutation (Mundry and Gierer, 1958) or to nullify its infection capacity (Schuster and Schramm, 1958).

Giant chromosomes

In all these studies on the effect of the genes, we have to do with the smallest objects of investigation, since the necessary evidence can only be brought from simple biological systems, mainly from micro-organisms and viruses. It is always doubtful whether the results obtained can be applied to the cells of superior organisms.

It is of great importance that certain highly differentiated cells exist as particularly favourable objects of investigation, which are equally suitable for the study of the mechanisms of the chromosomal subunits. These are the giant chromosomes, which occur in one typical form, for example in *Diptera*, i.e., in insects, and in another also typical form, as the meiotic "lampbrush chromosomes", for instance in the large nuclei of amphibian egg cells.

Giant chromosomes, especially in the salivary gland of *Chironomus*, are up to 25 μ wide and 300 μ long. Since homologous chromosomes are tightly paired, we find only a haploid number of chromosomes (Heitz and Bauer, 1933). Their remarkable size is due to their polyteny (Bauer, 1938; Bauer and Beermann, 1952; see also Beermann, 1962), i.e., the number of their chromonemata is many times that which is found in other nuclei and their DNA content can be as high as 512 times that of diploid cells (Alfert, 1954; Swift and Rasch, 1955). The chromonemata are visible in the light microscope as helically coiled fibrils. They display chromomere-like lumps which are situated always exactly next to one another, thus forming the typical "bands" (Figs. 17 and 18). This pattern of bands remains constant in each chromosome and in each chromosome section, a feature which we find in all tissues that contain these giant chromosomes (summarized by Beermann, 1962).

More detailed investigations showed that the single bands are capable of changing shape, namely, they can stretch out. Simultaneously, the longitudinal fibrils separate from one another, so that the chromosome gains in width at these points (Fig. 55). These widened portions are called "puffs". At some sites, the elongation of the longitudinal fibrils by stretching is particularly marked and the fibrils protrude from the chromosome in loops; they are then called "Balbiani rings" (Fig. 55) after their discoverer (Balbiani, 1881). These different stages of stretching, from the mere blurring of the band contours to the large Balbiani rings, turned out to be tissue specific differentiations (Beermann, 1952a and b; Breuer and Pavan, 1955; Mechelke, 1953; Becker, 1959). Concomitant with the separation of the chromosomal fibrils in the puffs, a Feulgen-negative substance is deposited at these sites (Breuer and Pavan, 1955), often in the form of minute droplets (Kimoto, 1958b, 1959), and from its staining and radioautographical behaviour we know it is RNA (Breuer and Pavan, 1955; Pelling, 1959) and protein (Clever, 1962). That RNA synthesis indeed occurs in these puff regions of the chromosomes has been demonstrated by radioautographic labelling studies with ^3H -uridine (Fig. 56) (Pelling, 1959). These stretched out sections are differentiated regions of particularly intense synthetic activity and are consequently the sign of an enhanced RNA synthesis (see, e.g., Mechelke, 1963). Since we know that the primary

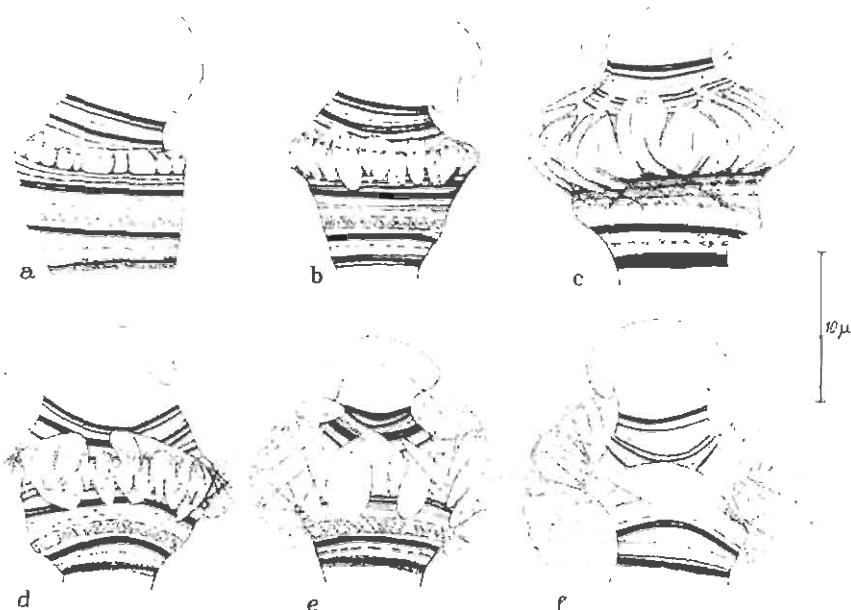


Fig. 55. Different stages in the stretching of a band in a giant chromosome of the salivary gland of *Chironomus*. Top row: Formation of a "puff". Bottom row: Formation of a Balbiani ring (see also Fig. 18). (From Beermann, W. (1952), *Chromosoma*, 5, 139-198.)

role of the gene is the formation of RNA on the DNA molecule (see p. 116), we can consider this RNA synthesis on the "puffs" to be a direct representation of the gene function.

During the development of the larvae, the differentiated pattern along the chromosomes changes (Clever, 1962). Every time a certain locus is activated, the corresponding region of the chromosome swells.

It is theoretically possible to derive from the giant chromosomes of *Diptera* a "map" of the unfolded bands in their relation to the cell function, analogous to the "gene maps". Of course, we should not forget that these are two completely different dimensions; however, these giant chromosomes, which incidentally do not occur only in *Diptera* (Hasitschka, 1956; Tschermark-Woess, 1956; and others), give us for the first time the opportunity of observing directly the functional unfolding of certain sections of the chromosomes.

The lampbrush chromosomes (Rückert, 1892), too, provide some important information about the chromosome function. They are large meiotic chromosomes in the oöcytes, mainly of amphibians, but also of plants (Grun, 1958). They have a central axial thread and brush-like lateral processes (Fig. 57). These lateral chains are loops that continue the central

Fig. 56. Incorporation of ^3H -uridine in a giant chromosome of *Chironomus*. Note the selective labelling of certain bands indicating that an RNA synthesis has taken place at that point. $\times 1260$. (Courtesy of G. Pelling.)



Fig. 56.

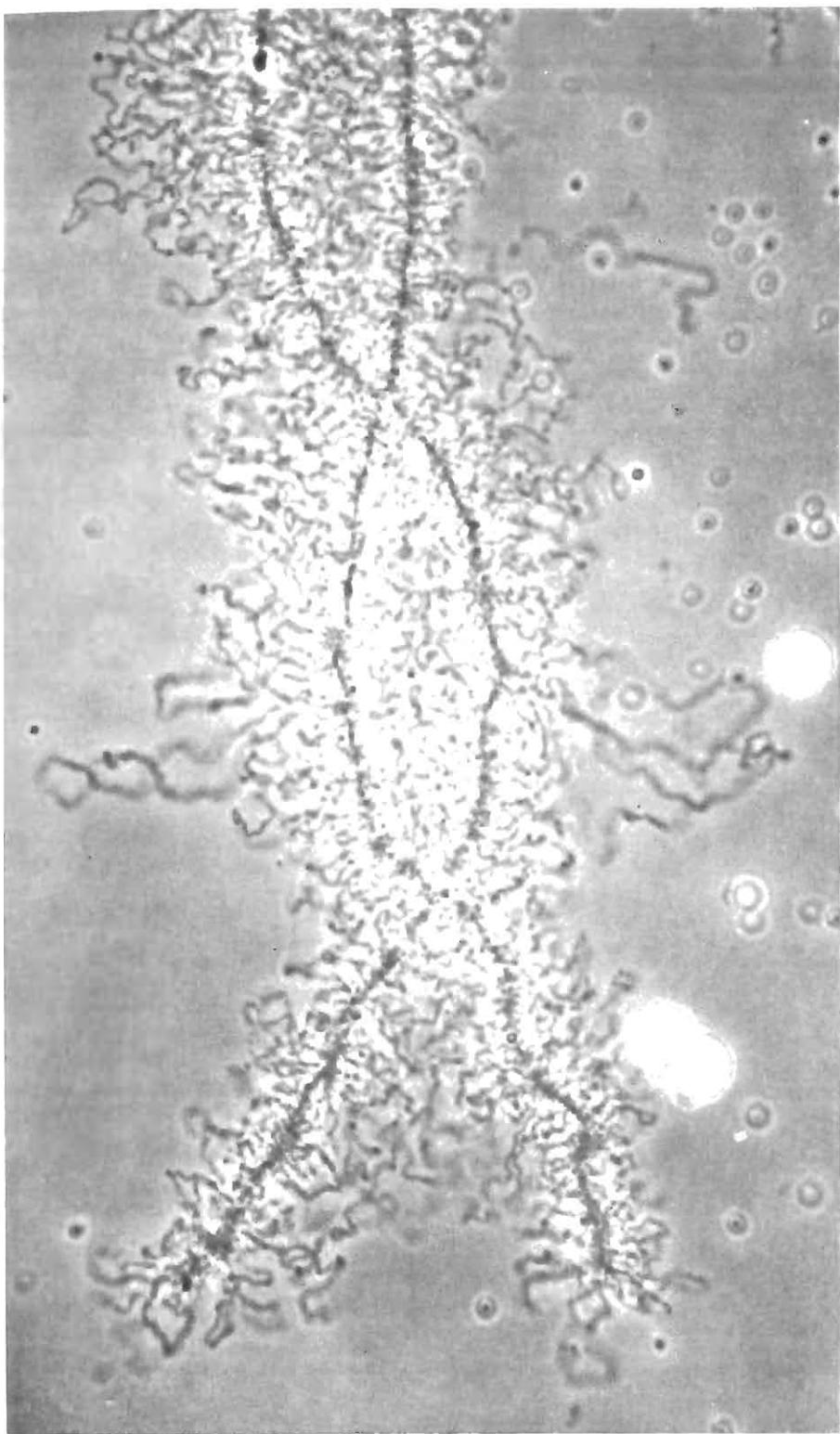


Fig. 57.

thread (Alfert, 1954; Gall, 1954*b*; Ris, 1957; Callan and Lloyd, 1960). This thread can be partly stretched out; however, this does not eliminate all the loops. As seen in the electron microscope, it consists, like other chromosomes, of bunches of fine helically coiled fibrils (Lafontaine and Ris, 1955; Nebel and Hacket, 1961). At the base of the loops there are densities, lumps, which have been interpreted as chromomeres and perhaps correspond to heterochromatin (Ris, 1957).

The loops proper consist of fine strands of DNA (Alfert, 1954) but mainly of RNA and protein (Gall, 1954), i.e., of those substances which we recognized as functional variables of the chromosomes. And indeed, we can see, surrounding the loops, a kind of "matrix", which multiplies periodically, separates itself from the loops and lies then free in the nuclear sap (Callan and Lloyd, 1960). Moreover, on these loops the nucleoli, too, are formed, which

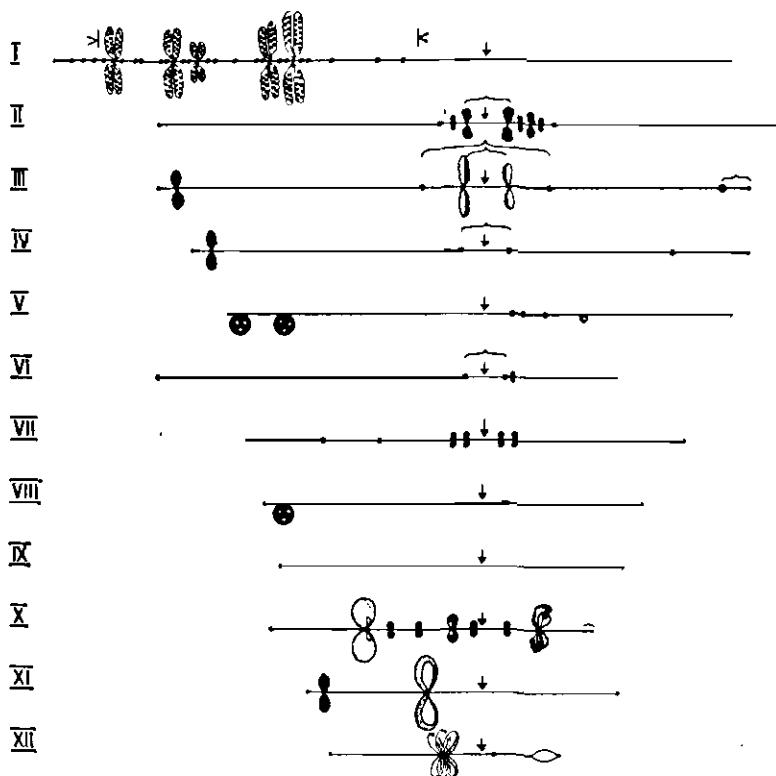


Fig. 58. Schematic drawing of the lampbrush chromosomes of *Triturus cristatus carnifex* showing the relative length of the 12 chromosomes and the typical loops, which make identification possible. The vertical arrows point to the location of the centromere. (From Callan, W. G. and Lloyd L. (1960), *New approaches in cell biology*. Acad. Press, London.)

Fig. 57. Phase contrast micrograph of an unfixed lampbrush chromosome from an oocyte of *Triturus viridescens*. $\times 700$. (Courtesy J. G. Gall.)

then detach themselves also and move to the nuclear membrane. Thus, the loops of the lampbrush chromosomes seem to be, like the stretched sections of the giant chromosomes in chironomids, the sign of a local function; further evidence of this fact would be equally authentic lateral loops of the Balbiani rings (Beermann, 1952a), which obviously resemble the loops of the lampbrush chromosomes. Each of the twelve chromosomes of *Triturus c. carnifex*, for example, is characterized by its typical loop pattern. Fig. 58 only shows the particularly striking loops (Callan and Lloyd, 1960); but it would not be difficult to establish a kind of "loop map" analogous to that of the distribution of the puffs and the Balbiani rings.

Review

Despite the variety of its aspects, the chromosome is a cell component of quite a uniform nature. Its linear structure, subdivided into hitherto not quite elucidated sequences of subunits, reflects the linear arrangement of the genes. The gene itself is no longer the last unit, but is subdivided, in certain cases, into several hundred sites. However, the linear principle of structure can be considered as a general rule and appears, for instance, in the banding pattern of the giant chromosomes and probably also in the loop structure of the lampbrush chromosomes. The variable stretchings of the bands are the direct indication of a circumscribed genetic activity, which, from present theories, consists primarily in RNA synthesis, with formation of an RNA pattern specific for the required function. The messenger RNA transmits the information of the DNA code to the structures of the cytoplasm. How this code of nucleic acids, primarily of the DNA, is arranged, is not yet fully elucidated. It is dependent on the secondary structure of the DNA, in which four typical bases determine in their sequential arrangements the specificity of each genetically active locus. The double helix, secondary structure of DNA, is probably responsible for the coiling of all chromosomal units. Euchromatin and heterochromatin probably differ from one another by their degree of spiralization. On the other hand, they represent, together with the primary and the secondary constrictions, i.e., with the spindle insertion on the centrosome and the nucleoli-organizing SAT segments, morphological particularities which characterize each separate chromosome as a constant individual, in the same way as its total length or the length of each one of its arms characterizes a given chromosome.

3. Reproduction of Nucleus and Cell

Up to now we were concerned with the nucleus and its components. However, we could understand its functions only in relation to the cytoplasm. Whether we considered metabolic activities in general or gene functions in particular, we were always reminded of the indivisible unity of the cell, which subsists and reproduces itself owing to the interplay of nucleus and cytoplasm.

Cell reproduction is to be now our main object of study. It concerns every part of the cell, but is most strikingly evident in the nucleus. We can even say that the reproduction of the nucleus appears to be the leading process, only later followed by the cell's reproduction. But, whereas most of the cytoplasmic organelles grow by way of a mere increase in substance, the main elements of the nucleus, the chromosomes, pass, at least during *mitosis*, through a definite cycle, which is the main characteristic of this type of reproduction. Consequently, the chromosomes always play the major role, no matter how disturbed and irregular the mitosis might be. Likewise during the reproductive cycle of metazoa with its additional "maturation divisions", the important factor is, during meiosis, the behaviour of the chromosomes, which are reduced to exactly half their original number. The reverse process, i.e., duplication or multiplication of the number of chromosomes leads to *polyploidy*, obtainable through certain *abnormal mitoses* or through an *endomitosis*. In an endomitosis, the chromosomes are generally not visible at all, and frequently only the following mitosis makes it possible to detect the result of an endomitosis. Equally invisible are *amitotic* processes, i.e., a "direct" type of nuclear division, which is being increasingly disputed.

MITOSIS

If it were not for mitosis, we would know very little about the chromosomes. For it is mainly during mitosis that they are recognizable as separate individuals, as "nuclear threads" (Strasburger, 1875; Flemming, 1880). They gave the whole process the name of "mitosis", or "thread metamorphosis" of the nucleus, which Flemming wanted to use instead of the older denomination "karyokinesis" (Schleicher, 1878), which again was a reference to the movement of the chromosomes. But before we examine in detail the origin of names and definitions, let us first give a brief survey of the process itself. It is fascinating indeed and no one who has been fortunate enough to observe the mitotic division of a cell in the microscope or at least in one of the good filmings of it, will ever forget it. Fig. 59 shows a scheme of the process.

Suddenly, the nucleus undergoes a peculiar change. There appear circumscribed densities, which give a stronger phase contrast than the environment. One can soon recognize threads clustered into clumps, seemingly at first with no beginning nor ending. This is the first phase of the mitotic nuclear division, or *prophase*. Soon the nuclear membrane disappears,

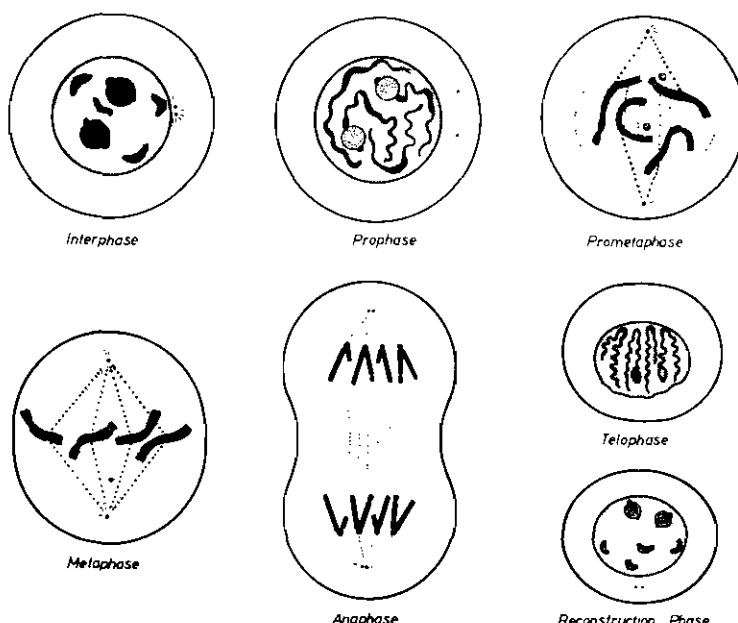


Fig. 59. Diagram of the stages of mitosis in animals. (after Grell, K. G.)

leaving the chromosomes free in the cytoplasm as well-defined loops often surrounded by a particularly light area. While the spindle forms itself as a structure of fine fibres, the chromosomes move with their spindle insertion to a median plane of the cell, called the "equatorial plane", and there they build the "equatorial plate". This movement occurs in *prometaphase*. The latter leads directly into *metaphase*, during which the spindle grows to its full size. The duration of the metaphase varies. Then, suddenly, the chromosomes move again and *anaphase* begins. Each longitudinal half of a chromosome moves to its respective spindle pole. Between the chromosome halves an *intermediary body* forms which is very similar to the spindle. Frequently the *cleavage* of the cell begins at the same time with a constriction of or at least a depression in the cell body at the previous equatorial plane. The cleavage of the cell is usually rapidly completed during *telophase*. Simultaneously, the spindle dissolves, its fibres disappear. The chromosome halves at the previous poles of the spindle contract to a compact clump. In the last phase, or *reconstruction phase*, the normal nuclear structures are formed anew, while the chromosomes disperse, new nucleoli appear and a new nuclear envelope is formed.

This is mitosis as originally defined by Flemming. We prefer to name this process mitotic division of the nucleus, or else to use the old word karyokinesis (Schleicher, 1878). For today the term mitosis implies more than the original definition by Flemming; it is now defined as a process of the whole cell. We speak of a mitotic cycle (e.g., Hughes, 1952). Such a cycle,

however, can be complete only if we include the period between the reconstruction phase and the next prophase. During this period of time, the mitotic processes of the nucleus lie at rest. This explains the frequent use of the expression resting nucleus—so misleading, since it applies precisely to the phase of its most intense metabolic activity; let us, therefore, avoid this term. Almost all synthetic processes which serve for the multiplication or, frequently, for the duplication of substances and structures halved during the cell division, occur in the period between the telophase and the next prophase, i.e., between two karyokineses. And if we consider today mitosis as a cycle, we must include in this cycle the phase between the karyokineses. We call it the *interphase* (Fig. 60).

In all differentiated cells, the period of time between two karyokineses is used not only to replicate the cellular substances, but also—or rather, chiefly—to perform its main cellular functions for the tissue and, eventually, for the whole organism. It is clear, therefore, that the highly differentiated parenchyma cells use but a fairly small portion of their interkaryokinetic time for interphase replications; it is no wonder that this interkaryokinetic period varies considerably in length. It always consists of a phase of replication of the cell's halved components—the interphase as a part of the mitotic cycle—and of a phase of differentiated cellular functioning, wedged in, so to speak, between two cycles—the *intermitosis* (Cowdry, 1953). In all meristematic tissues or, generally speaking, in all cells which divide in rapid succession, i.e., where one mitotic cycle directly follows the other, this interkaryokinetic period is identical with the interphase.

Yet, not all cells are capable of a new division. Many of them lose their multiplication power when they differentiate themselves, for example, the ganglion cells of the central nervous system of mammals, or the erythrocytes and granulocytes or the epithelial cells of the epidermis. Their mitosis was irrevocably their last and they are now in a state of *postmitosis* (Cowdry, 1953). This is shown in Fig. 60. Postmitosis may last only a few days, as in granulocytes; in ganglion cells it lasts as long as the organism lives. Certain types of cells seem to have an intermediary status between intermitotic and postmitotic cells, such as many mesenchymal cells, vascular endothelia, etc. They regain their division capacity under certain circumstances and have, therefore, been called “reversibly postmitotic cells” (Cowdry, 1953).

Yet it is clearer to classify all these cells among the intermitotic cells, for the only decisive element is their capacity to undergo mitosis. Whether this capacity is realized depends on many factors, among other things, on how long the organism lives.

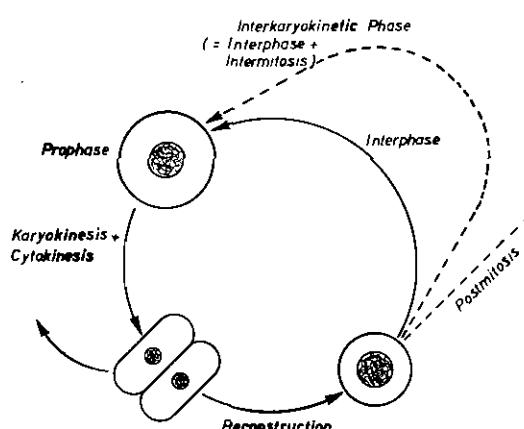


Fig. 60. Diagram of the stages of the mitotic cycle, showing the correlation of intermitosis and postmitosis.

This subdivision into interphase, intermitosis and postmitosis makes the old denomination of resting nucleus obsolete. If it is necessary to cover all phases with one expression, it is best to speak of *interkaryokinesis*, i.e., the period of time between two karyokineses. The mitotic cycle itself consists of two parts, which are essentially of equal importance: (1) the karyokinesis, or mitotic division of the nucleus followed by reconstruction and cytokinesis, (2) the interphase, i.e., the period of replication of the previously halved cell components.

Thus we have gone very far from the original conception of mitosis as nuclear division and we have included in it the more general process of growth. However, if we want to know the details of the processes involved, we must return at least temporarily to where we started. For the most important feature is, as we emphasized already, the change that occurs in the chromosomes, and apparently the only purpose of such a process as karyokinesis, which looks so complicated at first, is to distribute the material of the chromosomes absolutely evenly between both daughter cells.

Cyclic changes in the morphology of the chromosomes

There are two processes which we must consider separately. First, each chromosome divides itself longitudinally and second, each longitudinal half assumes a transportation shape, which gives it more mobility and makes a loss of chromosomal parts more difficult.

Changes in size and mass

The tight "packing" of chromosomes occurs simultaneously with a noticeable reduction of their total length. The extent of this contraction, however, cannot be determined with precision since there are no chromosome boundaries visible in the interkaryokinetic nucleus. The length of chromosomes can be measured in certain cases, but only during the last part of the contraction process, when the chromosomes become visible again at prophase. These measurements have been made on various objects, mainly on plants with relatively long chromosomes. The relation in length between prophase and metaphase chromosomes varies between 2:1 and 4:1, i.e., metaphase chromosomes may shorten by the factor of 4 (Sax and Sax, 1935; Colombo, 1952; and others). However, the process of contraction is still not completed at this stage. During the metaphase, the chromosomes continue to shorten and, at the beginning of anaphase, they reduce once more markedly in length (Bajer, 1959). Thus, the contraction reaches its maximum at the end of anaphase.

The real extent of the contraction is at least double that of the given measurements. It might be a fairly accurate estimate to gauge it as one-tenth of the chromosome's length, as in the case of the giant chromosomes in the antipodes of *Aconitum* (Tschermark-Woess, 1956). In meiosis, we must assume contractions down to one-fiftieth of the chromosome's length (Darlington and Upcott, 1939).

Parallel to this contraction we notice a considerable loss of water from the chromosomes (Kuwada, 1939), while in the surrounding cytoplasm the relative water content increases markedly with the condensation of the chromosomes, as could be demonstrated by measuring the viscosity (Carlson, 1946) and the refractive index (Ross, 1954). However, the loss of binding water from the chromosomes is by no means solely responsible for their contraction (p. 68). At any rate, we find here once more the particularly variable capacity of the chromo-

somal substance to bind water, as we pointed out already when we discussed the appearance of the nucleus (p. 69) and of the heterochromatin (p. 75) after fixation; also, we now understand why the whole metaphase chromosome has been described as temporarily heterochromatized (Kaufmann *et al.*, 1960).

Changes in spiralization

The analogy with heterochromatin can be drawn even further; for dehydration intensifies considerably and thus, reveals particularly clearly a structural feature apparently common to all chromosomes, that is, the spiral, or better, the gyre structure.

In our previous chapter, when we discussed the fine structure of the chromosomes (p. 108), we have met this gyre structure in many dimensions. Light microscope findings, however, have their limitations. In favourable specimens we may see, when the chromosomes become visible during early prophase, how they are twisted at first in irregular torsions, which then arrange themselves readily into a regular gyre. This major spiral is the *standard spiral* (Fig. 61) and the first part of prophase during which this spiralization appears for the first

time, is called spiral prophase. The number and diameter of these relatively large gyres remain, with a remarkable regularity, identical within each organism, and also even in individuals of the same species; thus, we can consider the shape of the standard spiral to be, to a certain extent, a specific feature of each species. In particularly large chromosomes, the strand of the standard spiral is composed of multiple sub-strands, which are themselves coiled in *minor spirals*. At prophase, another loose coiling of chromosomes can be frequently observed; it is the remainder spiral; that which remains from the standard spiral of the previous prophase (Darlington, 1935).



Fig. 61. Standard spiral of the mitotic chromosomes in the pollen tube cells of *Lilium candidum*. (Courtesy of H. Marquardt.)

True, the coiling mechanism varies; moreover, we cannot yet say down to which dimension we may assume that such a coiling takes place. However, we know for certain that the coiling is a general characteristic of the chromosomes and that the contraction of the prophase chromosomes represents an increase in this spiralization.

Chemical changes

The assumption must be that a change in the chemical composition of the chromosomal structure is responsible for the prophase contraction. Interferometric findings in the endosperm of plants have shown that metaphase chromosomes represent only about a quarter of the nuclear mass (Richards and Bajer, 1961), so that in the prophase there occurs mainly a loss or degradation of proteins. Concurrently, another substance seems to accumulate at the

chromosomes, namely, RNA or a certain form of RNA (Love, 1957, 1961). At least, according to staining evidence, chromosomes in the pro-, meta- and anaphase are rich in RNA, and at telophase the amount of RNA decreases. This RNA cycle at the chromosomes (Kaufmann *et al.*, 1960) has been found in many instances (Jacobson and Wegg, 1952; Boss, 1955b).

When the chromosomes join at telophase after their anaphase migration, they again incur a loss of RNA. In certain specimens one can observe how RNA, which obviously comes from the chromosomes, appears in the interzonal region, i.e., in the area between the separating chromosome halves (Jacobson and Webb, 1952; Boss, 1955b). The chromatin elimination, which occurs especially in certain insects (Bauer, 1933; Ris and Kleinfeld, 1952) is evidently a variant of the same process, only particularly intensified. What we have here is an accumulation of an elimination chromatin in the previous equatorial plane, occurring predominantly after the meiotic anaphase. There is evidence showing that this chromatin elimination is visible at the chromosomes as early as in prometaphase (Frolowa, 1935). It is undoubtedly free of DNA (Bauer, 1933) and its staining behaviour indicates that it consists of proteins and mainly of RNA (Ris and Kleinfeld, 1952).

Behaviour of the nucleolus

Since the nucleolus is the region of the nucleus which is the richest in RNA (p. 48), and since it generally disappears at this stage, it is but natural to assume that the supplement of RNA at the chromosomes is of nucleolar origin. It is a fact that the nucleolus does not compulsorily disappear. In many cases and in cells of the most different types, it is possible to see nucleolar material as an isolated structure during karyokinesis, most frequently in protists (Belar, 1926) and in several plants (Tischler, 1934-1951). Generally, if it remains outside the spindle, it dissolves gradually. Inside the spindle, it is liable to be transferred to the metaphase plate, together with the chromosomes; then, even before the beginning of anaphase, it stretches itself out like a drop towards one end or both ends of the spindle, and finally reaches both spindle poles, thus, eventually, entering the daughter nuclei (e.g., Schaede, 1928, 1929; Frew and Bowen, 1929; Bajer, 1953; Brown and Emery, 1957; see also further literature in Schrader, 1954). Persisting nucleoli, however, are in any case altered in their morphology, and certainly also in their substance (Geitler, 1938d) and it is still not clear whether or not these persisting nucleoli contain only parts of the original nucleolar substance, while another part lies, for example, at the chromosomes.

For, indeed, a transfer of nucleolar substance to the chromosomes has been postulated time and again by many authors (Berghs, 1909; de Litardière, 1921; Ludford, 1954; and others) and was even observed with different staining methods (e.g., Oura, 1953; Estable and Sotelo, 1954), and also in the electron microscope (Yasuzumi *et al.*, 1958). Some authors have denied the fact (e.g., Belar, 1928; Stich, 1956; Roth *et al.*, 1960). Yet, particularly in the rat liver, treated with thioacetamide, where nucleoli are known to be especially large (Rather, 1958; Altmann *et al.*, 1963), ribosomes have been seen, in the electron microscope, coating the chromosomes (Kleinfeld and Haam, 1959). Such findings corroborate the opinion that parts of the nucleoli which contain RNA, especially the nucleolonema, are transported to the spindle poles together with the chromosomes (Jacobson and Webb, 1952; Estable and Sotelo, 1954), and rejoin the nucleolar organizer during the post-telophase reconstruction (McClintock, 1934). It may possibly be that same portion of nucleolar substance which can

be traced by zinc staining and which, also, moves towards the poles on the chromosomes (Fuji, 1954).

The opposite concept, however, according to which the whole of the nucleolar substance is reconstructed anew by the chromosomes in the telo- or reconstruction phase (Stich, 1956; Roth *et al.*, 1960; and others), at the moment is difficult to reject completely. There can be no doubt that, during the post-telophase reconstruction, the nucleoli arise from small RNA pellets, which are to be found at first on or between the chromosomes (e.g., Frankel, 1937; Morrison and Lin, 1955) and then gather in the main nucleolus.

The problem of the chromosomal matrix

The concept of a deposit of part of the nucleolar substance on the chromosome is closely related to the problem of the chromosomal matrix. According to the definition which is the most accepted today, we understand by matrix of the chromosomes a substance that lies between the chromosomes and thus envelops each and every chromonema (Nebel, 1939). This is the way it is represented today in most of the schematic diagrams of chromosomes (Fig. 47). Another concept according to which the matrix, a DNA-containing envelope, surrounds a DNA-free chromosome strand (Serra, 1947; Schrader, 1954) is not quite consistent with the chromonemal fine structure of the chromosome (p. 108).

The problem concerning the matrix is mainly the difficulty of demonstrating it. It has been described only during karyokinesis, i.e., precisely at those stages in which the chromonemata probably lie close together and lose a great deal of their accessory proteins (Richards and Bajer, 1961). It is also particularly visible when the nuclei are put into a medium in which chromosomes shrink, so that at least several pictures of matrix represent only a shrinkage artifact (Ris, 1957).

The matrix has again and again been referred to as the cause of spiralization (Kuwada, 1939; Huskiss, 1941; and others). The theory was that the storage of this substance forces the passive chromosomes into a narrow coiling (Sax and Humphrey, 1934). However, since we are familiar with the molecular structure of DNA, the essential component of the chromosomes, such hypotheses become more and more meaningless. For molecular DNA is already a double spiral (Fig. 53). The tendency to coil seems, as we mentioned previously, to be a genuine characteristic of chromosomes and chromonemata (Darlington, 1935), connected with intensive water transfer. Thus, in this problem we have again reached the starting point of our discussion. Hydration of the macromolecules of the chromosomes appears to be of major importance and probably plays the key role. The loss of water during prophase may be concurrent with a loosening of intermolecular transverse bonds (Philpot and Stanier, 1957) which can experimentally be brought about by only a relatively small change in the ion balance (Davidson and Anderson, 1960), owing to the above-mentioned lability of chromosomal hydrations. This water loss is probably decisively responsible for the prophase condensation of the chromosomes; whether an adsorption of RNA-containing nucleolar material or of matrix substance plays a part here at all, is doubtful.

Longitudinal splitting

The "matrix" appears, however, to be of some significance in the longitudinal splitting of the chromosomes. The latter can be seen in the light microscope often as early as in the

prophase, nearly always in the metaphase and frequently also in the previous anaphase. Both halves of the chromosomes stay closely together even when, during metaphase, they lie parallel to each other without being twisted. True, the kinetochore, or spindle insertion, is generally not yet separated. But even when it is already divided, the chromatids are firmly held together (Fig. 62), until the beginning of anaphase, possibly by a matrix-like substance (Lima-de-Faria, 1959a).

Investigators have set the moment earlier when this longitudinal splitting is supposed to occur; first from metaphase to prophase, since in the latter they had already discovered the double nature of the chromosomes; then however to anaphase since more and more

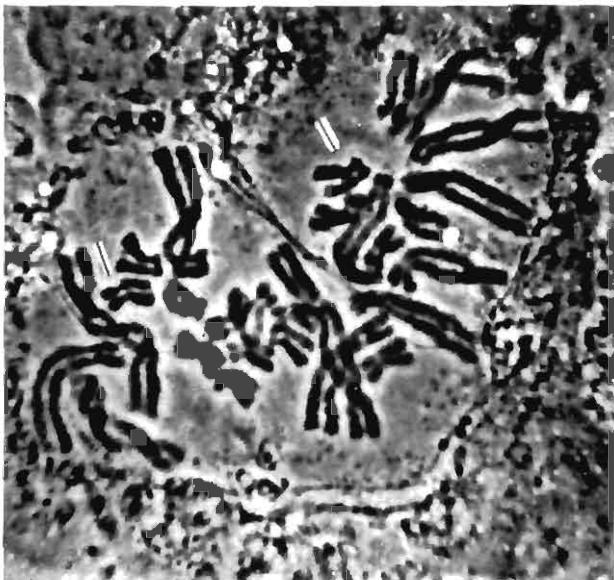


Fig. 62. Metaphase of *Haemanthus Katharinae*. Note that the chromatids lie close to each other, although the kinetochores are already divided (see the two white indicators). (From Lima-de-Faria, A. (1959), *Hereditas*, 45, 463-465.)

evidence came to light of anaphase chromosomes split into two chromatids (e.g. Heidenhain, 1907; Lundgardh, 1912), until finally photographs were published showing anaphase chromosomes with four strands (Nebel, 1939; Marquardt, 1941). According to certain radioautographic findings (Taylor *et al.*, 1957; Taylor, 1958a and b, 1962), the chromosomes behave as if they were composed of two strands (p. 110); however, other radioautographic observations (La Cour and Pelc, 1958) were consistent with the multiple strand hypothesis (p. 110) (see Mazia, 1961a; see there too for a detailed discussion of the problem). Let us not go here into the details of this question, which still has to be solved. One fact is certain: the longitudinal splitting of the chromosomes becomes visible during prophase, sometimes already during the previous anaphase, and we may assume that it has been prepared long before that.

Review

At the term of this discussion we are faced again with the same unsolved problem as when we investigated the fine structure of the chromosomes. This we can say for sure: the prophase condensation which forms the standard spiral and the minor spiral which is sometimes to be seen, are probably based on an inner tension of the chromosomes, caused by the

double helix DNA molecule. This spiralization is realized mainly by the dehydration processes at the beginning of prophase. It is still unclear whether an adsorption of RNA-containing material by the nucleolus plays a part in this and to what extent. It is a fact that the nucleolus generally always disappears at the time when the chromosomes condense and reappears with the decondensation at telophase. We will have to deal later, in the chapter on the mechanism of mitosis, with the question as to what triggers off the dehydration processes at prophase and hydration processes at telophase. We have explained first that the chromosomes undergo in the karyokinesis a cyclic change in form, which starts at prophase with a condensation, contraction and coiling, and ends at telophase with a dispersion, elongation and uncoiling. Simultaneously, the chromosomes split longitudinally. Now let us consider the transportation of these chromosome halves.

Kinetocentres

This transportation occurs roughly in three stages. First, after loosening of the prophase entanglement, the chromosomes align themselves equatorially at metaphase, then the longitudinal halves move towards two poles, which are as a rule situated at the exact opposite of each other. These poles are the centres of the movement of the chromosomes and we call them therefore *kinetocentres*. They determine also the orientation of the metaphase equatorial plane, which according to definition lies exactly in the middle between both poles, vertical to the abstract line connecting them, thus dependent on the position of the kinetocentres. Vice-versa, we define the kinetocentres as those cell components which determine direction and extent of the movement of the chromosomes. There are two kinetocentres in each typical mitotic karyokinesis, i.e., they are identical with the "mitosis centres" of Mazia (1961a).

The behaviour of the kinetocentres was described for the first time in the cleavage divisions of *Ascaris* of the horse (van Beneden and Neyt, 1887; Boveri, 1887, 1888, 1890). In this case, in the cytoplasm, already prior to the beginning of prophase there is a relatively large, strongly light-refracting body, which sometimes shines a little and seems to be the very source of this radiation. After staining with iron haematoxylin, one or two granules can be recognized, like dots, in the centre of this corpuscle (Fig. 63, left). During prophase, these centrosomes move towards the poles; as the process goes on, the radiation increases especially in the zone where the rays are directed against each other and the chromosomes seem to be caught in the middle between the centrosomes, thus forming the equatorial plate (Fig. 63, right). At this point, we can well recognize the radiation also on the living object. As the chromosomes start their anaphase migration, the polar bodies flatten to a smooth biconvex lens, the radiation dims down progressively and finally disappears completely. The centrosomes then constrict and divide themselves in the middle between their two granules, they go on reducing in size and during the interkaryokinesis they both lie close to each other near the nucleus, until they start growing again in the new prophase and emit new polar rays (Fig. 63, left). The terms used for the elements which participate in this cycle are manifold and lack uniformity. The two relatively large corpuscles, which seem to emit the rays, were called centrosomes (Boveri, 1888, 1890) and their substance, the centroplasm. The small granule inside the centrosomes is called centriole. Yet the terms centrosome and centriole are often used as synonyms (see Geitler, 1934). We shall, however, according to the

original definition given by Boveri (1900), distinguish both and always speak of the centrosome when a pole has the structure of a corpuscle. The centriole is then the small inner corpuscle in the middle of the centrosome, and at the same time it is also probably the granule which persists in the cytoplasm during interkaryokinesis. The centrosomes are special functional structures of the kinetocentres (see, e.g., Fig. 63), the centrioles are probably their self-reproductive components. The radiation in the area of the mitotic centrosomes is the polar radiation or astrosphere (Boveri, 1888, 1890) and consists of archiplasm, spheroplasm (Boveri, 1888, 1890) or kinoplasm (Strasburger, 1880).

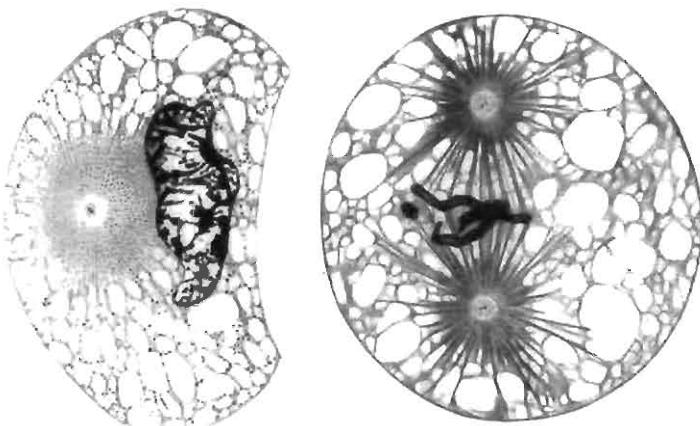


Fig. 63. Centrosomes, centrioles and astrospheres in the blastomere division of *Ascaris* of the horse. *Left*: interphase; *right*: metaphase. (From Boveri, Th. (1900), *Zellenstudien*, Vol. IV, Fischer, Jena.)

We have already described above the cleavage divisions of *Ascaris*, which became historical through the first descriptions made of the phenomenon by van Beneden and Neyt (1887) and Boveri (1888); from this example, we may ascribe to the kinetocentres the following general qualities: they undergo a cyclic change in form, which precedes the mitotic chromosome cycle and determines its dynamics. They utilize thereby the surrounding cell structures, which they arrange in rays. Synthesis and degradation of these structures show clearly that the functioning rhythm of the kinetocentres is in close correlation with the mitotic cycle. They can form more of themselves and are therefore permanent self-reproductive cell elements.

Such a listing of the properties of kinetocentres is, though hypothetical as yet, the necessary first step to all further explanations. For no other cell component is as disputed as the kinetocentres and for no other is it as difficult to give a generally accepted morphological definition. Indeed, in animals and plants there are a multitude of possible ways in which these elements can form themselves; one of the greatest problems, therefore, is that of too diversified a terminology, which in turn makes the uniformity of all these structures questionable. When we apply the more general term of kinetocentre to all these elements which determine the

direction and extent of the movement of the chromosomes, we are led by the conviction that their common function compels us to give them a common appreciation.

Let us go further and say that such kinetocentres probably have a function in every mitotic karyokinesis, albeit one can relatively rarely trace them down authentically. We are encouraged to make such a generalizing statement (see also Belar, 1926; Geitler, 1934; Mazia, 1961a) by the fact that all mitoses run according to the same principle, whether corpuscular pole elements can be demonstrated or not. Thus, several simple plants such as mosses and ferns have centrosomes only during spermatogenesis. In subsequent divisions these exist no more, but they appear again in the spermatogonia of the following generation. Other organisms lack kinetocentres during spermatogenesis, but display some clearly in other divisions. In principle, we must be prepared for the possibility that invisible kinetocentres exist, of which there are some clear-cut examples. In protozoans, for instance (see Geitler, 1934), the fusion of several unicellular organisms can bring about polyvalent nuclei. At the division of these macronuclei, there appear as many bipolar spindles as there were previously nuclei which coalesced, even though no kinetocentres are to be seen at any time. We can also mention, as another example, the blocking of the mitoses of the sea urchin by mercaptoethanol (Mazia *et al.*, 1960).

Thus we feel entitled, here too, to apply the principle we had established at the beginning, that is, to present, so far as possible, all cytological phenomena as variants of common principles of structure and function.

Shapes and fine structure

The difficulty of applying this principle here appears clearly as soon as we glance at the multiplicity of shapes which are to be found among kinetocentres. Similar to the above-described cleavage of *Ascaris* is the behaviour of the kinetocentres in the cell division of other egg cells, in the division of many spermatocytes and also in most mitoses of differentiated animal cells. However, in the latter, there has been relatively little evidence of corpuscular centrosomes, whereas the existence of interkinetic centrioles was already proved in early observations by Flemming (1891a), Hermann (1891) and Heidenhain (1894) and in the living or surviving cell (Boveri, 1900; von Bresslau, 1910; Huettner and Rabinowitz, 1933; Watts, 1952; and others). Thereby it was shown that not all kinetocentres have a spheric shape. They were often found to be small rods (Zimmermann, 1898; Schrader, 1947) which lie either crosswise over each other or together in the shape of a V (Costello, 1961).

The kinetocentres of certain large forms of protozoans can be particularly clearly seen, for instance in hypermastigina, the richly differentiated flagellates that live as intestinal parasites in termites or scabs (Cleveland, 1938, 1953). In certain species of these flagellates, the kinetocentres are not in the cytoplasm but in the nucleus. Belar (1926) and Grell (1956) have described such intranuclear kinetocentres also in other protozoans. In this case, the centrosome may, under certain circumstances, leave the nucleus at the beginning of the division (Jameson, 1920).

Compared with these particularly well demonstrable kinetocentres of certain protozoans, we find in most seed plants exactly the opposite situation. All attempts that were made to detect similar elements have failed, so that the few positive findings must be considered as misinterpretations (Tischler, 1934-1951). Consequently, it has been generally attempted to

distinguish between karyokineses with and without centres, i.e., between two fundamentally different types of division (Wassermann, 1929). One can see, however, how difficult it is to establish such a distinction, when one considers the case of the protozoans, which display all stages of kinetocentres, from the most clearly visible to the completely invisible (Belar, 1926).

Histochemically, kinetocentres are but slightly distinguishable from the surrounding cytoplasm. During their functional development in the karyokinesis, they appear lighter in the phase microscope than their environment. However, it is not possible to distinguish them with certainty from the surrounding polar radiation and, therefore, all data concerning RNA and protein content (Stich, 1954) or the incorporation of SH-rich proteins (Kawamura and Dan, 1958) can apply equally to the polar rays. We can trust the findings in interkaryokinetic cells to a greater extent in which the polar radiation is degraded or at least markedly reduced. Histochemistry of the markedly enlarged centrosphere regions of tuberculous epitheloid and giant cells, for example, shows a positive periodic acid leucofuchsin reaction to muco- or glycoproteins (Gedigk, 1954), in which it is interesting to note that the centrioles remain unstained as light dots (Fig. 64), i.e., they are probably almost free of these substances (Altmann, 1961a). Since the spindle too can be stained in the same way (p. 147), one may discuss whether it is not so much the centrosphere which is made visible here, but rather a mass of residual or newly accumulated spindle material.

Through the thin-sectioning technique of electron microscopy we have gained some essential insight into this matter. In the dimension of the electron microscope, we generally find two

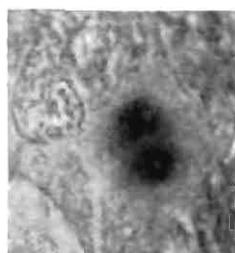


Fig. 64. PAS-positive staining of the astrosphere of the centrosomes in a brain tumour. Note that the centrioles remained unstained. (Courtesy H.-W. Altmann.)

corpuscles of a specific structure, each consisting of a cylinder averaging 500 m μ in length and 150 m μ in diameter. The wall of the cylinder is composed of nine tubule systems, which lie exactly at the same distance from one another and often consist of three or four separate tubules (Fig. 65, left). In longitudinal sections the tubules appear as longitudinal bands (Fig. 65, right). This structure of the centrioles was discovered in white blood cells (Bernhard and De Harven, 1956; Bessis and Breton-Gorius, 1957; Amano, 1957; Low, 1960; Bessis and Thiery, 1961) but the very same structure has also been found in many other cells of mammals and humans (e.g., Stoeckenius, 1957; Reale and Bucher, 1962).

Further, electron microscopy has shown that the marked translucency of the centriole region, noticeable in the phase contrast microscope and often also by staining, is caused by the lack of mitochondria, ribosomes and other cytoplasmic corpuscles (Fig. 126, left). All these particles lie, together with a special system of vesicles and canaliculi, the Golgi apparatus (p. 256), at a specific distance from the centriole. This close topographical relationship

between centriole and Golgi apparatus (Amano, 1954; Hanaoka, 1957; Stoeckenius, 1957a; Tanaka *et al.*, 1957; Yamada, 1957; Polycard *et al.*, 1958; and others) is certainly due to the orienting forces of the centrioles, which they exert on their environment even during interkaryokinesis. This arrangement of the cellular components may, in the electron microscope, appear as an oriented ray-like translucency of the cytoplasmic ground substance (Bessis and Thiery, 1961).

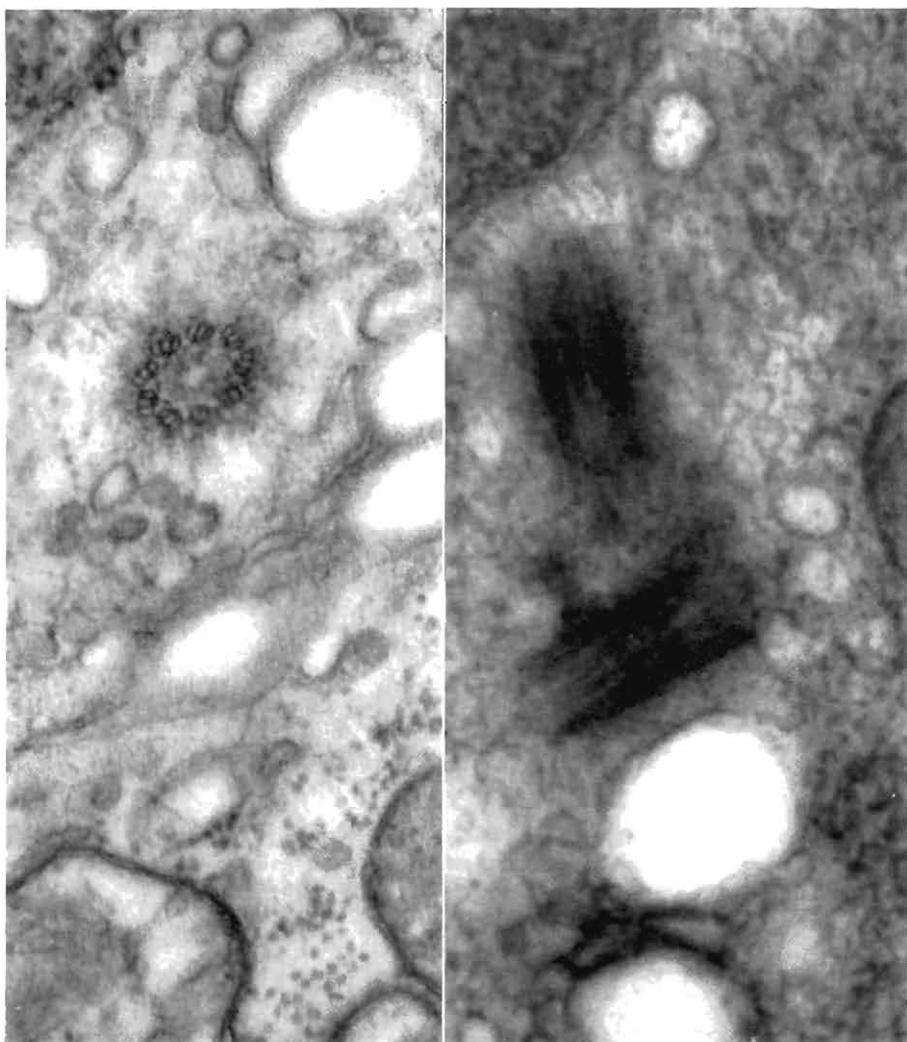


Fig. 65. Electron micrograph of centrioles in mouse lymphoblasts. *Left:* transverse section taken in the vicinity of the vacuoles of the Golgi apparatus; $\times 63,000$. *Right:* longitudinal section of the diplosomes; $\times 82,000$. (From Amano, S. (1957), *Cytologia*, **xx**, 193-212.)

Interkaryokinetic constancy

These findings in the electron microscope have also confirmed the theory, which light microscopists established many decades ago, that the centrioles are not generated anew for each karyokinesis but exist as permanent components throughout the whole life of the cell. This had already been asserted by the discoverers of the centrosomes, Boveri (1887, 1888) and van Beneden and Neyt (1887); it was proved by numerous experiments made on interphase as well as on intermitotic and postmitotic cells. The investigators always found at certain points of the cytoplasm two small granules, the diplosomes (Flemming, 1891b; Heidenhain, 1894; Zimmerman, 1898; and others); these were observed to persist throughout several divisions, not only in the cleavage divisions of *Ascaris* of the horse (see the classical observations by Boveri, 1887, 1888, and by van Beneden and Neyt, 1887) but also in the *Drosophila* egg (Huettner, 1933) and many other specimens (Pollister, 1933; Watts, 1952; and others); always with the same division and function cycle as described above. It is, moreover, typical that the centrioles divide not later than at the end of the anaphase, in most cases already during pro- or metaphase, that is, they are obviously produced only by other centrioles. The question has yet to be solved whether or not there exist any self-reproducing centrioles, such as, for example, in the artificial cytasters of the sea urchin (Morgan, 1896; Buchner, 1911; Dirksen, 1961).

The functional significance of the centrioles lies mainly or exclusively in the stimulation or control of movements. Of this there is evidence not only from their behaviour during mitosis, but also of their close relationship to the basal granules of the flagella and cilia. This relationship was noted at the same time, and independently, by both von Lennhossek (1898) and Henneguy (1898) and is supported by evidence mainly from observations on protists (Chatton and Lwoff, 1924; Belar, 1926; Cleveland, 1938, 1953; and others); thus, the close topographical relation between the basal granules of the flagellar apparatus and the centrioles may be considered the general rule (see, e.g., also Tanaka, 1955). Moreover, the centriole may emerge from the blepharoplast, the formative corpuscle of the flagellum (Andrew, 1925), or the basal granule may assume the role of the centrosome and, vice-versa, the centrosome the role of the basal granule (Ankel, 1924; Sotelo and Trujillo-Cenóz, 1958; and others). Electron microscopy, too, has shown that basal granules and centrioles have the same structure (Fawcett and Porter, 1954; Rouiller and Fauré-Fremiet, 1958; Nanney and Rudzinska, 1960).

There is also a close functional relationship between the centrioles and another structure, the kinetochores, or spindle insertions of the chromosomes (p. 141). According to Pollister and Pollister (1943), centrioles, kinetochores and basal granules are largely homologous. One structure can spring from another or take over the function of another (see also Mazia, 1961a). We shall return to this homology when we discuss the formation of the spindle, for it is a key to this problem. At this point, it is important to note that this homology, too, confirms the theory of the interkaryokinetic persistence of the kinetocentres which we can now consider, generally speaking, as typical movement organelles of the cells.

Polar rays

They are the first evident signs of the activity of the centrosomes at the beginning of karyokinesis and also the precursors of the mitotic spindle, which has certain features in

common with them. With the point of a fine needle it is possible to bend the polar rays sideways (Chambers, 1917) and they even prove to have a certain elasticity (see also Chambers and Chambers, 1961). Polarized light microscopy shows a birefringence of the polar rays (Schmidt, 1936; Swann, 1952; Inoué, 1953) and it seems likely that they are attached to the cell wall, at least in certain cells in which the polar rays go far into the cytoplasm or traverse the cytoplasm completely (Conklin, 1917a; Heilbrunn, 1920; Carlson, 1952).

All these observations point to the fact that polar rays are a reversible arrangement of the cytoplasm molecules, induced by the centrosomes. Maybe oriented molecular "long range forces" are here at work, as considered, for example, for the conglomerations of virus aggregates. The positive birefringence showed under polarized light would then be an "orientation birefringence" (Schmidt, 1937).

Hence, it is no wonder that the orienting effect of the centrioles also appears in the electron-microscopical dimension. In the blastomeres of the sea urchin which were thoroughly examined in the light microscope by Harris (1961), the formation of the polar rays starts with the accumulation of membranous, fine vesicular material in the area surrounding the centrioles. This material seems to be a portion of the endoplasmic reticulum (p. 241). It soon lines up in radially arranged canaliculi, which, however, still retain this fine vesicular character. At this point, we may again give certain credit to the old hypothesis, that polar rays are channels in which a liquid streams centripetally to the centrosomes. For, in the inter-karyokinetic cell, a liquid flows within the canaliculi of the endoplasmic reticulum which is in direct contact with the intercellular substance and which, under the influence of the centrioles, can apparently be oriented in the sense of a parallel arrangement of similarly oriented molecules (see also Pollister, 1941). As of yet, however, this is a mere hypothesis.

Review

Thus, we return to the function of the kinetocentres, from which we had deduced their definition, namely, that they direct and limit the movements of the chromosomes. As we know now, they start exerting their control force even before meta- and anaphase, as soon as the chromosomes become active again, at the beginning of prophase. This force orients the surrounding cytoplasm and probably the membranes of the endoplasmic reticulum in particular. However, in the area surrounding the labile endoplasmic canaliculi, the cytoplasm solidifies, so that the polar rays are able to dissociate the centrioles. During karyokinesis, the centrioles appear mostly as diplosomes within the cytoplasm, and are shown by the electron microscope to lie at a right angle with each other, often in the Golgi region. They consist of nine tubule systems equidistant from one another, each often having three tubules twisted around one another. All observations made hitherto indicate that they reproduce themselves in the process of a generative reproduction. The centrioles are the generative elements of the kinetocentres. It is to be assumed that homologous elements are also present in divisions where no centrioles can be demonstrated, for, as a general rule, kinetocentres are to be considered not as corpuscular, but as functional structures. They appear, in a general sense, to be movement organelles of the cell, since the basal granules of the flagella and cilia are identical with the centrioles or may derive from them. Further, centrioles are evidently closely related to the kinetochores of the chromosomes. This will become more apparent

in our following discussion of the mitotic spindle, the most characteristic structure of the mitotic division.

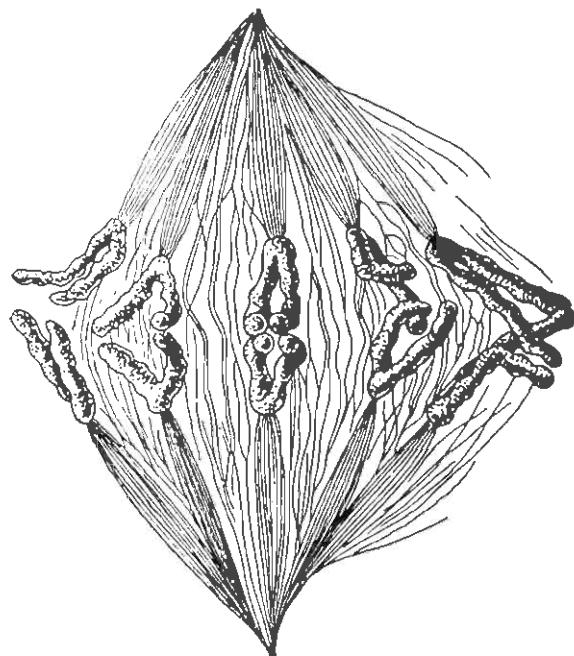
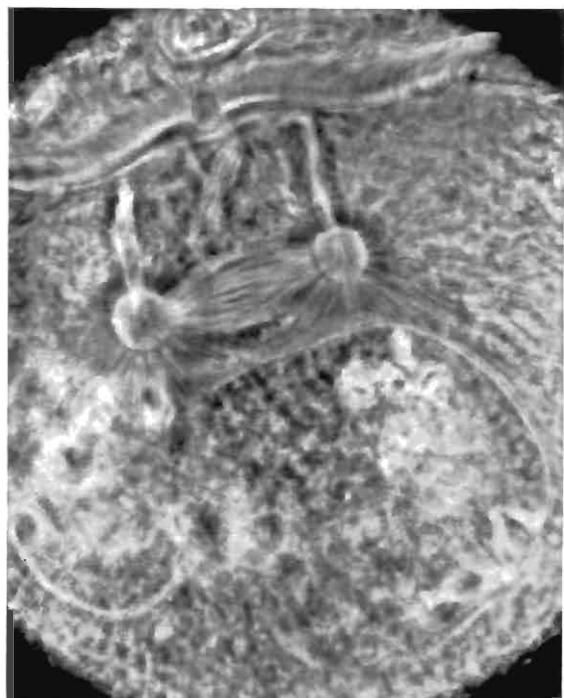
The spindle

The first detailed descriptions of mitosis (Strasburger, 1875, 1880; O. Hertwig, 1876, 1877; van Beneden, 1876; Bütschli, 1876; Balbiani, 1876) already gave data concerning the nuclear barrel or nuclear spindle. It was discovered even before the discovery of the chromosomes, which Flemming for the first time distinguished from the spindle as separate structures. He called chromatin those parts which were deeply stainable with carmine or haematoxylin, and achromatin those which were not, or only slightly, stainable; the mitotic spindle belonged to the latter (Hermann, 1891). This is the origin of the expression achromatic apparatus, still used today to describe the spindle including the pole structures. The spindle is an exclusive and typical structure of mitotic karyokinesis, thus making it possible to infer, from its presence, the very existence of mitosis. When, for example, in certain types of yeast, we see spindles appearing, we can deduce from this fact a mitosis-like type of cell division (Robinow, 1961). The bipolarity of the normal cell division manifests itself morphologically in the shape of the spindle.

Central spindle

The spindle is primarily the product of the kinetocentres. This is clearly shown by the mode of generation of the central spindle (see also Bonnevie, 1910). With the centrioles moving away from each other and the concurrent growth of the polar rays, it emerges in certain cells as a fibrous body (Fig. 66), so to speak, as a particularly enlarged centrodesmose

Fig. 66. Centrosomes from oöcytes of *Diaulula sandigenesis*. Top: division of the centriole with centrodesmose. Bottom: stages in the formation of a central spindle. (From McFarland, F. M. (1897), Zool. Jb., 10, 227-264.)



(Heidenhain, 1907). The primary central spindle, relatively small at first, appears *in vivo* as a light, nearly homogeneous body, free of cytoplasmic granules. It grows gradually during the prophase and becomes a structure often larger than the nucleus, in which meanwhile the chromosomes have taken shape. After the nuclear envelope has disappeared, the chromosomes arrange themselves separately in the equator of the spindle, equidistant from each other, like a peripheral belt (Fig. 68). This is how the complete central spindle emerges, for example in the spermatogonia of the salamander (Hermann,

Fig. 67. Intravitam phase contrast micrograph of a central spindle mitosis in *Barbula-nympha*. The large centrosomes are surrounded by polar rays. From the junction of these rays, the central spindle has emerged in the middle. Note below the nucleus with the nuclear envelope and the chromosomes which have just become visible. (From Cleveland, L. R. (1953), *Trans. Amer. Phil. Soc.*, 43, 809-869.)

Fig. 68. Typical structure of a central spindle at metaphase (lily pollen mother cell). The mantle fibres connect the poles with the chromosomes, the central fibres connect the poles to each other. (From Mottier, D. M. (1903), *Bot. Gaz.*, 35, 250-280.)

1891; Meves, 1897) or in the egg cells of molluscs (MacFarland, 1897), in the mitoses of the locust neuroblasts (Kawamura, 1960b), in certain human brain tumours (Altmann, 1961) and in the large, polymastigina flagellates (Fig. 67). It emerges, at least in most cases, *next to* the nucleus from cytoplasmic material; it is a typical cytoplasm spindle. Neither the nucleus nor its chromosomes are primarily concerned.

Only when the central spindle is completely developed, does it establish contacts with the chromosomes, thus displaying two sorts of fibres (Fig. 68). One type of fibre extends from one pole to the other; those are the continuous fibres or central fibres. They are the direct result of the orienting force of the centrioles. The second type of fibre establishes the relation between centrioles and chromosomes. They are called therefore chromosomal spindle fibres. Since the chromosomes, in the typical central spindle mitosis, adjoin the outer aspect of the spindle, the bundle of fibrils that comprises the chromosome fibres of the central spindle form a kind of mantle (Fig. 68) and have been called therefore mantle fibres (Hermann, 1891). In regard to their much discussed mechanical importance in the mechanism of anaphase (p. 158), they are also often called traction fibres (Drüner, 1895; Boveri, 1900; Bonnevie, 1906); however, this term is a rash anticipation of their function.

Kinetochores

The kinetochores are indispensable for the formation of the chromosomal spindle fibres. Their synonyms such as centromeres, spindle insertions and a whole series of other denominations (see the list summarized by Schrader, 1954), indicate clearly their ambivalence. They are essentially components of the chromosomes, yet their functional importance lies exclusively in their relation to the spindle, since they are the point of insertion of the spindle fibres. They are one of the longitudinally differentiated regions of the chromosomes and we mentioned already the primary constrictions of the chromosomes (p. 104). Their structure is complex. Especially during the meta- and anaphase of the chromosomes in plant cells, a small granule could be seen in the middle of the constrictions (Trankowsky, 1930; Koslov, 1937; Iwata, 1940; and others); it was also observed in certain amphibians (Schrader, 1936, 1939) and suggested a direct relationship to the chromosomes. It was termed spindle corpuscle or spindle granule. In the electron microscope it appears denser than its environment and is, therefore, clearly visible after fixation with osmium (Fig. 69). Histochemically, it contains DNA (Propach, 1940; Iwata, 1940; Coleman, 1940), and even the fine connection strands between the chromosomes and these spindle corpuscles can be Feulgen-positive (Lima-de-Faria, 1950, 1955, 1958). Further studies showed that the kinetochore region often has not one but two small granules (Fig. 70), which divide longitudinally during metaphase together with the chromosome. Since each chromatid often has its own spindle corpuscle, each kinetochore consists of four granules (Tjio and Levan, 1950).

Previously, investigators viewed the formation of the spindle only from the angle of the kinetocentres, merely considering the progressive extending of the polar rays in the direction of the chromosomes, and they could not understand how the spindle fibres find the kinetochores to insert themselves into them. The old term insertion point referred to this mechanism. However, a series of observations has been published since then, requiring a new interpretation.

It has been noticed long ago that if chromosomes break, fragments which contain no

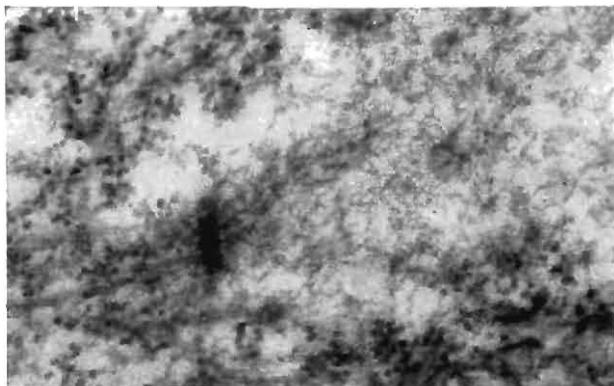


Fig. 69. Electron micrograph showing dense kinetochore from a sea urchin egg at metaphase. To the left of the kinetochore, the chromosomal spindle fibres are seen and to the right, in the lighter area, the fine fibrillar material of the chromosome. Approx. $\times 65,000$. (From Harris, P. (1962), *J. Cell. Biol.*, 14, 475-487.)

kinetochores dissociate from the chromosome package and thus get lost (these are the acentric chromosome fragments). On the other hand, fragments with kinetochores move absolutely normally to the poles.

This rule has many exceptions, especially in insects. For there, a kinetochore need not be located at any particular point; it may occupy the whole length of the chromosome (Schrader, 1935). If we break up these chromosomes, for instance by Roentgen irradiation, all the fragments, even the smallest, behave as centric chromosome fragments (Hughes-Schrader and Ris, 1941; Ris, 1942; Hughes-Schrader and Schrader, 1961; and others). We then speak of a diffuse kinetochore. The same observation was made on plants (Malheiros *et al.*, 1947; De Castro *et al.*, 1949) and on algae (Godward, 1954). Sometimes, the diffuse centromere is a mere fallacy; this is the case when a chromosome is a collective body of many small chromosomes, each of which has one centromere. This gives, for example in the maturation divisions of *Ascaris* of the horse, the same picture as in the diffuse centromere (Painter and Stone, 1935; Schrader, 1935; White, 1936).



Fig. 70. Chromosomes of *Allium cepa* showing two spindle corpuscles in the kinetochore region. (From Lima-de-Faria, A. (1958), *Int. Rev. Cytol.*, 7, 123-157.)

Actually, there is no insertion at all. Instead, the kinetochores are themselves the sites of formation of the chromosomal spindle fibres. Of this we have clear evidence, especially in the diffuse kinetochores; for, in pathological mitoses, each chromosome fragment is able to form its own small spindle (Hughes-Schrader and Ris, 1941). If there is no diffuse centromere, each chromosomal fragment may always develop its own spindle, provided it contains at least one kinetochore (Belling, 1927; Beams and King, 1938). This is one of the origins of multicentric spindles.

Yet the latter can appear also in normal, undisturbed mitoses. Such is the case, for example, in the plant *Drosera rotundifolia* (Rosenberg, 1899); in its root tips, each chromosome forms its own bipolar spindle. This is equally the case in insects (Hughes-Schrader, 1924, 1955;

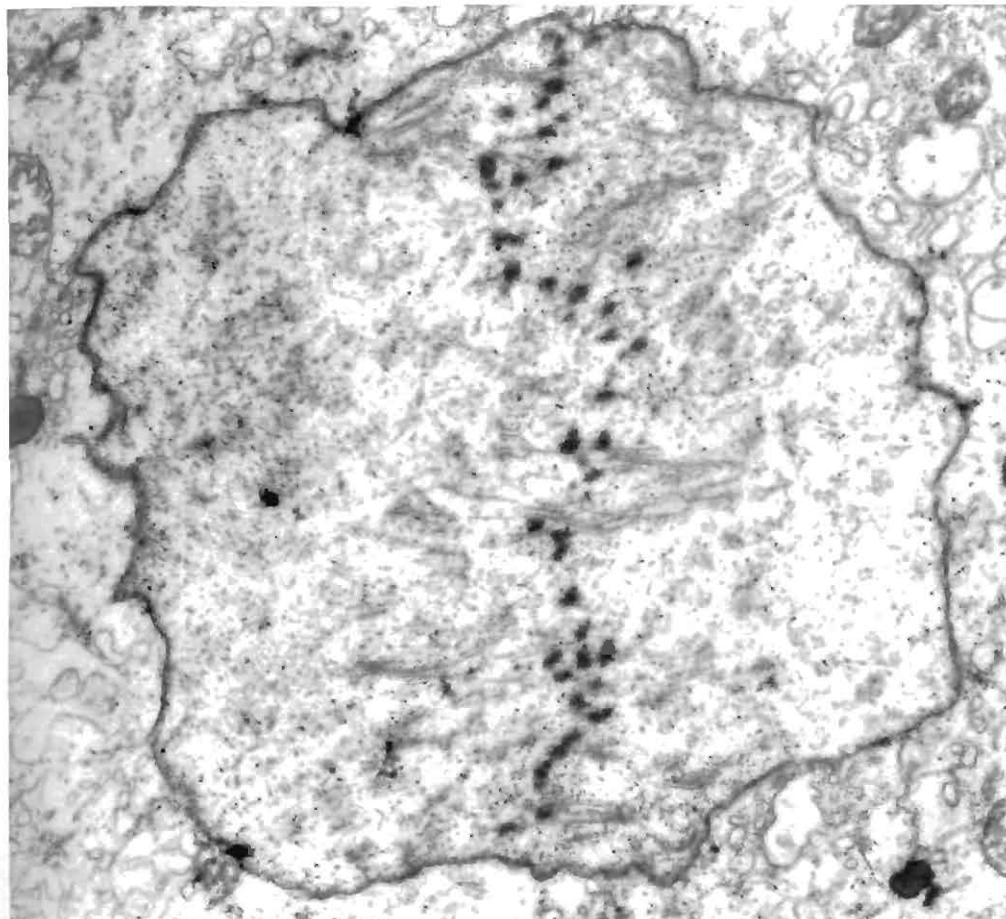


Fig. 71. Electron micrograph of a giant amoeba at metaphase, showing the chromosomes in the middle, the spindle fibres and a fine granulated substance. $\times 15,000$. (From Roth, L. E. and Daniels, E. W. (1962), *J. Cell. Biol.*, 12, 57-78.)

Dietz, 1959) or protozoans (Berkley, 1948), where this fact can be also demonstrated by electron microscopy (Fig. 71). Here we find spindles with blunt poles or "centrosomeless" spindles (see Belar, 1926; Dietz, 1959).

Thus, kinetocentres and kinetochores appear to have the same functions; they form the spindle fibres. This similarity extends so far that, at least in the case of the snail *Vinparus*, the kinetochores may at certain stages of spermatogenesis become kinetocentres and even kinetosomes of the flagella (Pollister and Pollister, 1943). Presumably, all three structures, kinetochores, kinetocentres and kinetosomes, are closely related to one another.

This fact is of significance for the mitotic spindle; for, in the same way as the formation of the central fibres is a result of the bipolar orientation of the cytoplasmic elements between two kinetocentres, similarly, the chromosomal spindle fibres emerge between the orientating forces of the two poles; one pole is the kinetocentre, the other, the kinetochore. Thus, the old question of how the spindle fibres insert themselves into the kinetochore is redundant.

The chromosomal spindle

Now we understand how the spindle is formed in the majority of karyokineses where there is neither a typical central spindle nor a spindle which emerges solely from the kinetochores. Since, in contrast to the central spindle, it generally includes the chromosomes right from the beginning, we call this type of spindle chromosomal spindle. The above-described differing spindles (Fig. 71) are merely extremely special cases of the same principle, since one may assume that kinetocentres—even though multicentric—are, here too, at work (Mazia, 1961a).

The formation of this spindle follows variable patterns according to the different cells. It depends on the shape and the primary location of the kinetocentres, on the extent of the polar rays and on the shape of the cell. It is often quite impossible to follow the different stages of its development and frequently, especially *in vivo*, the spindle appears suddenly when the conditions are favourable.

In large egg cells, that are about to divide after fertilization, it is fairly easy to follow the formation of the spindle. Once the centrosomes of what will later be the spindle poles have grown to a volume characteristic of their activated stage, the polar radiation embraces the prophase nucleus from all sides and forms a kind of central spindle, including, however, the nucleus. Then the nuclear membrane breaks down and at this point the chromosomal spindle fibres appear. In many cases, well supported by evidence, this process does not begin at the

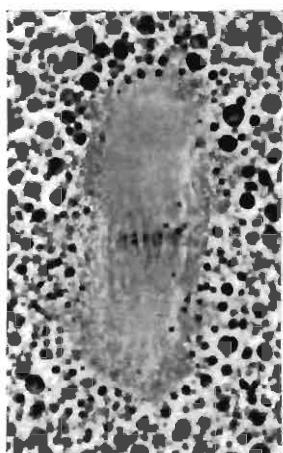


Fig. 72. Metaphase of a *Tubifex* egg, stained with haematoxylin erythrosin. The spindle fibres are visible only at the chromosomes (From Specht, W. (1961), *Z. Anat.*, 122, 266-288.)

centrosomes, but at the chromosomes, and continues from there in the direction of the poles (see, e.g., Specht, 1961). Thus, we may observe stages in which spindle fibres appear only in the chromosome region (Fig. 72). The spindle gradually expands in all directions and reaches its full size at the end of the metaphase.

In any case, the chromosomal spindle fibres are at least partly of nuclear origin. The central fibres, which extend from pole to pole, may, depending on the location of the kineto-centres, emerge from cytoplasmic or also from nuclear substance. The majority of mitotic spindles may combine substances from both these elements of the cell. Since both types of fibres result from orienting impulses from the kinetocentres or the closely related kineto-chores, we must consider them to be basically equal. The chromosomal spindle fibres are frequently bundled together (Fig. 68), which makes them all the more visible. However, all fibres are of a temporary nature and the duration of their presence as well as their location is strictly a function of the mitotic karyokinesis. The hypothesis that a permanent connection of fibres also exists during interkaryokinesis between kinetocentres and kinetochores (Lettré and Lettré, 1958; Resende *et al.*, 1959) may elucidate certain problems of karyokinesis (Lettré, 1961); however, it lacks as yet objective evidence.

Fine structure and composition

In the foregoing we have simplified to a great extent. We spoke of spindle fibres extending between the poles or from pole to chromosome—and now to analyse exactly the nature of these fibres, we are suddenly faced with the task of proving first of all that fibres here actually exist. For these structures are, indeed, most peculiar; they are present, at least in their typical shape, only during meta- and anaphase, and later vanish more or less completely, ready to appear again with their full characteristics in the next karyokinesis. This fact alone contradicts the theory that they are of a genuine fibrillar nature comparable to that of the connective tissue or muscle fibres. Moreover, these temporary connections are fairly unstable elements, which disappear under extreme temperatures of under 5°C and over 35°C (Inoué, 1952), and also, when exposed to mechanical (Seifritz, 1936; Marsland, 1951) or hydrostatic pressure (Pease, 1941, 1946) as well as in a strongly hypotonic medium (Lewis, 1934) or in mercaptoethanol of low molarity (Mazia, 1961*a* and *b*). They then reappear when the conditions become normal again.

Moreover, observations of the spindle fibres *in vivo*, without any pretreatment, is possible only in particularly good specimens, such as for example the polymastigina flagellates, demonstrated by Cleveland (1938, 1953, 1957*a* and *b*) (Fig. 67), or in relatively large, cytoplasm-rich cells (e.g., Hughes-Schrader and Ris, 1941; Cooper, 1941; Tahmisian, 1951; Nath *et al.*, 1954). In most cases we can only see a homogeneous, empty space (Wilson, 1899; Lundegardh, 1912; Chambers and Sands, 1923; Martens, 1929; Belar, 1929; and others), even in the phase contrast microscope (Austin and Smiles, 1948; Hughes and Swann, 1948; Ludford *et al.*, 1948; Zollinger, 1948*a*; and others).

Under polarized light we can demonstrate that the substances of which these fibres are composed are, indeed, longitudinally oriented. The spindle substances always show a positive birefringence parallel to the axis of the spindle (Runnström, 1928; Schmidt, 1937, 1939; Pfeiffer, 1939, 1951; Swann, 1951*a*, 1952; Inoué, 1953; Inoué and Bajer, 1961; and others). The same conclusion must be drawn from the observation that, after experimental

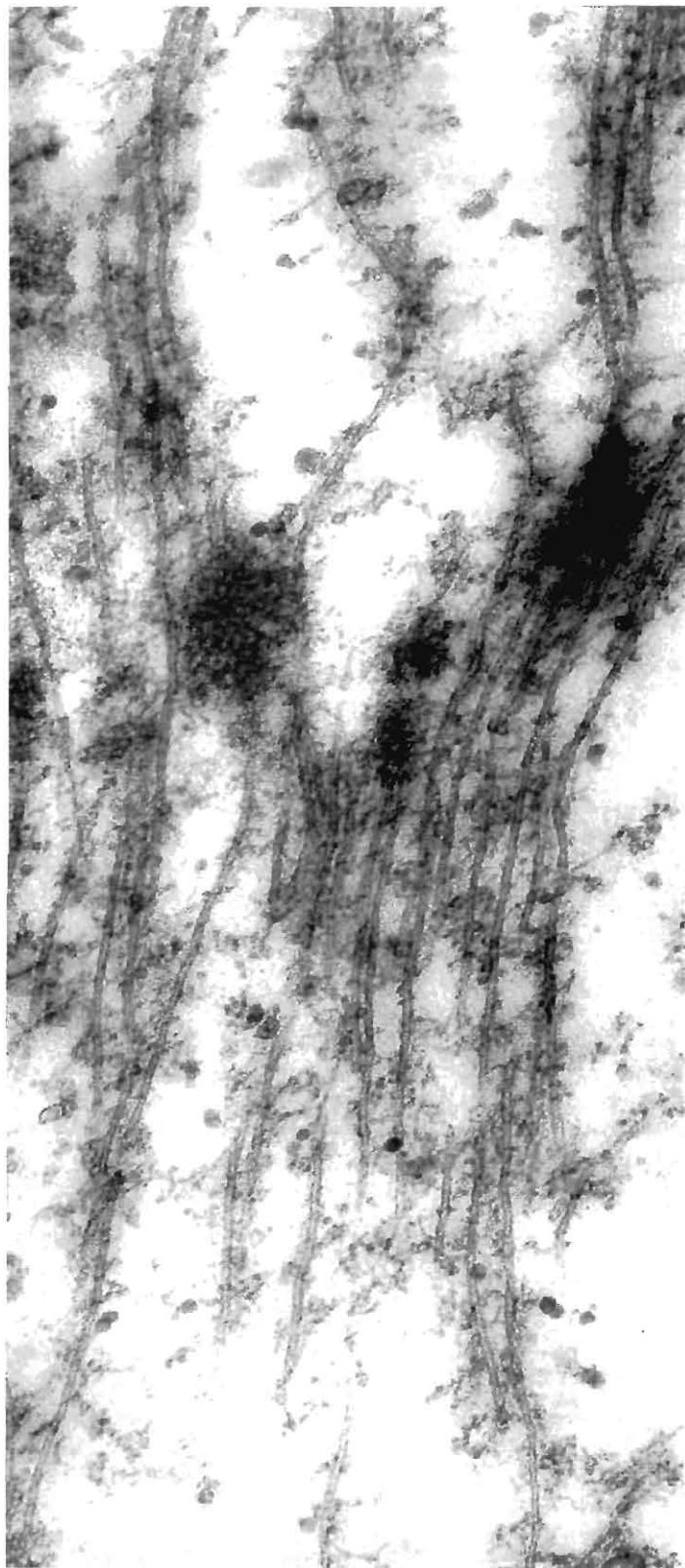


Fig. 73. Electron micrograph of the metaphase spindle fibres of a giant amoeba, showing ribosome-like particles and smaller granulated material at the spindle fibres. $\times 70,000$. (From Roth, L. E. and Daniels, E. W. (1962), *J. Cell. Biol.*, 12, 57-78.)

shrinkage in a hypotonic medium, clefts appear within the spindle running always parallel to the spindle axis and never obliquely, let alone vertically to it (Belar, 1927).

If this can be considered only as an indirect evidence of the longitudinal orientation of the spindle elements, thin-section electron microscopy has brought a clear confirmation of this. Comparable to the polar rays which are nothing else but radial channels, the spindle contains tubuli, surrounded by two filaments; these tubules connect, as chromosomal spindle fibres, the centrosomes with the kinetochores, and, as central fibres, they extend from pole to pole, passing between the anaphase chromosomes (Fig. 73). The particularly labile nature of the spindle fibres is again observed in the electron microscopical dimension, making it impossible from the start to distinguish them, for example, from the fibrils of connective tissue or muscle cells. Without the addition of bivalent cations to the osmotic acid, only small particles can be seen, but no threads (Roth and Daniels, 1962).

These fine fibrils, however, are not the only elements present in the spindle area. We frequently find between them granules of 30–40 m μ in diameter (Fig. 71 and 73) (Harris, 1962; Roth and Daniels, 1962); in certain cases, as for instance in the Walker carcinoma, large lamellae consisting of one or many layers are interspersed between the spindle fibres, which have the appearance of paired cisterns of the endoplasmic reticulum (Buck, 1961). These lamellae have, on their exterior aspect, ribosome-like granules and evidently play a part in the formation of the nuclear envelope at telophase. Similar ergastoplasmic elements have been found in many other cells within the spindle (Ruthmann, 1958; Gross *et al.*, 1958; Ito, 1960; Porter and Machado, 1960). Other, much smaller granules, averaging 2–6 m μ , surround the spindle fibres, for example in a giant amoeba, as cloudy ill-defined deposits (Fig. 73). This is possibly the same material as that of which the spindle fibres themselves are made, only in an unoriented state (Roth and Daniels, 1962).

What sort of a material is this?

All recent staining results (e.g., Stich, 1951a; Yokoyama *et al.*, 1953; Shimamura and Ota, 1956; Love and Liles, 1959) and the ultraviolet absorption (Davies, 1952) indicate that the spindle contains RNA; measuring of X-ray absorption (Stich and McIntyre, 1958) and chemical analysis (Zimmermann, A., 1960) show that this RNA accounts for 5–6% of the total spindle mass. However, it is not certain whether it is really a component of the spindle fibres. The inclusions of endoplasmic reticulum with their ribosomes are indeed fully sufficient to explain the above given RNA content. There are elements which are certainly not essential to the spindle, and these are the often demonstrated lipids (Shinke and Shigena, 1933) which equally may be attributed to the inclusions of endoplasmic reticulum (Mazia, 1961a). What is not clear, is the origin of PAS-positive substances in the spindle.

A great step forward was made when Mazia and Dan (1952) were able to isolate large quantities of spindles from sea urchin eggs and examine them chemically. The methods of investigation have been much improved since then (Mazia, 1955, 1960; Mazia *et al.*, 1961; Kane, 1962), however, with great difficulty, owing to the above-mentioned labile nature of the spindle fibres. The material obtained is to a large extent free of foreign elements and contains the kinetoctres with the polar rays, the spindle and the chromosomes (Fig. 74). This complex was called the mitotic apparatus.

The first finding made by means of this isolation technique has also been hitherto the most impressive. 90% of this mitotic apparatus consists of protein, mainly of only one type

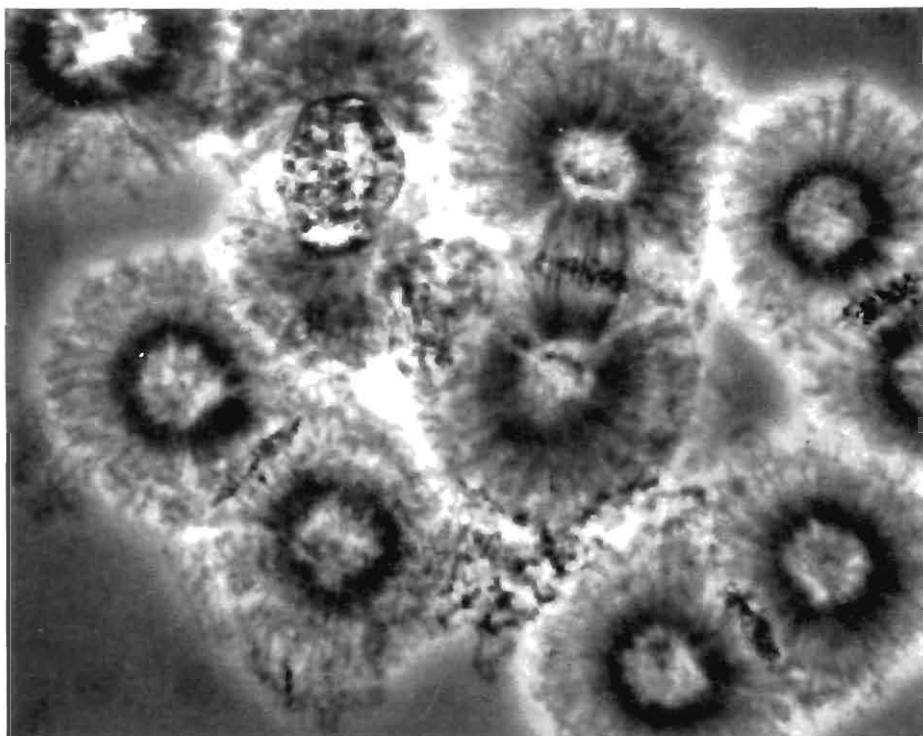


Fig. 74. Isolated "mitotic apparatus" of sea urchin eggs. (From Mazia, D. (1961), *The Cell*. Vol. III. Acad. Press, New York.)

of protein (Mazia and Dan, 1952) with a molecular weight of $315,000 \pm 20,000$ (Zimmermann, 1960), an isoelectrical point at pH 4.5 and an amino-acid ratio which is similar to that of the actin in the mammalian muscle or of the proteins in isolated flagella of flagellates (see Jones and Lewin 1960). With immune chemical methods, however, at least four different proteins were shown to be present in the mitotic apparatus (Went and Mazia, 1959; Went, 1959; Mazia, 1961a and b), which is not surprising, considering its heterogeneous composition. We may assume that proteins with SH-groups play a special part; for, in the sea urchin egg, the amount of SH-groups soluble in trichloracetic acid increases markedly during the formation of the spindle and decreases thereafter (Fig. 75). Even though Rapkin's original assumption (1931), that we may be dealing here with a glutathione cycle, has proved false (Sakai and Dan, 1959), the evidence of an SH cycle has been repeatedly confirmed (e.g., Sandritter and Krygier, 1959; Hase *et al.*, 1959; and others). Cytochemistry shows that the spindle fibres and the polar rays are rich in SH-groups whereas the inner part of the centrosomes display predominantly SS-groups (Kawamura, 1960a).

Since thiols usually have an effect on the polymerization processes of the cell and may establish intermolecular bonds, the possibility of a formation of fibrils cannot, in this case,

be excluded. Of course, other intermolecular bonds, for instance hydrogen bonds (Waugh, 1954), may be of equal or even greater importance.

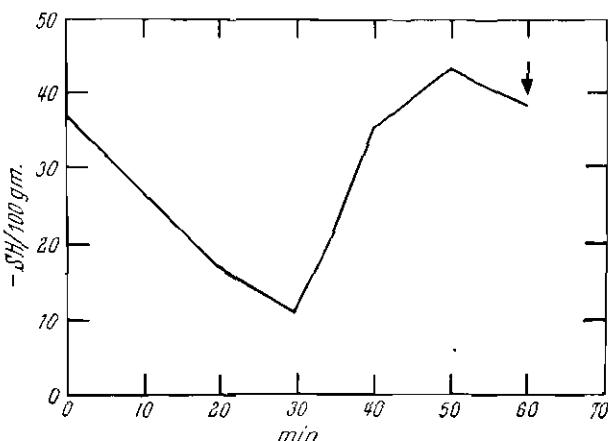


Fig. 75. Behaviour of the SH groups in the sea urchin egg. Decrease in number of the SH groups before the first division, and increase during the formation of the mitotic spindle. The arrow indicates the beginning of cytokinesis. (According to Rapkin's findings, after Mazia, D. (1961), *The Cell*, Vol. III. Acad. Press, New York.)

Review

We do not have yet a more precise knowledge of these intermolecular bonds which hold the spindle together as a relatively isolated structure. However, we know that the spindle is made of labile fibrils, which were demonstrated in the light and the phase contrast microscope, in the polarization microscope and, best of all, in the electron microscope. They are due to the orienting forces of the kinetocentres and kinetochores and consist probably, for the most part, of one single type of protein. This fact is true of the chromosomal spindle fibres, that connect the chromosomes with the poles as well as of the central fibres, that run from pole to pole. Both types of fibres are to be found in all forms of spindles, whether the mass of spindle substance comes from nuclear or from cytoplasmic material, whether we are dealing with a central spindle, on whose exterior aspect the chromosomal spindle fibres are attached as mantle fibres, or whether we have a chromosome spindle, which primarily envelops the chromosomes. The various types of spindles and the process of their development are an indication that kinetocentres and kinetochores—probably together with the kinetosomes or basal granules of the cilia and flagella—are homologous structures, the kinetochores playing, however, frequently the decisive part in the formation of the chromosomal spindle fibres.

Movements of the chromosomes

Two movement phases are to be distinguished (see Fig. 59). One directs the chromosomes out of the prophase entanglement into the equatorial plate; this is the prometaphase rearrangement (Flemming, 1880). The other distributes the chromosomes' longitudinal halves to the spindle poles; this is the chromosome migration at anaphase. Between both movement phases there is a phase of relative rest, the metaphase, in which the chromosomes remain

for a variable length of time. This phase is as necessary for the understanding of the movements of the chromosomes as are the introductory prophase and the concluding telophase.

Prophase

It begins with the condensation of the chromosomes, visible at first only in certain places, and ends with the first movements for rearrangement. The transition from interkaryokinesis occurs so gradually that it is impossible to determine the exact beginning of prophase. The breakdown of the nuclear envelope is often taken as a marker for the starting of karyokinesis. However, there are many karyokineses with intact nuclear envelope, such as in the heliozoon or the polymastigina flagellates (Cleveland, 1949, 1957a and b). Also, the disappearing of the nucleoli is no obligatory feature (p. 132). Lastly, even the condensation and coiling of the chromosomes cannot be considered as a general criterion for the beginning of prophase, since the chromosomes of the previously mentioned holomastigina flagellates are condensed and coiled during the whole interkaryokinesis. Evidently, there is no distinct separation of interkaryokinesis and prophase and the passage from telophase to nuclear reconstruction is equally imperceptible.

Thus the data on the duration of prophase vary to a great extent, ranging from 10 minutes in sarcoma cells (Mazia, 1961a) to 102 minutes in the locust neuroblasts (Carlson, 1938) over a series of intermediate time intervals (see Table 6). On the average, it takes a cell 40 to 45 minutes, from the beginning of prophase condensation, to reach the stage of the metaphase plate, i.e., including prometaphase.

TABLE 6. Duration of mitotic phases (in minutes) according to observations *in vivo*

	Prophase	Metaphase	Anaphase	Telophase
Chicken fibroblasts (After Lewis and Lewis, 1917)	30-60	2-10	2-3	3-12
Plant cells (After Martens, 1927)	36-45	7-10	15-20	20-35
Locust neuroblasts (After Carlson and Hollaender, 1948)	102	13	9	57
Salamander fibroblasts (After Hughes and Preston, 1949)	> 18	17-38	14-26	28
Plant endosperm (After Bajer and Molé-Bajer, 1953)	40-65	10-30	12-22	40-110
Sarcoma cells (After Mazia, 1961a)	10	44	5	18

The first impression one has of an early prophase, is that of a complicated entanglement and this is how Flemming (1882, 1892b, 1897) described it. He assumed that the chromatin existed as a continuous spireme, that is, as one uninterrupted thread. Only by further condensation do the chromosomes become separately visible. In many plant cells there is yet another stage prior to this, in which the chromosomes are still relatively little light-refracting,

but their spiral structure is particularly clear; this stage has, therefore, been called "spiral prophase" (e.g., Belar, 1930b; Straub, 1938).

Also previous to the chromosome condensation, the nucleus increases in size, presumably owing to the absorption of water from the surrounding cytoplasm. Moreover, by measuring the X-ray absorption in cyclops eggs, it has been possible to demonstrate a considerable increase in the dry weight of the nucleus prior to the beginning of the visible prophase (Stich and McIntyre, 1958); radioautography showed that the RNA turnover had concurrently increased in the nucleus (Moses and Taylor, 1955). This material is neither incorporated into nor released by the chromosomes; in other words, it is a non-chromosomal nuclear substance. Of this extra-chromosomal nuclear material are made those parts of the spindle which come from the nucleus, namely, in plants the majority of chromosomal spindle fibres, and in animals at least a great part of them. This is Boveri's archiplasm (1888), Hermann's achromatin (1891), Strasburger's kinoplasm (1900), Bleier's paragenoplastin (1930) or whatever the spindle substance might be called. It is interesting to note that in the amitotic division of the polyploid macronucleus of *Paramecium*, extra-chromosomal nuclear ground substances are also present (Schwartz, 1957).

The beginning of prophase has another characteristic. The nuclei frequently rotate inside the cell or execute a peculiar oscillating movement (Leone *et al.*, 1955; Moorhead and Hsu, 1956), of which we do not know the significance.

After the above-described increase in volume, the size of the nucleus often decreases suddenly, just before the beginning of the movements for rearrangement. This contraction stage, however, has hitherto been observed with certainty only in plant cells (Bajer, 1954; Bajer and Molé-Bajer, 1956); the contraction may compress the entangled prophase mass evenly on all sides or only at the poles. Simultaneously, the spindle fibres appear between kineto-centres and kineto-chores (Bajer and Molé-Bajer, 1956), which now take over the leading role in the chromosomal movements; nuclear envelope and nucleoli disappear, the rearrangement begins.

Prometaphase

One can only speak of rearrangement when a previous arrangement is being converted into a new one. For, in fact, the chromosomes at prophase are only seemingly clustered together in a complicated entanglement (Flemming, 1879, 1882). When for instance in the larva of the young salamander the chromosomes become separately visible at the beginning of karyokinesis, they appear in a very definite array. With their apex at the site of the kineto-chores, they are oriented to one side of the nucleus. Their apices lie closely together and form the pole region of the nucleus (Rabl, 1885). Of course, this "Rabl-orientation" (Fig. 76, left) cannot be generalized (see Manton, 1935; Wolf, 1940), although it has been observed in a great many specimens from animals and plants (Meves, 1898; Heidenhain, 1907; Belar, 1930b; Schneider, 1933, 1938; Geitler, 1935; and others); it corresponds, moreover, to the anaphase or telophase array from the previous karyokinesis (Schrader, 1954). And, indeed, after completion of the anaphase movements, the chromosomes evidently do not notably change their position within the nucleus, which is another evidence of the continuity of the chromosomes' individuality (p. 72). The diplosome can be found lying in the depression of this pole region, thus bearing the proof, so to speak, that it originated from one of the spindle

poles of the previous karyokinesis. However, it is also frequently connected with the nucleoli, for, in several tissue culture cells (Fell and Hughes, 1949) or in plant root meristems (see Fig. 29), the chromosomes are, in the early prophase, oriented radially toward the central nucleolus.

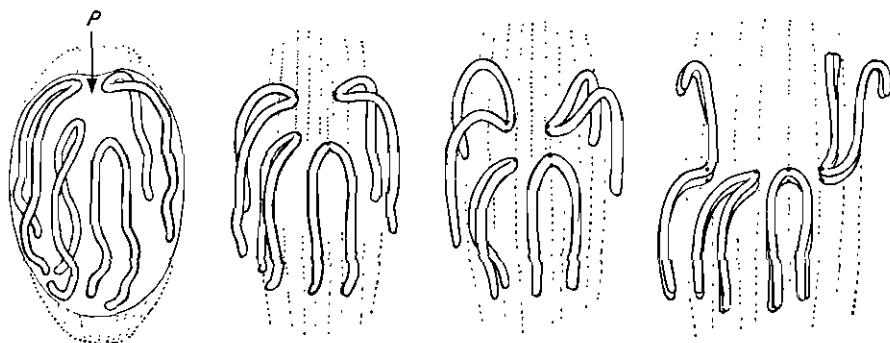


Fig. 76. Series of schemes showing the behaviour of the chromosomes during metakinesis. Note how the apex of the chromosomes bends from the polar region (P) towards the equatorial plane. (From Belar, K. (1930), *Z. Zellforsch.*, **10**, 73-134.)

This prophase arrangement of the chromosomes is the starting point of the prometaphase rearrangement or metakinesis (Wassermann, 1926, 1929). If the situation of the pole region at prophase corresponds to that of a later stage, the apices of the chromosomes have only to bend toward the middle of the cleavage area, in order to reach a median plane between the spindle poles, called the equatorial plane (Fig. 76). This process was observed many times in plant mitoses (Belar, 1930b; Schneider, 1933). In some cases, however, where the prophase pole region is not situated at the same site as one of the later spindle poles, it has been assumed that the preprophase nucleus rotates (Gurwitsch, 1926; Schneider, 1938) and such a preprophase rotation has, indeed, later been demonstrated many times (Hughes and Swann, 1948; Fell and Hughes, 1949; and others).

The more recent observations *in vivo* on plant cells during endosperm mitosis (Bajer and Molé-Bajer, 1956), on fibroblast cultures (Zirkle, 1957) on orthoptera (Nicklas, 1961) and especially on tipulid spermatocytes (Dietz, 1956; Bauer *et al.*, 1961), have complicated the picture to a great extent. We can note three particularities:

1. The rearrangement is not a uniform migration. It appears that the kinetochores oscillate backward and forward, getting closer first to one then to the other pole, and that they assume only fairly late an intermediate position between the poles (see also Schneider, 1933; Schrader, 1947; Zirkle, 1957; and others).
2. The arms of the chromosomes move also frequently and may be directed first toward one pole, then toward the other.
3. Every chromosome has its own, its individual behaviour, so to speak. This is true of the kinetochores as well as of the chromosome ends (Fig. 77). Only the general direction of the migrations is uniform, which brings the kinetochores into the equatorial plane and the chromosome ends longitudinally alongside the spindle axis.

Every attempt to explain the mechanism of rearrangement must take these particularities into consideration. For example, the theories of Lundgardh (1912) and Wassermann (1926, 1929, 1939) who believed that a bilateral pressure exerted on the entangled prophase mass pushes the chromosomes into the equatorial plate, as their condensation continues, has proved to be insufficient. On the contrary, the individual behaviour of the chromosomes during rearrangement leads us to assume that a force is exerted on each chromosome in particular, and this force most probably exerts itself via the chromosomal spindle fibres.

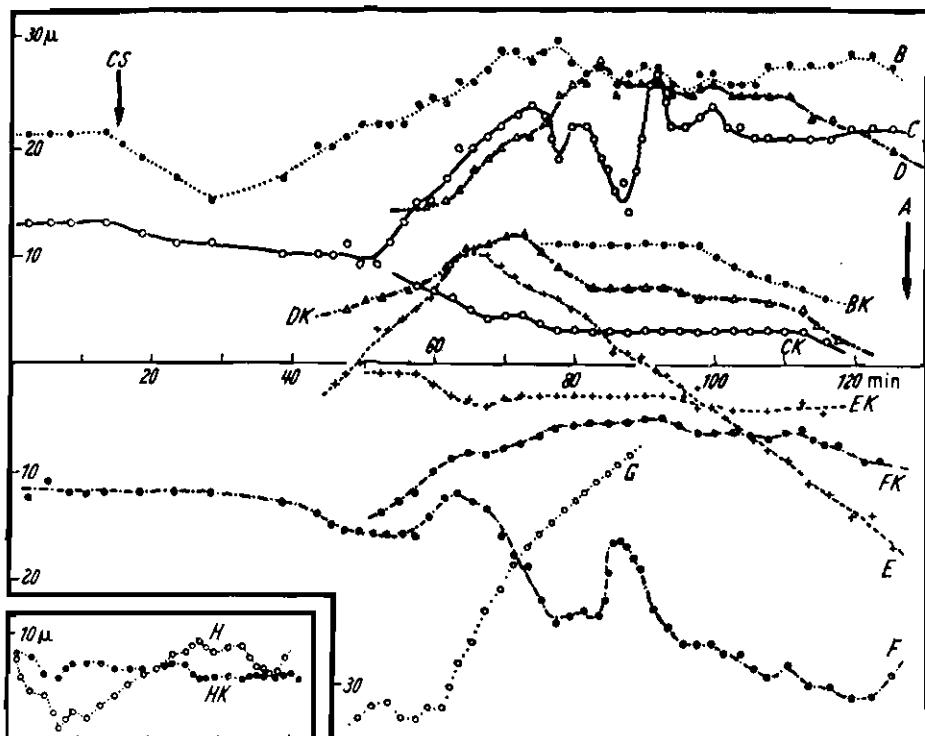


Fig. 77. Behaviour of chromosomes in endosperm of *Haemanthus Katharinae* and *Clivia cyathiflora* (H and HK), from the contraction stage (CS) to the beginning of anaphase (A). The curves depict the distance of the tips of the longer chromosome arms (B-D) and the kinetochores of these chromosomes (BK-FK) from the median line of the equatorial plate. The kinetochores oscillate backwards and forwards, and even more so the chromosome tips, some of which change direction (for instance E). (From Bajer, A. and Molé-Bajer, J. (1956), *Chromosoma*, 7, 558-607.)

Here is, indeed, the clue to the problem. We have discovered that the formation of the spindle fibres resulted from the complementary interaction of two orienting forces; now we must add that the latter always try to establish connections at the shortest possible distance, that is, in a straight line. Since the kinetochores are paired, so to speak, back to back, they

will, according to this principle of the shortest distance, assume quite naturally a median position between the spindle poles, namely, in the equatorial plane. This shortest possible distance may vary, depending on certain factors, such as for instance the spindle body itself which, in the case of typical central spindles, presses the chromosomes to the circumference of the equatorial plane, thus disposing them into a kind of garland (Fig. 68).

It evidently takes some time before both opposing chromosomal spindle fibres come to a state of balance (Östergren, 1949) and this may at first cause undirected movements of the kinetochores (Fig. 77) which can be considered the result of alternating pushing and pulling forces (Östergren, 1950a).

The part played by the kinetochores could also be clearly demonstrated. An ultraviolet irradiation concentrated specifically on the kinetochore may prevent (Uretz *et al.*, 1954; Zirkle, 1957) or at least complicate (Bajer and Molé-Bajer, 1961) the arrangement of the chromosomes involved. If we also include here the behaviour of meiotic chromosomes, kinetochores seem still to play the major role and all movements can be explained exclusively by a kinetochore activity; the movements then are caused by the constant incorporation of fibre substance by the chromosomal spindle fibres, counteracted in turn by a spindle contraction (Dietz, 1956, 1958).

In the meiosis of certain animals, particularly of insects, bivalent chromosomes show during prometaphase a most interesting behaviour. Prior to their migration into the equatorial plate, they often stretch out toward the axis of the spindle (Fig. 78). This "prometaphase stretching" (Hughes-Schrader, 1943, 1947) results from an apparent contraction of the chromosomal spindle fibres at a time when the chromosome arms are still firmly linked together; it is, so to speak, a premature activation of the anaphase mechanism, which soon ceases again, and by means of which the kinetochores reach the equatorial plane. It is quite possible that the polar traction forces, which we will discuss later, have their maximal intensity during prometaphase and are reduced to a minimum at metaphase (Dietz, 1956).

Metaphase

The prometaphase oscillations of the kinetochores, which are frequently quite extensive, are by no means followed by a metaphase period of inactivity. All observations on living cells, and, principally, the first filming of a karyokinesis by Michel (1943) with the aid of a phase contrast microscope, show that the chromosomes go on oscillating at the equatorial plane (see also Lewis, 1951; Hughes and Swann, 1948; Michel, 1950; Bajer, 1954; Bajer and Molé-Bajer, 1956; Moorehead and Hsu, 1956; Dietz, 1956, 1958; Bauer *et al.*, 1961; and others).

This is most easily understandable when we assume that the forces which forwarded the chromosomes into the metaphase plate during the rearrangement are still active and maintain each other in a state of bipolar balance. Such an explanation suggests itself readily and has been stated fairly often by many authors (Drüner, 1895; Meves, 1897); Östergren (1945, 1948, 1950a, 1951) then built up a theory from it. On account of the traction on the chromosomes by the kinetoctres, so evident for example in the prometaphase stretching (Fig. 78) and many other movements, an equilibrium would seem particularly plausible if the intensity of the traction increases, the more kinetocentre and kinetochore are distant from each other, i.e., the traction is particularly strong in the equatorial plane. When a chromosome moves

away from a pole, for instance during an oscillation, the traction force increases, causing the chromosome to swing rapidly back; incidentally this may result in an excessive increase of the opposing force. Yet, as we have already mentioned, this explanation, however tempting, can only be a hypothesis. Recent observations on living cells indicate that during metaphase, the chromosomes are subjected only to minimal forces (Dietz, 1956, 1958; Bauer *et al.*, 1961). There is evidence, too, from the oscillations of the chromosomes during metaphase; if they were the result of the action and counteraction of opposed forces, they would decrease progressively and cease completely after a while. This has, as yet, never been authentically observed.

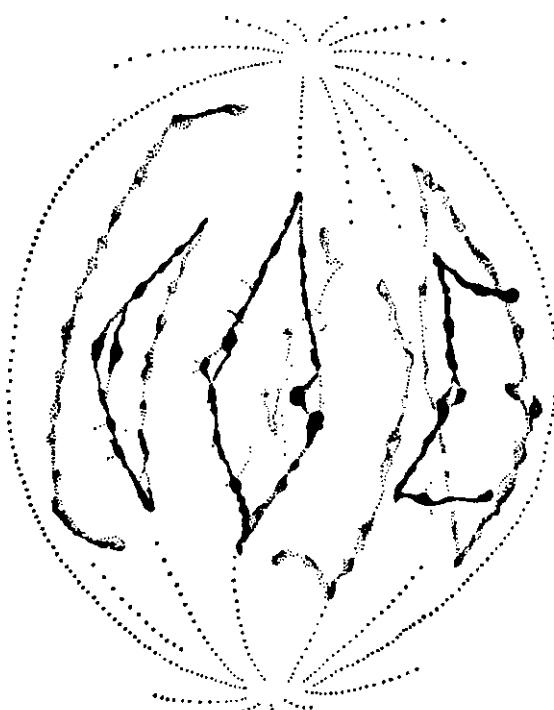


Fig. 78. "Prometaphase stretching" in the first meiotic division of a mantis. Migration of the homologous kinetochores to the opposite poles and stretching of the paired chromosomes. (From Hughes-Schrader, S. (1953), *Biol. Bull.*, **85**, 265-300.)

It is somewhat easier to explain why metaphase chromosomes never touch each other but often lie equidistant from one another. This can be the result of an electrostatic repulsion (Lillie, 1905; Darlington, 1937) or of surface tension (Schrader, 1947), but it can also be due to a special envelope, called pellicula, which surrounds the chromosomes when they are in a state of condensation. It is not yet clear whether there is, in addition, a kind of transversal equilibrium between the attachment of the chromosomes on the chromosomal spindle fibres and a centrifugal force (Östergren, 1945).

Anaphase

No chapter of cell research has been studied with as much intensity, and hardly any process has stimulated as many hypotheses as the movement of the chromosomes towards the poles, called anaphase. Since during this phase the final division process takes place, it has, ever since the discovery of mitotic karyokinesis, raised the most interest, especially as this mysteriously well-ordered movement follows basically the same pattern in all karyokineses; it is apparently everywhere subject to the same conditions.

We must first point out that the mechanisms of anaphase movements can be released only when the daughter chromatids of the metaphase chromosomes are separated from each other. This separation of the chromatids is in itself an independent process; for it can take place without anaphase and without the interkaryokinetic structures, for example in endomitosis (p. 221). We also know cases of monopolar karyokineses, for instance in the artificial parthenogenesis of sea urchin eggs, whereby the lack of a second pole prevents the anaphase movement, but the division of the chromatids comes to a perfect completion (Belar and Huth, 1933).

The traction mechanism

As a rule, kinetochores take the lead in the anaphase migration, drawing along the chromosome arms, so that they form an acute angle, especially in the case of a median spindle insertion. The tips of the daughter chromosomes or telomeres may, at this stage, stay still attached to each other for a long time (Melander, 1950). The picture resembles that of meiotic prometaphase stretching (p. 155). As to what causes these movements, we can refer to the same explanation as above, when we traced down the prometaphase stretching to what at least looks like a contraction of the chromosomal spindle fibres. We may add that the tension of the chromosome arms suggests the presence of a force which stretches them (Fig. 78).

And, indeed, we find much evidence of this in many details of the anaphase process. Thus, the two spindle corpuscles (Fig. 70) of each kinetochore are evidently drawn toward the poles at the beginning of anaphase, so that only a narrow thread connects them with the chromosome arms. This has been observed many times in animal (Grassé, 1939; Melander, 1950) as well as in plant chromosomes (Schrader, 1939; Propach, 1940; Iwata, 1940) and it clearly gives the impression that the chromosomes are pulled apart by an outward force; at the same time, however, they show a certain resistance to this separation.

The same interpretation can be given in the fairly frequent cases where chromosomes stick to each other as an effect of various chemicals or of ionizing rays; in such cases, the kinetochores proceed first in a well-ordered movement toward the poles, but the chromosomes adhere firmly to each other at some point or another. Thus, the chromosome arms get stretched out (Fig. 79) until one of them breaks. The broken off arms then snap rapidly back again. The result is a defect in the chromosome set, for now a portion of one chromosome is lacking in one of the sets and is supernumerary in the other one. Sometimes, fragments are broken off



Fig. 79. Adherences between two chromosomes of *Bellevalia romana*. Note that the adhering chromosomes are greatly stretched. (Courtesy E. Gläß.)

both chromosomes and remain in the equatorial plane or are rejected from the spindle. There can hardly be any other interpretation for such observations (see also Cornmann, 1944) but that a traction force is exerted on the chromosomes. This is why the chromosomal spindle fibres are frequently called traction fibres.

But what is the origin of this force?

Since the whole spindle is, generally speaking, like a flexible elastic body, it has been readily proposed to consider the spindle fibres as elastic threads (e.g., Shimamura, 1940; and others). On this conception was based Östergren's theory (1945, 1950a) of a mechanical equilibrium during metaphase (p. 155).

However, two series of facts make it impossible to apply this theory to anaphase. For one thing, the chromosome migration to the poles is much too slow to be the result of a mere traction. In the endosperm of plant cells, for example, it takes the chromosomes more than 30 minutes to complete their movement (Fig. 80)—in this case, however, the special conditions of preparation and observation must be taken into account. In other cells, the anaphase movement is executed more rapidly (Table 6) yet its speed does not exceed, for instance in *Tradescantia*, 0.7μ per minute, that is 0.012μ per second (Table 7). This is very slow as compared with the speed of an amoeboid movement, which averages 150μ per minute (Barber, 1939), or even with the movement of cilia (Pfeiffer, 1959; see Table 7). Also, the anaphase movement of the chromosomes occurs as a rule fairly evenly, that is, once a certain speed has been reached, it is maintained, with some variations, throughout the process (Fig. 80). If there were an elastic traction drawing the chromosomes to the kinetocentres, the speed should reduce, the nearer they are getting to the poles. On the other hand, the traction fibres would be expected to become thicker and thicker as anaphase progresses, since we would have to consider them as previously extended. However, this is not the case, neither in light nor in electron microscopic observations.

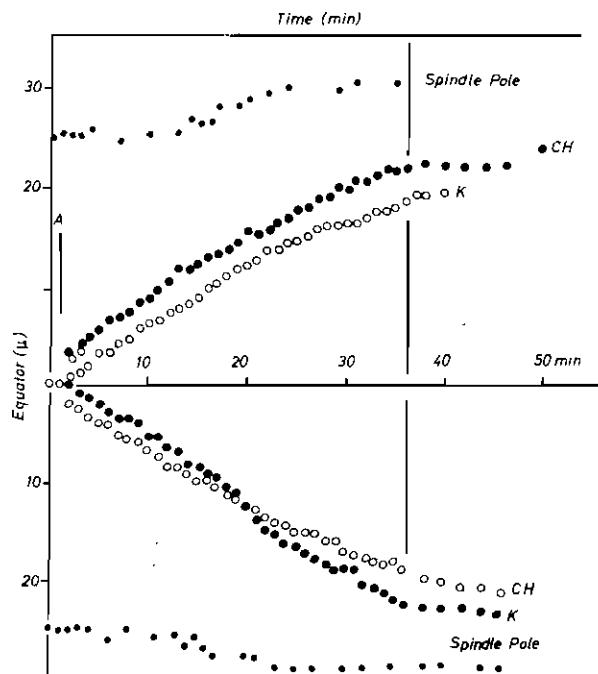


Fig. 80. Graphic representation of anaphase migration in *Haemanthus* endosperm, after the findings of Bajer. Migration of two groups of chromosomes (CH) and a pair of kinetochores (K). (From Mazia, D. (1961), *The Cell*, Vol. III. Acad. Press, New York.)

TABLE 7. Rates (in μ per sec.) of chromosome migration at anaphase and of other intracellular movements

Anaphase chromosomes of <i>Tradescantia</i>	0.0116
Anaphase chromosomes of heart fibroblasts	0.043
Anaphase chromosomes of other connective tissue cells <i>in vitro</i>	0.065-0.07
Increase in length of the pollen duct in <i>Datura</i>	0.53-0.916
Locomotion <i>Amoeba proteus</i>	2.1-4.6
Cytoplasmic streaming in <i>Tradescantia</i>	5.0
Terminal stroke of cilia in <i>Mytilus</i>	3.75×10^2

(Pfeiffer, H. H. from *Protoplasma* (1959) 51, 390)

This makes all contraction mechanisms improbable, which inferred an analogy between the shortening of the chromosomal spindle fibres and muscle contraction (Lettre, 1950; Lettre and Albrecht, 1951; and others). On the other hand, one cannot completely deny the relationship between the spindle fibres and the contractile proteins in general (see, e.g., Weber, 1958). Contractile proteins are widely distributed within the cells (see Frey-Wyssling 1955 for a summary). They determine partly the shape of the cell, not to mention the flagella and fibrils which are related to the mitotic array through the kinetocentres and display certain similarities with the spindle by virtue of their protein character. The source of energy for the movements of the flagella is evidently, as in the mitotic spindle, the fission of ATP; from this point of view at least, both can be compared to muscle contraction (Hoffmann-Berling, 1954a, 1961). The energy required for the latter is, however, $8 \cdot 10^{-13}$ erg (Pfeiffer, 1959), that is, 10^8 times lower than, for instance, in the cilia movement of *Paramecia* (Table 8).

TABLE 8. Comparison of the kinetic energy (in erg) of different cell movements

Anaphase movement of chromosomes in connective tissue cells of <i>Triturus cristatus</i>	8.52×10^{-13}
Same in hair cells of <i>Tradescantia virg.</i>	10.18×10^{-13}
Same in erythrocytes of <i>Triton sp.</i>	12.39×10^{-13}
Cytoplasmic movement in <i>Amoeba proteus</i>	8.0×10^{-9}
Forward stroke of the cilia tip of <i>Paramecium</i>	4.56×10^{-5}

(Pfeiffer, H. H. from *Protoplasma* (1959) 51, 390)

If, despite all these differences, there should be a traction mechanism, of whatever nature it might be, that moves the chromosomes, the poles must be fixed. This stability may come from the polar rays, which are similar to the spindle fibres; this was the old theory of van Beneden (1883), which was confirmed many times, at least in its principle (e.g., Inoué, 1952). It may also be due to the inner stability of the spindle fibres, whereas the central fibres (p. 141) maintain the distance between the poles against the anaphase movements of the chromosomes. This explains why in certain cases, during metaphase or early anaphase (Jacquez and Bieseile, 1954; Marakov, 1960), the spindle poles get closer to each other, as if here the inner stability of the spindle structure was not sufficient to counteract the traction.

Stretching of the spindle

However, the opposite phenomenon is more frequent by far. During anaphase the kineto-centres withdraw from each other and the spindle stretches itself. This has been observed time and again (e.g., by Hughes-Schrader, 1931; Hughes and Swann, 1948; Dietz, 1954; Boss, 1954) and exact measurements have been taken (Brumfield, 1940; Ris, 1942, 1943, 1949; Martin, 1953; Jacquez and Bieseile, 1954; Makino and Nakanishi, 1955; and others); it often happens that the whole cell stretches itself in the axis of the spindle, so that its transverse diameter diminishes (see, e.g., also Izutsu, 1960). Certain anaphases even appear to be based exclusively on such a stretching of the spindle, i.e., the distance between kineto-centres and kinetochores remains relatively constant, while the poles separate from each other (Ris, 1943; Short, 1946; Berkeley, 1948). Hence, in the majority of anaphases, the movement of the chromosomes to the poles is caused concurrently by a shortening of the chromosomal spindle fibres and by a stretching of the spindle (Fig. 81), the former being predominant at the beginning, the latter at the end of anaphase. Thus anaphase may, in certain cases, appear to be divided into two clear-cut stages (e.g., Hsu, 1955). It has also been possible to produce the stretching of the spindle experimentally, for instance in dead fibroblasts after they had been extracted with cold glycerine under simultaneous treatment with ATP (Hoffmann-Berling, 1954, 1955); the distance between chromosomes and poles remained thereby nearly constant (Fig. 82).

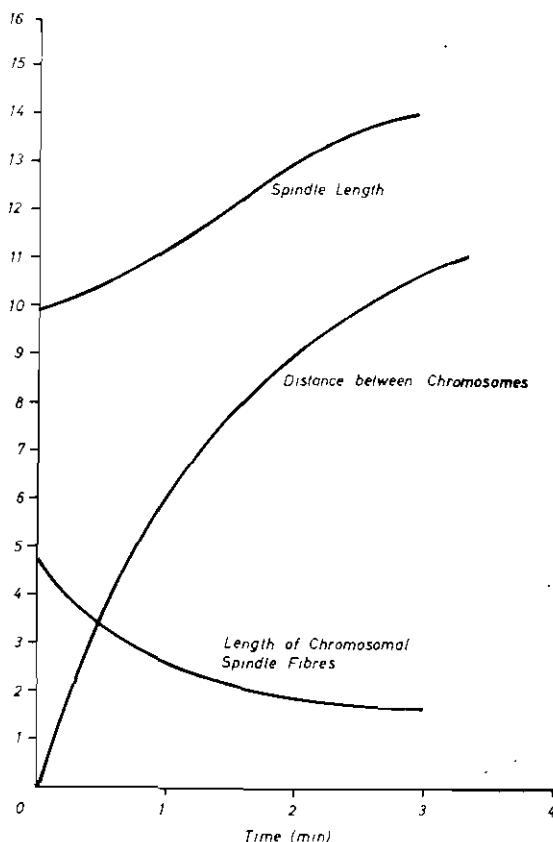


Fig. 81. Values of eight anaphases from chicken osteoblast cultures (mean values). Note that the stretching of the spindle and the shortening of the chromosomal spindle fibres occur nearly simultaneously. (From Hughes, A. F. W. and Swann, M. M. (1948), *J. exper. Biol.*, **25**, 45-70.)

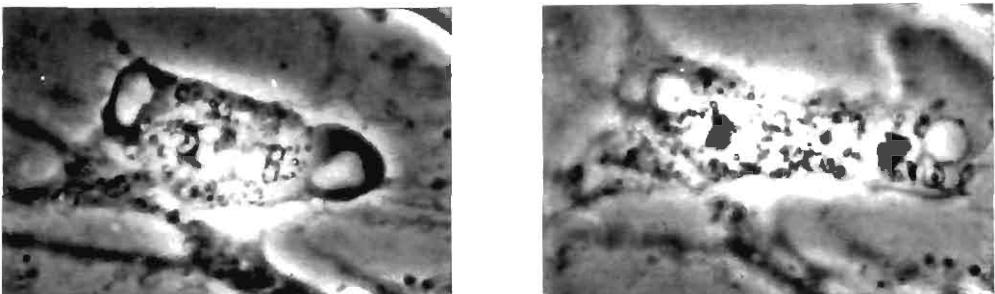


Fig. 82. Experimental stretching of the spindle with ATP in fibroblast culture cells after glycerin extraction. *Left:* cell at anaphase; *right:* the same cell after 12 minutes exposure to ATP. (From Hoffmann-Berling, H. (1954), *Biochim. biophys. Acta*, 15, 226-236.)

Movements of the individual chromosome

The stretching of the spindle is, at least in most karyokineses, only an additional and not a decisive factor, for the whole set of daughter chromosomes is not moved *en bloc* to the poles, but each chromosome behaves somewhat differently from the others. Especially in living cells, at the beginning of anaphase, one may always observe one or the other chromosome rushing forward or lingering behind (e.g., Moorehead and Hsu, 1956). Certain chromosomes move even at first to the wrong side. Others seem for a short while to move backwards towards the equatorial plane. In phase contrast films (Michel, 1943) taken in time-lapse, one has the impression that at times the chromosomes get into a kind of whirl, and then it is quite impossible to recognize any clear direction in their movements (see also Tischler, 1934-1951).

Yet, beyond this seeming incoherence, there is evidently a definite order. Sex chromosomes behave often differently from autosomes; they may arrive late in the metaphase plate and, in the anaphase, lag behind the autosomes (e.g., Hughes-Schrader, 1947; Das, 1958; Bauer *et al.*, 1961) or rush forward (e.g., Martin, 1953; Dietz, 1955; Das, 1956). In the early cell divisions of certain insects, single chromosomes are even eliminated; they either are ejected from the metaphase plate or else they build, in the course of anaphase, their own small spindle, and then degenerate (e.g., Reitberger, 1940). This also is an example of the movements of single chromosomes and the process certainly does not concern the spindle nor any lever (Belar, 1929); it is peculiar to the chromosomes themselves (Geyer-Duszynska, 1961).

Let us cite another example. In the first spermatocyte division of the fly *Sciara*, there are no bivalents and the division occurs according to a monopolar pattern (Merz *et al.*, 1926). Thus there is no metaphase plate either. During the early anaphase, one half of the chromosomes tends towards one pole. The other half goes away from the pole, reaches the opposite cell border and is stored in an appendix of the cell which is then pinched off. It is not left to chance what chromosome belongs to which group; all maternal chromosomes assemble at this single pole, all paternal chromosomes are rejected (Metz, 1933). Fig. 83 shows a diagram of the process.

We must admit that there can be only one interpretation of the fact; the anaphase migration

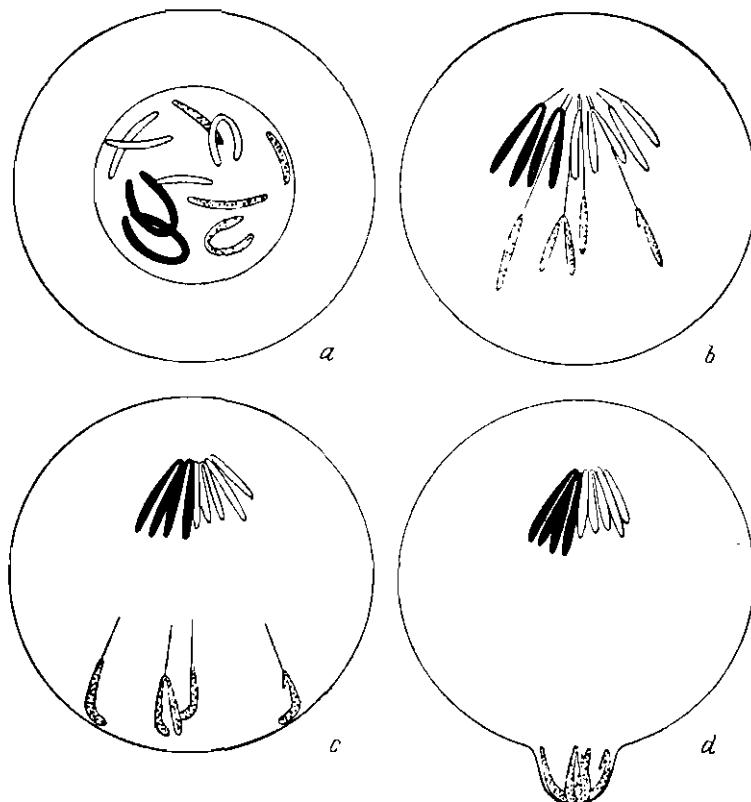


Fig. 83. Diagrammatic representation of the first monopolar spermatocyte division in *Sciaria*. The maternal chromosomes (black or white) reach the pole, the paternal chromosomes (dotted) are rejected from the pole and stored in an appendix of the cell at the opposite cell wall. (From Metz, C. W. (1933), *Biol. Bull.*, **64**, 333–347.)

is basically a process of the single chromosome and all traction or leverage mechanisms are exerted on each chromosome individually.

And lastly, the chromosomal spindle fibres are not merely contracted during the anaphase migration, let alone compressed, as one would have to expect in the case of a leverage mechanism; they are degraded. In the polarization microscope, the birefringence of the chromosomal spindle fibres decreases during anaphase (Schmidt, 1939; Hughes and Swann, 1948; Swann, 1951b, 1952) and concurrently the maximal birefringence shifts from the equator to the poles. Bajer (1961) observed in the endosperm of plants that the point of strongest birefringence, which is always to be found in the vicinity of the kinetochores (Fig. 84), migrates with the chromosomes to the poles (see also Inoué, 1953).

Such observations suggest the kinetochores take a leading role. In pursuing the concept of a traction mechanism, these microscopic findings, obtained under polarized light, have led to the conclusion that the kinetochores give the spindle fibres a contraction impulse

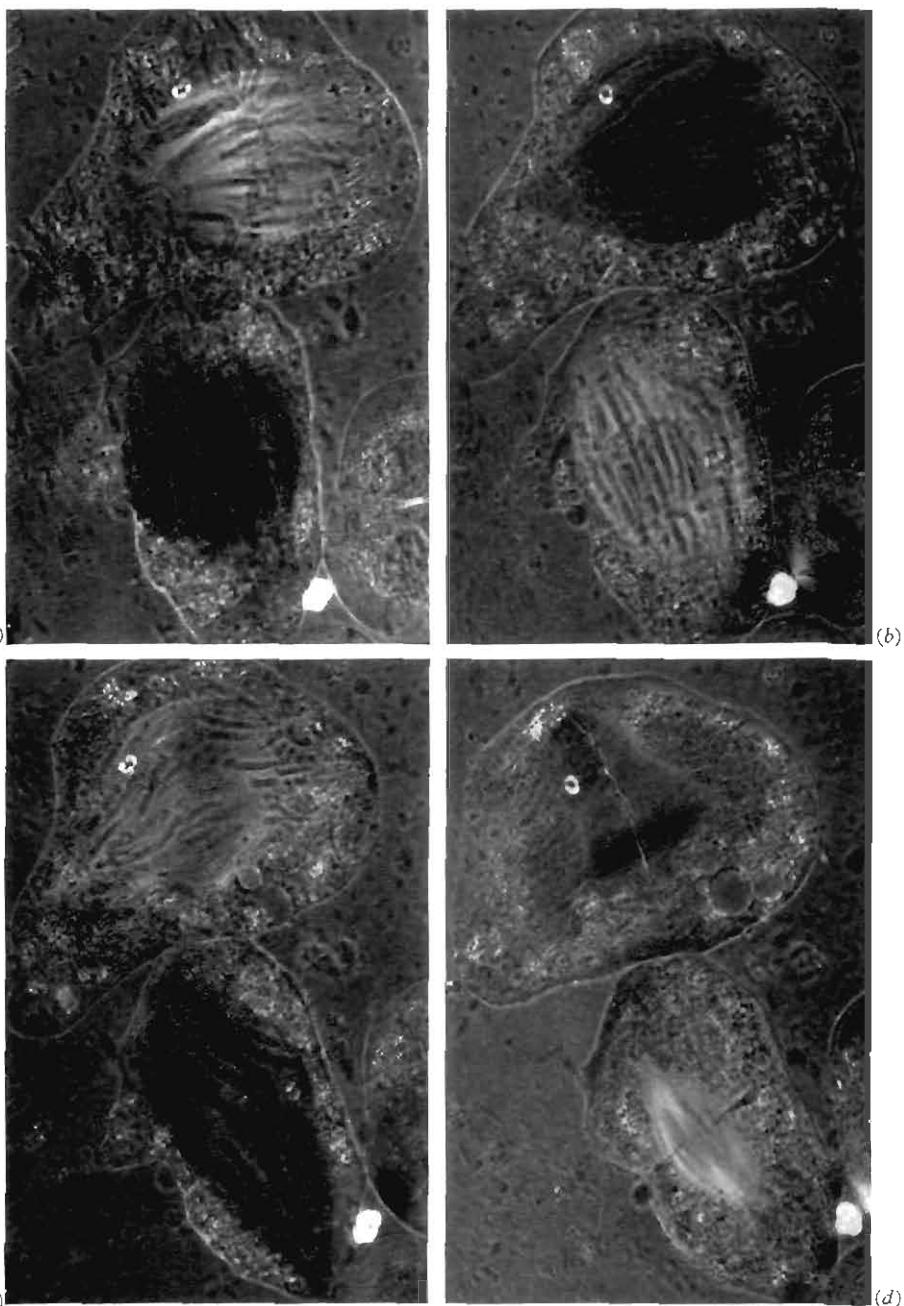


Fig. 84. Polarization micrograph with black and white compensation showing the birefringence of the spindle fibres in endosperm cells of *Haemanthus Katharinae*. (a) Early anaphase (b) Late anaphase. (c) Early telophase. (d) Formation of the cell plate with phragmoplast. (From Bajer, A. (1961), *Chromosoma*, 12, 64-71.)

(Schmidt, 1941; Cornman, 1944), perhaps under the form of a special substance, which the kinetochores pass on to the spindle fibres (Belar, 1929). Frequently, there has been observed a thickening of the chromosomal spindle fibres at the kinetochores (e.g., Schrader, 1934; Gross, 1935) which has been interpreted as a movement or migration substance (Loorbeer, 1934; Kupka and Seelich, 1948; Wada, 1950). True, it is difficult to prove these conceptions in all their details with certainty. The most important fact, however, is that, according to observations in the polarized light microscope, a change occurs during anaphase in the orientation of the chromosomal spindle fibres, i.e., the molecular orientation is reduced and this reduction moves in a wave towards the poles. From the explanations given above (p. 158), it may be a mechanism which absorbs energy and is possibly dependent on ATP. It may give the impression of a traction from the poles when the kinetochores climb their way up, so to speak, along the chromosomal spindle fibres, degrading that part of the fibre substance (Stich, 1954; Dietz, 1958) which does not remain as interzonal fibres (p. 167).

If we combine this conception of an autonomous movement of the chromosomes with the postulated attraction exerted by the kinetocentres and assume an additional impulse rising from between the separating chromosomes (see Wolf, 1960), we would have then once more an example of the interaction of kinetocentres and kinetochores; related and functionally complementary elements.

Spindle currents

However, this still does not explain all the findings. In certain spermatocyte divisions, for instance, the chromosomes were found to behave peculiarly. During the movement of the univalent sex chromosomes towards the poles, it was found that the kinetochores did not precede the chromosome arms; on the contrary, the chromosome arms preceded the kinetochores. In the autosomes, it happened that the arms of the chromosomes flapped back, so that the kinetochores became oriented towards the equatorial plate (Dietz, 1958). This cannot be explained by activities of the kinetochores alone. There must be other factors at work too.

Many of those additional mechanisms have been discussed in the past. Let us mention only a few. For example, in the reference to the similarity of the metaphase setting and the figure formed by magnetic or electrostatic force lines, magnetic or electrostatic changes within the single cell elements were held responsible for the movements of the chromosomes (for ref., see Wassermann, 1929; Milovidov, 1949; Schrader, 1954). Darlington (1937) had developed a "balance theory of mitosis", according to which the centres would repel each other by virtue of equal charges, but would be capable of attracting the chromosomes during anaphase. Such theories hardly corresponded to reality (see Geitler, 1938d; Schrader, 1954).

The assumption that additional transportation mechanisms at anaphase are based on currents inside the spindle seems to be more plausible. This was already a very early conception (Berthold, 1886). Especially since the behaviour of the cytoplasmic particles, such as mitochondria or plastids, are an indication of such an intracellular current.

For it is a fact that, at the beginning of the rearrangement, the mitochondria are moved to the periphery of the equatorial plate (e.g., Lundgardh, 1912; Belar, 1929; Hughes and Swann, 1948; Michel, 1950), thus forming a cuff-like garland. After anaphase, certain

mitochondria are shifted towards the poles, others may incidentally form the intermediary body (p. 167). If one puts a drop of oil into a dividing cell, it will also be deposited at first in the equatorial plane, undergo division there and both parts will move to the poles (Chambers, 1938; Chambers and Chambers, 1961). Other cytoplasmic components behave basically the same way, as in the mitosis of plants (Bajer and Molé-Bajer, 1956; and others). A particularly clear demonstration of this apparently passive movement could be made on the spindle of *Cyclops* eggs by means of DNA-containing granules (Stich, 1954). They too get inside the spindle, in contrast to the mitochondria. There, following the chromosomes, they are

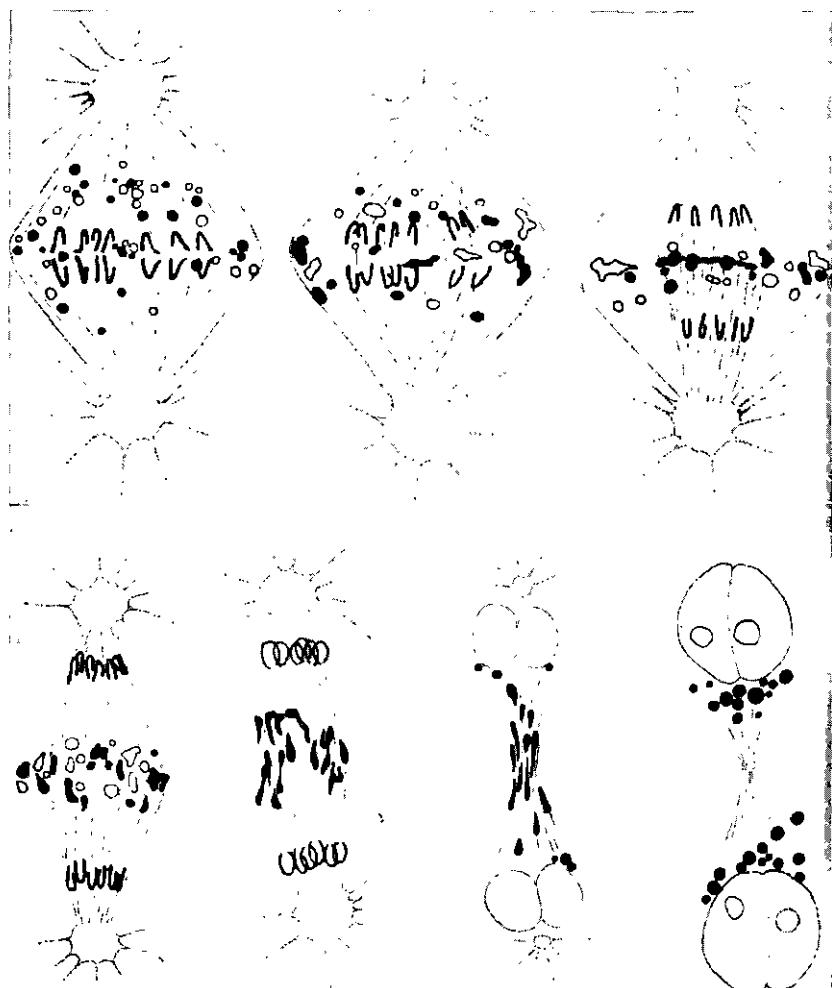


Fig. 85. Behaviour of DNA-containing granules in the spindle of *Cyclops* egg. Note that they reach the equatorial plane *after* the chromosomes and move to the poles also *behind* them. (From Stich, H. (1954), *Chromosoma*, 6, 199-236.)

forwarded into the equatorial plane and move towards the poles, still following the chromosomes, yet they do not get included into the daughter nuclei (Fig. 85). These and many older observations (e.g., von Erlanger, 1897; Conklin, 1902; Spek, 1918; Schneider, 1938) suggest a current, which flows exteriorly from the pole region to the equatorial plane, then turns into the centre of the cell and goes on flowing in the direction of the poles approximately in the area of the previous spindle axis, as an axial current. The origin of this current is still completely obscure.

Among the manifold processes which we mentioned for the anaphase, the active chromosome movements were, however, the most important and the motor of these movements must be assumed to lie in the kinetocentres and the kinetochores. Their mechanism is evidently determined by the principle of a multiple safety device; it involves the chromosomal spindle fibres, which direct the anaphase migration, but also the central fibres, which may cause the stretching of the spindle and thus push apart the daughter chromosomes; finally, the spindle current plays also a part as a supporting and supplementing factor.

Telophase

When they reach the poles, the chromosomes are condensed into a compact chromatin mass, in which no inner structure may be recognized at first. Thus, the chromosomes once more lose their morphological individuality for the sake of the newly growing nuclei. We have already explained (p. 130) how the processes of hydration and uncoiling influence each other in this case, and we have also shown the special part played in these processes by RNA. The reconstruction of the interkaryokinetic nuclei forms anew all the structures of the mother nucleus; euchromatin and heterochromatin reappear, and already at a very early stage the nucleoli take shape at characteristic sites. The new nuclear membrane appears early at the border between the uncoiling chromosomes and the cytoplasm (p. 64, see also Fig. 25). All this we can consider as well known to the reader, since we have already discussed the different parts of the nucleus and their relation to one another.

During the course of all these reconstruction processes, which take a variable amount of time, but generally less than prophase (Table 6), all accessory structures of karyokinesis, which had previously determined the picture of the cell, i.e., the polar rays as well as the spindle fibres, disappear. Despite the lack of precise investigations on this degradation, it can be easily understood as the cessation of the orientation given by the kinetocentres. Because the substance of these structures consists of transformed nuclear and cytoplasmic elements, it can return again to its original state or remain as small residual bodies except for that part of the spindle which was left between the parting anaphase chromosomes or was reconstituted partially, that is, the interzone region which, in plant cells, changes into phragmoplasts. This, however, belongs already to the next and last step, the division of the cell body, which will be discussed in the following chapter.

Review

The chromosome movements, rearrangement from the prophase into the metaphase plate and movement to the poles of the chromosome halves, seemed at first so simple, and yet we had to follow such complicated paths in order to come to a relative understanding of the

processes involved and of their probable mechanisms. Yet fairly abundant and relatively clear evidence has been brought to support the second stage of mitotic movements, i.e., the anaphase. Resulting from the functional interaction of kinetocentres and kinetochores, the anaphase migration appeared predominantly as a combined traction and pressure mechanism, in which the kinetochores played the major part, changing the orientation of the spindle fibres and probably degrading them. The kinetocentres are partners of the kinetochores and are, moreover, responsible for the simultaneous stretching of the spindle. However, their function is possibly to direct and maybe even to produce the spindle current, which supports and supplements the kinetochores. This is a multiple safety device. The pro-metaphase rearrangement—quite a complicated movement of the chromosomes, as compared to the migration to the poles—is completely determined by the concurrently arising mitotic spindle and is, consequently, also subject to the complementary interaction of kinetocentres and kinetochores. In both movements, rearrangement and anaphase, all chromosomes definitely behave as individuals even though they follow a common pattern. The rearrangement does not consist merely in the migration of the chromosomes into the equatorial plate, it also brings about the arrangement of the chromosomes into a symmetrical array and opposite each other. In the following prophase, this position is the basic position for the next rearrangement, which completes the karyokinetic cycle of the chromosomes.

Cytokinesis

The reconstruction of the daughter nuclei in the vicinity of the spindle poles is the last stage of karyokinesis. It is followed, as a rule, by the division of the cytoplasm, or cytokinesis.

If the sequence karyokinesis-cytokinesis is taken as the rule, we must mention the exceptions to this rule. By no means all nuclear divisions entail a division of the cell body and not every cytoplasmic division is directly preceded by a nuclear division. A dissociation of both division processes is best exemplified in the multinuclear symplasts, which can be found mainly in the formation processes of gametes or spores of animals and plants. Here, repeated nuclear divisions first generate multinuclear protoplasmic structures. It is only at a second stage that either uninuclear cells develop, or are preceded by multinuclear protoplasmic fragments, which subsequently are divided, in one or many stages, into uninuclear gametes. When a multinuclear mother cell generates a number of daughter cells in one step corresponding to the number of nuclei, we speak of a *simultaneous multidivision*. This kind of division is typical of a whole series of protists (see Grell, 1956), for example trypanosomes, and it is liable to leave behind a portion of the protoplast as a residual structure. The same process occurs, but in many stages, in the sporogenesis of plants and also in protists; it is then called *successive multidivision*. In both cases, the result is the same, namely, a multinuclear symplast is divided into uninuclear cells. Similar processes have been mentioned for example in the cleavage division of many insects, when discussing basic objections against the cell theory (p. 4).

Interzone structures and phragmoplast

We must bear in mind the possibility of dissociating karyokinesis and cytokinesis as we now proceed to examine each process in particular; this fact is of special importance in the

question as to whether the accessory structures of karyokinesis, i.e., the kinetocentres with their polar rays and the spindle, play a role in the division of the cytoplasm.

When we described the anaphase, we pointed out that the structures of the chromosomal spindle fibres are used up at the kinetochores, in a way which still remains obscure in the details (p. 163). We added, however, that similar fibrous structures could frequently be observed behind the kinetochores and that the direct connections between the poles, the central fibres, remained intact. Thus, the interzonal fibres between the parting chromosome sets consist basically of two components, the central fibres and fibres which establish a kind of retrograde connection of the kinetochore regions.

The nature of these retrograde connections has been much discussed and remains ultimately obscure. They have been interpreted as empty channels within the viscous spindle substance, which have stayed open behind the chromosomes (Ellenhorn, 1933) or else, as the chromosome envelope, the pellicula, stretched out by the anaphase migration of the chromosomes, and thus giving rise to the formation of very long fibres (Fig. 86) (e.g., Goldschmidt, 1923; Schrader, 1931, 1932, 1954; Carothers, 1936).

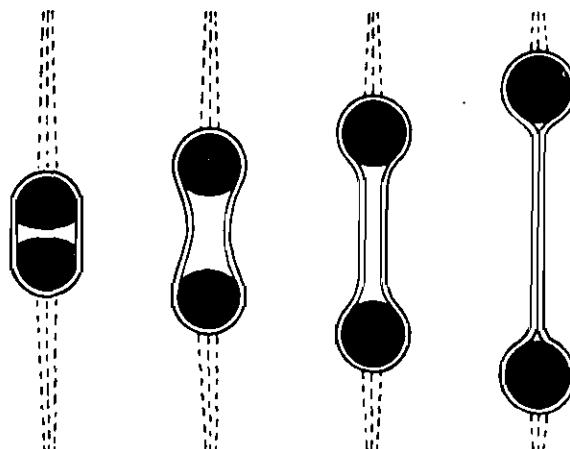


Fig. 86. Scheme showing how interzonal fibres may be produced by an anaphase stretching of the chromosome pellicula. (From Schrader, F. (1954), *Mitose*, Deuticke, Wien.)

In many cases, the formation of the interzonal fibres coincides with a progressive and typical transformation. Whereas, for example, the birefringence at the kinetochores, as observed in the polarization microscope, ceases at first, a new birefringence can be seen again during anaphase between the parting chromosomes, though of lesser intensity than in the spindle halves (Inoué, 1953). Later, birefringence (Hughes and Swann, 1948) and ultraviolet absorption (Davies, 1952) may increase again. Simultaneously, the interzonal fibres gather to form a relatively small bundle and build the intermediary body (Flemming, 1891b). The latter may become as large as the previous spindle. As it diminishes, the new cell wall develops from all sides and it often looks as if the intermediary body is squeezed in by the cell wall (e.g., Fry, 1937; Fawcett, *et al.*, 1959). As can be seen in the electron microscope, small cytoplasmic vesicles appear at this stage in the division plane, which are liable to order themselves into many chains; they are at their maximal size in the direct proximity of the intermediary body (Fig. 87). Their fusion brings about both walls of the daughter cell (Buck and Tisdale, 1962b).

Whereas in animal cells such intermediary bodies are probably the exception, the interzone structures, as a rule, disappearing rapidly, they are particularly important in seed plants (Tischler, 1934-1951; Mühlendorf, 1951). For in this case there appears between the telophase groups a well-defined body, the phragmoplast (Errera, 1888). Its inner structure is, like the spindle, of a fibrous nature, visible in the light microscope as well as in the electron microscope (Fig. 88), and appears to contain proteins with S-S bonds (Olszewska, 1961b).

Consequently, the phragmoplast was and still is often considered to be a special form of

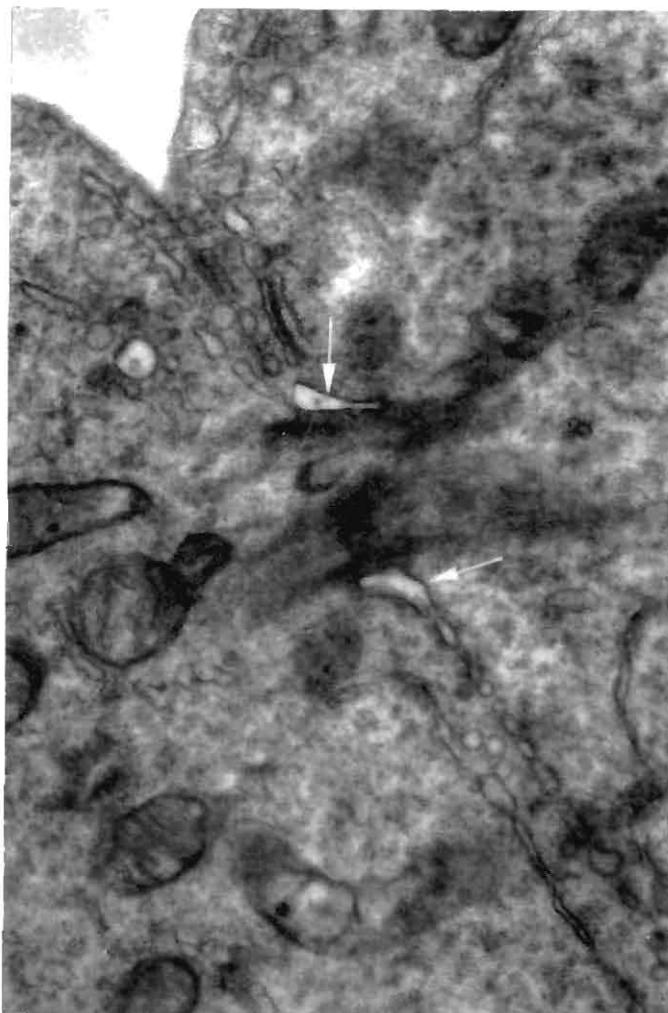


Fig. 87. Electron micrograph of a rat erythroblast showing the formation of the cell membrane by lamellar vesicles similar to the endoplasmic reticulum. $\times 36,000$. (From Buck, R. G. and Tisdale, J. M. (1962), *J. Cell. Biol.*, 13, 117-125.)

the spindle (Strasburger, 1880; Nemec, 1927; Wada, 1950). However, these must be markedly transformed spindle elements, for the formation of the phragmoplasts is concurrent with the absorption of labelled precursors of RNA and protein (Olszewska, 1960a). This suggests that phragmoplasts build their structure by way of an increase in substance somehow in relation to the ergastoplasm; and it is a fact that, during anaphase and telophase, elements of the endoplasmic reticulum enter the interzone structure and gather about the phragmoplast (Porter and Machado, 1960). Simultaneously, the phragmosomes appear in the division plane, covered by a membrane, and from these emerges a continuous cell plate in the centre of the phragmoplast, which expands centrifugally until it reaches the outer walls of the mother cell. Since it is a double plate from the beginning (Fig. 88), both primary cell membranes are thus formed already. A centripetal influx of substances from the environment and the wall of the mother cell itself then produces

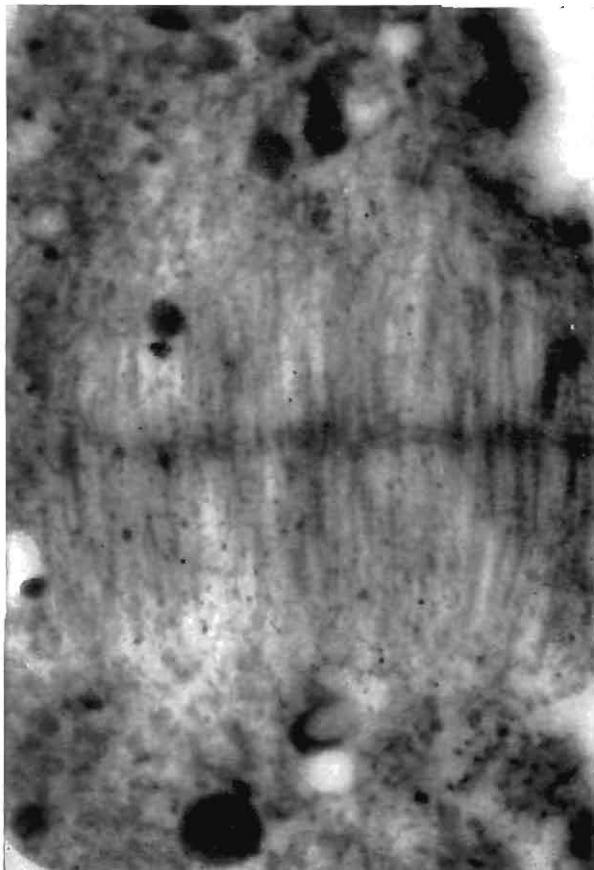


Fig. 88. Electron micrograph of a longitudinal section through a pollen mother cell of *Lilium lancifolium* at telophase II. Fibrillar inner structure of the phragmoplast. Formation of the cell plate as a double membrane. $\times 4500$. (From Sato, S. (1959), *Cytologia*, 24, 98-106.)

the fully developed compact cell wall which gradually becomes firm, first at the periphery, then progressively towards the centre (Sato, 1959). Thus, in plants, the new cell wall proper develops between the primary couple of cell plates, as had been already assumed by Strasburger (1875).

In large cells, in which the outer cell walls and the phragmoplasts are at a fair distance from each other, the edge of the phragmoplast is liable to expand circularly, and the phragmoplast is thicker on its edges than in its centre. It is in this phragmosphere (Beer and Arber, 1919) that the cell plate develops centrifugally. The phragmoplast proper has turned into a hollow cylinder and, seen from the poles, it looks like a cell plate ring (Went, 1887). It may

happen that the phragmoplast grows first on one side of the cell wall, builds there a part of the cell plate and then moves to the other side. Such a successive formation of the cell plate is no rarity in plants (ref. see Tischler, 1934-1951). But there is also an infinite number of variants with, in certain cases, centripetal instead of centrifugal cell plate formation and with all the intermediary stages up to the cleavage of the cell (Mühlendorf, 1951).

Significance of spindle and kinetocentres

The cell cleavage is, at least at first glance, a remarkably simple process. At telophase, a circular cleavage furrow forms itself in the equatorial plane, cutting in deeper and deeper until it finally divides the cell into two halves (Fig. 89). However, this process, too, can have many variations (see, e.g., Mühlendorf, 1951). It occurs differently in egg cells and in amoebae for instance; the pinching off of buds, e.g., in yeast cells, the so-called blastogenesis, is finally a special form of this same process. It is to this date still full of mysteries (see the summarizing surveys by Mühlendorf, 1951; Wolpert, 1960; Mazia, 1961a; Roberts, 1961; and others). Let us try and tackle these problems step by step.

The position and structure of the phragmoplasts suggest their connection with the spindle. With the cell cleavage we are faced again with the same question: does the spindle play a part in the process, for instance once more via the interzone structures?

The chronological sequence seems to deny it. The cell cleavage never starts before anaphase, as a rule even only at telophase, at a time when karyokinesis is completed and the spindle is being degraded. On the other hand, the direction of the cleavage is determined primarily by the spindle. This is true of normal mitoses, in which the division plane coincides exactly with the metaphase equatorial plane; but it is also true of special mitoses such as the differential neuroblast division in locusts (Kawamura, 1960b), where an unequal cell division

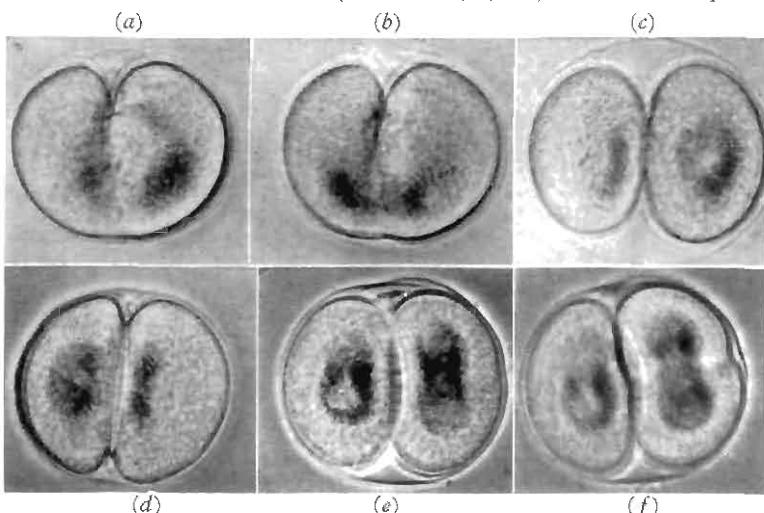


Fig. 89. Phase contrast micrograph of the cleavage divisions of a fertilized sea urchin egg. (a) and (b) Beginning constriction (top); (b) depression (bottom); (c) completed cytokinesis; (d) the larger cell (*at right*) grows and starts dividing again; (e) right cell at metaphase, left cell in preparation for metaphase; (f) the right cell begins cytokinesis with a circular constriction.

generates a small ganglion cell and a larger neuroblast. If one pushes the anaphase spindle with a microneedle against the side which normally generates the (larger) neuroblast, a markedly smaller cell is generated there, that is, the relation in size is inverted. On the other hand, Carlson (1952) has described on those same neuroblasts, how a considerable shifting of the whole spindle into one of the cell poles does not influence the direction of the cytokinesis.

This is an obvious discrepancy. The clue to the problem is probably to be found already in the older centrifugation experiments of Harvey (1934), made on sea urchin eggs. A shifting of the spindle during metaphase entails a shifting of the cell division plane. Later, the direction of the division cannot be changed any more. Thus, there exists a critical moment during karyokinesis, up to which the position of the future division plane is settled—and this is surely true of many mitoses (Mazia, 1961a).

Certainly, none of these findings is a direct proof of a connection with the spindle structure. The shiftings of the division plane may also result from the shifting of the kinetocentres. Thus the question once more arises whether the kinetocentres influence the cell cleavage in any way.

Let us start from the previously mentioned traction effect of the polar rays (p. 158). A system of fixed radii would already, according to Heidenhain's model of steel rails (1897), produce purely mechanically a depression in the median plane when both centres withdraw from each other (Fig. 90). At the same time, in a less rigid system, the radii which previously ran parallel to each other in the median region, would cross each other (see Dan and Dan, 1947), thus facilitating all the more a constriction, as the distance between the centres increases. Moreover, if one considers the contractile capacity of the polar rays, one may explain the cell cleavage from a purely mechanical point of view. In this case, the surface tension should decrease at the cell walls in the vicinity of the poles, while it would rise in the equatorial plane; this would amount to the equivalent of an equatorial contraction (Wolpert, 1960). Yet such a process would apply, strictly speaking, only to those types of cells in which large polar rays traverse the whole cytoplasm, i.e., almost solely to animal egg cells.

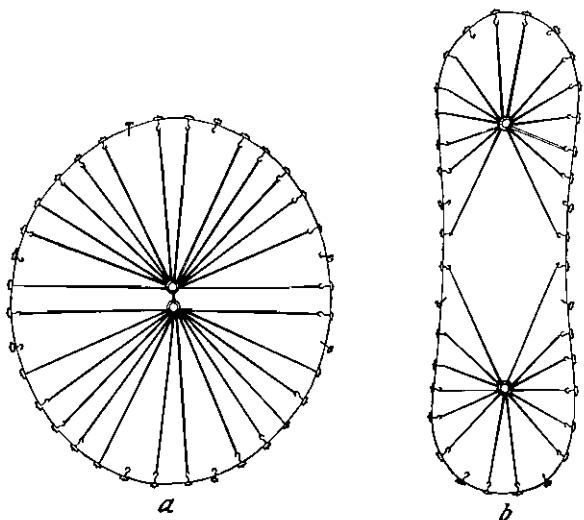


Fig. 90. Steel rod model showing the mechanism of cell cleavage. (a) Both centres being attached to each other, an even tension is exerted on the rubber radii; (b) if this attachment is loosened, the centres withdraw from each other. (From Heidenhain, M. (1897), *Morphol. Arch.*, 7, 281-365.)

There is, however, further evidence of a participation of the kinetocentres and their polar rays in cytokinesis. For instance, in many cells, the position of the division plane depends on the length of the polar rays; when the polar rays of one of the kinetocentres are shortened or depressed on one side, spindle and equatorial plane also get shifted towards the smaller system of polar rays, thus producing two cells of a different size (Dan *et al.*, 1952).

If one bisects an echinoderm egg at an acute angle to the equatorial plane during anaphase, the cleavage plane will still appear at the same site as before and two nucleate and two non-nucleate cellular elements will develop; any further cleavage is then possible only in those cellular elements which contain the kinetocentres (Chambers, 1919; see also Chambers and Chambers, 1961) (Fig. 91). That neither the nucleus nor the chromosomes play any major part in this process, has been demonstrated by many other observations, bringing clear evidence of a cytokinesis without participation of the nucleus (e.g., Harvey, 1936, 1960; Lorch *et al.*, 1953; Lettré and Siebs, 1955; De Terra 1960*b*; and others). Moreover, the preparatory role attributed by some to the nucleus in cytokinesis (see, e.g., Shapiro, 1961) is probably more appropriate for the kinetocentres than for the nuclear substance.

This preparation of cytokinesis leads us once more to the problem as to when a cell cleavage is finally determined and at what point it can still be influenced. The microdissection experiments of Chambers (1919) had shown that at anaphase the division plane is fairly well determined. We can even remove an anaphase spindle, and cytokinesis will still occur at the normal site and at a normal time (Hiramoto, 1956). Disturbances of the spindle with podophyllin (Cornman and Cornman, 1951) or colchicine (Swann and Mitchison, 1953) have also no effect on cytokinesis during anaphase; before that stage, however, the whole cell division is liable to be disturbed. The microdissection experiments of Scott (1960*a*) have also brought evidence of the fact that the division plane of the cell body is determined when the cell

stretches at anaphase. It is true, however, that the signal for cytokinesis to come into effect is given much later (Mazia, 1961*a*).

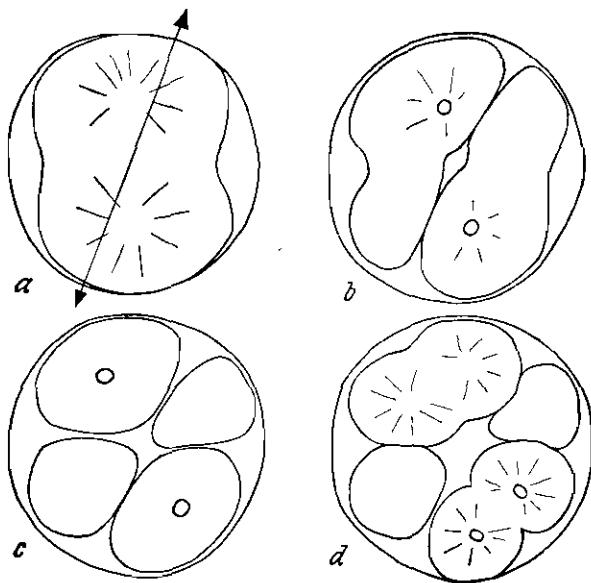


Fig. 91. Schematic drawing of microbisection experiments on starfish eggs. (a) Direction of the bisection; (b) constriction in the original equatorial plane; (c) result of cytokinesis; (d) a second cytokinesis occurs only in those parts of the cell which contain kinetocentres. (From Chambers, R. (1919), *J. gen. Physiol.*, 2, 49–68.)

Changes in the cell surface

This accomplishment of the cell division is always a matter of the cell wall itself, even though it is directed by intracellular factors such as the kinocentres. In the cytokinesis of an untreated sea urchin egg, the firmness of the cell wall increases sharply during meta- and anaphase, reaches its maximum at the end of anaphase and decreases equally sharply during cleavage of the cell (Mitchison and Swann, 1955). Therefore, it has been suggested that the cell surface is stretched primarily at the poles, and the stretching goes on like waves over the cell towards the division plane, where it finally releases a contraction. This is, indeed, well supported by evidence (see, e.g., Hiramoto, 1958).

Such processes of expansion and contraction are frequently interpreted as sol-gel changes (Marsland, 1957). The gelation in the furrow region could, for instance, be responsible for the contraction ring seen in phase contrast (Mota, 1959; Scott, 1960b) as well as in the electron microscope (Mercer and Wolpert, 1958) as a concentric thickening of the cell wall. This contraction ring is also thought to be the result of an active role played by the cell. For, according to many findings, ATP is as necessary for the contraction as it is for the normal tonus of the cell membrane (Lettré, 1952b). ATP can, in addition, counteract the inhibiting effect on cytokinesis of pressure or temperature (Landau *et al.*, 1955). Finally, in his telophase models, i.e., in dead fibroblasts, extracted with glycerine, in which cell cleavage had only just begun, Hoffmann-Berling (1954b, 1955) was able to bring the cytokinesis to completion by adding ATP (Fig. 92). Thus we are entitled, as in the case of the anaphase migration (p. 160), to assume a direct relation to contractile proteins (see also Weber, 1958). However, to what extent an active contraction of the cell membrane plays a part in the cleavage, is still very much disputed (see e.g., Wolpert, 1960; Roberts, 1961; Mazia, 1961a).

On the other hand, there is no doubt that cleavage necessitates the renewed formation of cell wall substance (Wolpert, 1960).

As of yet, it is not clear whether the latter is formed right there or whether it is brought secondarily to the cell wall. Mainly under the impression of the large polar rays of egg cells and the current that was thought to flow within them, it was frequently assumed that a cytoplasmic mitotic substance (Kopac,

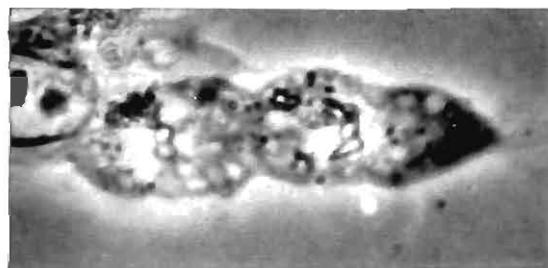


Fig. 92. Glycerin-extracted fibroblasts. *Top*: at the beginning of cytokinesis. *Bottom*: cleavage completed after administration of ATP. (From Hoffmann-Berling, H. (1954), *Biochim. biophys. Acta*, 15, 332-339.)

1951) is forwarded from the kinetocentres to the periphery of the cell (see also, Dan and Dan, 1947; Scott, 1961). Such a substance could be identical with the gelation factor found by Runnström (1961) in the sea urchin egg. For the building up of the cell wall, as for many synthetic processes, a primer molecule may be necessary (Lark and Lark, 1961).

As we pointed out many times, most of these investigations have been carried out on the echinoderm egg and their results apply, strictly speaking, only to this object. The multitude of parenchyma cells of the mammalian tissue for example has no such deep furrowings. In this case, cytokinesis appears as a kind of intermediary process between the cell cleavage discussed above and the phragmoplast division which we described at the beginning. Probably, part of the conditions required for the cleavage division are fulfilled. On the other hand we have here a process of reconstitution which is far more extensive than a cleavage; it is similar to the cell plate mechanism of the phragmoplast in seed plants (p. 169).

After cytokinesis, free living cells separate immediately from each other. This is due partly to the retraction of the separated cytoplasm halves, but partly also to an active movement of the cell which causes multiple bulges in the cytoplasm, thus giving the impression, when filmed with time-lapse technique, of a bubbling of the cell. These bubbling movements start as a rule as soon as anaphase (Boss, 1955b) and cease at the end of cytokinesis. Incidentally, it may be produced also experimentally, independently from the mitotic cycle (e.g., Lettré and Schleich, 1955; Dornfeld and Owzcarzak, 1958). We have here probably a special form of amoeboid movement which has its origin in changes in the condition of the cytoplasm (p. 275).

Review

Thus, cytokinesis can occur according to two patterns which seem, at a glance, to have little in common. On one hand, a structure can develop, which resembles the spindle but is probably largely independent as to its origin, namely, the phragmoplast. Within this structure, from deposits of substance, the primary cell plate arises, which is double from the beginning and will later build the cell walls. Such a process is typical of seed plant cells. On the other hand, the cell can constrict and divide itself circularly behind the parting chromosomes, as is characteristic of the cleavage divisions of the animal egg cell. Both types of division depend partly on the spindle but to a much greater extent on the kinetocentres, which determine as a rule the position of the division plane. The latter is determined, at the latest, during the early anaphase. The building up of a separating wall is concurrent with changes in the surface of the cell, which could be observed and measured especially in egg cells. These changes are, in fact, an alteration in the nature of the cortex, expanding like waves from the pole region to the division plane and ending in a kind of division ring. Despite all the differences between both types of division, there are many points in common which seem to indicate that they both follow the same basic lines.

Interphase

Cytokinesis terminates the division processes. One mother cell has generated two daughter cells, each of which contains, as a rule, one half of the mother cell, and with the replication, during the following interphase, of the material of each daughter cell, the mitotic cycle is

completed (p. 126). Thus, we visualize the interphase, on the one hand, as the growth phase of the cell. Yet, on the other hand, it is during interphase that all the other conditions required for new karyo- and cytokinesis must be established. This implies not only the provision of material, for example for the spindle, but mainly also the accumulation of the energy necessary for the division process. For most of the investigations in this field have shown that, during karyokinesis, the cell performs but a small exchange of material with its environment. Probably karyokinesis and cytokinesis can only rely on stored energy, and the necessary energy must be gained beforehand and made ready to be used.

DNA synthesis

When we discussed the fine structure and the composition of the nucleus, we discovered that DNA was an integral part of the chromosomes, to be considered as the gene substance itself (p. 114). This explains the special interest that was devoted to the question as to when, during the mitotic cycle, this gene substance is duplicated, in other words, when the single genes are duplicated.

The discovery of the morphological chromosome cycle led readily to the assumption that these replications take place at the beginning of prophase, since it is at this stage that the chromosomes take shape, are more strongly stainable and also reveal early on their double-nature by displaying two half chromatids, which subsequently separate from each other during anaphase as two daughter chromosomes. Yet, a reference to the multiple splitting of the chromosomes, which may in certain cases be traced back already to anaphase (p. 108), has been enough to prove that there is not necessarily a direct connection between chromosome splitting and genome replication.

The problem has been solved by two cytochemical methods, microspectrophotometry and radioautography. The first measures the quantity of light absorbed by DNA, either directly in ultraviolet light or indirectly in visible light after specific staining. Radioautography determines the duration and intensity of the DNA labelling by means of isotopes, best with ^3H -thymidine.

The first findings, measured by microspectrophotometry still with fairly unreliable instruments, seemed to confirm the assumption mentioned above. The absorption increased markedly, especially at prophase (Ris, 1947; Caspersson, 1950). However, it soon appeared that these findings did not correspond to an increase in DNA but were only the sign of an intensification of the non-specific absorption during the prophase condensation of the chromosomes. More detailed investigations showed that the DNA content remains unchanged from prophase to telophase, except for its splitting into two halves at anaphase (Walker and Yates, 1952; Grundmann and Marquardt, 1953; Patau and Swift, 1953; Patau and Srinivasachar, 1959; and others), and that the mitotic DNA content is twice as high as in most interphase nuclei. Consequently, it must have been replicated before the beginning of karyokinesis; in other words, the genetic material is already replicated when it enters nuclear division.

Much trouble has been taken to come to an agreement on the duration of interphase DNA synthesis. Timings vary from object to object (for ref. see, e.g., Bloch, 1958; Prescott, 1961*b*; and others). No doubt there are cell types in which the major part of interphase is used for the purpose of DNA synthesis. They belong to rapidly proliferating tissues such as

young tissue cultures (Walker and Yates, 1952), or the rapidly dividing neuroblasts of the locust embryo (Bergerard, 1955). In the root tip meristem of plants there is also a zone of rapid proliferation, in which almost the entire interphase is used for DNA synthesis (Grundmann and Marquardt, 1953; Woodard *et al.*, 1961a). Evidence of this is given by the congruent findings of both microspectrophotometry and radioautography (Fig. 93). In slower proliferating meristems, the middle or else the second half of interphase are stages of a particularly intensive DNA synthesis and can, therefore, be clearly seen (Howard and Pelc, 1951; Deeley *et al.*, 1957; Sisken, 1959). In binuclear meristem cells, the duration of DNA synthesis was found to be longer than in uninuclear cells (Howard and Dewey, 1960).

But even in unicellular organisms the findings are, to this date, still inconsistent. In the

particularly well investigated ciliate *Tetrahymena*, DNA synthesis begins in the macro-nucleus soon after the previous karyokinesis and is completed in the middle of the interphase or at the beginning of the second half of the interphase, at the latest (McDonald, 1958, 1962; Prescott, 1960). In contrast to this, it starts in the highly polyploid macronucleus of *Paramecium* only in the middle of interphase or even later and appears to continue through the prophase (Walker and Mitchison, 1957; Kimball and Barka, 1959; Woodard *et*

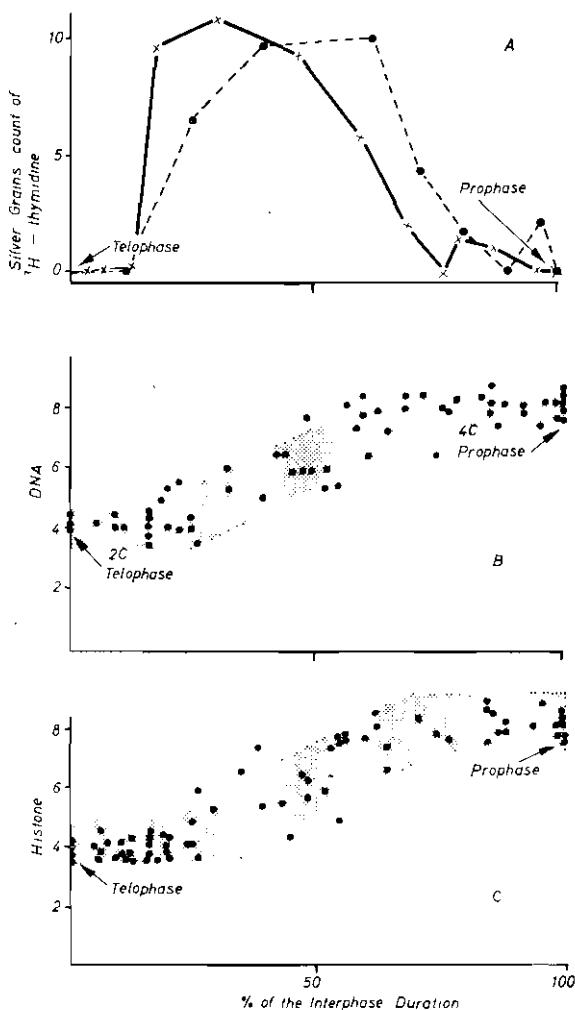


Fig. 93. Synthesis of DNA and histones in the zone of rapid proliferation of the *Vicia faba* root meristem. (A) Silver grains count following exposure to ^3H -thymidine. (B) DNA content determined by Feulgen cytophotometry. (C) Histone content determined by fast green cytophotometry at pH 8.1. (From Woodard, J. *et al.*, 1961, *J. biophys. biochem. cytol.*, 9, 445-462.)

al., 1961b). In the diploid micronucleus, it begins at the same time but is completed much earlier (Woodard *et al.*, 1961b).

In most mammalian tissues, the picture is fairly uniform. DNA synthesis extends over a relatively constant period of 6 to 8 hours (Quastler and Sherman, 1959; Looney, 1960; Schultze and Oehlert, 1960; Taylor, 1960a; Maurer and Koburg, 1961), also in the case of tumour cells (e.g., J. L. Edwards *et al.*, 1960; Johnson and Bond, 1961; Johnson, 1961; Defendi and Manson, 1961; Oehlert *et al.*, 1962); in the latter, the separation of labelled DNA into two equal portions by karyokinesis can be particularly clearly recognized (Fig. 94). Furthermore, DNA synthesis is fairly closely connected with the beginning of the

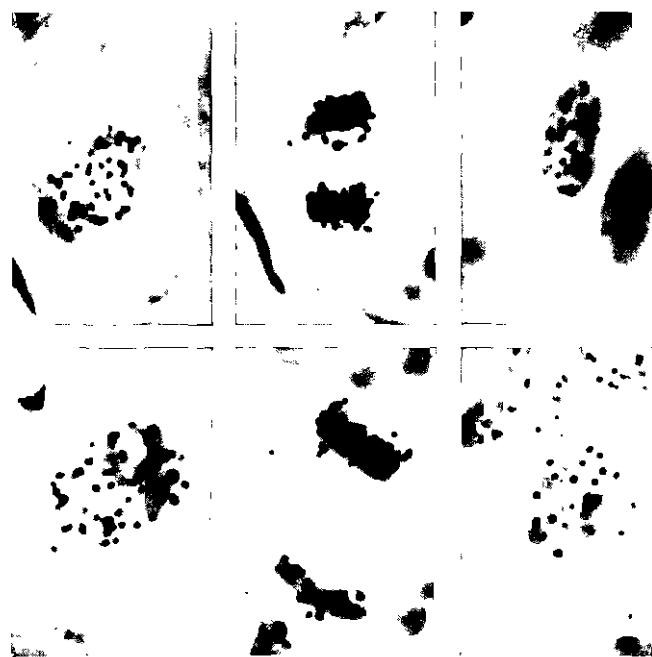


Fig. 94. Labelled nuclei of the basement cell layer in the mouse skin (top row) and in the solidifying Ehrlich carcinoma (bottom row), 6 hours after administration of ^{3}H -thymidine. *Left:* complete labelling of the intermitotic nuclei. *Middle:* labelled telophases; *Right:* extent of labelling reduced to one-half after mitosis. (From Oehlert, W. *et al.* (1962), *Beitr. path. Anat.*, 127, 63-78.)

following karyokinesis. Between the end of DNA replication and the next prophase there is an interval of 40 to 60 minutes, the quiescent premitotic phase (Firket, 1956, 1958; Quastler and Sherman, 1959; Maurer and Koburg, 1961; Sisken and Kinoshita, 1961). However, this prekaryokinetic interval (the expression "quiescent phase" is as incorrect as "premitotic", for this interval is part of the mitotic cycle as is the whole interphase) may last much longer, for example in the keratinizing squamous cell epithelium of the mouse (Koburg and Schultze, 1961), in tissue cultures (Painter and Drew, 1959) or in malignant tumour cells (Oehlert

et al., 1962). Still more variable is the period extending between the end of the reconstruction and the beginning of the next DNA synthesis; in intermitotic cells, it is liable to last days, weeks or months. This is the function phase of tissue cells.

These findings allow us to lay out a clear-cut classification of the interphase, as it occurs in the majority of vertebrate cells (Fig. 95). In the karyokinesis (K), DNA is divided into two equal portions. During a variably long interval (G_1), lasting weeks or months in intermitotic nuclei, the DNA content remains constant. Prior to the beginning of the next karyokinesis, DNA replicates within a period of 6 to 8 hours during the DNA synthesis time (S), which is followed by a prekaryokinetic interval of 40 to 60 minutes (G_2) prior to the next karyokinesis (K). The DNA synthesis time (S) has proved to be the most stable span of time within the interphase.

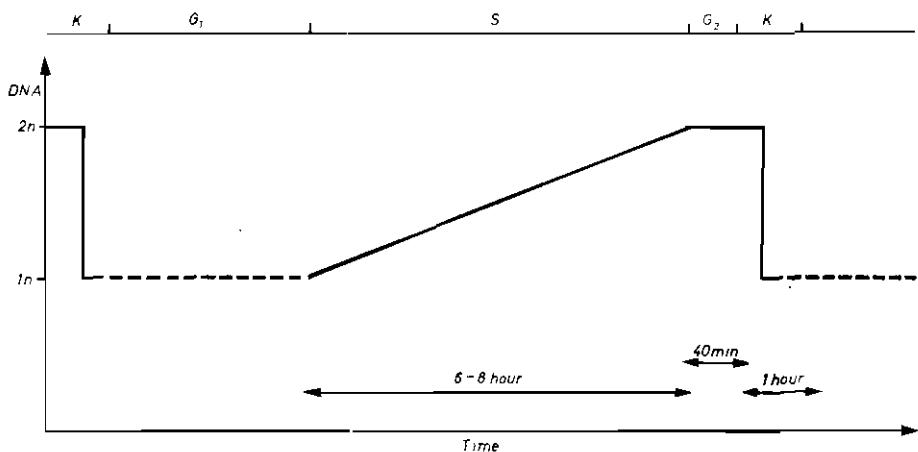


Fig. 95. Division of the interphase between two karyokineses (K) into presynthesis time (G_1), DNA synthesis time (S) and prekaryokinetic interval (G_2).

The investigations of Kornberg *et al.*, have outlined some important stages in DNA synthesis (see, e.g., Lehmann *et al.*, 1958a, 1958b; and others). A polymerase can combine the triphosphates of deoxyribonucleosides with an already present DNA primer in such a way that the DNA type of this primer will always be generated again (see, e.g., Bollum, 1960). The base ratio and, hence, the specificity of the newly formed DNA are determined by this DNA primer. Fig. 96 shows a diagram representing such a binding of free nucleosides; in this model the DNA double helix is assumed to be opened at the site of replication (see also Fig. 52). We discussed earlier the problems that arose from the model proposed by Watson and Crick for DNA (Fig. 53).

If the diagram in Fig. 96 is true, it is not possible that DNA synthesis occurs at the same time along the whole length of the chromosomes. Of course, a chromosome may perhaps consist of many double chains of DNA, wound around one another in a spiral, and in each double chain the DNA synthesis may take a different direction, begin at a different site or occur at a different speed (see, e.g., Painter, 1961). In fact, if cells are left only for a short

time in a ^{3}H -thymidine-containing medium, the single chromosomes are labelled only incompletely; thus it was inferred that certain parts of the chromosomes replicate in a sequence which repeats itself constantly (Taylor, 1960b) and this, also in man (Lima-de-Faria *et al.*, 1961). DNA synthesis frequently starts at the tips of the chromosome and from there proceeds, like two waves directed towards each other, to the kinetochore (Taylor, 1958a).

Quite a similar principle could be demonstrated by the striking experiment made on the ciliate *Euplotes eurystomus*. Its macronucleus is a crescent-shaped rod of 140 μ in length. DNA synthesis begins early during the interphase at the tips of this rod and two opposite waves move towards each other (Gall, 1959b; Prescott and Kimball, 1961). Radioautographs show this wave to be a zone labelled with ^{3}H -thymidine and the distal arms of the chromosomes appear markedly thicker than the proximal (Fig. 97a). Both these synthesis waves, described in early reports purely morphologically as reorganization bands, consist, as seen in the phase contrast microscope, of two transverse bands (Fig. 97b); to the already synthesized zone (D) a lighter band (R) joins itself, which seems optically empty and is probably highly hydrated. At any rate, the dry weight in this band is relatively low, as could be demonstrated by means of an interference microscope (Prescott and Kimball, 1961). Adjacent to this zone, we find a homogeneous compact band (S), which extends into the still unreplicated portion of the nucleus (U). Radioautography shows that only the light, homogeneous zone (R) is the site of DNA synthesis (Fig. 97c), in other words, the zone of maximal hydration (Prescott, 1962).

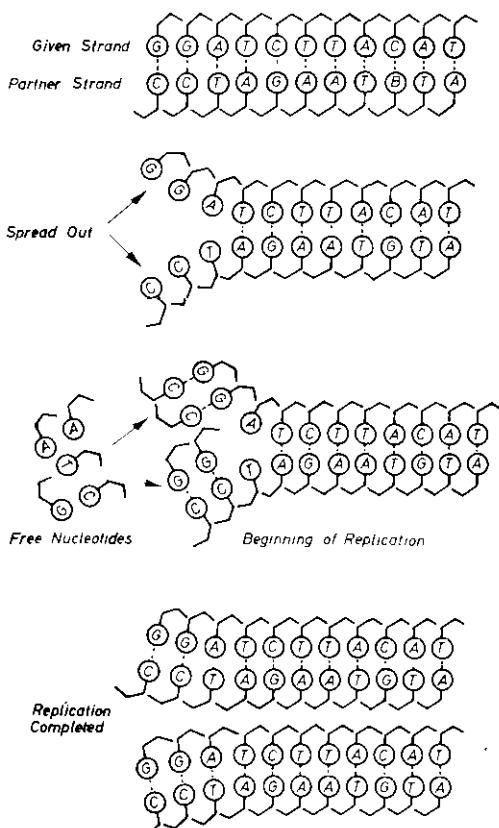


Fig. 96. Model showing the binding of the nucleotides to the DNA chain (DNA primer). (From Weidel, W. (1957), *Virus, die Geschichte vom geborgten Leben*, Springer, Berlin.)

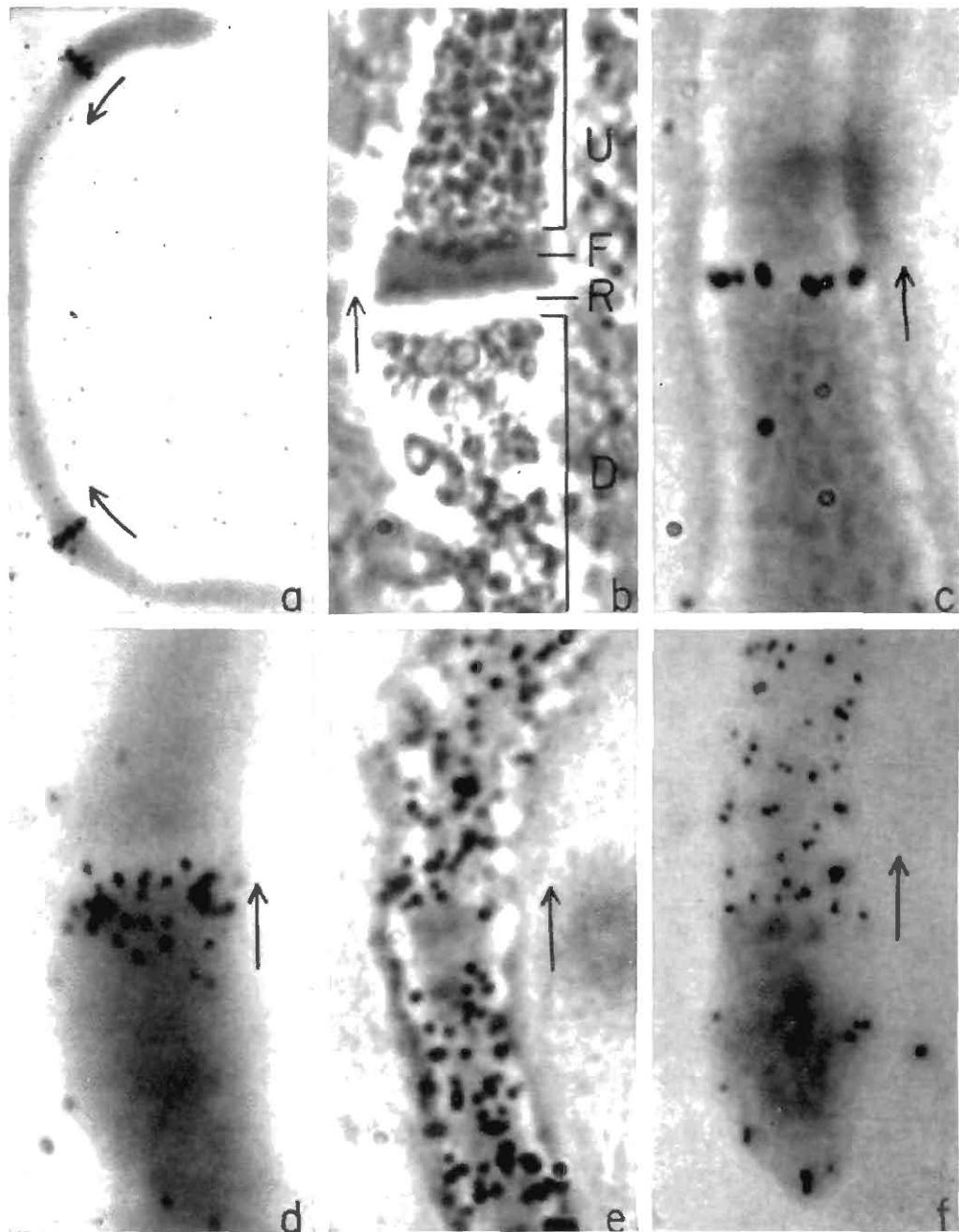


Fig. 97.

Protein and RNA synthesis, changes in the nuclear volume

This same light zone is, in the ciliate *Euplotes*, also the site of maximal absorption of ^3H -histidine (Fig. 97d); thus, one cannot but conclude that the corresponding protein component, the histone, is synthesized also at the same time as the DNA (Prescott, 1961b, 1962). This fact has been demonstrated in *Euplotes* (Gall, 1959b) but also in a great variety of organisms and with different techniques, mainly cytophotometry, using fast green dyes (Bloch and Godman, 1955; Alfert, 1958a; Woodard *et al.*, 1961a). The quantitative changes in fast green absorption values at pH 8.1 correspond then nearly exactly to those of Feulgen absorption (Fig. 93, bottom). In the root tip meristem of onions and the pollen grains of *Tradescantia*, the incorporation of ^3H -thymidine into the DNA and that of ^3H -arginine into the histones are synchronous (De, 1961).

The chromosomes contain other proteins (p. 93), which are also divided into two equal portions by karyokinesis and replicate in interphase. It has not been possible as yet to determine with the same exactitude at which moment the latter occurs. Probably protein synthesis as a whole is restricted to interphase or intermitosis; at least, it is reduced to a minimum during karyokinesis (Prescott and Bender, 1962). The interphase may largely reveal a steady incorporation of amino-acids, as for instance in the frequently mentioned ciliate *Tetrahymena* (Prescott, 1960) and of *Paramecium* (Woodard *et al.*, 1961b), where the nuclear dry weight increases accordingly (Kimball *et al.*, 1959). In the root tip meristem, the total protein weight increases particularly markedly after telophase and prior to prophase, parallel to the nuclear volume (Woodard *et al.*, 1961a).

Whereas the prophase nucleus is strongly hydrated, the telophase nucleus is largely dehydrated. Hence, the nuclear volume increases to many times its original value at telophase. In the meristem of root tips, this change occurs again in waves (Fig. 98); in other words, the nuclear volume rises after telophase relatively abruptly, increases then much slower for quite a while, until the preprophase swelling of the nucleus again causes a rapid increase which ends with the disintegration of the nuclear envelope (see, e.g., Grundmann and Marquardt, 1953; Dolezal and Tschermak-Woess, 1955; Woodard *et al.*, 1961a). In other objects with a rapid succession of karyokineses, the interphase increase in volume is nearly linear, for instance in *Tetrahymena* (Cameron and Prescott, 1961).

It is equally impossible to establish a general rule as to the duration and timing of RNA synthesis. With the exception maybe of yeast cells (Mitchison, 1957) and of certain tumour cells (Lauf *et al.*, 1962), it is suspended during a great portion of the karyokinesis

Fig. 97. The processes of synthesis in the macronucleus of *Euplotes eurystomus* (a) After 20 minutes exposure to ^3H -thymidine, labelling of two waves of DNA synthesis moving toward each other. (b) Phase contrast micrograph showing the different zones of the "reorganization band". F: compact front zone; R: light zone; U: still unreplicated zone; D: replicated zone of nucleus. (c) After 2 minutes exposure to ^3H -thymidine. (d) After 40 minutes exposure to ^3H -histidine, labelling of the lighter zone and the adjacent portions of the replicated zone. (e) After 30 minutes exposure to ^3H -uridine, RNA labelling in all parts of the nucleus except in the "reorganization band". (f) After 45 minutes exposure to ^3H -uridine and 8½ hours storage in a non-radioactive medium, no labelling in the "reorganization band" or in the already replicated parts of the nucleus; marked labelling in the still unreplicated nuclear portion. The arrow shows the direction of the synthesis waves. (From Prescott, D. M. (1962), *J. Histochem. Cytochem.*, 10, 145-153.)

(Feinendegen *et al.*, 1960; Prescott and Bender, 1962). But it can be demonstrated during the whole of interphase, in *Tetrahymena* especially during the second half of interphase (Prescott, 1960). Since RNA synthesis is a metabolic function of every interkinetic nucleus which promotes protein synthesis in the cytoplasm (p. 38), it is difficult to discern which part of it serves for replication of the nuclear material.

However, it is absolutely clear that RNA synthesis and DNA synthesis exclude each other at the same site. During the period of DNA synthesis, the formation of RNA is reduced throughout the whole nucleus (e.g., Taylor and McMaster, 1954; Moses and Taylor, 1955; Sisken, 1959; Nygaard *et al.*, 1960), as in the ciliate *Tetrahymena*, for example (Prescott, 1960). The self-replication of the DNA molecule is not compatible with a simultaneous induction of RNA molecules (Prescott, 1961*b*), since RNA is produced at the DNA according to the same mechanism as the new DNA chain (p. 116).

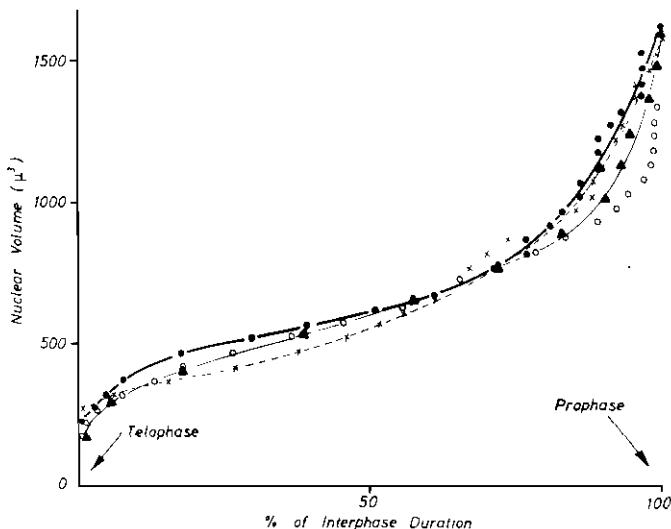


Fig. 98. Interphase increase in the nuclear volume of *Vicia faba* root meristem. (From Woodard, J. *et al.* (1961), *J. biophys. biochem. Cytol.*, **9**, 445-462.)

A particularly brilliant demonstration of this alternative between DNA and RNA syntheses was made, again on the elongate macronucleus of *Euplotes* (Prescott and Kimball, 1961; Prescott, 1962). ^3H -uridine is incorporated into RNA along the whole chromosome, with the only exception of the zone of the reorganization band, which, as we saw, was used for DNA synthesis (Fig. 97*e*). Yet RNA is synthesized in the region of the reorganization band and is delivered into the cytoplasm; for, if the ciliate is incubated for 45 minutes with ^3H -uridine and then kept for $8\frac{1}{4}$ hours in a non-radioactive medium, the fraction of the nucleus which contains the reorganization band is normally labelled, whereas the one behind it is hardly labelled at all (Fig. 97*f*). Apparently in this fraction the RNA material is lost as the DNA synthesis wave sweeps it away. After that, however, RNA production starts again (Fig. 97*e*).

Behaviour of the cytoplasm

The extent and intensity of cytoplasmic syntheses during interphase vary a great deal from case to case. For example, they may be influenced by the quantity of the surrounding nutritional elements. In a sufficiently nutritious medium, for example, the size of amoebae may increase nearly uniformly up to double the cell's size just before the end of cytokinesis. If, during cytokinesis, two daughter cells of unequal size arise, they grow at interphase up to the size of normal amoebae, before they enter a new division cycle. Consequently, replication is definitely the rule but by no means the condition *sine qua non* of every cell division (Prescott, 1955). There seems rather to be a sort of critical cell mass (Mazia, 1956) which must be reached during interphase. Considering the relative constancy of the nuclear volume, this is another way of referring to the "nucleocytoplasmic relationship" which was previously considered as a restricting factor in the interphase growth of the cell or as a promoting element for the following cell division (R. Hertwig, 1903a).

However, such general data do not give sufficient explanation. We must investigate more accurately, which elements of the cytoplasm must be synthesized. In the course of karyokinesis, the endoplasmic reticulum, that is, the system of tubules traversing the whole cytoplasm (see Fig. 1), undergoes typical changes. In the prophase, it loses part of its organized lamellar structure and turns into a system of vesicles, which appear to be largely discontinuous and which are, moreover, frequently deposited at the cell's periphery (Porter, 1954). In plant cells, large lamellae remain, which are forwarded to the region of the pole caps (Porter and Machado, 1960). The synchronous disintegration of the nuclear envelope as a part of the endoplasmic reticulum (p. 64) becomes quite understandable in this greater context. However, the cytoplasmic syntheses of interphase affect in this way not only the growth but also the reorganization of this endoplasmic reticulum. The Golgi apparatus, too, disintegrates during karyokinesis (Dalton, 1951). In amoebae, the pulsation of the nutrition vacuoles ceases (Chalkley, 1951); cilia and fimbria may get lost or are resorbed (Grell, 1956) and in more highly differentiated cells, such as in gland cells, the secretion granules disappear (e.g., Cohen and Berrill, 1936) or the specific elements of such cells are degraded. All these structures need to be manufactured anew after karyokinesis.

The behaviour of the mitochondria deserves special attention. Most of the quantitative studies have shown that the number of mitochondria is reduced during prophase, reaches a minimum at meta- and anaphase, and increases again after telophase (Frederic, 1954; Ågren, 1955; and others). No doubt, such quantitative data are difficult to interpret, since they may indicate on the one hand a disappearing or a disintegration of the mitochondria, but also, on the other hand, a gathering to greater mitochondrial complexes. And indeed we find, at least in tissue cultures or especially in locust spermatocytes, the mitochondria as long threads arranged like a cuff around the equatorial plate and stretched out during anaphase, thus participating in certain cases to the formation of the intermediary body between the telophase cells (p. 168) (Barer and Joseph, 1957). Their distribution occurs probably passively as a result of the cytoplasmic currents, mentioned previously (p. 163), independently from the position of the nucleus (Makino and Nakahara, 1955). Maybe larger cytoplasmic elements deposit as a rule on the outer surface of the spindle during metaphase, whereas smaller ones remain diffusely scattered (Gross and Mayer, 1953).

Respiration and glycolysis

The behaviour of the mitochondria leads us to the problem of mitotic energy. When and how does the cell gain the energy which is necessary for the movements and rearrangements of karyokinesis?

The most evident and the most efficient way of gaining this energy is by oxidative phosphorylation, for which the cell needs mitochondria, as they contain the necessary enzymes (p. 308). Hence, if even only a part of the mitochondria have undergone disintegration or structural changes and are thus inactivated in their functioning during karyokinesis (see, e.g., Lettré and Schleich, 1955), there can hardly be enough energy generated oxidatively during the period of nuclear and cytoplasmic divisions, which consume energy.

Also, it has always appeared that the respiration processes are markedly reduced during karyokinesis. This is true, for example, of the synchronous microsporocyte division of *Lilium* and *Trillium* (Erickson, 1947; Stern and Kirk, 1948), of the late cleavage divisions of egg cells (Zeuthen, 1947, 1955; and others), and also of *Tetrahymena* ciliates. On the other hand, interphase is very sensitive to a depletion of O₂ and the rate of mitoses following a lack of oxygen rises in direct correlation with the oxygen tension (Bullough and Johnson, 1951; Amoore, 1962a). Probably, an iron complex of the cell, independent of the respiration, plays a special part here (Amoore, 1962b).

The actual importance of glucose is most controversial. In experiments made mostly on the epidermis of the mouse ear, a direct correlation was found between blood sugar level and mitotic frequency (Bullough, 1949). It was also possible to establish a connection with the glycogen content (Bullough and Eisa, 1950), while the oxidative degradation of carbohydrates was considered to play an important role in the preparation of energy for karyokinesis. The latter is supposed to take place during an antephase, directly preceding the prophase (Bullough and Johnson, 1951). Chronologically, this antephase would coincide exactly with the prekaryokinetic interval (p. 177). However, these findings of Bullough *et al.*, could not be reproduced with certainty (Laws, 1952) and more accurate investigations of the mitotic frequency under the influence of intermediary products of the carbohydrate metabolism equally failed to confirm Bullough's observations (Gelfant, 1960a and b).

However, these investigations did show that karyokineses obey the all-or-none law, once the conditions are favourable (see Mazia, 1961a), so that single karyokineses may come to completion even after the death of the whole organism (Bullough, 1950). At the beginning of prophase, the cell has a pool of stored energy which saves it the necessity of having to produce energy during the division phases. However, this energy pool (Swann, 1957) is not filled up at a period immediately preceding prophase, i.e., during an antephase, but during the whole of the interphase, and, in part, probably even before the previous karyokineses; for, if respiration is impeded during one karyokinesis, the latter will still occur quite normally, yet the beginning of the next karyokinesis will be slowed down or completely stopped (Swann, 1954; Hamburger and Zeuthen, 1957; Scherbaum, 1960; and others). What kind of energy storage this is, is still open to speculation. Maybe ATP plays a part here (Plesner, 1958; Mazia, 1961a).

The mitotic cycle and its stimulation

We have now a certain knowledge of the interphase processes; there are still many gaps, but we can at least establish certain principles. The interphase includes nearly all the metabolic processes related to mitosis, whether it is the synthesis of those cell elements which have been halved by the division or the necessary processes for the production of energy. Both are the prerequisites of karyo- and cytokinesis; a mitosis does not start as late as the prophase condensation of the chromosomes, it begins already with the interphase. Since the prekaryokinetic interval or premitotic quiescent phase (p. 177) covers a relatively constant period of time, the beginning of karyokinesis seems, unless disturbances occur, to coincide with the beginning of DNA synthesis, which also proves to be largely constant in timing and duration. Consequently, the much discussed problem of the mechanism provoking cell division is not as much related to the division processes themselves as it is to the interphase processes, for they are the ones to determine the rhythm of mitosis.

Yet, this is only partly true; for there are many cells in which DNA replication occurs days or weeks before karyokinesis. We can mention here, for example, such tissues in which the nuclei become first polyploid or polytene and then are divided by karyokinesis; this is the case, for instance, during the development of plant galls of beans (Rasch *et al.*, 1959) or in the experimental cancerization in the rat liver (Grundmann, 1954, 1961b, 1962; Hobik and Grundmann, 1962). In exactly the same way, exposure of the alga *Chlorella* to intense light produces polyploid (or polytene) macronuclear light cells, which generate subsequently, once in the dark again, 16 small dark cells by way of a rapid succession of ploidy reducing divisions (Iwamura, 1955). In certain tissues there even seem to be two cell populations, one of which may undergo division right away, whereas the other one must accomplish beforehand a DNA synthesis (Gelfant, 1962). The former type of cell remains, consequently, during most of the intermitosis period, in the G₂ phase, the latter in the G₁ phase, as shown in Fig. 95.

A dissociation of the interphase syntheses and the karyokinesis may be produced experimentally by means of repeated heat shocks as, for instance, on the ciliate *Tetrahymena* (Scherbaum and Zeuthen, 1954). By raising the temperature for half an hour up to 33.5°C, it is possible to bring the divisions to a complete stop, whereas the DNA syntheses continue. A return to normal temperature will bring back synchronous divisions after an interval of 70 minutes (Fig. 99).

This also brings some light to the synchronous cell divisions which occur naturally. In fact, one would expect all cells, which originate from a common mother cell and live under the same conditions, to have the same division rhythm. By maintaining strict standard conditions, one may keep the ciliate *Tetrahymena*, for example, synchronous for four divisions (Scherbaum and Zeuthen, 1954), amoebae for five divisions (Prescott, 1955). But afterwards, the small differences in nutrition, respiration etc., which arise from the environment determine each cell's particular rhythm.

Such factors of the environment are also responsible for the daily rhythm of mitoses, which we find practically in all tissues. In the human epidermis, for example, this rhythm reaches a maximum during the first 4 hours after midnight (Scheving, 1959), in a regenerating mouse liver at 2 a.m. (Barnum *et al.*, 1954), whereas rat liver shows a maximal mitotic

frequency at 8 a.m. and 12 noon (Jackson, 1959). We find similar peaks in mouse ear epithelium (Bullough, 1948) and, according to Chu (1960) also in the connective tissue of the mouse, and its spinal ependyma shows clearly, according to Kulenkampff (1961), a maximum at 10 a.m. We must point out here that rodents are night animals, so that the morning peaks in the mitotic frequency occur during the period of rest of these animals; the case is the same for the human epidermis which has its maximal division frequency after midnight.

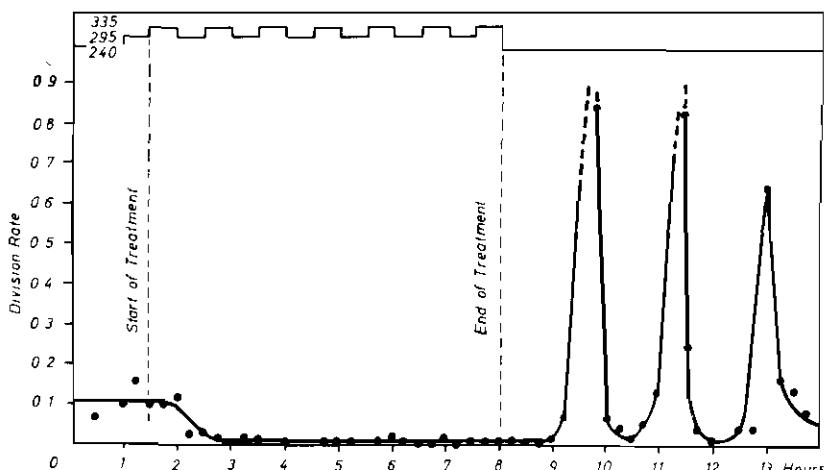


Fig. 99. Synchronous division of *Tetrahymena pyriformis* following heat shock. The upper line indicates the temperature levels, both vertical lines mark the beginning and the end of the heat shock. (From Scherbaum, O. and Zeuthen, E. (1954), *Expl. Cell Res.*, **6**, 221-227.)

Hence, we may, to a certain extent, generalize and say that most karyokineses of physiological growth occur when, in its daily rhythm, the cell's activity has come to a pause, such as in the case for example of the frog corneal epithelium, at 2 a.m., provided that the experimental exposure to light does not impede the physiological rhythm of the eye (Meyer, 1954). This rule applies also to the nightly mitotic peaks in plants (e.g., Karsten, 1918; Jensen and Kavaljian, 1958; for further reference see Tischler, 1934-1951) or in chlorophyll-containing flagellates (Leedale, 1959a) in which mitoses occur principally during the phase of darkness. An increase in functional activity, brought about for instance in the ependyma of mice, when the animals swim for several hours (Kulenkampff, 1961), delays or reduces the mitotic peak. Tissues which are in their function controlled by specific hormones may be affected by these hormones in their mitotic rhythm (Bullough, 1955; Dobrokhotov, 1961), however only *in vivo*, not *in vitro* (Gelfant, 1960a and b). It is experimentally possible to delay the division, for example, of starving plasmodia by adding just before the beginning of the division macronuclear nutrition substances, which require activity on the part of the cell in order to be assimilated. If, by contrast, these substances are given during the early interphase, some period of time before the cell undergoes karyokinesis, it is all the more ready to divide itself (Guttes and Guttes, 1962).

Thus, the cell's activity is on the whole a factor of delay for the beginning of mitosis. We may even go further and assume that cells involved in an intense activity do not start any mitosis any more (Peter, 1940, 1947; and others). We will return to this principle when we deal with amitosis.

However, cell activity is only one of the factors which affect the mitotic frequency. There are, in addition, determinants of all sorts, of which we know probably only a fraction (see, e.g., Swann, 1958; Wilson, 1959; Stich, 1960; and others). For instance, we have known for a long time that in the vicinity of wounds and necroses substances appear which promote the mitosis of the neighbouring cells. They were first found in various plants and called wound hormones. Since then, there has been evidence of similar effects in mammalian organisms (Altmann, 1949; Cain, 1961; Teir and Lahtiharju, 1961), where many factors are liable to play a part at the same time. At any rate, it is possible, in the skin of the mouse, to cause separately the proliferation of the epidermis and of the hair follicles, and it may be assumed that two different substances are responsible for this (Bullough and Laurence, 1960). Some findings would also suggest the presence of an inhibiting substance, which would be produced in the epidermis by the postmitotic cells and which is temporarily diminished by the infliction of wounds (Oehlert and Block, 1962). The fact that substances really exist which promote regeneration in wounds could be made plausible in an experiment on the rabbit lens epithelium. Following a small mechanical injury, a stimulation wave expands from the site of the wound at a speed of 17μ per hour, beginning with a DNA synthesis, detected by radioautography, and leading into karyokineses (Harding and Sriniwasan, 1961). Sometimes a second mitotic wave follows the first.

It is to be assumed that physiological growth, too, is determined by such substances (Oehlert and Block, 1962), which can be demonstrated also in plants, for example in growing fruit (Letham and Bolland, 1961), whereby, once more, accelerating substances may be bridled by retarding factors, thus maintaining the tissues in a state of equilibrium between stimulation to and restraining from mitosis. Such substances with antagonistic effects were found by Menkin (1959) (see also Menkin *et al.*, 1959) in homogenates of sea urchin ovaries; the accelerating factor appears to be here a uracil-containing dinucleotide, the retarding substance probably of polynucleotide (Menkin, 1959). In mouse tissues, thymidine was found to be the stimulating agent (Greulich *et al.*, 1961).

It was possible to isolate a specific hormone for cellular division from yeast cells and spermatozoans, the 6-furfurylamino purine, called by its discoverers (Miller *et al.*, 1955) kinetin. Its effect, for instance on the human epidermis, depends on the concentration; a maximal stimulation to mitosis is brought about with a low concentration of about 0.001 mg/100 ml culture medium; higher concentrations have no effect at all or even retard the beginning of mitosis (Orr and McSwain, 1957). This explains also its variable effect on tissue cultures (Lettré and Endo, 1956). The physiological action of kinetin cannot yet be fully evaluated. Its effect on *Paramecium*, for example (Guttmann and Back, 1958), on the salamander *Triturus* (Buckley *et al.*, 1962) and in many plants (Torrey, 1961) is, however, certain. Perhaps kinetin is really an ubiquitous factor for mitotic cell division.

Finally we find other mitosis-stimulating substances in the blood serum of mammals, for instance in the rat after partial hepatectomy (Friedrich-Freksa and Zaki, 1954). This factor is not species specific; it appears under the same form, for example, in the Syrian

hamster (Wrba *et al.*, 1960b), reaching its maximum 48 hours after partial hepatectomy (Bucher *et al.*, 1950; Wrba *et al.*, 1960a). If one considers that, according to old findings which have been found recently new confirmation (Inoué, 1962), the nervous system, too, has a regulating effect on the mitoses of metazoans, we can see that the range of determinants of mitosis includes nearly all factors which have some effect on the inner and outer milieu of an organism.

Review

Thus, it is apparent that mitosis is correlated with the general processes which regulate the organism. It is a reaction of the tissue to injuries and stimulations of the most different kind with the special goal of regeneration. It is commanded by the same exterior and interior factors, which determine also the whole metabolism of an organism, such as light and temperature, but also hormones and the local environment of a tissue. Moreover it requires a relative pause in the metabolism, as we can conclude mainly from the physiological daily rhythm. This is made clear by the observation that those cytoplasmic structures which deal with the cell's function are inactivated during karyokinesis or are even partly degraded. Thus, during karyokinesis, not only the nuclear material, because of its distribution into the chromosomes, but with it the whole cell is largely without function. Concurrently, the production of energy is reduced and the energy necessary for the division processes is stored before the beginning of karyokinesis, as a kind of reserve. Moreover, at interphase, all those synthetic processes take place by virtue of which karyokinetic division becomes a real process of growth. In the nucleus as well as in the cytoplasm, all important substances are replicated during the course of a normal mitotic cycle; in this process, DNA synthesis is of particular importance as a replication of the genomes and its beginning must be considered as the very beginning of the mitotic cycle. It occurs within a definite period of time, which is constant in mammals, before the prophase and concurrent with the replication of the histones. DNA and RNA synthesis exclude each other in time as well as in location. As a phase of synthesis or replication, the interphase bears the same importance as the whole karyokinesis. The substances manufactured during interphase, especially the replicated chromosomal material, is distributed by the relatively complicated mechanism of mitotic karyokinesis to two daughter cells, which can now replicate anew. This alternating cycle of synthesis and division constitutes the very nature of mitotic growth.

MEIOSIS

One of the characteristics of sexual reproduction is the union of two gametes to form one zygote, which has then twice the number of chromosomes present in the gametes. Once this fusion of both gametic nuclei, or karyogamy, was known, a compensation process was expected to take place which would reduce again the number of chromosomes. This compensation process is meiosis, a special kind of karyokinesis, whose mechanisms are partly the same as those of the mitotic cycle. However, its particularities require that we consider it separately; we shall restrict ourselves to the main facts.

Reduction of the number of chromosomes involves two nuclear divisions. The first begins with a pairing of the homologous parental chromosomes and it ends, after the chromo-

somes have separated, with their redistribution onto two haploid nuclei. In the second meiotic nuclear division, as in the mitotic karyokinesis, the chromosomes end up in two, likewise haploid, nuclei. In this sequence, the first nuclear division is a reduction division, the second an equation division. Since these lead, at least in most animals, to the formation of the egg cells and the sperm cells, they are also frequently called the first and second maturation division. Consequently, in most animals, the only cells to be haploid are the sex cells, or gametes and this is why such a meiosis is also called the gametic type of meiosis (Wilson, 1928). In inferior plants, i.e., in some algae and fungi, meiosis occurs soon after fertilization, so that here only the zygote is diploid. This zygote generates, by meiotic chromosome reduction, haploid spores, which grow to become a haploid thallus. The latter then mitotically produces new gametes. This is the zygotic type of meiosis. There is also a third intermediary type, characteristic of many superior plants and many ferns and mosses. In this case a diploid vegetation organism, the actual plant itself, produced by mitotic division and differentiation, forms diploid sporophytes; it is then in their sporangia that meiosis takes place. The main difference between this type of meiosis and the above-mentioned gametic type is that the haploid cells produced meiotically, the macro- and micro-spores, do not directly become gametes, but by a number of mitoses grow into female or male gametophytes, a part of which mature to be gametes. Inferior plants, that is, the fungi and algae, as we know them, have a specifically developed body, the gametophyte; superior plants, i.e., the organisms of our superior vegetable kingdom, have a fully developed diploid vegetation body. We can see how, with the evolution of superior plants, the diploid sporophyte acquired its special character, supplanting the haploid gametophyte. However, all three types of meiosis have in common the reduction to haploidy of the diploid chromosomal set brought about by fertilization, no matter how long a time might have elapsed since fertilization.

Premeiotic processes

We now understand in which way meiosis is connected with the whole cycle of the organism's life, yet we hardly know anything at all about the stimuli leading to meiosis. No doubt they are dependent on meiosis-stimulating substances to be found in the environment of the cell; the details of this process still being obscure. For example, certain symbiotic flagellates, *Trichonympha* living in the intestine of certain insects such as termites and *Cryptocercus*, pass through a sexual phase when the insect is moulting as a result of the action of the insect's growth and moulting hormone, ecdysone. Normally, these fully developed insects produce only very little ecdysone and the flagellates reproduce themselves asexually. But injections of ecdysone into the insect immediately stimulate several sexual cycles of the flagellates (Cleveland, 1959).

Other stimulants of meiosis are probably to be found in the cell itself, although we cannot exclude their dependence on exogenous factors. It is typical, for instance, that in animal as well as in plant cells, meiotic prophase shows a marked enlargement of the nucleus, which is threefold that which occurs at the beginning of mitotic karyokinesis (Beasley, 1938; and others). This is mainly an influx of water, which explains why meiotic prophase nuclei contain much free nuclear sap. The presence of this sap seems to condition the various

shiftings of the chromosomes during meiosis. Whether it causes meiosis to take place, is, however, quite uncertain. The same can be said of the observation that meiotic prophase nuclei contain relatively little RNA, or at least less than the mitotic, and Sinha (1960) concluded from this that a high RNA-DNA ratio induces a mitotic, and a low RNA-DNA ratio, a meiotic division.

The question as to what is cause and what effect seems to remain equally unsolved in the precocity theory of Darlington (1937). Darlington's basic theory was that the chromosomes are not yet replicated at the beginning of the meiotic prophase, so that they enter the prophase precociously. The cause of the beginning of meiosis is, consequently, a dissociation in time between the chromosomal and the extra-chromosomal division cycle (see also Darlington, 1958). However incisive this theory might be, it still says little about the factors causing meiosis.

In reference to this, it was pointed out that the interphase DNA synthesis is, as a rule, completed before the meiotic karyokinesis, as it is also before the mitotic karyokinesis, so that the precocity theory is no longer founded. And it is a fact that findings in spermatocytes of mice (Swift, 1950a), in *Tradescantia* (Swift, 1950b) and in *Lilium* (Taylor and McMaster, 1954) clearly indicate that such a DNA synthesis does take place prior to the beginning of meiosis; the DNA content replicates just before the meiotic prophase and is twice reduced by one-half by both meiotic divisions (Fig. 100). Furthermore, we had already, when discussing the mitotic chromosome cycle (p. 131), emphasized the fact that the longitudinal split that appears in the chromosome under the light microscope is probably merely the manifestation of a replication which took place long before. This is confirmed by all those observations which indicate that the anaphase chromosomes of the last premeiotic karyokinesis already consist of at least two chromatids (Nebel and Ruttle, 1936; Atwood, 1937; and others), which should be all the more and necessarily the case in the following meiotic

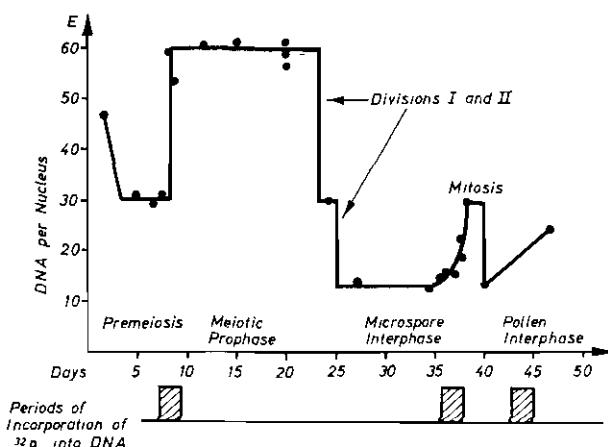


Fig. 100. Changes in nuclear DNA content at various stages of microsporogenesis in *Lilium longiflorum*. (From values given by Taylor, J. H. and McMaster, R. D. (1954), *Chromosoma*, 6, 489-521.)

prophase. And indeed, the chromosomes are unmistakably replicated in the first stage of the meiotic prophase, in the leptotene (see, e.g., Geitler, 1938*d*; Oehlkers and Eberle, 1957; and others).

The process of meiosis

At the beginning of meiosis, as we already mentioned, we notice a marked increase in the nuclear volume brought about mainly by an influx of water from the cytoplasm. Concomitantly, the chromosomes condense by way of contraction, coiling and loss of water, exactly as in the mitotic prophase. However, the condensation here is not nearly to the same extent as in the latter; the chromosomes remain longer and thinner and form a fairly even entanglement of threads which fills the nucleus. In many specimens, the chromomeres can be seen lined up like beads all along the chromosomes; it was, indeed, in this first stage of the meiotic prophase that the chromomeres were discovered for the first time (p. 113). Within the sap-filled nucleus, the chromosomes appear quite mobile and may assume various positions. In the pollen mother cells of *Lilium*, for example, they form, even after careful preparation, relatively dense masses which appear on one side of the nucleus; this is probably a typical *in vivo* artifact (Belar, 1930*a*). It is called synizesis. In the spermatocytes of certain animals, the chromosomes lie in a definite array at the so-called bouquet stage, i.e., the tips of the chromosomes, the telomeres, point towards the kinetocentres, whereas the other parts of the chromosomes extend into the nuclear space, a position which can appear again even at later prophase stages of meiosis, such as in pachytene (Hughes-Schrader, 1943). This is clear evidence of an attraction force between kinetocentres and telomeres (Schrader, 1954). The nucleoli always remain, in contrast to mitotic prophase (p. 129). This first stage of meiotic prophase, displaying very fine threads, is called *leptotene* (Fig. 101 (2) and (3)).

Without any clear-cut transition, condensation and coiling of the chromosomes continue during the following *zygotene* (Fig. 101 (4)). The chromosomes shorten and thicken, the chromomeres become more and more visible, and a process occurs, which is largely specific for meiosis. First, it can be noted that single fragments of the chromosomes run absolutely parallel to one another; these are generally the homologous chromosomes of the parental couple. Soon all chromosomes lie very closely paired next to each other (Fig. 101 (5) and (5a)); at this stage, all the different sections along the chromosomes' length, i.e., the heterochromatic areas, the primary and secondary constrictions, the satellites etc., always coincide exactly. This pairing of the chromosomes, or syndesis, may start simultaneously at several points along the chromosomes; but it may also start at the kinetochore and extend itself from there to the tips of the chromosome (procentric pairing), or, vice versa, start at the arms and reach the kinetochore only in the end (prototerminal pairing) (Darlington, 1937).

Once the pairing is completed, *pachytene* begins (Fig. 102 (1)); the chromosomes have become even shorter and, as indicated by the term *pachytene*, even thicker. Whereas the *zygotene* is a stage of mobility which leads to the pairing of the chromosomes, the *pachytene* is a period of temporary rest. If, at one point or another, the pairing has been left incomplete, it cannot be compensated any more. As a rule, the homologous chromosomes lie so close to each other, that they give the impression of being chromatids and the number of chromosomes, haploid. The homologues are frequently coiled several times around each other

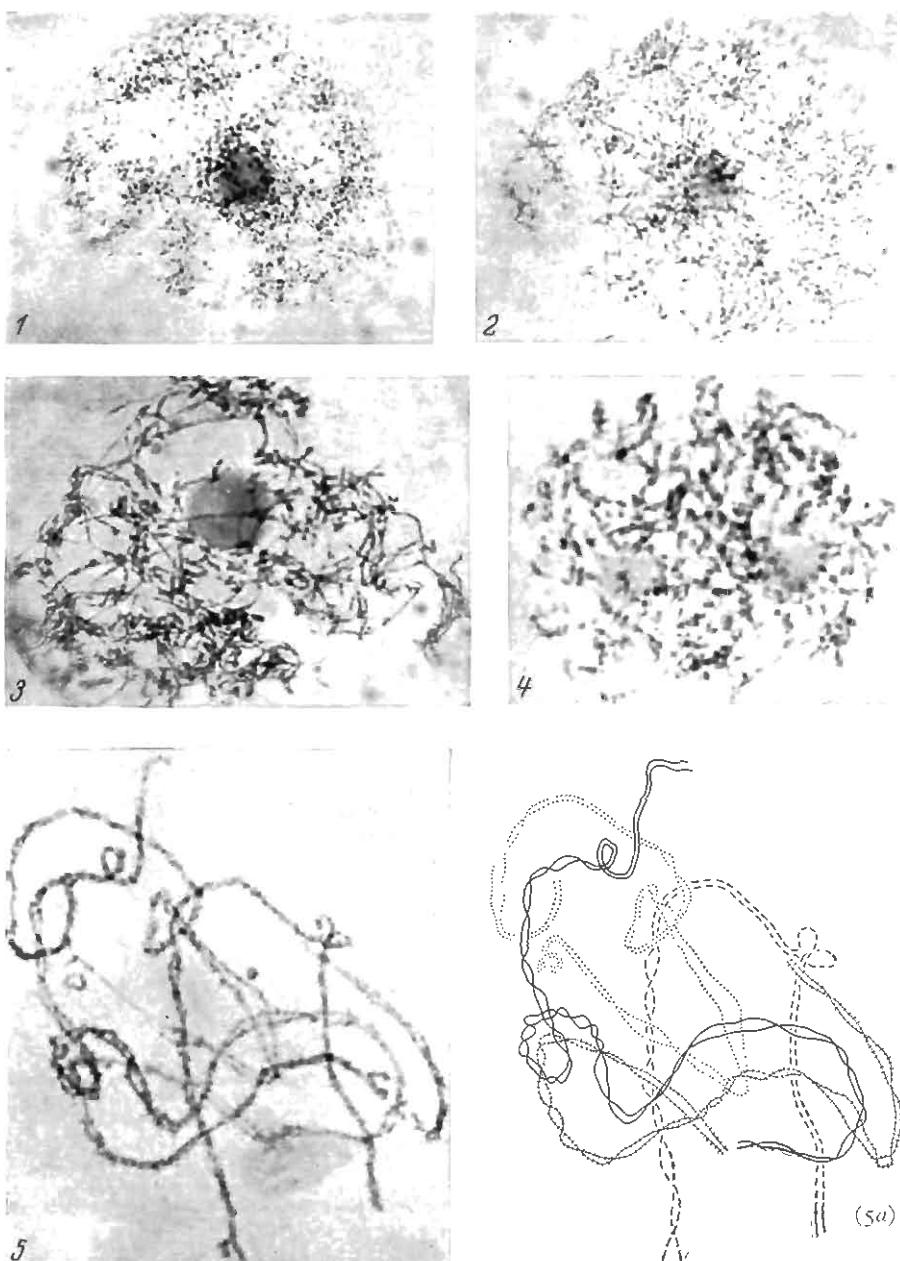


Fig. 101. Stages of meiosis in anthers of *Bellevalia romana*. (1) Interphase nucleus. (2) and (3) Leptotene. (4) Beginning of zygotene. (5) (5a) Diagram of (5). (From Oehlkers, F. (1956), *Das Leben der Gewächse*, Vol. I. Springer, Berlin.)

(relational coiling), thus, enhancing the apparent likeness to chromatids. The nucleolus can now often be seen to be particularly distinctly connected via its organizer (p. 44) with the one or the several chromosomes that bear nucleoli. It was in the pachytene that this close connection was discovered for the first time and this discovery was facilitated by the fact that the pachytene lasts quite a long time, especially in *Zea Mays*.

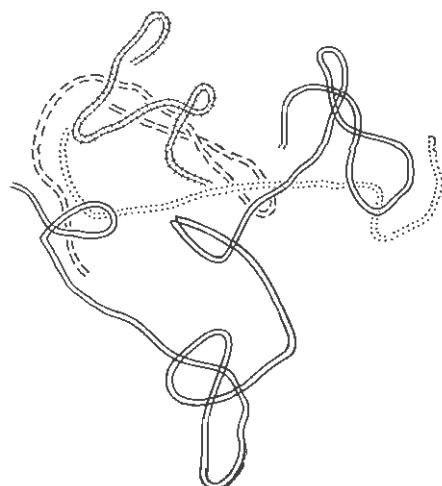
In the course of the following stage, or *diplotene*, the homologous chromosomes separate again from each other. This, however, presents difficulties since the coupled chromosomes adhere in many points. The quadruple strandedness of the paired chromosome often appears here quite clearly and consists of two chromosomes each with two chromatids and we can see how each chromatid of one chromosome crosses each chromatid of the other, building a *chiasma* (Fig. 102 (2) and (3)). Thus, the homologous chromosomes are bound to each other, and the arrangement of the bivalents, as paired chromosomes are also called, remains for a while. In the light microscope one has the impression that the crossing chromatids change partners at the chiasma and go over to the other chromosome. But this is only seemingly so; what actually takes place is an exchange of chromatid segments. At these chiasmata the chromatids having broken, have then been exchanged with the corresponding chromatid segment of the homologous chromosome and have secondarily healed again. A chiasma is, in consequence, the result of an exchange of segments; this is confirmed by the fact that at these points the chromatids are attached relatively firmly to their original partner. Chiasmata are the morphological indication of a rearrangement of the chromosomes, which takes place in the diplotene often under the limits of visibility (Oehlkers, 1956).

Frequency and position of these chiasmata vary from species to species. As a rule, the longer the chromosome, the more frequent are the chiasmata. In certain species, the chiasmata are to be found mostly at the tips of the chromosomes, i.e., terminally. Other interstitial chiasmata are located at other points along the arms of the chromosomes; they may either stay there or get secondarily shifted to the tips of the chromosomes; they are then terminalized. Whereas these processes of the diplotene can be relatively clearly observed in plants, they are largely invisible in animals; for example, in the spermiogenesis of certain insects or in the oogenesis of fishes and frogs, the chromosomes become barely visible at the beginning of the diplotene and are scattered more and more diffusely within the nucleus.

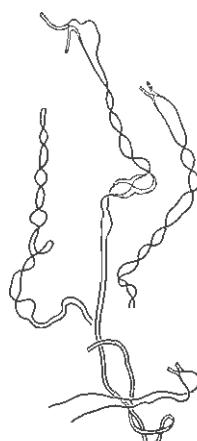
However, this is, here too, only temporary. The chromosomes soon are very well-defined again and even more strongly contracted and at this point the nucleolus disappears or loses its chromosomal attachment. During this *diakinesis*, the bivalents are very much shortened and are liable to withdraw very far from each other. Since, after terminalization, they still frequently adhere quite firmly together at the tips of the chromosomes, they may form rings (Fig. 103). In general, chiasmata which have not yet been disengaged at this point, are quite easily recognizable.

During late diakinesis, the spindle generally starts forming itself and, in certain insects, the homologous kinetochores separate from each other and move towards the spindle poles (Hughes-Schrader, 1943). This "prometaphase stretching" has been already discussed with the mechanisms of karyokinesis. It is, no doubt, an exception, but of a great theoretical importance (p. 154).

Diakinesis marks the end of meiotic prophase. The nuclear envelope now disappears, the spindle has emerged and the chromosomes migrate during *prometaphase I* into the



(1a)



(2a)



(3a)

equatorial plane. Now starts *metaphase I*. All these processes evidently follow the same rules as in the mitotic karyokinesis, with the following difference, however: the pairs of chromosomes gathered in the equatorial plane as far as their function is concerned, have undivided kinetochores still, which are not situated in the centre of the spindle but have the same orientation as the longitudinal axis of the spindle (Fig. 215 (2)). The gap between them depends apparently on the position of the nearest chiasmata, and they seem to repel one another actively.

At *anaphase I*, the remaining chiasmata disengage too, and the chromosomes move to the poles (Fig. 103 (3)), again basically in the same way as in mitotic karyokinesis, yet again with the typical difference that it is not the daughter chromatids which separate from each other as in the latter, but the homologous chromosomes. Tracing back the origin of these homologous chromosomes, one notices that it is by no means so, that all maternal chromosomes go to the one and all paternal to the other pole; on the contrary, the distribution seems to be a mere question of chance. Thus each newly formed genome consists of chromosomes of both parental genomes. In addition to the chiasmata, this is the second alteration of the chromosome sets, the "rearrangement of the genomes" (Oehlkers, 1956). It is connected at the same time with the halving of the number of chromosomes, the chromosome reduction; both anaphase halves are haploid.

Once the chromosomes have reached the poles, they gather to a loose mass and build a nuclear wall which is generally relatively thin. Thus starts *telophase I*. The main difference between this and mitotic telophase is the less compact clustering of the chromosomes, which each remain well-defined, generally even during the following *interkinesis* (Fig. 103 (4)). There is no real reconstruction phase nor any interphase. After a short interkinesis, a new division starts again; this is the second meiotic division, called also meiotic mitosis. Meanwhile, DNA is not replicated (Fig. 100) and there occur also probably no other synthetic processes, except maybe for the production of energy necessary for karyokinesis (p. 184), provided the energy required for the second division was not already made available before the beginning of the reduction division.

The differences between the *second meiotic division* and a mitotic karyokinesis can be summarized in four points:

1. The prophase is markedly shorter. In fact, the chromosomes enter this phase still largely condensed and coiled; at least this is always the case when there has previously been no interphase uncoiling but only a short interkinesis. Moreover, the nucleolus has not appeared again in the interkinesis, so that the corresponding rearrangement processes do not take place (p. 129).
2. The prophase chromosomes are to be found only in a haploid number, as a result of the previous reduction.
3. In many cases, their chromatids do not lie parallel to but very distant from each other, thus assuming the shape of an X, since they are attached at the kinetochores. The problem of a longitudinal splitting at prophase, which we had discussed when dealing with mitotic

Fig. 102. Further stages of meiosis in anthers of *Bellevalia romana*. (1) Pachytene. (1a) Diagram of (1). (2) Diplotene. (2a) Diagram of (2). (3) Very late diplotene. (3a) Diagram of (3). Note the chiasmata in (2) and (3). (From Oehlkers, F. (1956), *Das Leben der Gewächse*, Vol. I. Springer, Berlin.)

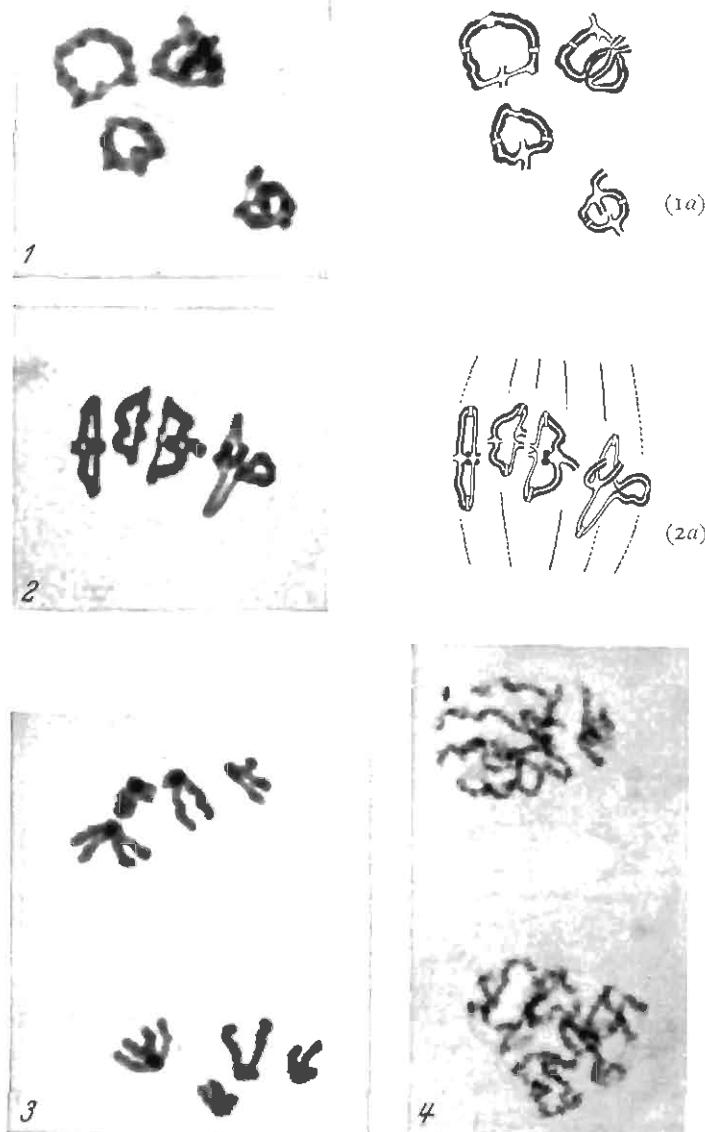


Fig. 103. Further stages of meiosis in anthers of *Bellevalia romana*. (1) Diakinesis. (1a) Diagram of (1). (2) Beginning of anaphase. (2a) Diagram of (2). (3) Telophase. (4) Interkinesis (From Oehlkers, F. (1956), *Das Leben der Gewächse*, Vol. I. Springer, Berlin.)

karyokinesis (p. 130), does not exist here. Furthermore, there is no trace here of any relational coiling, i.e., a loose coiling of the chromatids resulting from a previous karyokinesis.

4. The composition of the genomes is quite different from that at the beginning of the first meiotic division; for, first of all, the chiasmata, with their exchange of fragments, have changed the structure of the chromosomes, and secondly, the fortuitous distribution of the parental chromosomes in the first anaphase had already brought about a rearrangement of the genomes.

The prometaphase rearrangement is quite similar to a mitotic karyokinesis, apart from haploidy, with migration of the kinetochores into the equatorial plane of metaphase II, anaphase II, telophase II and the reconstruction phase, and we may assume that the same determining conditions are present. As a result there are four haploid nuclei, which by way of a double cytokinesis, are distributed onto four haploid cells, the *gonia*. In the spermiogenesis of animals they develop further and become the mobile spermatozoa. In the oögenesis of animals, the first meiotic prophase may be interrupted for quite a while in pachytene or in diplotene. This is when the long lampbrush chromosomes (Fig. 57) appear, the properties of which we discussed previously (p. 119). During oögenesis, the large egg cell does not divide itself into two cells of equal size at the end of the meiotic division; instead, it ejects a small nucleate cell, the first polar body (or directional body). The second meiotic division produces the second polar body in the same way, and since, synchronous with the second maturation division, the first polar body frequently divides itself, at the end of the egg cell's meiosis, there are three polar bodies. In plants, the development of the gonia is much more variable, depending on cyclic metagenesis, as we already pointed out (p. 189), but we will not discuss this further at this point.

Syndesis

The late phases of both meiotic divisions need no further explanation, since they correspond essentially to those of mitotic karyokineses. However, there are two particularities we must examine more closely in order to understand meiosis. First, the phenomenon of the prophase conjugation of pairs of chromosomes, the syndesis or synapsis and, secondly, the formation of chiasmata or, rather, the exchange of chromatid fragments which leads to their formation.

The pairing of the chromosomes during the first meiotic prophase is one of the most fascinating processes in cytology, and at the same time, one of the most obscure as to its causes. It is a fact that this pairing occurs according to an extremely exact pattern, whether it is procentric as we already mentioned, that is, whether it starts at the kinetochores, or whether it is proterminal, i.e., beginning at the tips of the chromosomes, or intermediary, occurring first at one or several points along the chromosome. Only homologous chromosomes alone are capable of an organized pairing; the result is a parallel arrangement of each eu- and heterochromatic chromosomal part, of the primary and secondary constrictions and of the satellites, if there are any. How precise this arrangement is, can be seen for example on the giant chromosomes of *Diptera* (p. 118) in which a similar, though somatic, pairing takes place, each strand being paired with its homologous strand. Exceptions occur only in pathological cases. The pairing unit is not the whole chromosome, nor even the eu- or the

heterochromatin, but those functional units which appear in the strands and which we know as gene groups (p. 119). Maybe even the gene itself is the pairing unit. If, following an injury to the chromosome by irradiation or exposure to a mutational agent, there occurs a translocation (p. 206), the chromosome fragments still get paired with their homologous fragments of chromosome. Thus, in certain cases, parts of different chromosomes may join and it is not uncommon that the success or failure of the meiotic synthesis is decisive for the reproductive fitness of a mutant brought forth by a translocation. The pairing of non-homologous chromosomes or chromosome fragments occurs only in exceptional cases.

The mechanism of synthesis is a problem which has yet to be solved. Many hypotheses were formulated and rejected; among them, the most outstanding were the physical theories, according to which the chromosomes are brought together by a difference in electrostatic surface charge (Lamb, 1907; Fabergé, 1942) or in surface bipolarity (Friedrich-Freska, 1940). It is possible that the attraction of the chromosomes to one another is a problem similar to that of the attraction between kinetochores and kinetocentres (p. 144). Possibly, the synthesis takes place in two phases. In the first phase, the chromosomes enter into contact at one point or another; this may happen accidentally, especially since the chromosomes at the beginning of meiotic prophase are fairly mobile, but it may also be the effect of "long-range forces". Once such a contact is established, the pairing can extend like a zip fastener all along the chromosomes, bringing the homologous points exactly together (see, e.g., Cooper, 1948). Most likely, the specific structure of the nucleoproteins, i.e., the specificity of the genes, can be considered as a determinant here, especially since the pairing involves structures down to this dimension.

Nature of the chiasmata

This close pairing of the chromosomes paves the way for the apparent crossing over of chromatid fragments during the meiotic prophase, or chiasmata (Janssens, 1909). We already mentioned two important facts in this context: (1) these chiasmata occur as a rule only between chromatid halves of homologous chromosomes; (2) they are the tangible sign of a morphological exchange of chromatid fragments.

Fig. 104 shows the formation of a chiasma and the behaviour of the chromosomal parts until the end of the second meiotic division. One may see, represented schematically, the pairing at zygotene, the exchange at pachytene (in this case, one longitudinal half of each of the chromatid halves is exchanged for that of another chromatid half), the emerging of the chiasma at diplotene and the distribution of the segments to the four division products during both anaphases. Moreover, at metaphase I, the terminalization of the chiasma is indicated.

There are many interior and exterior factors which have a bearing on the exchange of fragments. Among the interior ones, there is the age of the organisms. A study of the exchange frequency of a certain chromosomal fragment in the course of several weeks, e.g. in *Drosophila*, revealed a rate of 5.9% in the first ten days, of only 1.8% during the following 10 days and again 8% from the 20th to the 30th day (Bridges, 1915). The location of the investigated fragment was thereby of importance; it was situated in the vicinity of the kinetochore, which, as confirmed by many other observations, may control the exchange

(Beadle, 1932; Graubard, 1932; and others). Among the exterior determinants, temperature plays an extremely important part.

Moreover, it must be pointed out that the exchange of fragments is not restricted to the meiosis of gametes; it occurs also in somatic cells (Stern, 1936). Such a somatic exchange is even quite frequent in *Drosophila* (see, e.g., Kaufmann, 1934); it is likely to be the result of the unusual pairing of the homologous chromosomes in the differentiated tissue cells, such as in the salivary glands. Possibly the exchange of somatic fragments occurs on a much wider scale than we know now (see Swanson, 1960); it may even be a regular process of every karyokinesis (see Fig. 51). However, a close pairing of the chromosomes is always the first step.

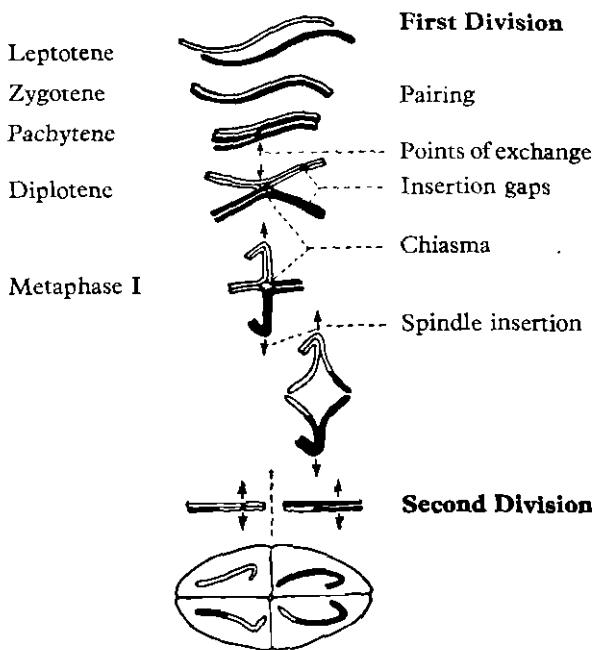


Fig. 104. Schematic representation of the pairing of chromosomes and the behaviour of the chiasma. (From Geitler, L. *Grundriß der Cytologie* (1934), Borntraeger, Berlin.)

The mechanism of the chiasmata is still widely discussed (for ref., see, e.g., Swanson, 1960; Rhoades, 1961; and others). Belling (1931, 1933) placed the whole process into the phase of reconstruction of the chromatids. He assumed that, during this reconstruction, first the chromomeres and only secondarily the chromonemata were generated, thus making it possible for chromosomes of non-sister chromatids to be joined together; this would make it conceivable that an exchange of fragments takes place at all levels. However, this hypothesis is completely rejected today, since it has been proved: (1) that the reconstruction of the chromosomal material occurs long before the pairing (Fig. 100) and (2), that chromomeres and genes are not identical, but that chromomeres are probably merely the result of a higher degree of coiling (see p. 114). Coiling of the chromosomes and of their subunits is the core of Darlington's "torsion hypothesis" (1935, 1937). It relies on the well-grounded observation that at the beginning of meiotic prophase the homologous chromosomes are wound around each other in the fashion of a relational coiling and attract one another two by two. These attraction forces, says the author, cease as soon as the new chromatids are formed and the homologous chromosomes then repel each other. However, he continues, since they

are bound together by the relational coiling, there occurs a torsion which is released by the break of both sister chromatids. The rejoining of the broken fragments then completes the exchange. This fairly simple theory found many advocates at first. Yet, it cannot account for the multiplicity of possible meiotic recombinations—not to mention the fact that all of Darlington's theories which are concerned with the mitotic behaviour of the chromosomes (1937) are ultimately based on electrostatic arguments. Thus, he postulates an attraction of the chromosomes or a repulsion, whenever it fits, so to speak, into his theories. Probably the decisive role must be attributed to intermolecular forces between the homologous genes or gene groups, as well as to mechanical factors, which exist beyond doubt, as indicated by the terminalization of the chiasmata.

Review

This survey of the problems of meiosis has shown, in spite of its fragmentary character, that cytology is in particularly close contact with genetics. One of the functions of meiosis, the reduction of a diploid to a haploid number of chromosomes, is accomplished during the first meiotic division, called therefore also reduction division. The second meiotic division is a haploid karyokinesis, in which the daughter chromatids originating from the first division are divided. The other function of meiosis is fulfilled in part during the long prophase of the first division. This is when the homologous chromosomes are paired, and during this pairing an exchange of chromatid fragments takes place, which appears morphologically in the form of a chiasma. It occurs invariably between two chromatid halves, more precisely between two non-sister chromatids. This is to a certain extent the cytological equivalent to the "crossing over" in genetics. It causes a rearrangement in the gene structure of the chromosomes. A rearrangement of the genomes takes place during the first meiotic anaphase, owing to the fortuitous distribution of the chromosomes onto both haploid daughter nuclei.

DISTURBANCES OF MITOSIS AND MEIOSIS

The great number of processes and mechanisms involved in the mitotic cycle and the meiotic divisions accounts for the great number of possible disturbances. Although we know that karyokinesis is a process which is essential for the conservation of the individual and the continuance of the species, and therefore a very stable process, the single phases of division are so adjusted to one another that anything hindering or disturbing one, hinders or disturbs all of them.

Since the chromosome arms are transported passively by the action of kinetocentres and kinetochores, all movement disturbances are ultimately due to an alteration of these movement centres. For instance, if some foreign substance arrests the chromosomes at metaphase, as is typical of colchicine, it is because it has paralysed the kinetochore and kinetocentre activity which, as we know, is responsible for the anaphase. The same thing occurs when the prometaphase rearrangement is impaired and no ordered metaphase plate can be formed. In general, such pathological karyokineses are usually considered the result of a *spindle disturbance*.

Other pathological karyokineses are brought about by damages done primarily to the

chromosomes. All these alterations we summarize as the consequence of *chromosomal injury*. Since karyokinesis chromosomes are relatively well packed (p. 127), these are mainly damages which the chromosomes suffered much earlier, during the previous intermitosis or interphase. However, by *interference with the interphase* we mean a different group of disturbances. In this case, we have an alteration of processes characteristic of the interphase, namely, the replications in nucleus and cytoplasm, the accumulation of energy available for karyokinesis and the stimulation to mitosis. One of the typical effects is that the beginning of karyokinesis may be retarded or fail. The division of the cell body we know to be a special and in many respects independent process; however, its close functional relations to the spindle and the polar rays range these *impairments of cytokinesis ultimately among the spindle disturbances*.

The above classification accounts for the most frequent disturbances of mitosis. We will now give merely a rough survey of the different kinds of alterations that may happen in each of the mitotic phases, and we will classify them according to the phases of mitosis and not to the causes of the disturbances. For the latter may vary a great deal and range from almost insignificant variations in the cell's metabolism to the lethal action, on the cell, of toxic substances or irradiation.

A great many investigators have devoted themselves to the study of such chemical substances, which cause the mentioned mitotic disturbances and, therefore, are called mitotic poisons. Definitions of this group of substances vary according to the method of the investigator. Whereas Lettré for instance (1946, 1951) considers them as those substances which prove *in vitro* to have a direct effect on the cell division in tissue culture, other authors build upon the fact that the damage depends on the concentration and term as *mitotic poison* all chemical compounds which disturb mitosis, even if their concentration is too low to have any tangible effect on interkaryokinetic nuclei (Hughes, 1952) or, at least, is too low to kill the cell (Cornman, 1954). We must doubt whether there exists any substance that has an absolutely specific effect on mitosis, as Eigsti and Dustin (1955) thought to be the prerequisite of an authentic mitotic poison.

Investigation of mitotic poisons has a practical goal, namely, chemotherapy of malignant tumours. This goal has been a great incentive. Two facts were known from the beginning: (1) malignant tumours are rich in mitoses, by virtue of their rapid growth; (2) the curative effect of ionizing rays on the malignant process of growth appears to be based partly on a disturbance or an arrest of the mitoses. Thus, it was clear that one had to find substances which would have the same impairing effect on the proliferations in tumours as ionizing rays, or even a stronger one, yet without the damaging side effects which those rays have on the rest of the organism. Perhaps one might even find chemical compounds this way which would check malignant growth exclusively. However, these hopes have not materialized as yet.

A bulk of data has been gathered from the above investigations which has become immense. There is hardly a group of chemical compounds which has not yet been investigated for its mitosis-disturbing capacities, and proved to be mitotic poisons according to the above-mentioned definitions. Bieseile (1958a) has described the most important of these substances and ordered them in a summarized survey. We will limit ourselves here to a short review of the mitotic disturbances, since the evidence brought by all these investigations may be elucidated from single examples.

Interference with interphase

Nearly all the factors which we described as regulating the mitotic frequency in mammalian tissues (see our discussion of interphase, p. 187) may retard the beginning of karyokinesis. Such is the case, for example, with many *hormones*. Cortisone checks, depending on the concentration, the mitoses of many mammalian tissues, e.g., in the skin, the regenerating liver (Roberts *et al.*, 1952) or in the lymphatic tissue of the rat (Grundmann, 1958*b*), not, however, in the exterior lachrymal gland of the rat (Isotalo and Teir, 1953). The effect of cortisone is probably very complex; it affects cytoplasm and nucleus alike and the impairment of the beginning of mitosis is only one of its effects (Grundmann, 1958*b*). It is not yet clear whether the major factor here is a disturbance of DNA and RNA synthesis, as has been assumed from the regenerating rat liver after partial hepatectomy (Einhorn *et al.*, 1953), or an interference with the interphase production of energy, the antephase (p. 184), as was concluded from observations on the chick embryo (Menon, 1962).

Adrenaline, too, was found to be connected with the metabolism of energy, and Lettré and Albrecht discovered its mitosis-arresting effect (1941); its oxidation product, adrenochrome, was found to have the same effect, and is also considered a mitotic poison (Lettré *et al.*, 1951). Adrenochrome checks the beginning of mitosis, for example in the mouse epidermis, at a concentration as low as 1 µg/ml (Bullough, 1955) and its action occurs probably via an inhibition of the hexokinase (Meyerhof and Randall, 1948). An isomer of adrenochrome, trihydroxy-N-methylindole, is also a poison of the mitochondria as well as of mitosis (Chèvremont and Chèvremont-Comhaire, 1953; Chèvremont, 1961*b*). Both effects are liable to be connected, the damage to the mitochondria impairing the interphase oxidative formation of energy and, consequently, the beginning of mitosis, by interfering with the succinic dehydrogenase-cytochrome-oxidase system (Chèvremont-Comhaire and Frederic, 1955).

Trypaflavine and other diaminoacridines also inhibit the beginning of mitosis; this was observed by Bucher (1939), then by Dustin (1947) and later confirmed many times (e.g., Lettré and Lettré, 1946; Schümmelfeder *et al.*, 1959). Monoaminoacridines have the same effect, for example on cultures of chick fibroblasts (Lasnitzki and Wilkinson, 1948). The strongest effect appeared to be that of 5-aminoacridine hydrochloride which could clearly inhibit mitosis at a concentration as low as 7 µg/ml (O'Connor, 1949). Whereas these experiments left respiration to a great extent unchanged, there was early evidence of a relation between the diaminoacridines and the nucleic acids (Lettré, 1948); they can form a fairly stable complex with the nucleic acids (Morthland *et al.*, 1954), whereby the highly polymeric DNA of ox spleen, for example, is bound to 9-aminoacridine with a much stronger bond than the low molecular weight yeast RNA (Irvin and Irvin, 1954). This explains why trypaflavine is also a typical poison of the chromosomes (Bauch, 1947), causing the chromosomes to adhere to each other; its effect can be traced down directly to damaging the chromosomes (Bauch, 1949).

We find the same combination of effects using various *analogues of the physiological purines*, as, for instance, 2,6-diaminopurine, which is related to adenine (Bieseile, 1954). The inhibition effect may be reversed by adenine and partly also by guanine (Miller, 1953; Nickell and Rhoads, 1955; Setterfield and Duncan, 1955). Examination of the root meristem of *Vicia*

faba after exposure to 2,6-diaminopurine reveals nuclei with diploid and nuclei with tetraploid DNA values, but no intermediary values (Setterfield and Duncan, 1955). Consequently, 2,6-diaminopurine inhibits the beginning of DNA synthesis, but is unable to interrupt the process of a DNA synthesis which has already started. Probably 2,6-diaminopurine is incorporated in the nucleoproteins instead of adenine. Other purines have basically the same action; 2-chloroadenine, for example, checks the mitotic growth of tumour cells at concentrations as low as 0.05 mMol, and 6-methylpurine even at the 0.005 mMol level (Biesele, 1958*b*, 1960).

Alkylating agents, such as nitrogen mustard, have been the object of a particularly intensive study because of their carcinostatic, as well as carcinogenic properties. They too are thought to have several effects on the cell and may produce typical chromosomal injuries. Their therapeutic effect relies on a destruction of the interphase nuclei (Grundmann, 1952). In smaller doses, however, they mainly impair the beginning of mitosis (Novick and Sparrow, 1949; Hohl, 1949), and certain investigators found they predominantly inhibited the synthesis of DNA (e.g., Swift, 1953). Maybe, the action they were found to have on guanine of the DNA molecule (Lawley, 1957) has even more general implications. The morphological changes noticeable in animal and human tumour cells are early a great enlargement of the nucleoli together with an excessive swelling of the nucleus (Fig. 105) and an increase in the RNA content in nucleus and cytoplasm (Grundmann *et al.*, 1960; Grundmann, 1963*b*), whereas the DNA content remains the same. On the one hand, these changes suggest a dissociated disturbance of the nucleic acid metabolism, similar to that which happens after Roentgen irradiation (Mitchell, 1942); on the other hand, there is an equally typical decrease of the NAD (=DPN, see p. 35) content in the same tumours (Holzer and Kröger, 1958*a*), although it occurs chronologically after the cytological alterations and the quantitative changes in the nucleoproteins (Grundmann *et al.*, 1960).

The derivatives of quinone have also been held responsible for a primary NAD injury (Holzer and Kröger, 1958*b*) and they too can retard the beginning of mitosis (Mitchell and Simon-Reuss, 1952; Druckrey *et al.*, 1953) and counteract various tumours as chemotherapeutic agents (Domagk, 1954). Their mechanism of action is complex too. On one hand, there is evidence to show that they block the SH groups (Lettré, 1952*a*), on the other hand, phosphorylations appear to play a major part; whereas 10^{-5} molar toluidydroquinone, toluquinone or an inorganic phosphate alone cannot impair mitoses, a combination of toluquinone and phosphate can clearly inhibit mitosis in chick fibroblast cultures (Friedmann and Simon-Reuss, 1954). Finally, a derivative like 2,5-diethylenimine benzoquinone has an inhibiting effect on respiration and fermentation, and therefore, on the energy metabolism of various tissues (see the above-mentioned NAD decrease); however, it has not been possible to connect this with the regression of tumours, which these same substances can bring about (Jühling, 1954).

Interferences with interphase are also typical of the cellular action of ionizing rays, mainly X-rays. The first radiobiological investigations on amphibian embryos (Bohn, 1903; Bardeen, 1907) and on the root meristem of *Vicia faba* (Koernicke, 1905) have already shown an inhibition of the process of growth, which was later studied in greater detail, mainly on the corneal epithelium of urodele larvae (Alberti and Politzer, 1923). Immediately after irradiation, the mitotic frequency decreases abruptly and a mitosis-free interval follows of 1 to

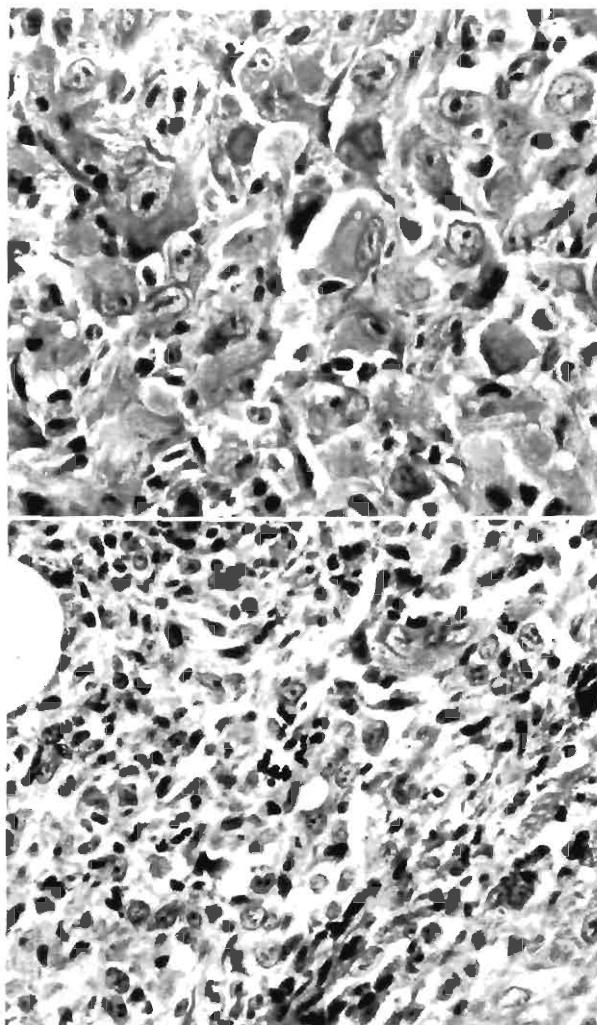


Fig. 105. Intense swelling of cell and nucleus, concurrent enlargement of the nucleoli in a Jensen's sarcoma of the rat, two days after curative injection of nitrogen mustard. Below: an untreated Jensen's sarcoma, same magnification. Staining: Galloxyanin chromalum.

3 days, depending on the kind of tissue and the dose of irradiation administered; in the *Vicia faba* root meristem, for example, this interval lasts 6 hours after 420 r, and 12 hours after 550 r (Jüngling and Langendorff, 1930).

There is still no answer as to what ultimately causes this impairment of the onset of mitosis, despite the particularly exhaustive studies concentrated on the effect of irradiation on the cell (see, e.g., Errera, 1957; Marquardt, 1957; Fritz-Niggli, 1959, 1960; Bacq and Alexander, 1961; and others). Incidentally, small doses of X-rays seem to act during the prekaryokinetic interval, in the G₂ phase (see Fig. 95) (Yamada and Puck, 1961).

Chromosome injury

No matter how overwhelming the action of ionizing rays is on interphase, the morphological and functional damages suffered by the chromosomes still play the major role. Strictly speaking, these are frequently nothing else but interferences with the interphase, since a great number of the chromosomal alterations which become visible during karyokinesis, are due to injuries inflicted upon them previously, during interphase or intermitosis. The early prophase is considered by many authors to be particularly sensitive to irradiation (Sax and Swanson, 1941; Koller, 1946).

The most basic and the most frequent consequence of irradiation on the chromosomes is the adherence of two daughter chromosomes to each other. When they withdraw from one another at anaphase, they remain attached at one or several points and form the typical anaphase bridges (Fig. 79). The rate of occurrence of such a "stickiness" depends on the dose administered (e.g., Koller, 1946; Marquardt, 1950; Carlson and Harrington, 1955). If the forces which forward the chromosomes to the poles are strong enough, one of the daughter chromosomes breaks, thus producing a chromosome fragmentation. If this happens immediately after or in the first few hours following irradiation, we have what is called a primary effect of the X-rays (Alberti and Politzer, 1923; Strangeways and Oakley, 1924; Marquardt, 1937, 1938). Particularly strong damage done to the chromosomes impairs the anaphase movement and the chromosomes form clumps. Frequently, pairs of telophase nuclei can be seen, connected by fine threads of chromatin similar to the anaphase bridges, called pseudoamitoses. Such cells are generally not viable and die after a short time.

When after the above described mitosis-free interval karyokineses become visible again, the so-called secondary effect appears. It too entails chromosome adherences and fragmentations as well as characteristic recombinations of chromosome fragments (Marquardt, 1938, 1950; Lea, 1947; Kaufmann, 1948; and others). However, whole fractions of chromosomes may also be broken off and ejected from the spindle. These either die or build their own micronuclei. The loss of a fairly large fragment of chromosome generally brings about the death of the cell shortly afterwards. The parts of the chromosome remaining on the backbone and containing the kinetochores enter the daughter nuclei, but may join at the fragmentation sites and form bicentric chromosomes, i.e., chromosomes with two kinetochores. If these two kinetochores orient themselves towards different poles, anaphase bridges appear again with all their consequences.

As a rule, the sites of chromosomal fragmentation recombine readily with other sites of chromosome breakage. This may occur in many ways. If both breaks are close to each other in one chromosome, the broken fragment can turn by 180° and be inserted again at the same place by means of an "inversion" (Fig. 106). The fragments may also be removed from the chromosome by "deletion" and the gap in the chromosome closes again by the rejoicing of the chromosomal end fragments (Fig. 106). The portion of chromosome thus removed from the chromosomal backbone may get lost or unite its ends to form a ring (Fig. 106). It is a common feature that recombinations of the open broken ends of a chromosome frequently lead to the formation of typical ring chromosomes. If breaks occur on different chromosomes, single fragments of chromosomes may be exchanged; this is known as "translocation" (Fig. 106).

The chromosomes are not always broken quite through. There are often only fragments of chromatid halves or quarters, which may basically incur the same fate as fragments of whole chromosomes. Here, too, we find removal of fragments (deletions), inversions and translocations. When breaks of two half chromatids occur at exactly the same site, we speak of isochromatid breaks (Darlington and Upcott, 1941).

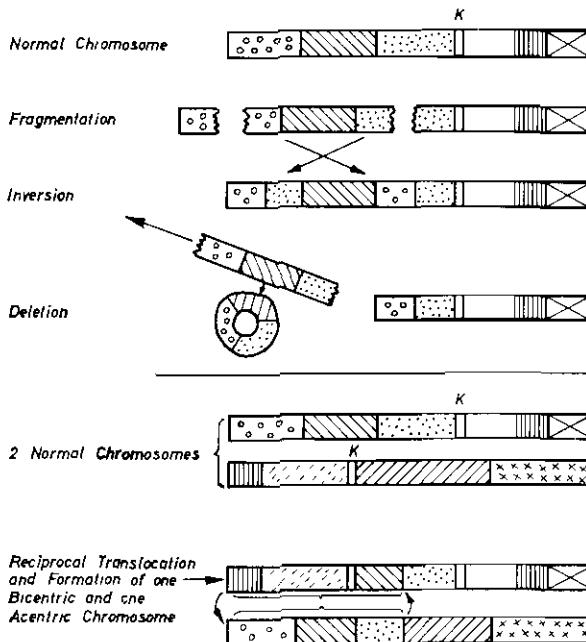


Fig. 106. Schematic representation of various chromosomal injuries (K: kinetochore).

All these chromosomal injuries which have been repeatedly analysed in detail in many organisms, particularly in plant large chromosomes, are directly related to the dose of irradiation administered, and depend also on the environment. In an almost pure O_2 atmosphere, the number of chromosome injuries is markedly higher than in nitrogen. The strongest effect is brought about by an increase of the oxygen tension from 0% to 21% (Giles, 1954).

Damages similar to those inflicted upon the chromosomes by ionizing rays appear after exposure to chemical substances (Bieseile, 1958a; Sharma and Sharma, 1960). Since we are always dealing here with defects or translocation in the genetic material, i.e., in the chromosomal substance, these substances are mutagens.

The first discovery of the chromosome mutagenic effect was made by Oehlkers with urethane (1943, 1953; Oehlkers and Linner, 1949). With this substance it was possible to produce, as with irradiation, breakage of chromosomes and chromatids, translocations and other recombinations. In *Vicia faba*, fragmentation seemed to occur most frequently on

SAT chromosomes in the vicinity of the kinetochore (Deufel, 1951). When urethane was used as a chemotherapeutic agent against malignant tumours, the same chromosome injuries were found again (Green and Lushbaugh, 1949; Boyland and Koller, 1954). In the Walker carcinoma of the rat, the effect of urethane was checked partially by thymine but not by uracil, which led to the conclusion that urethane directly affected DNA (Boyland and Koller, 1954).

The second substance whose mutagenic nature was discovered was mustard gas (Auerbach, 1943, 1951; Auerbach and Robeson, 1947), which also causes chromosomes to break (Darlington and Koller, 1947). Here, too, we find the most varied types of fragmentation and recombination (Koller, 1947), as after Roentgen irradiation, yet the effect is longer-lasting. The fragmentation rate still increases in the first five days after administration of the gas (Koller and Casarini, 1952). Mustard gas derivatives, such as nitrogen mustard and others, have been largely used in the chemotherapy of malignant tumours, always incurring serious damage of the chromosomes (Fig. 107), with partial stickiness of chromosomes, pycnoses of whole sets of chromosomes, fragmentations and removal of single fragments from the

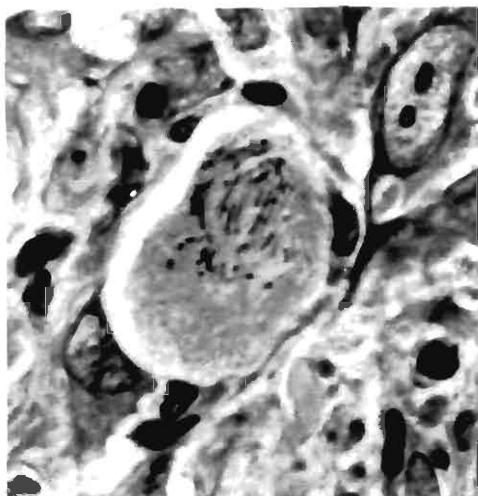


Fig. 107. Fundamental mitotic disturbance in a Jensen's sarcoma of the rat under the effect of nitrogen mustard. Haematoxylin eosin stain.

main backbone. Meanwhile, a great amount of other alkylating agents have been developed, with essentially the same effects. Thus, for example, ethylenimine (Plummer *et al.*, 1952; Bieseile *et al.*, 1952) or myleran, a 1,4-bis (methylsulphonyloxy)butane, causes chromosome fragmentations in the same way in various plant cells (Truhaut and Deyson, 1954) as well as in animal tumours (Tanaka *et al.*, 1955).

Purine and pyrimidine analogues have also been found to cause fragmentation and recombination of chromosomes. The discovery by Kihlman and Levan (1949) of the chromosome mutagenic effect of caffeine led to the investigation of many other purine derivatives. The lipid soluble 8-ethoxy-caffeine appeared to have the strongest effect. Caffeine itself as a water soluble substance seems to be mutagenic only during karyokinesis (Östergren and Wakonig, 1954). It is interesting to note that 8-ethoxy-caffeine, like the ionizing rays, needs oxygen for its full mutagenic effect; it proved ineffective in anoxia (Kihlman, 1955). Among purine derivatives, 2-aminopurine also has a strong damaging effect on chromosomes in tissue cultures (Bieseile *et al.*, 1952) and causes many fragmentations and adherences; it

may even produce in certain cases "three group metaphases" (Bieseile, 1961) in which parts of the chromosome sets lie in the equatorial plate and others at the poles, where they form clumps. 2-aminopurine may be incorporated in DNA instead of adenine and thus lead, in the bacteriophage T₄, to the transformation of an adenine-thymine base pair into a guanine-hydroxymethyl cytosine pair (Freese, 1959), in other words, to a mutation at the level of a single base pair.

Another substance analogous to a base, 5-bromo-2'-desoxyuridine, an analogue of thymidine, has a particular cytological effect; it stretches the primary and secondary constrictions of the chromosomes (p. 104) and causes, mainly there and at the telomeres (p. 106), breaks in the chromosomes and translocations (Hsu and Somers, 1961). Possibly a particularly large amount of thymidine is replaced by a 5-bromo-2'-desoxyuridine which had been incorporated in the DNA during the previous DNA synthesis. Similar damage inflicted specifically on the chromosome constrictions has been observed with many mutagenic substances, such as on *Tradescantia* with mustard gas (Darlington and Koller, 1947; Koller, 1947) or with urethane on the tomato (Gottschalk, 1951a, 1951b), where four-fifths of all breakages occur in the kinetochore region. 2,3-diepoxypropylether leads to chromosome injuries mainly in the zone of heterochromatin (Revell, 1953), which, generally, is the zone of predilection for the action of a whole series of substances. For instance, maleic hydrazide causes breakages in the chromosomes of the root meristem of *Vicia faba* with a predilection for the heterochromatin, and even for certain heterochromatic points of the long satellite chromosome on both sides of the kinetochore; of 202 observed chromosome injuries, 194 were located at this site (Darlington and McLeish, 1951; McLeish, 1953). Probably, both heterochromatic regions are particularly labile here, like the regions of loose coiling, which Marquardt (1952) found in the meiosis of *Oenothera* by cold experiments.

The foregoing entitles us to the assumption that the localization of the above discussed chromosome injuries depends on an uneven stability of the elements of the chromosomes' longitudinal structure, the primary and secondary constrictions and the heterochromatin being points of a particularly great fragility. However, the fundamental mechanism of breakage and recombination is yet to be elucidated. In many cases, the chromosomal injuries were found to be dependent on temperature (Kihlman, 1956). Probably, chromosomes or chromatids do not break according to the principle of all-or-nothing, but have potential and actual breaks (Thoday, 1953). This is true of mutagenic agents as well as of ionizing rays, whose linear ionization density is a decisive determinant of the variability of chromosomal injury (Kirby-Smith and Daniels, 1953; Swanson, 1955, 1960). Those regions of loose coiling, specific for the chromosomes, are the local factors of such damages. They are probably also particularly responsible for the spontaneous chromosome mutations which occur fairly frequently, mainly in the meiosis of plants (McClintock, 1941; Walters, 1951; Marquardt, 1952; for further ref., see Sharma and Sharma, 1960); these mutations, which entail breakages and recombinations, are often lethal, but may also possibly have an evolutionary significance.

Spindle disturbances

The injuries inflicted on the chromosomes are not restricted to the chromosomal structure, they also affect anaphase motility. Adhering or secondarily bicentric chromosomes (Fig. 106) impede the migration to the poles and may thus cause the death of the cell.

Such abnormal chromosome movements are of major importance in that group of pathological karyokineses which we classify under the general term of "spindle disturbances". We have already mentioned how inadequate this denomination is. When the activity of kinetochores and kinetocentres ceases, the spindle disappears. Spindle and chromosome movements have a common origin and if, in those mitotic disturbances we are about to discuss, the spindle and the chromosome movements are abnormal, then these anomalies are not the consequence of a spindle disturbance but of an inadequacy of the kinetochores or the kinetocentres. Considering how polyvalent the origins of these movements are, it is not surprising if we cannot, in any case, ascribe such a failing exclusively to the kinetocentres or to the kinetochores.

We can apply the foregoing directly to the most typical of the spindle disturbances, the arrest of the metaphase chromosomes by colchicine. This effect is so characteristic of colchicine that it is generally called "C-mitosis". It still occurs at a colchicine concentration as low as 10^{-8} molar (Ludford, 1936; Molé-Bajer, 1958). After an essentially normal, even though frequently prolonged prophase, the prometaphase rearrangement is incomplete and the chromosomes lie in the equatorial plane without the normal regular array. As they grow shorter, the longitudinal split which was previously hardly recognizable, becomes more and more visible, in other words, the daughter chromatids separate, but remain parallel to each other. Thus the number of chromosomes is replicated. At this point, segregation and migration of the daughter chromosomes fail to occur, i.e., there is no real anaphase. Instead, the chromosomes pass progressively to the interphase state. The result is a replication of the number of chromosomes, a polyploidization. Colchicine probably inhibits the orienting function of the spindle molecules; this would explain why in the sea urchin egg polar rays and spindle fibres disappear when exposed to colchicine (Mazia, 1955). Since the spindle fibres are a result of the kinetocentre-kinetochore function, the phenomenon is due to a disturbance in the function of both or of one of these elements.

Which metabolic factors are determinant here, is still open to question. It is very doubtful whether ATP plays a part in this case (Lettré, 1952b). Various factors may intensify the effect of colchicine, some non-specific, some more specific. Thus, for example, exposure of *Vicia faba* root tip meristem to heat may accelerate the metaphase arrest (Evans and Savage, 1959). Cortisone or DOCA, too, potentiate the colchicine effect, and have been called, therefore, "synergists of mitotic poisons" (Lettré *et al.*, 1951). Yet the question is still open as to whether colchicine directly attacks kinetochores and kinetocentres or primarily the surrounding cytoplasm (Druckrey *et al.*, 1957).

Many attempts have been made to elucidate the mechanism of action of colchicine by varying the colchicine molecule. Simultaneously, attempts have been made to inhibit the generalized toxic side effects of colchicine in order to utilize polyploidization for raising plants (see, e.g., a review of the subject by Eigsti and Dustin, 1955). This has led to the discovery of synthetic colchicine derivatives, such as N-methylcolchicamide, the effect of

which is four times stronger than that of colchicine (Lettré, 1952c). Demolcolcine, a diacetyl-methylcolchicine, also known as colcemide, is twenty times more effective and, above all, it is largely free of toxic side effects. With this substance, Sauaia and Mazia (1961) were able to follow in detail the progressive destruction of the spindle orientation in the sea urchin egg; hence, one may justly call colchicine and its analogues "depolarizing substances" (Sentain 1961). As a rule, its effects are of various degrees, ranging from simple disturbances in the movements of the chromosomes to apolar mitoses and finally to the complete destruction of the metaphase plate and a wide scattering of the chromosomes (Sentain, 1961).

Since oxidation of the SH groups into S—S bridges may in certain cases play a part in the linear orientation of the spindle molecule (p. 148), inhibiting substances of the SH groups were readily assumed to have the same effect as colchicine. And indeed, substances like monoiodacetamide do destroy the polar rays and the spindle (Brachet, 1947) and chloracetophenone may, in the way described above, produce large heteroploid nuclei or groups of nuclei. Almost all typical SH reagents, such as chloropicrine, monoiodoacetic acid, etc., and probably also ketones, reacting with SH groups, disturb the formation of the mitotic spindle (Lehmann *et al.*, 1954).

Among alkaloids having the same effect as colchicine, we must mention podophyllin. It equally arrests metaphases (Sullivan and Wechsler, 1947; Sentain, 1951) at even much lower concentrations. For a long time, the interest was centred on its cytostatic, tumour-inhibiting effect (Makino and Tanaka, 1953; and others), as is the case today with vincaleukoblastin, which is said to have, too, an effect similar to that of colchicine (Palmer *et al.*, 1960).

Similar results have been obtained with quite a different method. Relying on the hypothesis that RNA plays a certain role in the spindle, investigators undertook experiments with RNase. And, indeed, various mitotic anomalies appeared in RNase solutions such as chromosomes that were left behind, polyploidies and multicentric divisions (Kaufmann and Das, 1955). Moreover, injection of small doses of RNase into frog eggs, led to a rapid arrest of the divisions (Ledoux *et al.*, 1954) and disorientation of the spindle. The majority of cells, however, remain in the interphase stage. Their nuclei swell and the nucleoli lose their pyroninophilia. From these observations, a disintegration of RNA is beyond doubt; yet, on the other hand, Montgomery and Bonner (1959), in similar investigations on tadpole cells, could not detect any decrease in the ultraviolet absorption, although their experiments, too, revealed an arrest of metaphase. Brachet (1957) assumed that the cause of the RNase effect on mitosis was a disturbance in the formation or the replication of the centrosomes.

The latter unquestionably play a part, especially when the spindle is not bipolar but multipolar, for every spindle pole contains at least one kinetocentre. Such a multiplication of poles may be the result of a pathological repetition of the replication and division of the centrioles; however, it is more frequently due to nuclear divisions being retarded or suppressed while the kinetocentres go on dividing. This is the case, for instance, with the effects of colchicine and its derivatives. But there are also many odd spindles, tripolar ones for instance, which must have originated from a disturbance in the replication and division of the kinetocentres (Bergan, 1960).

Multipolar spindles are quite common. They can be found frequently in human malignant tumours (e.g., Koller, 1947), after irradiation, after colchicine (see above), caffeine, urethane (Fig. 108) and following administration of aliphatic, monovalent, saturated alcohols (Bartel-

mess, 1957) as well as after many other substances which do not impair the formation of the spindle nor the anaphase migration of the chromosomes (Bartelmess and Einlechner, 1959). We may even say that nearly all the possible damages inflicted upon karyokinesis can appear under this aspect (see, e.g., Lettré and Lettré, 1954). This applies also, for instance, to damages caused by extreme temperatures, extreme cold (Henley, 1950) or extreme heat (Davidson, 1958; and others). The formation of multipolar spindles generates, in turn, mostly multinuclear giant cells (Fig. 108).

This list of the spindle disturbances is far from being complete. All abnormal chromosome movements belong in this category, provided they do not result primarily from chromosomal injuries (p. 205). It is generally impossible to exclude the possibility of a primary chromosomal injury in chromosomes which have been left behind or ejected (Fig. 109) at meta- or anaphase. This is also the case with the above mentioned three group metaphase, observed by Parmentier and Dustin (1951) in the intestinal epithelia of mice after injections of phenols. This phenomenon was also observed in human pre-invasive atypical cervical epithelium by Hamperl (1954) and by many other investigators after administration of a number of mutagenic agents. The three group metaphase, too, is the sign of a complex cell injury rather than a pure spindle disturbance (Siebs, 1960).

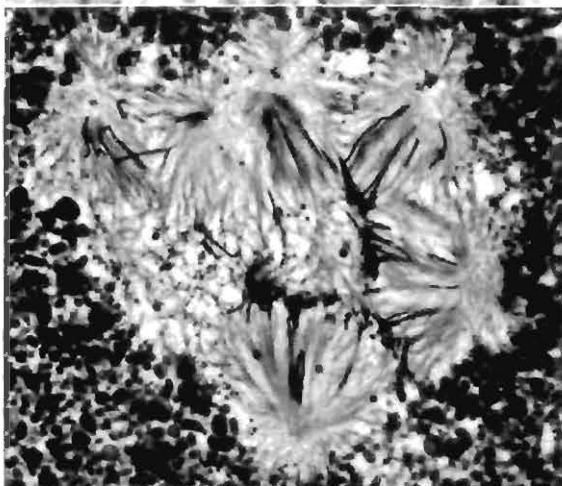
A similar difficulty arises if we want to distinguish the spindle disturbances from the *impairment of cytokinesis*, especially if one considers the close causative relationship between the spindle and cytokinesis, which makes it likely that many spindle disturbances inhibit also the cytoplasmic division. This is quite clear in the case of the C-mitosis, in which one single polyploid nucleus is generated and cytokinesis is regularly omitted. But also many other substances which induce a more or less marked spindle disturbance simultaneously inhibit the division of the cytoplasm; such is found with various phenol derivatives, when tested on the echinoderm egg (Krahl and Clones, 1936), which showed a strong increase in oxygen consumption concurrent with the inhibition of cytokinesis (see also a survey of the subject by Krahl, 1950). Further inhibition of the division of the cell body may also be induced by halogenates of benzene or toluene (Simonet and Guinochet, 1939) or by purines, e.g., adenine. Except for the factors discussed in the section on cytokinesis—such as the effects of pressure or temperature (p. 173)—a generalized injury to the cell and its division apparatus plays always the central part, not to mention the fact that many data on “poisons inhibiting cell division” do not contain any cytological details at all and it is often the absence of the easily controllable cytokinesis which is considered alone as a criterion of a drug effect.

Finally, we must consider as a special case the non-nucleate cytoplasmic fragments produced by treatment with various mitotic poisons (Albrecht, 1954; Lettré and Siebs, 1956), which is probably also a consequence of spindle disturbances in a larger sense.

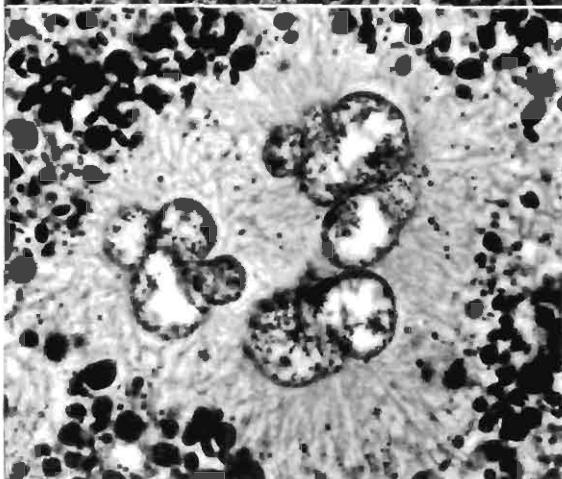
Mitotic and meiotic disturbances as the origin of human diseases

These aberrations of mitotic karyokinesis may basically occur in the same way in meiosis and are indeed found there on a large scale. Meiotic disturbances have acquired a great practical importance in human pathology during the past years, since certain diseases are evidently invariably connected with a change in the number of chromosomes. In other diseases, such a connection is assumed.

(a)



(b)



(c)

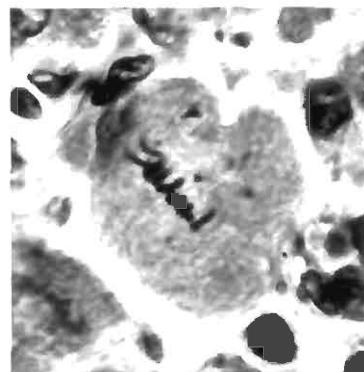


Fig. 109. Metaphase in a Jensen's sarcoma of the rat showing rejected chromosomes after administration of nitrogen mustard.

Haematoxylin eosin stain.

Fig. 108. Multipolar mitoses in the *Urodele* egg under the effect of phenyl urethane. (a) Metaphase. (b) Anaphase. (c) Post-telophase reconstruction and formation of a multinuclear giant cell. (From Sentain, P. (1961), *Path. Biol.*, 9, 445-466.)

The latter is the case for example in malignant tumours of mammals and of man. For more than 40 years, the discussion has been going on whether to impute these tumours to a genome alteration; it has become widely known as the "mutation theory of cancer" (Bauer, 1928, 1949). Apart from the evidence that a "new cell race" appears with completely different conditions of growth, suggesting the existence of a mutation as the inducing factor, such a connection is also indicated by the fact that many substances are mutagenic and carcinogenic at the same time. In which order of magnitude this carcinogenic mutation takes place, that is, whether it occurs at the level of the gene, of the chromosome or of the whole genome, is still quite unknown. It is still being discussed whether carcinogenesis is to be ascribed primarily to a mutation at all (see, e.g., Grundmann, 1961*b*, 1962).

Cytological analysis of malignant tumours in early investigations showed that the number of chromosomes varies a great deal (Hansemann, 1890), i.e., in most cases, their number was found to be abnormally large (for ref., see Hamperl, 1956; Oberling and Bernhard, 1961; and others). It appeared that most malignant tumours were aneuploid, yet that each particular tumour had its own aneuploidy; thus, there was no aneuploidy characteristic of all malignant tumours. There is now evidence of the fact that a stem line develops in the malignant tumour (see, e.g., Makino, 1957), in other words, a type of cell with a number of chromosomes characteristic of this particular tumour, and that this stem line is connected with a special intensity of growth. This had been assumed as early as in 1914 by Boveri, and was inferred 40 years later from various observations on tissue cultures (Moore *et al.*, 1956; Hauschka and Levan, 1958) and on the rat liver during carcinogenesis (Marquardt, 1958, 1959; Gläss, 1960). These findings are contradicted by the fact that there are undoubtedly diploid tumours (Wakonig, 1960) and that the malignant growth, even when preceded by polyploidization, begins from diploid cells; with this, chromosome counts (Bayreuther, 1960) and cytophotometric measurements of DNA content (Grundmann, 1954, 1961, 1962; Hobik and Grundmann, 1962) are concordant, which of course does not exclude the possibility of an injury at a submicroscopic level.

In the case of chronic myeloid leukaemia, a chromosomal alteration specific for this disease has been demonstrated; namely, the presence of a small chromosome fragment, a minute chromosome, which was discovered in Philadelphia (Nowell and Hungerford, 1960) and called therefore ¹Ph chromosome. It has been found regularly only in this disease, and only in the leukaemic leucocytes and not in other somatic cells (Baikie *et al.*, 1960). Actually, it is not an additional chromosome but a small autosome 21 or 22 of the Denver convention, become defective by a deletion or a translocation (see Fig. 45 and Table 5). If one counts chronic myeloid leukaemia among the tumour diseases, it is as yet the only case of neoplastic disease which is undoubtedly chromosomal and thus determined by mutagenic agents (see Nowell, 1962). Acute leukaemia seems frequently to contain hypo- or hyperdiploid cells, as seen in chromosome counts (e.g., Awano *et al.*, 1961) and measurements of DNA content (Gross *et al.*, 1961), although it is true that this might be the consequence of a cytostatic therapy (Hungerford, 1961).

Pathological changes of the smallest chromosomes in man are also responsible for mongolism, a grave congenital malformation with imbecility, several small malformations of the extremities and mongoloid slanting eyes. In contrast to normal human beings, these children have not 46 but 47 chromosomes and one of the minute chromosomes is to be found in



Fig. 110. Karyotype of a 2 year-old boy exhibiting mongolism with trisomy of the chromosome 21. Top: Normal disposition of the chromosomes. Bottom: Chromosomes ranged by size. (From Kosenow, W. and Pfeiffer, R. A. (1962), *Disch. med. W'schr.*, 87, 1413-1419.)

triplicate (Fig. 110). Consequently, this is generally a trisomy (Lejeune *et al.*, 1959). Mongoloid women may, though rarely, have children who can equally be mongoloid. Since the same small chromosome is present that undergoes qualitative changes in chronic myeloid leu-

Fig. 111. Karyotype of a 12 year-old girl with Ullrich Turner's syndrome of XO constitution. Top: Normal position of the chromosomes. Bottom: Chromosomes ranged by size. (From Kosenow, W. and Pfeiffer, R. A. (1962), *Dtsch. med. W'schr.*, 87, 1413-1419.)



kaemia, simultaneous occurrence of mongolism and leukaemia has often been observed (see, e.g., German *et al.*, 1962). Turner and Jennings (1961) found an 8 year-old boy with schizophrenia but no mongolism, yet with a trisomy of one of the small chromosomes; in other words, not every trisomy of these chromosomes leads to mongolism. On the other

hand, not every mongolism is induced by such a trisomy; it may appear with a total number of 46 chromosomes, when one autosome is only a monosome, i.e., present only once, and another one—which does not have to be one of the small chromosomes—is a trisome (e.g., Fraccaro *et al.*, 1960); in some cases, it is the result of translocation (Carter *et al.*, 1960; Böök *et al.*, 1961).

Trisomy has also been noted in the human chromosome 17 (Edwards *et al.*, 1960), and in chromosome 18 (Smith *et al.*, 1962). Both trisomies cause multiple malformations of the head, the extremities and also of the inner organs; the children, therefore, often only live a few days or weeks. Similar observations have been made on the medium-sized chromosomes 13–15 (Atkins and Rosenthal, 1961; Patau *et al.*, 1961; and others). These extra chromosomes disturb the gene balance in the genome.

When one haploid chromosome set is in excess, in other words, when the children are triploid, the consequence is a series of serious malformations (see, e.g., Delhanty *et al.*, 1961), which, however, may in certain cases be compatible with life (Böök and Santesson, 1960). This is quite remarkable, seeing that triploids have very rarely been observed in mammals and then with certainty only in the early stages of the embryo.

We find trisomy too in the sex chromosomes, mainly in the X chromosome. These XXX females are not "super females" as they are occasionally called (Jacobs *et al.*, 1959; Lenz, 1961a), but often females with an ovarian insufficiency and mental retardation; however, no definite clinical picture has been attributed as yet to the XXX complex (Johnston *et al.*, 1961). From genetic estimations, we may assume that such XXX females are more frequent than we know at present (Lenz, 1961b).

Two other diseases, also connected with a quantitative disturbance of the chromosomes, were discussed previously when we dealt with the sex chromatin (p. 79). These are Klinefelter's syndrome and Ullrich Turner's syndrome. In the first, the nucleus is chromatin positive and the male patients have, in addition to 44 autosomes, at least three heterosomes of the XXY type (Jacobs and Strong, 1959). In the Ullrich Turner's syndrome, no sex chromatin is to be found in the nucleus; the female patients have an XO heterosome constitution (Fig. 111). However, this rule, too, has many exceptions. Thus, Klinefelter's syndrome sometimes has 48 chromosomes, made up of 44 autosomes and 4 heterosomes of the type XYY or XXXY (e.g., Carr *et al.*, 1961). One XXXXY male, with 49 chromosomes, was half imbecile, sexually underdeveloped and showed many exterior malformations (Miller *et al.*, 1961).

The most evident explanation of such chromosome constitutions is a nondisjunction of the homologous chromosomes at meta- or anaphase, or, in the last of the above-mentioned cases, during meiosis. This process is well-known from older genetic experiments on *Drosophila*, in which such a nondisjunction, for instance of the X chromosome, induces XX egg cells, that lead to the zygotic XXY constitution (Bridges, 1916). These nondisjunctions occur in *Drosophila* also in the male X chromosome, and they occur spontaneously (see, e.g., Kelsall, 1961). Fig. 112 shows how nondisjunction of the heterosomes in man during meiosis can induce an XXX trisomy or the typical Klinefelter's syndrome XXY or the Ullrich Turner's syndrome with an XO constitution. Hence, trisomies of the autosomes result from the nondisjunction of autosomes in one of the two meiotic divisions (see, e.g., Koch, 1961).

Human sex chromosomes also frequently display mosaic formations, i.e., cells with two different chromosome constitutions appear next to each other. This was observed for instance in some cases of slight Turner's syndrome (Grouchy *et al.*, 1961) in which certain cells show an XO constitution characteristic of the disease and others an XX constitution. Another heterosome XYY/XO mosaic induces a dysgenesis of the female gonads without any mental disturbance (Cooper *et al.*, 1962). The formation of such a mosaic must be due to the fact that in the early division of the fertilized egg cell the replicated Y chromosome did not disjoin itself, but entered a single cell, whereas the other daughter cell received only one X chromosome and no Y chromosome.

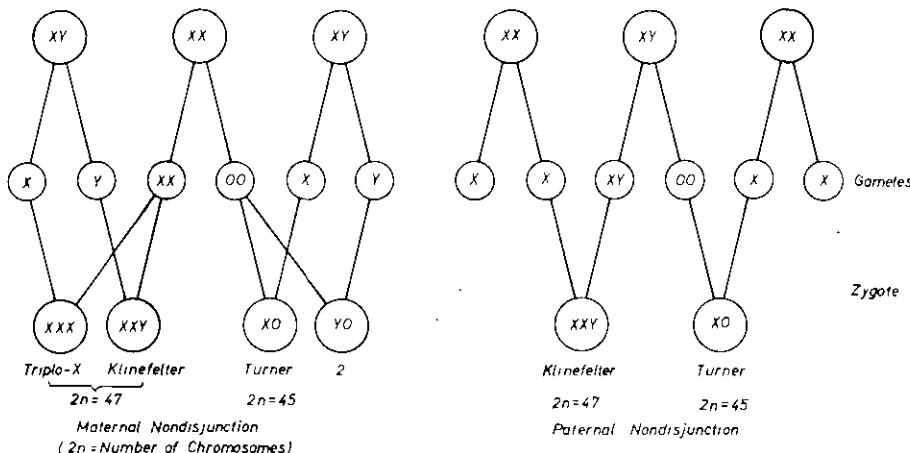


Fig. 112. Diagram showing the development of the chromosome constellations in Klinefelter's and Turner's syndrome by maternal or paternal nondisjunction. (From Koch, G. (1961), *Ärztl. Forsch.*, 15, I/354-I/366.)

Observations of anomalies in human chromosome numbers are accumulating. They have been made possible by new methods of preparation of cell cultures of the peripheral blood or, for instance, of the epidermis (for ref., see Nachtsheim, 1959, 1960; Kosenow, 1960; Koch, 1961; Lenz, 1961b; Kosenow and Pfeiffer, 1962; and others). We still know very little about the origin of these anomalies. In most mammals, the first stages of meiotic prophase up to diplotene occur in the oocytes shortly after birth; the later stages of meiosis, up to metaphase of the second meiotic division, take place during the few hours between the stimulation of the Graafian follicles and ovulation, whereas the second meiotic anaphase starts only after the egg cell has been fertilized by the spermatozoan. This accounts for the great variety of possible disturbances of oocyte meiosis and part of the nondisjunctions will probably take place after fertilization in the tube or the uterus. However, it is quite conceivable that a certain number of these anomalies and the resulting malformations are acquired and not genetic alterations. They can be induced by a great many peristatic factors, which, as we know, play an important part in the production of malformations (see, e.g., Büchner, 1961b).

Review

In most disturbances of mitosis and meiosis, we are confined to a mere description of the facts. We establish that this or that substance has a certain effect on the division of nucleus and cell; and observation of pathological karyo- and cytokineses does entitle us in many cases to infer the abnormal conditions of the division processes. However, the ultimate cause of mitotic disturbance remains generally obscure. Moreover, the number of substances damaging mitosis has meanwhile become so large that the term mitotic poison appears outdated; every substance having a direct or indirect influence on the cell, even oxygen or nitrogen, may impair mitosis. Its normal functioning requires an inner balance, the disturbance of which results in mitotic anomalies. The effect of high or low temperatures is an example of such a disturbance of balance. The accessory structures of karyo- and cytokinesis, the spindle and the polar rays, prove the most labile. Further, there are damaging factors, such as the ionizing rays, which affect the chromosomes at points of predilection, causing there chromosomal mutations; yet, their action is by no means restricted to the chromosomes; the cytoplasmic organelles, too, for instance the mitochondria, undergo fundamental changes in the same sense (p. 316). Also all those substances which we have seen interfering specifically with interphase or intermitosis affect the chromosome movements; this is the case, for example, with the typical spindle poisons. In other words, there is hardly any other field in cytology where a classification is less justified than in the case of the mitosis-disturbing substances. As we already often emphasized, the cell responds to every damage as a unit. A mitotic disturbance is only one of the possible aspects of such a reaction, even though it occurs particularly easily. Thus it does not surprise us to find that certain malformations in man are induced by disturbances of mitosis and meiosis, mainly by a loss of chromosomes or a nondisjunction of homologous chromosomes. Generally speaking, the cell is in a state of crisis during karyo- and cytokinesis in which previously inflicted damages become usually visible and effective, and frequently determine the fate of the cell.

POLYPLOIDY AND ENDOMITOSIS

All the facts that we have discussed in the foregoing, in connection with the division of the cell and its disturbances, confirm the law of constancy of the chromosome number, and it is the main function of the complicated processes of mitotic karyokinesis to maintain this constancy. The conjugation of two haploid germ cells at fertilization generates the diploid zygote, mother cell of all somatic cells in the organism, which are, in turn, mostly diploid like the zygote, i.e., they contain two sets of chromosomes. However, we already mentioned the fact that in many parenchymatous organs of mammals, apart from these diploid cells, other cells could be found, that have a higher DNA content; the DNA values of these cells are in an integral ratio to the DNA values of diploid cells. From the above emphasized close correlation between the number of chromosome sets and the respective DNA content, we can conclude that these somatic cells do not contain diploid nuclei with two chromosome sets, but that their chromosome sets have been multiplied. This is somatic polyploidy.

Occurrence of polyploidy

Polyploidy is to be found in almost all organisms, in plants as well as in animals or in humans. In mammals, the nuclei of the parenchyma cells are, as a rule, diploid directly after birth. A few days later, nuclei with higher DNA values, for instance in the liver (Fig. 113) have already appeared, indicating a polyploidy (Inamdar, 1958). From chromosome counts by Tanaka (1953), at least 80% of all nuclei in the liver of the rat embryo are diploid; according to Marquardt and Gläss (1957), 84% are. By contrast, in adult rats, the number of diploids is markedly reduced; according to Bieseile (1944), it amounts to 59%, according to Tanaka (1953), to 35% and according to Marquardt and Gläss (1957), to 28–58% of all nuclei; the others prove polyploid. Basically, the same can occur in other parenchymata. Extreme polyploidy can be found normally for example in the giant cells of bone marrow in mammals and man (Potter and Ward, 1940; Weicker and Nöller, 1951; and others),

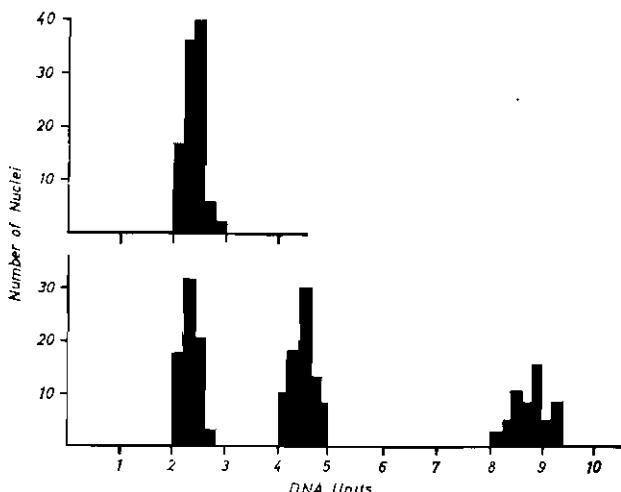


Fig. 113. Distribution of DNA content in the nuclei of the mouse liver, at birth (top) and 2 weeks later (bottom), showing how postnatal polyploidy develops. (From Inamdar, N. B. (1958), *J. Morphol.*, **103**, 65–90.)

and also, as a pathological feature, in many malignant tumours. However, it may be observed in tissue cultures of mammalian cells, such as in the mouse embryo (Levan and Hauschka, 1953; Levan and Hsu, 1961), where repeated "endoreplications" cause the homologous chromosomes to stay clustered together (Fig. 114).

In most plants, the cells of the root meristem are diploid. But in the proximal parts of the root, polyploid nuclei are the rule (Stomps, 1910; McLeish and Sunderland, 1961; and others) especially in the region of the vascular system (for ref., see, e.g., Geitler, 1941, 1953). In the course of investigations on protozoans, Hartmann (1909) noticed that multiple divisions of the nucleus can happen in a cell, one nucleus being apt to generate rapidly a large number of daughter nuclei. Consequently, such nuclei must have been previously of a complex nature. Hartmann called those nuclei which can divide themselves into several nuclear portions "polyenergid" nuclei (see also Hartmann, 1953), in reference to Sachs'

concept of polyenergids, which were meant, however, to characterize rather a functional multivalence. The macronuclei of protists are in most cases markedly polyploid (Grell, 1950b). In the macronucleus of *Paramecium*, the DNA content seems to indicate, for instance, a total of 860 genomes.

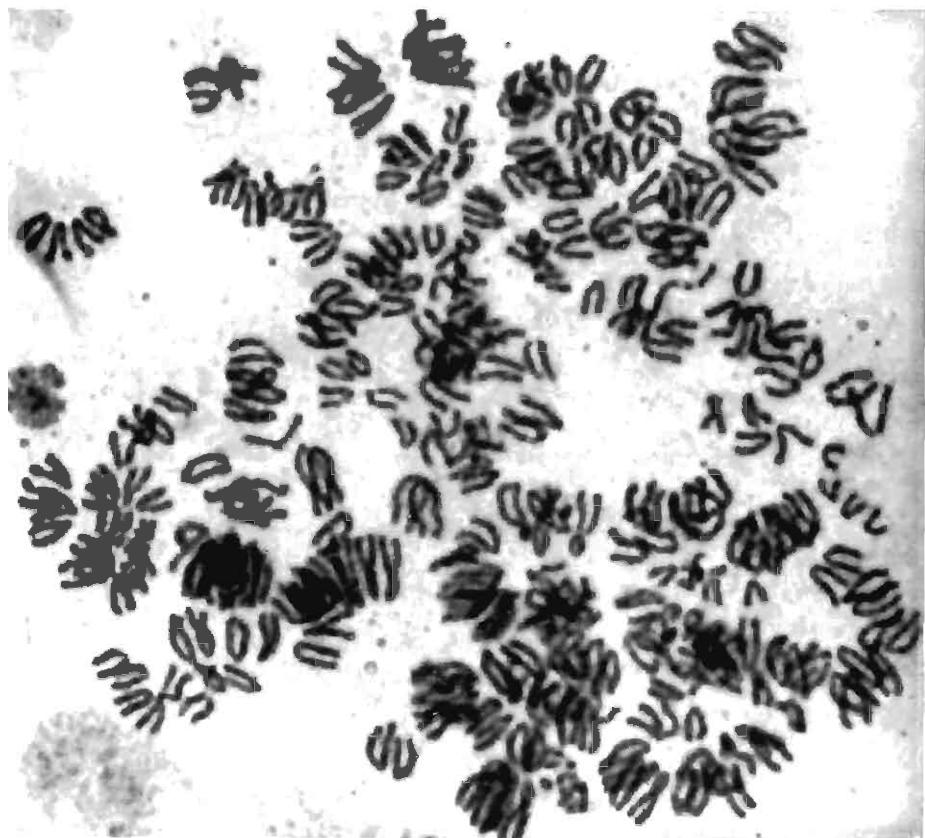


Fig. 114. Polypliod cell from a culture of mouse embryo cells at metaphase. The chromosomes, self-generated by endoreplication, stay clustered together. (From Levan, A. and Hsu, T. C. (1961), *Hereditas*, 47, 69-71.)

Divisions of larger nuclei into smaller ones were found by Holt (1917) and Berger (1938) also in the epithelium of the small intestine of *Culex pipiens*. During the development of the larvae, the nuclei grow without dividing themselves. Shortly after pupation, mitoses appear and chromosome counts show that the chromosomes may even be 64-ploid. Mitoses reduce polypliody down to an octoploid. Berger (1938) named this phenomenon "somatic reduction". It occurs in a similar way in several tissues, for example in the metamorphosis of butterflies (Risler, 1950), in the honeybee and in other insects (Risler, 1959); it is a general

fact that polyploid cells are to be found relatively frequently in insects. As early as in 1906, Wilson had observed numerous polyploid mitoses in the walls of the ovaries of a bug, in the Fallopian tubes and in the walls and interstitial cells of the testicle as well as in the adipose body (see Wilson, 1928). However, this list of polyploid nuclei is far from being complete. We shall encounter some other examples when we discuss the conditions of endomitotic polyploidization (p. 226).

Polyplody as a result of abnormal karyokineses

One of the first causes of polyplody to be considered are abnormal karyokineses such as the colchicine mitosis, in which the replicated chromosomes remain in the metaphase plate and the reconstructed nucleus is necessarily polyploid. It is a fact that with colchicine, polyploid nuclei may be produced experimentally.

Another source of polyplody is spindle fusion in binuclear or multinuclear cells. Normally, in binuclear liver cells, for example, two separate spindles develop. If two spindles which are adjacent to each other unite, only two daughter nuclei are generated, each with two sets of chromosomes. This is how for instance Beams and King (1942) explain the formation of tetraploid liver cell nuclei. Another possibility is the fusion of two interphase nuclei (see, e.g., Mechelke, 1952), that is, the retrograde process, so to speak, of the amitosis which will be discussed later (p. 228).

Polyplody may also occur if only one spindle is formed for the two diploid nuclei of a binuclear cell (see also Fell and Hughes, 1949). Despite the lack of certified data on the rate of occurrence of such spindle fusions, this explanation has been used often for the production of tetraploid nuclei in parenchyma cells (see, e.g., Himes *et al.*, 1957; Inamdar, 1958; Walker, 1959).

The process has been observed with the greatest authenticity in the antipode cells of *Caltha palustris*. According to Grafl (1941), these antipode cells become first binuclear by a series of mitoses. Then both nuclei undergo a common meta- and anaphase, so that the following generation of nuclei has two sets of chromosomes. The process repeats itself up to the formation of octoploid nuclei.

All these pathways leading to polyplloidization by a disturbance or a variation in karyokinesis have been classified by Hsu and Moorehead (1956) into four groups, according to their observations made by filming HeLa cells: (1) Interreplication, i.e., replication of the chromosomes at interphase without any visible change, especially without any break-down of the nuclear envelope. (2) Proreplication, in which the chromosomes condense within the nucleus and undergo replication at a prophase stage. (3) Metareplication, parallel to the C-mitosis described earlier. (4) Ana- or teloreplication, in which the chromosomes of one single nucleus or of two adjacent nuclei join at ana- or telophase.

Endomitosis

From these four possibilities, the first two are especially important. A process similar to that of Hsu's and Moorehead's (1956) "proreplication", with multiplication of the chromosomes within the intact nuclear envelope, had been described by Geitler (1938a) and called

"endomitosis". He carried out his first and decisive investigations on the nuclei of the water striders, in which he had earlier (1937) observed a polyploidy. This type of beetle has a totally heterochromatic X chromosome, that appears as a chromocentre in the "resting" nucleus (p. 77) and from the number of chromocentres one may infer the degree of ploidy of the nucleus (Geitler, 1937, 1938b). Aided by this circumstance, Geitler (1938a, 1939a) was then able to describe the process of intranuclear multiplication of the chromosomes, or endomitosis. Similarly to what happens in mitotic karyokinesis, the chromosomes form a spireme coiled into a clew (Fig. 115a) during "endoprophase" which loosens itself progressively as the condensation of the chromosomes increases (Fig. 115b and c). The nucleoli become

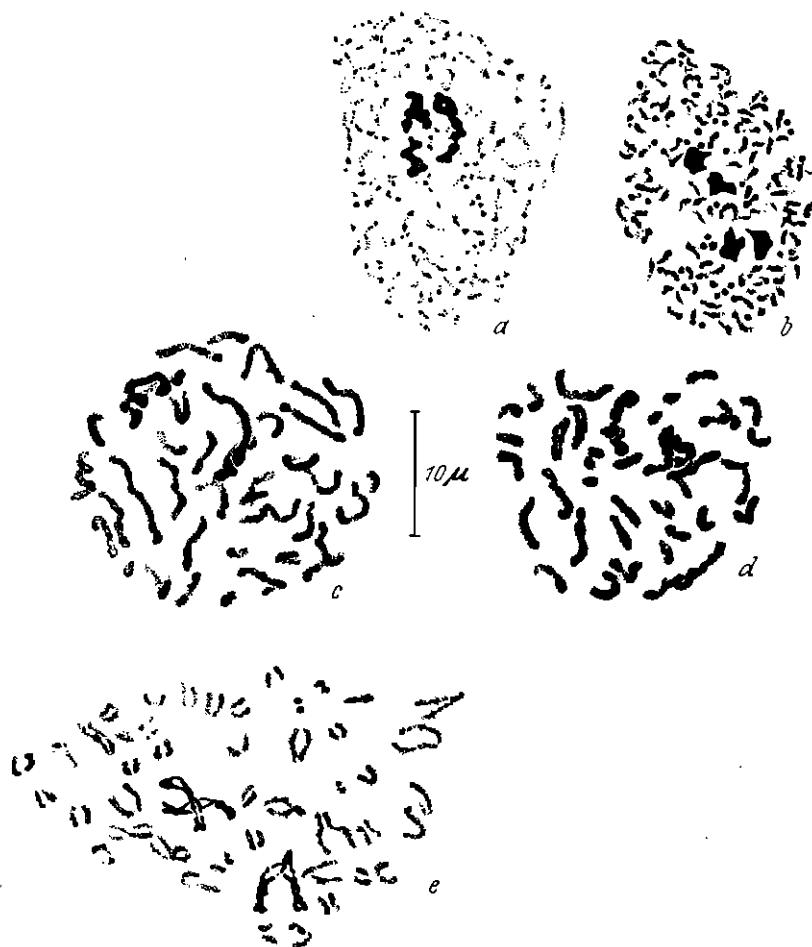


Fig. 115. Phases of endomitosis in an octoploid nucleus of *Gerris lateralis*. (a)-(c) Endoprophase. (d) Endometaphase. (e) Endotelophase. (From Geitler, L. (1939), *Chromosoma*, 1, 1-22.)

smaller, but do not disappear completely (Geitler, 1939b). There is no spindle nor spindle-like structure and all movements of the chromosomes in the metaphase plate or towards two poles fail to occur. The chromosomes remain scattered more or less regularly throughout the nucleus and go on condensing progressively; yet, the condensation at "endometaphase" (Fig. 115d) is never as extensive as during mitotic metaphase. Owing probably to the relatively slighter contraction of the chromosomes, the morphological difference between euchromatin and heterochromatin never disappears completely and the heterochromatic sex chromosomes may still, even at this phase of endomitosis, be distinguished from the euchromatic autosomes. A longitudinal cleft soon becomes visible along the chromosomes and the chromatids, which had split during the endometaphase contraction, withdraw from each other in a parallel array. This is "endoanaphase". In the process of this parallel dissociation, the daughter chromosomes separate but slightly, then decondense progressively at "endotelophase" (Fig. 115e) and enter finally the intermitosis or interphase stage. It often happens that the chromosome tips still cling to each other for quite a while, thus forming oval-shaped figures (Fig. 115e). The spindle insertion, which may sometimes be seen, remains inactive (Geitler, 1941); consequently, the process of endomitosis corresponds in a certain sense to that of a C-mitosis, which leads to the same result. However, in contrast to C-mitosis, the condensation of the chromosomes is not enhanced but attenuated; thus, we have here a variant chromosomal cycle. Geitler (1940, 1953) observed the same process in many other organisms, and he justly considers it the most frequent way to polyploidy. It does not exclude other mechanisms of polyploidization like those discussed above.

This basic scheme of endomitosis has a great many variants. In the roots of *Rhoeo discolor*, Dolezal and Tschermak-Woess (1955) found a series of endomitotic peculiarities which pertain to the heterochromatic structure of the chromosomes. During the endoprophase coiling, the heterochromatin is almost entirely degraded, revealing chromomere-like structures. Since, at the same time, the euchromatic parts of the chromosomes appear more clearly and are particularly noticeable as evenly distributed granules, the distinction between eu- and heterochromatin largely disappears. This is the "pulverization phase". When the heterochromatin becomes visible again, it has already formed "double chromocentres"; these then separate from each other, which could be the sign of a terminating endomitosis (see also Deufel, 1954). In *Rhoeo discolor*, however, there is no direct connection between the number of chromocentres and the degree of ploidy, for many chromocentres unite to form larger collective chromocentres. "Pulverization" of the chromocentres during endomitosis is evidently a most characteristic feature (Fig. 116), and could be observed in a whole series of organisms (Hasitschka-Jenschke, 1959).

In *Sauromatum guttatum*, the beginning of endomitosis is marked by a flattening of the chromocentres, which are "pulverized" at endometaphase and reconstituted at endotelophase (Tschermak-Woess, 1954). In contrast to the classical endomitosis in the water strider (Fig. 115), the single phases of endomitosis are much more difficult to distinguish. However, since the changes in the shape of the chromosomes occur rhythmically and are combined with a rhythmic volume increase of the nuclei, they can be considered the sign of genuine endomitoses. Pulverization of the chromocentres or appearance of double chromosomes alone is no absolute evidence of an endomitosis. The same can be said of endomitosis-like phenomena, which may be found for example in mouse ascitis tumours or in human carcinomas (Homann,

1952, 1954). In the water boatman *Corixa*, for instance, we also find changes in the shape of the chromosomes; as in endomitosis, they undergo condensation and pairing, i.e., homologous chromosomes or chromocentres lie next to each other, yet there is no division nor polyploidization of the chromosomes. Such "pseudoendomitoses" may occur in diploid as well as in polyploid nuclei (Lipp, 1953). However, the same water boatman may have also real endomitoses with a particularly strong condensation of the chromosomes (Lipp, 1953) and the "pseudoendomitoses" occur at the same time as the real endomitoses. Chromosome condensation evidently occurs independently from chromosome replication.

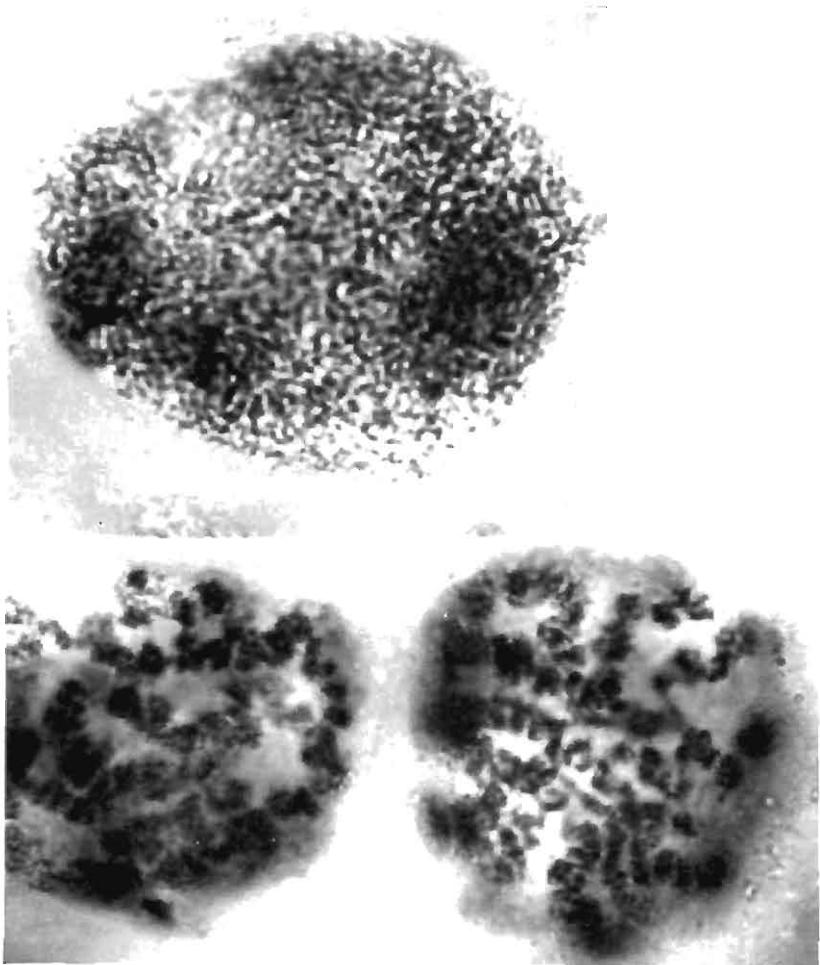


Fig. 116. "Pulverization" of the chromocentres during endomitosis of a 16-ploid antipode nucleus of *Errantis hiemalis*. Bottom: two intermitotic nuclei with giant chromosomes. (From Hasitschka-Jenschke, G. (1959), *Chromosoma*, 10, 229-267.)

Geitler (1953) classified the different types of endomitoses into three groups: (1) Endomitoses in which the stages of the process may be identified directly and authentically; (2) Nuclei in which only the result of endomitosis, namely, polyploidy, may be established with certainty; (3) Polyploid nuclei from which one may infer only with a certain degree of probability that an endomitotic polyploidization has taken place. From this broad concept of endomitosis, it is possible to interpret retrospectively as endomitoses several older findings, such as the formation of large nuclei in the ciliates (Piekarski, 1941). The first endomitoses appear to have been discovered by Vejdovski (1911, 12) in elongated nuclei of the circular musculature in worms. In this case, the chromosomes condense, replicate and separate, following the phases of endomitosis exactly.

The relationship of DNA replication to endomitosis was investigated for the first time by Tschermak-Woess (1959). Feulgen cytophotometry on epidermis cells of *Sauromatum guttatum* demonstrated that the amount of DNA doubles before the beginning of the endomitotic cycle as it does before mitosis. The young endointerphase nuclei had a DNA content of $2n$ or of twice ($4n$) or four times this amount ($8n$), and there were also numerous intermediary values indicating that a DNA synthesis was taking place. In contrast to this, nuclei examined during the endomitotic cycle showed exclusively DNA values of $4n$ and $8n$ and no intermediary values.

Polyteny

How different the processes of endomitosis may be in one and the same specimen has become clear in the blue bottle fly *Calliphora* (Bier, 1958). Its young nutritional cells show clear-cut endomitotic cycles. As the cells grow, the phases of this cycle are less and less distinguishable and can in the end only be deduced indirectly. In such "cryptoendomitoses", the chromosomes neither coil nor condense and the daughter chromatids do not segregate but build multistranded bundles of chromonemata, the polytene chromosomes. Polyteny occurs, in contrast to the above discussed polyploidy, when the newly generated chromonemata stay joined together. It may also be the result of an endomitotic cycle. The chromatid division without chromatid segregation in the anther tapetum of *Antirrhinum majus* was called "polymery" by Mechelke (1952). By "polymer mitoses" and by a mitotic karyokinesis these polymeric chromosomes may develop into "dimeric", i.e., normal chromosomes. Perhaps it would be better to speak of polymery only when the chromatids are duly segregated and not only divided, and to call polyteny the not particularly structured multistrandedness of large chromosomes. However, polyteny, polymery and polyploidy are ultimately only variants of the same principle, viz., a repeated replication of the chromosomes' longitudinal elements without their redistribution into different nuclei (Huskins, 1948).

Polyteny also occurs in the giant chromosomes of *Diptera*, which have revealed to us a great deal about the structural organization of the chromosomes (p. 118). Not only are they much longer but they are also much wider than normal chromosomes and may reach a diameter of 25μ . They consist of many longitudinal fibrils (Bauer, 1935), particularly recognizable by the Balbiani rings (Fig. 55). Paintner and Reindorp (1939) observed that in the youngest larvae the chromosomes are still of a fairly small and simple structure. Only as the larva develops do they grow to giant chromosomes, without showing any sign of division

processes or endomitotic phases. The degree of polyteny which they finally reach is 1024 times that of the haploid germ cells (Swift and Rasch, 1955) or even higher (Beermann, 1952b) and it may still be increased by infection with microsporidia (Keyl, 1960).

Polytene giant chromosomes have also been found in many other tissues, including plants. Hasitschka (1956) described them as they appear in the antipodes of *Papaver*, where they are a great deal smaller than in *Diptera*. Tschermak-Woess (1956) and Hasitschka-Jenschke (1957, 1959) observed them in the antipodes of several other plants too (Fig. 116).

Conditions in which endomitosis occurs and its significance

The occurrence of an endomitotic multiplication of the chromosome sets can be closely connected with that of a mitosis. This could be inferred from investigations, for example, on the scale formation of the flour moth (Henke and Pohley, 1952), in which endomitoses also take place and the number of endomitoses is inversely proportional to the number of mitoses. Yet, the sum of both endomitoses and mitoses remains constant. It is to be assumed that factors of the cytoplasmic metabolism are capable of stimulating or inducing both processes.

This could be demonstrated in detail for example in the larva epidermis of the culicide *Aedes aegypti* (Risler, 1959). Here, the epidermis nuclei are tetraploid in normal larvae; but if one reduces the food supply of the larvae, the nuclei remain diploid. The conditions are the same for the liver of mammals, in which the frequency of polyploid nuclei depends on the functional stimulation of the organ (p. 219). Additional injuries to the liver tissue, as caused by partial hepatectomy (Grundmann and Bach, 1960) or exposure to carcinogenic agents (Grundmann, 1954, 1961b, 1962; Hobik and Grundmann, 1962) increase the degree of polyploidy. Castration (Swartz *et al.*, 1960), thyroxine and growth hormone have the same effect (Geschwind *et al.*, 1960).

Although we still do not know the nature of each single substance that induces endomitosis, we may venture a generalizing statement and say that an endomitotic polyploidization always occurs when the cells are subject to an increased functional or differential stress (see also Geitler, 1953), in other words, when more gene material is necessary for the cell's activities. In consequence, polyploidization appears to be a most important process in the amplification of the nuclear function. This relationship is basically sufficient to explain nearly all the conditions in which polyploidization occurs. Even the high degree of polyteny in the salivary gland cells of *Diptera* may be interpreted as the sign of a functional strain.

There is another causative factor of endomitotic polyploidization, which is closely related to the foregoing, namely, differentiation. Organization of the tissue structures specific for each organ is in most cases combined with endomitotic polyploidization (Geitler, 1953). Some tissues are characterized by a uniform degree of polyploidy; in the water strider, for instance, the Malpighian tubes are 32- to 64-ploid, whereas the nuclei of the muscle cells are only diploid, those of the testicular septae 16-ploid etc. (see Geitler, 1938b, 1939a, 1939b). The adipose body is composed of cells of different degrees of ploidy and we find such a "polysomaty" also in many tissues of the superior mammals and man, as we noted already in the case of the liver, for example (p. 219). This is the result of a specific cell function which has been made possible by differentiation. The cells which retain their mitotic dividing capacity often remain diploid, whereas other parts of plants which have

lost their meristematic character reveal higher ploidy values. This is the case with the proximal inner layers of the root tips (Huskins and Steinitz, 1948), as well as of the trichomes and trichocytes of several angiosperms which show a regular pattern of even distribution from diploid up to 256-ploid nuclei (Tschermark-Woess, 1954).

Finally, endomitotic polyploidization is of great importance for the variation of the species. For instance, in the hamster, there are two types with a different ploidy; one species of hamster contains 11 chromosomes in the germ cells, the other 22 and the diploid somatic cells have accordingly 22 or 44 chromosomes. The second type, with the greater number of chromosomes, is the Syrian hamster. It presumably developed from the first type by a ploidy mutation. This kind of evolutionary polyploidization is very frequent in plants. It forms the basis of many of our culture plants of today. For instance, among the cereals, the hexaploid types are frequently cultivated today, whereas the original diploid types are hardly known any more, especially since they were far from being as productive as those with a higher degree of ploidy. We must assume that the polyploid types of cereals were bred progressively at some time or the other in the course of the millennia.

Multiplication of the chromosome sets of one and the same species, as was the case in the above described polyploidizations, is called autoploidy. However, we find also a pairing of chromosome sets belonging to different types of individuals; this is termed allopolyploidy, and its features are most important in all breeding experiments. The condition required for this is that the chromosomes are always related in such a way that a regular meiotic pairing is made possible; slight differences in the number of chromosomes may be disregarded in certain cases. Generally, plants with a higher degree of ploidy are much bigger; their leaves are often of a deeper green or the surface of the leaves is sturdier and more markedly grooved. Animals rendered polyploid experimentally are apt to have notably enlarged cells. Except for certain rare types of polyploid mutants in *Drosophila* breeds, polyploid races are to be found mostly in animals which reproduce themselves by parthenogenesis, such as crabs, parthenogenetic butterflies or arthropods and beetles, and in hermaphrodites of the most various types (Christensen, 1961). In mammals it is very difficult to produce ploidy mutations. We mentioned already, in the section on mitotic and meiotic disturbances, the existence of a spontaneous triploidy in man (p. 216). In all these polyploidizations, the accent must be laid not only on the possibility of pairing of the chromosomes at meiosis, but also on a reciprocal "harmony" of the genes, the "gene balance", which is an important factor of human malformations when changes occur in the number of the chromosomes.

Review

We have found that polyploidy is a most frequent phenomenon in animals and plants. It may be caused by different factors: abnormal mitoses, spindle fusions, nuclear fusions and, mainly, endomitosis or chromosome multiplication without nuclear division. This endomitosis, which may in certain cases be observed as a morphological cycle, can often only be detected by its result, polyploidy or polyteny. It is ultimately a simplified mitosis. Endomitotic polyploidization is closely connected with the cell and tissue function and with the differentiation processes and plays also an important role in the development of the species.

AMITOTIC DIVISION OF THE NUCLEUS**Definition**

Amitosis is not a good term, for it consists of a negation, indicating that it is *not* a mitosis, and a negation is hardly apt to characterize a phenomenon. Many attempts have been made to replace this term by a better one. The ending “-mitosis” implies the relationship to the indirect nuclear division, and this is why Flemming (1892b) called amitosis “direct nuclear division”, even though he had introduced the terms “mitosis” and “amitosis” in 1882. Evidently, he was not pleased with his own nomenclature and also spoke of “nuclear cleavage” or “holoschisis”. Other authors, such as Arnold (1883) or Jacobj (1942), favoured the expressions “nuclear fragmentation” or “nuclear segmentation”. However, these terms could be applied to a large variety of phenomena of the most different kinds.

We must make a sharp distinction between two processes: the amitotic nuclear division (Bucher, 1959) and the nuclear segmentation or nuclear fragmentation with simple pinching off of nuclear elements.

The necessity of defining more precisely the term “amitosis” by adding the phrase “nuclear division”, as we will do now in following Bucher (1959), appears clearly if we consider the manifold extensions to which the concept of amitosis has been subjected. Jacobj (1926), for example, always spoke of amitosis “when no exterior sign of division (not even an amitotic cleavage of the nucleus) can be seen, but only a process of growth of the nucleus following the rules of replication”. Yet, according to our definition, this would be no amitosis at all, but an endomitosis. Clara (1930) made an attempt to solve these definition problems; he used the term “endoschisis” for the inner process of division which corresponds practically to what we call today endomitosis, and “phenoschisis” for what we call amitotic nuclear division.

However confusing these concepts may seem at first, they lead us all the same directly to the definition of amitotic nuclear division, by distinguishing it from other phenomena. It is in fact a nuclear division which has nothing to do with the mitotic cycle; consequently, the chromosomes do not become visible and there are no karyokinetic accessory structures. Thus, expressed in modern terms, it corresponds exactly to Flemming’s first definition of amitosis (1882, 1892) and is identical with Clara’s “phenoschisis” (1930). Amitosis is more than just an “increase of the nuclear surface area in a resting nuclear structure” as Benninghoff (1922) wanted to define amitosis. In other words, it is not identical with the various invaginations and evaginations of the nuclear envelope, which sometimes bring about amitosis-like figures and for which Rössle (1926) proposed the term kalymmauxosis, i.e., segmentation and fragmentation of the nucleus.

Evidence of an amitotic nuclear division

The occurrence of nuclear segmentations and fragmentations is generally agreed upon. But whether there exists a real amitotic nuclear division analogous to the mitotic, is still very much a matter of controversy, and a number of biologists would like the concept eradicated. They are, as everyone, influenced by their own objects of investigation, and if, for

nstance, many botanists ascribe to amitosis a mere historical significance, at the utmost, it is due to the fact that plants very rarely do have clear-cut amitoses.

This scepticism finds justification also in the history of the theory of nuclear division (see also p. 28). For the 19th-century theory of "omnis nucleus e nucleo" had developed not from the discovery of mitosis, but from repeated observations of single, seemingly "direct" nuclear cleavages according to the scheme of Remak (1858), and this scheme is approximately the same as that of what we call today amitotic cleavage of the nucleus. When the first "thread metamorphoses" of the nucleus, the mitotic karyokineses, were described, they were considered an exception to the simple nuclear cleavage (e.g., Eberth, 1876). Once mitosis had been generally recognized as the typical and main form of nuclear division, investigators realized they had been deceived by the optical insufficiency of the microscopes. The pendulum swung to the other extreme and the general opinion was that there are no amitoses and what was called amitosis is either the result of inexact observations or a disturbance in the normal mitotic karyokinesis, i.e., a "pseudoamitosis". The pendulum has not swung back yet. Flemming (1897), one of the first pioneers of the doctrine of nuclear division, remained cautious. Wassermann (1926), in his thorough handbook contribution, indicated his many doubts and even the most recent exhaustive survey of the problem by Bucher (1959) is still not free of scepticism. Today, a few years later, we must make even further reservations, for many of the so-called amitoses depicted in these standard works must be interpreted today as "pseudoamitoses"; at least we lack evidence that they are strict amitotic cleavages of the nucleus. For example, the multinuclear buds in muscle cell cultures, which have been considered up to quite recently as amitotic, develop, according to Bassleer's radioautographic findings (1962), predominantly from the fusion of cells and not from amitotic nuclear divisions. The alternative between direct nuclear division and nuclear fusion hinders also the interpretation of every cell picture after fixation, where a typical amitotic constriction is claimed to be recognized. It is then no longer possible to tell which way the cell was proceeding.

The most elegant evidence for the occurrence of amitotic nuclear division is brought by direct observation and motion pictures, many of which have been published. Bucher (1959) classified them synoptically and added his own observations. He concluded by stating that "amitotic nuclear division has been demonstrated . . . from investigations on living cells". Later, however, he was more reserved in his statements (Bucher, 1962) and Wassermann (1962) summarized the discussions of the 4th international symposium of histology in Lausanne with the words: "The perfect demonstration of the process of amitosis in a motion picture . . . has still to be obtained."

Other sources of evidence are all the more valuable. They are to be found mainly in the numerical relations of the various classes of nuclear volume in mammalian tissues. For instance, in the mouse liver, after a hunger of 2 days, one may observe a significant decrease in the number of macronuclear and an increase in that of micronuclear epithelial cells, and yet, there is no visible sign of any mitoses (Phan and David, 1958). Following partial hepatectomy in the rat, the relative number of the diploid nuclei has already tripled by 30 minutes, also without any mitotic karyokineses (Grundmann and Bach, 1960; see also Busanny-Caspari, 1961). The same basic effect may be obtained in the rat liver by a mere laparotomy under ether anaesthesia, i.e., without the liver parenchyma being even reduced (Grundmann

and Pfeifer, 1963). In the rat kidney, 6 to 8 hours after withdrawal of an arterial clamp, many diploid micronuclei appear in the vicinity of necroses, again accompanied by a multiplication of the binuclear cells and again without any increase in the rate of mitoses (Cain, 1961). Exactly the same phenomenon occurs in the cat heart, where, in the vicinity of experimentally produced necroses, the number of the binuclear muscle fibres increases significantly (Grundmann, 1950). Since, in all these cases, mitoses fail to occur, the increase of the small nuclei at the expense of the larger ones can only be the result of an amitotic nuclear division.

Another direct evidence for the amitotic nuclear division is the behaviour of the nucleus in certain unicellular organisms, the ciliates. These organisms have a generative micronucleus, which reproduces itself mitotically, and a morphologically predominant macronucleus (e.g., Belar, 1926). The macronucleus is formed by endomitotic polyploidization (Grell, 1950b) and its division resembles the amitotic nuclear cleavage.

The process of amitosis

The simplest form of amitosis can be seen in the above-mentioned protozoans (Fig. 117). Without any apparent change in the nucleus or in the organelles of the cytoplasm, the nucleus moves to the centre of the cell, assumes first an oval, then an elongated shape and with the cleavage of the cell body, the nucleus too is divided into two apparently equal parts; two new individuals have been generated, evidently in the simplest possible way.

In many cases, the division of the nucleolus seemed to precede the amitotic cleavage of the

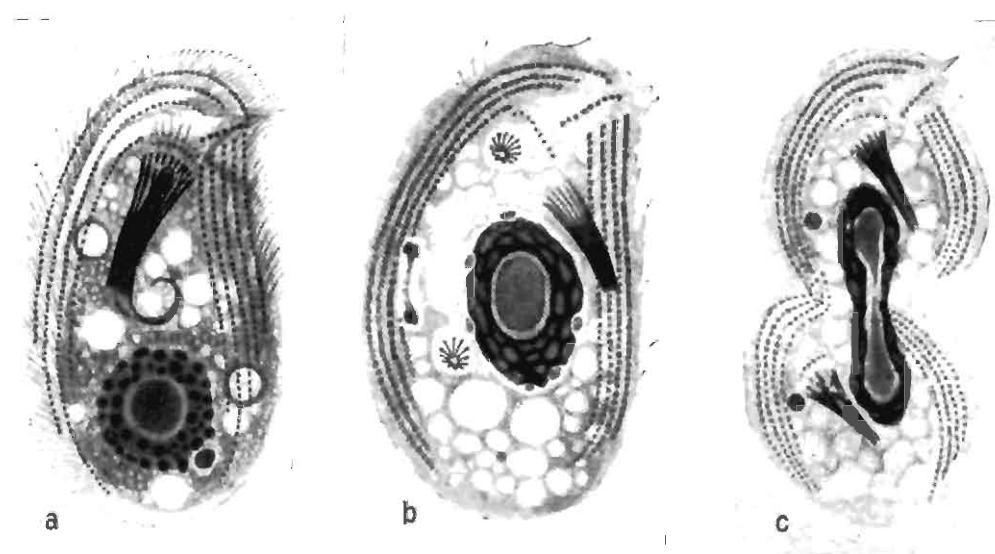


Fig. 117. Division of the macronucleus of a ciliate. (From Belar, K. (1926), *Erg. Zool.*, 6, 235-265.)

nucleus, and the division of the nucleoli have often been considered as the beginning of amitosis (e.g., Körner, 1935; Weed, 1937; Burkl, 1949). This was observed also many a time in tissue culture (e.g., Wendt, 1960) and indeed, in such organs as the mammalian liver, there are always nuclei with two nucleoli present, which do seem to be the signs of a certain bilateral symmetry, as if the nucleus was preparing here to divide into two halves. Of course, this remains as yet an "as if" statement.

On the other hand, nuclei which seem to prepare for an amitotic cleavage are always quite large, larger than the others, and they generally contain several nucleoli, often more than seven of them in the liver of rodents (Phan and David, 1958). As we may infer from the size of the nuclei and their DNA content, these nuclei are diploid. This observation, viz., that nuclei at the beginning of an amitosis are large and often rich in nucleoli, is also true of the inferior organisms, such as, for example, various forms of *Euglena* (Leedale, 1959b).

The behaviour of the nuclear chromatin during amitosis has previously been a frequent object of study, mainly because investigators hoped to elucidate from this the mechanism of amitosis. Thus a distinction was made between two types of mechanisms, "distraction" and "dissection" of the nucleus (v. Wasielewski, 1903, 1904). According to the former theory, the nuclear material gathers in two opposite areas of the nucleus; both areas then move away from each other while the bridge of material connecting them becomes narrower and narrower and finally breaks. In contrast to this, "dissection" would be the direct cleavage, vertically to the longitudinal axis, of the somewhat elongated nucleus, as is frequently the case, for example, of the nuclei of certain fungi, which divide amitotically (Robinow, 1957; Schmid, 1958).

It is true that the liver of mammals shows two different kinds of amitotic nuclear division (Grundmann and Pfeifer, 1963). The first starts with the two nucleoli assuming a position opposite to each other (Fig. 118a), while a lighter area appears in the centre between both of them. From the nuclear envelope, threadlike structures emerge, enhancing the impression of bilateral symmetry, since they arise midway between the nucleolar poles and radiate to the nucleoli (Fig. 118b and c). The bilateral symmetry is even more marked in those nuclei which show a median constriction, and here, too, a translucency of the nuclear substance may be recognized in the centre, in the area of the cleavage furrow (Fig. 118d-f). The other kind of nuclear division has a median division line, which bears some resemblance to the "cell plate" in cytokinesis; this plate has two layers which separate, thus dividing the nucleus (Fig. 119). The first kind of division would be a "distraction", the second a "dissection", according to Von Wasielewski's nomenclature (1903, 1904).

Of course, in all such classifications, extreme caution is required. For, on principle, this process may occur in the reverse direction, thus representing a nuclear fusion, which certainly exists. Moreover, the shape and the inner structures of the nucleus transform themselves quite markedly when the nuclear function is intensified, and yet, no direct nuclear division is involved.

This applies particularly to the many descriptions and photographs which show deep unilateral constrictions, mostly in the area of a central nucleolus, dividing the nucleus into two segments, which look most convincingly like two separate nuclei, according to the level of the section or observation. These pictures are found in tissue cultures, often in tumour cells (Atsumi, 1953; Homann, 1955) or in blood smears for instance of human acute leucosis,

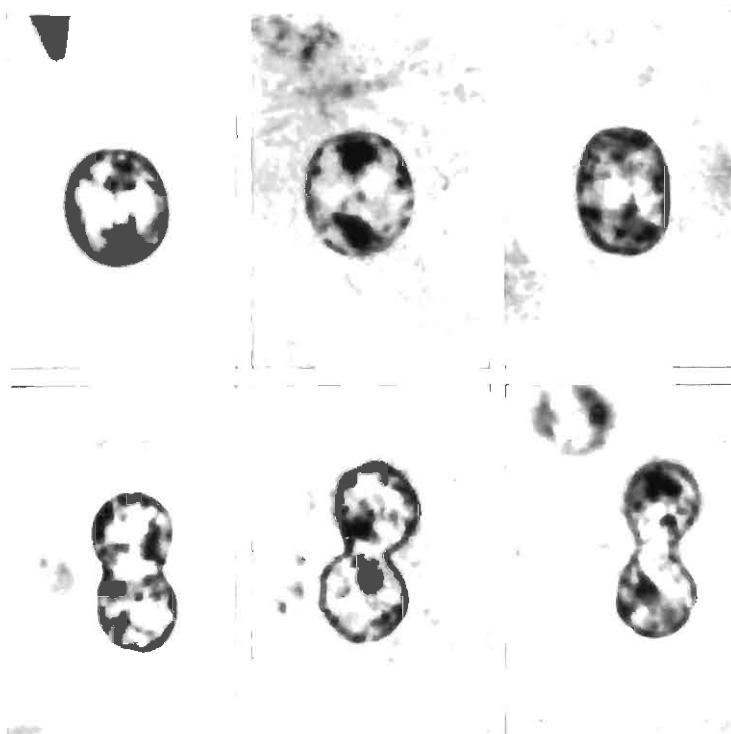


Fig. 118. Nuclei from a human liver in haemolytic anaemia. *Upper row*: bipolar orientation of the nucleoli and of the fibrillous chromatin. *Bottom row*: Micrographs of a nuclear cleavage. Form I of the amitotic nuclear division. Haematoxylin eosin. (From U. Pfeifer (1963), *Med. Diss.*, Freiburg.)

where there is no amitosis present, but functional forms of "nuclear polymorphism" (Gross *et al.*, 1962), which have been taken more than once for amitoses (Feyrter, 1960, 1961). In tissue cultures such deep furrows may also disappear even after hours or days, without a nuclear cleavage having ever taken place (Bucher, 1959). On the other hand, deep functional

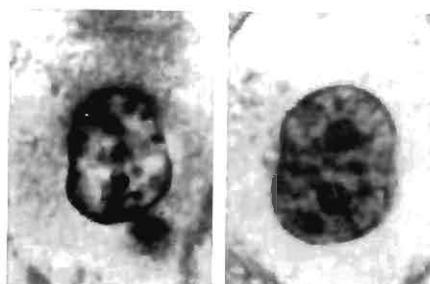


Fig. 119. Formation of a separative median line in nuclei of human liver cells. Form II of the amitotic nuclear division. Haematoxylin eosin. (From U. Pfeifer (1963), *Med. Diss.*, Freiburg).

constrictions in the area of the nucleolus may in some cases lead to a division of the nucleus, as for instance in certain types of ganglion cells (Correia, 1960; Müller, 1961a, 1961b).

Assumptions concerning the mechanism of amitosis

In the last above-mentioned instances, the mechanism is relatively easy to trace down. The depression reaches down to one of the nucleoli directly, and after the latter has "opened", there is only a narrow nuclear bridge left, which can then be divided. The only question is whether these constrictions and cleavages can really be interpreted as authentic amitoses, i.e., as simplified analogues of mitosis. And this mechanism certainly does not explain the typical amitoses, be it "distraction" or "dissection"; we can even say that the mechanism of amitosis cannot as yet be validly explained at all. Yet, it has, no doubt, some basic points in common with mitosis; from our observations described above (Fig. 118), the nuclear substance, that is, the chromosomal material, is divided into two halves and stored at both poles. This is, in a more simple form, the process of mitosis and why should not the mechanism here be essentially the same?

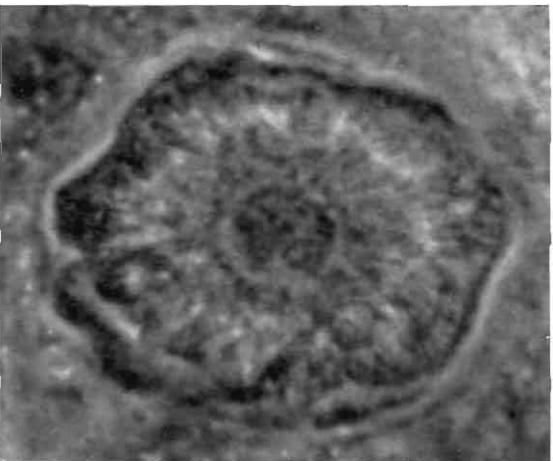
Let us consider the process more closely. In mitosis, the chromosomes are individualized, well-ordered and may be moved in opposite directions by means of the spindle fibres. In amitosis, there is no individualization and no order of the chromosomes nor are there any karyokinetic accessory structures. And yet, there seems to be some kind of order, viz., when the enlarged, generally polyploid nucleus stretches itself, one notices a bilateral symmetry of the chromatin including the nucleoli (Fig. 118, top), as if the chromosome sets withdrew from each other without completely breaking up the connection.

In one of the protozoan ciliates, whose macronucleus divides amitotically (see above), it was recently possible to analyse a process of division more closely. Kaneda (1960, 1961) found faintly visible "chromosomes" which order themselves into a kind of metaphase plate and execute an anaphase movement (Fig. 120b and c). There is no formation of any spindle. But, from the large interior body of the macronucleus (see Fig. 120a), there emerge threadlike bands, which insert themselves between the "chromosomes" and form, in addition, polar caps (Fig. 120d-f).

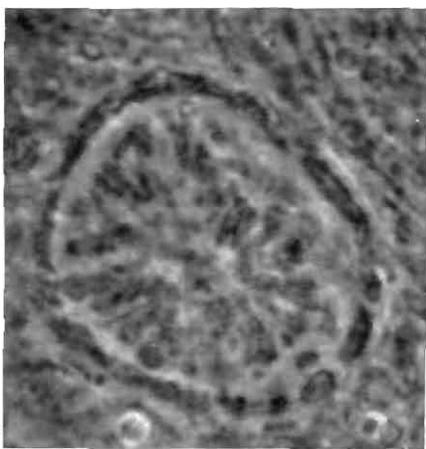
There are two ways of interpreting this whole process. On one hand, it looks as if the division of the ciliate macronucleus can no longer be regarded as an amitosis but proves to be a variation of mitosis. On the other hand, one may see in this division an example of other forms of amitosis. For, in the same way as all kinds of intermediary stages exist especially in protists, from the full typical mitosis to the concealed form of mitosis with a hardly visible spindle and intranuclear centrosomes (see Belar, 1926), so intermediary forms are also possible between the above-described division of a ciliate's macronucleus (Fig. 120) and the amitoses of liver cells (see Fig. 118). Perhaps there is an intranuclear centrosome mechanism in the amitoses of mammalian tissues, as in certain protists (Belar, 1926). This would, too, make an identical division of the daughter nuclei plausible.

No doubt it is an important fact that the nuclei which are about to undergo amitosis are usually polyploid. If amitosis is to be analogous to karyokinesis, one must expect at least that in amitosis whole genomes are sorted out. And indeed, several facts suggest such a "genome segregation" (Grell, 1950b). In the reproduction cysts of *Colpoda steinii*, each

(c)



(b)



(a)

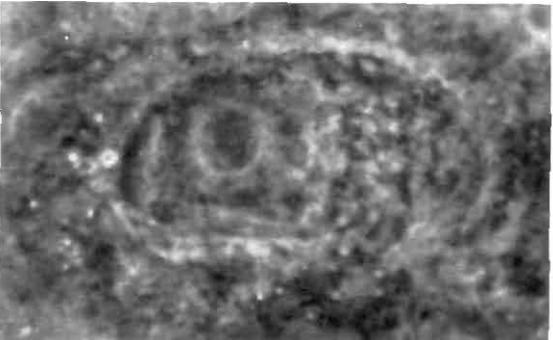
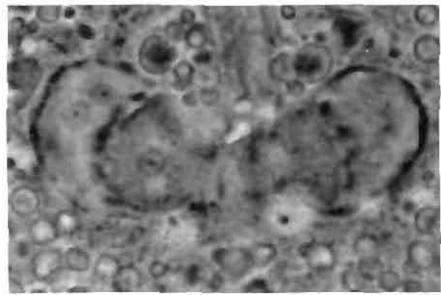
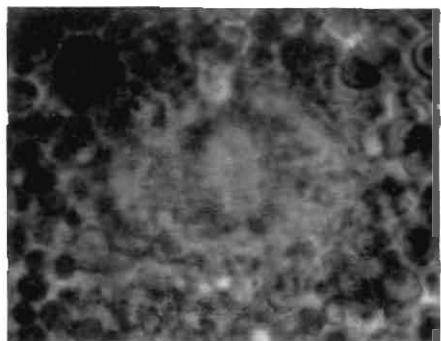


Fig. 120. Phase contrast micrograph of the ciliate *Chlamydomonas pedemus*, showing the division of the macronucleus. $\times 1000-1500$. (a) Interphase. (b) Formation of chromonema-like threads in a radial array, evidently corresponding to metaphase. (c) Cytolysis caused by adjunction of distilled water results in the conglomeration of the chromonemata into chromosome-like bundles. (d) and (e) Anaphase in different phase contrasts; the chromonemata are ordered vertically to the division plane. (f) Telophase and separation of both groups of chromonemata.

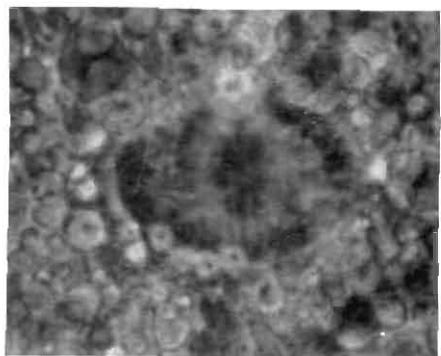
(From Kaneda, M. (1960, *Jap. J. Zool.*, 12, 477-491.)



(f)



(d)



(e)

single macronucleus divides into eight parts, which enter the four daughter animals two by two and build in each of them one daughter nucleus (Piekarski, 1939b). In the detached living *Dactylophryga* stages of a parasitic suctarium, the macronucleus is divided by successively pinched-off nuclear buds into macronuclei of daughter cells, which are morphologically and functionally absolutely identical with each other (Grell, 1950a). Each daughter nucleus contains at least one whole genome. The mother nucleus was, consequently, of a high degree of ploidy and the successive division into four daughter cells (p. 166) must be preceded by a segregation of the genomes. Probably, the elaboration of nuclear buds in certain yeast cells relies on a process which is essentially the same (Hashimoto *et al.*, 1958), especially since the nuclear constriction is independent from the cleavage of the cell body.

The condition required for such a "genome segregation" is that each single nuclear bud really contains a whole genome or a number of genomes; in other words, that the single genomes have been previously separated from each other. Such a "genome segregation" (Bauer, 1943) has been actually demonstrated, in rat liver which had also provided evidence for the occurrence of amitotic nuclear divisions (Grundmann and Bach, 1960). There, the separation of two or several genomes from one another is possible, by rearrangement of the chromosomes within the nucleus (Gläss, 1957). Fig. 121 shows that this genome segregation may occur as early as prophase and not always only after the prometaphase rearrangement. Moreover, for this prophase, the objection is not valid (Stern, 1958) that the "genome segregation" is a mere crushing artifact, especially since such an objection could be refuted in model experiments (Gläss, 1961a, 1961b). A nucleus which has undergone such a genome segregation has gone already a decisive step in an amitotic process.

Fig. 121. Diploid prophase of the rat liver showing segregation of a genome into two haploid chromosome sets. (From Gläss, E. (1957), *Chromosoma*, 8, 468-492.)



Result of an amitotic nuclear division

If the daughter nuclei contain the material from whole genomes, amitosis is equal and its result corresponds exactly to that of mitotic karyokinesis. But the question may be inverted, as follows: if amitosis, at least in the typical cases, does entail an equal division of the nuclear material, then it must rely on or be preceded by a process corresponding to the "genome segregation".

The simplest method seems to be the measuring of the nuclear volume. Are both daughter nuclei of equal size? If the question is easy to ask, a clear answer is much more difficult to

formulate. One must first make sure that the measured pairs of nuclei actually come from amitotic nuclear divisions. At this point we can very often only form conjectures; this is the case with most measurements of nuclear sizes on fixed and stained preparations as, for instance, on binuclear liver cells, where equal nuclear volumes may be usually found (Münzer, 1923; Jacobij, 1925; Clara, 1930, 1931; Bucher and Délèze, 1955; and others).

However, nuclear volume is the result of many factors (p. 89) and the quantity of genetic material, which alone determines whether the divided parts are equal or not, is only one of

these factors. Since we know today that DNA is the main substance in the genetic material and since the DNA in each nucleus can be measured by cytophotometry, it has been possible to tackle the problem of equal or unequal amitosis by this method.

The result as we have it now is quite clear; the typical amitotic nuclear division of the mammalian liver, which we are discussing here, is equal, at least to the same extent as mitotic karyokinesis (Grundmann and Pfeifer, 1963) (Fig. 122). In the macronucleus of the ciliate *Tetrahymena* too, it was found that the DNA contents after amitotic division were largely equal (McDonald, 1958).

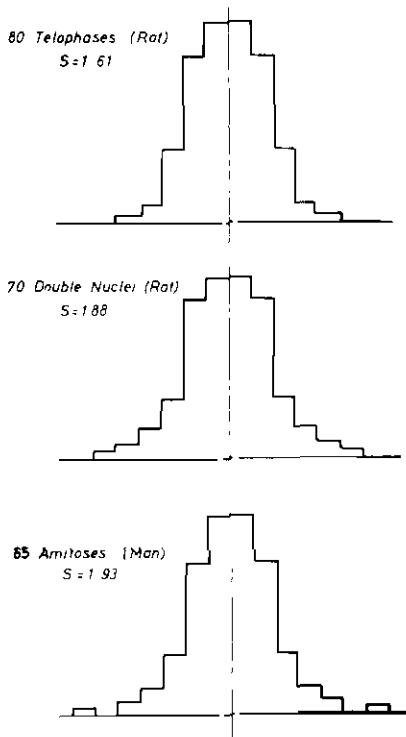


Fig. 122. Repartition of DNA content in the different liver cell nuclei. *Top*: In karyokinetic telophases. *Middle*: In binuclear epithelium cells during intermitosis. *Bottom*: During amitotic nuclear division. (From U. Pfeifer (1963), *Med. Diss., Freiburg*.)

There is, however, an essential difference between mitosis and amitosis. Whereas mitosis is directly followed, at least in most cases, by the division of the cell body (p. 166), in amitosis, division of the nucleus and cleavage of the cell are clearly dissociated. From many observations on fixed preparations (e.g., Ries, 1932; Graupner and Fischer, 1935; Clara, 1930; Leedale, 1959b; and others) and on living tissue cultures (Lewis, 1927; Bucher, 1958b; Wendt, 1959; and others), we see that nuclei generated amitotically often remain in one single cell body. This cell body, as was observed over a long period of several weeks, does not divide, even though sometimes there appear depressions in the cell wall or signs of a cleavage (e.g., Gey *et al.*, 1954).

By contrast, in the ciliates, division of the cell body follows amitotic cell cleavage directly

(Figs. 117 and 120). The case is the same with the amitotic cell cleavages in *Tachyblaston ephelotensis* (Grell, 1950a). It has been even possible to infer it indirectly, by calculations, from rat liver after partial hepatectomy (Grundmann and Bach, 1950; Busanny-Caspari, 1961) and in experimental carcinogenesis (Grundmann, 1954; Grundmann and Sieburg, 1962).

Nevertheless, the fact still remains that most amitoses in mammalian tissues produce binuclear cells. This is, at least temporarily, the typical result of an amitosis, and in consequence we speak of "amitotic nuclear division". By contrast, the regular result of a mitosis is the formation of two identical daughter cells.

Conditions of amitosis and their assumed significance

Accordingly, both processes have a different significance. For the purpose of mitosis is exclusively cell reproduction, growth; that of amitosis is presumably a different one. But in order to determine this, let us first investigate the circumstances under which amitotic nuclear divisions may appear.

Let us refer to the evidence for amitosis exposed above; for, such statements are only justified when it has been demonstrated to a fair degree of probability that these nuclear divisions are authentically amitotic. We had solid evidence for the occurrence of amitotic nuclear division directly following partial hepatectomy; the number of micronuclei increases, yet no mitosis has occurred (Grundmann and Bach, 1960; Busanny-Caspari, 1961). The case is apparently the same in the early regeneration of the kidney tubules after ischaemia (Cain, 1961).

Partial hepatectomy has three direct consequences for the single cell in the remaining third of the liver. First of all, for the immediate restoration of the parenchyma, the lost cells must be replaced, evidently by means of division of the remaining cells. Secondly, this serious operation causes considerable disturbance of the cell's metabolism, probably induced by resorption, and accompanied by hydration, massive fatty degeneration and many necroses (Grundmann and Bach, 1960; Ohlert, Häammerling and Büchner, 1962). Thirdly, the rest of the liver must engage in an increased metabolic activity, since the parenchyma is markedly reduced. In consequence, amitosis might be interpreted as an early phase of regeneration. However, it might also be the sign of a damage to the cell or of an increased strain on the cell's metabolism, and would thus have the purpose of raising the metabolic capacity of the nucleus.

Amitosis is certainly rightly interpreted as "division amitosis" (Benninghoff, 1922), i.e., as a phenomenon of growth, when it produces new unicellular individuals as, for instance, in the ciliates (Figs. 117 and 120). All the other observations may be interpreted in another way. It is beyond doubt that the postnatal phase in the growth of the human heart, which seems to proceed amitotically (Linzbach, 1955), is still a real growth and the nuclear constrictions, for instance, observed by Nieth (1949) in the heart of hypertensives, indicate an increase in the cell's substance. The significant increase in the rate of occurrence of double nuclei without any mitoses in the vicinity of hypoxic necroses in the cat heart (Grundmann, 1950) looks like the beginning of regeneration, even though unsuccessful. However, this very failure incites us to caution in the interpretation of amitosis as a general process of growth.

We only need to remember the problems discussed earlier, when we noted that in mammalian tissues, amitosis often remains without any cell cleavage and produces cells with two nuclei.

Amitosis as a process of growth, as a "division amitosis" (Benninghoff, 1922), as a "growth amitosis" (Clara, 1930) or as a "generative amitosis" (Bucher, 1959) occurs, as we noted, only in certain cases. Amitosis as a consequence of cell damage, or "degenerative amitosis", is possible in many cases, but lacks confirmative evidence. Thus, we are left with the third of the above-mentioned possibilities, namely, amitosis as the consequence of an increased metabolism of the cell.

General evidence for this is suggested by the observation that the frequency of occurrence of the double nuclei in the mouse liver depends on nutrition (Münzer, 1925; Phan and David, 1958). Excessive feeding of fat or protein (Noel, 1923; Schröter, 1937) as well as exclusive sugar diet (Wermel and Szinewa, 1934) raise the frequency of binuclear cells. In the mouse kidney with trypan blue it is possible to exert a strain on the function of the epithelium in proximal convoluted tubules and, thus, increase the number of binuclear cells there (Bucher and Gailloud, 1958). One may notice a similar increase in the cells' activity in the fibres of the human heart muscle directly after birth or in the case of a functional hypertrophy, where amitoses have been observed (Linzbach, 1947, 1955; Nieth, 1949; Hort, 1953; and others).

We have even stronger evidence. The follicle epithelial cells in the ovary of the louse and other insects divide mitotically only as long as the epithelium does not secrete. With polar differentiation and the beginning of secretion, the mitoses cease and amitotic cell divisions start taking place (Ries and van Weel, 1934; Fischer, 1936; Stefani, 1955). We could mention many similar observations. They all confirm this striking synchronization, viz., at the beginning of an intense activity of the cell, mitoses disappear and are replaced by amitoses; as if, under these conditions, the cell could not afford the degradation and rearrangement of functional structures in nucleus and cytoplasm, which a mitosis entails, and therefore turned to amitosis, which enables the cell to continue its activity. This antagonism between the cellular functions and mitosis and their close relationship to amitosis have been repeatedly emphasized for the last 70 years (Ziegler, 1891; Peter, 1940, 1947; Jacobj, 1942; Bucher, 1959). Thus the origin of the term "reaction amitosis" (Benninghoff, 1922), used to characterize amitosis as a reaction of the nucleus to a functional overstraining.

Further, observations on insect ovaries show that cell reproduction ceases when mitosis ceases; for the number of cells remains constant after secretion has begun, despite the amitotic nuclear divisions (Fischer, 1936). If we disregard the possibility that in other cases a cell cleavage may, after all, follow amitosis, we see in this particularly favourable example a clear difference between indirect and direct type of division; the first is "generative", the latter "reactive". It is consequently of no great importance whether one lays the stress on the increase in the surface area for metabolic exchanges between nucleus and cytoplasm, as is usually done (Flemming, 1891a; Conklin, 1917b; Benninghoff, 1922; Wassermann, 1929; Bucher and Gattiker, 1954; and others), or whether one takes into account the shortening and normalization of intranuclear transportation pathways which are liable to be abnormal in polyploid nuclei; the principle is the same.

Another fact is certain, namely, the close relationship between amitotic nuclear division and endomitotic polyploidization (Geitler, 1941; Grundmann, 1954; Gläss, 1957; Grund-

mann and Bach, 1960; Hobik and Grundmann, 1962; and others). Higher degrees of ploidy gained by endomitosis may be again reduced by an amitotic nuclear division. This mainly happens in the liver, where the degree of ploidy of the nuclei can be varied with particular ease. The newborn mammalian has exclusively diploid nuclei and polyploidizations appear after birth with the beginning of functional stimulation (Fig. 113). Aggravation of this stimulation, for instance by toxic substances, like carcinogenic agents, increases the polyploidy (Grundmann, 1954, 1961*b*) but at the same time creates favourable conditions for an amitotic nuclear division. When the functional stress diminishes, the conditions become normal again. In this whole process, in which the functional strain intensifies the regulation of the nuclear mass of the whole parenchyma, the constructive phase occurs via endomitosis, the degenerative mainly via amitotic nuclear division. It is not mitosis but endomitosis which is the antagonist of amitotic nuclear division, although it is also in many cases the very prerequisite of amitosis.

Abnormal amitoses—nuclear polymorphism

We must exclude from this authentic type of amitotic nuclear division all those cases where a disturbed mitosis merely gives the illusion of an amitotic nuclear division, viz., the so-called "pseudoamitoses" (Häcker, 1900) or "pycnomitoses" (Pfuhl, 1938). The similarity between the amitotic nuclear division and these pathological mitoses may be great and it may be very difficult to distinguish them (Conklin, 1917*b*; Wassermann, 1929; Tahmisian, 1952; Kolb, 1959; Lettré and Siebs, 1962; Roizman and Schlueder-Berg, 1962); it is advisable, when the case is doubtful, to consider it to be a pathological mitosis.

On the other hand, amitosis in a larger sense includes all those manifestations such as nuclear budding, nuclear segmentation or fragmentation, whereby one or several evaginations of the nuclear envelope are progressively pinched off and remain ultimately connected with the main nucleus only by fine threads. When these threads break, small paranuclei are formed. Nuclear buds (Maximow, 1908) may in certain cases bring about the death of the pinched-off paranuclei (Lipp, 1952); they must, of course, be distinguished from the micronuclei which appear after pathological mitoses (p. 205). In the case of many buds, we speak of nuclear segmentation, a feature which can occur in normal, histologically specific types of nuclei, such as for instance in the granulocytes of man and of superior mammals. No doubt functional factors play a great part in all these aberrations, and one is tempted to connect them with an increase in nuclear surface area (Benninghoff, 1922), insofar as they are not the sign of a pathological degeneration.

The distinction is particularly difficult to establish when deep evaginations and constrictions give the nucleus such a polymorphic aspect that it completely loses its typical normal shape. Such morphological changes have been found many times in tissue cultures (Benninghoff, 1923) but mainly after fixation and staining, and were called nuclear polymorphism (Bucher, 1959; Feyrer, 1960, 1961). This term certainly includes the buds, lobes, segments and fragments and the manifold intermediary forms which justify the use of a common denomination. Although they occur under pathological circumstances in the cell's life, their formal genesis seems to be the same as that of real amitoses: viz., a strongly intensified cellular metabolism, that is liable to go on, in the cellular agony even after the death of the

organism (Feyrter, 1961). This most evidently classifies nuclear polymorphism (with all its different aspects) under amitotic nuclear division, even if as an abnormal variant. However, the expression nuclear polymorphism should be restricted to these pathological manifestations; for not every nuclear budding nor every nuclear fragmentation has this meaning.

Normal nuclear divisions may also be considered to take one of two forms, meroamitosis and karyonomy. The former is said to consist of the segregation of a small nuclear bud, which is pinched off the mother cell together with the corresponding piece of cytoplasm (Thomas, 1935, 1938); this apparently only occurs in tissue cultures (Feyrter, 1961). The meaning and existence of the latter, the process of karyonomy, is questionable (Feyrter, 1951, 1960, 1961); this term is used to describe the observed fact that in reticular cells small pieces of the nucleus are chipped off and shifted peripherally into cell processes, where they remain. Finally, a critical re-examination is necessary of what has been boldly called "endocytogenesis" (Collin, 1924), that is, the elaboration, within the cytoplasm of the mother cell, of a new cell from an amitotically divided nucleus. Contrarily to all accepted views on the differentiation of tissues, this process is thought to produce new types of cells, such as for instance endocrine pancreas cells from exocrine (Florentin and Picard, 1936) or thymus lymphocytes from reticular cells (Törö, 1955, 1962).

Review

From all of the foregoing, we are not surprised to find that, in the course of these hundred years that the process has been known, amitosis has progressively lost its good reputation. Its interpretation has undergone a multiplicity of variations and has been at fault right from the beginning, since it was based on a negation, viz., that of being no mitosis. The concept embraces many single phenomena. Some types of amitoses have a generative character, as for example the amitoses of ciliates and suctoria. Most types of amitoses are reactive processes to an intense functional strain exerted on the cell and their purpose is to provide a better and easier activity of the nucleus. This classifies amitosis not among the proliferative processes, but among the functional manifestations of the cell, like endomitosis, which is the opposite process. However, the mechanism of the amitotic nuclear division seems to be, after all, closely related to mitotic karyokinesis, even though it operates for a great part below the limits of what can be observed today.

4. The Cytoplasm

Following the basic concept of this book, which is to try and understand the functions of the different parts of the cell from their structure and chemical composition, we can provide much clearer data in this chapter than in our previous discussions. The modern methods of preparation in biochemistry and the triumph of electron microscopy as a cytological procedure have given us most clear insights into the structure and functions of the cytoplasmic elements. We may consider today many problems as solved. However, the limitations of such solutions will be readily evident in the course of our discussion; we must also constantly bear in mind that these solutions rely on specific methods and that new methods are liable to bring new findings which may lead us, in turn, to consider today's acquisitions as obsolete. Even electron microscopy, which has profoundly renewed morphological investigations of the cytoplasm by the results it has been giving for the last 12 years, will be subject to the same evolution. For we cannot visualize any structures without using certain methods of preparation; the living state of the cell remains, at least to this date, far beyond what we can observe in the electron microscope. We must basically content ourselves, as in light microscopy, with representations equivalent to the living state.

Biochemical analyses, too, give only equivalents. Biochemistry has also been accumulating data during the past 10 years and is gaining us quite a solid knowledge in many fields. However, this knowledge, too, is subject to a rapid evolution, mainly because the methods here are often even more differentiated and because the dynamic processes of metabolism imply so many possible variants. The last 30 years have brought more than one example of the frequent changes that affect theories and concepts; this goes to show how wrong it is to consider today's interpretations as absolute.

The cytoplasm is a structure composed of a more or less homogeneous cytoplasmic *ground substance*, which contains the *mitochondria* and, in plant cells, also the *plastids*; it is traversed by a system of tubules and vesicles, the *endoplasmic reticulum*, which forms in certain areas of the cytoplasm a specific structure, the *Golgi apparatus*. Let us consider now separately each of these parts of the cytoplasm.

THE ENDOPLASMIC RETICULUM

We mentioned already that this denomination is not quite adequate; for it is, strictly speaking, no reticulum. This term dates back to the early stages of electron microscopical cytology. Porter *et al.* (1945, see also Porter and Thompson, 1947) found in thin sections through tissue-cultured macrophages a network of fine vesicles with multiple anastomoses. The vesicles of this reticulum were considered to be equivalents of the "microsomes" which Claude (1938, 1941) and other investigators had isolated by centrifugation and recognized as RNA-containing elements.

The normal representation in the electron microscope

We are certain today that the endoplasmic reticulum consists of a system of fine canaliculi, which extends in principle through the whole cytoplasm and is connected in many cases with the extracellular space as well as with the perinuclear space (Fig. 1).

This is, however, a much simplified view. For this schema has been demonstrated only for certain types of cells, for instance in plasma cells, where we find a direct connection with the extracellular space through pores in the cell wall (Fig. 123). Moreover, the canaliculi certainly do not form an absolutely continuous system in all cases. Insofar as the thin-sectioning technique allows any statement at all, we must assume that some vesicles remain at least isolated over a longer or shorter period of time (Porter, 1961).

On the other hand, we may be quite certain today that the endoplasmic reticulum is an essential part of the cell. It is to be found not only in all mammalian cells (ref., e.g., Haguenau, 1958), except for the non-nucleate erythrocytes and thrombocytes, but also in plant cells (Hodge, 1956; Whaley *et al.*, 1959; Porter and Machado, 1960; and others) including yeast (Hashimoto *et al.*, 1960) and algae (Sager and Palade, 1957). Whether the vesicle- or lamellae-shaped folds which were found in the cell envelope of certain bacteria (Ryter *et al.*, 1958; Kellenberger *et al.*, 1958; Glauert and Hopwood, 1960) may be interpreted as analogues to an endoplasmic reticulum, is still open to question.

For not all tubular or vesicular systems of the cytoplasm belong to the endoplasmic reticulum, and it is not always easy to establish a clear distinction. The endoplasmic reticulum must be distinguished from the structures of the Golgi apparatus (p. 256), which are generally gathered in a specific area; we must also discriminate it from the vesicular systems of the centriole and its centrosphere (p. 137); both these systems are, as a rule, free of endoplasmic reticulum. At the cell wall it is frequently impossible to separate it with certainty from pinocytotic vesicles (p. 282).

This uncertainty is due to the great many variations in shape of the endoplasmic reticulum. Sometimes it appears as vesicles or canaliculi of different length. In other cases there are lamellae lying in pairs behind each other like the sceneries of a theatre stage; but in cross-sections they have once again the appearance of canaliculi. The endoplasmic reticulum may also form sometimes a more or less well-ordered system of tubules. The diameter of the canaliculi and the size of the vesicles may vary. In liver cells, the double lamellae for example lie fairly close to each other (Fig. 4), thus leaving only a narrow space between them. In plasma cells, however, they build large sacs (Braunsteiner and Pakesch, 1955; Bernhard and Lepluis, 1955), filled with a material of a lesser electron density (Fig. 123).

Attached to the outside of the membranes of this endoplasmic reticulum, there often lie granules of a very constant size of 140–150 Å (Palade, 1955, 1956, 1958), at an equal distance of 150–450 Å or more from one another (Figs. 4, 123, 124). These granules were called after their discoverer "Palade granules". Their particularly high RNA content has been already demonstrated and they have therefore been named ribosomes (see for instance, Wendler-Deane and Porter, 1960). They are closely concerned with protein synthesis (p. 249) and are always present in large quantities in cells with an intense protein synthesis, i.e., mainly in liver cells and in glandular cells (see for instance Sjöstrand and Hanzon, 1954; Palade, 1958; Haguenau, 1958), but also in the ganglion cells of the central nervous system

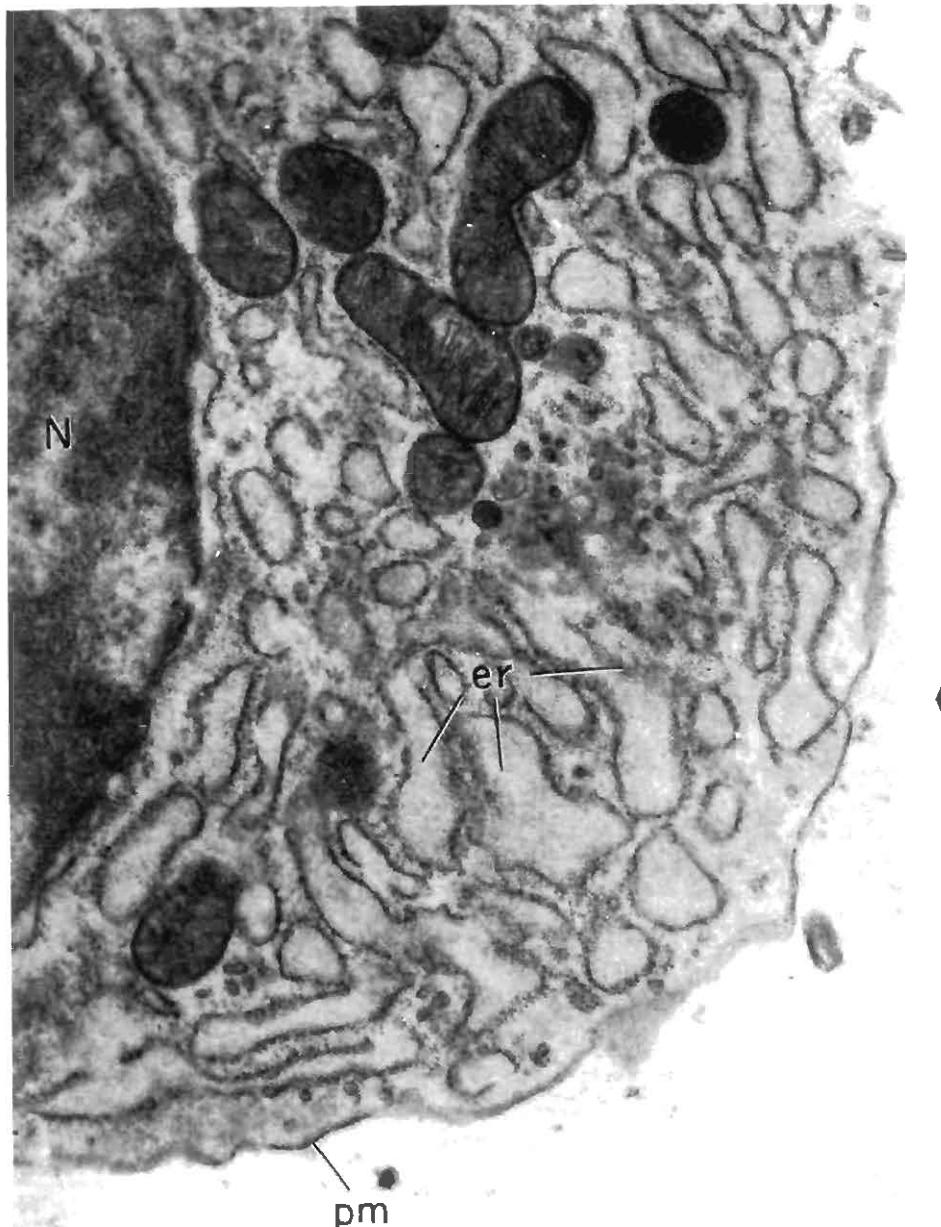


Fig. 123. Part of a plasma cell from an *Ambystoma* larva showing distended endoplasmic reticulum (er). Note that the plasma membrane (pm) extends directly into the endoplasmic reticulum at certain points indicated by the arrow. (From Porter, K. R. (1961), *The Cell*, Vol. II. Acad. Press, New York.)

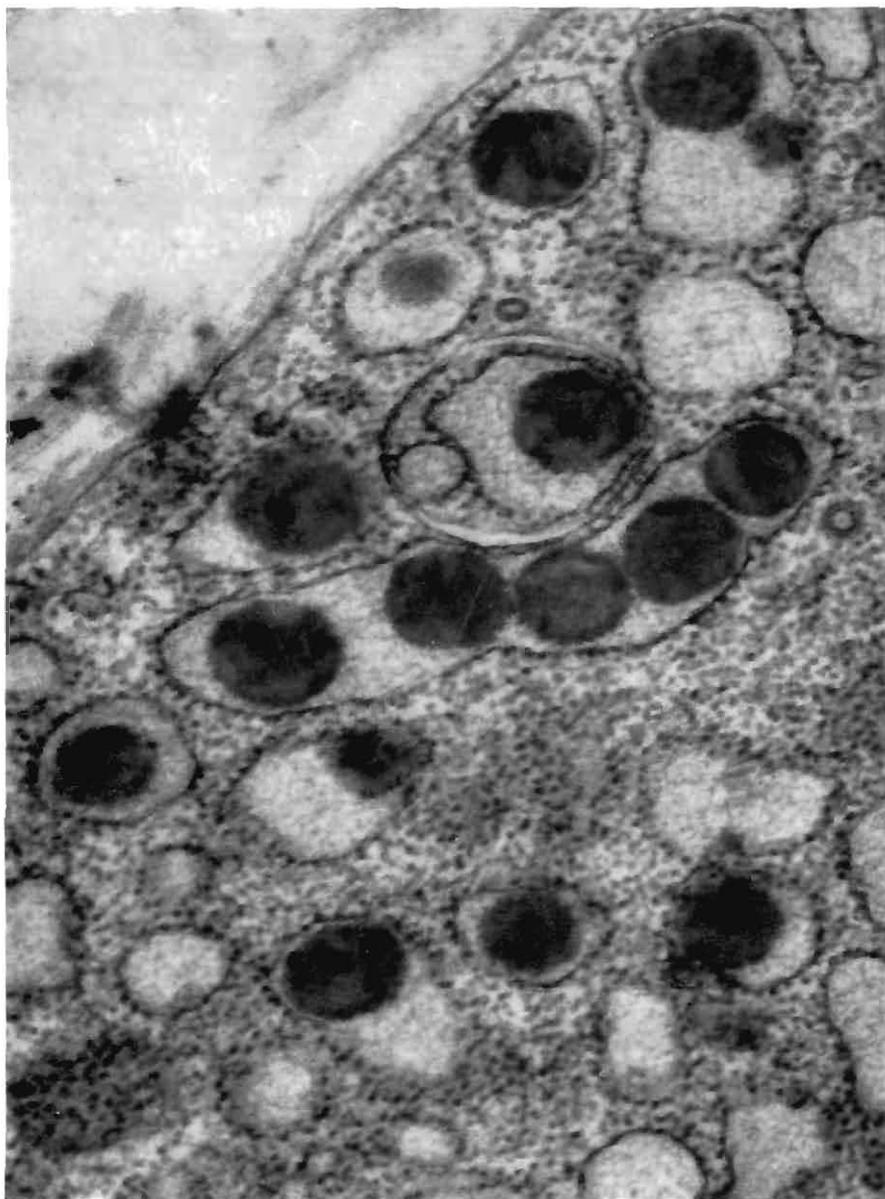


Fig. 124. Electron micrograph showing part of an exocrine cell from guinea-pig pancreas after 48 hours fasting, following 60 minutes of protein intake. Intracisternal granules can be seen in the distended endoplasmic reticulum, which is evenly settled with ribosomes. Note the presence of ribosomes also between the cisternae. $\times 70,000$. (Courtesy G. E. Palade.)

(see, for example, Hild, 1959; Niklowitz, 1962; and others). They do not lie exclusively against the membranes of the endoplasmic reticulum, they often fill the space between the vesicles and the canaliculi (Fig. 124). Consequently, they are ultimately elements of the cytoplasmic ground substance and not of the endoplasmic reticulum, for they only adhere to its outer surface and may detach themselves from it. From certain micrographs, one gains the impression that some of them even may be nothing but cross-sections of small threads coiled together (see, for instance, Wohlfahrt-Bottermann, 1959). Every time ribosomes are attached to the membranes of the endoplasmic reticulum, we speak of it as being rough-surfaced or granular.

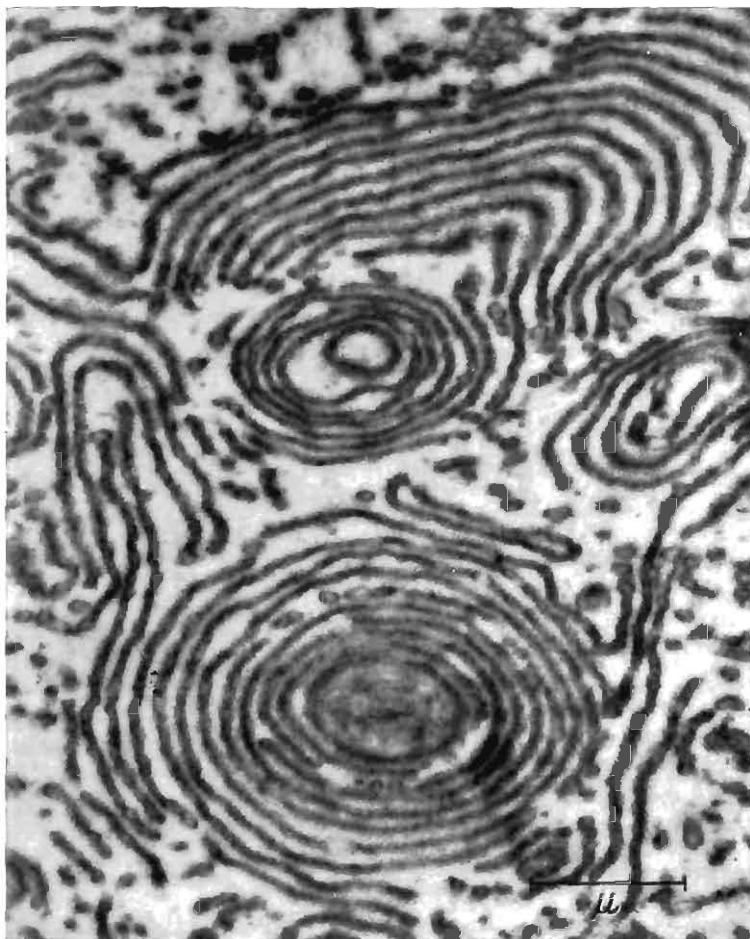


Fig. 125. Salivary gland cell from a *Chironomus* larva showing the concentric layers of lamellae of the smooth-surfaced endoplasmic reticulum and the concurrent formation of a paranucleus. (From Bernhard, W. et al. (1954), *Arch. Anat. Mikrosk.*, 43, 236.)

By contrast, the smooth-surfaced or agranular type of endoplasmic reticulum is free of ribosomes. Some cells have only this type, as is the case of the interstitial cells of the opossum testis, the striated muscles (Porter and Palade, 1957), many plant cells (see, e.g., Porter and Machado, 1960) or the pigment epithelium of the frog retina. In mammalian cells, rough-surfaced and smooth-surfaced components of the endoplasmic reticulum lie next to each other. In certain cells, the smooth-surfaced type is made up of concentric layers and forms more or less compact pellets, composed of lamellae arranged like the peels of an onion (Fig. 125). When still larger, granular or vesicular elements are found within these corpuscles they correspond morphologically to a large extent to the paranuclei (Nussbaum, 1882) described mainly in the pituitary gland but also in many other types of glandular cells (ref. see Haguenau, 1958; Altmann and Osterland, 1961; and others). To distinguish these types of corpuscles from other structures bearing the same name, they are called endoplasmic paranuclei (Haguenau, 1958). They may be found in the liver following a chronic injury of the cell, such as for example in experimental hepatomas of the rat (Fawcett and Ito, 1958) or after administration of thioacetamide (Altmann and Osterland, 1961; Thoenes and Bannasch, 1962) (Fig. 126). There is also another corpuscular aggregation of elements of the smooth-surfaced endoplasmic reticulum, namely, the myeloid elements in the pigment epithelium of the retina (Yamada, 1958; Porter and Yamada, 1960). Here too, the lamellae lie parallel and very close to one another. Possibly these myeloid structures play a part in the functioning of the retina.

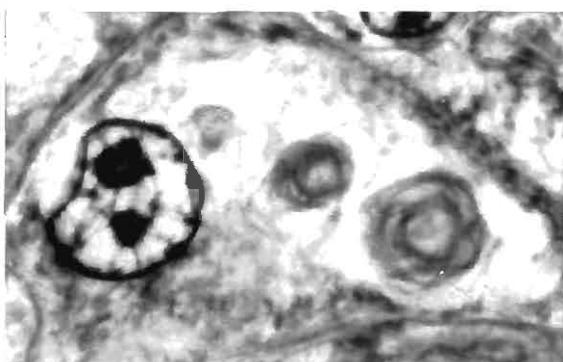


Fig. 126. Endoplasmic "paranucleus" in a rat liver after thioacetamide treatment. (Courtesy H.-W. Altmann.)

To a certain extent, shape and structure of the endoplasmic reticulum are, consequently, characteristic of each type of cell; cells having similar functions, as for instance glandular cells or other cells with high protein synthesis, have many canaliculi or lamellae with many ribosomes. Some have vesicles of various sizes, whereas others have a dense system of smooth-surfaced tubules. From this polymorphism, it is difficult to find a characteristic common to all which would enable us to establish a suitable morphological definition. The thickness of the membranes, averaging $5 \text{ m}\mu$, is the same in other cytoplasmic vesicles, such as in the Golgi complex, whose membranes were termed by Sjöstrand (1956) γ -cytomembranes to distinguish them from the α -cytomembranes of the endoplasmic reticulum and the β -cytomembranes of the cell walls.

The most constant criterion is their relation to the nuclear envelope. The perinuclear space (Fig. 1) is a part of this system of vesicles and tubules and one may even define the

endoplasmic reticulum, on the other hand, as an intracytoplasmic system, which is attached to the nuclear envelope (Porter, 1961). However, it also exists without there being a nuclear envelope, for instance during karyokinesis (p. 183).

The ergastoplasm and its functional and pathological changes

At the end of the last century, Garnier (1899) observed cytoplasmic filaments in the cells of exocrine glands, situated mostly at the base of the cell, which stained readily with basic stains and were closely connected with the production of the secretion granules. Garnier (1899) termed these basophilic basal filaments ergastoplasm, i.e., the plasma that produces something. It could be found as a rule in the cells of all exocrine glands and was easily recognized by its strong basophilia and its lamellar structure; the latter may be seen also in unstained preparations after dehydration in an acid medium (Ries, 1935) or in the phase contrast microscope (Rose and Pomerat, 1960). The electron microscope reveals it to be the rough-surfaced type of endoplasmic reticulum (for ref., see, e.g., Haguenaou, 1958; Kurosumi, 1961; and others), and it is frequently the case that all basophilic structures of the cytoplasm and all sections of the rough-surfaced endoplasmic reticulum are referred to as ergastoplasm.

The basophilic cytoplasmic structures vary a great deal already at the level of the light microscope and this variability is directly linked with the cell function. For example, the Nissl bodies of the ganglion cells, which are the cytochemical and morphological equivalents to the ergastoplasm, are, under functional stress, transported to the border of the cell and partly dissolved, and it makes no difference whether these cells are motor, sensory or vegetative ganglion cells (ref. see Altmann, 1955a; Attardi, 1957; Andres, 1961; and others). During the retrograde degeneration which follows the bisection of the axon, such a tigrolysis also occurs, i.e., a disintegration of the tigroid bodies (Nissl bodies) (e.g., Gersh and Bodian, 1943; Ortmann, 1952; Andres, 1961; and others). This process is generally called chromatolysis (Marinesco, 1896). It is concurrent with a decrease in the RNA and protein content (Hydén, 1943) and is the sign of a functional overstraining.

This may also be particularly well observed in the liver, especially in that of the rat and the mouse where dense basophilic areas are normally present in the cytoplasm, directly analogous to the Nissl bodies. A lack of protein in food is sufficient to make these bodies disappear (Stenram, 1952; Bharadwai and Love, 1959; David, 1959; and others). This chromatolysis is even more enhanced in all toxic liver injuries, whether they are caused by a typical liver cell poison such as carbon tetrachloride (Rosin and Doljanski, 1946; Stowell and Lee, 1950; Leduc and Wilson, 1958; and others) or by carcinogenic substances as dimethylaminoazobenzol (Opie, 1946, 1947; Grundmann, 1954; and others). Under exposure to alkylating diethylnitrosamine, it is possible to follow the process of chromatolysis step by step. The basophilic bodies leave the vicinity of the nucleus (Fig. 127, top right), are pushed gradually against the cell wall (Fig. 127, bottom left) and finally dissolve completely (Fig. 127, bottom right).

As can be seen in Fig. 127, chromatolysis often goes along with vacuolar changes in the cytoplasm, which mainly involve an alteration of the ground substance. Yet we see in the electron microscope that the endoplasmic reticulum is altered too; during the process of carcinogenesis in the rat liver cell under diethylnitrosamine, a vacuolation takes place in the

endoplasmic reticulum (Mölbert *et al.*, 1962). As the RNA content decreases in the cytoplasm (Hobik and Grundmann, 1962), the number of ribosomes diminishes and, under exposure to dimethylaminoazobenzol, the detachment of the ribosomes from the lamellae of the endoplasmic reticulum is the first reaction of the cell to the carcinogenic agent to be seen in the electron microscope (Porter and Bruni, 1959). Dimethylnitrosamine transforms the ergastoplasm in the same way (Emmelot and Benedetti, 1960).

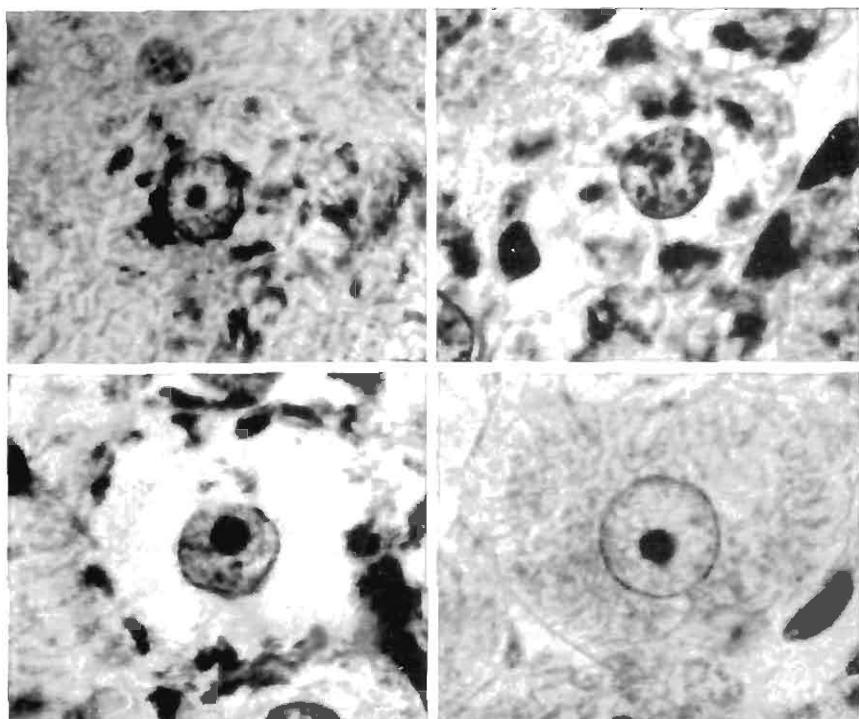


Fig. 127. Rat liver cells showing "chromatolysis" during the different stages of experimental carcinogenesis with diethylnitrosamine. Cresyl violet stain. $\times 1400$.

In all of the other above-mentioned chromatolyses, which are so different in respect to their origin, the electron microscope picture changes too. In extreme cases after aerogenic or metabolic hypoxia (Mölbert, 1957a; Büchner *et al.*, 1959; Hübner and Bernhard, 1961), intoxication by liver cell poisons such as carbon tetrachloride (Bassi, 1960), chloroform (C. Ruska, 1962) or thioacetamide (Thoenes and Bannasch, 1962), the ergastoplasm may largely disappear or turn into fine vacuoles. Roentgen irradiations in the mouse give basically the same picture (Braun, 1960). Consequently, what we have here is a sign that the cell has suffered an injury in its metabolism; in certain cases this might be an effect secondary to an insufficient energy supply, especially if one considers that the dissociation of respiration and phosphorylation brought about by thyroxine (Martius and Hess, 1951) may give basically

the same picture (Schulz *et al.*, 1956). In the rat pancreas, lack of exogenous protein induces dilatation of the endoplasmic reticulum (Weisblum *et al.*, 1962); this phenomenon is also particularly typical of experimental injury with ethionine (Seifert and Giesecking, 1961), which can induce saccular dilatation of the empty spaces between the endoplasmic canaliculi and partial dissolution of the membranes. Chromatogenesis after cessation of the damage often produces new lamellae fairly rapidly. In the shock-injured Purkinje cells of the rabbit cerebellum, for example, the reproduction of the membrane systems in the interlamellar space of the ergastoplasm plays the main role right from the beginning (Niklowitz, 1962) and we can observe a regular stacking up of membranes.

Protein synthesis

All of these morphological findings reflect functional alterations of the protein metabolism of the cell, for we know that the structures of the ergastoplasm or endoplasmic reticulum contain much RNA, and the investigations by Brachet (1941) and Caspersson (1941, 1950) have demonstrated the functional relationship between RNA and protein synthesis (p. 100).

Biochemical methods of investigation made it possible to gain for the first time clear evidence of this relationship; in his ultracentrifugation experiments (1938, 1941), Claude isolated from liver cells a fraction of small cytoplasmic particles, the microsomes. In this fraction were found proteins and a few phospholipids, but mainly a large amount of RNA. The identity of these microsomes with the basophilic structures of the ergastoplasm was readily demonstrated (ref. for example, Brachet, 1957, 1959a). Electron microscopic investigations of the microsomal fraction made it finally possible to establish its relationship to the elements of the endoplasmic reticulum; according to the type of embedding, the microsomal fraction contains vesicles, lamellae or granules (Chauveau *et al.*, 1955; Palade and Siekevitz, 1955), which could be identified as elements of the endoplasmic reticulum (Palade and Siekevitz, 1956; Novikoff, 1956). Attached to the membranes of the vesicles were found the typical 140–150 Å granules, analogous to the ribosomes of the rough-surfaced endoplasmic reticulum. They may be detached from the membranes and differentiated with desoxycholate (e.g., Hanzon and Toschi, 1959) and it now turns out that they contain a particularly large amount of RNA. In the rat liver, for example, one may isolate up to more than 60% of the whole cytoplasmic RNA content (Moulé *et al.*, 1960); the ribosomes contain up to 200 µg of RNA per mg of N (Chauveau *et al.*, 1962).

By contrast, the membranous lamellae of the endoplasmic reticulum are mainly composed of protein and phospholipids, but also contain large quantities of RNA, and this is apparently true of the membranes of the rough-surfaced as well as of those of the smooth-surfaced endoplasmic reticulum (Kuff *et al.*, 1956; Moulé *et al.*, 1960; Chauveau *et al.*, 1962). This could even be demonstrated *in situ* under the electron microscope; following exposure to hydrochloric or perchloric acid, ribosomes as well as membranes of the endoplasmic reticulum disappear and the picture in the electron microscope is reversed, for now, non-staining areas have appeared at the site of the endoplasmic reticulum (Bernhard and Leduc, 1960). Moreover, the protein component of the lamellae and membranes has a high specificity. This protein bears the antigenic property of the liver protein for example, partly as a constant feature, partly only temporarily (e.g., Vogt, 1960; Perlmann and Morgan, 1961).

The cytological conclusion drawn by Brachet (1941) and Caspersson (1941, 1950) that RNA and protein synthesis are closely linked together has been fully verified biochemically during the past 15 years, owing to the possibility of fractioning the cytoplasm by ultracentrifugal methods, which have become of increasing importance. The first clear evidence has been obtained with radioactively labelled amino-acids, which become visible after a very short time in the microsomal fraction, no matter whether the labelled amino-acids are administered *in vivo* or *in vitro* (Borsook *et al.*, 1949; Siekevitz and Palade, 1958; and others). The strongest reaction is located invariably in the ribosomes (Littlefield *et al.*, 1955; Siekevitz and Palade, 1960); in other words, where the RNA content is at its highest, there the incorporation of amino-acids is also the most intense.

Moreover, it was demonstrated that protein synthesis depends on the supply of energy. Evidence for this could be obtained *in vitro* by adding normally functioning mitochondria or ATP (Borsook *et al.*, 1950; Siekevitz, 1952); this, incidentally, confirms the old well-known observation, that proteins cannot form themselves from free amino-acids by merely reversing the process of proteolysis, but that energy is necessary to synthesize even the smaller peptides (Johnston and Bloch, 1951; Webster, 1953; and others).

The above-described incorporation of amino-acids into the microsomal fraction indicates a *de novo* synthesis of proteins. This explains that, for instance in bacteria with an exponential rate of growth, the incorporation of amino-acids proved irreversible (Rotman and Spiegelman, 1954; Hogness *et al.*, 1955). Tissues of plants and animals equally failed to bring any evidence of a mere exchange of amino-acids (Halvorson, 1953; Mandelstam, 1957), and, by combining radioautographic methods with the centrifugation technique, it was possible to demonstrate that newly-formed proteins always appear first in the ergastoplasm (Zalokar, 1960).

After having clarified these basic facts, investigators could then proceed to examine in greater detail the process of protein synthesis itself. Intracellular protein synthesis may be divided roughly into four stages (see, e.g., Zillig, 1958; Siebert, 1961; and others):

1. First, the amino-acids must be activated, i.e., they must be given a higher chemical potential.
2. The activated amino-acids are then transported to the site of synthesis.
3. There, synthesis of the peptide chains takes place.
4. The peptide chains must be detached from the site of synthesis and develop their typical three-dimensional folding, which is the sign of their specificity.

Let us consider each step separately.

1. The activation of the amino-acids occurs under the action of ATP and of activating enzymes found, after ultracentrifugation, in the supernatant of the microsomal fraction. To each amino-acid corresponds probably a specific activating enzyme, as could be demonstrated for instance with methionine in yeast (Berg, 1956) or with serine in the bovine pancreas (Webster and Davie, 1959). The structure of those activating enzymes is not yet fully elucidated. We know that they contain one SH group at an active site and that activations may produce thioesters of the amino-acids (Krimsky and Racker, 1952). ATP energy is made available not by a mere splitting of the phosphates with concurrent formation of ADP but by the elimination of inorganic pyrophosphates and the formation of AMP. Aminoacyl-adenylate emerges from the reaction. When produced synthetically, this substance forms free

amino-acids and ATP, in the presence of the activating enzyme and pyrophosphate (Holley, 1957); evidently, this is the reverse process of an activation of the amino-acids. The isolation of free aminoacyladenylate presents many difficulties (Hoagland *et al.*, 1956). It always appears closely bound to the activating enzymes and is more a prosthetic group of these enzymes rather than a real reaction product (Chantrenne, 1961). Free aminoacyladenylate would rapidly bring about non-ordered polypeptides; bound to the enzymes, it is protected from causing such reactions.

2. The transport of the activated amino-acids to the site of synthesis occurs via soluble RNA, which has a relatively low molecular weight of 20,000 to 40,000. Early investigations of this soluble RNA showed that it can bind different amino-acids. It is noteworthy that this binding is not a competitive but an additive process, which led to the conclusion that here, as in the case of the activating enzymes, a specificity exists between each amino-acid and its corresponding type of soluble RNA. This binding is effected via the aminoacyl group, the latter being bound to the ribose of the soluble RNA. Here the activating enzymes could be found, both specific of alanine, but one located in the nucleus, the other in the cytoplasm (Webster, 1960). The latter can bind alanine only to cytoplasmic RNA and not to nuclear RNA. The activating enzymes evidently have a kind of ambivalent specificity, directed on one hand to a single amino-acid, on the other hand to the corresponding type of soluble RNA.

This soluble RNA transports the activated amino-acids to the site of synthesis, the ribosomes, and this is why the soluble RNA is also called transfer RNA. The transfer was ascertained for the first time on rat liver homogenates (Hoagland *et al.*, 1957). This process too requires energy, for GTP (guanosine triphosphate) and, no doubt, also ATP must be present.

3. The formation of the peptide chains then takes place on the surface of the ribosomes, where the amino-acids are ordered according to a sequence proper to each protein. This sequence is genetically determined, since it is commanded by the chromosomes via the messenger RNA, which transmits to the cytoplasm the specific informations of the DNA. We had already stated this fact when we discussed the action of the genes (p. 116) and we had pointed out the part played here by a third type of RNA, the macromolecular ribosome RNA. This type of RNA does not seem to be determinant of the specificity of the protein synthesis, but is nevertheless necessary to the process itself.

Roughly speaking, we can conceive the soluble transfer RNA as transporting the activated amino-acids to certain points of the messenger RNA (adapter hypothesis), where contiguous amino-acids join to form a protein molecule via peptide bridges. Fig. 54 represents this hypothesis in part (Klingmüller, 1962). There is much evidence indicating that there are here no intermediary peptides, but that the amino-acids are linked to one another by a "zip fastener mechanism".

How does each amino-acid find its exact place in the "messenger RNA" and, consequently, its location in the polypeptide chain which is about to be formed? The answers to this question are still hypothetical. Crick (1957, 1958) assumes that each amino-acid of a protein is codified in the DNA by a triplet, i.e., by three bases (see p. 115). These triplets, according to the matrix mechanism, are transmitted by the DNA to the messenger RNA and direct the protein synthesis at the surface of the ribosomes, where the corresponding bases of the

transfer RNA bind themselves to these triplets via hydrogen bonds (Fig. 128). It is possible that specific enzymes select the amino-acids and bind them, as well as the transfer RNA, to the messenger RNA in the right sequence. Incorporating enzymes could be found in many different types of cells, mainly however in bacteria (Beljanski and Ochoa, 1958). In the presence of such enzyme systems, amino-acids release inorganic phosphate from nucleoside triphosphates, according to a certain selection; glycine reacts better with adenosine triphosphate (ATP) than with guanosine triphosphate (GTP); leucine reacts with uridine triphosphate (UTP) and with cytidine triphosphate (CTP); phenylalanine reacts only with cytidine triphosphate (CTP) (Beljanski, 1960). Many other factors have been found to be necessary for this stage of protein synthesis (for ref., see, e.g., Chantrenne, 1961).

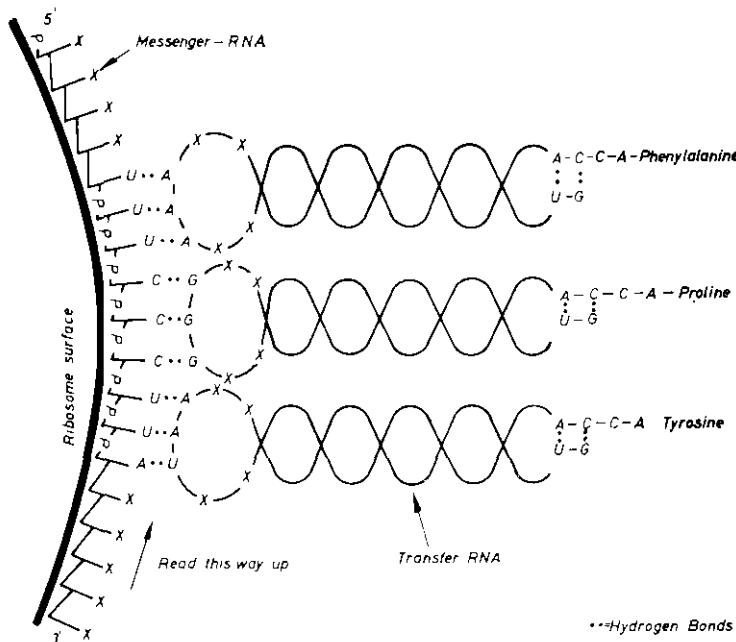


Fig. 128. Schematic diagram showing how the amino-acids with their transfer RNA are bound to the triplets of the messenger RNA. (From Harbers/Domaqk-Müller: *Nucleinsäuren*. Thieme, Stuttgart.)

4. In which sequence the secondary and tertiary structures of the proteins are built and what their determinants are, is also still unclear. For, indeed, proteins are characterized not only by the sequence of their amino-acids (primary structure) but also by their spatial arrangement (see, e.g., Karlson, 1962; Schöberl, 1962; and others). The secondary structure is the spatial arrangement of a peptide chain as for instance in the shape of a helix or a whirl. When several of these chains are bound together and form one molecule, we speak of tertiary structure.

The simplest explanation of the formation of secondary and tertiary structures was given by Perutz *et al.* (1960). According to him, the spatial folding after detachment of the polypeptide chains from the messenger RNA is a spontaneous process, which may be determined either by the sequence of amino-acids itself, or else by the local conditions in the cytoplasm, especially by the ions present or, generally speaking, by the pH. Since most proteins contain cystine, disulphide bonds may induce a folding, especially as disulphide bonds are of importance for the stability of the proteins and for several of their physiological properties. Globin gives a good example of the regular order in which different polypeptide chains lie adjacent to one another. Globin consists of two pairs of chains: two α -chains and two β -chains, which join, *in vitro* as well as *in vivo*, to form a fairly large molecule, always in the same typical pattern (Perutz *et al.*, 1960).

Now we understand the fundamental mechanism of protein synthesis at the endoplasmic reticulum or rather the ribosomes, let us point out once more that this synthesis is particularly intense in cells which produce protein and deliver it to their environment. We had mentioned this fact already when we studied the morphological aspects of the ergastoplasm, and it is fairly obvious that the production of gland secretions is closely connected with the endoplasmic reticulum (see also Birbeck and Mercer, 1961). The process itself varies considerably from one type of gland to another and many of its details need yet to be elucidated. In many cases, secretion is also in close connection with the Golgi complex (Palay, 1958a); therefore we shall examine in greater detail the mechanism of secretion when we discuss the Golgi apparatus.

It is of interest here to note that in secretory cells, the first substances that may be called prosecretion granules are often found in canaliculi of the endoplasmic reticulum which have extended to form cisterns (Fig. 124). This is not only true of protein-rich prosecretion granules from the pancreas (Palade, 1956; Burkl, 1959), in which chymotrypsinogen may possibly be synthesized directly at the ribosomes (Siekevitz and Palade, 1960); it is equally true of the thyroid gland (Wissing, 1960) and also of other protein secreting glands (see, e.g., Brandes and Portela, 1960; Moericke and Wohlfarth-Bottermann, 1960), and evidently also of the fat corpuscles of the mammary gland (Bargmann and Knoop, 1959; Hollmann, 1959). Furthermore, the proteins which have been synthesized at the ribosomes must, by and large, pass through the membranes of the endoplasmic reticulum, which presumably requires an active transport mechanism and a great amount of energy (see, e.g., Hirsch, 1960). In the further development of the prosecretion granules, the Golgi apparatus plays an active part, at least in many gland cells; it probably also plays a part in neurosecretion (Palay, 1960b; Scharrer and Brown, 1961; Murakami, 1962) which occurs mainly in the hypothalamus (for ref., see, e.g., Scharrer and Scharrer, 1954). More will be said of this later (p. 262).

Other functions

Analysis of the microsomal fraction has revealed that, next to those components which are necessary for the protein synthesis, the endoplasmic reticulum evidently also contains a large amount of other enzymes, the action of which extends to the most varied stages of metabolism (for ref., see, e.g., Leybold and Staudinger, 1962). The microsomal fraction

contains a particularly large amount of hydroxylases, in other words, of enzymes, which, when exposed to oxygen and a hydrogen donator, perform a substrate oxidation with concurrent formation of a hydroxyl group. We find hydroxylases for the formation of steroids in the microsomal fraction of endocrine glands which produce steroid hormones. Several hydroxylases detoxicate foreign substances, for example drugs, which may have entered the cell; they can be found in the liver, again mainly in the microsomal fraction. Another typical enzyme of the microsomal fraction of the liver is glucose-6-phosphatase, which is contained exclusively in the membranes of the endoplasmic reticulum and not in the ribosomes (Chauveau *et al.*, 1962). In liver microsomes, a cytochrome could also be identified (Strittmatter and Ball, 1952), namely, cytochrome b₅.

However, all these enzymes from the microsomal fraction may be only in part considered as components of the endoplasmic reticulum. Not to mention the fact that there are always small particles of mitochondria and of the Golgi apparatus present in the ultracentrifugate. This method hardly enables us to discriminate the corpuscular elements of the endoplasmic reticulum from those of the cytoplasmic ground substance, and it is quite probable that many of the enzymes found in the microsomal fraction should better be attributed to the ground substance of the cytoplasm (p. 266). This is surely equally true of a large amount of those enzymes, which are specific for a given tissue. And, after all, the ribosomes, as we have mentioned already, do not belong at all to the endoplasmic reticulum but to the cytoplasmic ground substance.

A consistent relationship was found to exist, in the rodent liver, between the endoplasmic reticulum and the production of glycogen. When, after fasting, the glycogen reserves of the liver have largely been consumed, the smooth-surfaced endoplasmic reticulum becomes hypertrophied and new glycogen granules appear in the vicinity of its membranes; these granules then gather in the ground substance of the cytoplasm and build larger masses (Porter and Bruni, 1960; Porter, 1961). It is by and large in the same way that the smooth-surfaced component of the endoplasmic reticulum expands when exposed to thioacetamide; it is equally accompanied by a loss of glycogen and must probably be interpreted in the same way (Thoenes and Bannasch, 1962).

An important, specific and partly well-definable function of the endoplasmic reticulum is intracellular transport. If the continuity of the system of canaliculi extending from the perinuclear into the extracellular space, which has been observed many a time, can be assumed to be a general feature of all cells, all extracellular substances may this way come into close contact with the cytoplasm and even also with the nucleus. Vice-versa, cellular products from the endoplasmic membranes may rapidly, following this same pathway, be delivered to the cell's environment, as in the plasma cell (Fig. 123); the protein-containing material to be secreted is produced in its large endoplasmic cisterns and delivered to the environment through the cell pores, a phenomenon which can be observed sometimes in the light microscope (Hamperl, 1962). An intracellular, intracanalicular transport of absorbed material has been identified particularly clearly in the gastro-intestinal tract, where the epithelial cells contained fat pellets they had absorbed in their endoplasmic system of canaliculi (Palay and Karlin, 1959; Palay, 1960a), even in the direct vicinity of the nucleus (Fig. 129). Yet it is still not clear whether this material had previously entered the cytoplasm following pinocytosis (p. 281) or directly through pores of the cell wall (Porter, 1961).

Finally, considering the large surface area of the intracytoplasmic system of membranes and the part played in general by the electrostatic potentials of membranes, it is probable that the endoplasmic reticulum can also be an accumulator of potentials and a transmitter of stimuli to the inside of the cell (Ruska *et al.*, 1958).

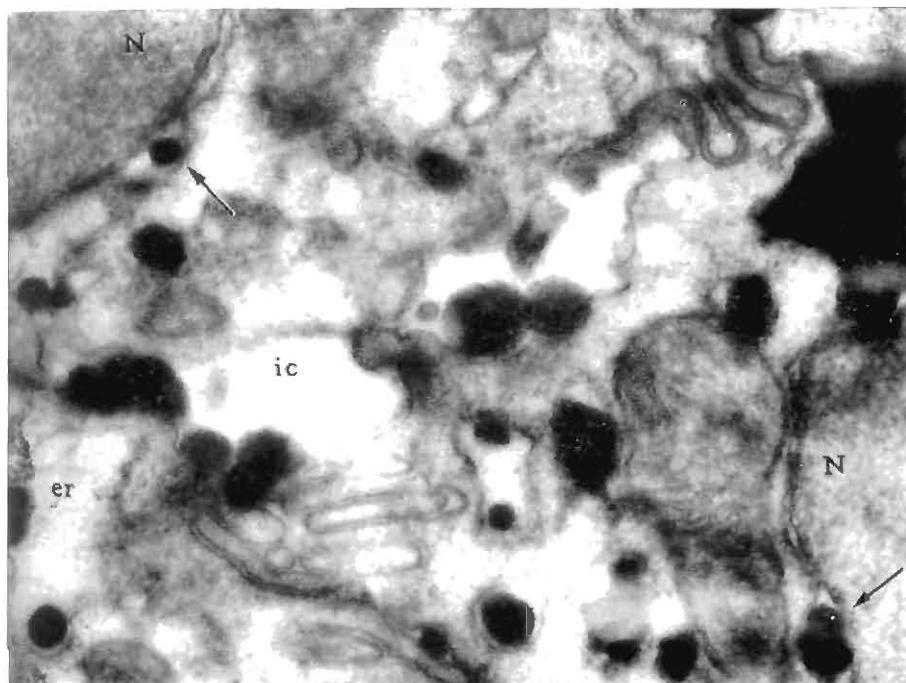


Fig. 129. Numerous fat pellets in the cisternae of the endoplasmic reticulum (er) and in the perinuclear space (arrow). (From Palay, S. L. (1960), *J. biophys. biochem. Cytol.*, 7, 391-392.)

Review

The multiplicity of the morphological aspects of the cytoplasm, connected with its large possibilities of differentiation, allow but few generalizing statements. The endoplasmic reticulum is, indeed, a typical case exemplifying the principle of executive organelles of the nuclear genes. Information which is released from the genes and transmitted by messenger RNA affects first of all the components of the endoplasmic reticulum, where it induces protein synthesis specific for that particular tissue. In several stages, the amino-acids are activated, then conjugated to a soluble transfer RNA and, together with the latter, they link themselves into polypeptide chains attached to the messenger RNA with the help of the ribosome RNA, which is quantitatively the largest portion of RNA. This process takes place at the ribosomes, i.e., 140-150 Å granules of the cytoplasmic ground substance, which adhere to the exterior surface of the lamellae or the membranes of the endoplasmic reticulum.

When there are no ribosomes, we speak of smooth-surfaced or agranular endoplasmic reticulum, which may be found for example in cells synthesizing steroids, but also functionally connected with the synthesis of glycogen. The rough-surfaced endoplasmic reticulum corresponds, in the electron microscope, to the basophilic ergastoplasm of glandular cells, which, as can be seen in the light microscope, may disappear under functional stress and, especially, under pathological strain (chromatolysis). It is built up again when the cell regenerates (chromatogenesis). From observations in the electron microscope, this variability mainly concerns the ribosomes. Since the sites of protein metabolism are also transport channels, the endoplasmic reticulum represents a fairly closed functional unit, although it barely seems possible to separate it completely from the cytoplasmic ground substance, precisely in regard to its function.

THE GOLGI APPARATUS

Beside the endoplasmic reticulum there is a second system of lamellae and vesicles, commonly called Golgi apparatus, Golgi region, Golgi element or Golgi system. All these terms are hardly justified; for, the "apparato reticulare interno" which was described by Golgi (1898) as surrounding the nucleus, now designates a circumscribed region of the cytoplasm, which has but little in common with Golgi's first description. The Golgi apparatus, however, has become a firm, definite concept and the term can hardly be changed any more. Such changes have been attempted some 150 times during the past 60 years, according to the progress of science (see Hirsch, 1939, 1955) but they failed every time.

Evidence from light microscopy

Golgi (1898) noticed in the nervous system of the cat and the owl a network which can be outlined by silver impregnation and appears to surround practically the whole circumference of the nucleus and extend through large parts of the cytoplasm. Much trouble was then taken to identify similar networks in other cells, which succeeded in many cases (for ref. see, e.g., Hirsch, 1939). It was soon noted that these same structures were able to reduce osmium tetroxide, which reaction, too, makes them visible as black masses; this last method is obviously more specific than Golgi's silver impregnation, and this is why they are now called osmiophilic bodies, or, in reference to their presumed lipid content, lipochondria (for ref., see Baker, 1953, 1957; Hirsch, 1955; and others). A closer comparison of both methods revealed that Golgi's original network was clearly the result of a precipitation of silver nitrate between the lipochondria, as the consequence of an overstaining, and that it was, therefore, a mere artifact. The osmiophilic bodies are composed of a multitude of vesicles of different sizes; they appear more or less empty inside for they contain a chromophobic substance and are bound by a stainable envelope, the chromophilic substance. The Golgi network appeared now to be a kind of negative image of the osmiphobic substance (Fig. 130, left). Baker (1953) described, beside the lipochondria, other osmiophilic structures, the osmiophilic platelets and the lepidosomes, i.e., slender corpuscles frequently curved or stacked on top of one another (Fig. 130, right). True, the methods of preparation and the scope of observations in light microscopy appear today rather crude; yet the evidence of

light microscopy has been essentially confirmed by electron microscopy, even though the nomenclature is different.

Before electron microscopy, it was doubtful whether the Golgi apparatus existed at all. Instead of the Golgi network, vacuoles were frequently found, which stained with neutral red *intra vitam*, in certain cases also with methylene blue or nile blue sulphate, and which were taken for the Golgi system (Parat and Painlevé, 1924). This vacuome hypothesis had to be modified many times (e.g., Hibbard, 1945) and was the object of much controversy (for ref., see Bensley, 1951; Dalton, 1961) focused mainly on the artifact problem, until finally more and more doubt was cast upon the very existence of the object of the discussion.

However, light microscopical studies of the osmiophilic bodies showed that they could be distinguished clearly from stored fat droplets and also from the mitochondria and the ergastoplasm, especially in secretory cells (ref. see Hirsch, 1955; Zeiger, 1955). Osmiophilic structures were then found in the epithelial cells of the gastro-intestinal tract, in blood cells and in many other cells, and finally, phase contrast microscopy made it possible to make

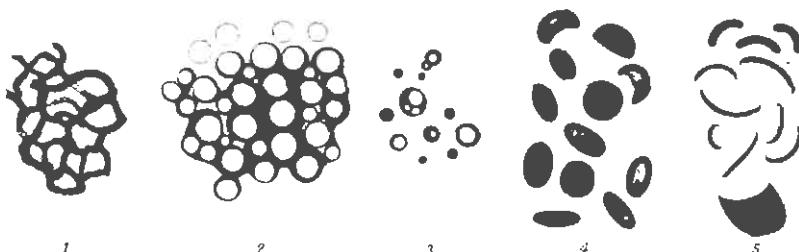


Fig. 130. Various aspects of the Golgi system as seen in the light microscope. (1) Golgi's "apparato reticulare interno". (2) Osmiophobic vesicles surrounded by osmiophilic material, which may be mistaken for a network. (3) The lipochondria. (4) Osmiophilic platelets. (5) Lepidosomes. (From Baker, J. R. and Hirsch, G. C. (1956), *Hdb. allg. Path.*, Vol. II/1, 92-212, Springer, Berlin.)

observations *in vivo* of the Golgi apparatus (Hirsch, 1948; Hanaoka, 1954, 1957; and others). Thus, beside vesicles, it has been possible to identify canaliculi (Gryzcki, 1960). Phase contrast microscopy has shown that the plasma cells contain a particularly large Golgi structure (Stobbe, 1958). It is frequently closely related to the centriole, which in certain cases is located in the middle of the Golgi region or at its periphery.

By comparing the observations made *in vivo* by phase contrast microscopy with the findings made available by silver impregnation, it was possible to elucidate the nature of the Golgi formations. It appeared that Golgi's (1898) silver impregnation technique affected the endoplasmic reticulum, that is, the space between the Nissl bodies; and these are homologous with the ergastoplasm (Malhotra, 1959; Sharma and Manocha, 1962). Thus, the result obtained has nothing in common with the Golgi apparatus as we conceive it today; nor do lipid granules or mitochondria play any important part in the formation of such structures.

The fundamental functional relationships of the Golgi apparatus have been demonstrated at the light microscopical level. First, it has been found out that the Golgi apparatus and the dictyosomes of the spermatozoa and the invertebrates are homologous (Gatenby, 1917;

Hirschler, 1918). The latter contain, too, a substance impregnable by silver, but they have the shape more of a crescent. They stain with various dyes and appear in the living cell as well-defined corpuscles (Brice *et al.*, 1946; Gresson, 1950). Furthermore, during spermatogenesis, the Golgi apparatus has been found in constant relationship to the acrosome, which is apparently produced under the influence of the Golgi apparatus (Bowen, 1929). Finally, evidence has been gained quite early suggesting that the Golgi apparatus was involved in the intracellular secretion (Nassonov, 1924*a*; Ludford, 1925*b*; Hirsch, 1939; and others). A cyclic change in form was seen to take place, especially in exocrine pancreas cells (Sluiter, 1944; Hirsch, 1948, 1955; and others); an hour after stimulation of the fasting mouse with pilocarpine, an increased number of enlarged osmophilic structures appeared in the Golgi region, after the secretion granules had been discharged; 2 hours later, the first new secretion granules became visible, and when some 16 hours later the cell again contained the normal number of secretion granules, the Golgi apparatus had returned to its original proportions. This experiment could be repeated indefinitely and always followed the same basic pattern. It indicated clearly that there are vesicular structures in the cell, which are probably related to the formation of the secretion granules.

Evidence from electron microscopy

The thin-sectioning technique of electron microscopy put the whole Golgi controversy on a new level (see, e.g., Dalton and Felix, 1954, 1957; Sjöstrand, 1954*a*, 1956; Sjöstrand and Hanzon, 1954; Haguenau and La Cour, 1954; Lacy and Challice, 1957; Dalton, 1961; and others). It was now ascertained that there existed a Golgi apparatus in nearly all cells, and that it always had the same aspect despite all variations in size. It is seen to consist of three elements (Fig. 131): vacuoles of different sizes, generally grouped together; flattened, smooth-surfaced sacs, in a lamellar array; and very small vesicles, which have apparently detached themselves from the edges of the lamellar sacs (see also Mollenhauer and Zebrun, 1960).

The extent and size of the different components vary. Epidermis cells, lymphocytes and certain tumour cells have a very small Golgi apparatus with only a few small vacuoles and scattered systems of lamellae (see, e.g., Howatson and Ham, 1955; Low, 1960). Other carcinoma cells (see e.g., Haguenau and Bernhard, 1955; Oberling and Bernhard, 1961), but mainly gland cells of the most different types (see, e.g., Wessel, 1960) and certain blood and plasma cells (Tanaka *et al.*, 1957; Stoeckenius, 1957*a*; and others) have quite an extended Golgi apparatus with large vacuoles and a great number of lamellar sacs grouped together. The centriole is frequently included in the Golgi region or lies in its direct vicinity; this is also the case with many other cells and has been interpreted as the sign of a functional relationship between both structures (e.g., Gusek, 1959).

That these electron microscopic structures were indeed the very same Golgi apparatus seen in the light microscope, could be ascertained by a secondary osmium staining. In principle, all three components can reduce osmium tetroxide (Dalton and Felix, 1956) yet each one with a different affinity and intensity. The strongest impregnation is found on the lamellar sacs and the small vesicles which obviously have budded off from them. These investigations confirmed, at an electron microscopic level, the homology between the Golgi

apparatus and the dictyosomes; the latter, too, are made of lamellar sacs, vesicles and vacuoles of different sizes and have this same aspect in animal cells (Dalton and Felix, 1956; Hovasse, 1957) as well as in plant cells (Buvat, 1957). Finally, electron microscopy has also elucidated in greater detail the close relation between the Golgi apparatus of spermatocytes and the acrosome (Horstmann, 1961). In human spermogenesis, the acrosome emerges between the nucleus and the Golgi apparatus within a vacuole adjacent to the nucleus, and part of the Golgi vacuoles open into the acrosome vacuole, so that a transfer of substance seems possible.



Fig. 131. Electron micrograph of part of a plasma cell of mouse spleen, showing a well-defined Golgi apparatus, mitochondria and distended endoplasmic reticulum. $\times 22,800$. (Courtesy of F. Miller.)

Thus, electron microscopy has fully confirmed the early light microscope evidence of the existence of a separate osmophilic Golgi apparatus and we may very well correlate the different aspects of the osmophilic substance, as Baker (1951, 1953) described them, with the electron microscope findings; the large and the small vesicles in Fig. 130 correspond to the vacuoles at the electron microscope level, the lepidosomes correspond to the group of lamellar sacs in Fig. 131. We shall deal later with Baker's lipochondria (1951, 1953).

If one compares the lamellar sacs of the Golgi apparatus with the lamellae of the endoplasmic reticulum, which also frequently lie together in groups (Fig. 4), it is difficult to notice any basic difference in morphology. The thickness of the membranes is the same; the only difference is that in the Golgi apparatus they lie closer together and build complexes with sharper contours. *But they may also separate from each other, like the lamellae of the endoplasmic reticulum, and form cistern-like cavities.* As a rule, the membranes of the Golgi apparatus are free of ribosomes and one is inclined to consider the Golgi apparatus as a part of the smooth-surfaced endoplasmic reticulum. The cover cells of the mucous membrane of the stomach always show a well-defined Golgi apparatus in the light microscope which fails to appear in the electron microscope. By secondary osmium staining, intracytoplasmic vacuoles are blackened, which otherwise must be attributed to the smooth-surfaced, agranular endoplasmic reticulum (Hally, 1960). Here, the Golgi apparatus stainable by osmium does seem to be merely a special form of differentiation of the endoplasmic reticulum (Dalton, 1961).

Beside its osmophilic character, the Golgi complex has also a biochemical particularity, which distinguishes it from the endoplasmic reticulum; Golgi material which has been isolated by a special centrifugation technique contains hardly any RNA at all, but mainly phospholipids, i.e., the substance of the membranes, and especially a high specific concentration of acid phosphatase (Kuff and Dalton, 1960), viz., nucleoside diphosphatase (Novikoff and Goldfischer, 1961). Maybe the Golgi apparatus is connected with the lysosomes (p. 290), which typically contain the enzyme acid phosphatase (De Duve *et al.*, 1955) and are found to be frequently located very near the Golgi apparatus (Novikoff *et al.*, 1961).

Relationship to secretion

From the few morphological features which the Golgi apparatus and the endoplasmic reticulum have in common, one is tempted to infer that they also have some common function. We had already pointed out, when we examined the evidence brought in the light microscope, that the exocrine cells of the mouse pancreas showed a regular function cycle of the Golgi apparatus which is closely connected, in time and localization, with the production of the secretion granules. On the other hand, we had found out that the secretion products were formed in the cisternae and the membranes of the endoplasmic reticulum (p. 253); for it is there and in the ribosomes which are attached to them that the protein synthesis takes place (p. 249). Which part does the Golgi apparatus play in this process?

We must note first that secretions also exist which do not involve the Golgi apparatus, for instance, in the oviduct glands of the hen (Hendler *et al.*, 1957), in the subepidermal gland cells of planarians (Klug, 1960) or in the salivary glands of aphids (Moericke and Wohlfarth-Bottermann, 1960). Moreover, the primary secretion granules apparently emerge as a rule

between the membranes and the lamellae of the endoplasmic reticulum. If, in most cases, this cannot be directly demonstrated in the electron microscope, it is because with the present technique of preparation for electron microscopy, proteins at a low concentration remain invisible as a faint opacity, as for instance in the cisterns of plasma cells (Fig. 123). In exceptional cases only, the endoplasmic reticulum contains compact protein drops, such as in the exocrine cells of the pancreas in the starving guinea-pig shortly after refeeding (Fig. 124). Yet, these same drops appear much more frequently in the sacs and vacuoles of the Golgi apparatus. How they get into the Golgi vesicles has not yet been definitely elucidated. Either they are dissolved in the canaliculi of the endoplasmic reticulum and reconstituted in the vesicles of the Golgi apparatus, or else they migrate along the system of canaliculi and pass directly into the Golgi system. This last hypothesis, confirmed by older findings (Hirsch, 1939), is the most probable. Hirsch was able to observe, after 3 hours, pro-enzyme granules of the exocrine pancreatic tissue moving from the base of the cell to the Golgi region.

The Golgi region is where the zymogen granules develop. As soon as 20 minutes following intravenous injection of ^3H -leucine, the radioactivity is found to be particularly intense in the Golgi apparatus; this could be demonstrated by combined electron microscopic and radioautographic investigations (Caro, 1961). The secretion product is evidently condensed in the Golgi apparatus and may in certain cases even accumulate there, as is indicated by the quite early radioactivity of the Golgi apparatus (see also Caro and Palade, 1961). In addition, certain processes of rearrangement may take place. Lastly, the secretion granules detach themselves from the Golgi apparatus (Burkl, 1959), are surrounded by a membrane and stored in the cytoplasm as the final zymogen granules, ready to be discharged into the lumen under the action of a secretion stimulant. Hirsch (1960) represented the whole process schematically (Fig. 132).

The part played by the Golgi apparatus in the secretory process has been ascertained also

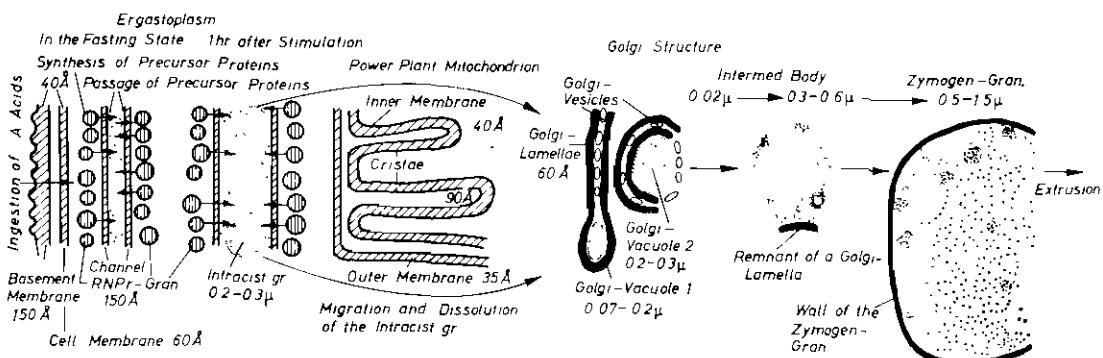


Fig. 132. Diagram of secretion mechanism in the exocrine pancreas cell. (From Hirsch, G. C. (1960), *Naturwissenschaften*, **47**, 25-35.) Recent investigations by G. C. Hirsch (see *Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas*, Schattauer, Stuttgart, 1964) have shown that intracisternal granules only appear under excessive pressure in the endoplasmic reticulum and that the Golgi structure in this diagram does not correspond to the electron microscopical findings. (Addendum on review.)

in a great many other gland cells; in the thyroid gland of salamanders, for example, the Golgi apparatus increases markedly in size after injection of a thyrotropic hormone and contains many secretion droplets, up to 1 μ in size (Herman and Fitzgerald, 1961). Similar drops appear in the Golgi apparatus of the cells in the frontal lobe of the rat pituitary gland, (Farquhar, 1961), whereas in the rat thyroid gland, the Golgi apparatus evidently shows only a slight reaction (Ross, 1960). In human sweat glands (Munger, 1961), in the medulla of the toad suprarenal gland and in other cells, specific droplets appear electively in the Golgi apparatus (Robertis and Sabatini, 1960); similarly we can first find the vitelline protein in gastropods (Worley and Moriber, 1961), the milk protein in the mouse mammary gland (Wellings and Deome, 1961) and the trap secretion in insectivorous plants in the enlarged structures of the Golgi apparatus (Schnepp, 1961).

The findings concerning the neurosecretions are absolutely similar. The first authentic secretion precursors are always found between the lamellae of the Golgi apparatus, no matter what the object of study is, be it the nucleus praopticus of the goldfish (Palay, 1960b), the supraesophageal ganglion of the earthworm (Scharrer and Brown, 1961), the hypothalamus of the mouse (Murakami, 1962) or any other neurosecretory cells (see, e.g., Bern *et al.*, 1961). Their protein is probably synthesized in or at the membranes of the endoplasmic reticulum (Scharrer and Brown, 1961); but then, it condenses into visible granules in the Golgi apparatus and the ready neurosecretion droplets are stored like the other secretory products between the lamellae of the endoplasmic reticulum.

Another substance, melanin, also appears first in the Golgi apparatus. This could be demonstrated equally well in the cells of malignant melanomas in man (Wellings and Siegel, 1959; David and Velhagen, 1961; Rose and Staehlin, 1961) and in fish hybrids (Stolk, 1960). Although it is evident that here again the structural elements derive from the endoplasmic reticulum, the elaboration of these elements into the brown pigment takes place in the Golgi apparatus, which may swell to the size of the cell nuclei, surrounded by the lamellae of the endoplasmic reticulum and the mitochondria. In tissue cultures, this appears particularly clearly in the phase contrast microscope (Fig. 133). Other protein-containing cell products appear also primarily in the Golgi apparatus. This is the case with the chondrocytes of the rabbit ear, in which the Golgi vacuoles contain some granular material, locally arranged in bands; this material is considered to be collagen precursor (Sheldon and Kimball, 1962). Living osteoblasts contain droplets rich in proteins and polysaccharides in their Golgi vesicles (Rose, 1961). True, not every secretion passes via the Golgi apparatus; its functions are even taken over in part by the endoplasmic reticulum, by the smooth-surfaced system of membranes. Yet all of these findings (see also surveys by Palay, 1958; Kurosumi, 1961) confirm the special role played by the Golgi apparatus in the secretion process and, consequently, in the formation of protein-rich cell products (Zeigel and Dalton, 1962)—not, however, in that of lipid products (Bargmann and Knoop, 1959b).

The discharge of the cell's secretions or other products into the environment or, rather, into the transport channels may occur through an opening in the vacuoles. This type of secretion is called merocrine or eccrine secretion in opposition to the holocrine type of secretion, in which the whole cell is degraded, and to the apocrine secretion, which is believed to consist in a discharge of cytoplasmic elements (see Kurosumi, 1961). Yet, whether this last type of secretion exists at all, is most doubtful (Bargmann *et al.*, 1961).

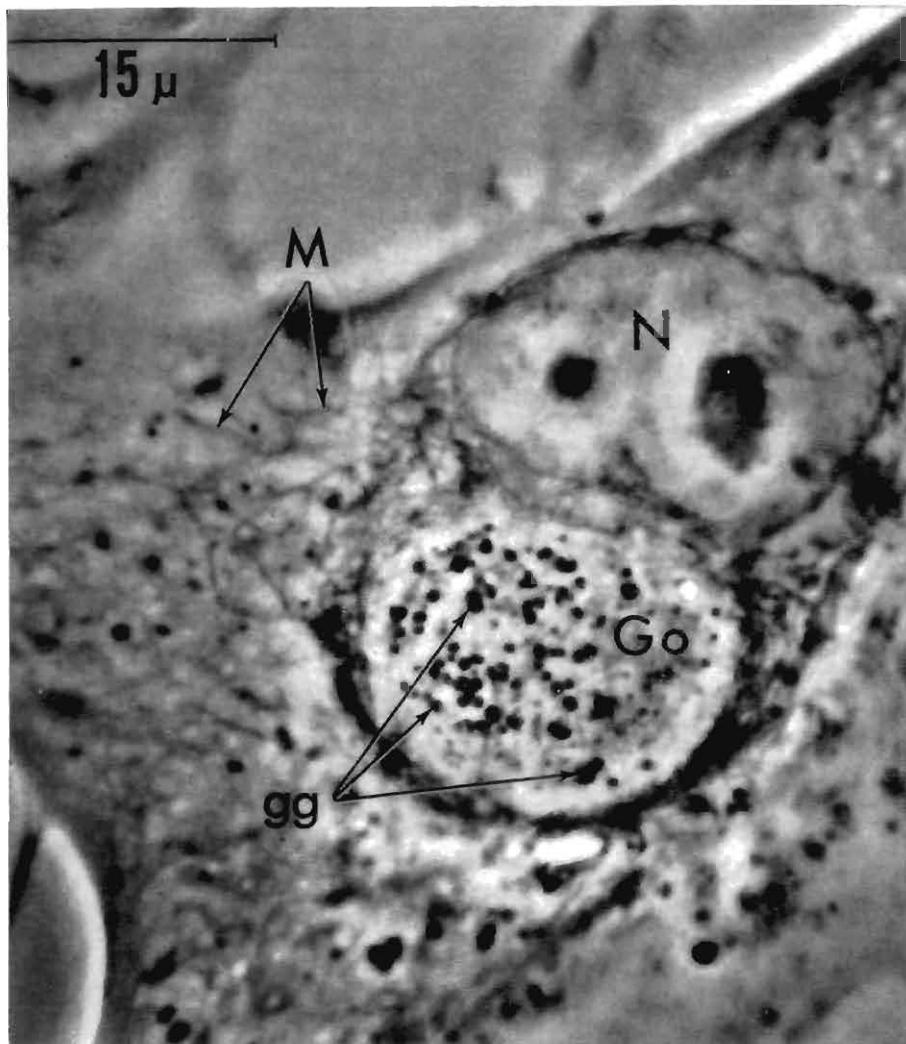


Fig. 133. Melanoma cell from tissue culture showing large Golgi apparatus (Go) with many dark granules (gg). Adjacent, the nucleus (N) and filamentous mitochondria (M) in the cytoplasm. (From Rose, G. G. and Stehlin, J. S. (1961), *Cancer Res.*, 21, 1455-1460.)

Other possible functions

Everything seems to indicate that the part it plays in the secretion process is not the only function of the Golgi apparatus. We have already mentioned examples of other cell products, the formation of which is evidently also connected with the Golgi apparatus, for instance

collagen. It is possible that preamyloid is produced in the same way (Battaglia, 1961) and then precipitated extracellularly (Letterer *et al.*, 1960; Fruhling *et al.*, 1961). The Golgi apparatus seems to be equally involved in the construction of the cell walls of root caps in plants (Mollenhauer *et al.*, 1961). It is perhaps a formation centre for the lamellae of the endoplasmic reticulum.

Further, the Golgi apparatus takes an active part in the incorporation of substances from the environment. In intestinal epithelium cells, it always appears markedly enlarged in the light microscope whenever there is an increase in protein or fat resorption (Adamston, 1958, 1959). In the electron microscope, too, the epithelia of the mouse small intestine after 24 hours fasting showed a very small collapsed Golgi apparatus with only few vacuoles (Fig. 134, above). After 40 minutes oil feeding, the Golgi vacuoles had greatly increased in number and contained fat droplets of different sizes (Fig. 134 below). We cannot neglect that some of the fat droplets are found in the cytoplasmic ground substance into which the lipid substance had been probably first absorbed. Their topographical relationship to the Golgi apparatus, however, is quite clear in the electron microscope (Dalton, 1961) and the Golgi apparatus must be also considered as a region of predilection for the storage of fat (see also Palay and Karlin, 1956). These fat-containing vacuoles were probably the lipochondria in Baker's series of osmophilic substances (1951), as represented in Fig. 130.

The Golgi apparatus has yet another function. It is a fact, observed long ago, that in certain protozoans this structure or one of its homologues is located in the vicinity of the contractile vacuole (Nassonov, 1924) and therefore participates in the intracellular water transfer system (e.g., Gatenby and Singh, 1938; Gatenby *et al.*, 1955). Refractometric and interference microscopic investigations on the epithelial cells of the mouse intestine have led to the same conclusions (Kruszynski and Ostrowski, 1959). In addition, the condensation of the cellular secretion products in the Golgi vacuoles is also concomitant with a loss of water whereas the anabolism of absorbed material is generally accompanied by a hydration process. We have, therefore, a fairly coherent, albeit still hypothetical, picture of the principal function of the Golgi apparatus; namely that of an organelle for local hydration and dehydration processes.

Review

Thus, the Golgi apparatus, still frequently considered a questionable cell organelle, has become clearly delineated in its concept and function. Even though Golgi's original description was based on an artifact, it did bring to attention a special cell structure, which has the property of reducing silver nitrate or osmium tetroxide and shows specific characteristics in the electron microscope; consequently, it is to be differentiated from the endoplasmic reticulum, even though it is composed, like the latter, of vacuoles and lamellae and may in certain cases be directly connected with it. A function common to both systems is the formation of secretory and other cell products; the proteins of these products are synthesized in or at the endoplasmic reticulum but are frequently condensed in the Golgi apparatus and form secretion granules or other cell products. This is the case with many exocrine and endocrine secretions, as well as with collagen, and, in a remarkable way, with the precursors of neurosecretions, which are condensed in the Golgi apparatus and possibly

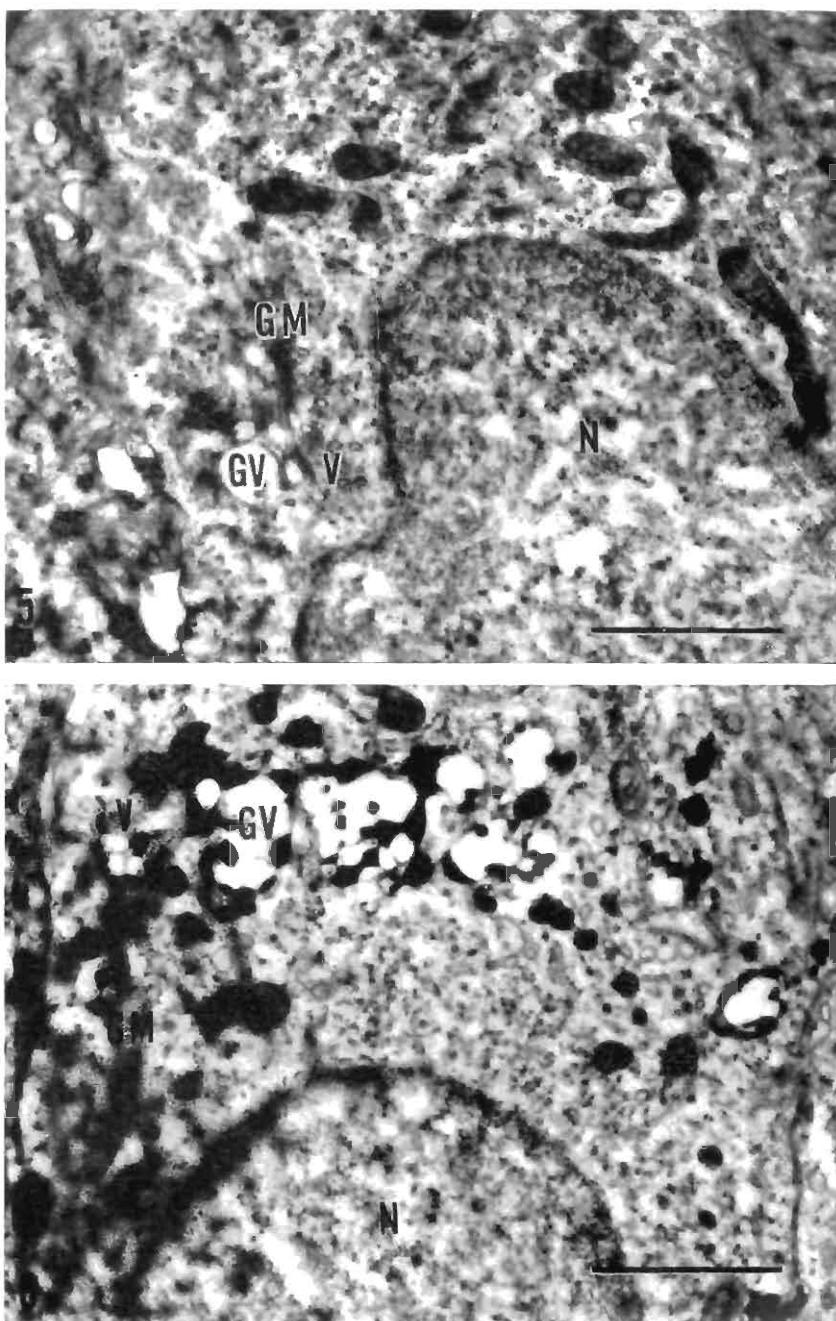


Fig. 134. Golgi apparatus from glandular cells of mouse duodenum, after 24 hours fasting (top) and 40 minutes oil feeding (bottom). Note that the oil concentrates in the Golgi vacuoles (GV) and not in the small vesicles (V) nor between the membranes (GM). N = cell nucleus.
(From Dalton, A. J. (1961), *The Cell*, Vol. II, 603-619. Acad. Press, New York.)

metabolized there. These observations may be largely united by the hypothesis that the Golgi apparatus is an organelle of the intracellular water transfer system.

CYTOPLASMIC GROUND SUBSTANCE

Endoplasmic reticulum and Golgi apparatus have a close structural and functional relationship to the substances which surround and enclose them; consequently, they are closely connected with those parts of the cytoplasm which lie outside the canaliculi and vesicles on the exterior aspect of the membranes and lamellae. This is the matrix of the cytoplasm or cytoplasmic ground substance. Since it generally appears in the living cell as completely homogeneous, it is often called hyaloplasm, as opposed to the paraplasma or paraplastic structures. The latter designate intracytoplasmic deposits, such as pigments, fat drops, crystals, etc. They are to be found in many cells and are often the sign of an injury or a disease of the cell. In a larger sense, virus particles also belong to the paraplasma.

Owing to the great variability of the paraplasma, all biochemical investigations of the cytoplasmic ground substance have given the most variable results. In general, the material of this ground substance is thought to be identical with the supernatant of ultracentrifugation sedimentation. But here, we are faced again with the same problems as those mentioned before; for example, the ribosomes lie in a special sedimentation fraction, the microsomal fraction, but are elements of the cytoplasmic ground substance and only a part of them adheres to the exterior aspect of the membranes of the endoplasmic reticulum (see Figs. 4 and 124). The same can be said of other elements of the cytoplasmic ground substance and, probably, a large number of the enzymes which are isolated in the microsomal fraction (p. 253) belong to the ground substance and not to the endoplasmic reticulum. There is no doubt that the ground substance of the cytoplasm is the site of the glycolytic production of energy.

Normal representation in the electron microscope

From a morphological point of view, the concepts are often too vaguely defined, particularly since some authors include the endoplasmic reticulum and its components in the cytoplasmic ground substance (see, e.g., Brachet, 1957; Porter, 1961). The problem is the same under pathological conditions which may affect both structures.

Electron microscopy has hitherto considered the cytoplasmic ground substance as its stepchild, so to speak, as can be seen by the lack of precision in the above-mentioned example of a definition. This is mainly due to the fact that the object of study seems to be of no interest, since, when one excludes all paraplastic deposits, the cytoplasmic ground substance seems in the electron microscope to be completely structureless (see, e.g., Zetterquist, 1956; and others). On the other hand, 40–50 Å “opaque particles” have often been found in this structureless matrix (Sjöstrand and Rhodin, 1953; Sjöstrand, 1954), which could not be explained at first. In certain animals such as amoebae, fibrillar structures have been described, having a diameter of 150–200 Å, and also vesicles and granules of the same dimension (Lehmann, 1952, 1958); in plant cells, cytonemata have been observed, bearing the same structure (e.g., Strugger, 1956, 1957). The very measurements of their size, as mentioned

above, indicate that the fibres do not really belong to the cytoplasmic ground substance, but rather to the endoplasmic reticulum, as could be ascertained, for instance, in the pea root meristem (Sitte, 1958).

Wohlfarth-Bottermann (1959, 1961) was the first to prove authentically that the cytoplasmic ground substance, as seen in the electron microscope, is by no means homogeneous but has a specific structure. This he demonstrated most clearly on the amoeba *Hyalodiscus simplex*. Phase contrast microscopy shows *in vivo* that the amoeba is composed of an almost round endoplasm with many granules and vacuoles (Fig. 135, left) and of a relatively poorly structurated, hyaline ectoplasm (Fig. 135, right). The first, according to the nomenclature of colloid chemistry, is a plasmasol, the second a solidified plasmagel. As seen in the electron microscope, the endoplasm consists of a great number of membranes and vesicles which belong to the endoplasmic reticulum as described above, and contains in addition typical

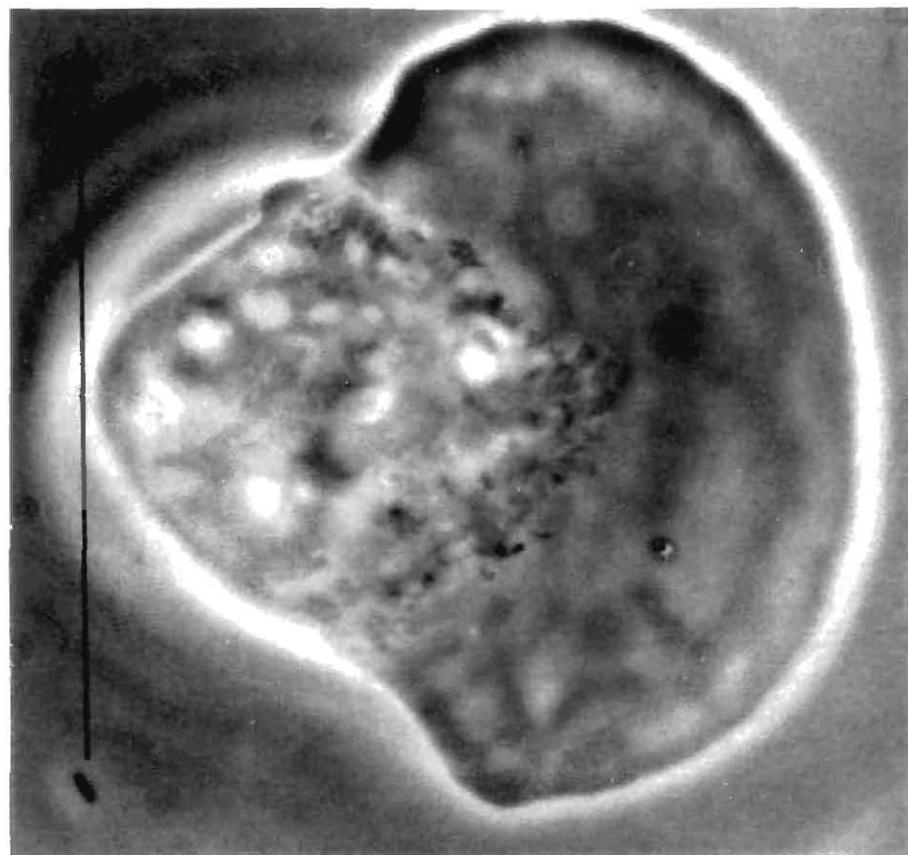


Fig. 135. Phase contrast micrograph *intravitalm* of amoeba *Hyalodiscus simplex*. Left, the granular endoplasm; right, the hyaloplasm. (From Wohlfarth-Bottermann, K. E. (1959), *Verh. disch. Ges. Zool.*, 393-419.)

mitochondria and many other lamellar structures (Fig. 136, right). The picture is, accordingly, basically the same as in the cytoplasm of mammalian parenchyma cells.

The gelled ectoplasm has no membranes nor lamellae, but only single vesicles; however, its main characteristic is a fairly regular texture of granules and filaments, which varies in thickness (Fig. 126, left), and, consequently, the same texture found also in the endoplasm between the membranes is considered a typical component of the cytoplasmic ground substance. Wohlfarth-Bottermann (1961) investigated this granular filamentous texture in detail with various fixation techniques and in various amoebae; the result was always essentially the same. However, the picture is not constant, but shows three possible variations: first, the filamentous granular structure can be so badly defined that the cytoplasmic ground substance seems almost homogeneous. Secondly this ground substance may contain globular elements, measuring 50–100 Å in diameter. Finally, there is frequently a network of filamentous structures, apparently manifoldly interwoven. These variants may occur next to one another in one and the same cell. They are typical equivalents and are quite certainly no irregular artifacts. They are rather the sign of varying conditions within the cytoplasmic ground substance, obviously as a result of the sol-gel changes connected with amoeboid movements (Wohlfarth-Bottermann, 1961).

These findings have, in principle, a threefold significance:

1. Presumably, the cytoplasmic ground substance of other organisms has the same structure (Wohlfarth-Bottermann, 1961). It was demonstrated to be the same in *Paramecium*, for example (Schneider, 1959).

2. It is possible, experimentally, to transform the sol state of the endoplasm into the gel state of the ectoplasm by using dehydrating salts, such as for instance Na_2SO_4 . The membranous vesicular structures of the endoplasm disappear, i.e., the membrane structure of the endoplasm transforms itself into the granular filamentous texture of the ectoplasm, which closely surrounds the mitochondria and congeals them as if they were put "on ice" (Wohlfarth-Bottermann, 1959). Vice-versa, hydrating salts, such as for instance KSCN, bring about the solation of the ectoplasm into endoplasm and the electron microscope shows the formation of membranes typical of the latter (Fig. 137).

3. We may infer from these findings that the subelectron microscopical structure of the cytoplasmic ground substance exists. To judge from the globular elements of 50–100 Å in diameter, there are spheroproteins, which apparently have a very variable relation to each other. We must assume that they may, by aggregating, build filamentous or membranous structures, which are liable to disintegrate rapidly again into their globular elements, thus conditioning the rapid transformation of the ground texture. We do not yet know the details of this process. The best explanation given to this date is that of Frey-Wyssling (1955), although it is ultimately based on a series of findings and ideas which are no more valid today. He too, regarded the various states of the cytoplasm, the "reticular, the granular, the fibrillar and the lamellar aspect", as variants of the aggregation of globular particles which

Fig. 136. Portion of amoeba *Hyalodiscus simplex*, $\times 45,500$. Fine structure of ectoplasm (EK) and endoplasm (EN) with pinocytotic vesicles (PB) and mitochondria (M). The short arrows indicate membranes that have the structure of the cell wall, the white arrow indicates simple membranes of the endoplasmic reticulum. (From Wohlfarth-Bottermann, K. E. (1960), *Protoplasma*, 52, 58–107.)

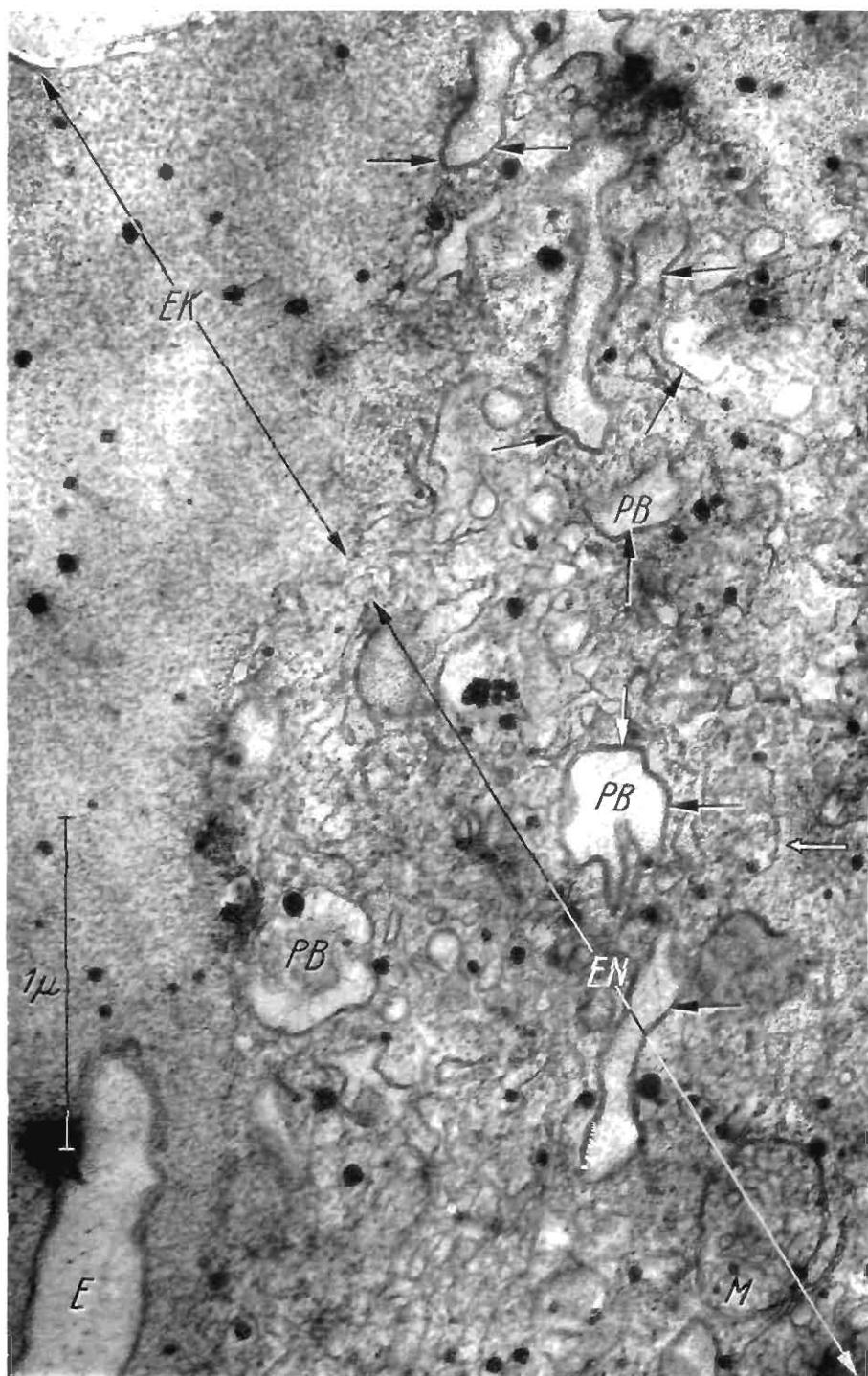


Fig. 136

are probably highly hydrated, i.e., which contain much water. They may condense to a hyaline mass, build reticular structures or arrange themselves linearly into fibrils, like beads on a thread, or into flat lamellae.

This is particularly easy to demonstrate, when the intermolecular cohesion forces of the globular macromolecules or macromolecular groups are not evenly distributed throughout

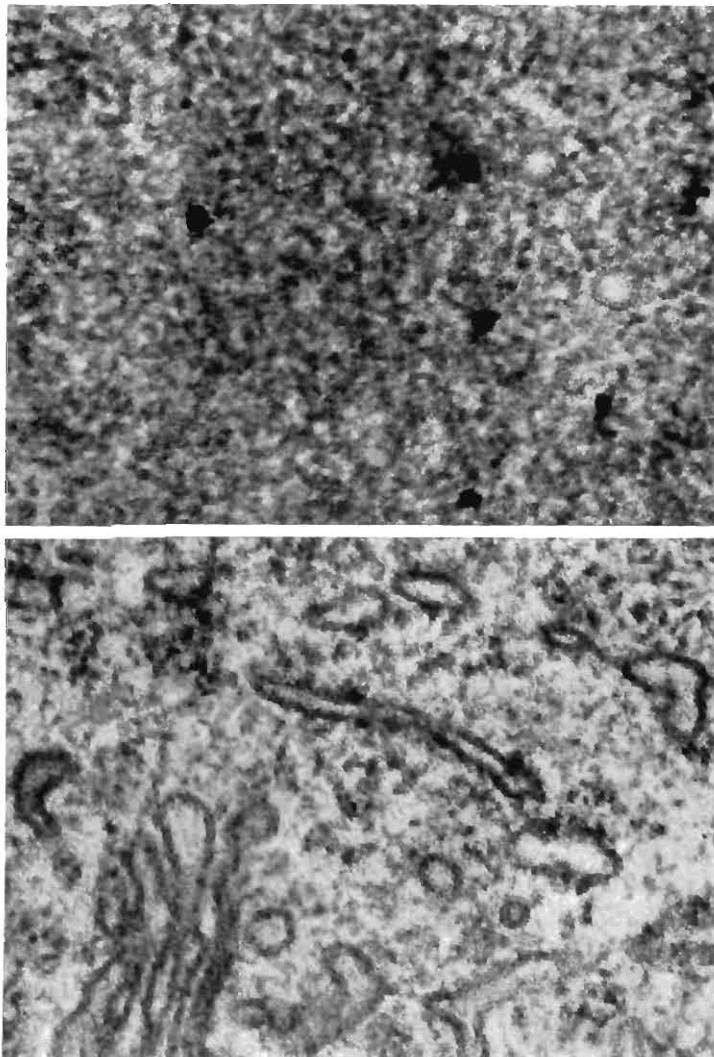


Fig. 137. Transformation of hyaloplasm (*top*) into endoplasm (*bottom*) 3·5 minutes after administration of a 0·2% potassium thiocyanate solution. (Courtesy K. E. Wohlfarth-Bottermann.)

the whole surface of the sphere, but are arranged into a polar pattern, so that certain points of their surface are preformed for the intermolecular cohesion. Frey-Wyssling (1938) gave these points the general name of "attachment points" or "attachment regions" and applied this concept first of all to the network of filamentous particles. The findings in the electron microscope prompted him later to apply it mainly to spheric macromolecules (Frey-Wyssling, 1955), the number and arrangement of which determine the different possibilities of aggregation. If the globular protein particles have, for example, two polar attachment points, a linear aggregation of the molecules occurs into an (elementary) fibril (Fig. 138a). Three such attachment points, evenly interspaced, form a porous film (Fig. 138b). If there are four of them, groups of four molecules appear (Fig. 138c), as has been demonstrated in certain protein crystals. If the globular proteins are all closely adjacent to each other, it means that there are 12 attachment points (Fig. 138d), in other words, that all the points of contact between the spheres become attachment points.

We may readily conclude that in the last of the cases mentioned above, a very compact and strongly gelled cytoplasmic ground substance develops which hardly shows any structures at all. The less attachment points are active, the looser is the arrangement of the globular proteins, and the more water is stored, which, as we may see from Fig. 138, leads automatically to the formation of lamellae, membranes or fibrils. Thus, when under the action of hydrating salts (see Fig. 137) a transformation of ectoplasm into endoplasm occurs (Wohlfarth-Bottermann, 1959), there are evidently four such attachment points saturated with water

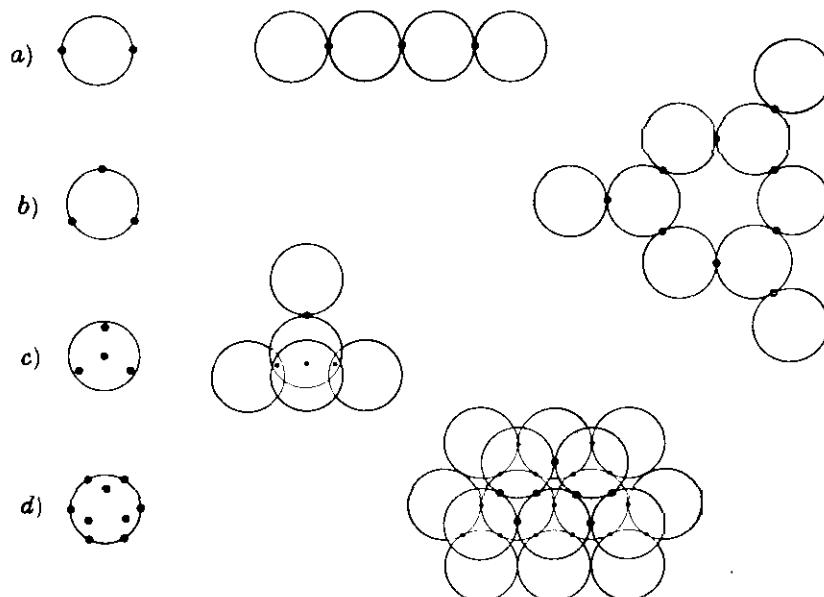


Fig. 138. Aggregation of spheric macromolecules by way of "attachment points". (a) Chains of spheres. (b) Porous film. (c) Crystalloid structure. (d) Densest complex of spheres with 12 attachment points. (From Frey-Wyssling, A. (1953), *Submicroscopic Morphology of Protoplasma*, 2nd Ed. Elsevier, Amsterdam.)

molecules, and the observed membranes and fibrils are formed by those attachment points whose binding forces are still active. Dehydrating salts, according to Fig. 138*d*, bring all groups of globular proteins together; the filamentous membranous structures are then lost.

Of course, this is all hypothesis. Yet it explains many observations and gives moreover an idea at the molecular level of what causes this variability of the structures of the cytoplasmic ground substance. The intracytoplasmic membranes which electron microscopy today is able to demonstrate particularly clearly, are most variable, labile aggregates of phospholipids; this cannot be emphasized enough. But this does not diminish the basic importance of these membranes (see., e.g, Fawcett, 1961); yet it is a warning not to overestimate the electron microscopic picture which after all is nothing but a snapshot. For it is ultimately only an equivalent to the relation existing at a given time between liquid and solid phase, as colloidal chemistry used to call it, i.e., between water and protein-containing dry mass, which is composed mainly of macromolecules with their typical properties (Staudinger and Staudinger, 1954).

Intracellular water transport

These properties of the macromolecules are a decisive factor of the dynamics inherent to all the processes of life. For the cell lives in a constant exchange with its environment. It must absorb construction material and discharge the waste products of its metabolism. In superior organisms, this exchange is promoted by an elaborately ordered system of extracellular streams, for instance the blood- or lymphstreams. Inferior organisms, on the other hand, accomplish this exchange in a far less favourable and less stable medium and are, therefore, much more endangered. Yet, here too, absorption and discharge of material occur via water streams and the water exchange between the cells and between cell and environment is one of the chief processes of life.

Within the cell, the water is not bound in a fixed state either but is subject to constant changes. Every process of synthesis or hydrolysis at any point of the cell is concurrent with a local transfer of water; thereby, the water content of active growing cells is higher than that of non-active cells. In humans, animals and plants, the relative water content decreases with age. In certain individuals such as plants, for instance, there are daily variations of the water content, showing an increase at night and a decrease during the day. The latter is due to a loss of water by physiological transpiration, and the loss is compensated again at night by the suction capacity of the roots (Kramer, 1949; Wilson *et al.*, 1953). A certain water content is the necessary condition of all metabolic processes; if this falls below a minimal level, the cell or the whole organism dies.

The water is maintained within the cell, on one hand, by osmotic forces, that is, as a result of soluble molecules, or the ions of these molecules. On the other hand, the cell contains water-binding forces, mainly in its macromolecules. The proteins and nucleoproteins are such hydrophilic colloids, whose capacity to bind water is exerted mainly on the hydrophilic side chains of the amino-acids. Thus, the —OH, —NH₂ and —COOH— groups, for example, are hydrophilic. One OH— group of an organic molecule shows three free valencies, which can bind three water molecules (Pauling, 1939). The other groups mentioned above have similar properties.

These cursory remarks explain that the intracellular water equilibration depends for a large part on the water-binding macromolecules (for ref., see, e.g., Kramer, 1955; Stocking, 1956). When this water-binding capacity is lost because of a deterioration or even the destruction of the molecular protein structure, binding water is set free, and may in certain cases be visible morphologically as an expulsion vacuole (Klemm, 1895).

Coagulative necrosis

A typical example of such an intracellular water transfer is the coagulative necrosis in which the proteins irreversibly turn into lumps and congeal. The binding water thus released forms a great number of such expulsion vacuoles, as can be observed for example in the liver (Altmann, 1949; Kettler, 1954) and in the fibres of the heart muscle (Grundmann, 1950). Coagulative necrosis can be caused by many different factors: intoxications of all sorts, relative or complete lack of oxygen or food and even electric current or high temperatures (e.g., Gössner, 1960). Viruses may also lead to an irreversible coagulation of the cytoplasm and bring about the death of the cell. In the human liver, one can often see single cells detached from the bundle of trabeculae, rounded up and necrotic; they have a pycnotic nucleus and a strongly eosinophilic cytoplasm, characteristic of the dehydration (Fig. 139).

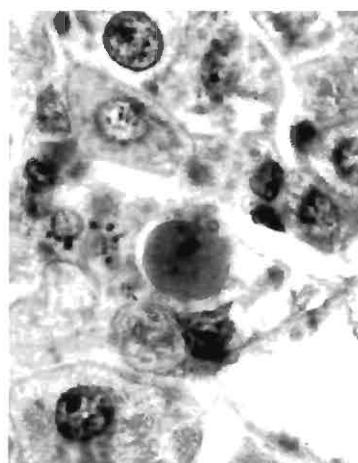


Fig. 139. Coagulative necrosis in an epithelium cell of the rat liver following exposure to the carcinogen diethylnitrosamine. Haematoxylin eosin stain.

Such coagulative processes do not always destroy the whole cell. They may also be restricted to circumscribed plasma areas and are then generally the result of a local deterioration (Altmann, 1955a). They induce smaller or larger portions of cytoplasm to condense and form lumps; these portions then separate from the rest of the cytoplasm and are subsequently either degraded intracellularly or ejected from the cell. These necrotic portions are frequently surrounded by a border of expulsion vacuoles, especially if the swelling of the cytoplasm was enhanced by a preceding influx of water. The causes of the damages range again from the most various poisons (Councilman, 1898) including carcinogenic substances (Grundmann, 1961b; Grundmann and Sieburg, 1962) to virus diseases (for ref., see, e.g., Altmann, 1955). As in generalized necrosis, the disintegrating portions of cytoplasm are, because of the extensive degradation of their RNA and the increased denseness of their structure, strongly eosinophilic. Electron microscopy shows a dense, structureless mass of

granules and filaments in such partial or local necroses (see, e.g., Miller, 1958), in some cases together with remnants of the endoplasmic reticulum (Oberling and Rouiller, 1956).

Reversible increase in denseness of structures

Increased denseness of the cytoplasmic structures is not always irreversible and does not always result in the death of this particular part of the cell. There are, under normal as well as under pathological conditions, many alterations in the water transport in single parts of the cell or in the whole cell. In the last case, the cell may be atrophic as we see it in old age and also following a lack of water or nutrient. The larger the cell, the more noticeable is the atrophy, as for example in the big motor ganglion cells of the human spinal cord after a fairly long inactivity of the musculature. The cells then lead *only a vita minima* and their metabolism is reduced exclusively to the maintenance of the structures necessary to life.

Yet, normal tissue too contains such reversibly condensed cells, noticeable in the liver, for example, by their strong eosinophilic character and their pycnomorphic nucleus. They have been called dark liver cells (Podwysszki, 1886) and sometimes also shock cells (Helmke, 1939); some authors frequently mistake them for single necrotic cells. And, indeed, in many instances of liver damage we find their number increased (for ref., see Altmann, 1955); yet, this condition must be regarded as reversible. When, in the case of such an atrophy, the whole cell shrinks, there has evidently been more than just a transfer of water within the cell; water has also leaked into the environment. This entitles us to classify this condition among the intracellular as well as among the intercellular disturbances in water transport.

Cytoplasmic streaming

One of the typical consequences of intracellular water transport is cytoplasmic streaming, best observed in plant cells; there, it compensates, so to speak, for the rigidity of the cell wall and the lack of association with an extracellular fluid in contrast to the close association found in animal cells. We have not yet elucidated all the details of this process at a molecular level (see Kamiya, 1959). We know for certain that the streaming depends on the viscosity of the cytoplasm, which varies a great deal according to the type of cell and is subject to many exogenous and endogenous influences (for ref., see Heilbrunn, 1928). This viscosity is inversely proportional to the water content; the less free water there is in the cytoplasm, the higher the viscosity. Moreover, living protoplasm does not behave like a simple colloidal solution of proteins or of proteins mixed with lipids. By virtue of the laws proper to living structures, a series of particularities are present which can be explained only in part. A phenomenon typical of a great number of cells is for instance the subdivision in an outer cortex with a higher viscosity and a relatively liquid inner zone (see Fig. 135). Both zones react differently (see also p. 267). In amoebae, for example, small quantities of calcium solidify the cortex even more, but make the inner cytoplasm still more liquid (Heilbrunn, 1958). Marine egg cells react in the same manner. Many data (e.g., Kamiya and Kuroda, 1956) seem to indicate that the interplay of forces at the boundary between the sol-like endoplasm and the gel-like cortex may be the origin of cytoplasmic streaming. Investigators found that this very boundary often had a still lower viscosity than the inside of the cell (Kamiya and Kuroda, 1958), which indicates a constant change in the viscosity, depending on the cytoplasmic streaming.

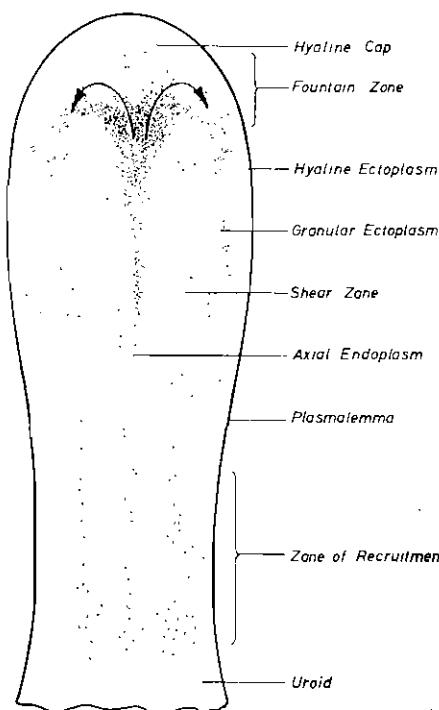
In more highly differentiated plant cells, the cytoplasm moves along like bands, following the cell wall quite closely, thus enclosing the large central vacuole of these cells. We speak here of a rotating movement or simply of rotation. If, in addition, the cytoplasm flows through channels across the vacuole, we use the term circulation (Lundegardh, 1922). Yet the cytoplasmic movement may also be random (digressing or sliding movement) (Nägeli, 1855) and its intensity subject to many variations. From the rotation streaming, there may suddenly spring a side stream like a fountain and pour into the central plant vacuole, which changes the rotation into a circulation (Berthold, 1886). These old observations have been repeated and extended many times, thus elucidating in greater detail the mechanisms of these movements (Kamiya, 1959). These movements may be enhanced by chemical substances (Fitting, 1925, 1936) and by exposure to light (Moore, 1888), and also by ultraviolet irradiation (Nothmann-Zuckerkandl, 1915; Noethling and Rochlin, 1931). No doubt contractile proteins (Tanaka, 1958) and ATP as a source of energy play a decisive part (Goldacre and Lorch, 1950; Goldacre, 1952; Kamiya, 1961; and others), at least in many protozoans. Which transformation processes take place here between the cytoplasmic structures, is still largely unknown. The rhizopods of *Foraminifera*, which display several currents, often seemingly directed against each other (e.g., Jahn and Rinaldi, 1959), are seen in the electron microscope to consist of several subunits containing many single channels, in each one of which the current seems to be possible only in one direction (Wohlfarth-Bottermann, 1961).

Amoeboid movement

The functional significance of cytoplasmic streaming lies first in the transport of nutrition and waste products (De Vries, 1885), but, in many unicellular organisms, this streaming is also utilized for locomotion (Kamiya, 1959), as, for example, in amoeboid movement. The explanation of its origins is still to this date based on conjectures; it is presumed that, beside sol-gel changes, streaming mechanisms and contraction processes play a part in various ways and following various patterns (for ref., see De Bruyn, 1947; Allen, 1961). All theories, however, are based on the common fact that the ectoplasm is a relatively solid gel and the endoplasm a more fluid sol (see p. 267) and that the anterior tip, which is oriented in the same direction as the movement of the amoeba, bears an ectoplasmic cap. The amoeba *Hyalodiscus simplex*, for example (Fig. 135), flows, pushing a large piece of hyaline ectoplasm and trailing along the endoplasm as a hump. While it moves along, frequently small protrusions of the endoplasm into the ectoplasm appear like tiny hernial sacs, and they give the impression of parts of the endoplasm being pushed into the ectoplasm by the cytoplasmic streaming (Wohlfarth-Bottermann, 1960). Exogenous impedance to the amoeboid movement brings about a retraction of the frontal hyaloplasm, which is converted to endoplasm, whereas, at another point, the endoplasm is converted to a new ectoplasm, thus giving the movement its new direction. A conversion of endoplasm to ectoplasm and vice-versa seems all that is needed to explain the movement of an amoeba. We have already acquainted ourselves with the nature of this process in examining the fine structure of the cytoplasm (p. 268).

This was also the basic idea of Goldacre's and Lorch's theory (1950), viz., that a reversible sol-gel transformation contracts the ectoplasm (see also Goldacre, 1952). Mast (1926, 1931) had already much earlier, in comprehensive studies, described such transformations. He assumed that parts of the plasmasol, i.e., the endoplasm penetrate into the hyaline plasma

cap and that, after a new boundary layer has formed between endoplasm and ectoplasm, a new plasma cap develops. If such a flowing of the endoplasm is not as extensive as assumed by Mast (1926, 1931) and if it only produces small evaginations, this theory could very well agree with the above-mentioned observations by Wohlfarth-Bottermann (1960).



There is a modern theory (Allen, 1961; Allen *et al.*, 1962) which is based on exhaustive studies of the behaviour of cytoplasmic particles in centrifugation experiments (Allen 1960) and on the observation of particles adherent to the surface of the amoeba (Griffin and Allen, 1960). According to this theory, an amoeba can be subdivided longitudinally into several zones (Fig. 140), the most important of which in regard to its function is the inner axial endoplasm. In an anterior fountain zone this passes into the granular ectoplasm and shows a sudden increase in viscosity there, which cannot be explained merely by a sol-gel transformation. Allen (1961*a*, 1961*b*) suggests a contraction mechanism in the fountain zone,

Fig. 140. Subdivisions of the amoeba in a longitudinal section. (From Allen, R. D. (1960), *J. biophys. biochem. Cytol.*, 8, 379-397.)

which pumps forward the other parts of the amoeba when the axial endoplasm advances into the granular ectoplasm. The accumulation of cytoplasm found by Käppner (1961*a*) in the amoeba *Chaos chaos* prior to the formation of the pseudopodia could be interpreted in the same manner.

Injection, in the tail of an amoeba, of a 1-3% solution of ATP accelerates the cytoplasmic streaming. The same injection in the advancing pseudopodium reverses the direction of the streaming. In a 1×10^{-1} to 1×10^{-3} molar solution of ATP, all pseudopodia retract rapidly (Käppner, 1961*b*). This shows that amoebae, like other cells, are able to contract actively. Whether these findings entitle us to draw conclusions on the mechanisms of amoeboid movement, is still conjectured.

Intercellular water transport

As we mentioned already, the water content of a cell depends roughly, on one hand, on the water-binding capacity of the intracellular macromolecules, on the other hand, on the osmotic

pressure conditions. This applies to the degree of local hydration within the cell, as we outlined in the previous chapter. This applies even more to the whole cell.

Most measurements confirm the fact that the osmotic pressure in mammals is much higher in the cell than in the surrounding blood (Elliott, 1946; Pichotka *et al.*, 1964; Opie, 1954; Burck and Netter, 1960; and others); depending on the method employed, the osmotic pressure of the cell is one to three times that of the blood. Consequently, to maintain itself in a state of balance with the environment, the cell needs a mechanism which keeps up this intracellular hypertonicity. The nature of this mechanism has been the object of various explanations; some thought it to be an active, energy-consuming transport of water against the concentration gradient (Robinson, 1953; Opie, 1954); others suggested cyclic changes in the electrostatic resistance of membranes (Teorell, 1953). Hypertonicity has been regarded as an artifact, caused, for example, by products of metabolic degradation, which rapidly appear during the preparation of the small pieces of organs after extraction, and which, no doubt, have a great osmotic activity (Conway *et al.*, 1955). This opinion has been overthrown by the observation that such degradation products, mostly of low molecular weight, diffuse rapidly; hence, they can hardly be at the origin of intracellular hypertonicity (Swan and Miller, 1960).

Of major importance for the intercellular water exchange is the distribution of sodium and potassium. Normally, to a high potassium and a low sodium concentration inside the cell correspond a low potassium and a high sodium concentration outside the cell. However, the internal potassium concentration is not as high as the external sodium concentration, and the result of this is a Donnan equilibrium (Netter, 1959). Potassium influx and sodium efflux are energy-consuming cell activities (Berliner *et al.*, 1951; Ling, 1952, 1955; for further ref., see Burck, 1962), the cell possibly taking its energy from ATP; how this happens is, however, not yet fully elucidated (see, e.g., Netter, 1961). In the normal cell, the inward directed osmotic forces have the same intensity as those directed outward, so that there is no net transfer of water. This equilibrium is disturbed experimentally for instance in plasmolysis, in which the cytoplasm of the plant cell detaches itself from the rigid wall following depletion of water. If, on the other hand, there is an uptake of water into the intact cell, the uptake of sodium increases; yet this sodium uptake is only to a certain extent correlated with the water content (Rixon and Stevenson, 1958). The cell loses concomitantly a part of its potassium, and a new equilibrium is established.

Vacuolar degeneration

Yet, with an insufficient supply of energy this equilibrium collapses. Sodium flows into the cell in large quantities, parts of the intracellular potassium leave the cell and, according to the osmotic concentration gradient, water penetrates into the cell. This disturbance in the cellular distribution of ions and the resulting swelling of the cell was termed by Burck (1962) dysionicity as opposed to the normal isoionicity. Every injury to the cell which disturbs the cell's osmolarity by such a dysionicity causes an influx of water into the cell.

This fact has, indeed, been observed in the course of a great many investigations. In regard to the causes of the inhibition of cellular respiration, the emphasis has been laid in the past few years, either on aerogenous hypoxia (Fig. 141) or on a disturbance in respiratory metabolism (for ref., see Büchner, 1944, 1957a; Altmann, 1949, 1955a; Kettler, 1954;

Becker, 1959; and others). This process is basically the same in all the other toxic damages which check the production of energy or hinder its utilization (for ref., see Altmann, 1955; Manuelides, 1958; Becker, 1959). In the liver, accordingly, we find the same picture in cases of shock, for example, following burns (Zinck, 1940) or the administration of histamine (Gloggengiesser, 1944).

Let us classify these alterations according to their degree of severity. Hypoxic damages begin to show after a relatively minor and short disturbance in the respiration and they consist, in the electron microscope, of small vesicles in the cytoplasmic ground substance (Fig. 142); these are much smaller than for instance the mitochondria (see also Bassi *et al.*, 1960; Hübner and Bernhard, 1961). It is frequently quite difficult to discriminate these vesicles in the cytoplasmic ground substance from enlarged cisternae of the endoplasmic reticulum. However, they rapidly grow bigger, especially in the central parts of the liver lobes, and form then those vacuoles adjacent to the nucleus which are typical of the light microscopical picture (Pichotka, 1942; Büchner, 1944, 1947; Altmann, 1949; Grundmann, 1950; Becker and Neubert, 1959; and many others). They appear even if the lack of oxygen is of short duration, and nearly all the epithelial cells in the liver which are in the vicinity of the central veins may be swelled by these often fairly large vacuoles (Fig. 141). In the electron

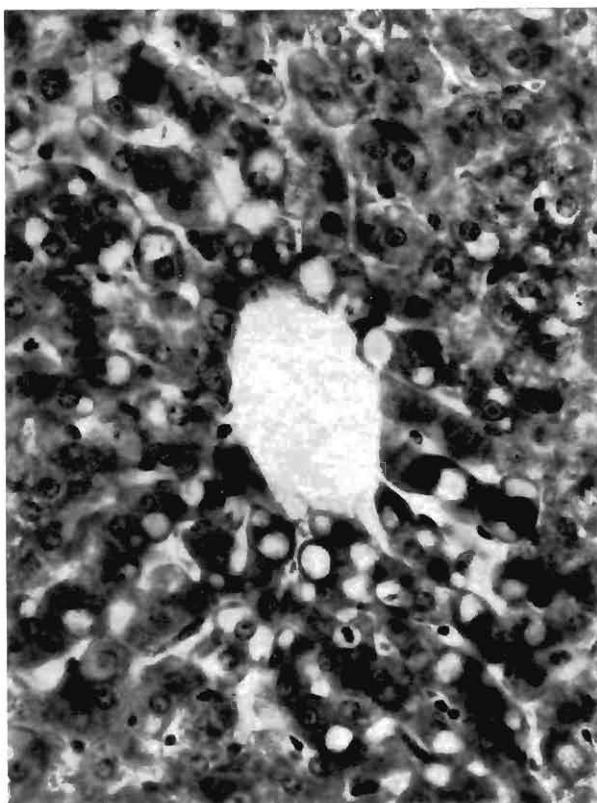


Fig. 141. Rat liver cell showing, after a short period of aerogenous hypoxia, optically empty vacuoles in the vicinity of the nucleus. (From Pichotka, J. (1942), *Beitr. path. Anat.*, 107, 117.)

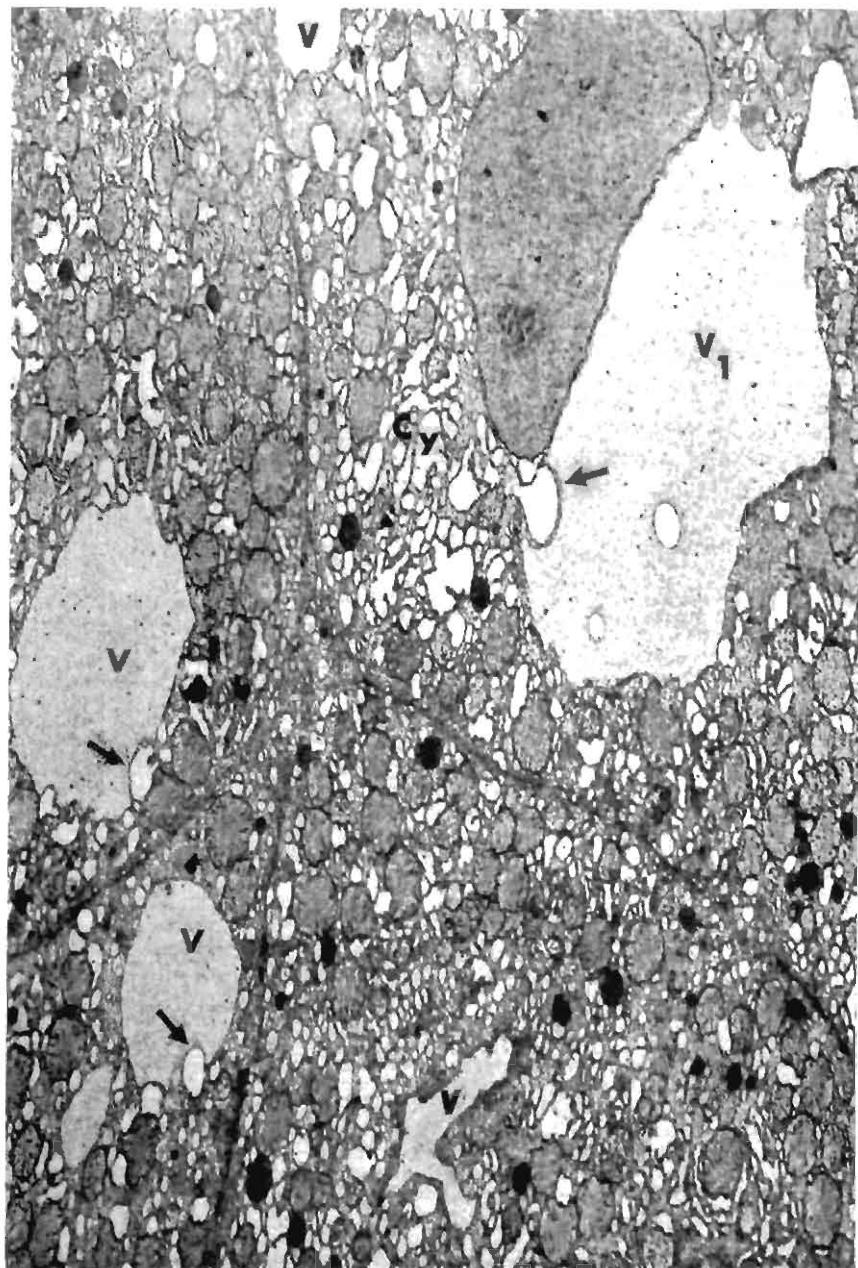


Fig. 142. Vacuoles (V) in liver cells of the rat after temporary obstruction of the blood flow; adjoining them (arrows), the distended cisternae of the endoplasmic reticulum (Cy). (From Hübner, G. and Bernhard, W. (1961), *Beitr. path. Anat.*, 125, 1-30.)

microscope, these vacuoles are recognizable by their size (Mölbert, 1957a; Mölbert and Guerritore, 1957) and also by a fine granulation within them (Hübner and Bernhard, 1961); thus, they may be easily distinguished from the ergastoplasmic cisternae, which are mostly optically empty and often either indent the vacuoles or lie inside them (Fig. 142). Incidentally, this distinguishes the hypoxia vacuoles also from the autolysis vacuoles, which appear with a similar rapidity and may, in the light microscope, occupy the entire cytoplasm (e.g., Buckley, 1962); in the electron microscope, they too are empty.

Even large hypoxic vacuoles are completely reversible (Altmann, 1949; Grundmann, 1950; Becker and Neubert, 1959; Hübner and Bernhard, 1961); the water accumulated in the vacuoles can leave the cell again quite rapidly. The extrusion of the water which penetrated into the cell in the shape of a vacuole adjacent to the nucleus must be interpreted as a sign that the cell defends itself against any excessive intrusion of water. This explains why vacuolized cells do not show any major change in their NAD content (Duspiva and Franken, 1957) nor in the activity of their catalase, uricase and succinic dehydrogenase (Bassi *et al.*, 1960). By contrast, in such vacuolized liver epithelium cells, the protein synthesis, as measured by the incorporation of ¹⁴C-labelled amino-acids, is markedly reduced (Bernelli-Zazzera and Guidotti, 1958; Bernelli-Zazzera *et al.*, 1960); yet it is possible that this is less due to a disturbance of the cytoplasmic ground substance than to one of the endoplasmic reticulum, since it may be equally damaged in the process (Fig. 142).

Vesicular degeneration

When the cell cannot defend itself any more against hypoxic damages, vacuoles are no longer extruded and the cytoplasm is then more or less diffusely occupied by fluid-containing vesicles. The vacuolar degeneration has become vesicular (Fischer-Wasels, 1922). It appears as a typical primary alteration in the liver, for example with carbon tetrachloride (Cameron and Karunaratne, 1936; Remy and Tethrüggen, 1950; Eger, 1954; and others). Among multiple necroses and morphologically intact epithelium cells, there are extensively swollen liver cells with many vesicles in their cytoplasm. The picture in other intoxications is basically the same, for example in liver damage due to phosphorus (Laird, 1933; and others), to chloroform (Fischer-Wasels, 1922) or to carcinogenic agents (see for ref. Altmann, 1955a). In all these cases, the fluid can induce such an extensive swelling of the cell body that there only remains a narrow border of cytoplasm against the inner aspect of the cell wall; the nucleus or nuclei float then in the cell as if in a lake (Fig. 143). The electron microscope reveals, long before these light microscopical alterations become apparent (Grundmann and Sieburg, 1962), lighter areas in the cytoplasm, which appear before other structures are altered (Mölbert *et al.*, 1962). These alterations too are still reversible and light microscopy often shows in these vesicles a protein which may, after condensation, produce hyaline drops.

In certain cases, however, this vesicular degeneration destroys very large parts of the cytoplasm. This can be observed particularly well in heart muscle fibre, in which the more or less diffuse water influx is concurrent with a partial dissolution of the fibrils, called fibrilolysis (Grundmann, 1950). It occurs in cases of experimental oxygen deficiency (Büchner and Lucadou, 1934; Grundmann, 1950), in anaemia (Opitz, 1935; Schubothe and Altmann, 1950) or in the vicinity of myocardial infarctions in humans (Saram, 1957). Since a certain

potassium concentration is necessary for the structural maintenance of the myosin molecule in the muscle fibres, we must attribute to the potassium influx at least the same importance as to the potassium efflux (Grundmann, 1950). For electron microscopy shows that a potassium deficiency in the heart muscle fibre is concomitant with a slackening and a progressive dissolution of the myofibrils (Poche, 1958). By contrast, it is probably only an osmotic mechanism that produces the vesicles in the microvilli of the rat jejunum after administration of hypotonic or hypertonic salt solutions (Millington and Finean, 1962).

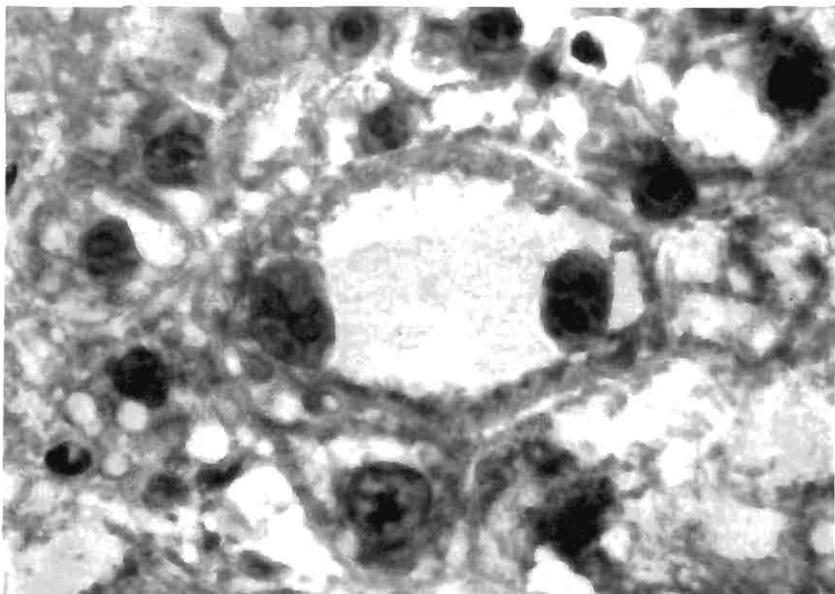


Fig. 143. Massive influx of water in a cell of the rat liver after administration of diethyl-nitrosamine. (From Grundmann, E. and Sieburg, H. (1962), *Beitr. path. Anat.*, **126**, 57-90.)

Finally, this vesicular degeneration may perhaps occur for quite different reasons. We find it, for example, in the epithelia of proximal convoluted tubules of the frog kidney after injections of casein sodium, which causes a tubular polyuria. Here, vesicular degeneration is possibly the result of too slow a water extrusion when the cell is flooded (Heinzel, 1960). This mechanism, according to this theory, is radically different from that mentioned above, for it is based on a quantitative disturbance of a physiological mechanism and not on a primary pathological alteration. Every living cell depends on the constant water exchange which it performs with its environment, particularly since it has to take up nutritive elements as well as the water.

Pinocytosis

Such an ingestion process is indeed visible and is called pinocytosis. The expression was used by Lewis (1931) to designate the fact that certain cells drink, that is, they can take up water in the shape of small droplets and assimilate them. Although Lewis (1931, 1937) was

able to demonstrate his observations on macrophage tissue cultures by using time-lapse photography, his discovery remained for a long time without noticeable response. It was only 20 years later that his observations were confirmed (Frederic and Chèvremont, 1952; Gey *et al.*, 1954; and others). The same fate befell Mast and Doyle (1934), who independently from Lewis had discovered a similar process in *Amoeba proteus*.

The uptake of fluid droplets always follows the same basic pattern, but shows many variants. In tissue-culture cells, the typical object of study for pinocytosis, the drop is encircled by the membranes of the pseudopodia, becomes gradually surrounded by the cytoplasm and is thus progressively incorporated into the cell as a vacuole. Thus, the vacuole rounds itself up, gets smaller and smaller and may disappear completely. The same process is to be found in tumour cells (Gey *et al.*, 1954), in leucocytes, monocytes and histiocytes (Bessis and Bricka, 1952; Wittekind, 1960; and many others). In amoebae, it is frequently somewhat different; an undulated channel forms, from the inner end of which small vesicles are pinched off and penetrate into the inside of the cell (Fig. 144). Each channel is bounded by a part of the cell membrane, since it was formed by an invagination of this membrane (Brandt, 1958). A pinocytosis channel lasts a few minutes, and there may be 50 to 100 such channels present at the same time, each with a diameter of 1–2 μ . Larger channels have been observed, too, and even large invaginations of the cytoplasm showing all the transitional stages up to phagocytosis of fairly large nutriment particles.

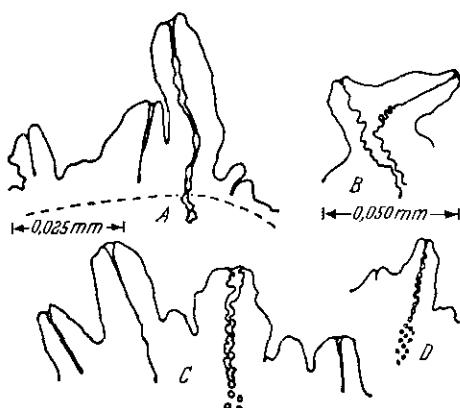


Fig. 144. Pinocytotic channels in *Amoeba proteus*. Note the formation of vesicles at the inner ends of the channels. (From Mast, S. O. and Doyle, W. L. (1934), *Protoplasma*, **20**, 555.)

However, it has only been as a result of electron microscopy that pinocytosis has become a subject of interest. Cells with microvilli, lining for example the intestinal or kidney epithelia as a brushborder, always show small vesicles or channels penetrating into the cytoplasm at their bases; at first, they are still connected with the extracellular space (Fig. 145), but then they detach themselves from it and migrate progressively into the inside of the cell (see, e.g., Clark and Wochner, 1958; Miller, 1960; and others). In cells without any such microvilli, pinocytosis occurs according to the same basic pattern (see surveys by, e.g., Holter, 1959; Policard and Bessis, 1959; and, further, Staubesand, 1960; Staubesand and Schmidt, 1960; Elbers and Bluemink, 1960; Weiling, 1961; and others).

Most observations are concordant with Bennett's hypothesis (1956) that pinocytosis starts with the adsorption of molecules or ions on the surface of the cell membrane. A local reduction of the tension at the surface brings about an invagination at the adsorption site; thus, the adsorbed particles reach the inside of the cell, surrounded by a part of the cell

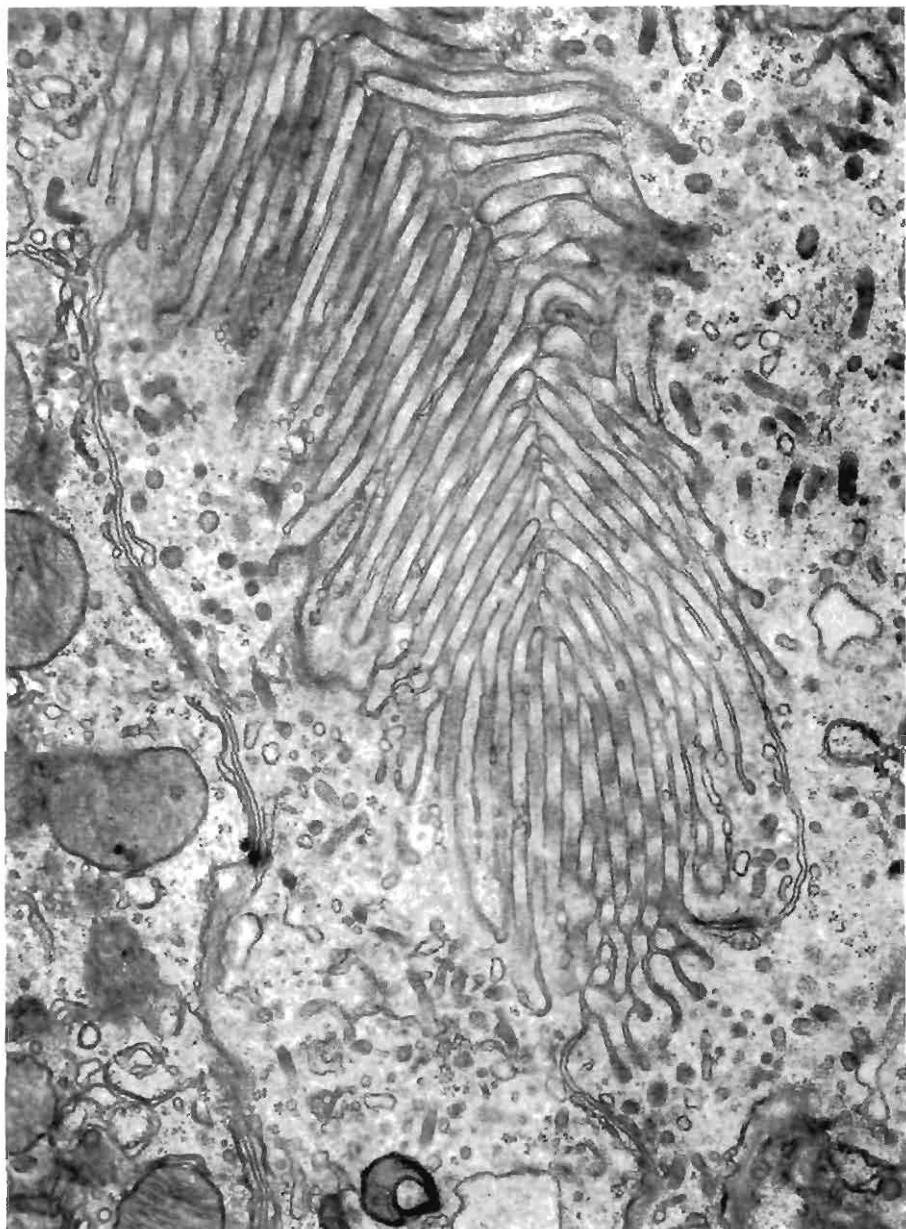


Fig. 145. Electron micrograph of the apical cell region in a proximal tubule cell of the mouse kidney. Note the numerous pinocytotic vesicles which are pinched off at the base of the microvilli. $\times 24,000$. (Courtesy of F. Miller.)

membrane which dissolves sooner or later. Experiments on amoebae have shown that fluorescent protein, for example, adheres to the surface (Brandt, 1958; Schumaker, 1958). The surface here, as seen in the electron microscope, consists of a plasmalemma, 200 Å thick, from which hairlike evaginations, up to 2000 Å in length, emerge; these can adsorb for example ferritin molecules (Brandt and Pappas, 1960, 1962). Thorium dioxide (thorotrust) is adsorbed in the same way, and it is easy to follow the pinocytotic vesicle with its thorotrust content entering the cell (Brandt and Pappas, 1962) and being pinched off by the cytoplasm (Fig. 146). Since the thorotrust cannot be incorporated into the cell, it remains in the vacuoles. The boundaries of these vacuoles become more and more irregular and the thorotrust is finally deposited into and eliminated with the so-called defaecation vacuole, which serves the amoeba to get rid of its metabolic waste products and of infiltrated bacteria (Brandt and Pappas, 1962). The absorption of ferritin granules in vesicles is quite similar and has been

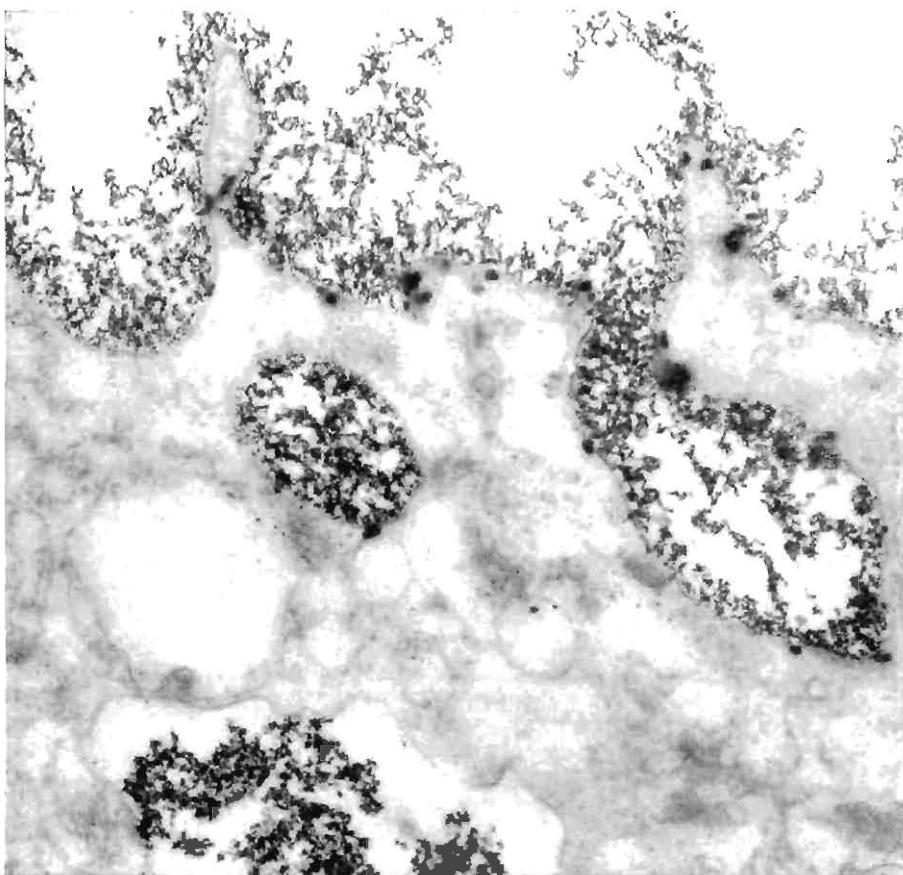


Fig. 146. Thorotrust particle in the pinocytotic vesicles of an amoeba. (From Brandt, P. W. and Pappas, G. D. (1962), *J. Cell. Biol.*, 15, 55-71.)

called by Polycard and Bessis (1958) rhopheocytosis (see also Bessis, 1959; Heilmeyer *et al.*, 1962). By contrast, the injection of ferritin plus an antibody complex into ascites tumour cells induces the formation, in the cytoplasm, of a complicated network of pinocytotic channels; these contain ferritin granules which, unlike thorotrast, are absorbed by the cell, whereby the pinocytotic channels run directly into the cisternae of the endoplasmic reticulum (Eastou *et al.*, 1962).

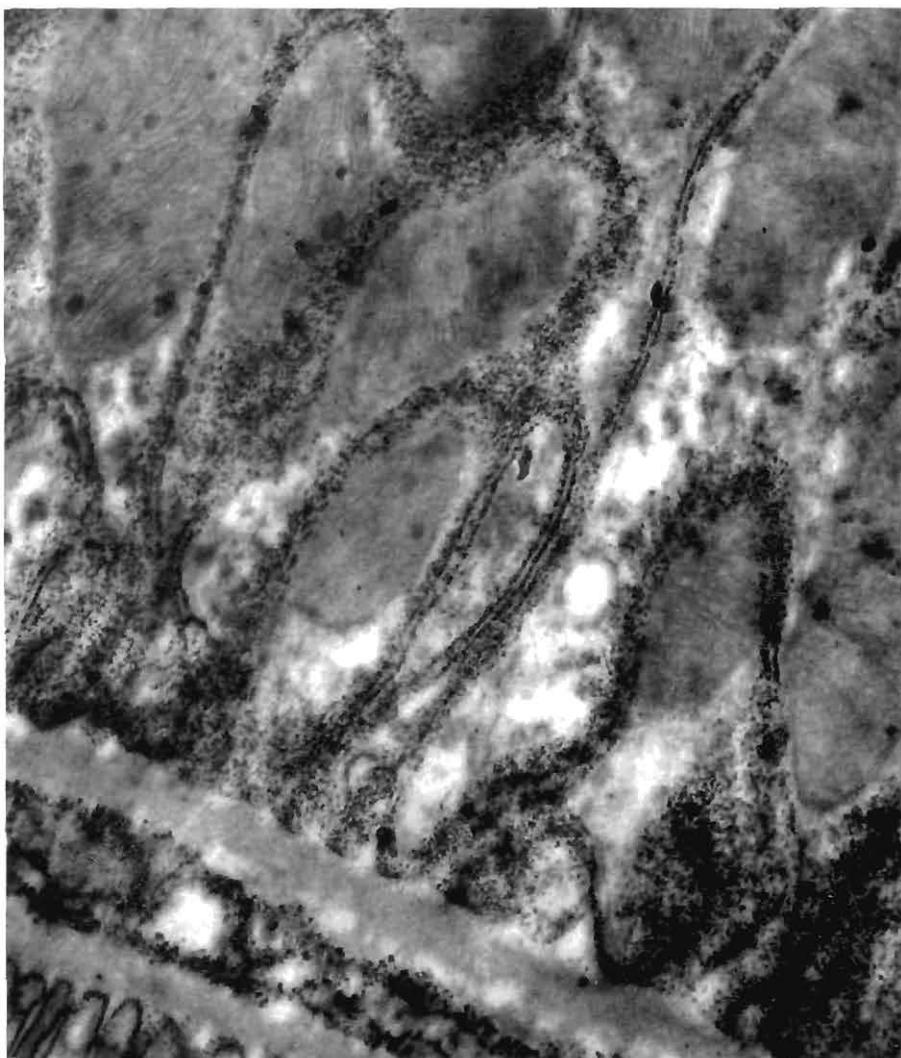


Fig. 147. Electron micrograph of tubular epithelial cells of the mouse kidney, alkaline phosphatase made visible with lead salt. The lighter band below is the basement membrane.
× 54,000. (Courtesy E. Mölbert.)

In summary, pinocytosis occurs in three phases (Nachmias and Marshall, 1961): (1) adhesion of the substance to the cell's surface by means of an exchange of ions; (2) formation of vesicles by way of a hitherto not completely elucidated contraction of the cytoplasm; this process is independent from the cellular metabolism; (3) assimilation within the cell, i.e., absorption of the vesicle into the cytoplasm under the action of hydrolytic enzymes (p. 288) and, in certain cases, storage or discharge of the absorbed material. The pinocytotic vacuole bears, accordingly, a direct resemblance to the nutrition vacuole of the amoeba.

The significance of pinocytosis can, to this date, be evaluated only in part. We know for certain that along this pathway not only water, but also amino-acids, proteins and other substances of high-molecular weight, as well as toxins and antitoxins, can penetrate into the cell (e.g., Chapman-Andresen and Holtzer, 1960; Holter, 1959, 1961; Felton and Pomerat, 1962; and others). Thus, it represents one aspect of active membrane transport, or, at least, pinocytosis transfers by active transport through the cell membrane into the inside of the cell body, where the cell membrane is dissolved. No doubt the conditions for fermentation are better inside the cell than at the cell wall; moreover, the invagination carries with it into the inside of the cell the specific properties of the cell wall, such as, in many epithelia, alkaline phosphatase, located in kidney epithelia (Mölbert *et al.*, 1960) and in other cells (Clark, 1961) mainly in the cell membrane (Fig. 147). Perhaps it is possible to make a rough distinction between the active transport of molecules through the membranes, a problem which is being much investigated today (see, e.g., Lefèvre, 1954; Ussing, 1961; Netter, 1961; Järnefelt, 1961; Heinz, 1961; and others), and the equally active absorption of high-molecular weight substances through pinocytosis.

Moore and Ruska (1957) discovered another process, closely related to pinocytosis, and termed it cytopempsis. It is based on the observation that endothelial cells and sometimes even mesothelia frequently contain many vesicles, some small, some large (Fig. 148), which are evidently used more as a means of passage through the cell than for a liquid absorption (see, e.g., Staubesand and Schmidt, 1960; Staubesand, 1960). In nephrosis of the human kidney, that is, in a state of marked liquid and protein transfer, the cover cells of the glomeruli are full of vacuoles (Farquhar *et al.*, 1957); experiments in animals show that these vacuoles serve, among other things, for the transfer of ferritin molecules through the capillary wall of the glomerulus (Farquhar and Palade, 1960). Such observations point to the need of revising many theories regarding permeability. The pathology of permeability (Eppinger, 1949) is no longer an argument against cellular pathology but a part thereof.

Phagocytosis

Pinocytosis, originally regarded as a mere absorption of water by the cell, serves also, as we just mentioned, for the absorption of high-molecular and also, no doubt, of dissolved low-molecular weight nutriments; hence, it is a kind of phagocytosis on a small scale. Phagocytosis proper is a more comprehensive concept and a more specific activity of the cell. For, whereas pinocytotic processes were spotted in many cells of the most varied structure and functions, phagocytosis remains a property of differentiated cells, that is, certain cells, for example in a mammalian organism, are differentiated to perform the task of phagocytosis; this is the case, for example, with the granulocytes or the cells of the reticuloendothelial system (Aschoff,

1924) and the system of the macrophages which are related to it. Phagocytosis can serve two functions: first, the absorption of nutritive elements, second, the destruction of living or lifeless foreign bodies. As an example of the first, we have the protozoans, in this case particularly the amoebae; for the second, the mammalian granulocytes.

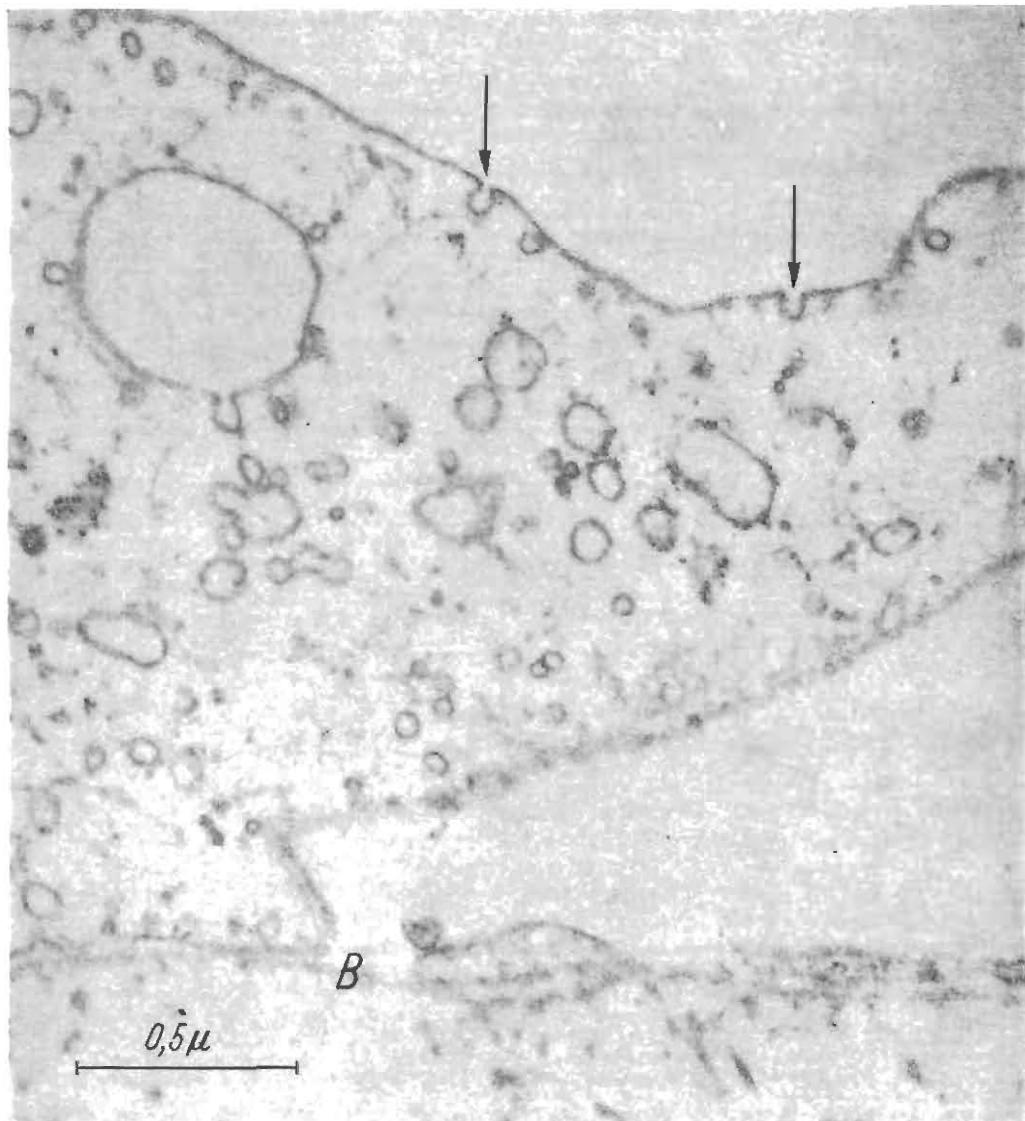


Fig. 148. Cover cell of the gall bladder of *Bufo bufo* showing numerous pinocytotic vesicles. The arrows point to two pocket-shaped depressions in the cell wall (B = basement membrane). (Courtesy of J. Staubesand.)

Phagocytosis in amoebae follows in principle four patterns (Rhumbler, 1898).

1. A foreign body may be incorporated into the amoeba without the latter suffering any particular change in shape. This mere incorporation can be explained by a particularly strong adhesion of the amoeba to the surface of the foreign body; Rhumbler (1898) showed that a glass thread coated with shellac will be absorbed completely into a drop of chloroform. The shellac then leaves the surface of the thread passing into the chloroform phase, and the thread now uncoated is rejected from the drop.

2. The foreign body may be encircled actively by the amoeba's pseudopodia, which also necessitates a strong adhesion of the surface of the foreign body to the amoeba. Between this absorption by engulfing and the above-described incorporation, there are many intermediary stages.

3. The term circumvallation is used when the amoeba by stretching out several pseudopodia surrounds the foreign body from different sides and finally traps it. This gives the impression of a purposeful action, yet from older studies by Rhumbler (1898) we know that it is also a question of changes in the surface tension.

4. The best-known form of phagocytosis is the invagination. At the point of contact with the foreign body, a recess appears, in which the foreign body is invaginated; the cytoplasm of the amoeba then pinches off the invagination and closes itself again. Thus, the foreign body lies inside a phagocytotic vacuole, where it is gradually degraded.

When, for example, alveolar macrophages of the mouse lung phagocytize particles of India ink, they use the principle of invagination (Karrer and Cox, 1960). The surface of the macrophages becomes strongly serrated from many cytoplasmic processes. At the point of contact with the ink, the cell boundary invaginates and the particles adhering to the cell surface are encircled by the cytoplasm as the cell membrane joins behind them; they then form a vacuole and, later, compact inclusion bodies of different sizes. The absorption of bacteria by macrophages follows basically the same pattern (Braunsteiner *et al.*, 1960; Nelson *et al.*, 1962). Here, too, contact with the cell membrane is followed by its invagination and the formation of a vacuole surrounded by the membrane; in this vacuole, the bacteria are finally dissolved. If, on the other hand, the membrane of the phagocytotic vacuole is dissolved prior to this, the cell perishes. Phagocytosis of fat droplets by human thrombocytes also starts with an invagination of the cell membrane (Schulz and Wedell, 1962), and here, too, the phagocytized fat particles are stored in vesicles. Reticular cells react in the same manner when they phagocytize India ink or proteins (Arai, 1960). By contrast, the absorption of particles of erythrocytes by the macrophages of an ascites carcinoma occurs according to the principle of engulfing, the pseudopodia embracing the fragments of erythrocytes and enclosing them in a vacuole (Essner, 1960).

Following phagocytosis, there comes a digestive phase in which the cell assimilates the absorbed material, mainly by means of hydrolytic enzymes. These are found most frequently in the area of the phagocytotic and of the pinocytotic vacuoles. In planarians, acid phosphatase is encountered in the nutrition vacuoles as soon as 5 minutes after nutriment intake.

Fig. 149. Demonstration of acid phosphatase (indicated by arrows) in the mouse liver in the vicinity of phagocytized dextran drops (dv). (From de Man, J.C.A. *et al.* (1960), *J. Ultrastruct.*, 4, 43-57.)

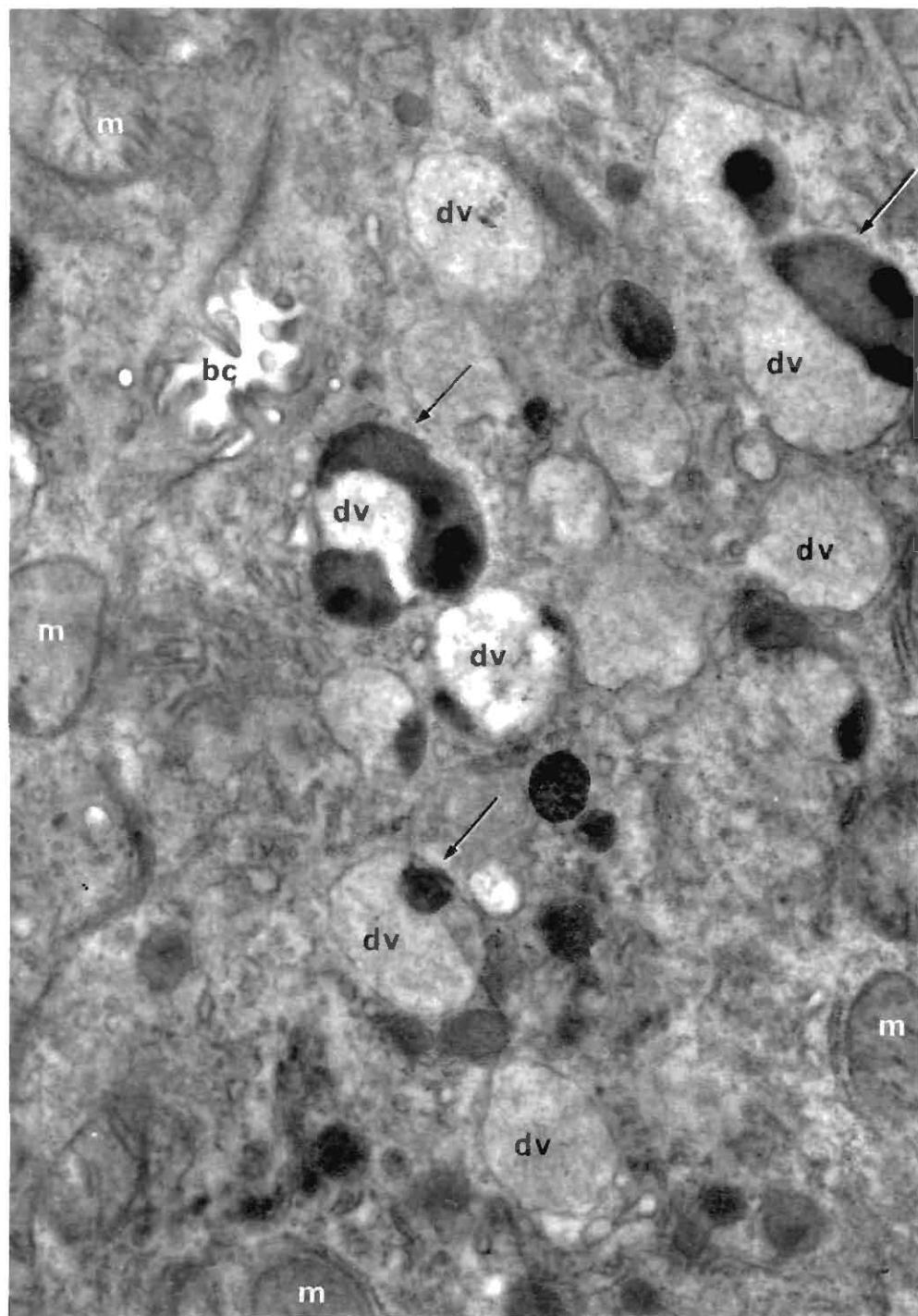


Fig. 149

Its activity reaches its maximum 1 or 2 days later and disappears after 7 days. It is the same with aminopeptidase and β -glucuronidase (Rosenbaum and Rolon, 1960). This activity of the acid phosphatase in the vicinity of pinocytotic and phagocytotic vacuoles has recently been an object of particular study and it was found that not only is it inevitably present (e.g., Essner, 1960; Müller and Törö, 1962; for further ref., see Novikoff, 1961b), but it is sometimes concomitant with a non-specific esterase (e.g., Müller *et al.*, 1962). It is particularly intense in the granules of the neutrophilic granulocytes of the rabbit and of humans (Cohn and Hirsch, 1960) and the digestive process following phagocytosis is, in these cells, concurrent with a reduction, that is, with a consumption of the granules (Hirsch and Cohn, 1960).

The lysosomes

These granules, rich in acid phosphatase, are frequently called lysosomes. The name was coined by de Duve *et al.* (1955) to designate a particular centrifugate, characterized by its great quantity of acid phosphatase. It is meant to emphasize the special lytic activity of this fraction. Meanwhile, in this fraction, beside the acid phosphatase, at least nine types of acid hydrolases were found (see, e.g., Novikoff, 1961b): cathepsin A and B, acid desoxyribonuclease, acid ribonuclease, β -glucuronidase, arylsulfatase A and B, phosphoprotein phosphatase, β -galactosidase, β -N-acetylglucosamidase and α -mannosidase. The composition of the fractions varies a great deal according to the type of cell. Nevertheless, de Duve (1959, 1963) regards the lysosomes as a group of particles on its own, having specific properties.

Morphological investigations in the light as well as in the electron microscope have shown the presence of granules with an intense acid phosphatase activity, predominantly in the granulocytes, but also in other cells, as, for example, the pericanalicular granules of the liver (Essner and Novikoff, 1961). There, the acid phosphatase activity, which can be labelled with lead phosphate, is often to be found in the periphery of the lipofuscin granules. Similar corpuscles of various sizes also lie in the vicinity of phagocytized dextran vacuoles; such is the case in epithelial cells (Fig. 149) or in Kupffer cells of the mouse liver, in which the localization of these corpuscles indicates that they too are related to acid phosphatase (de Man *et al.*, 1960). The term giant lysosomes was used by Blinzingier and Hager (1961) to designate 12 μ inclusion bodies, surrounded by a membrane, in the macrophages in experimental colimeningitis. However, in such electron dense granules, the cytochemical reaction to acid phosphatase varies (Holt and Hicks, 1961), and perhaps these dense corpuscles have many different origins, such as the degradation of mitochondria or other parts of the cytoplasm (Ashford and Porter, 1962).

Hydrolases may also be found in great quantity on the membranes of those nutrition and pinocytotic vacuoles (Barka, 1962) which digest biological structures at an acid pH. It is possible that the membranes play a special role here and that the membranes of the lysosomes prevent the cell from digesting itself under the action of the hydrolytic enzymes (de Duve, 1963). But it is also possible that the acid phosphatase exists in the lysosomes under an inactive form and is only activated in the presence of pinocytotic or phagocytotic vacuoles. This is suggested, for example, by the observations made by Rosenbaum and Rolon (1960) on planarians (p. 288), in which an acid phosphatase activity appears only when nutrient

particles are available and phagocytosis begins. It would be expected that in necroses the lysosomes increase greatly in number, and, in the liver, the number of the electron microscopically dense corpuscles does increase markedly, for example after ligation of the bile duct, or intoxication with carbon tetrachloride or dimethylaminoazobenzol, or after glucagon infusion (Ashford and Porter, 1962; see for further ref. Novikoff, 1961). Since, in certain cases, the Golgi apparatus also plays a part in the absorption and assimilation of material (p. 264), it is no surprise that an acid phosphatase activity is revealed under certain conditions. In the chick para-erythroblasts, infected with erythroblastosis viruses, lysosome-like structures were found directly adjacent to the Golgi apparatus; they seemed here and there to extend into the membranes of the Golgi apparatus (Benedetti and Leplus, 1958). Perhaps there are parts of the Golgi apparatus in the lysosome fraction.

The limits of the lysosome concept (de Duve *et al.*, 1955; de Duve, 1959, 1963; Novikoff, 1961b) still have to be established. However, it is no longer a hypothesis that a close relation between the hydrolytic enzymes and the nutrition and pinocytotic vacuoles, which serve for the assimilation of the absorbed material, exists.

Deposits

After they have been assimilated, most of the absorbed substances remain in the cell; many of them are subjected to cellular metabolism, as nutritional elements. Others form the most varied types of deposits, the so-called paraplasma, partly called pigment because of its particular colour. Among the deposits, there are also proteins, which are not assimilated, i.e., mainly foreign protein, and fats, which lie as a rule in the cell in the shape of drops, as well as the more diffuse glycogen.

Pigments rich in iron

Iron is the most important pigment in cellular pathology. It is stored in the human parenchyma cells in the most varied pathological conditions, as a golden brown pigment, with fairly coarse granules, and, in the form of haemosiderin, it stains blue with the Berlin blue reaction. Haemosiderin is an aggregate pigment (Gedigk and Strauss, 1953; Schreiber *et al.*, 1960; and others), containing considerable amounts of protein, carbohydrates, lipids and also copper and calcium besides iron (Bessis and Breton-Gorius, 1962). Its iron content varies a great deal from tissue to tissue and the values range from 1·5% (Ludewig, 1959) to 37% (Wöhler, 1960). Haemosiderin is not a clearly defined substance but a most variable complex. As a rule, it contains ferritin, a protein with a mean iron content of 23% in the form of ferric hydroxide phosphate. By means of reducing agents such as cystine, one can detach the iron components and obtain the protein components of ferritin, called apoferritin. The ferritin molecule is generally hexagonal and has a maximal diameter of 100–110 Å and a side length of 70–80 Å (Bessis and Breton-Gorius, 1960). The iron component is bound at many points to the apoferritin (Richter, 1959) which forms a kind of protection colloid (see, e.g., Gedigk, 1958a).

Ferritin has a characteristic fine structure (Fig. 150), due to the arrangement of the iron in the molecule. It was first assumed that the iron components were located at the four corners of a square (Farrant, 1954). Yet the various aspects of the ferritin molecule are

better accounted for if one considers them as the projection of an octahedron (Fig. 150) (Kerr and Muir, 1960; Bessis and Breton-Goriüs, 1960, 1962). Absorption of the iron generally occurs via rhopheocytosis, which is similar to pinocytosis (p. 285); in reticulum cells and histiocytes it also occurs via phagocytosis of erythrocytes or their fragments and probably the iron is then passed on directly to the erythroblasts, often adjacent to the reticulum cells (Bessis, 1959).

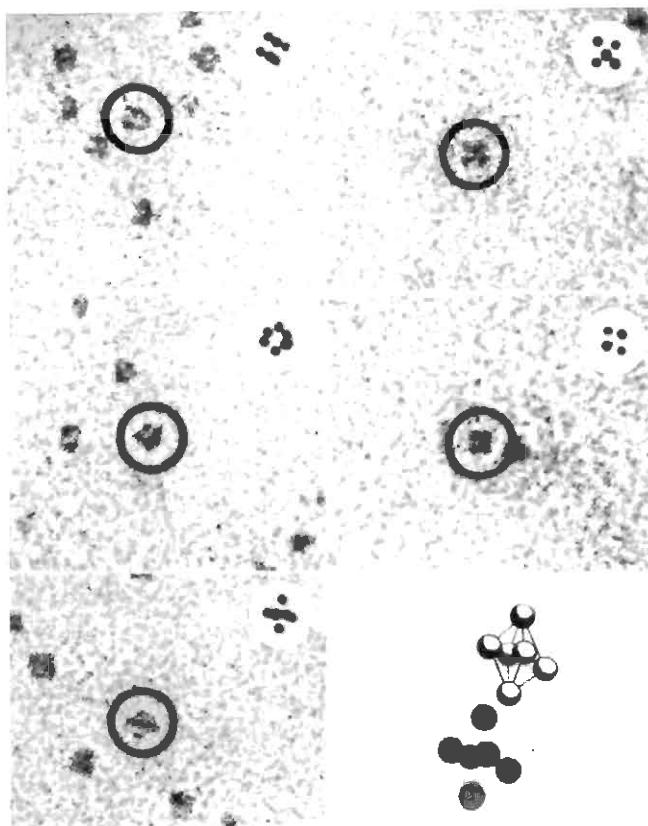


Fig. 150. Electron micrographs showing various aspects of the ferritin molecule. Bottom right, model of the resulting aspect of the ferritin molecule. $\times 450,000$. (From Bessis, M. C. and Breton-Goriüs, J. (1962), *Blood*, 19, 635-663.)

In the reticulum cells, erythroblasts etc., the ferritin molecules are partly scattered throughout the cytoplasm and partly confined to granules, visible in the electron microscope (Fig. 151). These granules are called "siderosomes" (Richter, 1958; and also Lindner, 1958). They originate in connection with the rhopheocytotic vacuoles, by a process of condensation, confluence and rearrangement, whereby the iron hydroxide micelles are wrapped in a cytoplasmic substance (Wessel and Gedigk, 1959; and others). They can grow to form quite

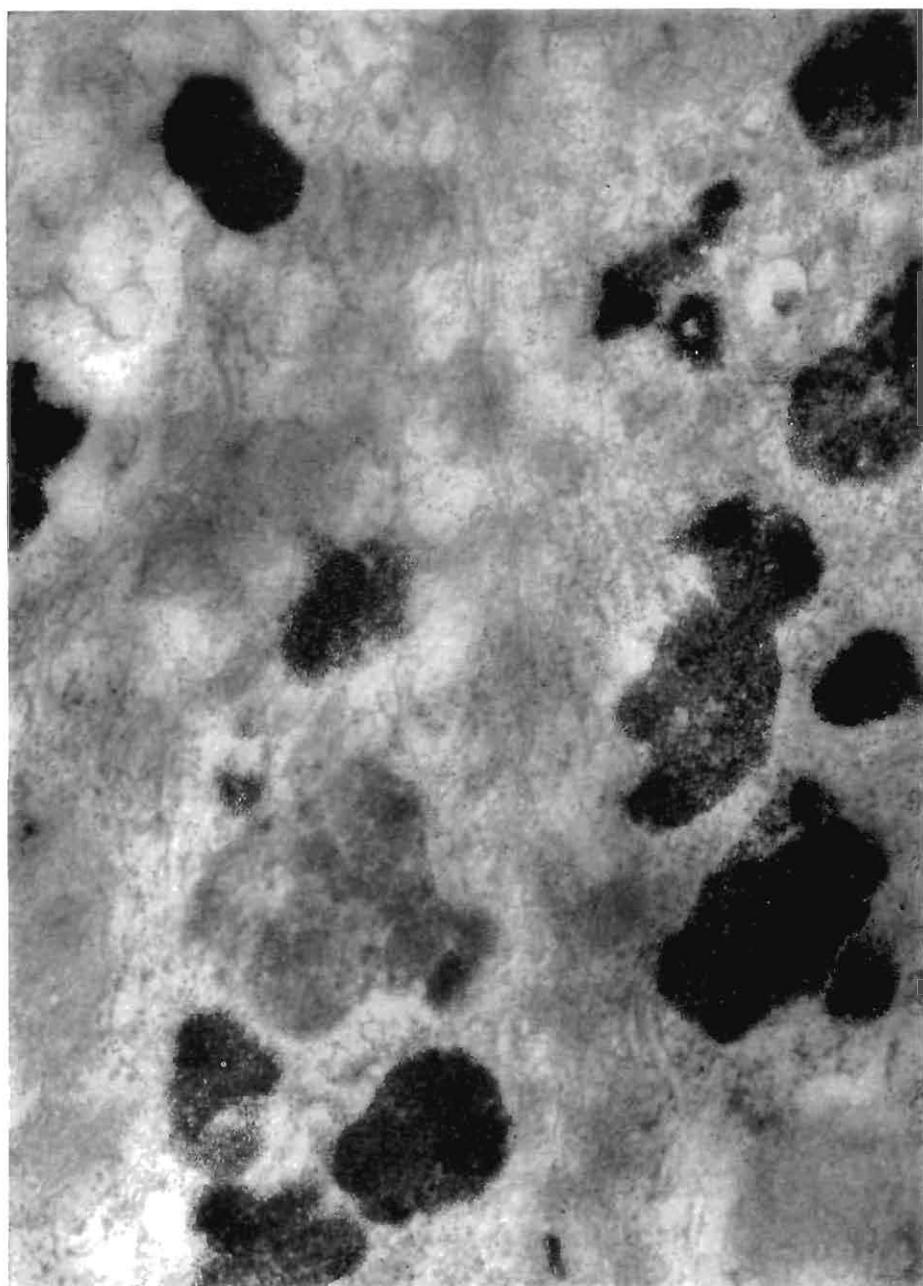


Fig. 151. "Siderosomes" in erythroblasts in a case of sideroachrestic anaemia. $\times 52,600$.
(Courtesy E. Mölbert.)

large aggregates and sometimes the ferritin granules are stacked in a pseudo-crystalline arrangement, as for instance in macrophages of the brain tissue after traumatic necroses; in this last case, the stacking arrangement comes from the impregnation of myelin structures (Hager, 1960a).

Cytosomes

Siderosomes are a special variety of cytosomes. This name was used for the first time by Schulz (1958b) to designate those cytoplasmic corpuscles in general, which appear in the most various types of cells, and are mostly round or oval in shape and distinguishable from the mitochondria mainly by their regular texture and their lack of cristae or tubules. The basic structure of the cytosomes is said to be similar to that of the microbodies, corpuscles of a generally oval shape, 0.3–0.5 μ in size, which have an equally finely granulated or a homogeneous texture and a simple outer membrane, 50 Å thick. Their relationship to the mitochondria will be dealt with later (p. 320). That the latter can, in some cases, represent the first stage in the development of the cytosomes, could be demonstrated on chick myeloblast cultures (Weissenfels, 1962a).

The cytosomes are evidently typical storage organelles, for they can absorb, besides iron, many other substances, predominantly proteins, which are liable to swell them up tremendously. In tissue cultures, their number depends to a large extent on the quantity of ingested nutrition protein (Weissenfels, 1962a). It is not always easy to distinguish them for sure from other similar granules. We know for certain that they are involved in several metabolic processes (e.g., Schiebler and Knoop, 1959; Schulz and De Paola, 1958; and others). But evidently their main feature is their capacity of storing absorbed substances, and perhaps the much described phagosomes (see, e.g., Straus, 1959a, 1961), which absorb for example phagocytized protein, may be interpreted essentially in the same way. Yet they are less likely to be identical with the lysosomes (p. 290) (see Trump, 1961).

Iron can be absorbed by the mitochondria too (Bessis and Breton-Gorius, 1957, 1960, 1962; Heilmeyer *et al.*, 1962; and others), appearing in the shape of ferritin granules or as the more diffused micelles ferrugineuses typically between the cristae (see also Bessis, 1959, 1961). It is possible that under the influence of the mitochondrial enzymes the iron components of the ferritin are transferred into the erythroblasts and incorporated there to the porphyrin (Heilmeyer *et al.*, 1962).

Pigments devoid of iron

Generally, however, metallic substances are mostly stored in the cytosomes, and their deposition in the mitochondria is very rare. For example tellurium, which gives a well-contrasted image in the electron microscope (Hager, 1960b), seems partly amorphous and partly has the appearance of fine needles within the cytosomes. Crystalline substances, such as silicate, are mainly stored in the cytoplasmic ground substance (Nemetschek *et al.*, 1961), but also frequently in the cytosomes; melanin, which appears in its first, morphologically observable stages in the Golgi apparatus (Fig. 133), is also stored in the cytosomes, and develops into the mature melanin granules there (e.g., David and Velhagen, 1961).

Where the lipogenic pigments, i.e., lipofuscin, ceroid pigment and haemofuscin and similar substances (Gedigk, 1958b; and others) are stored, is still unknown. They appear

predominantly in disturbances of the lipid and protein metabolism, for example in areas of haemorrhagic necroses of fat tissue (Gedigk and Fischer, 1958). As to their origin, they are accumulations of unsaturated lipids within protein-rich sections of the cytoplasm. The ceroid pigment is produced, for instance, after phagocytosis of fat-containing particles of decomposing cells, as is the similar "vitamin E deficiency pigment" which appears in the muscle fibres whenever the intermediary metabolism of unsaturated lipids is disturbed (Gedigk and Fischer, 1959). All these pigments are a mixture of lipids, lipoproteins, proteins and perhaps also certain carbohydrates (Stammler, 1959; and others). In the electron microscope, the granules are strongly osmophilic because of their lipid content and, in the liver for example, they are 0·2–0·4 μ in size. They can fuse and form larger corpuscles, up to 1·5 μ in size, which are well recognizable in the light microscope as "old age pigment". Whether they are derived from cytosomes, from mitochondria by a process of degenerative transformation (Gadrat *et al.*, 1960) or from the lysosomes (Essner and Novikoff, 1960), is still unknown.

Hyaline drops

Protein pellets can evidently be stored either diffusely in the cytoplasmic ground substance or in the cytosomes, whereby it is probable that larger drops have practically lost all trace of their possible cytosomic origin. These hyaline drops can also be derived, as mentioned before, from condensed protein-rich vacuoles. On the other hand, in the kidney epithelia, these drops are mostly protein resorbed from the primary urine; the protein is here transported in an intracellular system of canaliculi and stored in the cytoplasm (Miller, 1960). In tissue cultures the storage of protein is essentially the same (Gropp, 1960) but here again we do not know how this process is related to the cytosomes. Other protein drops may result from a secretory process, in which the actual secretion has been inhibited, thus leading to the formation of large drops; the Russell's corpuscles of the plasma cells, for instance, are obviously due to a kind of secretory obstruction (Apitz, 1940). This is indicated by the fact that the same picture can be found in many exocrine and endocrine glands (for ref., see Altmann, 1955a).

Glycogen and lipids

Glycogen and lipids are not stored in the cytosomes but generally directly in the cytoplasmic ground substance. Glycogen forms small granules, visible in the electron microscope; it is noteworthy that, in Gierke's disease, these are smaller in the nucleus than in the cytoplasm (Fig. 36) although they retain the same basic structure (Sheldon *et al.*, 1962). They can be demonstrated in the electron microscope by means of a lead impregnation technique (Drochmans, 1960; Porter, 1961) or by partial staining with Best's carmine in an ammoniated solution (Themann, 1960, 1961). Lipids are likewise found at any point in the cytoplasmic ground substance; there, small droplets gather and form larger ones, which can cause a marked swelling of the cell body. As a rule, it is possible to deduce, from the size of the fat drops, the duration and intensity of the process which brought about the fatty degeneration. It may be, on the one hand, an increased fat intake from the environment; a typical case of this is the fattening diet, in which the size of the fat cells is a rough yardstick for the nutritional condition (see, e.g., Beste, 1961). On the other hand, it may be an

insufficient energy supply and a diminution of the binding capacity of the cytoplasmic ground substance due to toxins, hypoxia or other disturbances of the organism (see, e.g., Deane, 1958). For, indeed, even under normal conditions, the cytoplasmic ground substance contains finely dispersed fats, as in certain parenchyma cells (see, e.g., Sinapius, 1961). As soon as the fat becomes visible in the light microscope or electron microscope, it is the sign of pathological conditions in the cell.

Whereas neutral fats have a tendency to gather and form large drops, phosphatides or the cholesterol and their esters form a number of little droplets, which may fill the whole cell. This is how, after extraction of the lipids, the honeycomb structure of the foam cells is formed, which can be found in the epithelium in the most varied cases of lipidosis and also in the connective tissue as pseudoxanthoma cells. The difference with the fat storage in large vesicles is so characteristic that one may, in regard to morphology, infer with certainty the previous content of the vesicles.

Virus replication in the cytoplasm

It has been known for a long time that plant cells have crystalloid protein deposits. Particular interest was devoted to the crystals of cacti (Molisch, 1885) which may appear in great number in the epidermis. They were long interpreted as storage material. Today we know that they are aggregates of virus particles (see, e.g., Milicic, 1960; Thaler, 1961).

Large virus aggregates may be also observed in nuclei (Fig. 38), yet this is a rarity. The majority of viruses multiply in the shape of much smaller corpuscles, either in the nucleus (p. 81), or more frequently in the cytoplasm. It is not rare to find a synthesis of the virus particles occurring only at the cell membrane. Many viruses fail to show anatomically any early intracellular stage of development, corresponding to the virus which is seen extracellularly in the electron microscope, and yet, they must have originated in a cell.

We must distinguish between two processes: (1) The origin and maturation of the viral particles; (2) The reaction of the cytoplasm to the virus disease.

Most cytoplasmic viruses originate in the cytoplasmic ground substance, frequently in several stages of development. The enteroviruses have been investigated thoroughly in this regard, as for instance the ECHO virus, type 9 (Rifkind *et al.*, 1961). In the earliest stages it appears to consist of small granules, gathered in compact masses and again, like the nuclear viruses (Fig. 37), in a crystalline array (Fig. 152a and b). Cytochemically, they contain RNA. In analogy to other cytoplasmic viruses, such as the yellow fever virus of the rhesus monkeys (Bearcroft, 1960) or the Mengo virus (Franklin, 1962), these early particles lie mainly in the vicinity of the nucleus and by no means do they always appear directly in the area of the

Fig. 152. Replication of the ECHO 9 virus in cell cultures of monkey kidney. (a) Electron micrograph showing dense granules (g) in the cytoplasm, which are located mainly in vicinity of the strongly indented nucleus (N); the arrows point to virus aggregates lying in a linear array. $\times 16,000$. (b) Electron micrograph representing light and dark virus aggregates and fibrils in the cytoplasmic ground substance. Note the cistern-like cavities of the distended endoplasmic reticulum (er) and the swollen mitochondria (m). $\times 43,000$. (c) Electron micrograph of virus groups between two cell walls. The arrow points to a group of viruses in the cytoplasm. $\times 92,000$. (From Rifkind, R. A. *et al.* (1961), *J. exper. Med.*, **114**, 1-12.)

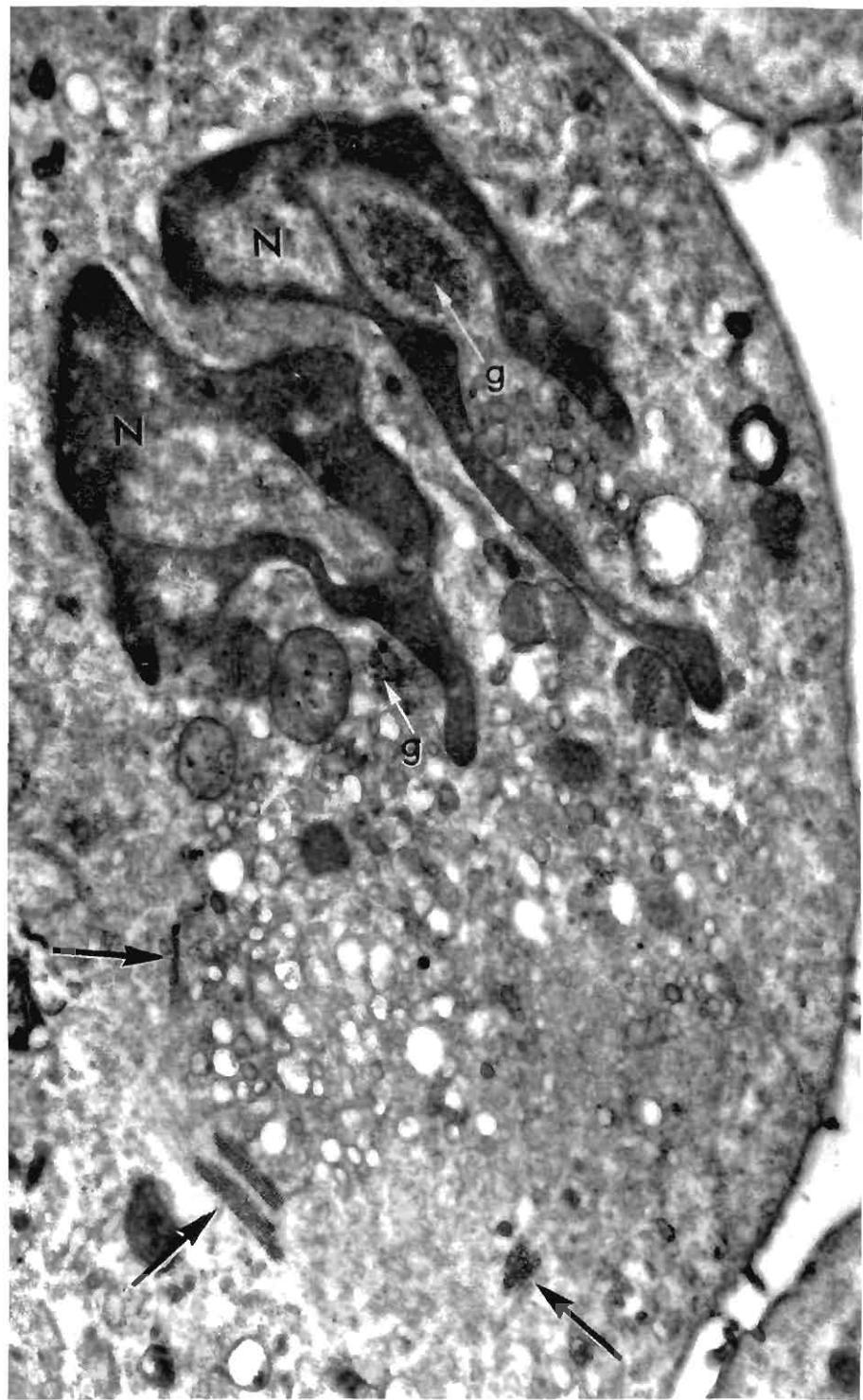


Fig. 152 (a)

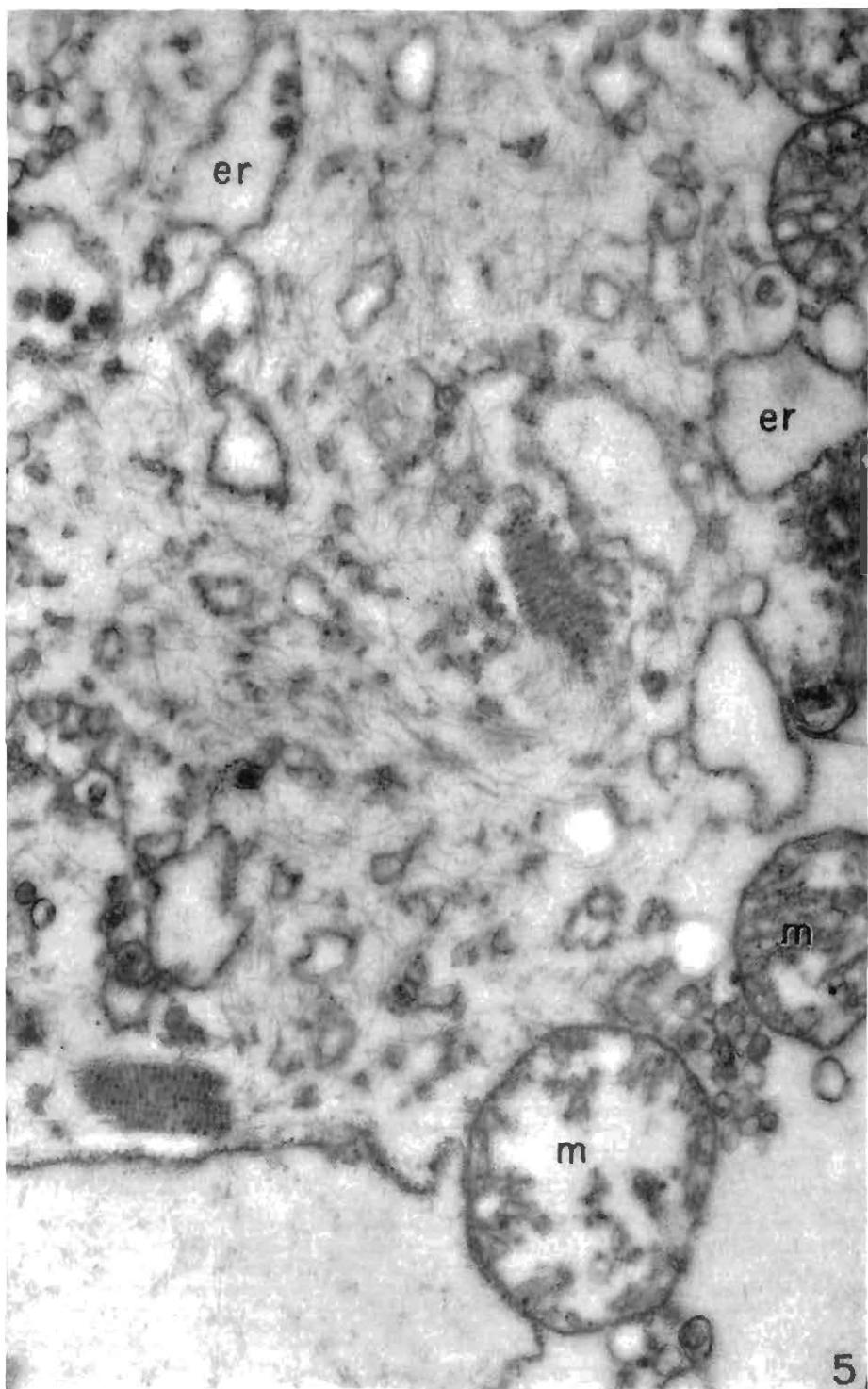


Fig. 152 (b)

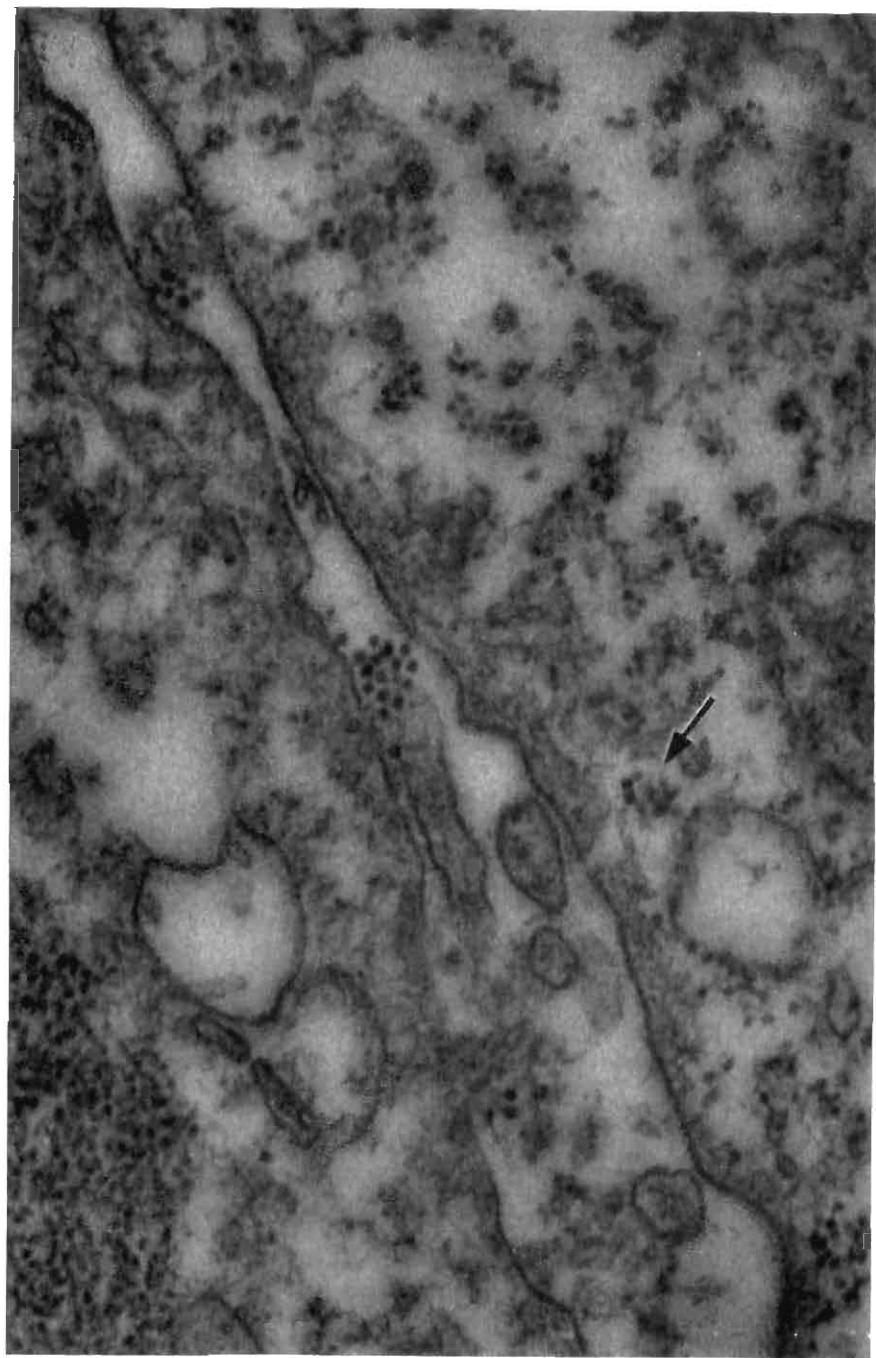


Fig. 152. (c).

likewise RNA-rich ergastoplasm. The primary reaction of the nucleus is an enlargement of the nucleoli, the folding in of the nuclear wall and a transfer of the chromatin to the border of the nucleus (p. 86); it corresponds, in most cytoplasmic viruses, to the reaction of the nucleus to nuclear viruses and must be interpreted, here also, as the sign of an intensification of nuclear function.

Long fine fibrils appear at the same time as the small particles of the ECHO-9 virus, and separate from each other the granules which are lined up in rows. In the cytoplasm there are more of these fibrils; they form a crisscross pattern having the appearance of a loose network. The small virus particles are partly dense, partly lighter inside and have a fine membrane (Fig. 152b). At a later stage, these groups of particles can build hexagonal figures, and each separate particle is surrounded by six fibrils, once again with a hexagonal shape.

Fig. 152c shows the cell membrane of two cells, both with a loose cytoplasmic ground substance and many virus particles grouped together, part of which were released from the cell through the cell membrane together with pieces of the finely granulated material adhering to them. These particles lie dispersed in the intercellular space. One group of virus particles, indicated by the arrow, lies directly under the wall of the right cell. Soon these cells burst and die, thus leaving the virus particles in masses free to infect other cells.

This type of virus replication of the ECHO-9 virus (Rifkind *et al.*, 1961) is only an example of the replication of cytoplasmic viruses. Like the nuclear viruses (p. 80), they too generally consist of two components, one of which is assumed to be protein and the other nucleoprotein. At any rate, the signs of cell injury are typical, namely, swelling and vacuolation of the cytoplasmic ground substance (see, e.g., Nunez-Montiel *et al.*, 1961), frequently accompanied by strong alterations in the mitochondria (Bearcroft, 1960; Chandra and Toolan, 1961; and others). In many cases it has been possible to observe how virus particles enter the cell. It starts with an adsorption at the cell surface and continues with a vesicular phagocytosis or pinocytosis (Dales and Siminovitch, 1961; Dales, 1962; and others). The receptivity of a cell for a particular virus depends primarily on the adsorption capacity, more precisely on the presence of specific lipoproteins of the cell wall, which may be also responsible for the eclipse, i.e., for the latent phase following infection (Syverton, 1961).

We can touch on the subject only briefly. Virus infection is a real disease of the cell, often ending with the cell's death. Yet, how many viruses are latent in the cells and wake up suddenly after a long period of time, we do not know. It also still remains to be elucidated how many of the particles described as virus particles are really authentic viruses or virus aggregates. At any rate, in most cases, the matrix for the development of the cytoplasmic viruses is the cytoplasmic ground substance in the direct vicinity of the nucleus.

Review

In addition to the part it plays in glycolysis, the cytoplasmic ground substance is the matrix of the synthetic processes which take place within the cytoplasm. Its normal picture in the electron microscope is not very impressive at first, yet it is subject to tremendous variations in morphology and function. It is mainly involved in the intracellular and intercellular water transport, a certain interior and exterior equilibrium of ions being necessary for its normal functioning, and in many pathological conditions this equilibrium is upset. As a

result of the intracellular water transport, we found cytoplasmic streaming and, in close connection with it, amoeboid movement, and also, under pathological conditions, an irreversible structural condensation (coagulative necrosis). Pathological hydration leads to the formation of vacuoles or even, if disintegration continues, to vesicular degeneration of the cytoplasm. The normal intercellular water transport, necessary for the cell's subsistence, occurs partly invisibly, partly through the absorption of small drops (pinocytosis). Since small and large molecules are absorbed too at the same time through the membrane and into the cell, the water transport is here a special variant of the active membrane transport. Uptake of foreign substances, whether for the purpose of detoxication or for the cell's nutrition, is performed by means of phagocytosis, which is followed by intracellular digestion. This is carried out mainly by hydrolytic enzymes which are present in large quantities in the lysosome fraction and perhaps even represent independent cytoplasmic organelles. Absorbed substances are stored diffusely throughout the cytoplasmic ground substance or condensed in special corpuscles, the cytosomes. Such is the case with many metals, for instance iron, which may be found, however, also in the mitochondria. The majority of pigments is stored in the cytosomes. Only carbohydrates and lipids are to be always found in the cytoplasmic ground substance, even under pathological conditions; but foreign proteins appear in the cytosomes, which may swell or fuse and form hyaline drops. A special case is that of the viruses of the cytoplasm, which develop in the cytoplasmic ground substance as foreign nucleoprotein-protein complexes which are able to destroy the cell.

MITOCHONDRIA

In the cytoplasmic ground substance we find, beside the structures we know already, those granules which Benda (1902) called threadlike granules or mitochondria because of their tendency to form threads. Benda had demonstrated them in all kinds of cells and recognized them as being essential components of the cell. Since then, many similar observations have been made, which broadened the scope of our knowledge by using special staining procedures (aniline-fuchsin picric acid, alizarin-crystal violet, iron haematoxylin etc.); however, these observations were based on non-specific staining reactions and are thus no longer important. The only exceptions were the vital dyes, such as the Janus green dye, used for the first time by Michaelis (1900); this dye was soon recognized as being specific for the mitochondria (Bensley, 1911; Bensley and Bensley, 1941; for further ref., see Cooperstein *et al.*, 1960), yet it never became widely used in cytology; for, the existence of the elements observed remained doubtful for a long time and their demonstration was regarded as an artifact.

The function of the mitochondria remained likewise obscure. Some workers assumed that they represented intermediary stages in the development of secretion granules, yolk pellets or fibrils (Meves 1918); others considered them to be the sign of bacterial symbiosis. Since they are apparently transferred from one cell to the other in mitosis, Benda (1902) had regarded them as possible carriers of the genes. Thus, the meaning of the mitochondria remained, up to the middle of our century, obscure to a large extent; mitochondria were also called chondriosomes and the entire system of mitochondria within a cell was termed the chondriome (Meves, 1907).

For no other cell component did the revelation come as suddenly as for the mitochondria.

After ultracentrifugation had made it possible to separate the mitochondria from the other cell components, as the fraction of large granules (Claude, 1944), it was soon discovered that they were the sites of biological oxidations (for ref., see, e.g., Hirsch 1955; Hogeboom *et al.*, 1957; Schneider, 1959; and others); thus, from the huge amount of differentiated findings on the function of the mitochondria, one fact was beyond doubt, viz., the central role that they play in the whole of the oxidative metabolism of the cell (see, e.g., Hackett, 1955; Brachet, 1957; Novikoff, 1961a; and others).

Following the discovery of ultracentrifugation technique, electron microscopy appeared as a cytological method, bringing light rapidly into the basic problems of morphology (Palade, 1952, 1953; Sjöstrand, 1954a; and others). It confirmed the fact that mitochondria are of universal occurrence in all cells. At the same time, however, it revealed that they have a specific structure, namely two exterior double membranes and a typical, albeit variable, interior system of membranes (for ref., see, e.g., Sjöstrand, 1956; Green, 1958; Rouillet, 1960; Novikoff, 1961a; and others). Consequently, mitochondria today may be defined on the one hand as cytoplasmic corpuscles having a specific electron microscopical structure, and on the other hand, as the main carriers of biological oxidations.

Representation in the light microscope

Benda's (e.g., 1902) description of the mitochondria as small granules, arranging themselves easily in chains, has been confirmed by many investigations made on living cells. Mitochondria are not rods of a fixed shape; they appear, *in vivo*, as being mostly threads of different length (Fig. 133) which often change shape, sometimes almost stretched out, sometimes undulated or rolled into a ball. They apparently also vary in length; several granular or rod-shaped particles can gather and form a thread and this thread may break up again into small elements (Gey *et al.*, 1954a; Frederic, 1954). Mitochondria are also liable to join end to end, end to side, or side to side; they may also join to form loops (Tobioka and Bieseile, 1956), in certain cases several microns long. They move here and there within the cell (Lettré, 1954; Chèvremont, 1956; Rose, 1957) and can apparently enter into direct contact with the nucleus, often precisely at the points where the nucleolus adheres to the inner wall of the nucleus (Frederic and Chèvremont, 1952; Frederic, 1958). This seems to indicate that the mitochondria have a movement of their own.

Light microscopical staining procedures, such as for example staining with iron haematoxylin after HCl hydrolysis, also reveal the most varied pictures. Mitochondria appear as granular or filamentous structures, frequently as large clumps; they may also disintegrate into elements almost as fine as dust (see, e.g., Altmann, 1955a). These are in most cases the result of mitochondria clumping together so that details are no longer discernible; in some cases, however, these pictures are just artifacts from overstaining. One may avoid such artificial aggregations by staining unfixed cells. This is possible for example with Janus green B. Its staining mechanism is largely known. Purified dehydrogenase systems are able to reduce Janus green, i.e., all those systems which are capable of transforming reduced flavoprotein back into flavoprotein (Lazarow and Cooperstein, 1953). It is true that these flavoproteins exist also in other cell components, but only in mitochondria do they appear together with enough cytochrome, that the dye may be reoxidized into its greenish-blue form. Thus,

regular intracellular conditions are necessary for a positive reaction; in isolated cell fractions the reaction is non-specific (Cooperstein *et al.*, 1960).

Since the Janus green B dye *intravital* frequently provokes only a very weak reaction and is often moody, attempts have been made to find other similar but more stable reactions. The nadi reaction is likewise based on the presence of oxidizing enzymes (Ehrlich, 1885), probably on the action of cytochrome oxidase (Nachlas *et al.*, 1958). The latter was varied many times in order to obtain more stable staining reactions (Burstone, 1959, 1960). Much use is made today of the tetrazolium salts, which are precipitated as formazan under the action of certain enzymes and give very clear pictures. These are reactions of the tetrazolium salts with the electron transport system of the mitochondria (p. 309), again in close connection with cytochrome oxidase, but also with the succinic dehydrogenase and the cytochrome C reductase (for ref., see Rudolph, 1960; Novikoff, 1961). This multivalence reduces the specificity of the formazan formation, since in the rat liver and pancreas, for instance, reductases are active also extramitochondrially. Moreover, formazan has a strong affinity for the surface of fat droplets, for example in the adrenal cortex (Nachlas, *et al.*, 1957) but also for the lipofuscin granules of the liver and for other lipid deposits (Novikoff *et al.*, 1961a). However, Novikoff and Essner (1960) succeeded, with cold formaldehyde calcium fixation, in staining DPNH and TPNH tetrazolium reductase almost electively, obtaining a fairly specific staining of the mitochondria. These can be demonstrated, for example, with DPNH as a substrate, very distinctly in the rat heart muscle between the fibrils (Fig. 153); yet again, they do not appear as single mitochondria, but only as aggregates.

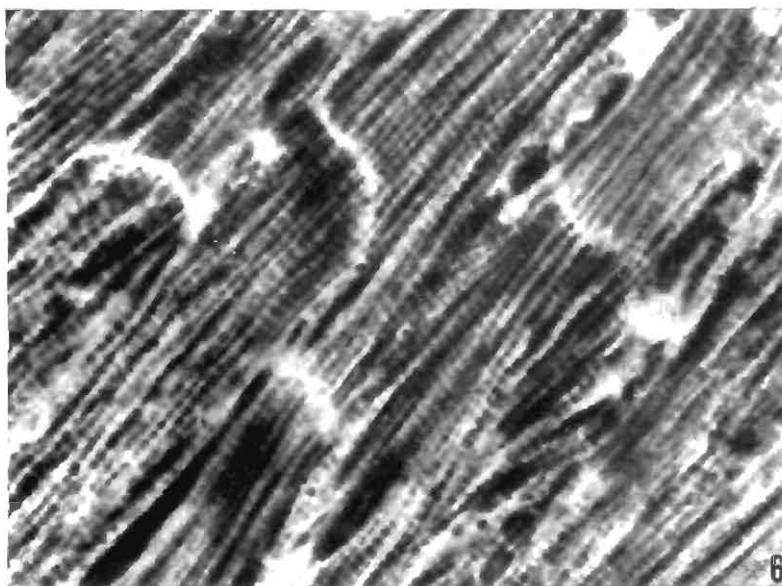


Fig. 153. Rat cardiac muscle. The mitochondria are stained with Nitro-BI and DPNH serving as a substrate. (From Novikoff, A. B. (1961), *The Cell*, Vol. II, Acad. Press, New York.)

Normal representation in the electron microscope

The first reliable criteria for the recognition and morphological definition of the mitochondria were given by electron microscopy (see, e.g., Palade, 1952; Sjöstrand, 1954a, 1956; Dalton and Felix, 1957; Green, 1958; Novikoff, 1961; Vogell, 1963; and many others). Yet, there is no one typical picture but a great many.

The basic structure is, however, common to all mitochondria; they are all bound by two membranes, the inner one of which shows many various infoldings. In the mitochondria of the kidney tubules, each of these membranes is approximately 40 Å thick and between both membranes there is a space of approximately 70 Å, so that the double membranes have a total thickness of approximately 150 Å (Sjöstrand, 1956). Their structure is comparable to a large extent with that of the unit membranes (Robertson, 1959), that is, they consist of two opaque layers and one less dense inner layer (Rhodin, 1954; Low and Freeman, 1958). Between the infoldings of the inner membrane, in the interior of the mitochondrial body, there is a finely granulated substance, the mitochondrial matrix, of a variable electron density.

The first results of electron microscopical thin-sectioning technique suggested that the inner double plates, called by Palade (1952) "cristae mitochondriales", are separate structures which adhere to the inner aspect of the outer double membrane (Sjöstrand, 1956). Meanwhile it has been ascertained (Low, 1956) that all these inner structures project from the inner layer of the outer membrane. These cristae mitochondriales are lamellae, which in the epithelial cells of the kidney tubule extend more or less parallel to one another. Thiel's model (1959) gives a rough idea of the resulting spatial arrangement in a mitochondrion (Fig. 154). However, such models should be regarded only as representing one moment of the mitochondrion's structural arrangement and should not make us consider the mitochondrion as a fixed body. Observations on living cells have brought evidence of the strong variability



Fig. 154. Model of a mitochondrion. (From Thiel, A. (1959), *Dtsch. med. Wschr.* **84**, 2038-2045.)

in size and shape of the mitochondria (p. 302). Therefore, the mitochondrion need not necessarily have the shape represented in Fig. 154. *In vivo*, many mitochondria appear as being long filaments, at least in tissue culture cells where they can be observed in the living state. It is improbable that the long mitochondria get fragmented by preparation, since mitochondria fixed with osmium and imbedded in methacrylate keep their shape as filaments (Borysko and Sapranaukas, 1954). The electron microscope picture is the result of many cross sections of the filaments and it mainly depends on the direction of the cutting plane whether the mitochondrion appears round, oval or long (Wessel, 1960). We have already mentioned the fact that many mitochondria may also be short rods or even pellets.

Both osmiophilic layers of the outer membrane seem to cross each other many times, that is, the outer boundary is formed alternatively by the one or the other membrane. Thus the cristae can, on principle, protrude from both layers, depending on which of them lies on the inside (Chandra, 1962). Moreover, in many pictures, the outer and inner layer seem to come to a dead end in the cytoplasmic ground substance, i.e., in the mitochondrial matrix. The inner processes are even more subject to variations. In many cells they extend perpendicularly to the longitudinal axis, as shown in Fig. 154. In other cells, for example in nerve cells or in certain spermatids (Powers *et al.*, 1956; Grassé *et al.*, 1956), they run parallel to the longitudinal axis and in kidney epithelial cells, partly longitudinally, partly transversely, partly also obliquely. In the interstitial cells of the opossum testicle, only fairly short villi (Fawcett, 1959) project into the matrix. Typical of the epithelial cells of mammalian adrenals and of other steroid secreting cells are tubules that are twisted many times around one another. They may be found likewise in the mitochondria of protozoans (Rudzinska and Porter, 1953; Belt and Pease, 1956) and of plants which have villous or lamellar inner structures (Hackett, 1955; Sunn, 1960; and others).

Besides these many variations in shape (see summary by Rouiller, 1960) there are also many differences in the number of cristae, villi or tubules, the clearest evidence being given by the muscle cells. Poorly active muscle fibres, such as the smooth, tonus-maintaining muscle fibres, contain relatively few mitochondria with cristae in a loose array (Bennett and Porter, 1953; Edwards and Ruska, 1955; and others). In active muscle cells, for instance, in the heart of mammals, or especially in the wing muscles of insects (Fig. 5), mitochondria are very dense and have many cristae or tubules lying closely one next to the other (Moore and Ruska, 1957; Vogell, 1963; and others).

The density of the matrix is generally inversely proportional to the number of membranous inner structures, whereby dense and less dense areas may lie directly next to one another. Sometimes electron dense granules are found in the matrix. This is typical for example of the kidney epithelia. They are thought to be related to intracellular cation exchange, but further details are unknown.

There is much discussion about whether or not other cell organelles can derive from the mitochondria. This transformation is quite certain in the case of the middle piece sheath of spermatozoa (Grassé *et al.*, 1956). Mitochondria are also the origin of certain paranuclei (p. 246) (Yasuzumi and Tanaka, 1958). Other transformations are improbable. The granules of the neutrophilic granulocytes, for example, are another kind of element, perhaps related to the lysosome (p. 290). Equally improbable is a transformation of mitochondria into plastids.

Biochemistry and function

Mitochondria are the carriers of the main respiratory enzymes. We noted this previously and we related the specificity of the Janus green B or the nadi reaction in light microscopical *intravitam* stainings to the activity of enzymes of oxidative metabolism, i.e., to electron transport (p. 302). Thus we have already defined the most essential part played by mitochondria in the metabolism. Let us now examine some detailed aspects of it.

Isolation of enzymes by electron microscopy

From the bulk of the foregoing, we see that we have, on one hand, a small group of cytochemical staining reactions, which make it possible, via relatively specific enzyme reactions, to isolate electively the mitochondria at the light microscopical level. On the other hand, electron microscopical thin-sectioning technique has revealed a finely structured system of membranes, which is so specific of mitochondria, that it enters into their definition. The next question is whether any enzymes are subordinated to this system of membranes and if so, what kind of enzymes.

The answer can be best found by combining the cytochemical reactions with electron microscopy. However, there are many difficulties to surmount, and the cytochemical procedures of light microscopy must be transformed in many respects, in order to make the final product visible in the electron microscope. These techniques are now in a stage of rapid development. Moreover, it is not always possible to infer for sure the nature of the enzyme involved; therefore, we shall examine here only a few early findings.

By *in vivo* administration of potassium tellurite (K_2TeO_3) or by incubation of small pieces of tissue with potassium tellurite, it was possible to demonstrate for example in heart muscle the presence of a reaction product reduced by dehydrases, probably by enzymes of the succinic dehydrogenase system, and thus to localize these activities at the membranous systems of the mitochondria using the electron microscope (Barnett and Palade, 1958). With tetrazolium salts, the same group of enzymes has been found at the same site (Sedar and Rosa, 1961), the nitroformazan deposits lying mainly on the cristae mitochondriales, and occasionally also at other points of the cytoplasm. The number of cristae with a positive reaction is relatively small. In the liver using the electron microscope it was possible to demonstrate a dehydrogenase activity (Théret and Jézequel, 1961).

In the cristae mitochondriales of heart muscle enzymatic activity which splits orthophosphate from glucose-1-phosphate was demonstrated with greater anatomical precision; this orthophosphate, in turn, can be precipitated at the site of the reaction with lead, that is, with an element of high electron density (Fig. 155). This activity is also found in the cell membrane and on small electron dense corpuscles (von Deimling *et al.*, 1960). There is a great deal of evidence to suggest that this is a muscle phosphorylase which may be found by ultracentrifugation in the supernatant and not in the mitochondrial fraction (Hers *et al.*, 1951).

Adenosinetriphosphatase has proved to be an enzyme which is not located on the membranes but electively in the mitochondrial matrix (Lazarus and Barden, 1962). The membranous systems of the mitochondria lack this enzyme although it appears on the membranes of the endoplasmic reticulum, most likely in the ribosomes (Fig. 156).

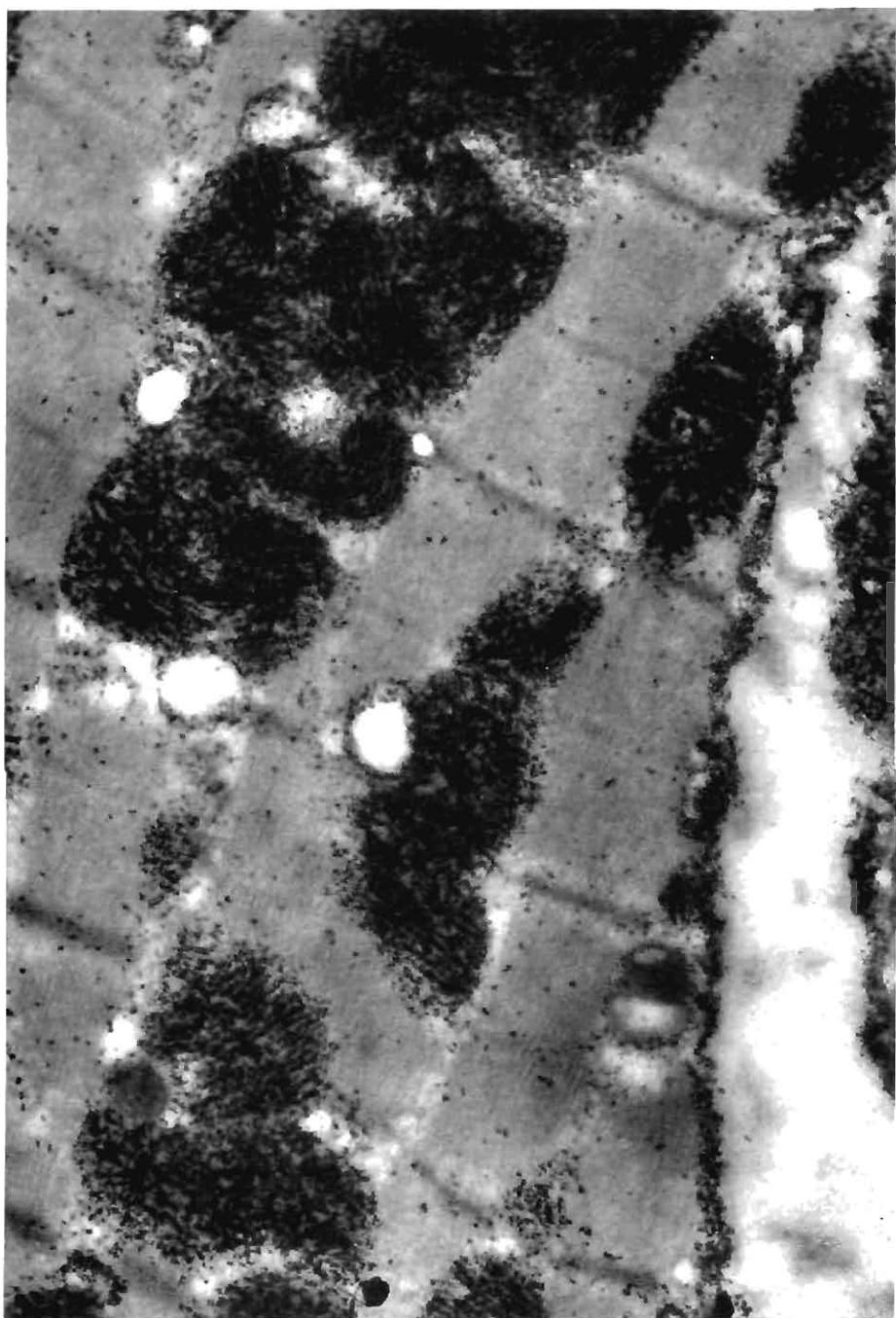


Fig. 155. Electron micrograph demonstrating, on the cristae mitochondriales of the rat heart muscle, a glucose-1-phosphate splitting enzyme. $\times 38,000$. (From Mölbert, E. et al. (1960), *Proc. Eur. Conf. Elektron. Micr.*, **160**, Vol. II, 635-650.)

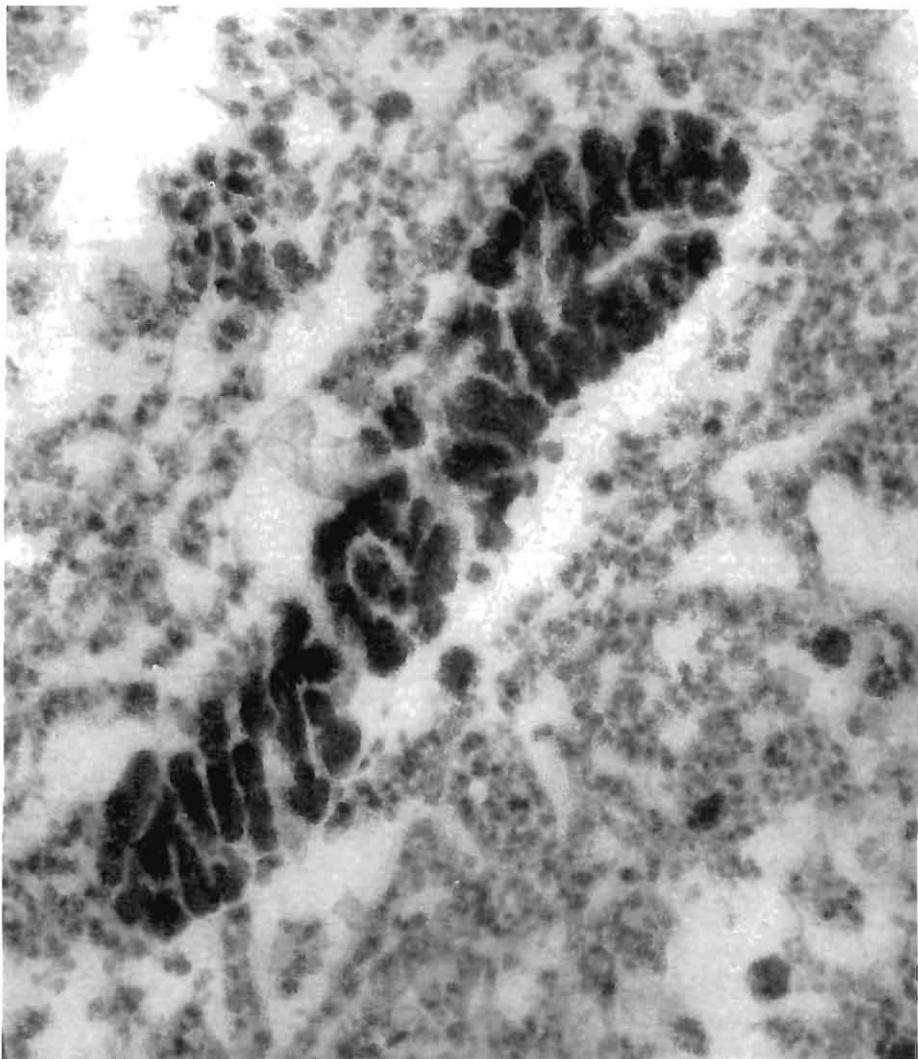


Fig. 156. ATPase activity between the cristae of a mitochondrion in an exocrine cell of the rabbit pancreas. Note the lesser activity in the ribosomes. (From Lazarus, S. S. and Barden, H. (1962), *J. Histochem. Cytochem.*, **10**, 285-293.)

Biochemical multi-enzyme systems

The techniques of enzyme biochemistry which are more advanced have provided the basis of our present knowledge concerning the functions of the mitochondria. During the past 20 years, many enzymes of the Krebs citric acid cycle have been found in the mitochondria (Schneider and Hogeboom, 1956; Schneider, 1959), i.e., a largely closed system of

multi-enzymes, called cyclophorase (Green, 1958). Some of these enzymes can be found in the supernatant; however, the whole cycle occurs only in the mitochondria (see Karlson, 1962). Further, mitochondria contain cytochrome oxidase and the whole respiratory chain, such as succinic dehydrogenase, DPNH oxidase and the cytochromes b, c and a. Mitochondria are, moreover, the main site of the coupling of phosphorylation with oxidation (Siekevitz, 1952); they are also the site of localization of those enzymes, which catalyze the oxidation of amino-acids (Claude, 1944) and fatty acids (Schneider, 1948; Green and Wakil, 1960; and others). Since the normal cell acquires its energy mainly by oxidation, mitochondria are the cell's power stations; they have, in regard to their enzymes, essentially the same structure in all cells (see, e.g., Klingenberg, 1963).

Oxidative phosphorylation and the electron transport depend on closely related multi-enzyme systems and it is likely that the enzymes are ordered along the membranes in a definite sequence, and that this sequence is of a great importance for the orderly functioning of the metabolic processes (Green, 1958; Lehninger, 1960; Green and Hatefi, 1961; Green and Oda, 1961). In suspensions of isolated mitochondria, the whole of the electron transport could be demonstrated from the substrate right up to O_2 via flavoprotein, cytochrome b, cytochrome c_1 , cytochrome c and cytochrome a (Chance and Williams, 1956).

As a general rule, the process of oxidative phosphorylation may be divided into three major reactions (Fig. 157). The first reaction is the transport of electrons from one carrier of a low oxidation-reduction potential (AH_2) to an oxidized carrier of a higher potential (B).

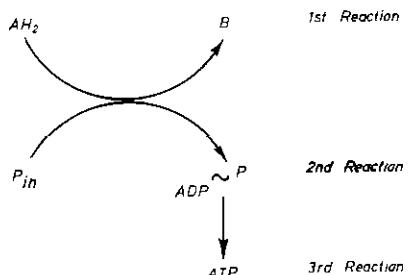


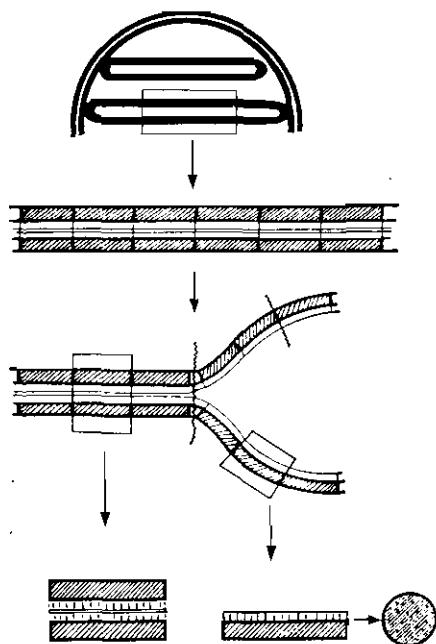
Fig. 157. The three main reactions of oxidative phosphorylation (see text).

This process paves the way for the second reaction, the transformation of an inorganic phosphate (P_{in}) into an energy-rich organic phosphate ($\sim P$). In the third reaction, the energy-rich phosphate group is transferred to adenosinediphosphate (ADP), thus producing adenosinetriphosphate (ATP). All these reactions take place in the mitochondria (see also Green, 1963).

The ATP produced in the mitochondria is the source of energy of many reactions which take place in the cytoplasm and inside the mitochondria as well; for example, the synthesis of the mitochondrial phosphatids (McMurray *et al.*, 1957) and proteins (Bates *et al.*, 1958), also probably of fatty acids (survey by Schneider, 1959). Possibly protein synthesis in the cytoplasm, which is the main function of the ribosomes and the endoplasmic reticulum (p. 249), depends on the energy production of the mitochondria. Moreover, amino-acids are in certain instances synthesized in the mitochondria, which contain a series of the

necessary enzymes, such as several transaminases (Hird and Roswell, 1950), glutamic acid dehydrogenase (Hogeboom and Schneider, 1953), glutaminase and other enzymes (for ref., see Novikoff, 1961a). Mitochondria are also likely to be involved in the synthesis of porphyrin and haeme (Sano *et al.*, 1958).

Certain lipoproteins, such as the coenzyme Q or cytochrome c play a key role in the processes of electron transport. They are probably inserted between the protein-rich elements of the respiratory chain (Karlsson, 1962), in the systems of membranes of the mitochondria. We must picture such a membrane as a polymer of several thousands of subunits, each of which contains a complete respiratory chain. And, indeed, it has been possible to break up the membranes of the mitochondria into such small subunits (Green and Hatefi, 1961); yet it appears that the smallest unit which performs electron transport and couples it with ATP synthesis (see Fig. 157) has itself a complicated structure. Since mitochondrial membranes are, from electron microscope evidence, double lamellae (p. 304), the smallest function unit of the electron transport is probably merely one partner of this double lamella;



but, for the coupling of electron transport and ATP formation, a double unit, that is, a portion of the double membrane, is necessary (Green and Oda, 1961). Fig. 158 gives a schematic representation of the division of mitochondrial membranes into such double units and shows how by separating the membranes from each other, it is possible to separate the electron optical units and, consequently, to inhibit ATP formation. However, whether it is actually true that both electron optical membranes as protein layers (in Fig. 158 obliquely hatched) are separated practically only by two layers of lipids (in Fig. 158 white or cross-hatched) is open to question.

Fig. 158. Schematic representation of separation of coupled mitochondrial units. (From Green D. E. and Oda, T. (1961), *J. Biochem.*, **49**, 742-757.)

Morphological evidence of the mitochondrial function

It is much more difficult to find any morphological evidence of these manifold functional relations that the mitochondria maintain in nearly all metabolic processes in the cytoplasm, by virtue of their key position in the oxidative phosphorylation and electron transport. In fact, the mitochondria are situated within the cytoplasmic ground substance, and thus, in the

matrix of all cytoplasmic synthetic processes; moreover, via possible pores in their membranes and by virtue of their capacity to exchange fluid and material with their environment (see, e.g., Borst, 1963), they are morphologically closely connected with all the processes within the cytoplasmic ground substance. Their motility (p. 302), of which practically nothing certain is known to this date, enables them to migrate to all structures whose metabolism needs their action. Such is the case, for example, with the regeneration of the endoplasmic reticulum, which, after partial hepatectomy, takes place electively in the direct vicinity of the mitochondria (Bernhard and Rouiller, 1956), or with neurosecretion in the neurohypophysis, which equally shows a topographical relation to the mitochondria (Bargmann *et al.*, 1957). Similar observations made in the rabbit submandibular organ have led to the conclusion that there is even a transfer of prosecretion from the inside of the mitochondria into the cytoplasm (Kurosumi *et al.*, 1961). Intramitochondrial alterations, too, have been reported to be connected with the secretion mechanism. This is the case, for example, in the adrenal cortex. When activated by the adrenocorticotropic hormone of the hypophysis, it causes not only a multiplication of the number of mitochondria but also an increase in size with accentuation of their vesicular structure (Ashworth *et al.*, 1959; Mölbert and Arnesen, 1960). The zona fasciculata or zone of the most intense steroid synthesis is already under normal conditions an area where the mitochondria are the largest and also the most thickly settled with vesicular elements (Sabatini and de Robertis, 1961).

We already mentioned (p. 305) the direct connection existing between the size and number of the mitochondria (or rather, the number of cristae) on one hand, and their metabolic activity in relation to smooth and striated muscles, on the other. The largest mitochondria, having probably the largest surface area of inner membranes, are to be found in locust wing muscles (Fig. 5), where the mitochondria, with highly twisted tubules, are tightly packed between the tracheoles and the muscle fibrils. This association provides the shortest possible connection between the aerogenous oxygen of the tracheoles, the energy-producing membranes of the mitochondria and the energy-consuming muscle fibrils (Vogell, 1963). Mattisson and Birch-Andersen (1962) have established in invertebrates a close correlation between the number and inner differentiation of the mitochondria on one hand, and the capacity of the cytochrome system on the other.

The relation to fat metabolism seems to be indicated, morphologically, by a particularly frequent accumulation of mitochondria on the surface of intracytoplasmic fat drops, especially noticeable in the mammalian liver and in the guinea-pig pancreas, after starvation (Palade, 1958). It is doubtful whether mitochondria are able to transform themselves directly into fat drops (Duncan and Hild, 1960). There is also some disagreement on whether or not mitochondria are involved in the formation of intracytoplasmic protein drops. It is quite certain that in kidney epithelia which have absorbed many protein drops, the number of mitochondria (ref., see Altmann, 1955a) and, concomitantly, the activity of the succinic dehydrogenase (e.g., Wallace, 1960) is markedly reduced. The striking abundance of mitochondria in the kidney epithelia (Rhodin, 1958) has, no doubt, its origin in the function of these cells, even though excretion in the rat kidney is not accompanied by any major changes in the electron microscopical structure of the mitochondria (Rollhäuser and Vogell, 1957).

Pathological changes

Swelling

Intensification of the cell's activity can, evidently, also cause a damage to the chondriome. For example, after intensive stimulation via the peripheral nerves, reduced thiols appear in the cerebral cortex of the rat, with swelling of the mitochondria and a reduction of the activity of succinic dehydrogenase and NAD diaphorase. After regeneration of the cerebral cortex, the activities of these enzymes return to normal (Portugalov *et al.*, 1962). As a rule, swelling is the most frequent reaction of the mitochondria, perhaps even the most frequent reaction of the cell to injury in general. In the hypophysis of teleostei, it is evidently a normal process (Bargmann and Knoop, 1960). However, we must regard it, in most cases, as the sign of a relatively minor cell damage, known for a long time as cloudy swelling (Ernst, 1915; Zollinger, 1948c; Cameron, 1952). An analogue of this cloudy swelling may be observed *intravitam* in the phase contrast microscope; as a consequence of the lack of oxygen and nutrition connected with the processing of the tissue, one can see the previously finely granulated mitochondria forming small vesicles, which are liable to fill the whole cell (Fig. 159), finally giving the cytoplasm a honeycomb-like structure (Altmann, 1955a).

This swelling corresponds in the electron microscope to a rounding up of the section plane of the mitochondria, which produces a more than threefold increase in surface area

(Schulz *et al.*, 1956). The swelling brings about a retraction of the cristae which project then frequently only as small stubs into the inside of the mitochondrion (Fig. 160). Often the cristae dissolve, and the mitochondria then seem empty, especially if the matrix too is dissolved becoming noticeably lighter in colour. In other cases, it remains as a finely granulated material or even becomes denser (Braun, 1958).

The causes of such a swelling of the mitochondria in the cell are manifold. They range from an extreme nutritional deficiency (e.g., Gansler and Rouiller, 1956; David, 1960b) through all the

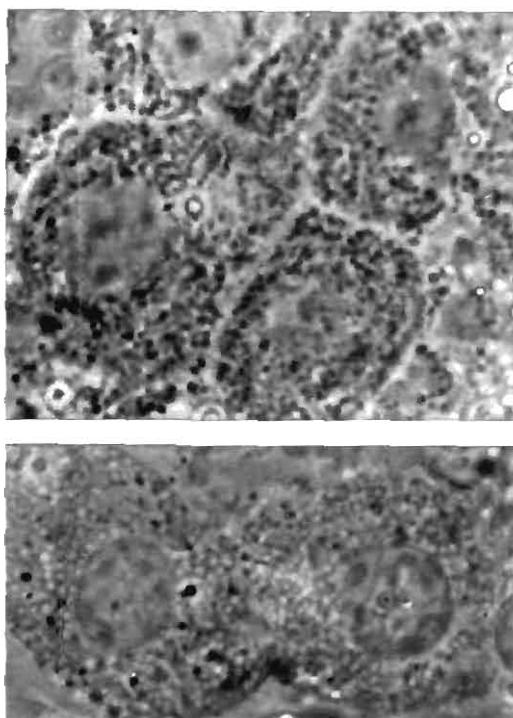


Fig. 159. Phase contrast micrograph showing swelling of mitochondria in epithelial cells of the white rat liver after 1 hour immersion in tap water. (From Altmann, H.-W. (1956), *Hdb. allg. Path.*, Bd. II/1, 419-612, Springer, Berlin.)

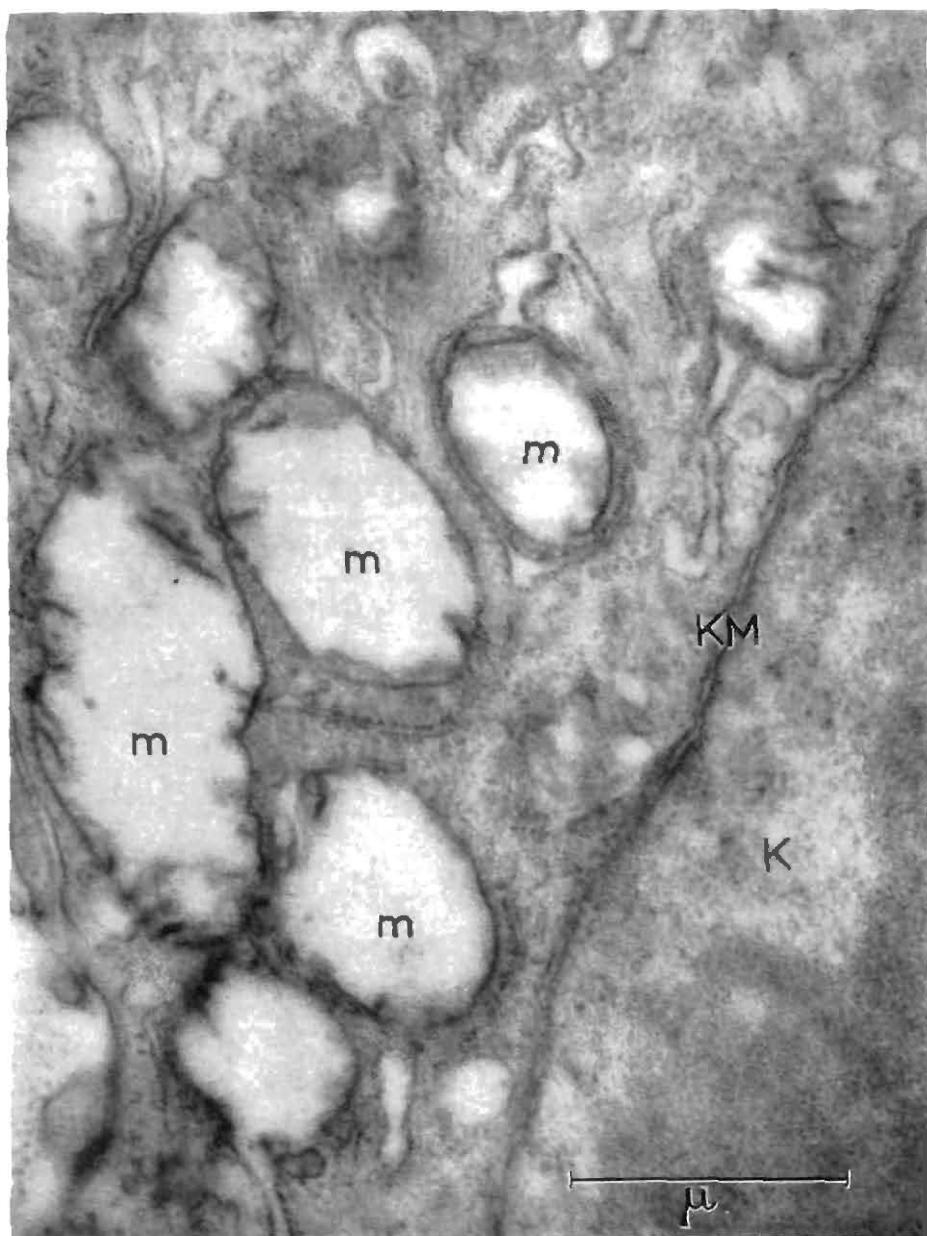


Fig. 160. Electron micrograph of mitochondrial swelling in tumour cells. The cristae remain as mere stubs. $\times 35,000$. (From Bernhard, W. (1957), *Klin. Wschr.*, 35, 251-261.)

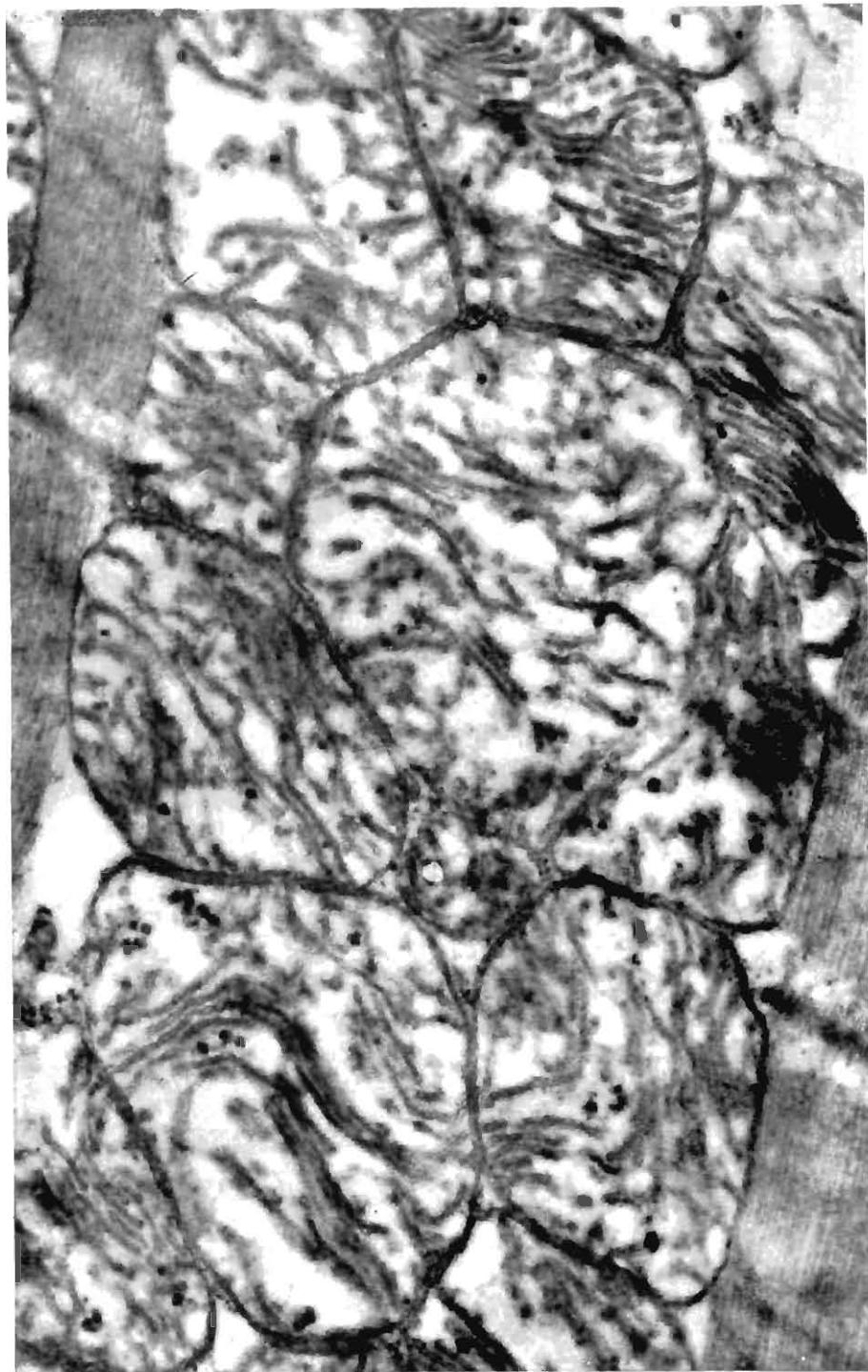


Fig. 161

various intoxications (e.g., Poche, 1958; further ref., see Rouiller, 1960) to hypoxia; in this last case, the swelling of the mitochondria with concurrent translucency of the matrix is particularly characteristic, as for example in the liver or the alveoli of the lung (Mölbert, 1957a; Mölbert and Guerritore, 1957; Schulz, 1959; Hübner and Bernhard, 1961). In cardiac muscle (Fig. 161) the same may be found (Bryant *et al.*, 1958; Büchner *et al.*, 1959) as also after experimental hypertrophy of the rabbit heart, in which it is the sign of an overstrained and eventually insufficient mitochondrial function (Mölbert and Iijima, 1958). This observation has thrown a new light on the disturbances in the metabolism of the myocardium, typical of coronary insufficiency (Büchner, 1959b). A disturbance in the mitochondrial function might also be responsible for a failure of the kidney tubules, where the same changes also occur (Rotter *et al.*, 1962). Our review of the factors causing a swelling *in vivo* is far from being exhaustive (for further ref., see Altmann, 1955a; Rouiller, 1960; David, 1961; Novikoff, 1961a), but additional causes are Roentgen irradiation (Braun, 1958, 1960), administration of dextran (Gabler, 1960) and overdoses of thyroxine (Poche, 1957).

All these alterations are reversible, as long as the outer membrane of the mitochondria remains intact. In the regenerative process after an injury, the mitochondrial matrix first condenses and, progressively, normal cristae form again either through new infoldings of the membranes, or through apposition of matrix material.

Biochemical alterations

One fact is important for our understanding of the whole swelling process, viz., that the earliest alterations evidently take place on the cristae. The osmiphobic layer of the cristae expands and small vesicles appear between the osmophilic layers, which fuse and partly destroy the cristae (Luft and Hechter, 1957; Lever and Chappell, 1958; and others). This is precisely the process which, according to Green and Oda (1961), leads to the dissolution of the smallest functional units of the membrane systems (Fig. 158), thus separating the respiratory chain from phosphorylation (see Fig. 157). The uncoupling of respiration from oxidative phosphorylation is one of the most discussed effects of thyroxine (Martius and Hess, 1951) and it is thyroxine which causes the primary fragmentation of the cristae and the typical swelling of the mitochondria (Poche, 1957).

Many biochemical investigations have been undertaken to obtain a more precise knowledge of this swelling process. They have led essentially to the conclusion that the intramitochondrial mechanism which normally checks the inflow of water into the body of the mitochondria, depends on the energy supply, in other words, that a normal oxidative phosphorylation is indispensable to a normal mitochondrial structure (Lehnninger, 1960; Emmelot *et al.*, 1960). On the other hand, as we can clearly see in Fig. 158, the swelling of the cristae in turn causes a definite disturbance in oxidative phosphorylation (see also Ernster, 1959). Thus, we have a vicious circle here. Energetic insufficiency causes the cristae mitochondriales to swell, which, in turn, inhibits oxidative phosphorylation at the site of the swelling. We are faced here most vividly with one of the general principles of biology, that the function of the cell

Fig. 161. Electron micrograph of the rat heart muscle showing swelling of mitochondria under carbon tetrachloride. $\times 68,000$. (From Büchner F., *et al.* (1959), *Beitr. path. Anat.*, 121, 145-169.)

and of its elements maintains the structures and, vice-versa, the integrity of the structures is the necessary condition for the good functioning of the cell.

Further, swelling and reconstruction of the mitochondria have been used as typical examples to elucidate mitochondrial oxidation mechanisms. It appears that the swelling *in vitro* is also possible with an intact respiratory chain (Chappell and Greville, 1960) and that cyanide or other inhibitors of respiration check the swelling brought about by phosphate, thyroxine or calcium (Hunter *et al.*, 1956; Lehninger *et al.*, 1959; and others). On the other hand, ATP can bring about a contraction of the mitochondria with a measurable efflux of water (Chappell and Perry, 1954), especially in a buffered potassium chloride medium, less so in sucrose (Lehninger, 1961). These contraction mechanisms of the mitochondria induced by ATP are to a large extent independent of respiration and phosphorylation and also occur in the presence of respiratory inhibitors such as cyanide or dinitrophenol (Lehninger, 1960). They are probably the effect of a contractile protein in the outer membrane of the mitochondria, or maybe of phosphoprotein components (Lehninger, 1961). The latter are also probably involved in the swelling process; for, according to Judah's observations (1960), phosphoprotein turnover and mitochondrial swelling are quantitatively parallel and the phosphoproteins of the mitochondria take their phosphorus directly from the oxidative phosphorylation system (Ahmed *et al.*, 1961).

Several other factors are thought to be responsible for the swelling and contraction processes. These are a soluble ATP-ADP exchange enzyme as well as several factors designated by the letters M, C, R and U (Lehninger, 1961). The last-mentioned factor is thought to have a fatty acid composition and bring about the swelling of the mitochondria in the presence of Ca^{++} ions or thyroxine (Wojtczak and Lehninger, 1961). The effect of osmosis on the swelling of the mitochondria (Tedeschi 1961; for older ref., see Altmann, 1955a) has probably nothing to do with all these factors; it happens to the mitochondria as it does to all other bodies surrounded by a semi-permeable membrane.

The energetic factors of mitochondrial swelling are the mitochondrial coenzymes, mainly NAD (DPN). This coenzyme is very tightly attached to the cristae mitochondriales and its absence is a very sure sign of mitochondrial injury (Hunter and Ford, 1955). Every swelling of the mitochondria facilitates this loss of coenzymes (Kaufman and Kaplan, 1960); this, in turn, makes the energy supply more difficult. Once again there is a vicious circle in the same sense as in the previously mentioned close relationship of structure and function (see Siebert, 1961).

We must also note that mitochondria in malignant tumours have an essentially normal structure (Novikoff, 1961; Oberling and Bernhard, 1961) and a normal set of enzymes (e.g., Aisenberg, 1961*a* and *b*). However, they are often smaller and especially more labile and their NAD seems to adhere more loosely to the structures (see, e.g., Siebert, 1961). The swelling of isolated tumour mitochondria, which is easier to induce in certain instances than in normal mitochondria (Arcos *et al.*, 1960; and others) is, however, less readily reversible (Hawtrey and Silk, 1959), whereas, on the other hand, hepatoma mitochondria swell in the presence of thyroxine much less than normal liver mitochondria (Emmelot *et al.*, 1960).

The fact that tumour cells do not lack any of the enzymes of the biological oxidations and that there is only occasionally a slight diminution in the number of the normally super-

abundant respiratory enzymes (see e.g., Siebert, 1961), contradicts Warburg's observation (1926, 1955) that, in the cells of malignant tumours, anaerobic glycolysis is largely predominant. We know for certain that ascites tumour cells, for example, have an intact respiratory chain and are capable of a normal phosphorylation. Respiration of the tumour cells is not impaired (Weinhouse, 1955) and the structural relations of their enzymes are normal (Chance and Hess, 1959*a* and *b*). Perhaps the predominance in tumour cells of glycolysis over respiration is due to the reduction of the intracellular ADP concentration (Novikoff, 1961*a*).

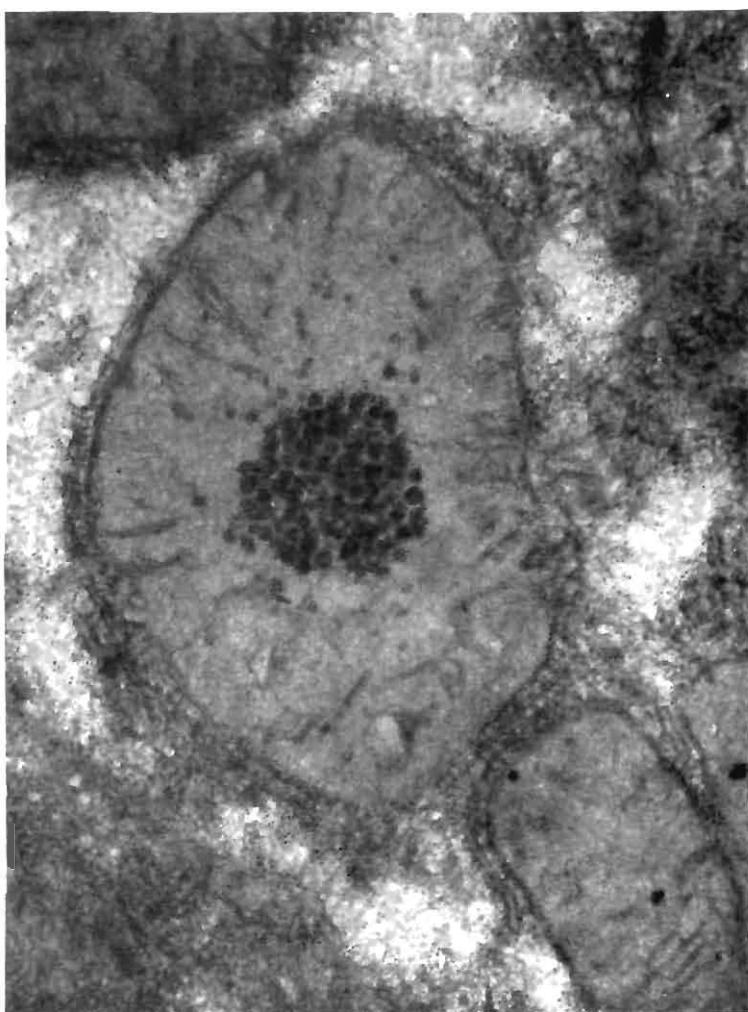


Fig. 162. Granules in a mitochondrion of the mouse liver at refeeding after fasting. (From David, H. (1961), *Acta biol. med. germ.*, 7, 311-321.)

Other structural alterations and destructions

The swelling is not the only pathological alteration of the mitochondria. Quite often there are well-defined or diffusely scattered deposits, some of which we have mentioned already (p. 306). We have also mentioned the electron dense granules, which are particularly numerous in renal epithelial cells (p. 305). Similar deposits are to be found in the duodenal epithelial cells after intake of food rich in potassium or sodium (Weiss, 1955). Intramitochondrial bodies also appear in the skin after exposure to carcinogens, as a result of cytotoxic damage to the cell (Nakai *et al.*, 1962). After intraperitoneal injection of silica gel, finely granulated deposits of silicic acid appear in the rat kidney between the cristae mitochondriales; they can swell up and form structures as large as 1200 Å (Policard *et al.*, 1961). The normal intramitochondrial granules of the matrix may, for instance in the mouse liver after fasting and refeeding, be replicated in the shape of numerous coarse granules (David, 1961); these may then be mistaken for virus inclusions (Fig. 162). A diffuse and almost even increase in substance of the matrix can be found in the liver after total body irradiation (Braun, 1958) and can be produced artificially at an elevated pH in the fixed preparation.

Schulz (1958a, 1959) described a peculiar transformation of the intramitochondrial structures in the alveolar epithelium of the lungs. Its origin is related to an increase in the CO₂ content of the ventilated air in respiration. It starts with a swelling of the osmiphobic interspace between the cristae mitochondriales, whereby the osmophilic layers of the neighbouring cristae lie passively adjacent to one another and grow larger and larger until they form broad lamellae, up to 200–700 Å in width, that give a variable contrast (Fig. 163). This lamellar transformation is an irreversible degenerative process with dissolution of the outer membranes (see arrows in Fig. 163) and, ultimately, decomposition of the mitochondria. The agglutination, in the adrenals, of cristae mitochondriales into bands, following a formalin stress, is maybe an early form of this type of degeneration (Mölbert and Arnesen, 1960).

Mitochondria in old tissue cultures of chicken heart myoblasts often display exceptionally long cristae, which extend longitudinally in an undulating pattern and can coalesce at many points (Weissenfels, 1961). In markedly enlarged mitochondria they can surround an empty centre like a necklace and are probably the result of an excessive growth of the cristae. Similar concentric layers of cristae can be found under other conditions, for instance in the rat heart in experimental myocardial infarction (David, 1961). A special change in shape is like that of a key that certain mitochondria assume, whereby both ends become thicker, the mitochondria stretch in length and become excessively narrow in the middle (Munger, 1958; David, 1961; David and Kettler, 1961). Many of these alterations result in a degeneration of the mitochondria and the dissolution of the outer membranes too, whereby the matrix forms either electron dense rings or clusters (e.g., Bargmann and Knoop, 1956). The mitochondria can also break apart (David, 1961) or, after dissolution of the outer membranes, the cristae can lie scattered throughout the cytoplasmic ground substance (Lever, 1956). It is possible that mitochondria may also be dissolved in the vacuoles of the Golgi apparatus. At least, this is how one may interpret the cytolyosomes which are, like the lysosomes, rich in acid phosphatase (p. 290) but still show remnants of the mitochondrial inner structures (Novikoff and Essner, 1962).

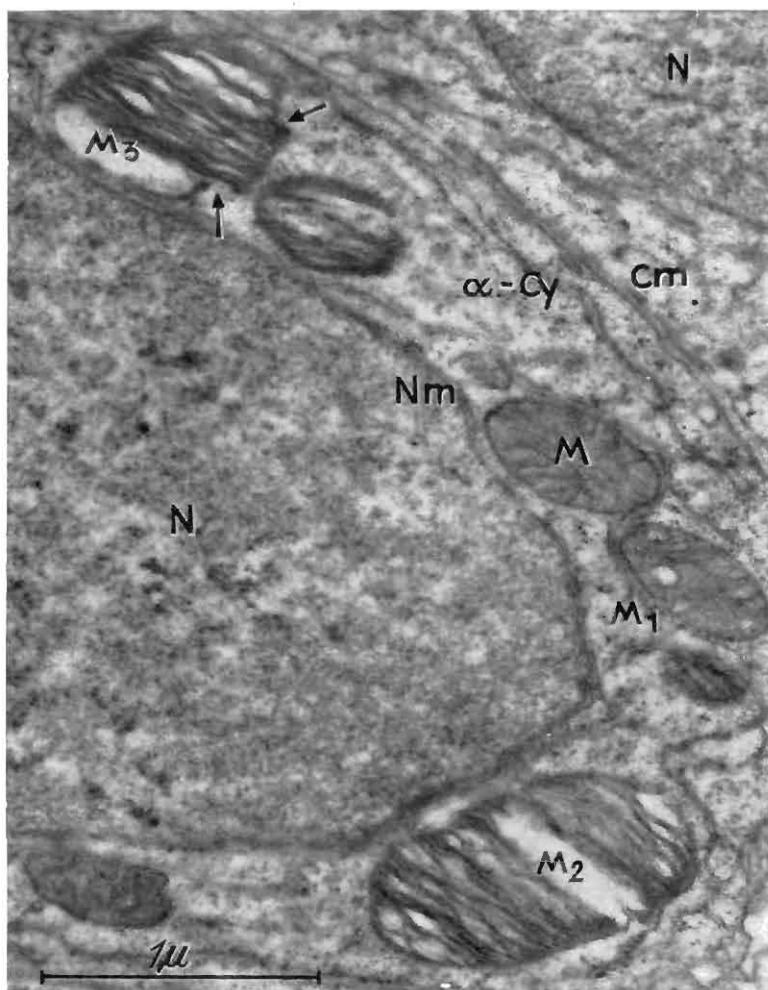


Fig. 163. Different stages (M , M_1 , M_2 , M_3) of the lamellar transformation of the mitochondria in the lung of the rat embryo. (From Schulz, H. (1958), *Beitr. path. Anat.*, **119**, 45-70.)

The problem of regeneration

The destruction of the mitochondria is sometimes followed by the death of the whole cell. In many cases, however, the cells survive and the chondriome regenerates. The details of such a regeneration are still not fully understood. The regenerating capacity of the chondriome is quite remarkable. Centrifugation of *Arbacia* eggs, for example, yields, after breaking of the eggs into two halves, egg cells which do not contain any mitochondria in the first cleavage division, at least not at a light microscopical level. There are no mitochondria in the

blastula either. But at the gastrula stage, Janus green dye stains perfectly normal mitochondria (Harvey, 1953). These observations have often been considered proof that mitochondria are manufactured anew. However, electron microscopy has revealed that the parts of *Arbacia* eggs which seem free of mitochondria in the light microscope, contain mitochondria (Gross *et al.*, 1960). None of the other findings suggesting a *de novo* formation of the mitochondria is valid (Novikoff, 1961), so that this theory has been practically abandoned today (Rouiller, 1960).

Most probably mitochondria are able to divide and indeed phase contrast microscopical observations *in vivo* have brought a great deal of evidence to support this fact, especially for instance in connection with mitosis (Chèvremont and Frédéric, 1954; Wohlfarth-Bottermann, 1957). The chondriome is a most labile system, probably continuously involved in functional transformation (p. 301).

On the other hand, practically all cell elements have been regarded as precursors of newly generated mitochondria: the membranes of the endoplasmic reticulum or of the Golgi apparatus (Lever, 1956), the cell wall, the nuclear wall (Hoffman and Grigg, 1958) and also the nucleoli (Ehret and Powers, 1955). A nuclear origin is not so impossible to conceive, especially since in trypanosomes, for instance, a body appears called the kinetoplast or kinetonucleus, which consists of a combination of nuclear and mitochondrial material (Steinert, 1960). The radioautographic findings of Chèvremont and Baekeland (1960) and of Chèvremont *et al.* (1960), in cultures of chicken embryo fibroblasts are also of great interest. They showed that after treatment with desoxyribonuclease a Feulgen positive DNA appears in the mitochondria which can incorporate ^3H -thymidine. Of course, these experiments do not indicate what kind of rearrangements are effected by the desoxyribonuclease. From these findings, it is impossible to infer either a mitochondrial capacity to synthesize DNA (p. 98) or an authentic relationship between chondriome and nucleus.

Following partial hepatectomy, or intoxication with carbon tetrachloride or other poisons, a great abundance of small corpuscles of a more or less round shape appear in the liver; they have a relatively high electron density (Gansler and Rouiller, 1956). They were first described in the kidney (Rhodin, 1954) and called microbodies (see Fig. 4). During the regeneration subsequent to partial hepatectomy, they form cristae-like internal structures; thus they were thought to be precursors of authentic mitochondria (Rouiller and Bernhard, 1956; Weissenfels, 1958a, 1958b; Rouiller, 1960). Dense corpuscles quite similar to these have been found by Hudson and Hartmann (1961) in the hypoglossal nuclei of small rodents following unilateral neurotomy, and here too cristae-like internal structures can be seen. Simultaneously, the size and, especially, the number of the mitochondria increase significantly (Hudson *et al.*, 1961). However, the relation between these dense microbodies and the mitochondria is still open to question, mainly because of the basic difficulty of obtaining morphologically seriated representations.

Perhaps the regeneration rate of mitochondria in normal cells is fairly high. At least, as judged from isotope studies, there are four components of the rat liver mitochondria, soluble and insoluble protein, lipid and cytochrome c, which have an average biological half time of only 10·3 days (Fletcher and Sanadi, 1961). Whether during that period of time whole mitochondria degenerate or whether only parts are replaced, such as single pieces of membranes, cannot be decided yet with certainty.

Review

Mitochondria, albeit one of the best investigated organelles of the cell, are still full of enigmas. Their origin is still obscure and it is uncertain whether they belong to those parts of the cell which reproduce themselves by replication. Their structure includes an outer double membrane and lamellar or tubular internal structures, and this structure is closely related to their function. These membranous septa contain well-ordered multi-enzyme systems: namely, the citric acid cycle, the enzymes of the respiratory chain and of oxidative phosphorylation. Oxidative production of energy is the main function of the mitochondria. Moreover, mitochondria are involved in the metabolism of proteins and lipids. Every alteration in their structure, such as swelling or even destruction, entails a reduction of their function and vice-versa, every functional disturbance damages their structure. Probably, the osmophilic membranes of the cristae mitochondriales are composed of minute functional units, each of which has one respiratory chain, and the coupling of these membranes into double membranes makes a transfer of energy to the ADP-ATP system possible. Although mitochondria are very numerous within one cell, they together form a closed function unit, the chondriome.

PLASTIDS

In plant cells there are, besides the mitochondria as producers of energy, other organelles, the plastids. These are corpuscles, generally round or disc-shaped, already well visible at a light microscopic level. They usually contain pigments and are, therefore, also called chromatophores. The chloroplasts, which contain the green colouring matter in plants, chlorophyll, are the site of photosynthesis, the fundamental biological process which makes life on earth possible. Carbon dioxide taken from the air is converted into sugar and oxygen with a concurrent hydrolysis of water (see p. 14).

Animal cells are ultimately dependent for their supply of energy on the carbohydrates produced by the photosynthesis of plants, even when they draw them secondarily from other animal tissues. What gives this reaction its special character is not so much the "assimilation of carbon dioxide"—the animal organism is capable of that too—but rather the utilization of sunlight for the synthesis of carbohydrates. In other words, light energy—more than 600,000 calories per glucose molecule—is converted into chemical energy, a process in which the ADP-ATP system plays a great part. At the same time, oxygen is released.

Our knowledge of the chloroplasts being specific carriers of chlorophyll dates back to the middle of the last century. At that time, Mohl (1855), Meyer (1883) and Schimper (1885) established an accurate concept of the specificity and fine structure of the plastids. Since then, light and especially electron microscopical investigations have elucidated the principles of their fine structure and the relation between their structure and their function.

Morphology

The plastids may be differentiated into three types: the chloroplasts, the leucoplasts and the chromoplasts. They are closely related to one another. Leucoplasts can generate chloroplasts and chromoplasts, chloroplasts can generate chromoplasts.

The chloroplasts, carriers of the green colouring matter in leaves, are actually only a special aspect of the chromatoplasts. However, their special role in the total metabolism and their specific fine structure require separate attention. They are at the same time the prototype of the plastids and most of the problems and findings to be dealt with in the course of this chapter concern the chloroplasts. In superior plants they lie in the cytoplasmic ground substance next to one another in groups of 20 or 30 or more and are noticeably equal in size and shape. Algae, by contrast, have highly differentiated chloroplasts. Algal cells often contain only one single chromatophore, which may assume different shapes; sometimes it is extremely flattened, sometimes spherical or disc-shaped and at times it has the shape of a bowl or that of a plate. As a rule, the chromatophore in algae is quite large and in terms of its size one of the main elements of the cell. It can extend from one end of the cell to the other as a coiled band or form a network with many anastomoses.

These plastids in algae contain one or several small pellets, the pyrenoids, structures very rich in protein, whose function is still unknown. They are capable of dividing (Sharp, 1934; Johnson, 1956), but they can also, contrarily to what was believed earlier, be generated anew in the chromatophores (Szejnman, 1933) and are probably one of the main depositories for starch. Thus, the nuclear granules of the pyrenoids may appear, in the light microscope as well as in the electron microscope, surrounded by a variably thick envelope of stored assimilation product, which often takes the shape of a kind of rosette. Electron microscopically, pyrenoids appear traversed in various ways by the stroma lamellae of the chromatophores (Leyon, 1954). In *Euglena gracilis* the matrix material of the chromatoplasts is considerably denser in the pyrenoid region and here too there are lamellae extending throughout the pyrenoid region (Gibbs, 1960).

Leucoplasts do not contain any pigment; they are not white, but of a colourless transparency. Their structure corresponds to that of the chloroplasts, yet they contain no granules rich in chlorophyll. All those cells of green plants which are not reached by sunlight because of their location in the plant body contain leucoplasts, that is, their plastids lack the stimulation for the production of the green pigment of leaves. These plastids have developed an enormous storage capacity instead and are able to store large quantities of carbohydrates (mainly in the form of starch), fats and proteins.

Starch-storing leucoplasts are called amyloplasts. The starch grain which they contain can be several times the size of the original leucoplast, whose substance often surrounds the starch grain as a thin and hardly visible membrane. When the membrane ruptures, there are only a few scattered remnants of the leucoplast left and the starch grain lies more or less free in the cytoplasmic ground substance. It is composed, as seen in the light microscope and in the electron microscope too, of concentric layers (Buttrose, 1960), as a result of successive deposits of new substance. The starch molecules lie in rings next to one another. Once the starch is degraded, the leucoplast can shrink to its original size again.

Lipid-storing leucoplasts are the elaioplasts. They usually coalesce into a group of oil-containing vesicles. If the leucoplasts have stored protein, they are called aleurone plastids or proteoplasts. However, lipids and proteins may also be stored, in plants as in animal cells, within the cytoplasmic ground substance, that is, without being primarily bound to a cytoplasmic organelle.

The chromoplasts, devoid of chlorophyll, always contain carotene, xanthophyll and other

pigment substances which form crystals and, hence, determine the shape of the chromoplasts (Straus, 1961b). Morphologically, we can distinguish three types of chromoplasts (Frey-Wyssling and Kreutzer, 1958): (1) chromoplasts with carotenoid crystals, visible microscopically; (2) chromoplasts with spherical inclusions; (3) chromoplasts with filaments of an orange pigmentation. Chloroplasts can, by undergoing certain internal transformations, develop irreversibly into chromoplasts. Their carotene content can be 50% higher than the plastids' dry weight (Straus, 1954). In addition, they contain approximately 15% proteins, a fair amount of lipids and some RNA, generally less than 1%.

Whereas the chromoplasts of superior plants do not develop any physiological photoactivity, those in unicellular organisms contain chlorophyll among other pigment substances, and therefore are capable of photosynthesis. They are the yellow or brown pheoplasts of the brown algae or diatoms and the red rhodoplasts of the red algae. The red colour is to be attributed here to phycoerythrine, a protein substance (Strain, 1949; Lemberg and Legge, 1949; for ref., see Granick, 1961). Bacteria, when capable of photosynthesis, contain minute particles measuring 110 µ when dry (Schachmann *et al.* 1952); there can be up to 3000 such particles in one bacterium (see also Wilkinson and Duguid, 1960).

Structure of the chloroplasts

Chloroplasts, the most important type of plastids, are in all plants lentil-shaped corpuscles averaging 4–6 µ in the greater diameter (Möbius, 1920). Their colourless ground substance contains many green 0·4 to 0·8 µ grana (Fig. 164) which are the carriers of chlorophyll. This structure had already been described by Meyer (1883) and Schimper (1885) and it was confirmed as being a general principle by Heitz's (1936) investigations (see, e.g. Granick, 1955).

Electron microscopic thin-sectioning technique has provided the details (for ref., see Mühlethaler, 1955; Granick, 1961; and others). The inside of the chloroplasts consists of a homogeneous stroma, of a lesser electron density, containing a system of virtually parallel lamellae. These lamellae extend through the whole length of the chloroplasts and are not directly connected with the cell wall (Fig. 6). In the grana the lamellae are much more densely packed. When the section plane is perpendicular to the lamellae, the ends of the large stroma

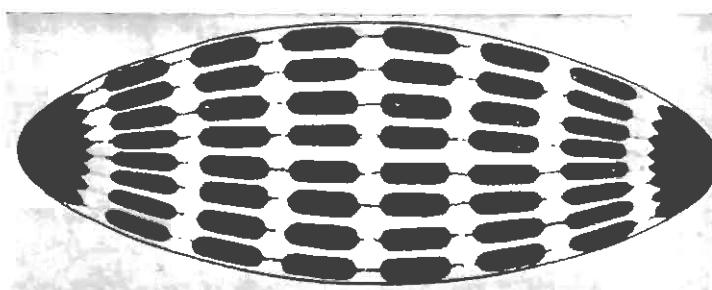


Fig. 164. Schematic representation of a chloroplast with grana and grana lamellae. (From Strugger, S. (1951), *Ber. Dtsch. Bot. Ges.*, **64**, 69.)

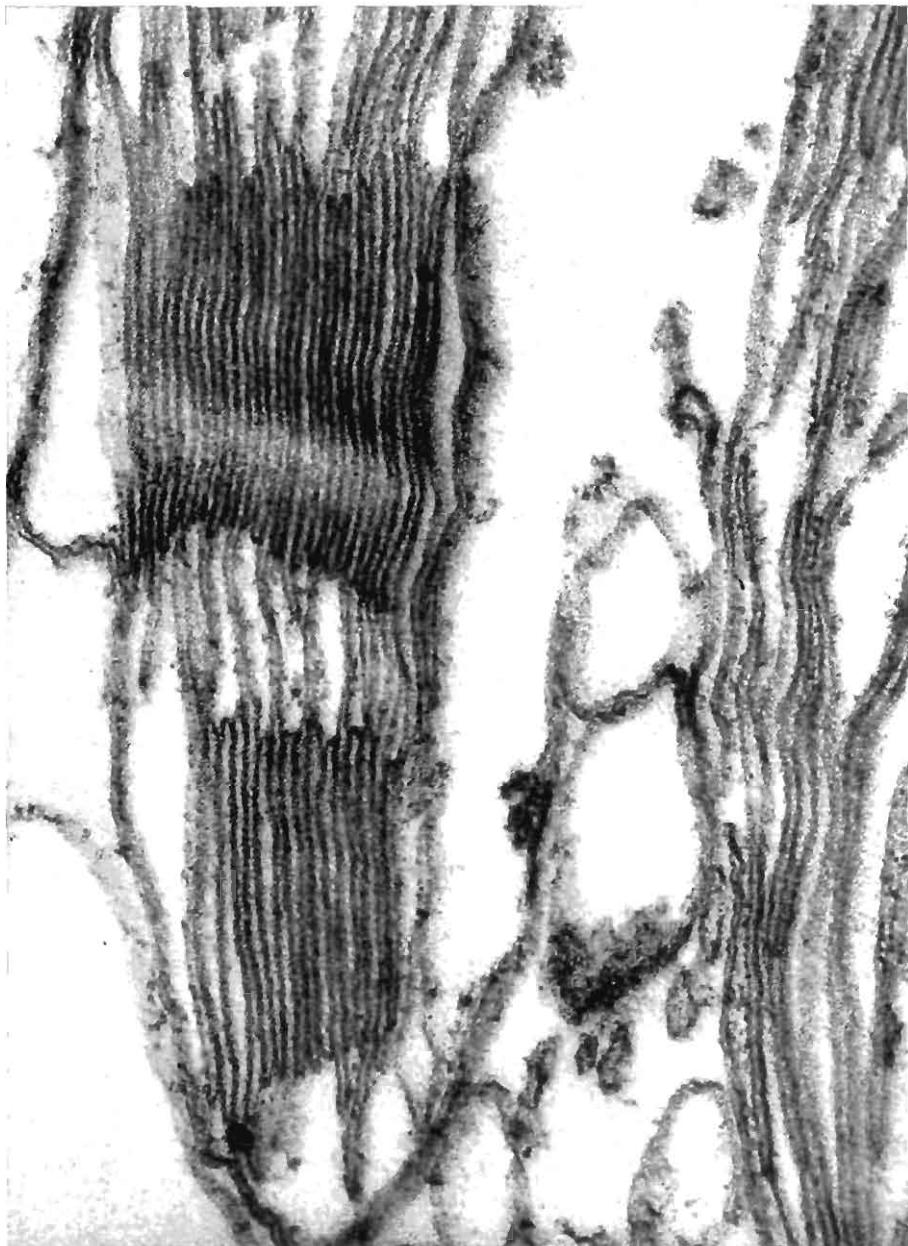


Fig. 165. Electron micrograph showing "lamellar pattern" in spinach chloroplasts. $\times 110,000$.
(From Wehrmeyer, W. (1961), *Z. Naturf.*, **16b**, 627-628.)

lamellae, which extend through the whole chloroplast, and also those of the small grana lamellae can be seen to converge two by two like the arms of a U. They may be considered, perhaps, as vesicles flattened and pressed against one another (Mühlthaler, 1960). Each stroma lamella is 30–35 Å thick and the space between two such lamellae is of 65–70 Å.

The system of lamellae probably represents a closed unit, stroma lamellae and grana lamellae being closely related to one another; that is, each grana lamella represents a local adhesion of two stroma lamellae (Heitz, 1960, 1961). It is most likely that stroma lamellae can turn into grana lamellae, although both may stay coupled for a long distance and then separate again from each other (Wehrmeyer, 1961). The bending over of stroma lamellae in the grana area is clearly visible in the electron microscope (Fig. 165). Moreover, lamellae in the grana area can evidently also branch out and display all kinds of arrangements (Gibbs, 1960; Weier and Thomson, 1962). Menke (1961) proposed the term thylacoids to designate all these closed circuit double lamellae which represent a structural principle common to almost all chloroplasts. Chloroplasts without grana are traversed by groups of two or several thylacoids in a regular pattern. In grana-rich chloroplasts there are stacks of thylacoids of the same diameter as the whole chloroplast and others which are of the same diameter as the grana. This leads to the formation of specific structures.

This structural pattern makes it easier to classify the lamellae of the granaless chloroplasts of inferior plants (Menke, 1961; Kawamatu, 1961), since all the various chloroplast structures (see, e.g., Granick, 1961) are mere variants of the same basic principle.

We can generalize this principle even further. The retinal rods in vertebrates, for example, contain the same double lamellae as the chloroplasts (Fig. 166), with a partly lamellar, partly tubular principle of structure, common to light receptors in different cell-types (Wolken, 1961).

In the homogeneous stroma of the chloroplasts, we can often see inclusion bodies (Fig. 6), especially starch grains or lipid drops of great electron density. They vary in size and arrangement (see, e.g., Murakami and Ueda, 1960).

Particularly large inclusion bodies have received the name magnoglobuli (Falk, 1960). They must be distinguished from degradation products of the lamellae that occur in the case of damage to or destruction of the chloroplasts, as for instance under the action of streptomycin or leucomycin (Drawert and Mix, 1961b). Iron deficiency reduces the size and the granular differentiation of the

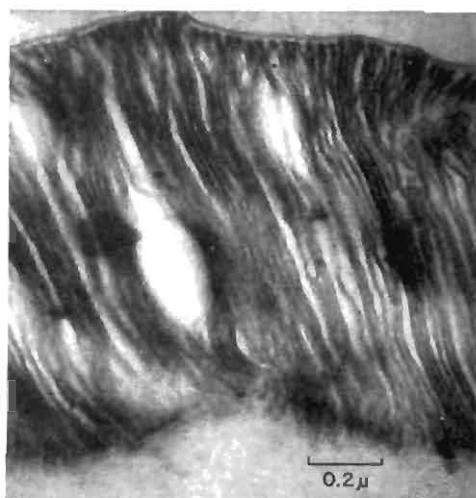


Fig. 166. Electron micrograph of photoreceptor structures in rods of the cat retina. (From Wolken, J. J. (1961), *Int. Rev. Cytol.*, **11**, 195–218.)

chloroplasts (Lamprecht, 1961a). Extreme iron deficiency can produce agranular chloroplasts, in which the double lamellae dissolve progressively (Lamprecht, 1961b). When, in chloroplasts that are kept in a nicotine solution, an experimental vacuolization can be induced, this very fact is taken as a sign of their good functioning (Lindner, 1959).

Composition and function of the chloroplasts

The function of the chloroplasts is photosynthesis. It is dependent on the chlorophyll, contained mainly in the grana of higher plants but which occurs also, in many granaless chloroplasts of algae, in the longitudinal lamellae.

Chemical structure

Chlorophyll is a porphyrin similar to haemoglobin, containing magnesium instead of iron. It occurs nearly always in two forms, chlorophyll *a* and *b*, which differ only by the side chains of the second ring.

The chloroplasts contain, on the average, 4–7% chlorophyll, 40–50% protein and 25–35% lipids (Menke, 1938; see also Rabinowitch, 1945). Roughly speaking, the substance of the homogeneous stroma is composed predominantly of proteins, that of the lamellae, of lipoproteins where the chlorophyll is also located. Several authors (von Euler *et al.*, 1934; Wolken and Schwertz, 1953; and others) have calculated that one chloroplast of a green plant contains $1-2 \times 10^9$ chlorophyll molecules and this estimate agrees with the evidence found in various species (Mühlethaler, 1955).

The chlorophyll molecule has a shape like a tadpole with a large head and a long (phytol-) tail. Fluorescence microscopy shows that it forms a one-layered film, in which the molecules lie one next to the other; thereby, the isoelectric rings form a series of tetrads and the phytol-tail extends into the adjacent layer of lipids (Fig. 167). Thus, there are always one lipid layer and one protein layer lying on top of each other and between the protein and the lipid layer there is the narrow film of the porphyrin heads of the chlorophyll (Wolken and Schwertz, 1954).

Chromoplasts are, indeed, rich in lipids (see above) and display a very high metabolism of long chain fatty acids (Sissakian, 1958). Protein synthesis too is considerable, particularly in association with the formation of chlorophyll and photosynthesis (see, e.g., Heber, 1962). The amino-acid composition of the structural protein of the lamellae is well known. It represents a complex of various enzyme proteins (Menke and Jordan, 1959), and structural proteins of different species evidently differ only in single amino-acids (Weber, 1959). The connection between the lamellar proteins and lipids seems fairly labile, since dehydration is enough to produce degenerations visible radiographically. The considerably smaller bacterial chromatophores mentioned above are also, according to the findings of Bergeron and Fuller (1961), made of one (inner) layer of lipids and one (outer) layer of proteins.

Last but not least, chloroplasts contain small quantities of RNA, often less than 1% (for ref., see, e.g., Granick, 1961); the RNA content here is somewhat higher in the dark than when exposed to an intense light (Szarkowski and Golaszewski, 1961), that is, it is subject to functional fluctuations. From spinach chloroplasts, it was even possible to isolate ribosomes with an RNA content of 45%, which corresponds essentially to the ribosomes of the

endoplasmic reticulum (for ref., see Lyttleton, 1962). The chloroplasts of the ciliate *Chlamydomonas* contain beyond doubt a Feulgen positive DNA, which is seen in the electron microscope as a structure of fine threads (Ris and Plaut, 1962); in other words, these chloroplasts have a kind of "nucleoplasm" analogous to that of bacteria or green algae.

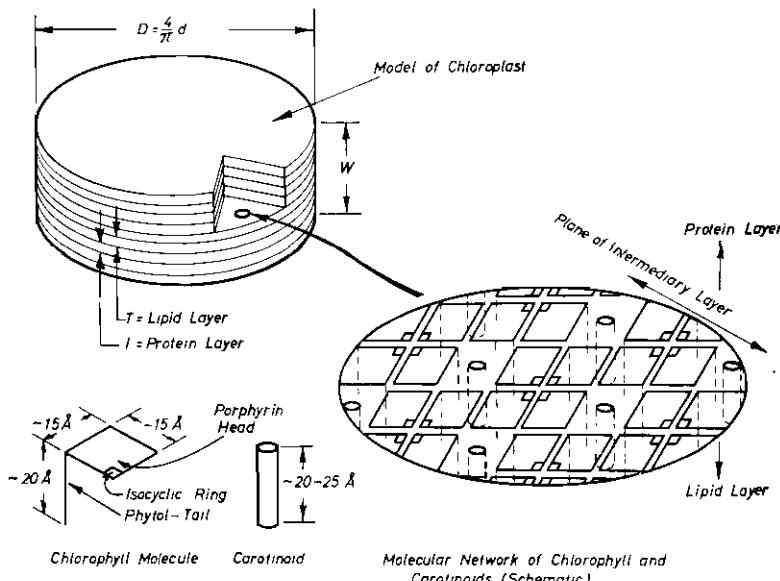


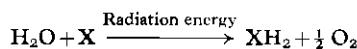
Fig. 167. Schematic representation of the molecular structure of an alga chloroplast. (From Wolkcn, J. J. and Schwertz, F. A. (1953), *J. gen. Physiol.*, **37**, 111.)

Enzymes and photosynthesis

Photosynthesis is divided into three reactions.

(α) Formation of ATP from inorganic phosphate and ADP, known as photophosphorylation. As the coupling of electron transport and ATP formation in mitochondria (p. 309), it occurs via a transport of electrons along a cytochrome chain, stimulated in this case by the absorption of a light quantum. This cytochrome chain is, however, certainly built differently from the respiratory chain of mitochondria. Here, too, inorganic phosphate is absorbed to form ATP from ADP. The details of the process of electron transport are still largely unknown. Evidently, cytochromeoxidase plays no part in it (Jagendorf, 1955). The whole process is of a cyclic nature; part of the energy-rich electrons return to the chlorophyll molecule, where their stimulation for the light absorption had come from. Evidence for this came from the discovery of Arnon *et al.* (1954), that isolated chloroplasts need light to produce ATP but can do so without mitochondria.

(β) Photolytic splitting of the water according to the scheme:



The different stages of the reaction are only partly known (see Karlson, 1962). The H^+ ion of the water must be reduced; perhaps it takes up that electron from the chlorophyll which is stimulated by light. The first reaction resulting from this process, the formation of the first stable bond, XH_2 , as seen in the scheme above is the hydrated triphosphopyridine nucleotide ($TPNH + H^+$). The OH^- ion remaining from the water must now donate one electron. This electron returns, according to the aforementioned cyclic character of photosynthesis, to the chlorophyll. Simultaneously, the following reaction takes place:



Photolysis of water is an extremely endo-ergonic process, i.e., energy-consuming (see, e.g., Warburg *et al.*, 1959). Photophosphorylation and photolysis of water occur in close relation to the chlorophyll, that is, in the grana or on their membranes. Oxygen is released with the production of ATP and $TPNH + H^+$, which provide energy for the synthesis of carbohydrates.

(γ) The formation of carbohydrate from CO_2 has been summarized by Arnon (1961) in the following diagram (Fig. 168). The figures I-III represent the three phases of this

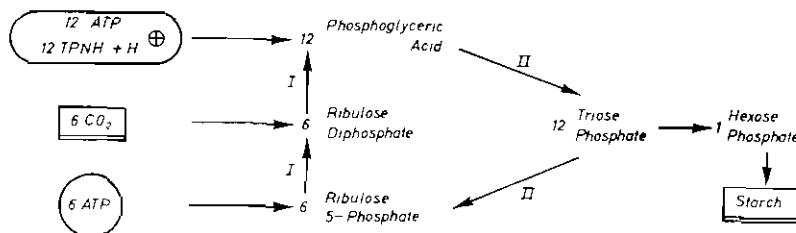


Fig. 168. Diagram of the carbohydrate cycle in chloroplasts. (From Arnon, D. I. (1961), *Biological Structure and Function*, Vol. II, 339-408. Acad. Press, New York.)

cyclic process. In phase I, ribulose-5-phosphate is phosphorylated to ribulose diphosphate, which binds one molecule of CO_2 and forms two molecules of phosphoglyceric acid. In phase II, phosphoglyceric acid is reduced to triose phosphate and, in phase III, triose phosphate is converted partly again into ribulose-5-phosphate and partly into hexose phosphate and hence into starch. Chloroplasts contain the main enzymes necessary for this process (Racker, 1954; Vishniac, 1955); probably, the last stages, at least, do not occur directly on the lamellae but in the stroma of the chloroplasts.

Function and structure

Photosynthesis is based on the action of chlorophyll and the production of chlorophyll is a result of photosynthesis. The close relation existing here between photosynthesis and protein synthesis has been already pointed out (p. 326). We had also mentioned the fact that the plastids of the inner parts of the cell, which are not exposed to sunlight, contain no chlorophyll and are, therefore, leucoplasts (p. 322). Thus, in principle, light and photosynthesis are necessary for the formation of chlorophyll and of the grana. Neither do green

algae contain much chlorophyll in the dark, but after a short exposure to light, chlorophyll and the lamellar structures of the plastids appear at the same time.

The plastids of bean leaves kept in the dark do not contain any lamellae but complexes of short tubes (Fig. 169a). If these leaves are exposed to light for a short time, vesicles appear in less than two minutes, which readily arrange themselves in concentric circles. After few

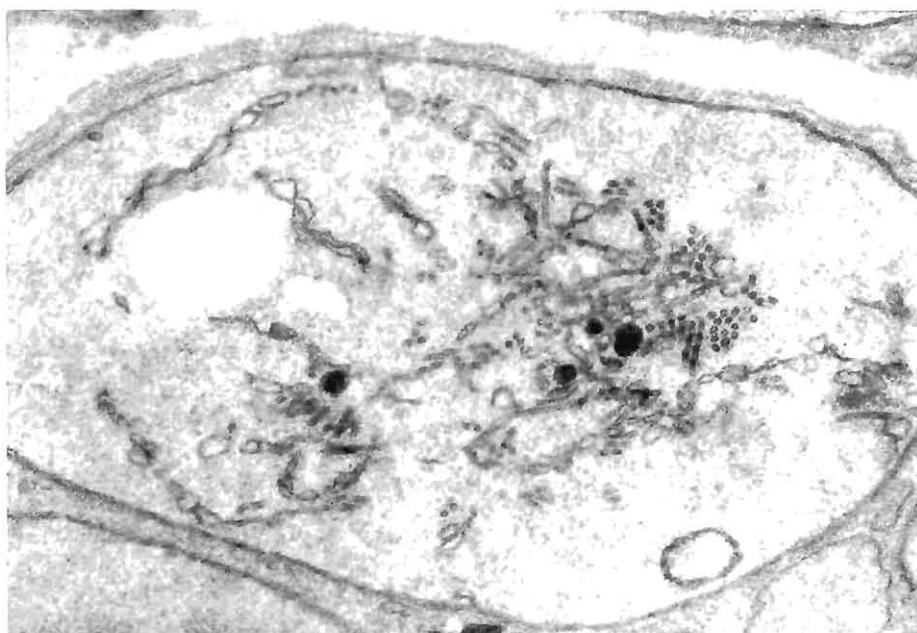


Fig. 169a. Electron micrograph of a 16-day-old leaf of *Phaseolus vulgaris*, grown in the dark, showing plastids with tubules sectioned longitudinally and transversely. $\times 40,000$. (Courtesy D. v. Wettstein.)

hours exposure, the grana start appearing again anew (Fig. 169b). In the first phase of the circular arrangement, protochlorophyll is transformed into chlorophyll and in the second phase, the chlorophyll content increases progressively in the plastids (Wettstein and Kahn, 1960; see also Eilam and Klein, 1962). Since, for example in *Zea mays*, low temperature also brings about concentric rings, which turn into lamellae at a normal temperature (Klein, 1960), the formation of the lamellae depends not only on photosynthesis but also on other, temperature-determined syntheses, in all likelihood of protein. There are also daily variations in the capacity to form chlorophyll (Mitrakos, 1959), whereas seasonal differences are to be encountered only in the quantities of starch and lipids stored (Parker and Philpot, 1961).

In connection with their function, chloroplasts perform movements within the cell of which not much is known. That such movements exist, was demonstrated by Noll (1883), who noticed in phosphorescent moss that the chloroplasts always gather at the focus of the convex lens built by the cell. These changes in the location and the orientation of the chloroplasts

can be found in all kinds of experiments and in many species (for ref., see Haupt, 1959); in algae, the chromatophore, which sometimes has a plate-like shape, turns on its longitudinal axis when exposed to light (Senn, 1908). This process in particular has been in recent years the object of accurate investigations (Haupt, 1959; Haupt and Thiele, 1961). The chromatophore, when exposed to a red light with linear polarization, orients itself in the direction of the oscillation plane (Haupt, 1960); this is to a certain extent dependent on the temperature (Mugele and Haupt, 1961) or, if used, ultraviolet irradiation (Haupt *et al.*, 1959). This

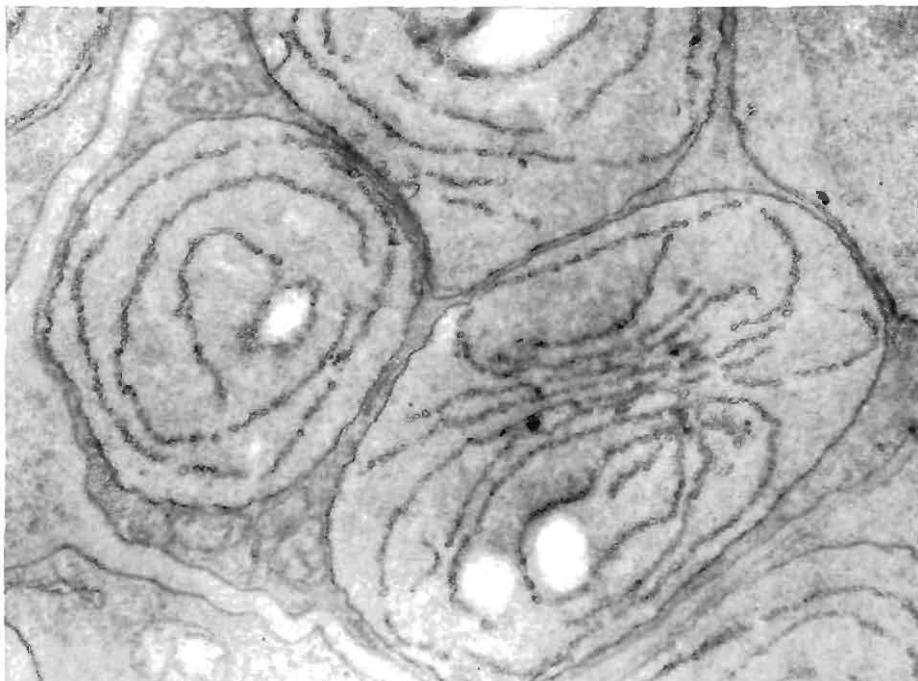


Fig. 169b. Electron micrograph of the plastids of a 16-day-old bean leaf grown in the dark, taken after 8½ hours exposure to light. Note the formation of vesicles and the rearrangement into primary layers. $\times 32,000$. (From v. Wettstein, D. and Kaban, A. (1960), *Proc. Eur. Conf. on Electron Microscopy*, Vol. II, 1051-1054, Delft.)

positive phototaxis is brought about by the absorption of light by the phytochrome, the pigment of the reversible light red/dark red reaction mechanism in algae (Haupt, 1959); in other words, the turning induced by light red light is partly neutralized again by dark red light. The photoreceptors of these movements, the phytochromes, do not lie directly on the chloroplasts but are evidently located in the cytoplasmic ground substance (Bock and Haupt, 1961). In addition to this positive phototaxis to red light, the opposite reaction, that is, a turn from the frontal to the less absorbent profile position, can also be obtained by using a more intensive short-wave irradiation. This negative phototaxis of the chloroplasts is probably based on quite a different primary process (Haupt and Schönfeld, 1962).

Formation of the plastids

Plastids are generated only by other plastids. They are self-reproducing cell organelles like the chromosomes and the centrioles. This fact had been already stated by Meyer (1883) and Schimper (1885) and has been confirmed since then. If one destroys them experimentally, for example with certain antibiotics, they are irremediably lost, even if the cell goes on living for some time.

In algae, the plastids are quite large and often of different shapes and the process of their division is easy to follow. In superior plants, the evolution is far more complicated. In the cells of the root tip meristem, for example, there are no mature plastids, but instead 0·4–0·9 μ colourless corpuscles, the proplastids (Fig. 170a). They appear in the electron micro-



Fig. 170a. Electron micrograph of *Elodea* proplastids, showing the inner membrane layer starting to infold. $\times 15,000$. (From Mühlthaler, K. (1960), *Disch. med. Wschr.*, 85, 1063–1065.)

scope to be surrounded by a double membrane and they contain a granular stroma, sometimes with several small vesicles. Here are the first signs of a differentiation of the internal structure, starting at the outer walls; small infoldings appear at first, which soon become larger under favourable light and stretch out, forming loose lamellae (Fig. 170b). These lamellae soon unite more compactly at certain points, thus forming the first granule areas; concomitantly, the first stroma lamellae appear, extending through the whole length of the chloroplasts (Fig. 170c). More lamellae are formed and, by juxtaposition of these lamellae

into grana and the pinching off of the grana lamellae, the chloroplast finally reaches its full maturation, provided that it is constantly or intermittently exposed to light. If the proplastids are left in the dark, the aforementioned complexes of small vesicles reappear (Fig. 169a); they develop from the small vesicles of the proplastids, but can transform themselves into a system of lamellae if exposed to light (Fig. 169b). The complexes of small vesicles in Fig. 169a are, therefore, also called prolamellar bodies (Hodge *et al.*, 1956), identical with the primary grana, which Strugger (1954) regarded as persistent grana, from which the chloroplast-containing grana were supposed to develop. Since then, electron microscopy has shown that these grana form themselves anew and that there is no continuity between those that preceded them (see, e.g., Mühlthaler, 1960).



Fig. 170b. Electron micrograph showing the formation of lamellar systems in proplastids under exposure to light. $\times 30,000$. (From Mühlthaler, K. (1960), *Disch. med. Wschr.*, 85, 1063-1065.)

The non-lamellated proplastids can also transform themselves into leucoplasts; these, in turn, can develop into immature lamellar plastids or, by a reversible process, into starch-containing amyloplasts. The still immature lamellar proplastids then grow to become either mature chloroplasts or other chromoplasts or even, by storing proteins, aleurone plastids. Mature chloroplasts can become chlorophyll-free chromoplasts or, by absorption of many lipids, elaioplasts. In the autumn, chloroplasts degenerate when the leaves wither, and the colourful aspect of autumn leaves is due to various qualitative shifting of both types of chlorophyll and partial transformation of the chloroplasts into other forms of chromoplasts. Thus, there are many different possibilities of development and transformation. The initial structures are always the colourless proplastids of the meristem cells.

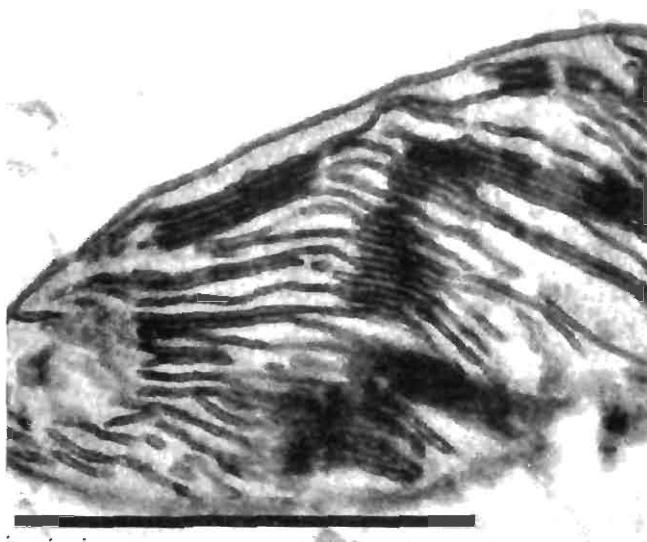


Fig. 170c. Electron micrograph of a young chloroplast showing some differentiated grana sections as well as still incomplete stroma lamellae. $\times 60,000$. (From Mühlthaler, K. (1960), *Dtsch. med. Wschr.*, 85, 1063-1065.)

The plastidome

Development and maturation of the plastids is determined by the nucleus and its genomes. Thus, there are many mutants of chlorophyll, for example in wheat (Highkin, 1950), where one mutant contains the normal total quantity of chlorophyll but only chlorophyll *a* and no chlorophyll *b*. In a white mutant of wheat (Stubbe and v. Wettstein, 1956) there are absolutely no lamellae. In other mutants, the plastids contain too little chlorophyll and the leaves are then of a faded or light green colour. If the gene or chromosome mutations do not take place before the meristematic tissue is formed, chromosomal chimeras can appear with green and white areas next to one another.

Further, extrakaryotic heredity, viz., hereditary transmission through cytoplasmic genes, can readily be observed in chloroplasts. Correns (1909) found in *Mirabilis jalapa* a typical case of extrakaryotic heredity in the form of a variatio albomaculata, i.e., a plant sort with spotted leaves. Crossing between green and spotted plants gave only green plants, when green was taken as female and spotted as male parent. But if a spotted plant was used as female parent, the offsprings were green, spotted and white. Mendel's law of the uniformity of the F_1 generation, reciprocity included, did not hold in this instance. This heredity contrary to Mendelian laws was apparent in the chlorophyll content and, consequently, in the colour of the leaves, i.e., in the plastids.

This is a typical instance of maternal heredity, since it was the female germ cell which had determined the hereditary transmission. In the zygote, all the elements of the cytoplasm

come from the maternal egg cell, the plastids too. Since, in the above-described experiments on *Mirabilis jalapa*, the formation of the plastids was disturbed by mutation, an inherited disturbance of the plastids seemed most probable; in other words, these mutated plants had, besides chlorophyll-containing plastids, leucoplasts which could not be transformed into chloroplasts, but were transmitted unchanged from cell to cell, even under exposure to light. This can be considered as a typical aspect of plastidome heredity (see Oehlkers, 1956), since the hereditary material contained in the plastids is called plastome or plastidome.

Other authors (e.g., Rhoades, 1943, 1946) explain Correns' experiments by the presence of a gene outside the plastids in the cytoplasm. The fact that hereditary characters can be located also in the cytoplasm is still much discussed and is designated by the term plasmatic heredity in opposition to the plastidome heredity; the cytoplasmic hereditary material located outside the nucleus and the plastids is called plasmon. However, this does not bring us much further; for, apart from the centrioles and the plastids, we do not know for sure of any other self-reproducing elements in the cytoplasm, as would be necessary for a plasmon heredity.

Plastidome heredity, on the other hand, has been ascertained in several organisms and has been proved to be completely independent from the chromosomal hereditary process (for ref., see, e.g., Granick, 1961).

Review

We now understand the special role played by the plastids. As self-reproducing elements, they carry hereditary characters. Moreover, they manufacture and carry chlorophyll, thus being the only site of photosynthesis. The three steps of photosynthesis, photophosphorylation, photolysis of water and transformation of CO₂, are the three steps in the transformation of light energy into chemical energy. The chloroplasts display the same structure in all green plants; they have a lamellar internal structure showing compact masses, the grana, likewise made of lamellae; in superior plants, these grana contain the chlorophyll. In inferior plants and chlorophyll-containing protozoans, the chloroplasts appear under various aspects, but are all built according to the lamellar principle, evidently also common to all photoreceptors. The chloroplasts derive from the proplastids of the meristem cells by infoldings of the outer membrane or agglomeration of small vesicles. Chloroplasts can turn into elaioplasts by taking up lipids; they may also become other chromoplasts by producing other pigment substances. Proplastids may be converted to leucoplasts and these, in turn, may become starch-containing amyloplasts or protein-storing aleurone plastids. By virtue of the pigment substance they contain, the chromoplasts, including the chloroplasts, are responsible for a great many of the colours we see in nature; they play a part in the beauty of a flower.

CONCLUSION

We have now reached the end of the way which led us from the origins of the cell and the beginning of cytology through the different branches of this science. We have encountered many problems; some solved, others still to be solved. Many of them we have had to leave aside, for a "General Cytology" must follow a certain course, since it is meant to be a master

key for specific problems. Some of the assertions in this book will soon become outdated by the supply of new evidence which will bring a greater insight into phenomena still obscure at this date. Some conceptions will be replaced by better ones, others will be confirmed by the discovery of new facts. Certain theories can be expected to last. Many investigations are still incomplete.

However, maybe this book succeeded in showing behind many a problem the characteristic common not only to the different parts of one cell, but to all cells and all the individuals made of these cells. This common unit is proper to all cellular structures as the category of life. It seems to lie beyond the methods available for its investigation, for these methods can only be in search of mechanisms. Each chapter of this book gives evidence of this unity of origin and life, evolution and death.

References

- ABBÉ, E. (1886). Über neue Mikroskope. *S.-B. Jenaischen Ges. Med. Naturwiss.*, 107-128.
- ACKERMAN, G. A. (1961). Histochemistry of the centrioles and centrosomes of the leukemic cells from human myeloblastic leukemia. *J. biophys. biochem. Cytol.*, **11**, 717-719.
- ADAMSTONE, F. B. (1958). Response of the Golgi apparatus of absorptive cells of the intestinal epithelium of the rat to the ingestion of protein. *Amer. J. Anat.*, **103**, 437-465.
- (1959). Reaction of the Golgi apparatus of intestinal epithelial cells of the rat to the ingestion of a neutral fat or fatty acid. *J. Morphol.*, **105**, 293-316.
- AFZELIUS, B. A. (1955). The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope. *Exp. Cell Res.*, **8**, 147-158.
- ÅGRELL, I. (1955). A mitotic rhythm in the appearance of mitochondria during the early cleavages of the sea urchin embryo. *Exp. Cell Res.*, **8**, 232-234.
- ÅGRELL, I., & WELIN-BERGER, E. (1957). A diurnal cycle in the mitotic activity of ascites tumour cells in mice. *Nature (Lond.)*, **180**, 705-706.
- AHMED, K., JUDAH, J. D., & GALLAGHER, C. H. (1961). Electron transport system and phosphoproteins of mitochondria. *Nature (Lond.)*, **191**, 1309-1310.
- ATSENBERG, A. C. (1961a). Studies on normal and neoplastic mitochondria. I. Respiration. *Cancer Res.*, **21**, 295-303.
- (1961b). Studies on normal and neoplastic mitochondria. II. Phosphorylation. *Cancer Res.*, **21**, 304-308.
- ALBERTI, W., & POLITZER, G. (1923). Über den Einfluß der Röntgenstrahlen auf die Zellteilung. *Arch. mikr. Anat.*, **100**, 83-109.
- ALBRECHT, M. (1954). Chromatinfreie Cytoplasmateilung von Promyelocyten und Myelocyten unter Einwirkung von Mitosegiften in vitro. *Z. Krebsforsch.*, **60**, 16-18.
- ALFERT, M. (1950). A cytochemical study of oogenesis and cleavage in the mouse. *J. cell. comp. Physiol.*, **36**, 381-410.
- (1954). Composition and structure of giant chromosomes. *Int. Rev. Cytol.*, **3**, 131-175.
- (1958a). Variations in cytochemical properties of cell nuclei. *Exp. Cell Res., Suppl.*, **6**, 227-235.
- (1958b). Cytochemische Untersuchungen an basischen Kernproteinen während der Gametenbildung, Befruchtung und Entwicklung. *Coll. Ges. Physiol. Chem.*, **9**. Springer, Berlin.
- ALFERT, M., & BERN, H. A. (1951). Hormonal influence on nuclear synthesis. I. Estrogen and uterine gland nuclei. *Proc. nat. Acad. Sci. (Wash.)*, **37**, 202-205.
- ALFERT, M., & BERN, H. A., & KAHN, R. H. (1955). Hormonal influence on nuclear synthesis. IV. Karyometric and microspectrophotometric studies of rat thyroid nuclei in different functional states. *Acta Anat. (Basel)*, **23**, 185-205.
- ALFERT, M., & GESCHWIND, I. J. (1953). A selective staining method for the basic protein of cell nuclei. *Proc. nat. Acad. Sci. (Wash.)*, **39**, 991-999.
- ALFERT, M., & GOLDSTEIN, N. O. (1955). Cytochemical properties of nucleoprotein in Tetrahymena pyriformis; a difference in protein composition between macro- and micronuclei. *J. exp. Zool.*, **130**, 403-419.
- ALFERT, M., & SWIFT, H. (1953). Nuclear DNA constancy: A critical evaluation of some exceptions reported by Lison and Pasteels. *Exp. Cell Res.*, **5**, 455-460.
- ALLEN, E. H., & SCHWEET, R. S. (1960). Role of transfer ribonucleic acid in hemoglobin synthesis. *Biochim. biophys. Acta (Amst.)*, **39**, 185-187.
- ALLEN, R. D. (1960). The consistency of Amoeba cytoplasm and its bearing on the mechanism of amoeboid movement. II. The effects of centrifugal acceleration observed in the centrifuge microscope. *J. biophys. biochem. Cytol.*, **8**, 379-397.
- (1961). Structure and function in amoeboid movement. In: *Proc. I. IUB/IUBS Symp.*, Vol. II. Acad. Press, London.
- ALLEN, R. D., COWDEN, R. R., & HALL, P. J. (1962). Syneresis in amoeboid movement: Its localization by interference microscopy and its significance. *J. Cell Biol.*, **12**, 185-189.
- ALLFREY, V. G. (1954). Amino acid incorporation by isolated thymus nuclei. I. The role of deoxyribonucleic acid in protein synthesis. *Proc. nat. Acad. Sci. (Wash.)*, **40**, 881-885.

- ALLFREY, V. G., DALY, M. M., & MIRSKY, A. E. (1955). Some observations on protein metabolism in chromosomes of non-dividing cells. *J. gen. Physiol.*, **38**, 415-424.
- ALLFREY, V. G., MEUDT, R., HOPKINS, J. W., & MIRSKY, A. E. (1961). Sodium-dependent "transport" reactions in the cell nucleus and their role in protein and nucleic acid synthesis. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 907-932.
- ALLFREY, V. G., & MIRSKY, A. E. (1955). On the supposed contamination of thymus nuclear fractions by whole cells. *Science*, **121**, 879-880.
- (1957). The role of desoxyribonucleic acid and other polynucleotides in ATP synthesis by isolated cell nuclei. *Proc. nat. Acad. Sci. (Wash.)*, **43**, 589-598.
- (1958). Biochemical properties of the isolated nucleus. In: *Subcellular particles*. Edited by T. HAYASHI. Ronald Press, New York.
- ALTMANN, H.-W. (1949). Über Leberveränderungen bei allgemeinem Sauerstoffmangel nach Unterdruckexperimenten bei Katzen. *Frankfurt. Z. Path.*, **60**, 376-494.
- (1952). Über den Funktionsformwechsel des Kerns im exokrinen Gewebe des Pankreas. *Z. Krebsforsch.*, **58**, 632-645.
- (1955a). Allgemeine morphologische Pathologie des Cytoplasmas. Die Pathobiosen. In: *Handbuch der allgemeinen Pathologie*, Vol. II/1, edited by BÜCHNER, F., LETTERER, E., & ROULET, F. Springer, Berlin.
- (1955b). Zur Morphologie der Wechselwirkung von Kern und Cytoplasma. *Klin. Wschr.*, **33**, 306-314.
- (1957). Allgemeine Morphologie der Zelle. In: *Handbuch der gesamten Hämatologie*, 2nd edition, Vol. I, edited by HEILMEYER, L., & HITTMAR, A. Urban & Schwarzenberg, München.
- (1959). Zur Pathologie des zellären Zentralapparates. *Zbl. Path.*, **99**, 415.
- (1961a). Ein Beitrag zur Pathologie des zellulären Zentralapparates. Nach Beobachtungen an einem Hirntumor. *Virchows Arch. path. Anat.*, **334**, 132-159.
- (1961b). Über Cocciden als "Kerneinschlüsse". Beobachtungen an Cyclospora caryolytica Schaudinn. *Virchows Arch. path. Anat.*, **334**, 236-242.
- ALTMANN, H.-W., & GRUNDMANN, E. (1955). Phasenkontrasttierischer Zellkerne. *Beitr. path. Anat.*, **115**, mikroskopische Untersuchungen zur Vitalstruktur 313-347.
- ALTMANN, H.-W., & MÉNY, R. (1952). Der Funktionsformwechsel des Zellkerns im exokrinen Pankreasgewebe. *Naturwissenschaften*, **39**, 138-139.
- ALTMANN, H.-W., & ÖSTERLAND, U. (1961). Über cytoplasmatische Wirbelbildungen in den Leberzellen der Ratte bei chronischer Thioacetamidvergiftung. *Beitr. path. Anat.*, **124**, 1-18.
- ALTMANN, H.-W., STÖCKER, E., & THOENES, W. (1963). Über Chromatin und DNS-Synthese im Nucleolus. Elektronenmikroskopische, autoradiographische und lichtmikroskopische Untersuchungen an Leberzellen von Ratten. *Z. Zellforsch.*, **59**, 116-133.
- ALTMANN, R. (1894). *Die Elementarorganismen und ihre Beziehungen zu den Zellen*. Veit, Leipzig.
- ALTSCHUL, R. (1948). On nuclear proliferation and nuclear size. *Anat. Rec.*, **100**, 517-533.
- AMANO, M., & LEBLOND, C. P. (1960). Comparison of the specific activity time curves of ribonucleic acid in chromatin, nucleolus and cytoplasm. *Exp. Cell. Res.*, **20**, 250-253.
- AMANO, S. (1954). Structure and function of the central body and the nucleolus. Extension-fiber theory of the mitotic mechanism. *Acta Sch. med. Univ. Kyoto*, **32**, 5-27.
- (1957). The structure of the centrioles and spindle body as observed under electron and phase contrast microscopes.—A new extension-fiber theory concerning mitotic mechanism in animal cells. *Cytologia (Tokyo)*, **22**, 193-212.
- AMANO, S., DOHI, S., TANAKA, H., UCHINO, F., & HANAOKA, M. (1956). The structure of the nucleus studied by electron microscopy in ultra-thin sections with special reference to the chromonema. An advocacy of "subchromonema" and "protochromonema". *Cytologia (Tokyo)*, **21**, 241-251.
- AMANO, S., & TANAKA, H. (1957). Dynamic structure of the nuclear membrane, with special reference to its disappearance and reappearance in mitosis as observed under the electron microscope. *Acta haemat. jap.*, **20**, 319.
- AMBROSE, J. E., & BAJER, A. (1959). *Hereditas*, **45**, 579-596.
- AMMOORE, J. E. (1962a). Oxygen tension and the rates of mitosis and interphase in roots. *J. Cell Biol.*, **13**, 365-371.
- (1962b). Participation of a non-respiratory ferrous complex during mitosis in roots. *J. Cell Biol.*, **13**, 373-381.
- ANDERSON, E., & BEAMS, H. W. (1956). Evidence from electron micrographs for the passage of material through pores of the nuclear membrane. *J. biophys. biochem. Cytol.*, Suppl. **2**, 439-444.
- ANDERSON, N. G., & WILBUR, K. M. (1952). Studies on isolated cell components. IV. The effect of various solutions on the isolated rat liver nucleus. *J. gen. Physiol.*, **35**, 781-796.
- ANDRÉ, J., ROUILLER, CH. (1957). L'ultrastructure de la membrane nucléaire des ovocytes de l'Araignée (*Tegenaria domestica* Clerk). In: *Proc. Stockh. Confer. on Electr. Micr. Acad. Press*, New York.

- ANDRES, K. H. (1961). Untersuchungen über morphologische Veränderungen in Spinalganglien während der retrograden Degeneration. *Z. Zellforsch.*, **55**, 49-79.
- ANDREWS, J. M. (1925). Morphology and mitosis in *Trichomonas termopsisidis*, an intestinal flagellate of the termite, *Termopsis*. *Biol. Bull.*, **49**, 69-85.
- ANKEL, W. E. (1924). Der Spermatozoendimorphismus bei *Bythinia tentaculata* L. und *Viviparus* L. *Z. Zellenlehre*, **1**, 85-166.
- APITZ, K. (1940). Die Paraproteinosen (Über die Störungen des Eiweißstoffwechsels bei Plasmocytom). *Virchows Arch. path. Anat.*, **306**, 631-699.
- ARAI, S. (1960). An electron microscope study on the phagocytosis of the reticuloendothelial cells. *Sapporo med. J.*, **17**, 299-318.
- ARCOS, J. C., GRIFFITH, G. W., & CUNNINGHAM, R. W. (1960). Fine structural alterations in cell particles during chemical carcinogenesis. II. Further evidence for their involvement in the mechanism or carcinogenesis. The swelling of rat liver mitochondria during feeding of amino azo dyes. *J. biophys. biochem. Cytol.*, **7**, 49-60.
- ARMSSTRONG, J. A., & PEREIRA, H. G. (1960). Significance of cytopathic effects observed during the growth of adenovirus. *Exp. Cell Res.*, **21**, 144-150.
- ARNDT, E. A. (1960). Die Aufgaben des Kerns während der Oogenese der Teleosteer. *Z. Zellforsch.*, **51**, 356-378.
- ARNOLD, J. (1879). Beobachtungen über Kernteilungen in den Zellen der Geschwülste. *Virchows Arch. path. Anat.*, **78**, 279-301.
- (1883). Beobachtungen über Kerne und Kernteilungen in den Zellen des Knochenmarkes. *Virchows Arch. path. Anat.*, **93**, 1-38.
- ARNON, D. I. (1961). Photosynthetic phosphorylation and the energy conversation process in photosynthesis. In: *Proc. I. IUB/IUBS Symp.*, Vol. II. Acad. Press, London.
- ARNON, D. J., ALLEN, M. B., & WHATLEY, F. R. (1954). Photosynthesis by isolated chloroplasts. *Nature (Lond.)*, **174**, 394-396.
- ASCHOFF, L. (1924). Das reticulo-endotheliale System. *Ergebn. inn. Med. Kinderheilk.*, **26**, 1-117.
- ASHFORD, T. P., & PORTER, K. R. (1962). Cytoplasmic components in hepatic cell lysosomes. *J. Cell Biol.*, **12**, 198-202.
- ASHWORTH, C. T., RACE, G. J., & MOLLENHAUER, H. H. (1959). Study of functional activity of adrenocortical cells with electron microscopy. *Amer. J. Path.*, **35**, 425-437.
- ASSIS, L. M. DE, EPPS, D. R., BOTTURA, C., & FERRARI, I. (1960). Chromosomal constitution and nuclear sex of a true hermaphrodite. *Lancet*, ii, 129-130.
- ATKINS, L., & ROSENTHAL, M. K. (1961). Multiple congenital abnormalities associated with chromosomal trisomy. *New Engl. J. med.*, **265**, 314-318.
- ATSUMI, A. (1953). Studies of amitosis with the Yoshida sarcoma. *Gann*, **44**, 21-30.
- ATTARDI, G. (1957). Quantitative behaviour of cytoplasmic RNA in rat Purkinje cells following prolonged physiological stimulation. *Exp. Cell Res.*, Suppl. **4**, 25-53.
- ATWOOD, S. (1937). The nature of the last premeiotic mitosis and its relation to meiosis in *Gaillardia*. *Cellule*, **46**, 389-410.
- AUERBACH, C. (1943). Chemically induced mutation and rearrangements. *Drosophila Information Service*, **17**, 48.
- (1951). Problems in chemical mutagenesis. *Cold Spr. Harb. Symp. quant. Biol.*, **16**, 199-214.
- AUERBACH, C., & ROBESON, J. M. (1947). Tests of chemical substances for mutagenic action. *Proc. roy. Soc. Edinb.*, B **62**, 284-291.
- AUSTIN, C., & SMILES, J. (1948). Phase-contrast microscopy in the study of fertilization and early development of the rat egg. *J. roy. micr. Soc.*, **68**, 13-19.
- AVERY, O., MCLEOD, C., & McCARTY, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *J. exper. Med.*, **79**, 137-158.
- AWANO, I., TSUDA, F., TOSHIMA, S., & KOKUBUM, K. (1961). Research on the genetic mechanism of leukemo-genesis (chromosome in the human- and mouse-leukemia and virus-like particles in the leukemic tissue). *Tohoku J. exper. Med.*, **74**, 1-17.
- BACCARINI, J. (1908). Sulle cinesi vegetative del "Cynomorium coccineum L.". *Nuovo Giorn. Bot. Ital. N.*, Ser. **15**, 189-203.
- BACHMANN, R. (1948). Zwischenhirnstudien II. *Z. Naturforsch.*, **3b**, 51-55.
- BACQ, Z. M. & ALEXANDER, P. (1961). *Fundamentals of radiobiology*. Pergamon Press, Oxford.
- BAIKIE, A. G., COURT-BROWN, W. W., BUCKTON, K. E., HARDNEN, D. G., JACOBS, P. A., & TOUGH, I. M. (1960). A possible specific chromosome abnormality in human chronic myeloid leukaemia. *Nature (Lond.)*, **188**, 1165-1166.
- BAILEY, F. W. (1920). The formation of the cellplate in the cambium of higher plants. *Proc. nat. Acad. Sci. (Wash.)*, **6**, 197-200.

- BAIRATI, A., & LEHMANN, F. E. (1952). Über die submikroskopische Struktur der Kernmembran bei Amoeba proteus. *Experientia*, **8**, 60–61.
- BAJER, A. (1953). Observations on spindle structure and persisting nucleoli. *Acta Soc. Bot. Pol.*, **22**, 653–666.
- (1954). Cine-micrographic studies on mitosis in endosperm I. *Acta Soc. Bot., Pol.*, **23**, 383–412.
- (1959). Change of length and volume of mitotic chromosomes in living cells. *Hereditas*, **45**, 579–596.
- (1961). A note on the behaviour of spindle fibres at mitosis. *Chromosoma (Berl.)*, **12**, 64–71.
- BAJER, A., & MOLÉ-BAJER, J. (1953). Influence of extreme temperatures on mitosis in vivo. I. Hymenophyllum. *Acta Soc. Bot. Pol.*, **22**, 267–298.
- (1956). Cine-micrographic studies on mitosis in endosperm. II. Chromosome, cytoplasmic and Brownian movements. *Chromosoma (Berl.)*, **7**, 558–607.
- (1961). UV microbeam irradiation of chromosomes during mitosis in endosperm. *Exp. Cell Res.*, **25**, 251–267.
- BAKER, J. R. (1945). *Cytological Technique*. Methuen, London.
- (1951). The absorption of lipoid by the intestinal epithelium of the mouse. *Quart. J. micr. Sci.*, **92**, 79–86.
- (1953). Nouveau coup d'œil sur la contraverte du "Golgi" I. Les techniques du "Golgi" et les objets qu'elles révèlent. *Bull. Micr. appl.*, Sér. **2**, **3**, 1–8.
- (1957). The Golgi controversy. *Symp. Soc. exp. Biol.*, **10**, 1–10.
- BAKER, J. R., & CALLAN, H. G. (1950). Heterochromatin. *Nature (Lond.)*, **166**, 227–228.
- BALBIANI, E. G. (1881). Sur la structure du noyau des cellules salivaires chez les larves de Chironomus. *Zool. Anz.*, **4**, 637–641 and 662–666.
- BALBIANI, M. (1876). Sur les phénomènes de la division du noyau cellulaire. *C.R. Acad. Sci. (Paris)*, **83**, 831–834.
- BALLENTINE, R. (1939). The intracellular distribution of reducing systems in the Arbacia egg. *Biol. Bull.*, **77**, 328.
- BALTUS, E. (1954). Observations sur le rôle biochimique du nucléole. *Biochim. biophys. Acta (Amst.)*, **15**, 263–267.
- (1960). Mise en évidence d'une ribonucléase latente dans les nucléoles isolés. *Arch. int. Physiol.*, **68**, 671.
- BANCHER, E. (1938). Mikrochirurgische Kernstudien. *Protoplasma (Wien)*, **31**, 301–310.
- BANK, O. (1939). Abhängigkeit der Kernstruktur von der Ionenkonzentration. *Protoplasma (Wien)*, **32**, 20–30.
- BARBER, H. N. (1939). The rate of movement of chromosomes on the spindle. *Chromosoma (Berlin)*, **1**, 33–50.
- BARDEEN, C. R. (1907). Abnormal development of toad ova fertilized by spermatozoa exposed to the Roentgen rays. *J. exp. Zool.*, **4**, 1–44.
- BARER, R., & DICK, D. A. (1957). Interferometry and refractometry of cells in tissue culture. *Exp. Cell Res.*, Suppl. **4**, 103–135.
- BARER, R., & JOSEPH, S. (1957). Phase-contrast and interference microscopy in the study of cell structure. *Symp. Soc. exp. Biol.*, **10**, 160.
- BARER, R., JOSEPH, S., & MEEK, G. A. (1959). The origin of the nuclear membrane. *Exp. Cell. Res.*, **18**, 179–182.
- (1960). The origin and fate of the nuclear membrane in meiosis. *Proc. roy. Soc. (Lond) B.*, **152**, 353–366.
- (1961). Membrane inter-relationships during meiosis. In: *Electron Microscopy in Anatomy*, edited by BOYD, JOHNSON and LEVER. Arnold, London.
- BARGMANN, W. (1942). Über Kernsekretion in der Neurohypophyse des Menschen. *Z. Zellforsch.*, **32**, 394–400.
- BARGMANN, W., FLEISCHHAUER, K., & KNOOP, A. (1961). Über die Morphologie der Milchsekretion. II. Zugleich eine Kritik am Schema der Sekretionsmorphologie. *Z. Zellforsch.*, **53**, 545–568.
- BARGMANN, W., & KNOOP, A. (1956). Vergleichende elektronenmikroskopische Untersuchungen der Lungenkapillaren. *Z. Zellforsch.*, **44**, 263–281.
- BARGMANN, W., KNOOP, A., & THIEL, A. (1957). Elektronenmikroskopische Studie an der Neurohypophyse von *Tropidonotus natrix* (mit Berücksichtigung der pars intermedia). *Z. Zellforsch.*, **47**, 114–126.
- BARGMANN, W., & KNOOP, A. (1959a). Elektronenmikroskopische Untersuchungen an Plazentartzotten des Menschen. (Bemerkungen zum Syncytiumproblem). *Z. Zellforsch.*, **50**, 472–493.
- (1959b). Über die Morphologie der Milchsekretion. Licht- und elektronenmikroskopische Studien an der Milchrüse der Ratte. *Z. Zellforsch.*, **49**, 344–388.
- (1960). Vakuolenbildung und Mitochondrien. *Z. Zellforsch.*, **51**, 456–466.
- BARIGOZZI, C. (1942). I fenomeni cromosomici nelle cellule semantiche di *Artemia solina* Leach. *Chromosoma (Berl.)*, **2**, 251–292.
- (1950). A general survey on Heterochromatin. *Port. Acta biol.*, **A**, 593–620.

- (1952). La struttura microscopica del nucleo durante il riposo. *Experientia (Basel)*, **8**, 133-136.
- (1954). The structure of the resting nucleus. In: *Fine structure of cells, Symp. 8. Congr. Cell Biol.*
- BARIGOZZI, C., & FELETIG, P. (1950). Heteropycnosis in human chromosomes. *Nature (Lond.)*, **166**, 36-37.
- BARKA, T. (1962). Cellular localization of acid phosphatase activity. *J. Histochem. Cytochem.*, **10**, 231-232.
- BARNARD, E. A., & BELL, L. G. E. (1960). Selective cytochemical demonstration of arginine groups in nucleoprotein in the cell nucleus. *Nature (Lond.)*, **187**, 508-509.
- BARNES, B. G., & DAVIS, J. M. (1959). The structure of nuclear pores in mammalian tissue. *J. Ultra-struct. Res.*, **3**, 131-146.
- BARNUM, C. P., JARDETZKY, C. D., & HALBERG, F. (1954). Nucleic acid synthesis in regenerating liver. *Tex. Rep. Biol. Med.*, **15**, 134-147.
- BARR, M. L. (1959). Sex chromatin and phenotype in man. Disagreement between nuclear sex and phenotype raises questions about the cause of sex anomalies. *Science*, **130**, 679-685.
- (1960). Sexual dimorphism in interphase nuclei. *Amer. J. hum. Genet.*, **12**, 118-127.
- BARR, M. L., & BERTRAM, E. G. (1949). A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature (Lond.)*, **163**, 676-677.
- BARR, M. L., BERTRAM, L. F., & LINDSAY, H. A. (1950). The morphology of the nerve cell nucleus, according to sex. *Anat. Rec.*, **107**, 283-297.
- BARNETT, R. J., & PALADE, G. E. (1958). Applications of histochemistry to electron microscopy. *J. Histochem. Cytochem.*, **6**, 1-12.
- BARSKI, G., & CORNEFERT, F. (1958). Aspects distinctifs des lésions cellulaires causées in vitro par différents types d'adénovirus. Étude de 20 souches appartenant à six types d'adénovirus. *Ann. Inst. Pasteur (Paris)*, **94**, 724-731.
- BARSKI, G., & ROBINEAUX, R. (1959). Evolution of herpes simplex cellular lesions observed in vitro by phase contrast microcinematography. *Proc. Soc. exp. Biol. (N. Y.)*, **101**, 632-636.
- BARTA, E. (1926). Deficient oxydation as a cause of giant cell formation in tissue cultures of lymph nodes. *Arch. exp. Zellforsch.*, **2**, 6-30.
- BARTH, L. G., & BARTH, L. J. (1954). *The energetics of development*. Columbia Univ. Press. New York.
- BARTHELMESS, A. (1957). Chemisch induzierte multipolare Mitosen. *Protoplasma (Wien)*, **48**, 546-561.
- BARTHELMESS, A., & EINLECHNER, J. (1959). Chemisch induzierte multipolare Mitosen II. *Protoplasma (Wien)*, **51**, 325-337.
- BASSI, M. (1960). Electron microscopy of rat liver after carbon tetrachloride poisoning. *Exp. Cell Res.*, **20**, 313-323.
- BASSI, M., BERNELLI-ZAZZERA, A., & CASSI, E. (1960). Electron microscopy of rat liver cells in hypoxia. *J. Path. Bact. (Edinb.)*, **79**, 179-183.
- BASSLEER, R. (1962). Étude de l'augmentation du nombre de noyau dans des bourgeons musculaires cultivés in vitro. Observations sur le vivant, dosages cytophotométriques et histoautoradiographies. *Z. Anat.*, **123**, 184-205.
- BATES, H. M., CRADDOCK, V. M., & SIMPSON, M. V. (1958). The incorporation of valine-1-C¹⁴ into cytochrome c by rat liver mitochondria. *J. amer. chem. Soc.*, **80**, 1000.
- BATTAGLIA, S. (1961). Zur Amyloidgenese. *Klin. Wschr.*, **39**, 795-798.
- BAUCH, R. (1947). Trypaflavin als Typus der Chromosomengifte. *Naturwissenschaften*, **34**, 346-347.
- (1949). Selektive Speicherung von Trypaflavin durch die Nukleoproteide der Chromosomen. *Biol. Zbl.*, **68**, 113-118.
- BAUD, CH. A. (1949). Morphologie submicroscopique de la membrane nucléaire. *Bull. Histol. Techn. micr.*, **26**, 99-100.
- (1953). Recherches sur la structure de la membrane nucléaire. *Acta anat. (Basel)*, **17**, 113-174.
- BAUER, H. (1933). Die wachsenden Oocytenkerne einiger Insekten in ihrem Verhalten zur Nuklealfärbung. *Z. Zellforsch.*, **18**, 254-298.
- (1935). Der Aufbau der Chromosomen aus den Speicheldrüsen von Chironomus Thummi Kiefer. *Z. Zellforsch.*, **23**, 280-313.
- (1936). Beiträge zur vergleichenden Morphologie der Speicheldrüsenchromosomen. *Zool. Jb. (Allgem. Zool.)*, **56**, 239-276.
- (1938). Die polyploide Natur der Riesenchromosomen. *Naturwissenschaften*, **26**, 77.
- (1943). Chromosomenforschung. *Fortschr. Zool.*, **7**, 256-287.
- BAUER, H., & BEERMANN, W. (1952). Die Polytanie der Riesenchromosomen. *Chromosoma (Berl.)*, **4**, 630-648.
- BAUER, H., DIETZ, R., & RÖBBELEN, CH. (1961). Die Spermatocytenteilungen der Tipuliden. III. Mitt. Das Bewegungsverhalten der Chromosomen in Translokationsheterozygoten von *Tipula oleracea*. *Chromosoma (Berl.)*, **12**, 116-189.
- BAUER, K. F. (1953). *Organisation des Nervengewebes und Neurencytiumtheorie*. Urban & Schwarzenberg, München.

- BAUER, K. F., & MÜLLER, E. (1959). Die Zellenlehre. *Med. Grundlagenforsch.*, **2**, 167-193.
- BAUER, K. H. (1928). *Mutationstheorie der Geschwulstentstehung*. Springer, Berlin.
- (1949). *Das Krebsproblem*. Springer, Berlin.
- BAYREUTHER, K. (1952). Extrachromosomal feulgenpositive Körper (Nucleinkörper) in der Oogenese der Tipuliden. *Naturwissenschaften*, **39**, 71.
- (1960). Chromosomes in primary neoplastic growth. *Nature (Lond.)*, **186**, 6-9.
- BEADLE, G. W. (1932). A possible influence of the spindle fibre on crossing over in *Drosophila*. *Proc. nat. Acad. Sci. (Wash.)*, **18**, 160-165.
- (1957). The role of the nucleus in heredity. In: *The chemical basis of heredity*. Hopkins, Baltimore.
- BEAMS, H. W. (1951). The effects of ultracentrifugal force on the cell with special reference to division. *Ann. N. Y. Acad. Sci.*, **51**, 1349-1364.
- BEAMS, H. W., & KING, R. L. (1942). The origin of binucleate and large mononucleate cells in the liver of rat. *Anat. Rec.*, **83**, 281-295.
- (1938). An experimental study on mitosis in the somatic cells of wheat. *Biol. Bull.*, **75**, 189-207.
- BEARCROFT, W. G. C. (1960). Cytological and cytochemical studies on the liver cells of yellow-fever-infected rhesus monkeys. *J. Path. Bact. (Edinb.)*, **80**, 19-31.
- BEASLEY, J. O. (1938). Nuclear size in relation to meiosis. *Bot. Gaz.*, **99**, 865-871.
- BEAVER, D. L. (1961). The ultrastructure of the kidney in lead intoxication with particular reference to intranuclear inclusions. *Amer. J. Path.*, **39**, 195-208.
- BECK, CH. (1956). Reaktionen der Kerne der äußeren Haarzellen beim Meerschweinchen auf adäquate Reize. *Arch. Ohr-, Nas.- u. Kehlk.-Heilk.*, **170**, 81-98.
- BECK, CH., & MICHLER, H. (1960). Feinstrukturelle und histochemische Veränderungen an den Strukturen der Cochlea beim Meerschweinchen nach dosierter Reintonbeschallung. *Arch. Ohr-, Nas.- u. Kehlk.-Heilk.*, **174**, 496-567.
- BECKER, F. F., & GREEN, H. (1960). The effects of protamines and histones on the nucleic acids of ascites tumor cells. *Exp. Cell. Res.*, **19**, 361-375.
- BECKER, H. J. (1959). Die Puffs der Speicheldrüsenchromosomen von *Drosophila melanogaster*. I. Mitt. Beobachtungen zum Verhalten des Puffmusters im Normalstamm und bei zwei Mutanten, giant und lethal-giant-larvae. *Chromosoma (Berl.)*, **10**, 654-678.
- BECKER, V. (1959). Morphologisches Äquivalentbild des äußeren und inneren Sauerstoffmangels. *Med. Grundlagenforsch.*, **2**, 341-383.
- BECKER, V., & NEUBERT, D. (1959). Über die Entstehung der hydropisch-vakuolären Zellentartung. *Beitr. path. Anat.*, **120**, 319-354.
- BEER, R., & ARBER, A. (1920). On multinucleate cells; A historical study (1879-1919). *J. roy. micr. Soc.*, **23**.
- BEERMAN, W. (1952a). Chromomerenkonstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. *Chromosoma (Berl.)*, **5**, 139-198.
- (1952b). Chromosomenstruktur und Zelldifferenzierung in der Speicheldrüse von *Trichocladius vitripennis*. *Z. Naturforsch.*, **7b**, 237-242.
- (1956). Nuclear differentiation and functional morphology of chromosomes. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 217-230.
- (1960). Der Nukleolus als lebenswichtiger Bestandteil des Zellkerns. *Chromosoma (Berl.)*, **11**, 262-296.
- (1961). Ein Balbiani-Ring als Locus einer Speicheldrüsenmutation. *Chromosoma (Berl.)*, **12**, 1-25.
- (1962). Riesenchromosomen. *Protoplasmatologia*, **VI D**. Springer, Wien.
- BELAR, K. (1922). Untersuchungen an *Actinophrys sol* Ehrenberg. I. Die Morphologie des Formwechsels. *Arch. Protistenk.*, **46**, 1..
- (1924). Die Cytologie der Microspermie bei freilebenden Rhabditisarten. *Z. Zellforsch.*, **1**, 1-21.
- (1953). 1924. Zit. nach HARTMANN, M.: *Allgemeine Biologie*. Fischer, Stuttgart.
- (1926). Der Formwechsel der Protistenkerne. *Erg. Zool.*, **6**, 1-420.
- (1927). Beiträge zur Kenntnis der indirekten Kernteilung. *Naturwissenschaften*, **15**, 725-734.
- (1928). Die cytologischen Grundlagen der Vererbung. In: *Hdb. d. Vererbungswiss.*, Vol. I, Borntraeger, Berlin.
- (1929). Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von *Chorthippus (Stenobothrus) linatus* Panz. *Arch. Entwickl.-Mech. Org.*, **118**, 359-484.
- (1930a). Über die reversible Entmischung lebenden Plasmas. *Protoplasma (Wien)*, **9**, 209-244.
- (1930b). Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen an den Staubfädenhaaren und Blattmeristemzellen von *Tradescantia virginica*. *Z. Zellforsch.*, **10**, 73-134.
- BELAR, K. & HUTH, W. (1933). Zur Teilungsautonomie der Chromosomen. *Z. Zellforsch.*, **17**, 51-66.
- BELJANSKI, M. (1960). Protein biosynthesis by a cell-free bacterial system. III. Determination of new peptide bonds requirement for the "amino acid incorporation enzyme" in protein biosynthesis. *Biochim. biophys. Acta (Amst.)*, **41**, 104-110.

- BELJANSKI, M., & OCHOA, S. (1958). Protein biosynthesis by a cell-free bacterial system. II. Further studies on the amino acid incorporation enzyme. *Proc. nat. Acad. Sci. (Wash.)*, **44**, 1157-1161.
- BELLING, J. (1927). The attachments of chromosomes at the reduction division in flowering plants. *J. Genet.*, **18**, 177-205.
- (1928). The ultimate chromomeres of *Lilium* and *Aloe* with regard to the number of genes. *Univ. Calif. Publ. Bot.*, **14**, 307-318.
- (1931). Chiasmas in flowering plants. *Univ. Calif. Publ. Bot.*, **16**, 311-338.
- (1933). Crossing over and gene rearrangement in flowering plants. *Genetics*, **18**, 388-413.
- BELT, W. D., & PEASE, D. C. (1956). Mitochondrial structure in sites of steroid secretion. *J. biophys. biochem. Cytol.*, Suppl. **2**, 369-372.
- BENDA, C. (1902). Die Mitochondria. *Ergebn. Anat. Entwickl.-Gesch.*, **12**, 743-781.
- BENEDEN, E. VAN (1876). Recherches sur les Dicyemides. *Bull. Acad. roy. Méd. Belg.*, Ser. **2**, **41**, 1160-1205.
- (1883). Recherches sur la maturation de l'œuf, la fécondation et la division cellulaire. *Arch. Biol. (Liège)*, **4**, 265-641.
- BENEDEN, E. VAN, & NEYT, A. (1887). Nouvelles recherches sur la fécondation et la division mitosique chez l'Ascaride mégalocéphale. *Bull. Acad. roy. Méd. Belg.*, Ser. **3**, 215-295.
- BENEDETTI, L. E., & LEPLUS, R. (1958). Cytologie de l'erythroblastose avaire. (Étude au microscope électronique). *Rev. Hémat.*, **13**, 199-230.
- BENNETT, H. S., & PORTER, K. R. (1953). An electron microscope study of sectioned breast muscle of the domestic fowl. *Amer. J. Anat.*, **93**, 61-105.
- BENNETT, L. L., SIMPSON, L., & SKIPPER, H. E. (1960). On the metabolic stability of nucleic acids in mitotically inactive adult tissues labelled during embryonic development. *Biochim. biophys. Acta (Amst.)*, **42**, 237-243.
- BENNETT, S. (1956). The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping. *J. biophys. biochem. Cytol.*, **2**, Suppl., 99-103.
- BENNINGHOFF, A. (1922). Zur Kenntnis und Bedeutung der Amitose und amitotischähnlicher Vorgänge. *S.-B. Ges. Förd. ges. Naturw.*, Marburg, 45-68.
- (1923). Beobachtungen über Umformungen der Bindegewebszellen. *Arch. mikr. Anat.*, **99**, 571-605.
- (1950). Funktionelle Kernschwellung und Kernschrumpfung. *Anat. Nachr.*, **1**, 50-52.
- (1951). Vermehrung und Vergrößerung von Nervenzellen bei Hypertrophie des Innervationsgebietes. *Z. Naturforsch.*, **6b**, 38-41.
- BENSBERG, R. R. (1951). Facts versus artefacts in cytology: The Golgi apparatus. *Exp. Cell Res.*, **2**, 1-9.
- BENSCH, K. G., & KING, D. W. (1961). Incorporation of heterologous desoxyribonucleic acid into mammalian cells. *Science*, **133**, 381-382.
- BENSLEY, R. R. (1911). Studies of the pancreas of the guinea pig. *Amer. J. Anat.*, **12**, 297-388.
- BENSLEY, R. R., & BENSLEY, S. H. (1941). Handbook of histological and cytological technique. Chicago.
- BERG, P. (1956). Acyl adenylates: The interaction of adenosine triphosphate and L-methionine. *J. biol. Chem.*, **222**, 1025-1034.
- BERG, W. (1934). Über den mikroskopisch nachweisbaren Übergang von Stoffen aus dem Cytoplasma in den Kern der Leberzelle. *Z. mikr.-anat. Forsch.*, **35**, 146-180.
- BERGAN, P. (1960). On the blocking of mitosis by heat shock applied at different mitotic stages in the cleavage divisions of *Trichogaster trichopterus* var. *sumatranaus* (Teleostei: Anabantidae). *Nytt Magas. Zool.*, **9**, 37-121.
- BERGER, C. A. (1938). Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. *Carnegie Inst. Wash. Publ.*, **476**, 209-232.
- BERGERARD, J. (1955). Synthèse de l'acide thymonucléique au cours du cycle mitotique des neuroblastes et des cellules nerveuses embryonnaires d'un insecte *Clitumnus extradentatus* Br. (Phasmidae). *C.R. Acad. Sci. (Paris)*, **240**, 564-567.
- BERGERON, J. A., & FULLER, R. C. (1961). The photosynthetic macromolecules of *Chlorobium thiosulfatophilum*. In: *Proc. I. IUB IUBS Symp.*, Vol. **II**, Acad. Press, London.
- BERGHS, J. (1909). Les cineses somatiques dans le Marsilia. *Cellule*, **25**, 73-84.
- BERKELEY, E. (1948). Spindle development and behaviour in the giant amoeba. *Biol. Bull.*, **94**, 169-175.
- BERLINER, R. W., KENNEDY, T. J., & ORLOFF, L. (1951). Relationship between acidification of the urine and potassium metabolism. *Amer. J. Med.*, **11**, 274-282.
- BERN, H. A., NISHIOKA, R. S., & HAGADORN, I. R. (1961). Association of elementary neurosecretory granules with the Golgi complex. *J. Ultrastruct. Res.*, **5**, 311-320.
- BERNAL, J. D., & FANKUCHEN, A. (1937). Structure types of protein "crystals" from virus-infected plants. *Nature (Lond.)*, **139**, 923-924.
- BERNAL, J. D., & FANKUCHEN, I. (1941). X-ray and crystallographic studies of plant virus preparations. I. II. III. *J. gen. Physiol.*, **25**, 111-165.
- BERNARD, C. (1865). *Introduction à l'étude de la médecine expérimentale*. Baillière, Paris.

- BERNELLINI-ZAZZERA, A., CARADONNA, D., & CASSI, E. (1960). Studies on the inhibition of protein synthesis in vacuolated rat liver cells. *Exp. Cell. Res.*, **20**, 592-595.
- BERNELLINI-ZAZZERA, A., & GUIDOTTI, G. (1958). Protein synthesis in vacuolated rat liver cells. *Exp. Cell Res.*, **14**, 614-618.
- BERNHARD, W. (1958). Ultrastructural aspects of nucleocytoplasmic relationship. *Exp. Cell Res.*, Suppl. **6**, 17-50.
- BERNHARD, W., & BAUER, A. (1954). Nouvelles acquisitions sur l'ultrastructure des cellules tumorales. *Symp. 8. Congr. of Cell. Biol.*, Leiden.
- BERNHARD, W., BAUER, A., GROPP, A., HAGUENAU, F., & OBERLING, CH. (1955). L'ultrastructure du nucléole de cellules normales et cancéreuses. Étude au microscope électronique. *Exp. Cell Res.*, **9**, 88-100.
- BERNHARD, W., HAGUENAU, F., & OBERLING, CH. (1952). L'ultrastructure du nucléole de quelques cellules animales, révélée par le microscope électronique. *Experientia (Basel)*, **8**, 58-59.
- BERNHARD, W., & DE HARVEN, E. (1956). Sur la présence dans certaines cellules de Mammifères d'un organite de nature probablement centriolaire. Étude au microscope électronique. *C. R. Acad. Sci. (Paris)*, **242**, 288-290.
- BERNHARD, W., & LEDUC, E. (1960). Essais de cytochimie ultrastructurale. Action sur l'ergastoplasm. *C. R. Acad. Sci. (Paris)*, **250**, 3411-3413.
- BERNHARD, W., & LEPLUS, R. (1955). La méthode des coupes ultrafines et son application à l'étude de l'ultrastructure des cellules sanguines. *Schweiz. med. Wschr.*, **85**, 897-899.
- BERNHARD, W., & ROUILLER, C. (1956). Close topographical relationship between mitochondria and ergastoplasm of liver cells in a definite phase of cellular activity. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 73-78.
- BERNSTEIN, M. H., & MAZIA, D. (1953a). The desoxyribonucleoprotein of sea urchin sperm. I. Isolation and analysis. *Biochim. biophys. Acta (Amst.)*, **10**, 600-606.
- (1953b). The desoxyribonucleoprotein of sea urchin sperm. II. Properties. *Biochim. biophys. Acta (Amst.)*, **11**, 11-68.
- BERTALANFFY, F. D. (1961). Diagnostic reliability of the acridine orange fluorescence microscope method for cytodiagnosis of cancer. *Cancer Res.*, **21**, 422-426.
- BERTHOLD, G. (1886). *Studien über die Protoplasma mechanik*. Felix, Leipzig.
- BESSIS, M. (1949). Etudes sur les cellules sanguines au microscope à contraste de phase et par la méthode de l'ombrage (avec une étude particulière des mégacaryocytes). *Rev. Hémat.*, **4**, 294-349.
- (1959). Erythropoiesis, as seen with the electron microscope. In: *The kinetics of cellular proliferations*. Grune and Stratton, New York.
- (1961). Neue signifikante Beiträge der dynamischen Zytologie zur Hämatologie. In: *Fortschritte der Hämatologie*, Vol. II, edited by TOCANTINS, L. M. Thieme, Stuttgart.
- BESSIS, M., & BRETON-GORIUS, J. (1957). Le centriole des cellules du sang. Étude à l'état vivant et au microscope électronique. *Bull. Micr. appl. Sér.*, **2**, 54-56.
- (1960). Aspects de la molécule de ferritine et d'apoferritine au microscope électronique. *C. R. Acad. Sci. (Paris)*, **250**, 1360-1362.
- BESSIS, M., & BRICKA, M. (1952). Aspect dynamique des cellules du sang. Son étude par la microcinématographie en contraste de phase. *Rev. Hémat.*, **7**, 407-435.
- BESSIS, M., & THIERY, J.-P. (1961). Electron microscopy of human white blood cells and their stem cells. *Int. Rev. Cytol.*, **12**, 199-241.
- BESSIS, M. C., & BRETON-GORIUS, J. (1962). Iron metabolism in the bone marrow as seen by electron microscopy: A critical review. *Blood*, **19**, 635-663.
- BESTE, W. (1961). Die Größe der Fettzellen und ihre Abhängigkeit vom Ernährungszustand. *Virchows Arch. path. Anat.*, **334**, 243-250.
- BETHE, K. (1943). Entwicklung und Regeneration von Acicularia Schenckii. *Z. indukt. Abstamm.- u. Vererb.-L.*, **81**, 252-270.
- BETHE, A. (1903). *Allgemeine Anatomie und Physiologie des Nervensystems*. Thieme, Leipzig.
- BHARADWAJ, T. P., & LOVE, R. (1959). Cytology of rat liver cells during starvation and refeeding. *J. Nat. Cancer Inst.*, **23**, 695-715.
- BHATTACHARYYA, T. K., & GHOSH, A. (1961). On the occurrence of Feulgen positive nucleoli in adrenocortical cells of the pigeon. *Naturwissenschaften*, **48**, 389-390.
- BIER, K. (1958). Beziehungen zwischen Wachstumsgeschwindigkeit, endometaphasischer Kontraktion und der Bildung von Riesenchromosomen in den Nährzellen von Calliphora. *Z. Naturforsch.*, **13b**, 85-93.
- (1960). Der Karyotyp von Calliphora crythrocephala Meigen unter besonderer Berücksichtigung der Nährzellchromosomen im gebündelten und gepaarten Zustand. *Chromosoma (Berl.)*, **11**, 335-364.
- BIERLING, R. (1960). Die Wirkung von Aktinomycinen auf menschliche Gewebe in vitro. *Z. Krebsforsch.*, **63**, 519-522.
- BIESELE, J. J. (1944). Chromosome complexity in regenerating rat liver. *Cancer Res.*, **4**, 232-235.

- (1954). Assay of carcinolytic and carcinostatic agents. *Ann. N. Y. Acad. Sci.*, **58**, 1129-1146.
 — (1958a). *Mitotic poisons and the cancer problem*. Elsevier, Amsterdam.
 — (1958b). Studies on mitosis in purin-treated tissue cultures. In: *Frontiers in Cytology*. Yale Univ. Press, New Haven.
 — (1960). Action of certain antimetabolites as mitotic poisons. In: *Fundamental aspects of normal and malignant growth*, edited by NOWINSKI. Elsevier, Amsterdam.
 — (1961). On the mechanisms of antimitotic action, as studied with cancer cells and antimetabolites. *Path. et Biol.*, **9**, 466-473.
 — (1962). Experimental and therapeutic modification of mitosis. *Cancer Res.*, **22**, 779-787.
- BIESELE, J. J., BERGER, R. E., & CLARKE, M. (1952). Tissue culture screening of purines and purine nucleosides for selective damage to mouse sarcoma cells. *Cancer Res.*, **12**, 399-406.
- BIRBECK, M. S. C., & MERCER, E. H. (1961). Cytology of cells which synthesize protein. *Nature (Lond.)*, **189**, 558-560.
- BJERKNES, V. (1902). *Hydrodynamische Formkräfte*. Barth, Leipzig.
- (1909). *Die Kraftfelder*, Vieweg, Braunschweig.
- BLEIER, H. (1930). Untersuchungen über das Verhalten der verschiedenen Kernkomponenten bei der Reduktionsteilung von Bastarden. *Cellule*, **40**, 85-144.
 — (1931). Zur Kausalanalyse der Kernteilung. *Genetica*, **13**, 27-76.
 — (1939). Mechanismus der Kernteilung. *Arch. exp. Zellforsch.*, **22**, 257-261.
- BLINZINGER, K., & HAGER, H. (1961). Elektronenmikroskopische Beobachtungen bei Spätstadien von experimenteller bakterieller Meningitis. *Verh. Dtsch. Ges. Path.*, **45**, 357-362.
- BLOCH, D. P. (1958). Changes in the desoxyribonucleoprotein complex during the cell cycle. In: *Frontiers in Cytology*. Yale Univ. Press, New Haven.
- BLOCH, D. P., & GODMAN, G. C. (1955). Evidence of differences in the desoxyribonucleoprotein complex of rapidly proliferating and nondividing cells. *J. biophys. biochem. Cytol.*, **1**, 531-550.
- BLOCH, D. P., MORGAN, C., GODMAN, G. C., HOWE, L., & ROSE, H. M. (1957). A correlated histochemical and electron microscopic study of the intranuclear crystalline aggregates of adenovirus (RJ-APC-Virus) in HeLa cells. *J. biophys. biochem. Cytol.*, **3**, 1-7.
- BOCH, H. E., LAUMEN, F., & HÜBNER, K. (1953). Klinische Möglichkeiten des Cytohepatogramms. *Schweiz. Z. all. Path.*, **16**, 429-435.
- BOCK, G., & HAUPP, W. (1961). Die Chloroplastendrehung bei Moutiag. III. Die Frage der Lokalisierung des Hellrot-Dunkelrot-Pigmentsystems in der Zelle. *Planta*, **57**, 518-530.
- BÖÖK, J. A., GUSTAVSON, K. H., & SANTESSON, B. (1961). Chromosomal abnormality in a Mongolism-like syndrome. *Acta paediat. (Uppsala)*, **50**, 240-248.
- BÖÖK, J. A., & SANTESSON, B. (1960). Malformation syndrome in man associated with triploidy (69 chromosomes). *Lancet*, **i**, 858-859.
- BOGAROCHE, R., & SIEGEL, B. V. (1961). Some metabolic properties of the nucleolus as demonstrated by recent radioisotope experiments. *Acta anat. (Basel)*, **45**, 265-287.
- BOHN, G. (1903). Influence des rayons du radium sur les animaux en voie de croissance. *C. R. Acad. Sci. (Paris)*, **136**, 1012-1013.
- BOIVIN, A., VENDRELY, R., & VENDRELY, C. (1948). L'acide désoxyribonucléique du noyau cellulaire, dépositaire des caractères héréditaires; arguments d'ordre analytique. *C. R. Acad. Sci. (Paris)*, **226**, 1061-1063.
- BOLLUM, F. J. (1960). Desoxyribonucleic acid synthesis by mammalian enzymes: Some observations on primers. In: *The Cell Nucleus*. Butterworths, London.
- BOLONGARI, A. (1959). Recenti vedute sulla struttura, sull'ultrastruttura, sullo stato fisico-chimico e sulla attività funzionali del nucleolo. *Arch. Zool. It.*, **44**, 53-139.
 — (1960). Precisazioni sul nucleolonema. *Experientia (Basel)*, **16**, 307-308.
 — (1961). Vedute attuali sul nucleolo e sull'ergastoplasma degli ovociti e delle cellule tumorali. *Atti Soc. pelorit.*, **7**, 1-104.
- BONNIEVE, K. (1906). Untersuchungen über Keimzellen. I. Beobachtungen an den Keimzellen von *Enteroxenos östergrenii*. *Jena. Z. Med. Naturw.*, **41**, N.F., 34, 229-428.
 — (1910). Über die Rolle der Zentralspindel während der indirekten Zellteilung. *Arch. Zellforsch.*, **5**, 1-35.
- BOOS, J. (1954). Mitosis in cultures of newt tissues. I. A critical study of methods and material. *Exp. Cell Res.*, **7**, 215-224.
- BOPP-HASSENKAMP, G. (1959a). Untersuchungen über die submikroskopische Fibrillenstruktur von Kern und Nukleolen. *Z. Naturforsch.*, **14b**, 188-194.
 — (1959b). Lichtmikroskopische und elektronenoptische Untersuchungen über den Aufbau pflanzlicher Chromosomen im Pachytän der Meiosis. *Protoplasma (Wien)*, **50**, 243-268.
- BORSOOK, H., DEASY, C. L., HAAGEN-SMITH, A. J., KEIGHLEY, G., & LOWY, P. H. (1949). The incorporation of labelled lysine into the protein of guinea pig liver homogenate. *J. biol. Chem.*, **179**, 689-704.

- (1950). Incorporation of C₁₄ labelled amino acids into proteins of fractions of guinea pig liver homogenate. *Fed. Proc.*, **9**, 154-155.
- (1952). Incorporation in vitro of labelled amino acids into proteins of rabbit reticulocytes. *J. biol. Chem.*, **196**, 669-694.
- BORST, P. (1963). Hydrogen transport and transport metabolites. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- BORSTEL, R. C. VON, & REKEMEYER, M. L. (1958). Division of a nucleus lacking a nucleolus. *Nature (Lond.)*, **181**, 1597-1598.
- BORYSKO, E., & BANG, F. B. (1951). Structure of the nucleolus as revealed by the electron microscope. A preliminary report. *Bull. Johns Hopkins Hosp.*, **89**, 468-473.
- BORYSKO, E., & SAPRANAUSKAS, P. (1954). A new technique for comparative phase contrast and electron microscope studies of cells grown in tissue culture with an evaluation of the technique by means of the time-lapse cinematography. *Bull. Johns Hopkins Hosp.*, **95**, 68-79.
- BOSCHKE, F. L. (1956). Zur Entstehung des Lebens. *Dtsch. med. Wschr.*, **85**, 1953-1960.
- Boss, J. (1954). Mitosis in cultures of newt tissues. II. Chromosome pairing in anaphase. *Exp. Cell Res.*, **7**, 225-231.
- (1955a). The pairing of somatic chromosomes: A survey. *Tex. Rep. Biol.*, **13**, 213-221.
- (1955b). Mitosis in culture of newt tissues. IV. The cell surface in late anaphase and the movements of ribonucleoprotein. *Exp. Cell. Res.*, **8**, 181-187.
- (1959). The contribution of the chromosomes to the telophase nucleus in cultures of fibroblasts of the adult crested newt *Triturus cristatus carnifex*. *Exp. Cell Res.*, **18**, 197-216.
- BOVERI, TH. (1887). *Zellenstudien*, I. Fischer, Jena.
- (1888). *Zellenstudien*, II. Die Befruchtung und Teilung des Eies von *Ascaris megalcephala*. Fischer, Jena.
- (1890). *Zellenstudien*, III. Über das Verhalten der chromatischen Kernsubstanz bei der Bildung der Richtungskörper und bei der Befruchtung. Fischer, Jena.
- (1900). *Zellenstudien*, IV. Über die Natur der Centrosomen. Fischer, Jena.
- (1904). *Die Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns*. Fischer, Jena.
- (1905). *Zellenstudien*, V. Über die Abhängigkeit der Kerngröße und Zellenzahl der Seeigel-Larven von der Chromosomenzahl der Ausgangszellen. Fischer, Jena.
- (1910). Über die Teilung zentrifugierter Eier von *Ascaris megalcephala*. *Arch. Entwickl.-Mech. Org.*, **30**, Part II, 101-125.
- (1914). *Über die Entstehung maligner Tumoren*. Fischer, Jena.
- BOWEN, R. H. (1929). The cytology of glandular secretion. *Quart. Rev. Biol.*, **4**, 299-324, & 484-519.
- BOYLAND, E., & KOLLER, P. C. (1954). Effects of urethane on mitosis in the Walker rat carcinoma. *Brit. J. Cancer*, **8**, 677-684.
- BRACHET, A. (1910). La polyspermie expérimentale comme moyen d'analyse de la fécondation. *Arch. Entwickl.-Mech. Org.*, **30**, 261-303.
- BRACHET, J. (1940). La détection histochimique des acides pentosenucléiques. *C. R. Soc. Biol. (Paris)*, **133**, 88-90.
- (1941). La détection histochimique et le microdosage des acides pentosenucléiques. *Enzymologia*, **10**, 87.
- (1941). La localisation des acides pentosenucléiques dans les tissus animaux et les œufs d'amphibiens en voie de développement. *Arch. Biol. (Liège)*, **53**, 207-257.
- (1947). *Embryologie chimique*. Masson, Paris.
- (1955). Recherches sur les interactions biochimiques entre le noyau et le cytoplasme chez les organismes unicellulaires. I. Amoeba proteus. *Biochim. biophys. Acta (Amst.)*, **18**, 247-268.
- (1957). *Biochemical Cytology*. Acad. Press, New York.
- (1959a). Ribonucleinsäure und Proteinsynthese. In: *Handbuch der Histochemie*, Vol. III/1, edited by GRAUMANN, W., & NEUMANN, K. Fischer, Stuttgart.
- (1959b). New observations on biochemical interactions between nucleus and cytoplasm in Amoeba and Acetabularia. *Exp. Cell Res.*, Suppl. **6**, 78-96.
- (1961). Nucleocytoplasmic interactions in unicellular organisms. In: *The Cell*, Vol. II. Acad. Press, New York.
- BRACHET, J., CHANTRENNE, H., & VANDERHAEGHE, F. (1955). Recherches sur les interactions biochimiques entre le noyau et le cytoplasme chez les organismes unicellulaires. II. Acetabularia mediterranea. *Biochim. biophys. Acta (Amst.)*, **18**, 544-563.
- BRANDES, D., & PORTELA, A. (1960). The fine structure of the epithelial cells of the mouse prostate. I. Coagulating gland epithelium. *J. biophys. biochem. Cytol.*, **7**, 505-510.
- BRANDT, P. W. (1958). A study of the mechanism of pinocytosis. *Exp. Cell Res.*, **15**, 300-313.
- BRANDT, P. W., & PAPPAS, G. D. (1960). An electron microscopic study of pinocytosis in ameba. I. The surface attachment phase. *J. biophys. biochem. Cytol.*, **8**, 675-687.
- (1962). An electron microscopic study of pinocytosis in Ameba. *J. Cell Biol.*, **15**, 55-71.

- BRAUN, H. (1958). Über Strukturveränderungen der Lebermitochondrien nach Röntgenbestrahlung. *Naturwissenschaften*, **45**, 18–19.
- (1960). Elektronenoptische Untersuchungen an Zellen des Dünndarmepithels nach Röntgenbestrahlung. *Exp. Cell Res.*, **20**, 267–276.
- BRAUNSTEINER, H., & PAKESCH, F. (1955). Electron microscopy and the functional significance of a new cellular structure in plasmocytes: A review. *Blood*, **10**, 650–564.
- BRAUNSTEINER, H., FIALA, Y., PAKESCH, F., & AUERSWALD, W. (1958). Über das intrazelluläre Verhalten des Poliomyelitisvirus nach Infektion von Zellkulturen. *Klin. Wschr.*, **36**, 1128–1132.
- BRAUNSTEINER, H., EIBL, M., & PAKESCH, F. (1960). Elektronenmikroskopische Beobachtungen über Bakterienphagozytose durch Makrophagen. *Wien. Z. inn. Med.*, **41**, 373–388.
- BRENNER, S., JACOB, F., & MESELSON, M. (1961). An unstable intermediate RNA carrying informations from genes to ribosomes for protein synthesis. *Nature (Lond.)*, **190**, 576–581.
- BRESSLAU, E. VON (1910). Über die Sichtbarkeit der Centrosomen in lebenden Zellen. *Zool. Anz.*, **35**, 141–145.
- BREUER, M. F., & PAVAN, C. (1955). Behaviour of polytene chromosomes of Rhynchosciara angelae in different stages of development. *Chromosoma (Berl.)*, **7**, 275–280.
- BRICE, A. T., JONES, R. P., & SMYTH, J. D. (1946). Golgi apparatus by phase-contrast microscopy. *Nature (Lond.)*, **157**, 553–554.
- BRIDGES, C. B. (1915). A linkage variation in *Drosophila*. *J. exp. Zool.*, **19**, 1–21.
- (1916). Non-disjunction as a proof of the chromosome theory of heredity. *Genetics*, **1**, 1–52 & 107–163.
- (1935). Salivary chromosome maps. *J. Hered.*, **26**, 60–64.
- BRIDGES, P. N. (1942). A new map of the salivary gland III chromosome of *Drosophila melanogaster*. *J. Hered.*, **33**, 403–408.
- BRICK, L. G., COOMBS, J. S., & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.*, **117**, 431–460.
- BROGHAMER, W. L. jr., & CHRISTOPHERSON, W. M. (1961). An interferometric study of the anhydrous nuclear mass of exfoliated cells from experimental cervical cancer. *Cancer (Philad.)*, **14**, 378–383.
- BROWN, M. S. (1954). A comparison of pachytene and metaphase pairing in species hybrids of *Gossypium*. *Genetics*, **39**, 962–963.
- BROWN, R. (1833) Observations on the organs and mode of fecundation in Orchideae. *Trans. Linn. Soc.*, **16**, 685.
- BROWN, W. V., & EMERY, W. H. P. (1957). Persistent nucleoli and grass systematics. *Amer. J. Bot.*, **44**, 585–590.
- BRÜSCHKE, G., & HERRMANN, H. (1962). Das numerische Verhalten der Anhängsel des Leukozytenkernes im Alter. *Dtsch. Gesundh.-Wes.*, **17**, 1–4.
- BRUM, N., LAGUARDIA, A., & SÁEZ, F. A. (1959). A study on sex chromatin. *Texas Rep. Biol. Med.*, **17**, 73–81.
- BRUMFIELD, R. T. (1940). Anaphase movement in *Allium cernuum*. *Science*, **91**, 97–98.
- BRUN, J., & CHEVASSU, D. (1958). L'évolution des structures chromatiniennes dans les cellules nourricières des ovocytes chez *Drosophila melanogaster* Meigen. *Chromosoma (Berl.)*, **9**, 537–558.
- BRUYN, P. P. H. DE (1947). Theories of amoeboid movement. *Quart Rev. Biol.*, **22**, 1–23.
- BRYANT, R. E., THOMAS, W. A., & O'NEAL, R. M. (1958). An electron microscopic study of myocardial ischemia in the rat. *Circulat. Res.*, **6**, 699–709.
- BUCHER, N. L. R., SCOTT, J. F., & AUB, J. C. (1950). Regeneration of the liver in parabiotic rats. *Cancer Res.*, **10**, 207.
- BUCHER, O. (1939). Zur Kenntnis der Mitose VI. Der Einfluß von Colchicin und Trypaflavin auf den Wachstumsrhythmus und auf die Zellteilung in Fibrocyten-Kulturen. *Z. Zellforsch.*, **29**, 283–322.
- (1952). Zur Analyse von Kerngrößenfrequenzkurven. Zugleich ein Beitrag zur Frage der Polyploidieerzeugung in Gewebekulturen. *Experientia (Basel)*, **8**, 201–204.
- (1955). Karyometrische Untersuchungen an Gewebekulturen in vitro. XVII. Kern- und Kernkörperchengröße in verschiedenen rasch wachsenden Bindegewebekulturen. *Z. Anat.*, **118**, 531–542.
- (1958a). Gibt es eine Amitose? *Z. mikr.-anat. Forsch.*, **64**, 100–109.
- (1958b). Zur Entstehung zweikerniger Zellen in Bindegewebekulturen. (Zugleich ein Beitrag zur Frage der Amitose.) *Z. mikr.-anat. Forsch.*, **64**, 174–191.
- (1959). Die Amitose der tierischen und menschlichen Zelle. *Protoplasmatalogia*, **VI/E** 1. Springer, Wien.
- (1962). Introduction au problème de l'amitose. *Acta anat.*, **48**, 173–174.
- BUCHER, O., & GATTIKER, R. (1954). Karyometrische Untersuchungen an Gewebekulturen. X. Über die zweikernigen Bindegewebzellen in vitro. *Z. mikr.-anat. Forsch.*, **60**, 308–323.
- BUCHER, O., & DÉLÈZE, J. (1955). Recherches complémentaires sur les cellules binucléées (foie et épithélium de transition). *Anat. Anz.*, **102**, 1–20.

- BUCHER, O., & GAILLOUD, C. (1958). Zum Verhalten der Zellkerne bei verschiedenen Funktionszuständen der Nierenkanälchen. *Bull. schweiz. Akad. med. Wiss.*, **14**, 254-272.
- BUCHER, O., & KLOTI, R. (1955). Karyometrische Untersuchungen an Gewebekulturen. XV. Mitt.: Über das intermitotische Kernwachstum. *Z. Zellforsch.*, **42**, 193-212.
- BUCHNER, P. (1910). Von den Beziehungen zwischen Centriol und Bukettstadium. *Arch. Zellforsch.*, **5**, 215-228.
- (1911). Die Reifung des Seesterneies bei experimenteller Parthenogenese. *Arch. Zellforsch.*, **6**, 577-612.
- BUCK, R. C. (1961). Lamellae in the spindle of mitotic cells of Walker 256 carcinoma. *J. biophys. biochem. Cytol.*, **11**, 227-236.
- BUCK, R. C., & TISDALE, J. M. (1962a). The fine structure of the mid-body of the rat erythroblast. *J. Cell. Biol.*, **13**, 109-115.
- (1962b). An electron microscope study of the development of the cleavage furrow in mammalian cells. *J. Cell. Biol.*, **13**, 117-125.
- BUCKLEY, I. K. (1962). Cellular injury in vitro: Phase contrast studies on injured cytoplasm. *J. Cell. Biol.*, **14**, 401-420.
- BUCKLEY, W. B., WITHUS, E. R., & BERGER, C. A. (1962). Kinetin, as a mitotic stimulant in *Triturus viridescens*. *Nature (Lond.)*, **194**, 1200.
- BÜCHNER, F. (1941). Das Problem der Form in der Pathologie. *Beitr. path. Anat.*, **105**, 319-336.
- (1944). Die pathogenetische Bedeutung des allgemeinen Sauerstoffmangels. *Verh. dtsch. path. Ges.*, **20**-38.
- (1955). Entwicklungslinien und Grenzen der Cellularpathologie. *Klin. Wschr.*, **33**, 289-294.
- (1957a). Die Pathologie der cellulären und geweblichen Oxydationen, die Hypoxydosen. In: *Handbuch der allgemeinen Pathologie*, Vol. IV/2, edited by BÜCHNER, F., LETTERER, E., & ROULET, F. Springer, Berlin.
- (1957b). Die Morphologie der Virushepatitis, insbesondere der posthepatitischen Narbenprozesse in der Leber. *Verh. dtsch. Ges. inn. Med.*, **63**, 155-176.
- (1959a). *Stoffwechsel und Struktur in der modernen Pathologie*. Freiburger Univ.-Reden N.F. Hft. **29**, Schulz, Freiburg.
- (1959b). Die Veränderungen der Ultrastruktur der Herzmuskelzelle bei Störungen der Aerobiose. Ein Beitrag zum Problem der Koronarisuffizienz. *Ärztl. Forsch.*, **13**, 1307-1314.
- (1960). Die Bedeutung der Morphologie für die moderne Medizin. *Dtsch. med. Wschr.*, **85**, 1665-1669 & 1683-1686.
- (1961a). Die experimentelle Kanzerisierung der Parenchymzelle in der Synopsis klassischer und moderner morphologischer Methoden. *Verh. dtsch. Ges. Path.*, **45**, 37-59.
- (1961b) *Allgemeine Pathologie* 4th edition, Urban & Schwarzenberg, München.
- BÜCHNER, F., GRUNDMANN, E., & OEHLMER, W. (1961). Die experimentelle Kanzerisierung der Parenchymzelle. *Dtsch. med. Wschr.*, **86**, 1845-1850.
- BÜCHNER, F., & v. LUCADOU, W. (1934). Elektrocardiographische Veränderungen und disseminierte Nekrosen des Herzmuskels bei experimenteller Coronarisuffizienz. *Beitr. path. Anat.*, **93**, 169-197.
- BÜCHNER, F., MÖLBERT, E., & THALE, L. (1959). Das submikroskopische Bild der Herzmuskelzelle nach toxischer Hemmung der Aerobiose. *Beitr. path. Anat.*, **121**, 145-169.
- BÜRGER, M. (1961). Über das Sex-Chromatin in diploiden und tetraploiden Parenchymkernen menschlicher Leberpunkte. *Beitr. path. Anat.*, **125**, 173-188.
- BÜTSCHLI, O. (1876). Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zellteilungsvorgänge der Eizelle, die Zelleteilung und die Konjugation der Infusorien. Abh. Senckenberg. Naturforsch. Ges., **10**.
- BULLOUGH, W. S. (1948). Mitotic activity in the adult male mouse, *Mus musculus* L. The diurnal cycles and their relation to waking and sleeping. *Proc. roy. Soc. B*, **135**, 212-233.
- (1949). The effect of a restricted diet on mitotic activity in the mouse. *Brit. J. Cancer*, **3**, 275-282.
- (1955). Hormones and mitotic activity. *Vitam. and Horm.*, **13**, 261-292.
- (1950). Completion of mitosis after death. *Nature*, **165**, 493.
- BULLOUGH, W. S., & EISA, E. (1950). The diurnal variations in the tissue glycogen content and their relation to mitotic activity in the adult male mouse. *J. exp. Biol.*, **27**, 257-263.
- BULLOUGH, W. S., & JOHNSON, M. (1951). The energy relations of mitotic activity in adult mouse epidermis. *Proc. roy. Soc. B* **138**, 562-575.
- BULLOUGH, W. S., & LAURENCE, E. B. (1960). The control of mitotic activity in mouse skin. *Exp. Cell Res.*, **21**, 394-405.
- BUNGENBERG, DE JONG, H. G. (1932). Die Koazervation und ihre Bedeutung für die Biologie. *Protопlasma (Wien)*, **15**, 110-173.
- BURCK, H. C. (1962). Die Beziehungen zwischen intrazellulärem osmorischen Druck und zellulärem Wasserwechsel. *Klin. Wschr.*, **40**, 761-765.

- BURCK, H. C., & NETTER, H. (1960). Das osmotische Verhalten als Kriterium für den Funktionszustand von Leberschnitten. *Klin. Wschr.*, **38**, 359-366.
- BURGOLD, H., & SPREER, F. (1960). Zur Bewertung heterochromatischer Kernstrukturen der Granulozyten bei der blutzellmorphologischen Geschlechtsbestimmung. *Klin. Wschr.*, **38**, 465-474.
- BURKL, W. (1949). Die Amitose als generative Teilungsform bei primitiven Erythroblasten. *Z. Zellforsch.*, **34**, 584-609.
- (1959). Sekretbildung im Pankreas. *Wien. Z. inn. Med.*, **40**, 241-249.
- BURNS, V. W. (1961). Relations among DNA and RNA synthesis and synchronized cell division in *L. acidophilus*. *Exp. Cell Res.*, **23**, 582-594.
- BURSTONE, M. S. (1959). Histochemical localization of oxidase activity in the mitochondria of the human heart. *Nature (Lond.)*, **184**, Suppl. 7, 467-477.
- (1960). Histochemical demonstration of cytochrome oxidase with new amine reagents. *J. Histochem. Cytochem.*, **8**, 63-70.
- BUSANNY-CASPARI, W. (1961). Die Rolle der Amitose bei experimentell gesteuerter Leberregeneration. *Verh. disch. Ges. Path.*, **45**, 155-157.
- BUSSE-GRAWITZ, P. (1946). *Experimentelle Grundlagen zu einer modernen Pathologie*. Schwabe, Basel.
- BUTTROSE, M. S. (1960). Submicroscopic development and structure of starch granules in cereal endosperms. *J. Ultrastruct. Res.*, **4**, 231-257.
- BUVAT, R. (1957). Formation de Golgi dans les cellules radiculaires d'*Allium cepa* L. *C. R. Acad. Sci. (Paris)*, **244**, 1401-1403.
- CAIN, H. (1961). Karyologische Befunde bei Regenerationsvorgängen in der Niere. *Verh. disch. Ges. Path.*, **45**, 174-178.
- CAJAL, S. R. y (1935). Die Neuronenlehre (Transl. by D. Miskolczy). In: *Bumke-Foersters Handbuch der Neurologie*, Vol. I. Springer, Berlin.
- CALLAN, H. G. (1952). A general account of experimental work on amphibian oocyte nuclei. *Symp. Soc. exp. Biol.*, **6**, 243-255.
- CALLAN, H. G., & LLOYD, L. (1960). Lampbrush chromosomes. In: *New Approaches in Cell Biology*, edited by WALKER. Acad. Press, London.
- CALLAN, H. G., & TOMLIN, S. (1950). Experimental studies on amphibian oocyte nuclei. I. Investigation of the structure of the nuclear membrane by means of the electron microscope. *Proc. roy. Soc. B* **137**, 367-378.
- CALVIN, M. (1956). Die chemische Evolution und der Ursprung des Lebens. *Naturwissenschaften*, **43**, 387-393.
- CAMERON, G. R. (1952). *Pathology of the cell*. Oliver and Boyd, Edinburgh.
- CAMERON, G. R., & KARUNARATNE, W. A. R. (1936). Carbon tetrachloride cirrhosis in relation to liver regeneration. *J. Path.*, **42**, 1-21.
- CAMERON, I. L., & PRESCOTT, D. M. (1961). Relations between cell growth and cell division. V. Cell and macronuclear volumes of *Tetrahymena pyriformis* HSM during the cell life cycle. *Exp. Cell. Res.*, **23**, 354-360.
- CANELAKIS, E. S., & HERBERT, E. (1961). s-RNA synthesis. IV. The effect of inorganic pyrophosphate and a method for the extraction of s-RNA. *Biochim. biophys. Act (Amst.)*, **47**, 78-85.
- CANNON, H. G. (1923). On the nature of the centrosomal force. *J. Genet.*, **13**, 47-78.
- CARLSON, J. G. (1938). Mitotic behaviour of induced chromosome fragments lacking spindle attachments in the neuroblasts of the grasshopper. *Proc. nat. Acad. Sci. (Wash.)*, **24**, 500-507.
- (1946). Protoplasmic viscosity changes in different regions of the grasshopper neuroblast during mitosis. *Biol. Bull.*, **90**, 109-121.
- (1952). Microdissection studies of the dividing neuroblast of the grasshopper, *Chortophaga viridifasciata* (de Geer). *Chromosoma (Berl.)*, **5**, 199-220.
- CARLSON, J. G., & HARRINGTON, N. G. (1955). X-ray-induced "stickiness" of the chromosomes of the Chortophaga neuroblast in relation to dose and mitotic stage at treatment. *Radiat. Res.*, **2**, 84-90.
- CARLSON, J. G. & HOLLANDER, A. (1948). Mitotic effects of ultraviolet radiation of the 2250 Å region, with special reference to the spindle and cleavage. *J. cell. comp. Physiol.*, **31**, 149-173.
- CARNEIRO, J., & LEBLOND, C. P. (1959). Continuous protein synthesis in nuclei, shown by radioautography with H^3 -labelled amino acids. *Science*, **129**, 391-392.
- CARO, L. G. (1961). Electron microscopic radioautography of thin sections: the Golgi zone as a site of protein concentration in pancreatic acinar cells. *J. biophys. biochem. Cytol.*, **10**, 37-45.
- CARO, L. G., & PALADE, G. E. (1961). Le rôle de l'appareil de Golgi dans le processus sécrétoire. Étude autoradiographique. *C. R. Soc. Biol. (Paris)*, **155**, 1750-1762.
- CAROTHERS, E. E. (1936). Components of the mitotic spindle with special reference to the chromosomal and interzonal fibers in the Acrididae. *Biol. Bull.*, **71**, 469-491.
- CARPENTIER, P. J., STOLTE, L. A. M., & VISCHERS, G. V. (1955). Sexing nuclei. *Lancet*, **ii**, 874-875.
- CARR, D. H., BARR, M. L., PLUNKETT, E. R., GRUMBACH, M. M., MORIHIMA, A., & CHU, E. H. Y.

- (1961). An XXXY sex chromosome complex in Klinefelter subjects with duplicated sex chromatin. *J. clin. Endocrin.*, **21**, 491-505.
- CARRIÈRE, R., LEBLOND, C. P., & MESSIER, B. (1961). Increase in the size of liver cell nuclei before mitosis. *Exp. Cell Res.*, **23**, 625-628.
- CARTER, C. O., HAMERTON, J. L., POLANI, P. E., GUNALP, A., & WELLER, S. D. V. (1960). Chromosome translocation as a cause of familial mongolism. *Lancet*, **ii**, 678-680.
- CASPERSSON, T. (1939). Über die Rolle der Desoxyribonucleinsäure bei der Zellteilung. *Chromosoma (Berl.)*, **1**, 147-156.
- (1940). Die Eiweißverteilung in den Strukturen des Zellkerns. *Chromosoma (Berl.)*, **1**, 562-604.
- (1941). Studien über den Eiweißumsatz der Zelle. *Naturwissenschaften*, **29**, 33-43.
- (1950). *Cell growth and cell function*. Norton, New York.
- CASPERSSON, T., & HOLMGREN, H. J. (1934). Variationen der Kerngröße während der verschiedenen Phasen der Leberarbeit. *Anat. Anz.*, **79**, 53-59.
- CASPERSSON, T., & SCHULTZ, J. (1939). Pentose nucleotides in the cytoplasm of growing tissues. *Nature*, **143**, 602-603.
- (1940). Ribonucleic acid in both nucleus and cytoplasm and the function of the nucleolus. *Proc. Nat. Acad. Sci. (Wash.)*, **26**, 507-515.
- CASPERSSON, T., & THORSSON, K. G. (1953). Virus und Zellstoffwechsel. *Klin. Wschr.*, **31**, 205-212.
- CASTRO, D. DE, CÁMARA, A., & MALHEIROS, N. (1949). X-rays in the centromere problem of *Luzula purpurea* Link. *Genetica Iberica*, **1**, 49-54.
- CERRONI, R. E., & ZEUTHEN, E. (1962). Inhibition of macromolecular synthesis and of cell division in synchronized Tetrahymena. *Exp. Cell Res.*, **26**, 604-605.
- CHALKLEY, H. W. (1951). Central of fission in Amoeba proteus as related to the mechanism of cell division. *Ann. N. Y. Acad. Sci.*, **51**, 1303-1310.
- CHAMBERS, R. (1914). Some physical properties of the cell nucleus. *Science*, **40**, 824-827.
- (1917). Microdissection studies. II. The cell aster: a reversible gelation phenomenon. *J. exp. Zool.*, **23**, 483-504.
- (1919). Changes in protoplasmic consistency and their relation to cell division. *J. gen. Physiol.*, **2**, 49-68.
- (1924). The physical structure of protoplasm as determined by microdissection and injection. In: *General Cytology*, edited by COWDRY. Univ. Press, Chicago.
- (1938). Structural and kinetic aspects of cell division. *J. cell. comp. Physiol.*, **12**, 149-165.
- (1951). Microsurgical studies on the kinetic aspects of cell division. *Ann. N. Y. Acad. Sci.*, **51**, 1311-1326.
- CHAMBERS, R., & CHAMBERS, E. L. (1961). *Explorations into the nature of the living cell*. Harvard Univ. Press, Cambridge/Mass.
- CHAMBERS, R., & FELL, H. B. (1931). Microoperations on cells in tissue cultures. *Proc. roy. Soc. B*, **109**, 380-403.
- CHAMBERS, R., & SANDS, H. C. (1923). A dissection of the chromosomes in the pollen mother cells of *Tradescantia virginica* L. *J. gen. Physiol.*, **5**, 815-819.
- CHANCE, B., & HESS, B. (1959a). Metabolic control mechanism. I. Electron transfer in the mammalian cell. *J. Biol. Chem.*, **234**, 2404-2412.
- (1959b). Metabolic control mechanism. II. Crossover phenomena in mitochondria of ascites tumor cells. *J. biol. Chem.*, **234**, 2413-2415.
- CHANCE, B., & WILLIAMS, G. R. (1956). The respiratory chain and oxidative phosphorylation. *Advanc. Enzymol.*, **17**, 65-134.
- CHANDRA, S. (1962). The reversal of mitochondrial membrane. *J. Cell Biol.*, **12**, 503-513.
- CHANDRA, S., & TOOLAN, H. W. (1961). Electron microscopy of the H-1 virus. I. Morphology of the virus and a possible virus-host relationship. *J. Nat. Cancer Inst.*, **27**, 1405-1459.
- CHANTRENNNE, H. (1961). *The biosynthesis of proteins*. Pergamon Press, Oxford.
- CHAPMAN-ANDRESEN, C., & HOLTZER, H. (1960). The uptake of fluorescent albumin by pinocytosis in Amoeba proteus. *J. biophys. biochem. Cytol.*, **8**, 288-291.
- CHAPPELL, J. B., & GREVILLE, G. D. (1960). Mitochondrial swelling and electron transport. I. Swelling supported by ferricyanide. *Biochim. biophys. Acta (Amst.)*, **38**, 483-494.
- CHAPPELL, J. B., & PERRY, S. V. (1954). Biochemical and osmotic properties of skeletal muscle mitochondria. *Nature (Lond.)*, **173**, 1094-1095.
- CHATTON, E., & LWOFF, A. (1924). Un protéomyxe, *Cinetidomyxa nucleoflagellata* n. g. n. sp. à cinétide intracytoplasmique, et sa multiplication. *C. R. Soc. Biol.*, **91**, 584-588.
- CHAVEAU, J., GAUTIER, A., MOULÉ, Y., & ROUILLER, CH. (1955). Étude morphologique et biochimique de la fraction "microsomes" des cellules du foie et du pancréas de rat. *C. R. Acad. Sci. (Paris)*, **241**, 337-339.
- CHAVEAU, J., MOULÉ, Y., ROUILLER, C., & SCHNEEBELLI, J. (1962). Isolation of smooth vesicles and free ribosomes from rat liver microsomes. *J. Cell Biol.*, **12**, 17-29.

- CHAYEN, J. (1959). The quantitative cytochemistry of DNA and its significance in cell physiology and heredity (Symposium). *Exp. Cell Res.*, Suppl. 6, 115-131.
- CHAYEN, J., GAHAN, P. B., & LA COUR, L. F. (1959). The masked lipids of nuclei. *Quart. J. micr. Sci.*, **100**, 325-337.
- CHÈVREMONT, M. (1956). *Notions de Cytologie et Histologie*. Desver, Liège.
- (1961a). Le mécanisme de l'action antimitotique. *Path. et Biol.*, **9**, 973-1004.
- (1961b). La préparation à la mitose. Quelques modalités de son inhibition par des substances antimitotiques. *Chemotherapia (Basel)*, **2**, 191-209.
- CHÈVREMONT, M., & BAECKELAND, E. (1960). Étude histoautoradiographique de l'incorporation de thymidine tritiée dans des cellules traitées par du trihydroxy-N-méthylindole. Synthèse cytoplasmique d'acide désoxyribonucléique. *C. R. Acad. Sci.*, **251**, 1097-1099.
- (1961). Étude histoautoradiographique de l'incorporation d'uridine tritiée en culture de tissus dans des fibroblastes normaux ou soumis à l'action de substances antimitotiques. *Arch. Biol. (Liège)*, **72**, 461-484.
- CHÈVREMONT, M., BAECKELAND, E., & CHÈVREMONT-COMHAIRE, S. (1959). Étude histoautoradiographique de l'incorporation de thymidine tritiée dans les cellules somatiques traitées vivantes par une désoxyribonucléase acide. Synthèse cytoplasmique d'acide désoxyribonucléique. *C. R. Acad. Sci. (Paris)*, **249**, 1392-1394.
- (1960). Contribution cytochimique et histoautoradiographique à l'étude du métabolisme et de la synthèse des ADN dans des cellules animales cultivées *in vitro*. II. Étude des acides désoxyribonucléiques dans des cellules animales soumises vivantes à l'action de désoxyribonucléases neutre ou acide. Synthèse et accumulation cytoplasmiques d'ADN. *Biochem. Pharmacol.*, **4**, 67-78.
- CHÈVREMONT, M., BASSLEER, R., & BAECKELAND, E. (1961). Nouvelles recherches sur les acides désoxyribonucléiques dans des cultures de fibroblastes refroidies puis réchauffées. Étude cytophotométrique et histoautoradiographique. Localisation cytoplasmique d'ADN. *Arch. Biol. (Liège)*, **72**, 501-524.
- CHÈVREMONT, M., & CHÈVREMONT-COMHAIRE, S. (1953). Recherches sur le trihydroxy-N-méthylindole et l'adrenochrome en culture de tissus. I. Action sur la croissance et la mitose. II. Action sur le chondriome. *Arch. Biol. (Liège)*, **64**, 399-437.
- CHÈVREMONT, M., & FREDERIC, J. (1954). Contribution à l'étude des chondriosomes vivants. *Symp. 8 Cong. of Cell Biol.*, Leiden.
- CHÈVREMONT-COMHAIRE, S., & CHÈVREMONT, M. (1956). Action de températures subnormales suivies de réchauffement sur l'activité mitotique en culture de tissus. Contribution à l'étude de la préparation à la mitose. *C. R. Soc. Biol. (Paris)*, **150**, 1046-1049.
- CHÈVREMONT-COMHAIRE, S., & FREDERIC, J. (1955). Contribution à l'étude de la mitose dans des cellules vivantes privées d'oxygène, en culture des tissus. *C. R. Soc. Biol. (Paris)*, **149**, 211-213.
- CHRISTENSEN, B. (1961). Studies on cyto-taxonomy and reproduction in the Euchytraeidae—with notes on parthenogenesis and polyploidy in the animal kingdom. *Hereditas*, **47**, 387-450.
- CHU, C. H. U. (1960). A study of the subcutaneous connective tissue of the mouse, with special reference to nuclear type, nuclear division and mitotic rhythm. *Anat. Rec.*, **138**, 11-25.
- CHU, E. H. Y. (1960). The chromosome complements of human somatic cells. *Amer. J. hum. Genet.*, **12**, 97-103.
- CLARA, M. (1930). Untersuchungen an der menschlichen Leber. II. Über die Kerngrößen in den Leberzellen. Zugleich über Amitose und über das Wachstum der stabilen Elemente. *Z. mikr.-anat. Forsch.*, **22**, 145-219.
- (1931). Das Wachstum der Leberzellen und die Entwicklung der Leberläppchen beim Schwein. *Anat. Anz.*, **72**, 219-227.
- CLARK, S. L. (1961). The localization of alkaline phosphatase in tissues of mice, using the electron microscope. *Amer. J. Anat.*, **109**, 57-84.
- CLARK, W. H. JR. (1960). Electron microscope studies of nuclear extrusions in pancreatic acinar cells of the rat. *J. biophys. biochem. Cytol.*, **7**, 345-352.
- CLAUDE, A. (1938). Concentration and purification of chicken tumor I agent. *Science*, **87**, 467-468.
- (1941). Particulate components of the cytoplasm. *Cold Spr. Harb. Symp. quant. Biol.*, **9**, 263-270.
- (1961). Morphologie et organisation de constituants nucléaires dans le cas d'un carcinome rénal de la souris. *C. R. Acad. Sci. (Paris)*, **252**, 4186-4188.
- CLAUDER, A. (1944). The constitution of mitochondria and microsomes and the distribution of nucleic acid in the cytoplasm of a leukemic cell. *J. exp. Med.*, **80**, 19-29.
- CLAUSS, H. (1959). Das Verhalten der Phosphorylase in kernhaltigen und kernlosen Teilen von Acetabularia mediterranea. *Planta*, **52**, 534-542.
- CLAUSS, H., & WERZ, G. (1961). Über die Geschwindigkeit der Protein-Synthese in kernlosen und kernhaltigen Zellen von Acetabularia. *Z. Naturforsch.*, **16b**, 162-165.
- CLELAND, K. W. (1961). Deoxyribonucleic acid content of nuclei dividing amitotically. *Nature (Lond.)*, **191**, 504-505.

- CLEVELAND, L. R. (1935). The intranuclear achromatic figure of *Oxymonas grandis* sp. nov. *Biol. Bull.*, **69**, 54-65.
 — (1938a). Morphology and mitosis in Tetranymppha. *Arch. Protistenk.*, **91**, 442-451.
 — (1938b). Mitosis in Pyrsomymppha. *Arch. Protistenk.*, **91**, 452-455.
 — (1938c). Origin and development of the achromatic figure. *Biol. Bull.*, **74**, 41-55.
 — (1949). The whole life cycle of chromosomes and their coiling systems. *Trans. Amer. Phil. Soc.*, **39**, 1-100.
 — (1953). Studies on chromosomes and nuclear division. *Trans. Amer. Phil. Soc., N.S.*, **43**, 809-869.
 — (1957a). Types and life cycles of centrioles of flagellates. *J. Protozool.*, **4**, 230-241.
 — (1957b). Achromatic figure formation by multiple centrioles of Barbulanympha. *J. Protozool.*, **4**, 241-248.
 — (1959). Sex induced with ecdysone. *Proc. nat. Acad. Sci. (Wash.)*, **45**, 747-753.
- CLEVER, U. (1961). Genaktivitäten in den Riesenchromosomen von *Chironomus tentans* und ihre Beziehungen zur Entwicklung. I. Genaktivierungen durch Ecdyson. *Chromosoma (Berl.)*, **12**, 607-675.
 — (1962). Untersuchungen an Riesenchromosomen über die Wirkungsweise der Gene. *Mit. med. Nordmark*, **14**, 438-452.
 — (1963). Einige Bemerkungen über die Regulation von Genaktivitäten in Riesenchromosomen. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- COHEN, A. (1957). Electron microscopic observations of *Amoeba proteus* in growth and inanition. *J. biophys. biochem. Cytol.*, **3**, 859-866.
- COHEN, A., & BERRILL, N. J. (1936). Cell division and differentiation in the growth of specialized vertebrate tissue. *J. Morph.*, **60**, 243-259.
- COHEN, L. (1937). Structure of the interkinetic nucleus in the scale epidermis of *Allium cepa*. *Proto-plasma (Wien)*, **27**, 484-495.
- COHN, Z. A., & HIRSCH, J. G. (1960). The influence of phagocytosis on the intracellular distribution of granule-associated components of polymorphonuclear leucocytes. *J. exp. Med.*, **112**, 1015-1022.
- COLEMAN, L. C. (1940). The cytology of *Veltheimia viridifolia* Jacq. *Amer. J. Bot.*, **27**, 887-895.
- COLLIN, R. (1924). Sur l'endocytogénèse. *C. R. Soc. Biol. (Paris)*, **90**, 1419-1421.
- COLOMBO, G. (1952). L'accorciamento dei cromosomi nella mitosi e nella meiosi. *Experientia*, **8**, 15.
- CONANDON, J., & DE FONBRUNE, P. (1939). Ablation du noyau chez une amibe. Réactions cinétiques à la piqûre de l'amibe normale ou dénudée. *C. R. Soc. Biol. (Paris)*, **130**, 740-744.
- CONGER, A. D. (1955). How oxygen causes increase in chromosomal aberration yield. *Genetics*, **40**, 568.
- CONGER, A. D., & FAIRCHILD, L. M. (1952). The induction of chromosomal aberrations by oxygen. *Proc. nat. Acad. Sci. (Wash.)*, **38**, 289-299.
- CONKLIN, E. G. (1902). Karyokinesis and cytokinesis in the maturation, fertilization, and cleavage of *Crepidula* and other Gastropoda. *J. Acad. natur. Sci. Philadelphia II. Scr.*, **12**.
- (1917a). Effects of centrifugal force on the structure and development of the eggs of *Crepidula*. *J. exp. Zool.*, **22**, 311-419.
- (1917b). Mitosis and amitosis. *Biol. Bull.*, **33**, 396-436.
- (1951). Cleavage and differentiation in marine eggs. *Ann. N. Y. Acad. Sci.*, **51**, 1281-1286.
- CONWAY, E. J., GEOGHEGAN, H., & McCORMACK, J. I. (1955). Autolytic changes at zero centigrade in ground mammalian tissues. *J. gen. Physiol.*, **30**, 427-437.
- COOPER, D. C. (1952). The transfer of desoxyribose nucleic acid from Tapetum to the microsporocytes at the onset of meiosis. *Am. Naturalist*, **86**, 219-229.
- COOPER, K. W. (1941). Visibility of the primary spindle fibers and the course of mitosis in the living blastomeres of the mite, *Pediculopsis grammum*. *Proc. nat. Acad. Sci. (Wash.)*, **27**, 480-483.
- (1948). The evidence for long range specific attractive forces during the somatic pairing of dipteran chromosomes. *J. exp. Zool.*, **108**, 327-336.
- (1949). The cytogenetics of meiosis in *Drosophila*. Mitotic and meiotic autosomal chiasmata without crossing over in the male. *J. Morph.*, **84**, 81-122.
- (1959). Cytogenetic analysis of major heterochromatic elements (especially x and y) in *Drosophila melanogaster*, and the theory of "heterochromatin". *Chromosoma (Berl.)*, **10**, 535-588.
- COOPER, H. L., KUPPERMANN, H. S., RENDON, O. R., & HIRSCHHORN, K. (1962). Sex-chromosome mosaicism of type XY^Y XO. *New Engl. J. Med.*, **266**, 699-702.
- COOPERSTEIN, S. J., DIXIT, P. K., LAZAROW, A., & JACKSON, J. A. (1960). Studies on the mechanism of Janus green B staining of mitochondria. IV. Reduction of Janus green B by isolated cell fractions. *Anat. Rec.*, **138**, 49-66.
- COPELAND, D. E., & DALTON, A. J. (1959). An association between mitochondria and the endoplasmic reticulum in cells of the pseudobranch gland of a teleost. *J. biophys. biochem. Cytol.*, **5**, 393-396.
- CORNMAN, I. (1944). A summary of evidence in favour of the traction fiber in mitosis. *Amer. Nat.*, **78**, 410-422.
- (1954). The properties of urethan considered in relation to its action on mitosis. *Int. Rev. Cytol.*, **3**, 113-130.

- CORNMAN, I., & CORNMAN, M. E. (1951). The action of podophyllin and its fractions on marine eggs. *Ann. N. Y. Acad. Sci.*, **51**, 1443-1488.
- CORREIA, J. C. (1960). Le comportement du noyau et des nucléoles pendant l'amitose de certaines cellules nerveuses de Mesencephalon chez la souris albinos. *Arch. Sci. (Genève)*, **13**, 89-101.
- CORRENS, C. (1900). G. Mendels Regel über das Verhalten der Nachkommenschaft der Rassenbastarde. *Ber. dtsch. Bot. Ges.*, **18**, 158-168.
- (1909). Vererbungsversuche mit blaß(gelb)grünen und buntblättrigen Sippen bei Mirabilis Jalapa, Urtica pilulifera und Lunaria annua. *Z. Vererbungsl.*, **1**, 291-329.
- COSSEL, L. (1961). Elektronenmikroskopische Befunde an den Leberepithelzellen bei Virushepatitis. *Acta hepatosplenol. (Stuttg.)*, **8**, 333-356.
- COSTELLO, D. P. (1961). On the orientation of centrioles in dividing cells, and its significance: a new contribution to spindle mechanics. *Biol. Bull.*, **120**, 285-312.
- COTTE, G. (1959). Observations au microscope électronique d'appareils nucléolaires dans la surrénales du cobaye, du hamster et du chat. *Z. Zellforsch.*, **50**, 232-237.
- COUNCILMAN, W. T. (1890). Report of etiology and prevention of yellow fever. *U.S. Marine Hosp.*, **151**.
- COWDRY, E. V. (1934). The problem of intranuclear inclusions in virus diseases. *Arch. Path.*, **18**, 527-542.
- (1953). Cells and their behaviour. In: *Pathology*, edited by ANDERSON. Mosby, St. Louis.
- CRICK, F. H. C. (1957). The structure of nucleic acids and their role in protein synthesis. *Biochem. Soc. Symp.*, **14**, 25.
- (1958). On protein synthesis. *Symp. Soc. exp. Biol.*, **12**, 138-163.
- CRICK, F. H. C., BARNETT, L., BRENNER, S., & WATTS-TOBIN, R. J. (1961). General nature of the genetic code for proteins. *Nature*, **192**, 1227-1232.
- CULLING, CH., & VASSAR, P. (1961). Desoxyribose nucleic acid. A fluorescent histochemical technique. *Arch. Path.*, **71**, 76-80.
- DALES, S. (1960). A study of the fine structure of mammalian somatic chromosomes. *Exp. Cell Res.*, **19**, 577-590.
- (1962). An electron microscope study of the early association between two mammalian viruses and their hosts. *J. Cell Biol.*, **13**, 303-322.
- DALES, S., & SIMINOVITCH, L. (1961). The development of vaccinia virus in Earle's L strain cells as examined by electron microscopy. *J. biophys. biochem. Cytol.*, **10**, 475-503.
- DALGAARD, O. Z., & PEDERSEN, K. J. (1959). Renal tubular degeneration. Electron microscopy in ischaemic anuria. *Lancet*, **ii**, 484-488.
- DALTON, A. J. (1951). Cytoplasmic changes during cell division with reference to mitochondria and the Golgi substance. *Ann. N. Y. Acad. Sci.*, **51**, 1295-1302.
- (1961). Golgi apparatus and secretion granules. In: *The Cell*, Vol. II. Acad. Press, New York.
- DALTON, A. J., & FELIX, M. D. (1954). A study of the Golgi substance and ergastoplasm in a series of mammalian cell types. *Symp. 8, Congr. of Cell Biol.*, Leiden.
- (1956). A comparative study of the Golgi complex. *J. biophys. biochem. Cytol.*, **2**, Suppl., 79-84.
- (1957). Electron microscopy of mitochondria and the Golgi complex. *Symp. Soc. exp. Biol.*, **10**, 148-159.
- DALY, M. M., & MIRSKY, A. E. (1955). Histones with high lysine content. *J. gen. Physiol.*, **38**, 405-413.
- DAN, K. (1943). Behaviour of cell surface during cleavage. VI. On the mechanism of cell division. *J. Faculty of Science, Tokyo Imp. Univ.*, **IV**, **6**, 323-368.
- DAN, K., & DAN, J. C. (1947). Behaviour of the cell surface during cleavage. VII. On the division mechanism of cells with eccentric nuclei. *Biol. Bull.*, **93**, 139-162.
- DAN, K., ITO, S., & MAZIA, D. (1952). Study of the course of formation of the mitotic apparatus in Arbacia and Macraea by isolation techniques. *Biol. Bull.*, **103**, 292.
- DAN, K., YAMAGITA, T., & SUGIYAMA, M. (1937). Behaviour of the cell surface during cleavage. I. *Protoplasma (Wien)*, **28**, 66-81.
- DANIELLI, J. F. (1958). Some theoretical aspects of nucleocytoplasmic relationships. *Exp. Cell Res.*, Suppl. **6**, 252-267.
- (1960). Cellular inheritance as studied by nuclear transfer in Amoebae. In: *New Approaches in Cell Biology*, edited by WALKER. Acad. Press, London.
- DANIELLI, J. F., LORCH, I. J., ORD, M. J., & WILSON, E. G. (1955). Nucleus and cytoplasm in cellular inheritance. *Nature (Lond.)*, **176**, 1114-1115.
- DARLINGTON, C. D. (1935). The internal mechanics of the chromosomes. I-III. *Proc. roy. Soc. B*, **118**, 33-96.
- (1937). *Recent advances in cytology*, 2nd edition. Churchill, London.
- (1940). The origin of iso-chromosomes. *J. Genet.*, **39**, 351-361.
- (1958). *Evolution of genetic systems*. Oliver and Boyd, Edinburgh.
- DARLINGTON, C. D., & KOLLER, P. C. (1947). The chemical break of chromosomes. *Heredity*, **1**, 187-221.

- DARLINGTON, C. D., & LA COUR, L. (1938). Differential reactivity of the chromosomes. *Ann. Bot.*, **2**, 615-625.
- (1940). Nucleic acid starvation of chromosomes in Trillium. *J. Genet.*, **40**, 185-213.
- DARLINGTON, C. D., & MCLEISH, J. (1951). Action of maleic hydracide on the cell. *Nature (Lond.)*, **167**, 407-408.
- DARLINGTON, C. D., & THOMAS, P. T. (1937). The breakdown of cell division in a Festuca-Lolium derivative. *Ann. Bot.*, **1**, 747-761.
- DARLINGTON, C. D., & UPCOTT, M. B. (1939). The measurement of packing and contraction in chromosomes. *Chromosoma (Berl.)*, **1**, 23-32.
- (1941). Spontaneous chromosome change. *J. Genet.*, **41**, 297-338.
- DARWIN, C. (1859). *The origin of species*. London.
- DAS, C. (1956). On the behaviour of sex-chromosomes during meiosis in two species of Indian heteroptera (Hemiptera). *Proc. Nat. Acad. Sci. (Wash.)*, **26**, 340-346.
- (1958). Studies on the structure and behaviour of chromosomes of Ranatra elongata (Fabr.). (Nepidae: Hemiptera, Heteroptera). *Cellule*, **59**, 203-210.
- DATTA, M. (1960). The effect of visible light on the mitotic spindle. *Exp. Cell Res.*, **21**, 261-273.
- DAVID, H. (1959). Korrelationen zwischen Größe und Zahl der Nukleolen und zytoplasmatischer RNS in den Lebern verschiedener Tiere während und nach absolutem Hunger. *Verh. dtsch. path. Ges.*, **417**-421.
- (1960a). Über einen Nucleolus mit Membran. *Z. Zellforsch.*, **53**, 50-54.
- (1960b). Zur Mitochondriengröße in den Lebern von Hungermäusen. Mikroskopische und submikroskopische Untersuchungen. *Acta biol. med. germ.*, **4**, 159-165.
- (1961). Submikroskopische Strukturveränderungen der Mitochondrien und ihrer Bestandteile. *Acta biol. med. germ.*, **7**, 311-321.
- DAVID, H., & KETTLER, L. H. (1961). Degeneration von Lebermitochondrien nach Ammoniumintoxikation. *Z. Zellforsch.*, **53**, 857-866.
- DAVID, H., & VELHAGEN, K. H. (1961). Zur Pigmentbildung in den Zellen des malignen Melanoblastoms des menschlichen Auges. *Verh. dtsch. path. Ges.*, **45**, 214-218.
- DAVIDSON, D. (1958). The effects of heat shocks on cell division. *Chromosoma (Berl.)*, **9**, 216-228.
- DAVIDSON, D., & ANDERSON, N. G. (1960). Chromosome coiling: Abnormalities induced by polyamines. *Exp. Cell Res.*, **20**, 610-613.
- DAVIDSON, J. D., & FREEMAN, B. B. (1955). The effects of anti-tumor drugs upon P^{32} -incorporation into nucleic acids of mouse tumors. *Cancer Res.*, **15**, 31-37.
- DAVIDSON, W. M., & SMITH, D. R. (1954). Morphological sex difference in the polymorphonuclear neutrophil leukocytes. *Brit. med. J.*, **1954/1**, 6-7 & 34-35.
- DAVIES, H. G. (1952). The ultra-violet absorption of living chick fibroblasts during mitosis. *Exp. Cell Res.*, **3**, 453-461.
- DAVIES, H. G., DEELEY, E. M., & DENBY, E. F. (1957). Attempts at measurement of lipid, nucleic acid and protein content of cell nuclei by microscope-interferometry. *Exp. Cell Res.*, Suppl. **4**, 136-149.
- DAVIS, J. M. G. (1960). The ultrastructure of the mammalian nucleolus. In: *The Cell Nucleus*. Butterworths, London.
- DAVISON, P. F. (1957). Chromatography of histones. *Biochem. J.*, **66**, 708-712.
- DAVISON, P. F., & BUTLER, J. A. V. (1954). The fractionation and composition of histones from thymus nucleoprotein. *Biochim. biophys. Acta (Amst.)*, **15**, 439-440.
- DAWE, C. J., KILHAM, L., & MORGAN, W. D. (1961). Intranuclear inclusions in tissue cultures infected with rat virus. *J. nat. Cancer Inst.*, **27**, 221-235.
- DAY, M. F., FARRANT, J. L., & POTTER, C. (1958). The structure and development of a polyhedral virus affecting the moth larva, *Pterolocera amplicornis*. *J. Ultrastruct. Res.*, **2**, 227-238.
- DE, D. N. (1961). Autoradiographic studies of nucleoprotein metabolism during the division cycle. *Nucleus*, **4**, 1-24.
- DE, P., CHATTERJEE, R., & MITRA, S. (1962). Nucleolar localization of succinic dehydrogenase. *J. Histochem. Cytochem.*, **10**, 6-7.
- DEANE, H. W. (1958). Intracellular lipids: Their detection and significance. In: *Frontiers in Cytology*. Yale Univ. Press, New Haven.
- DEELEY, E. M., DAVIES, H. G., & CHAYEN, J. (1957). The DNA content of cells in the root of *Vicia faba*. *Exp. Cell Res.*, **12**, 582-591.
- DEFENDI, V., MANSON, L. A. (1961). Studies of the relationship of DNA synthesis time to proliferation time in cultured mammalian cells. *Path. et Biol.*, **9**, 525-528.
- DEIMLING, O. v., MÖLBERT, E., & DUSPIVA, F. (1960). Elektronenmikroskopischer Nachweis eines Glukose-1-phosphatpfaltenden Enzyms im Herzmuskel der Albinoratte. *Beitr. path. Anat.*, **123**, 127-143.
- DEITCH, A. D. (1961). An improved Sakaguchi reaction for microspectrophotometric use. *J. Histochem. Cytochem.*, **9**, 477-483.

- DELBRÜCK, M., & STENT, G. S. (1957) On the mechanism of DNA replication. In: *The chemical basis of heredity*. Hopkins, Baltimore.
- DELBANTY, J. D. A., ELLIS, J. R., & ROWLEY, P. T. Triploid cells in a human embryo. *Lancet*, **1961/1**, 1286.
- DEMEREĆ, M. (1955). What is a gene?—Twenty years later. *Amer. Nat.*, **89**, 5–20.
- DENUES, A. R. T. (1958). Chromosomes: Their constitution and function. In: *Frontiers in Cytology*, Yale Univ. Press, New Haven.
- DERWORT, A., & DETERDING, K. (1959). Vakuolisierte Lymphocyten bei familiärer amaurotischer Idiotie und ihre diagnostische Bedeutung. *Nervenarzt*, **30**, 442–448.
- DEUFEL, J. (1951). Untersuchungen über den Einfluß von Chemicalien und Röntgenstrahlen auf die Mitose von *Vicia faba*. *Chromosoma (Berl.)*, **4**, 239–272.
- (1954). Zytologische Untersuchungen an sezernierenden Zellen. *Naturwissenschaften*, **41**, 41–42.
- DEVILLERS, Ch., & ROSENBERG, J. (1953). Les premières phases du développement de l'œuf de *Salmo iridens* en anaérobiose. *C. R. Soc. Biol. (Paris)*, **237**, 1561–1562.
- DEYSSON, G., & LONGEVIALLE, M. (1962). Contribution à l'étude biochimique de la mitose: La "gélation" mitotique est-elle analogue à la coagulation sanguine? *Biol. med.*, **51**, 42–110.
- DIEFENBACH, H., & FEDERLIN, K. (1955). Vergleichende Kernmessungen an lebensfrischen und verschiedenen fixierten Leberzellen. *Frankfurt. Z. Path.*, **66**, 16–23.
- DIETZ, R. (1954). Multiple Geschlechtschromosomen bei den Ostracoden *Notodromas Monacha*. *Chromosoma (Berl.)*, **6**, 397–418.
- (1955). Zahl und Verhalten der Chromosomen einiger Ostracoden. *Z. Naturforsch.*, **10b**, 92–95.
- (1956). Die Spermatocytenteilungen der Tipuliden. II. Mitt. Graphische Analyse der Chromosomenbewegungen während der Prometaphase I im Leben. *Chromosoma (Berl.)*, **8**, 183–211.
- (1958). Multiple Geschlechtschromosomen bei den cypriden Ostracoden, ihre Evolution und ihr Teilungsverhalten. *Chromosoma (Berl.)*, **9**, 359–440.
- (1959). Centrosomenfreie Spindelpole in Tipuliden Spermatocyten. *Z. Naturforsch.*, **14b**, 749–752.
- DIHLMANN, W. (1959). Zur Frage geschlechtsspezifischer Kernstrukturen in den Lymphocyten. *Acta haemat. (Basel)*, **22**, 345–352.
- DIRKSEN, E. R. (1961). The presence of centrioles in artificially activated sea urchin eggs. *J. biophys. biochem. Cytol.*, **11**, 244–247.
- DITTUS, P. (1940). Histologie und Cytologie des Interrenal-organs der Selachier unter normalen und experimentellen Bedingungen. Ein Beitrag zur Kenntnis der Wirkungsweise corticotropen Hormons und des Verhältnisses von Kern und Plasma. *Z. wiss. Zool.*, **154**, 40–124.
- DOBELL, C. (1914). Cytological studies on three species of amoeba—*A. lacertae* Hartmann, *A. gelebae* n. sp., *A. fluvialis* n. sp. *Arch. Protistenk.*, **34**, 139–189.
- DOBROKHOTOV, V. N. (1961). Regulation of rhythmic changes in mitotic activity in various tissues of the organism. *Path. et Biol.*, **9**, 507–509.
- DOBZHANSKY, T. (1944). Distribution of heterochromatin in the chromosomes of *Drosophila pallidipennis*. *Amer. Nat.*, **78**, 193–213.
- DOLEŽAL, R., & TSCHERMAK-WOESS, E. (1955). Verhalten von Eu- und Heterochromatin und interphasisches Kernwachstum beim *Rheoe discolor*; Vergleich von Mitose und Endomitose. *Österr. Bot. Z.*, **102**, 158–185.
- DOLLAY, D. H. (1925) The general morphology of pancreatic cell function in terms of the nucleo-cytoplasmic relation. *Amer. J. Anat.*, **35**, 153–197.
- DOMAGK, G. (1954). Weitere Beobachtungen an Yoshida-Tumoren der Ratte. *Verh. dtsch. Ges. Path.*, **38**, 338–346.
- DORNFELD, E. J., OWCZARZAK, A. (1958). Surface responses in cultured fibroblasts elicited by ethylene-diamine-tetraacetic acid. *J. biophys. biochem. Cytol.*, **4**, 243–250.
- DOUNCE, A. L. (1943). Enzyme studies on isolated cell nuclei of rat liver. *J. biol. Chem.*, **147**, 685–698.
- (1959). The state of DNA in the resting-cell nucleus. *Ann. N. Y. Acad. Sci.*, **81**, 794–799.
- DOUNCE, A. L., GAMOW, G., SPIEGELMAN, S., NEWMARK, P., HARKER, D., & M. SOODAK. (1956). Nucleoproteins ("Round-table-discussion"). *J. cell. comp. Physiol.*, **47**, Suppl. 1, 103–112.
- DOUTRELIGNE, J. (1933). Chromosomes et nucléoles dans les noyaux du type euchromocentrique. *Cellule*, **42**, 29–100.
- DRAWERT, H., & MIX, M. (1961a). Licht- und elektronenmikroskopische Untersuchungen an Desmidaceen. V. Mitt. Über die Variabilität der Chloroplastenstruktur bei *Micrasterias rotata*. *Planta*, **56**, 648–665.
- (1961b). Licht- und elektronenmikroskopische Untersuchungen an Desmidaceen. VI. Mitt. Der Einfluß von Antibiotica auf die Chloroplastenstruktur bei *Micrasterias rotata*. *Planta*, **57**, 51–70.
- DROCHMANS, P. (1960). Electron microscope studies of epidermal melanocytes, and the fine structure of melanin granules. *J. biophys. biochem. Cytol.*, **8**, 165–180.

- DRUCKREY, H., DANNEBERG, P., & SCHMÄHL, D. (1953). Zellteilungshemmende Gifte. *Arzneimittel-Forsch.*, **3**, 151-161.
- DRUCKREY, H., SCHMÄHL, D., & KAISER, K. (1957). Versuche mit zellteilungshemmenden Giften an unbefruchteten Seeigelzellen. *Naturwissenschaften*, **44**, 430-431.
- DRÜNER, L. (1895). Studien über den Mechanismus der Zellteilung. *Jenaer Z. N. F.*, **22**, 271-344.
- DUBLIN, W. B., GREGG, R. O., & BRODERS, A. C. (1940). Mitosis in specimens removed during day and night from carcinoma of large intestine. *Arch. Path.*, **30**, 893-895.
- DUJIN, C. VAN, JR. (1960). Nuclear structure of human spermatozoa. *Nature (Lond.)*, **188**, 916-919.
- DUNCAN, D., & HILD, W. (1960). Mitochondrial alterations in cultures of the central nervous system as observed with the electron microscope. *Z. Zellforsch.*, **51**, 123-135.
- DURREE, W. R. (1950). Chromosome physiology in relation to nuclear structure. *Ann. N.Y. Acad. Sci.*, **50**, 920-953.
- DUSPIVA, F., & FRANKEN, F. H. (1957). Untersuchungen über den Stoffwechsel bei akuter Hypoxie. I. Die stationäre Konzentration von oxydiertem und reduziertem Diphosphopyridinucleotid in Leber und Herzmuskel von Meerschweinchen während kurzfristiger Hypoxämie. *Beitr. path. Anat.*, **118**, 38-51.
- DUSTIN, P. JR. (1947). Some new aspects of mitotic poisoning. *Nature (Lond.)*, **159**, 794-797.
- DUTCHER, T. F., & FAHEY, J. L. (1960). Intranuclear localization of 185 Gamma macroglobulin in macroglobulinemia of Waldenström. *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 452-455.
- DUTROCET, R. J. H. (1837). *Mémoires pour servir à l'histoire anatomique et physiologique des végétaux et des animaux*. Baillière, Paris.
- DUVE, C. DE (1959). Lysosomes, a new group of cytoplasmic particles. In: *Subcellular particles*. Ronald Press, New York.
- (1963). Structure and functions of lysosomes. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- DUVE, C. DE, PRESSMAN, B. C., GIANETTO, R., WATTIAUX, W., & APPELMANS, F. (1955). Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat liver tissue. *Biochem. J.*, **60**, 604-617.
- DYSON, J. (1949). A transmission-type interferometer microscope. *Nature (Lond.)*, **164**, 229.
- EASTON, J. M., GOLDBERG, B., & GREEN, H. (1962). Demonstrations of surface antigens and pinocytosis in mammalian cells with ferritin-antibody conjugates. *J. Cell Biol.*, **12**, 437-443.
- EBERTH, C. (1876). Über Kern- und Zellteilung. *Virchows Arch. path. Anat.*, **67**, 523-541.
- EDSTRÖM, J. E. (1960). Composition of ribonucleic acid from various parts of spider oocytes. *J. biophys. biochem. Cytol.*, **8**, 47-51.
- EDSTRÖM, J. E., & BEERMANN, W. (1962). The base composition of nucleic acids in chromosomes, puffs, nucleoli, and cytoplasm of Chironomus salivary gland cells. *J. Cell Biol.*, **14**, 371-379.
- EDSTRÖM, J. E., GRAMPP, W., & SCHOR, N. (1961). The intracellular distribution and heterogeneity of ribonucleic acid in starfish oocytes. *J. biophys. biochem. Cytol.*, **11**, 549-557.
- EDSTRÖM, J. E., & KAWIAK, J. (1961). Microchemical deoxyribonucleic acid determination in individual cells. *J. biophys. biochem. Cytol.*, **9**, 619-626.
- EDWARDS, G. A., & RUSKA, H. (1955). The function and metabolism of certain insect muscles in relation to their structure. *Quart. J. micr. Sci.*, **96**, 151-159.
- EDWARDS, J. E., HARNDEN, D. G., CAMERON, A. H., CROSSE, V. M., WOLFF, O. H. A new trisomic syndrome. *Lancet*, 1960/I, 787-790.
- EDWARDS, J. L., KOCH, A. L., YOUNIS, P., FREESE, H. L., LAITE, M. B., & DONALDSON, J. T. (1960). Some characteristics of DNA synthesis and the mitotic cycle in Ehrlich ascites tumor cells. *J. biophys. biochem. Cytol.*, **7**, 273-282.
- EGELHAAF, A. (1961). Über die Wirkungsweise der Gene. *Klin. Physiol.*, **1**, 205-243.
- EGER, W. (1954). Zur Pathologie des zentralen und peripheren Funktionsfeldes des Leberläppchens. *Zbl. Path.*, **91**, 255-267.
- EHRENCHEB, L. (1946). Morphology and chemistry of the metaphase spindle. *Hereditas (Lund)*, **32**, 15-36.
- EHRET, C. F., & POWERS, E. L. (1955). Macronuclear and nucleolar development in Paramecium bursaria. *Exp. Cell Res.*, **9**, 241-257.
- EHRICH, W. (1936). Die polymere Kerngröße als Ausdruck der Krebsanaplasie. *Z. Krebsforsch.*, **44**, 308-324.
- EHRICH, P. (1885). *Das Sauerstoffbedürfnis des Organismus*. Hirschwald, Berlin.
- EICHNER, D. (1959). Kernmessungen an Schilddrüse und Niere der Ratte unter experimentellen Bedingungen. *Z. mikr.-anat. Forsch.*, **66**, 85-95.
- EIGISTI, O. J., & DUSTIN JR. P., (1955). *Colchicine—in agriculture, medicine, biology and chemistry*. Iowa State College Press.
- ELAM, Y., KLEIN, S. (1962). The effect of light intensity and sucrose feeding on the fine structure in chloroplasts and on the chlorophyll content of etiolated leaves. *J. Cell. Biol.*, **14**, 169-182.

- EINHORN, S. L., HIRSCHBERG, E., & GELLMORN, A. (1953). Effects of cortisone on regenerating rat liver. *J. gen. Physiol.*, **37**, 559-574.
- ELBERS, P. F., & BLUEMINK, J. G. (1960). Pinocytosis in the developing egg of *Limnaea stagnalis* L. *Exp. Cell Res.*, **21**, 619-622.
- ELKELES, G. (1961). Die Idee der Zellenstehung aus nichtzellulären Elementen im Lichte der Ergebnisse der Mikrobiologie und biologischen Zellforschung. *Zbl. Path.*, **102**, 311-315.
- ELLENHORN, J. (1933). Experimental-photographische Studien der lebenden Zellen. *Z. Zellforsch.*, **20**, 288-308.
- ELLIOT, K. A. C. (1946). Swelling of brain slices and the permeability of brain cells to glucose. *Proc. Soc. exp. Biol.*, **63**, 234-236.
- EMMELLOT, P., & BENEDETTI, E. L. (1960). Changes in the fine structure of rat liver cells brought about by dimethylnitrosamine. *J. biophys. biochem. Cytol.*, **7**, 393-396.
- EMMELLOT, P., BOS, C. J., BROMBACHER, P. J., & REYERS, I. H. M. (1960). Swelling of fresh and aged liver mitochondria as effected by succinate adenine nucleotides and thyroxin. *Nature (Lond.)*, **186**, 556-558.
- ENDERLIN, M. (1953). Phasenmikroskopische Untersuchungen über Umgebungseinwirkungen auf die quergestreiften Muskelfasern. *Acta anat. (Basel)*, **17**, 1-46.
- EPPINGER, H. (1949). *Permeabilitätspathologie*. Springer, Wien.
- EPSTEIN, M. A. (1957). The fine structure of the cells in mouse sarcoma 37 ascitic fluids. *J. biophys. biochem. Cytol.*, **3**, 567-576.
- ERICKSON, R. O. (1947). Respiration of developing anthers. *Nature (Lond.)*, **159**, 275-276.
- ERLANGER, R. v. (1897). Beobachtungen über die Befruchtung und ersten zwei Teilungen an den lebenden Eiern kleiner Nematoden. II. *Biol. Zbl.*, **17**, 339-346.
- ERNST, P. (1915). Die Pathologie der Zelle. In: *Handbuch der allgemeinen Pathologie*, Vol. III/1. Hirzel, Leipzig.
- ERNSTER, L. (1959). Distribution and interaction of enzymes within animal cells. *Biochem. Soc. Symp. London*, **16**, 54-71.
- ERRERA, L. (1888). Über Zellformen und Seifenblasen. *Bot. Zbl.*, **34**, 395-399.
- ERRERA, M. (1955). Action of ionizing radiations on cell constituents. *Symp. on Radiobiology*. Butterworths, London.
- ERRERA, M., HELL, A., & PERRY, R. P. (1961b). The role of the nucleolus in ribonucleic acid- and protein synthesis. II. Amino acid incorporation into normal and nucleolar inactivated HeLa cells. *Biochim. biophys. Acta (Amst.)*, **49**, 58-63.
- ERRERA, R. P., HELL, A., & ERRERA, M. (1961a). The role of the nucleolus in ribonucleic acid- and protein synthesis. I. Incorporation of cytidine into normal and nucleolar inactivated HeLa cells. *Biochim. biophys. Acta (Amst.)*, **49**, 47-57.
- ESSNER, E. (1960). An electron microscopic study of erythrophagocytosis. *J. biophys. biochem. Cytol.*, **7**, 329-334.
- ESSNER, E., & NOVIKOFF, A. B. (1960). Human hepatocellular pigments and lysosomes. *J. Ultrastruct. Res.*, **3**, 374-391.
- (1961). Localization of acid phosphatase activity in hepatic lysosomes by means of electron microscopy. *J. biophys. biochem. Cytol.*, **9**, 773-784.
- ESTABLE, C., & SOTELO, J. R. (1951). Una nueva estructura celular: El nucleolonema. *Inst. Invest. Cien. Biol. Publ.*, **1**, 47-68.
- (1954). The behaviour of the nucleolonema during mitosis. *Symp. 8 Congr. of Cell Biology, Leiden*.
- EULER, H. von, BERGMANN, B., & HELLSTRÖM, H. (1934). Über das Verhältnis von Chloroplastenzahl und Chlorophyllkonzentration bei *Elodea densa*. *Ber. dtsch. bot. Ges.*, **52**, 458-462.
- EVANS, H. J., & SAVAGE, J. R. K. (1959). The effect of temperature on mitosis and on the action of colchicine in root meristem cells of *Vicia faba*. *Exp. Cell Res.*, **18**, 51-61.
- EVANS, W. L. (1956). The effect of cold treatment on the desoxyribonucleic acid (DNA) content in cells of selected plants and animals. *Cytologia (Tokyo)*, **21**, 417-432.
- FABERGÉ, A. C. (1942). Homologous chromosome pairing: The physical problem. *J. Genet.*, **43**, 121-144.
- FALK, H. (1960). Magnoglobuli in Chloroplasten von *Ficus elastica* Rox B. *Planta*, **55**, 525-532.
- FARQUHAR, M. G. (1961). Origin and fate of secretory granules in cells of the anterior pituitary gland. *Trans. N. Y. Acad. Sci.*, Ser. **2**, **23**, 346-351.
- FARQUHAR, M. G., VERNIER, R. L., & GOOD, R. A. (1957). Studies on familial nephrosis. II. Glomerular changes observed with the electron microscope. *Amer. J. Path.*, **33**, 791-817.
- FARQUHAR, M. G., & PALADE, G. E. (1960). Segregation of ferritin in glomerular protein absorption droplets. *J. biophys. biochem. Cytol.*, **7**, 297-304.
- FARRANT, J. L. (1954). An electron microscopic study of ferritin. *Biochim. biophys. Acta (Amst.)*, **13**, 569-576.

- FAUTREZ, J. (1960). Corrélation entre le volume du noyau et sa teneur en ADN. *Z. mikr.-anat. Forsch.*, **66**, 210-224.
- FAUTREZ, J., & LAQUERRIÈRE, R. (1957). Teneur en acide désoxyribonucléique et volume des noyaux des cellules hépatiques chez l'homme. *Exp. Cell Res.*, **13**, 403-405.
- FAUTREZ-FIRLEYFN, N. (1950). Expulsion d'acide thymonucléique hors du noyau de certaines cellules de l'ovaire d'*Artemia salina* L. *C. R. Soc. Biol. (Paris)*, **144**, 1127-1128.
- FAWCETT, D. W. (1959). In: *Developmental Cytology*, Vol. VIII. Ronald, New York.
- (1961). The membranes of the cytoplasm. *Lab. Invest.*, **10**, 1162-1188.
- FAWCETT, D. W., & ITO, S. (1958). Observations of the cytoplasmic membranes of testicular cells, examined by phase contrast and electron microscopy. *J. biophys. biochem. Cytol.*, **4**, 135-142.
- FAWCETT, D. W., ITO, S., & SLAUTERBACK, D. B. (1959). The occurrence of intercellular bridges in groups of cells exhibiting synchronous differentiation. *J. biophys. biochem. Cytol.*, **5**, 453-460.
- FAWCETT, D. W., & PORTER, K. R. (1954). A study of the fine structure of ciliated epithelia. *J. Morph.*, **94**, 221-281.
- FEINENDEGEN, L. E., BOND, V. P., SHREEVE, W. W., & PAINTER, R. B. (1960). RNA and DNA metabolism in human tissue culture cells studied with triated cytidine. *Exp. Cell Res.*, **19**, 443-459.
- FELDHERR, C. M. (1962). The nuclear annuli as pathways for nucleocytoplasmic exchanges. *J. Cell Biol.*, **14**, 65-72.
- FELDHERR, C. M., & FELDHERR, A. B. (1960). The nuclear membrane as a barrier to the free diffusion of proteins. *Nature (Lond.)*, **185**, 250-251.
- FELIX, K. (1959). Struktur und Funktion der Kerne tierischer Zellen. *Naturwissenschaften*, **46**, 29-35.
- FELL, H. B., & HUGHES, A. F. (1949). Mitosis in the mouse: A study of living and fixed cells in tissue cultures. *Quart. J. micr. Sci.*, **90**, 355-380.
- FELSCH, G. (1961). Beitrag zur Entstehung der drumsticks. *Folia haemat. (Lpz.)*, **78**, 230-236.
- FELSENFELD, G., & RICH, A. (1957). Studies on the formation of two- and three-stranded polyribonucleotides. *Biochim. biophys. Acta (Amst.)*, **26**, 457-468.
- FELTON, H. M., & POMERAT, C. M. (1962). Antigen-antibody reactions in relation to pinocytosis. I. Cell injury and repair following *Staphylococcus* toxin and antiserum. *Exp. Cell Res.*, **27**, 280-291.
- FETNER, R. H. (1962). Ozone-induced chromosome breakage in human cell cultures. *Nature (Lond.)*, **194**, 793-794.
- FEULGEN, R., & ROSENBECK, H. (1924). Mikroskopisch-chemischer Nachweis einer Nukleinsäure vom Typus der Thymo-Nukleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. *Z. physiol. Chem.*, **135**, 203-248.
- FEYRTER, F. (1951). *Die Pathologie der vegetativen nervösen Peripherie*. Maudrich, Wien.
- (1960). Über den Kernpolymorphismus einiger Zellarten in der Leiche und im Operat. *Frankfurt. Z. Path.*, **70**, 740-756.
- (1961). Über Kernpolymorphismus und amitotische Kernteilung. *Med. Welt.*, 2038-2045.
- FICQ, A. (1955). Étude autoradiographique du métabolisme de l'oocyte d'*Asterias rubens* au cours de la croissance. *Arch. Biol. (Liège)*, **66**, 509-524.
- (1959). Radioautographic studies on nuclear activity in the liver. *J. Histochem. Cytochem.*, **7**, 215-223.
- (1961). Localization of different types of ribonucleic acids (RNA's) in amphibian oocytes. *Exp. Cell Res.*, **23**, 427-429.
- FICQ, A., & ERRERA, M. (1959). Metabolic processes in cell nuclei. *Exp. Cell Res.*, Suppl. **7**, 145-155.
- FINAMORE, F. J. (1961). The nucleic acids—metabolic key to development? *Quart. Rev. Biol.*, **35**, 117-122.
- FIRKET, H. (1956). Mesures cytophotométriques de la synthèse d'acides désoxyribonucléiques dans les cultures de tissus soumises au refroidissement puis réchauffement. *C. R. Soc. Biol. (Paris)*, **150**, 1050-1053.
- (1958). Recherches sur la synthèse des acides désoxyribonucléiques et la préparation à la mitose dans des cellules cultivées "in vitro" (Étude cytophotométrique et autoradiographique). *Arch. Biol. (Liège)*, **69**, 1-166.
- FISCHER, H. (1934). Größenänderungen von Kern und Nukleolus im Blattgewebe. *Planta*, **22**, 767-793.
- FISCHER, H., FERBER, E., & RÖWE, K. (1959). Quantitative Bestimmung von Aminosäuren aus hochspannungselektrophoretisch getrennten Gemischen durch automatische Extinktionsschreibung. *Z. physiol. Chem.*, **313**, 174-183.
- FISCHER, H., & WAGNER, L. (1954). Die Wirkung niedermolekularer (basischer) Proteine auf Zellen und Organismen. *Naturwissenschaften*, **41**, 532-533.
- FISCHER, I. (1936). Über den Wachstumsrhythmus des Folikelepithels der Läuse und Federlinge und seine Beziehungen zum Arbeitsrhythmus der Zelle und zur Amitose. *Z. Zellforsch.*, **23**, 219-243.
- FISCHER-WASELS, B. (1922). Experimentelle Untersuchungen über die blasige Entartung der Leberzelle und die Wasservergiftung der Zelle im allgemeinen. *Frankfurt. Z. Path.*, **28**, 201-250.

- FISHER, W. D., ANDERSON, N. G., & WILBUR, K. M. (1959). Studies on nuclei. I. Physical properties of deoxyribonucleoprotein from rat thymus in 1 M sodium chloride. *Exp. Cell Res.*, **18**, 100-111.
- FITTING, H. (1925). Untersuchungen über die Auslösung von Protoplasmaströmung. *Jb. wiss. Bot.*, **64**, 281-388.
- (1936). Über die Auslösung von Protoplasmaströmung durch chemische Wirkstoffe. *Fortschr. dtsch. Wiss.*, **12**, 160-161.
- FITZGERALD, P. J., & VINIJCHAIKUL, K. (1959). Nucleic acid metabolism of pancreatic cells as revealed by cytidine-H³ and thymidine-H³. *Lab. Invest.*, **8**, 319-329.
- FLEMMING, W. (1879). Beiträge zur Kenntnis der Zelle und ihrer Lebenserscheinungen. *Arch. mikr. Anat.*, **16**, 302-436.
- (1880). Beiträge zur Kenntnis der Zelle und ihrer Lebenserscheinungen. *Arch. mikr. Anat.*, **18**, 151-259.
- (1882). *Zellsubstanz, Kern- und Zellteilung*. Vogel, Leipzig.
- (1891a). Über Teilung und Kernformen bei Leukozyten und über deren Attraktionssphären. *Arch. mikr. Anat.*, **37**, 249-298.
- (1891b). Neue Beiträge zur Kenntnis der Zelle. II. Theil. *Arch. mikr. Anat.*, **37**, 685-751.
- (1892a). Über Unsichtbarkeit lebendiger Kernstrukturen. *Anat. Anz.*, **7**, 758-764.
- (1892b). Zelle. *Erg. Anat.*, **1**, 43-82.
- (1897). Zelle. *Erg. Anat.*, **6**, 184-283.
- FLETSCHER, M. J., & SANADI, D. R. (1961). Turnover of rat liver mitochondria. *Biochim. biophys. Acta (Amst.)*, **51**, 356-360.
- FLORENTIN, P., & PICARD, D. (1936). Recherches sur le pancréas endocrine. *Rev. franç. Endocr.*, **14**, 1-27.
- FONTANA, F. (1781). *Traité sur le venin de la vipère*. Florence.
- FOSTER, T. S., & STERN, H. (1958). Soluble deoxyribonucleic compounds in relation to duplication of deoxyribonucleic acid. *Science*, **128**, 653-654.
- FOX, S. W., & HARADA, K. (1961). Synthesis of uracil under conditions of a thermal model of pre-biological chemistry. *Science*, **133**, 1923-1924.
- FOX, S. W., HARADA, K., & VEGOTSKY, A. (1959). Thermal polymerization of amino acids and a theory of biochemical origins. *Experientia (Basel)*, **15**, 81-84.
- FRACCARO, M., KAIJSER, K., & LINDSTEN, J. Chromosomal abnormalities in father and mongol child. *Lancet*, **1960/1**, 724-727.
- FRANKEL, O. H. (1937). The nucleolar cycle in some species of Fritillaria. *Cytologia (Tokyo)*, **8**, 37-47.
- FRANKENBERGER, Z. (1961). J. E. Purkyně und die Zellenlehre. *Nova acta Leopoldiana, N.F.*, **24**, 47-55.
- FRANKLIN, R. M. (1962). A cytochemical description of the multiplication of mengovirus in L-929 cells. *J. Cell Biol.*, **12**, 1-15.
- FREDERIC, J. (1953). Rapports de la membrane nucléaire avec les nucléoles et les chondriosomes. *C. R. Ass. Anat.*, **72**, 111-115.
- (1954). Action of various substances on the mitochondria of living cells cultivated in vitro. *Ann. N. Y. Acad. Sci.*, **58**, 1246-1263.
- (1958). Recherches cytologiques sur le chondriome normal ou soumis à l'expérimentation dans des cellules vivantes cultivées in vitro. *Arch. Biol. (Liège)*, **69**, 167-349.
- FREDERIC, J., & CHÈVREMONT, M. (1952). Recherches sur les chondriosomes de cellules vivantes par la microscopie et la micro-cinématographie en contraste de phase. *Arch. Biol. (Liège)*, **63**, 109-131.
- FREESE, E. (1959). The specific mutagenic effect of base analogues on phage T4. *J. molec. Biol.*, **1**, 87-105.
- FRENSTER, J. H., ALLFREY, V. G., & MIRSKY, A. E. (1960). Metabolism and morphology of ribonucleoprotein particles from the cell nucleus of lymphocytes. *Proc. nat. Acad. Sci. (Wash.)*, **46**, 432-444.
- (1961). In vitro incorporation of amino acid into the proteins of isolated nuclear ribosomes. *Biochim. biophys. Acta (Amst.)*, **47**, 130-137.
- FREUNDLICH, H. (1927). Neuere Fortschritte der Kolloidchemie und ihre biologische Bedeutung. *Protoplasma (Wien)*, **2**, 278-299.
- (1937). Colloidal structures in biology. *J. Phys. Chem.*, **41**, 1151-1161.
- FREW, P. E., & BOWEN, R. H. (1929). Nucleolar behaviour in the mitosis of plant cells. *Quart. J. micr. Sci.*, **73**, 197-214.
- FREY-WYSSLING, A. (1938). *Submikroskopische Morphologie des Protoplasmas und seiner Derivate*. Bontraeger, Berlin.
- (1955). Die submikroskopische Struktur des Cytoplasmas. *Protoplasmatalogia*, **II/A2**. Springer, Wien.
- FREY-WYSSLING, A., & KREUTZER, E. (1958). The submicroscopic development of chromoplasts in the fruit of Capsicum annuum L. *J. Ultrastruct. Res.*, **1**, 397-411.
- FRICK, G. G. (1961). Der Erythrozyt als Beispiel biologischer Zweckmäßigkeit. *Schweiz. med. Wschr.*, **91**, 1245-1249.

- FRIEDE, R. L., & PAX, R. A. (1961). Mitochondria and mitochondrial enzymes. A comparative study of localization in the cat's brain stem. *Z. Zellforsch., Abt. Histochem.*, **2**, 186-191.
- FRIEDMANN, E., & SIMON-REUSS, I. (1954). Mitotic inhibition induced by phosphates. *Experientia (Basel)*, **10**, 494.
- FRIEDRICH-FRESKA, H. (1940). Bei der Chromosomenkonjugation wirksame Kräfte und ihre Bedeutung für die identische Verdopplung von Nukleoproteinen. *Naturwissenschaften*, **28**, 376-379.
- (1961). Genetik und biochemische Genetik in den Instituten der Kaiser-Wilhelm-Gesellschaft und der Max-Planck-Gesellschaft. *Naturwissenschaften*, **48**, 10-22.
- FRIEDRICH-FRESKA, H., & ZAKI, F. G. (1954). Spezifische Mitose-Auslösung in normaler Rattenleber durch Serum von partiell hepatektomierten Ratten. *Z. Naturforsch.*, **9b**, 394-397.
- FRITZ-NIGGLI, H. (1959). *Strahlenbiologie Grundlagen und Ergebnisse*. Thieme, Stuttgart.
- (1960). Allgemeine Strahlenbiologie. In: *Handbuch der allgemeinen Pathologie*, Vol. **X/I**, edited by BÜCHNER, F., LETTERER, E., & ROULET, F. Springer, Berlin.
- FROLOWA, S. L. (1935). Cytologie der künstlichen Parthenogenese bei Bombyx mori L. *Biol. Zurnal*, **4**, 275-308.
- FRÜHLING, L., KEMPF, J., & PORTE, A. (1961). Sur les modifications histologiques liées à la formation des dépôts de substance amyloïde. Étude au microscope électronique. *C. R. Soc. Biol. (Paris)*, **155**, 1563-1566.
- FRY, H. J. (1937). Studies on the mitotic figure VI. Midbodies and their significance for the central body problem. *Biol. Bull.*, **73**, 565-590.
- FRY, R. J., LESHER, S., & KOHN, H. I. (1961a). Estimation of time of generation of living cells. *Nature (Lond.)*, **191**, 290-291.
- (1961b). Age effect on cell-transit time in mouse jejunal epithelium. *Amer. J. Physiol.*, **201**, 213-216.
- FUJI, T. (1954). Notes on the presence of zinc in nucleoli and in the sperm middle-piece in some marine forms. *Ann. Zool. Jap.*, **27**, 115-117.
- GABE, M., & ARVY, L. (1961). Gland Cells. In: *The Cell*, Vol. **V/2**, edited by BRACHET & MIRSKY. Acad. Press, New York.
- GABELER, G. (1960). Über die Transformation des Chondrioms bei Speicherung makromolekularer Stoffe (Dextran, Periston). Elektronenmikroskopische Untersuchungen am Reticuloendothel der Rattenmilz. *Virchows Arch. path. Anat.*, **333**, 230-240.
- GADRAT, J., PLANEL, H., GUILHEM, A., & IZARD, J. (1960). Contribution de la microscopie électronique à l'étude des lipopigments du foie et du cortex surrénal. *Path. et Biol.*, **8**, 697-708.
- GALL, J. G. (1954a). Observation on the nuclear membrane with the electron microscope. *Exp. Cell Res.*, **7**, 197-200.
- (1954b). Lampbrush chromosomes from oocyte nuclei of the newt. *J. Morph.*, **94**, 283-351.
- (1955). Problems of the structure and function in the amphibian oocyte nucleus. *Symp. Soc. exp. Biol.*, **9**, 358-370.
- (1956). Small granules in the amphibian oocyte nucleus and their relationship to RNA. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 393-396.
- (1959a). The nuclear envelope after KMnO₄ fixation. *J. biophys. biochem. Cytol.*, **6**, 115-118.
- (1959b). Macronuclear duplication in the ciliated protozoan Euplotes. *J. biophys. biochem. Cytol.*, **5**, 295-308.
- (1961). Centriolereplication. A study of spermatogenesis in the snail Viviparus. *J. biophys. biochem. Cytol.*, **10**, 163-193.
- GAMOW, G. (1954). Possible mathematical relation between deoxyribonucleic acid and proteins. *Kgl. Danske Videnskab. Selkab. Biol. Med.*, **22**, 1-13.
- GAMOW, G., RICH, A., & YCAS, M. (1956). The problem of information transfer from the nucleic acids to proteins. *Advanc. biol. med. Phys.*, **4**, 23-68.
- GANSLER, H., & ROUILLER, C. (1956). Modifications physiologiques et pathologiques du chondriome. *Schweiz. Z. allg. Path.*, **19**, 217-243.
- GARNIER, CH. (1899). Contribution à l'étude de la structure et du fonctionnement des cellules glandulaires séreuses. Du rôle de l'ergastoplasmé dans la sécrétion. *Thesis Nr. 50, Nancy*.
- GATENBY, J. B. (1917). The cytoplasmic inclusions of the germ-cells. *Quart. J. micr. Sci.*, **62**, 555-611.
- GATENBY, J. B., & SINGH, B. N. (1938). The Golgi apparatus of Copromonas subtilis and Euglena spec. *Quart. J. micr. Sci.*, **80**, 567.
- GATENBY, J. B., DALTON, A. J., & FELIX, M. D. (1955). The contractile vacuole of parazoa and protozoa, and the Golgi apparatus. *Nature (Lond.)*, **176**, 301-302.
- GAULDEN, M. E. (1960). The nucleolus and mitotic activity. In: *The Cell Nucleus*. Butterworths, London.
- GAULDEN, M. E., & PERRY, R. P. (1958). Influence of the nucleolus on mitosis as revealed by the ultraviolet microbeam irradiation. *Proc. nat. Acad. Sci. (Wash.)*, **44**, 553-559.

- GAVOSTO, F., & RECHEMANN, R. (1954). In vitro incorporation of glycine- I^{-14}C in reticulocytes. *Biochim. biophys. Acta (Amst.)*, **13**, 583-586.
- GAY, H. (1956). Nucleocytoplasmic relations on *Drosophila*. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 257-268.
- GAYLORD, W. H. (1958). Cellular reaction during virus infections. In: *Frontiers in Cytology*. Yale Univ. Press, New Haven.
- GEDIGK, P. (1954). Zur Histochemie des Zentralapparates der Zelle. *Virchows Arch. path. Anat.*, **325**, 366-378.
- (1958a). Die funktionelle Bedeutung des Eisenpigmentes. *Ergebn. allg. Path. path. Anat.*, **38**, 1-45.
- (1958b). Zur Kenntnis lipogener Pigmente. *Verh. dtsch. path. Ges.*, **42**, 430-434.
- GEDIGK, P., & STRAUSS, G. (1953). Zur Histochemie des Hämosiderins. *Virchows Arch. path. Anat.*, **324**, 373-390.
- GEDIGK, P., & FISCHER, R. (1958). Über die Entstehung des Ceroidpigmentes bei der hämorrhagischen Fettgewebsnekrose. *Virchows Arch. path. Anat.*, **331**, 341-370.
- (1959). Über die Entstehung von Lipopigmenten in Muskelfasern. Untersuchungen beim experimentellen Vitamin-E-Mangel der Ratte und an Organen des Menschen. *Virchows Arch. path. Anat.*, **332**, 431-468.
- GEIGER, R. S. (1958). Effect of adrenaline on neurofibril formation in adult mammalian cortical neurones in tissue culture. *Nature (Lond.)*, **182**, 1674-1675.
- GEITLER, L. (1934). *Grundriß der Cytologie*. Borntraeger, Berlin.
- (1935). Beobachtungen über die erste Teilung im Pollenkern der Angiospermen. *Planta*, **24**, 361-386.
- (1937). Die Analyse des Kernbaus und der Kernteilung der Wasserläufer *Gerris lateralis* und *Gerris lacustris* (Hemiptera Heteroptera) und die Somadifferenzierung. *Z. Zellforsch.*, **26**, 641-672.
- (1938a). Die Entstehung der polyploiden somatischen Zellkerne durch wiederholte Chromosomenteilung ohne Spindelbildung und Kernteilung. *Naturwissenschaften*, **26**, 722-723.
- (1938b). Über den Bau des Ruhekerns mit besonderer Berücksichtigung der Heteropteren und Dipteren. *Biol. Zbl.*, **58**, 152-179.
- (1938c). Über das Wachstum von Chromozentrenkernen und zweierlei Heterochromatin bei Blütenpflanzen. *Z. Zellforsch.*, **28**, 133-153.
- (1938). *Chromosomabau*. Borntraeger, Berlin.
- (1939a). Die Entstehung der polyploiden Soma kerne der Heteropteren durch Chromosamenteilung ohne Kernteilung. *Chromosoma (Berl.)*, **1**, 1-22.
- (1939b). Das Heterochromatin der Geschlechtschromosomen bei Heteropteren. *Chromosoma (Berl.)*, **1**, 197-229.
- (1940). Kernwachstum und Kernbau bei zwei Blütenpflanzen. *Chromosoma (Berl.)*, **1**, 474-485.
- (1941). Das Wachstum des Zellkerns in tierischen und pflanzlichen Geweben. *Ergebn. Biol.*, **18**, 1-54.
- (1953). Endomitose und endomitotische Polyploidisierung. *Protoplasmatologia*, VI/C. Springer, Wien.
- GELFANT, S. (1960a). A study of mitosis in mouse ear epidermis in vitro. III. Effects of glycolytic and Krebs cycle intermediates. IV. Effects of metabolic inhibitors. *Exp. Cell Res.*, **19**, 65-82.
- (1960b). A study of mitosis in mouse ear epidermis in vitro. V. Effects of hormones. *Exp. Cell Res.*, **21**, 603-615.
- (1962). Initiation of mitosis in relation to the cell division cycle. *Exp. Cell Res.*, **26**, 395-403.
- GEORGIEV, G. P., MANTIEVA, V. M., & ZBARKY, I. B. (1960). RNA fractions in cell nuclei isolated by phenol and by sucrose-glycerophosphate. *Biochim. biophys. Acta (Amst.)*, **37**, 373-374.
- GEORGIEV, G. P., & CHENTSOV, J. S. (1962). On the structural organization of nucleolo-chromosomal ribonucleoproteins. *Exp. Cell Res.*, **27**, 570-572.
- GEORGII, A., MEHNERT, H. (1961). Über Leberveränderungen durch Thioacetamid bei gleichzeitiger Belastung mit blutzuckersenkenden Substanzen der Biguanidreihe. *Beitr. path. Anat.*, **124**, 278-294.
- GERBAULET, K., BRÜCKNER, J., & MAURER, W. (1961). Autoradiographische Untersuchungen über den Einfluß einer Röntgen-Ganzkörperbestrahlung auf die Eiweißsyntheserate im Zellkern. *Naturwissenschaften*, **48**, 526-527.
- GERMAN, J. L., DEMAYO, A. P., & BEARN, A. G. (1962). Inheritance of abnormal chromosome in Down's syndrome (mongolism) with leukemia. *Amer. J. hum. Genet.*, **14**, 31-43.
- GERSH, J., & BODIAN, D. (1943). Some chemical mechanism in chromatolysis. *J. cell. comp. Physiol.*, **21**, 253-274.
- GESCHWIND, I. I., ALFERT, M., & SCHOOLEY, C. (1960). The effects of thyroxin and growth hormone on liver polyploidy. *Biol. Bull.*, **118**, 66-69.
- GEY, G. O., SHAPRAS, P., BANG, F. B., & GEY, M. K. (1954a). Some relations of inclusion droplets (Pinocytosis-Lewis) and mitochondrial behavior in normal and malignant cells. *Symp. 8 Congr. of Cell Biol.*, Lciden.

- GEY, G. O., BANG, F. B., & GEY, M. K. (1954b). Responses of a variety of normal and malignant cells to continuous cultivation, and some practical applications of these responses to problems in the biology of disease. *Ann. N. Y. Acad. Sci.*, **58**, 976-999.
- GEYER-DUSZYNSKA, I. (1961). Spindle disappearance and chromosome behaviour after partial-embryo irradiation in Cecidomyiidae (Diptera). *Chromosoma (Berl.)*, **12**, 233-247.
- GIBBS, S. P. (1960). The fine structure of *Euglena gracilis* with special reference to the chloroplasts and pyrenoids. *J. Ultrastruct. Res.*, **4**, 127-148.
- GIERER, A. (1961). Molekulare Grundlagen der Vererbung. *Naturwissenschaften*, **48**, 283-289.
- GILES, N. H. (1954). Radiation-induced chromosome aberration in *Tradescantia*. In: *Radiation biology*, Vol. II. McGraw, New York.
- GLÄSS, E. (1956). Die Verteilung von Fragmentationen und achromatischen Stellen auf den Chromosomen von *Vicia faba* nach Behandlung mit Schwermetallsalzen. *Chromosoma (Berl.)*, **8**, 260-284.
- (1957). Das Problem der Genomsonderung in den Mitosen unbehandelter Rattenlebern. *Chromosoma (Berl.)*, **8**, 468-492.
- (1960). Die Chromosomenzahlen in der durch Buttergelbfütterung krebsig entarteten Rattenleber. *Z. Krebsforsch.*, **63**, 362-371.
- (1961a). Weitere Untersuchungen zur Genomsonderung. I. Modellversuche zum Phänomen der Genomsonderung. *Chromosoma (Berl.)*, **12**, 410-421.
- (1961b). Weitere Untersuchungen zur Genomsonderung. II. Die Anordnung der Chromosomen in den Wurzelspitzenmitosen von *Bellevalia romana*. *Chromosoma (Berl.)*, **12**, 422-432.
- GLAUERT, A. M., & HOPWOOD, D. A. (1960). The fine structure of *streptomyces coelicolor*. I. The cytoplasmic membrane system. *J. biophys. biochem. Cytol.*, **7**, 479-488.
- GLINOS, A. D. (1960). Environmental feedback control of cell division. *Ann. N. Y. Acad. Sci.*, **90**, 592-602.
- GLOGGENGIESSEN, W. (1944). Experimentelle morphologische und systematische Untersuchungen über die seröse Entzündung der Leber, nebst Beiträgen experimenteller Leberschäden durch Bakterien, Bakterientoxine und mechanisch-operative Eingriffe. *Virchows Arch. path. Anat.*, **312**, 64-115.
- GODMAN, G. C., MORGAN, C., BREITENFELD, P. M., & ROSE, H. M. (1960). A correlative study by electron and light microscopy of the development of type 5 adenovirus. II. Light microscopy. *J. exp. Med.*, **112**, 383-402.
- GODWARD, M. B. E. (1954). The "diffuse" centromere or polycentric chromosomes in *Spirogyra*. *Ann. Bot. N. S.*, **18**, 143-156.
- GÖSSNER, W. (1960). Studien zur Nekrose und Autolyse in vivo. *Verh. dtsch. Ges. Path.*, 204-209.
- GÖSSNER, W., SCHNEIDER, G., SIFFS, M., & STEGMANN, H. (1951). Morphologisches und humorales Stoffwechselgeschehen in Leber, Milz und Blut im Verlauf der experimentellen Amyloidose. *Virchows Arch. path. Anat.*, **320**, 326-373.
- GOLDACRE, R. J. (1952). The folding and unfolding of protein molecules as a basis of osmotic work. *Int. Rev. Cytol.*, **1**, 135-164.
- GOLDACRE, R. J., & LORCH, I. J. (1950). Folding and unfolding of protein molecules in relation to cytoplasmic streaming, amoeboid movement and osmotic work. *Nature (Lond.)*, **166**, 497-500.
- GOLDSCHMIDT, R. (1923). Kleine Beobachtungen und Ideen zur Zellenlehre. IV. *Arch. exp. Zellforsch.*, **17**, 167-184.
- GOLDSTEIN, L. (1958). Localization of nucleus-specific protein as shown by transplantation experiments in Amoeba proteus. *Exp. Cell Res.*, **15**, 635-637.
- GOLDSTEIN, L., & PLAUT, W. (1955). Direct evidence for nuclear synthesis of cytoplasmic ribose nucleic acid. *Proc. nat. Acad. Sci. (Wash.)*, **41**, 874-880.
- GOLGI, C. (1898). Sur la structure des cellules nerveuses. *Arch. ital. Biol.*, **30**, 60-71.
- GOTHE, H. D., & HINRICHSEN, K. (1959). Die Chromatinstruktur der Granulocytenkerne in ihrer Beziehung zu den geschlechtsspezifischen Kernanhängsgebilden. *Klin. Wschr.*, **37**, 506-511.
- GOTTSCHALK, W. (1951a). Untersuchungen am Pachytän normaler und röntgenbestrahlter Pollenmutterzellen von *Solanum lycopersicum*. *Chromosoma (Berl.)*, **4**, 298-341.
- (1951b). Der Vergleich von Röntgen- und chemisch induzierten Chromosomenmutationen im Pachytän von *Solanum lycopersicum*. *Chromosoma (Berl.)*, **4**, 342-358.
- GRAFL, I. (1941). Über das Wachstum der Antipodenkerne von *Caltha palustris*. *Chromosoma (Berl.)*, **2**, 1-11.
- GRAHAM, M. A., & BARR, M. L. (1952). A sex difference in the morphology of metabolic nuclei in somatic cells of the cat. *Anat. Rec.*, **112**, 709-723.
- (1959). Sex chromatin in the opossum, *Didelphys virginiana*. *Arch. anat. micr. Morph. exp.*, **48**, 111-121.
- GRANICK, S. (1955). Plastid structure, development and inheritance. In: *Handbuch der Pflanzenphysiologie*, Vol. I. Springer, Berlin.
- (1961). The Chloroplasts: Inheritance, structure, and function. In: *The Cell*, Vol. II. Acad. Press, New York.

- GRASSÉ, P. P. (1939). Études de mécanique cellulaire: centromères et centrosomes dans la mitose de certains flagellés. *C. R. Soc. Biol. (Paris)*, **131**, 1015-1018.
- GRAUBARD, M. A. (1932). Inversion in *Drosophila melanogaster*. *Genetics*, **17**, 81-105.
- GRAUPNER, H., & FISCHER, I. (1935). Die Entwicklung und Degeneration der Melanophoren von *Atherina mocho*. *Z. Zellforsch.*, **22**, 434-444.
- GREEN, D. E. (1958). Mitochondrial structure and function. In: *Subcellular particles*, edited by HAYASHI, T. Ronald Press, New York.
- (1963). Enzymatic organization of the mitochondrion. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- GREEN, D. E., & WACKIL, S. J. (1960). In: *Lipid Metabolism*, Wiley, New York.
- GREEN, D. E., & HATEFI, Y. (1961). The mitochondrion and biochemical machines. *Science*, **133**, 13-19.
- GREEN, D. E., & ODA, T. (1961). On the unit of mitochondrial structure and function. *J. Biochem. (Tokyo)*, **49**, 742-757.
- GREEN, E. U., CALL, I. O., & EVANS, N. J. (1959). Mitotic and intermitotic periods in *Rana pipiens* tadpoles and embryos. *J. exp. Zool.*, **140**, 519-561.
- GREEN, J. W., & LUSHBAUGH, C. C. (1949). Histopathologic study of the mode of inhibition of cellular proliferation by urethane: Effect of urethane on Walker rat carcinoma 256. *Cancer Res.*, **9**, 199-209.
- GRELL, K. G. (1950a). Der Generationswechsel des parasitischen Suktors Tachyblaston ephelotensis Martin. *Z. Parasitenk.*, **14**, 499-534.
- (1950b). Der Kerndualismus der Ciliaten und Suktorten. *Naturwissenschaften*, **37**, 347-356.
- (1956). *Protozoologie*. Springer, Berlin.
- GRELL, S. M. (1946). Cytological studies in *Culex*. I. Somatic reduction divisions. II. Diploid and meiotic divisions. *Genetics*, **31**, 60-94.
- GRESSON, R. A. R. (1950). A study of the male germ-cells of the rat and mouse by phase-contrast microscopy. *Quart. J. micr. Sci.*, **91**, 73-78.
- GREULICH, R. C., CAMERON, I. L., & THRASHER, J. D. (1961). Stimulation of mitosis in adult mice by administration of thymidine. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 743-748.
- GREW, N. (1682). *The anatomy of plants*. Rawlins, London.
- GRIFFIN, J. L., & ALLEN, R. D. (1960). The movement of particles attached to the surface of amoebae in relation to current theories of amoeboid movement. *Exp. Cell Res.*, **20**, 619-622.
- GROOTD, M. DE, DEROM, F., LAGASSE, A., SEBRUYNS, M., & THIERY, M. (1958). Fine structure of the nuclear envelope of carcinoma cells. *Nature (Lond.)*, **182**, 1030-1031.
- GROPP, A. (1960). Untersuchungen an Zellen in der Gewebekultur als Beitrag zu Fragen der Stoffaufnahme. *Verh. dtsch. Ges. Path.*, 220-225.
- GROS, F., HIAT, H., GILBERT, W., KURLAND, G. G., RISEBROUGH, R. G., & WATSON, J. D. (1961). Unstable ribonucleic acid revealed by pulse labelling of *Escherichia coli*. *Nature (Lond.)*, **190**, 581-585.
- GROSS, F. (1935). Die Reifungs- und Furchungsteilungen von *Artemia salina* im Zusammenhang mit dem Problem des Kernteilungsmechanismus. *Z. Zellforsch.*, **23**, 522-565.
- GROSS, P. R., PHILPOTT, D. E., & NASS, S. (1958). The fine structure of the mitotic spindle in sea urchin eggs. *J. Ultrastruct. Res.*, **2**, 55-72.
- (1960). Electron microscopy of the centrifuged sea urchin egg, with a note on the structure of the ground cytoplasm. *J. biophys. biochem. Cytol.*, **7**, 135-142.
- GROSS, R., & MAYER, A. (1953). Zum Verhalten der Granulationen während der Zellteilung in blutbildenden Geweben. *Z. Krebsforsch.*, **59**, 371-380.
- GROSS, R., & GÖDIGK, P. (1959). Die eosinophilen Leucocyten. In: *Physiologie und Physiopathologie der weißen Blutzellen*, edited by BRAUNSTEINER, H. Thieme, Stuttgart.
- GROSS, R., GRUNDMANN, E., & BREHMKE, H. (1961). Zur Frage der Proliferation undifferenzierter leukämischer Zellen. *Folia haemat. (Ffm.)*, **6**, 358-363.
- GROSS, R., GRUNDMANN, E., BREHMKE, K., KAHLSTORF, I., & BOCK, U. (1962). Art und Intensität der Zellvermehrung bei akuten Leukosen. (Nach morphologischen und cytophotometrischen Untersuchungen.) *Klin. Wschr.*, **40**, 392-400.
- GROUCHY, J. DE, LAMY, M., FREZAL, J., & RIBIER, J. (1961). XX/XO mosaics in Turner's syndrome. Two further cases. *Lancet*, **I**, 1369-1371.
- GRUITHUISEN, F. (1811). *Organozoönomie*. Lentner, München.
- GRUN, P. (1958). Plant lampbrush chromosomes. *Exp. Cell Res.*, **14**, 619-621.
- GRUNDMANN, E. (1950). Histologische Untersuchungen über die Wirkungen experimentellen Sauerstoffmangels auf das Katzenherz. *Beitr. path. Anat.*, **111**, 36-76.
- (1952). Karyologische und karyometrische Untersuchungen an Lymphknotenpunktaten bei Stickstoff-Lost-Therapie. *Z. exp. Med.*, **118**, 489-523.
- (1953). Beitrag zur DNS-Synthese im Interphasenkern und zum Thema "Ruhekern gift-Wirkung". *II. Freibg. Symp.*, 187-190.

- (1954). Beiträge zur Krebsentstehung in der Rattenleber an Hand mikrophotometrischer DNS-Messungen. *Verh. dtsch. path. Ges.*, **38**, 362–370.
- (1958a). Die Bildung der Lymphocyten und Plasmazellen im lymphatischen Gewebe der Ratte. *Beitr. path. Anat.*, **119**, 217–262.
- (1958b). Experimentelle Untersuchungen über die funktionelle Cytomorphologie der lymphatischen Strukturen bei Entzündung sowie unter Cortison und DOCA. *Beitr. path. Anat.*, **119**, 377–432.
- (1959). Über die Unterscheidung von zwei Lymphocytentypen im Phasenkontrastmikroskop. *Virchows Arch. path. Anat.*, **332**, 17–24.
- (1961a). Distribution of deoxyribonucleic acid in the cell nucleus. *Nature (Lond.)*, **190**, 359–360.
- (1961b). Die Zytogenese des Krebses. *Dtsch. med. Wschr.*, **86**, 1077–1084, 1095–1096.
- (1962). Die Krebsentwicklung als intrazelluläres Problem, dargestellt am Beispiel des Diäthyl-nitrosamin-Krebses der Rattenleber. *Mitt. GBK Nordrh.-Westf.*, **2**, 587–633.
- (1963a). Interferenzmikroskopische Trockenmassenbestimmungen an Thymus- und Leberzellkernen der Ratte. *Acta histochem. (Jena)*.
- (1963b). Die Mikrospektrophotometrie im sichtbaren Spektralbereich. *9 Freiburger Symp.*, 32–46.
- GRUNDMANN, E., & BACH, G. (1960). Amitosen, Endomitosen und Mitosen nach partieller Hepatektomie. *Beitr. path. Anat.*, **123**, 144–172.
- GRUNDMANN, E., & MARQUARDT, H. (1953). Untersuchungen an Interphaserkernen des Wurzelmeristems von *Vicia faba*. I. Mittl. Desoxyribonucleinsäure-Gehalt und Größe der Kerne. *Chromosoma (Berl.)*, **6**, 115–134.
- GRUNDMANN, E., KRÖGER, H., & HOLZER, H. (1960). Vergleichende histologische, cytophotometrische und biochemische Untersuchungen über die Wirkung von Endoxan auf das Jensen-Sarkom der Ratte. *Klin. Wschr.*, **38**, 546–548.
- GRUNDMANN, E., HILLEMANNS, H. G., & RHA, K. (1961). Cytophotometrische Untersuchungen am menschlichen Portioepithel während der Krebsentwicklung. II. Die Kernfärbbarkeit mit Fastgreen und mit Gallacyaninchromalaun. *Z. Krebsforsch.*, **64**, 403–417.
- GRUNDMANN, E., & STEIN, P. (1961a). Über das organspezifische Chromatin im normalen und im carcinomatösen Parenchymzellkern. *Verh. dtsch. path. Ges.*, **45**, 93–97.
- (1961b). Untersuchungen über die Kernstrukturen in normalen Geweben und im Carcinom. *Beitr. path. Anat.*, **125**, 54–76.
- GRUNDMANN, E., & HOFMEIER, G. (1962). Kern-Trockenmassen und Kern-Volumina in der regenerierenden Rattenleber. *Naturwissenschaften*, **49**, 235–236.
- GRUNDMANN, E., & SIEBURG, H. (1962). Die Histogenese und Cytogenese des Lebercarcinoms der Ratte durch Diäthylnitrosamin im lichtmikroskopischen Bild. *Beitr. path. Anat.*, **126**, 57–90.
- GRUNDMANN, E., & PFEIFER, H. U. (1963). Zum Problem der Amitose in Leberzellen (Kernzählungen, cytophotometrische DNS-Messungen und karyomorphologische Beobachtungen).
- GRZYCKI, S. (1960). The effect of different fixatives on the Golgi structure examined by immersion refractometry in phase contrast illumination. *Cytologia (Tokyo)*, **25**, 108–111.
- GURWITSCH, A. (1926). Das Problem der Zellteilung physiologisch betrachtet. Springer, Berlin.
- GUSEK, W. (1959). Topographie und Ultrastruktur von Zentral-Apparat und Golgi-Komplex in einem Inseladenom. *Tumori*, **3**, 1788–1797.
- GUTTES, E., & GUTTES, S. (1962). Cell growth and mitosis in *Physarum polycephalum*. I. The effect of an increased nutrient supply upon the onset of mitosis. *Exp. Cell Res.*, **26**, 205–209.
- GUTTMANN, R., & BACK, A. (1958). Effects of kinetin on cell division in *Paramecium caudatum*. *Nature (Lond.)*, **181**, 852.
- HAASE, R. (1959). Strukturbildung in der Natur und der zweite Hauptsatz der Thermodynamik. *Med. Grundlagenforsch.*, **2**, 717–746.
- HACKETT, D. P. (1955). Recent studies on plant mitochondria. *Int. Rev. Cytol.*, **4**, 143–196.
- HADEK, R., & SWIFT, H. (1962). Nuclear extrusion and intracisternal inclusions in the rabbit blastocyst. *J. Cell Biol.*, **13**, 445–451.
- HÄCKER, V. (1900). Mitosen im Gefolge amitotischer Vorgänge. *Anat. Anz.*, **17**, 9–20.
- HÄMMERLING, J. (1931). Entwicklung und Formbildungsvermögen von *Acetabularia mediterranea*. *Biol. Zbl.*, **51**, 633–647.
- (1934a). Regenerationsversuche an kernhaltigen und kernlosen Zellteilen von *Acetabularia Wettsteinii*. *Biol. Zbl.*, **54**, 650–665.
- (1934b). Über formbildende Substanzen bei *Acetabularia mediterranea*, ihre räumliche und zeitliche Verteilung und ihre Herkunft. *Arch. Entwickl.-Mech. Org.*, **131**, 1–81.
- (1943). Entwicklung und Regeneration von *Acetabularia crenulata*. *Z. Vererbungsl.*, **81**, 84–113.
- (1953). Nucleo-cytoplasmic relationships in the development of *Acetabularia*. *Int. Rev. Cytol.*, **2**, 475–498.
- (1957). Nucleus and cytoplasm in *Acerabularia*. *8 Congr. Internat. Botan. (Paris)*, **10**, 87–103.
- (1958). Über die wechselseitige Abhängigkeit von Zelle und Kern. *Z. Naturforsch.*, **13b**, 440–448.

- (1959). Spirogyra und Acetabularia. *Biol. Zbl.*, **78**, 703-709.
- HÄMMERLING, J., & STICH, H. (1956). Einbau und Ausbau von 32P im Nucleolus (nebst Bemerkungen über intra und extranukleare Proteinsynthese). *Z. Naturforsch.*, **11b**, 158-161.
- HÄMMERLING, J., CLAUSS, H., KECK, K., RICHTER, G., & WERZ, G. (1958). Growth and protein synthesis in nucleated and enucleated cells. *Exp. Cell Res. Suppl.*, **6**, 210-226.
- HÄMMERLING, J., & HÄMMERLING, CH. (1959a). Kernaktivität bei aufgehobener Photosynthese. *Planta*, **52**, 516-527.
- (1959b). Über Bildung und Ausgleich des Polaritätsgefälles bei Acetabularia. *Planta*, **53**, 522-531.
- HAEMPEL, O. (1956). Pränatale Geschlechtsvorhersage beim Menschen. *Wien. med. Wschr.*, **106**, 802-805.
- HAENEL, U. (1950). Eine Methode zur Beurteilung des Funktionszustandes von Knochenmarkszellen. *Schweiz. med. Wschr.*, **80**, 1089-1090.
- HAGER, H. (1960a). Elektronenmikroskopische Befunde zur Cytopathologie der Abbau- und Abräumvorgänge in experimentell erzeugten traumatischen Hirngewebsnekrosen. *Naturwissenschaften*, **47**, 427-428.
- (1960b). Elektronenmikroskopische Untersuchungen über die vitale Metallspeicherung im zentralnervösen Gewebe bei experimenteller chronischer Tellurvergiftung. *Arch. Psychiat. Nervenkr.*, **201**, 53-64.
- HAGUENAU, F. (1958). The ergoplasm. Its history ultrastructure and biochemistry. *Int. Rev. Cytol.*, **7**, 425-483.
- HAGUENAU, F., & LACOUR, F. (1954). Cytologie électronique de tumeurs hypophysaires expérimentales, leur appareil de Golgi. *Symp. 8 Congr. of Cell Biol.*, Leiden.
- HAGUENAU, F., & BERNHARD, W. (1955). L'appareil de Golgi dans les cellules normales et cancéreuses de vertébrés. Rappel historique et étude au microscope électronique. *Arch. Anat. micr. Morph. exp.*, **44**, 27-45.
- HALE, A. J. (1958). *The Interference microscope in biological research*. Livingstone, Edinburgh.
- (1960). The interference microscope as a cell balance. In: *New Approaches of Cell Biology*, edited by WALKER. Acad. Press, London.
- HALL, B. D., & SPIEGELMAN, S. (1961). Sequence complementarity of T₂-DNA and T₂-specific RNS. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 137-146.
- HALLER, A. V. (1757). *Elementa physiologiae corporis humani*. I. Bousquet, Lausanne.
- HALLY, A. D. (1960). Electron microscopy of the unusual Golgi apparatus within the gastric parietal cell. *J. Anat. (Lond.)*, **94**, 425-431.
- HALVORSON, H. (1953). Studies on protein and nucleic acid turnover in growing cultures of yeast. *Biochim. biophys. Acta (Amst.)*, **27**, 267-276.
- HAMBERGER, C. A., & HYDEN, H. (1945). Cytochemical changes in the cochlear ganglion caused by acoustic stimulation and trauma. *Acta oto-laryng. (Stockh.) Suppl.* **61**.
- HAMBURGER, K., & ZEUTHEN, E. (1957). Synchronous divisions in Tetrahymena pyriformis as studied in an inorganic medium. *Exp. Cell Res.*, **13**, 443-453.
- HAMERTON, J. L. (1961). Sex chromatin and human chromosomes. *Int. Rev. Cytol.*, **12**, 1-68.
- HAMPERL, H. (1954). Three group metaphases and carcinoma in situ of the cervix uteri. *Acta un. int. Cancr.*, **10**, 128-131.
- (1956). Die Morphologie der Tumoren. In: *Handbuch der allgemeinen Pathologie*, Vol. VI/3. Springer, Berlin.
- (1962). Zur Frage der Sekretion der Plasmazellen. *Klin. Wschr.*, **40**, 1-3.
- HANAOKA, M. (1954). Phase contrast microscopic studies on the fine structure of the Golgi body in plasma cells. *Acta haemat. jap.*, **17**, 421.
- (1957). Vacuoles endoplasmiques, centrosome et corps de Golgi de différentes cellules sanguines à l'état vivant. Étude en contraste de phase. *Rev. d'Hématol.*, **12**, 148-168.
- HANNAH, A. (1951). Localization and function of heterochromatin in *Drosophila melanogaster*. *Advanc. Genet.*, **4**, 87-125.
- HANSCHKE, H. J., & HOFFMEISTER, H. (1960). Die zellkernmorphologische Geschlechtsbestimmung beim Bronchialkarzinom der Frau. *Zbl. allg. Path. path. Anat.*, **101**, 99-100.
- HANSEMANN, D. v. (1890). Über asymmetrische Zellteilung in Epithelkrebsen und deren biologische Bedeutung. *Virchows Arch. path. Anat.*, **119**, 299-326.
- HANZON, V., & TOSCHI, G. (1959). Electron microscopy on microsomal fractions from rat brain. *Exp. Cell Res.*, **16**, 256-271.
- HARDING, C. V., & FELDHERR, C. (1958). Semipermeability of the nuclear membrane. *Nature (Lond.)*, **182**, 676-677.
- HARDING, C. V., & SRINIVASAN, B. D., (1961). A propagated stimulation of DNA synthesis and cell division. *Exp. Cell Res.*, **25**, 326-340.
- HARRIS, H. (1956). The relationship between the respiration and multiplication of rat connective tissue cells in vitro. *Brit. J. exp. Path.*, **37**, 512-517.

- (1959). Turnover of nuclear and cytoplasmic ribonucleic acids in two types of animal cells with some further observations of the nucleolus. *Biochem. J.*, **73**, 362-369.
- (1961). Formation of the nucleolus in animal cells. *Nature (Lond.)*, **190**, 1077-1078.
- HARRIS, P. (1961). Electron microscope study of mitosis in sea urchin blastomeres. *J. biophys. biochem. Cytol.*, **11**, 419-431.
- (1962). Some structural and functional aspects of the mitotic apparatus in sea urchin embryos. *J. Cell Biol.*, **14**, 475-487.
- HARRIS, P., & JAMES, T. W. (1952). Electron microscope study of the nuclear membrane of Amoeba proteus in thin sections. *Experientia (Basel)*, **8**, 384-385.
- HARTMANN, M. (1909). Polyenergide Kerne. Studien über multiple Kernteilungen und generative Chromosomen bei Protozoen. *Biol. Zbl.*, **29**, 481-487, 491-506.
- (1953). *Allgemeine Biologie*, 4th edition. Fischer, Stuttgart.
- HARTMANN, O. (1918a). Über den Einfluß der Temperatur auf Größe und Beschaffenheit von Zelle und Kern im Zusammenhang mit der Beeinflussung von Funktion, Wachstum und Differenzierung der Zellen und Organe. *Arch. Entwickl.-Mech. Org.*, **44**, 114-195.
- (1918b). Experimentelle Untersuchungen über den Einfluß höherer Temperatur auf Morphologie und Zytologie der Algen. *Arch. Entwickl.-Mech. Org.*, **44**, 589-642.
- (1919). Über den Einfluß der Temperatur auf Plasma, Kern und Nukleolus und zytologische Gleichgewichtszustände (Zellphysiologische Experimente an Pflanzen). *Arch. Zellforsch.*, **15**, 177-248.
- HARTREY, A. D., & SILK, M. H. (1959). Mitochondria of the Ehrlich ascites tumour cell-swelling characteristics. *Brit. J. Cancer*, **13**, 566-576.
- HARVEN, E. DE, & BERNHARD, W. (1956). Étude au microscope électronique de l'ultrastructure du centriole chez les vertébrés. *Z. Zellforsch.*, **45**, 378-398.
- HARVEY, E. B. (1932). The development of half and quarter eggs of Arbacia punctulata and of strongly centrifugated whole eggs. *Biol. Bull.*, **62**, 155-167.
- (1934). Effects of centrifugal force on the ectoplasmic layer and nuclei of fertilized sea urchin eggs. *Biol. Bull.*, **66**, 228-245.
- (1936). Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata. *Biol. Bull.*, **71**, 101-122.
- HARVEY, E. B., cit. nach NOVIKOFF, A. B. (1953). *J. Histochem.*, **1**, 265.
- HARVEY, E. B. (1960). Cleavage with nucleus intact in sea urchin eggs. *Biol. Bull.*, **119**, 87-89.
- HASE, E., MORIMURA, Y., & TAMIYA, H. (1957). Some data on the growth physiology of Chlorella studied by the technique of synchronous culture. *Arch. Biochem. Biophys.*, **69**, 149-165.
- HASE, E., MIHARA, S., OTSUKA, H., & TAMIYA, H. (1959). Sulfur-containing peptide-nucleotide complex isolated from Chlorella and yeast cells. *Arch. Biochem. Biophys.*, **83**, 170-177.
- HASHIMOTO, T., CONTI, S. F., & NAYLOR, H. B. (1958). Nuclear changes occurring during bud-formation in *Saccharomyces cerevisiae* as revealed by ultra-thin sectioning. *Nature (Lond.)*, **182**, 454.
- HASHIMOTO, T., GERHARDT, P., CONTI, S. F., & NAYLOR, H. B. (1960). Studies on the fine structure of microorganisms. I. Morphogenesis of nuclear and membrane structures during ascospore formation in yeast. *J. biophys. biochem. Cytol.*, **7**, 305-308.
- HASITSCHKA, G. (1956). Bildung von Chromosomusbündeln nach Art der Speicheldrüsenchromosomen, spiralierte Ruhekernchromosomen und andere Struktureigentümlichkeiten in den endopolyploidischen Riesenkernen der Antipoden von Papaver Rhoeas. *Chromosoma (Berl.)*, **8**, 87-113.
- HASITSCHKA-JENSCHKE, G. (1957). Die Entwicklung der Samenanlage von Allium ursinum mit besonderer Berücksichtigung der endopolyploidischen Kerne in Synergiden und Antipoden. *Öst. Bot. Z.*, **104**, 1-23.
- (1959). Vergleichende karyologische Untersuchungen an Antipoden. *Chromosoma (Berl.)*, **10**, 229-267.
- HAUGH, H. (1959). Die Zelldichte und ihre Bedeutung für die Hirnrinde. *Dtsch. Z. Nervenheilk.*, **178**, 648-667.
- HAUPT, W. (1959). Die Chloroplastendrehung bei Mougeotia. I. Über den quantitativen und qualitativen Lichtbedarf der Schwachlichtbewegungen. *Planta*, **53**, 484-501.
- (1960). Die Chloroplastendrehung beim Mougeotia. II. Die Induktion der Schwachlichtbewegung durch linear polarisiertes Licht. *Planta*, **55**, 465-479.
- HAUPT, W., MUGELE, F., & MÜLLER, D. (1959). Auslösung der Chloroplastendrehung bei Mougeotia durch UV-Strahlung. *Naturwissenschaften*, **46**, 409.
- HAUPT, W., & THIELE, R. (1961). Chloroplastenbewegungen bei Mesotaenium. *Planta*, **56**, 388-401.
- HAUPT, W., & SCHÖNFELD, I. (1962). Über das Wirkungsspektrum der "negativen Phototaxis" der Vaucheria-Chloroplasten. *Ber. dtsch. bot. Ges.*, **75**, 14-23.
- HAUSCHKA, T. S., & LEVAN, A. (1958). Cytologic and functional characterization of single cell clones isolated from the Krebs-2 and Ehrlich ascites tumors. *J. nat. Cancer Inst.*, **21**, 77-135.
- HEAGY, F. C., & ROPER, J. A. (1952). Deoxyribonucleic acid content of haploid and diploid Aspergillus conidia. *Nature (Lond.)*, **170**, 713-714.

- HEATH, J. E. (1954). The effect of cobalt on mitosis in tissue culture. *Exp. Cell Res.*, **6**, 311-320.
- HEBER, U. (1962). Protein synthesis in chloroplasts during photosynthesis. *Nature (Lond.)*, **195**, 91-92.
- HEBERER, G. (1927). Die Idiomerie in den Furchungsmitosen von Cyclops viridis. *Z. mikr.-anat. Forsch.*, **10**, 163-206.
- HEILBERG, K. A. (1929). Mitosemessungen im Geschwulstgewebe. *Z. Krebsforsch.*, **29**, 234-238.
- HEIDENHAIN, M. (1894). Neue Untersuchungen über die Zentralkörper und ihre Beziehungen zum Kern- und Zellenprotoplasma. *Arch. mikr. Anat.*, **43**, 423-758.
- (1897). Neue Erläuterungen zum Spannungsgesetz der zentrierten Systeme. *Morphol. Arb.*, **7**, 281-365.
- (1907). *Plasma und Zelle*. Fischer, Jena.
- (1923). *Formen und Kräfte in der lebendigen Natur. Vorträge und Aufsätze über Entwicklungsmechanik*. Springer, Berlin.
- HEILBRUNN, L. V. (1920). An experimental study of cell division. *J. exp. Zool.*, **30**, 211-237.
- (1928). *The colloid chemistry of protoplasm*. Borntraeger, Berlin.
- (1956). *The dynamics of living protoplasma*. Acad. Press. New York.
- HEILMEYER, L., MERKER, H., MÖLBERT, E., & NEIDEHARDT, M. (1962). Zur Mikromorphologie der hereditären hypochromen sideroachrestischen Anämie. *Acta haemat. (Basel)*, **27**, 78-95.
- HEINZ, E. (1961). Aktiver Transport von Aminosäuren. *Colloquium ges. physiol. Chem. (Mosbach)*, **12**, 167-185.
- HEINZEL, W. (1960). Über die tubuläre Polyurie der Froschniere. *Verh. dtsch. path. Ges.*, **44**, 305-309.
- HEITZ, E. (1928). Das Heterochromatin der Moose. *I. Jb. wiss. Bot.*, **69**, 762-818.
- (1929). Heterochromatin, Chromocentren, Chromomeren. *Ber. Dtsch. bot. Ges.*, **47**, 274-284.
- (1931a). Die Ursache der gesetzmäßigen Zahl, Lage, Form und Größe pflanzlicher Nukleolen. *Planta*, **12**, 775-844.
- (1931b). Nukleolen und Chromosomen in der Gattung Vicia. *Planta (Berlin)*, **15**, 495-505.
- (1933a). Die Herkunft der Chromozentren. *Planta (Berlin)*, **18**, 571-636.
- (1933b). Über totale und partielle somatische Heteropyknose sowie strukturelle Geschlechtschromosomen bei Drosophila funebris (Cytologische Untersuchungen an Dipteren II). *Z. Zellforsch.*, **19**, 720-742.
- (1934). Über α - und β -Heterochromatin sowie Konstanz und Bau der Chromomeren bei Drosophila. *Biol. Zbl.*, **54**, 588-609.
- (1935). Chromosomenstruktur und Gene. *Z. indukt. Abstamm.- u. Vererb.-L.*, **70**, 402-447.
- (1936). Gerichtete Chlorophyllscheiben als strukturelle Assimilationseinheiten der Chloroplasten. *Ber. dtsch. bot. Ges.*, **54**, 362-368.
- (1956). Die Chromosomenstruktur im Kern während der Kernteilung und der Entwicklung des Organismus. *Conference on Chromosomes*. Tjeenk Willink. Zwolle.
- (1960). Das Gemeinsame im lamellaren Muster der Chloroplasten mit und ohne Grana. *Z. Naturforsch.*, **15b**, 755.
- (1961). Das lamellare Dünn-Dick-Muster der Chloroplasten von Chlamydomonas, Euglena, Fucus, Vaucheria. *Z. Zellforsch.*, **53**, 444-448.
- HEITZ, E., & BAUER, H. (1933). Beweise für die Chromosomenstruktur der Kernschleifen in den Knäuelkernen von Bibio hortulanus L. (Cytologische Untersuchungen an Dipteren, I.). *Z. Zellforsch.*, **17**, 67-82.
- HELLMANN, B., & HELLERSTRÖM, C. (1959). Diurnal changes in the function of the pancreatic islets of rats as indicated by nuclear size in the islet cells. *Acta endocr.*, **31**, 267-281.
- HELMKE, K. (1939). Über den Zellkollaps. *Virchows Arch. path. Anat.*, **304**, 255-270.
- HELWEG-LARSEN, H. F. (1952). Nuclear class series. *Acta path. microbiol. scand. Suppl.*, **42**, 1-139.
- HENDLER, R. W., DALTON, A. J., & GLENNER, G. G. (1957). A cytological study of the albumin-secreting cells of the hen oviduct. *J. biophys. biochem. Cytol.*, **3**, 325-330.
- HENKE, K., & POHLEY, H. J. (1952). Differentielle Zellteilungen und Polyploidie bei der Schuppenbildung der Mehlmotte Ephestia kühniella Z. *Z. Naturforsch.*, **7b**, 65-79.
- HENLEY, C. (1950). Cytological abnormalities produced by experimental temperature shock on Triturus torosus embryos. *Biol. Bull.*, **98**, 1-18.
- HENNEGUY, L. F. (1898). Sur les rapports des cils vibratiles avec les centrosomes. *Arch. de l'anat. microscop.*, **1**, 481-496.
- HERMAN, L., & FITZGERALD, P. J. (1961). The fine structure of the Golgi body following thyroid stimulation and pancreatic regeneration. *Trans. N. Y. Acad. Sci.*, Ser. 2, **23**, 332-345.
- HERMANN, F. (1891). Beitrag zur Lehre von der Entstehung der karyokinetischen Spindel. *Arch. mikr. Anat.*, **37**, 569-586.
- HERS, H. G., BERTHET, J., BERTHET, L., & DUVE, C. DE (1951). Le système hexose phosphatasique. III, Localisation intercellulaire des ferment, par centrifugation fractionnée. *Bull. Soc. Chim. biol. (Paris)*, **33**, 21-41.

- HERSHEY, A. D. (1955). An upper limit to the protein content of the germinal substance of bacteriophage T2. *Virology*, **1**, 108-127.
- HERSHEY, A. D., & CHASE, M. (1952). Independent functions of viral protein and nucleic acid in growth of bacteriophage. *J. gen. Physiol.*, **36**, 39-56.
- HERSHEY, A. D., & BURGH, E. (1956). Genetic significance of the transfer of nucleic acid from parental to offspring phage. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 91-101.
- HERTL, M. (1957). Zum Nucleolus-Problem. *Z. Zellforsch.*, **46**, 18-51.
- HERTWIG, G. (1929). Allgemeine mikroskopische Anatomie und Organisation der lebenden Masse. In: *Handbuch der mikroskopischen Anatomie des Menschen*, Vol. I/1. Springer, Berlin.
- HERTWIG, O. (1876). Beiträge zur Kenntnis der Bildung, Befruchtung und Teilung des tierischen Eies. I. *Morph. Hb.*, **1**, 347-432.
- (1877). Beiträge zur Kenntnis der Bildung, Befruchtung und Teilung des tierischen Eies. II. *Morph. Jb.*, **3**, 1-86.
- (1893). *Die Zelle und die Gewebe*. Fischer, Jena.
- HERTWIG, R. (1903a). *Über das Wechselverhältnis von Kern und Protoplasma*. München.
- (1903b). Über Korrelation von Zell- und Kerngröße und ihre Bedeutung für die geschlechtliche Differenzierung und die Teilung der Zelle. *Biol. Zbl.*, **23**, 49-62, 108-119.
- HETT, J. (1937). Weitere Befunde über den Austritt von Kernsubstanzen in das Protoplasma. *Z. Zellforsch.*, **26**, 473-480.
- HEWSON, W. (1777). *Experimental enquiries. Part the third*. Johnson, London.
- HIBBARD, H. (1945). Current status of our knowledge of the Golgi apparatus in the animal cell. *Quart. Rev. Biol.*, **20**, 1-19.
- HIEZL, H. A. (1959). *Die zellkernmorphologische Geschlechtserkennung in Theorie und Praxis*. Hüthig, Heidelberg.
- HIGHKIN, H. R. (1950). Chlorophyll studies on barley mutants. *Plant Physiol.*, **25**, 294-306.
- HILD, W. (1959). Das Neuron. In: *Handbuch der mikroskopischen Anatomie des Menschen*, Vol. IV/4. Springer, Berlin.
- HILL, R., & WITTINGHAM, C. P. (1957). *Photosynthesis*. Methuen, London.
- HIMES, M., HOFFMAN, J., POLLISTER, A. W., & POST, J. (1957). Origin of polyploid nuclei in rat livers during regeneration following carbon tetrachloride poisoning. *J. Mt. Sinai Hosp.*, **24**, 935-938.
- HINRICHSEN, K., & GOTHE, H.-D. (1958). Morphologische und statistische Untersuchungen an Zellkernen von Ratten und Mäusen zur Frage einer cytologischen Geschlechtsdiagnostik. *Z. Zellforsch.*, **48**, 429-449.
- HINTON, T. (1942). A comparative study of certain heterochromatin regions in the mitotic and salivary gland chromosomes of *Drosophila melanogaster*. *Genetics*, **27**, 119-127.
- HINTZSCHE, E. (1945). Statistische Probleme aus der Kerngrößenforschung. *Experientia (Basel)*, **1**, 103-110.
- (1946). Polyploidie und Amitose in Geweben von Säugetieren (mit Demonstrationen). *Arch. Klaus-Stift. Vererb. Forsch.*, **21**, 299-303.
- HINTZSCHE, E., & TANNER, E. (1937). Über Beziehungen zwischen Nahrungsaufnahme und Kerngröße des Darmepithels. *Z. mikr.-anat. Forsch.*, **42**, 165-192.
- HIRAMOTO, Y. (1956). Cell division without mitotic apparatus in sea urchin eggs. *Exp. Cell Res.*, **11**, 630-636.
- (1958). A quantitative description of protoplasmic movement during cleavage in the sea-urchin egg. *J. exp. Biol.*, **35**, 407-424.
- HIRD, F. R. J., & ROSWELL, E. V. (1950). Additional transaminations by insoluble particle preparations of rat liver. *Nature (Lond.)*, **166**, 517-518.
- HIRSCH, G. C. (1939). *Form- und Stoffwechsel der Golgi-Körper*. Borntraeger, Berlin.
- (1948). Dynamik der Sekretionssysteme. *Verh. dtsch. Ges. Zool.*, 226-232.
- (1955). Allgemeine Stoffwechselmorphologie des Cytoplasmas. In: *Handbuch der allgemeinen Pathologie*, Vol. II/1. Springer, Berlin.
- (1960). Die Fließbandarbeit in der exokrinen Pankreaszelle bei der Produktion von Enzymen. Mit einem Exkurs über Ergastoplasma und Golgi-Körper. *Naturwissenschaften*, **47**, 25-35.
- HIRSCH, J. G., & COHN, Z. A. (1960). Degranulation of polymorphonuclear leucocytes following phagocytosis of microorganisms. *J. exp. Med.*, **112**, 1005-1014.
- HIRSCHLER, J. (1918). Über den Golgischen Apparat embryonaler Zellen. Untersuchungen an Embryonen von *Limnaeus stagnalis* L. *Mollusca*. *Arch. mikr. Anat.*, **91**, 140-181.
- (1935). Über eine Reihe von auf ihre fusionale Natur verdächtiger Zelleinrichtungen. *Erg. Fortschr. Zool.*, **8**, 329-414.
- (1942). Osmiumschwärzung perichromosomal Membranen in den Spermatocyten der Rhyncho-ten-Art *Palomena viridissima* Poder. *Naturwissenschaften*, **30**, 105-106.
- HIRSHFIELD, H. I. (1959). Nuclear control of cytoplasmic activities. *N. Y. Acad. Sci.*, **78**, 647-654.

- HIRSHFIELD, H. I., TULCHIN, N., & FONG, B. A. (1960). Regeneration and cell division in two protozoan species. *Ann. N. Y. Acad. Sci.*, **90**, 523-528.
- HOAGLAND, M. B., KELLER, E. B., & ZAMECNIC, P. C. (1956). Enzymatic carboxyl activation of amino acids. *J. biol. Chem.*, **218**, 345-358.
- HOAGLAND, M. B., ZAMECNIC, P. C., & STEPHENSON, M. L. (1957). Intermediate reactions in protein biosynthesis. *Biochim. biophys. Acta (Amst.)*, **24**, 215-216.
- HOBIK, H. P., & GRUNDMANN, E. (1962). Quantitative Veränderungen der DNS und der RNS in der Rattenleberzelle während der Carcinogenese durch Diäthylnitrosamin. *Beitr. path. Anat.*, **127**, 25-48.
- HODGE, A. J. (1956). Effects of the physical environment on some lipoprotein layer systems and observations on their morphogenesis. *J. biophys. biochem. Cytol.*, **2**, Suppl., 221-228.
- HODGE, A. J., MCLEAN, J. D., & MERCER, F. V. (1956). A possible mechanism for the morphogenesis of lamellar systems in plant cells. *J. biophys. biochem. Cytol.*, **2**, 597-608.
- HÖHN, K. (1961). Erscheinungsform und Ursprung des Lebens. *Naturewiss. Rdsch.*, **14**, 251-261.
- HÖPKER, W. (1953). Über den Nukleolus der Nervenzelle. *Z. Zellforsch.*, **38**, 218-229.
- HOFF-JØRGENSEN, E., & ZEUTHEN, E. (1952). Evidence of cytoplasmic deoxyribosides in the frog's egg. *Nature (Lond.)*, **169**, 245-246.
- HOFFMAN, H., & GRIGG, G. W. (1958). An electron microscope study of mitochondria formation. *Exp. Cell Res.*, **15**, 118-131.
- HOFFMANN-BERLING, H. (1954a). Die Bedeutung des Adenosintriphosphat für die Zell- und Kern teilungsbewegungen in der Anaphase. *Biochim. biophys. Acta (Amst.)*, **15**, 226-236.
- (1954b). Die glycerin-wasserextrahierte Telophasezelle als Modell der Zytokinese. *Biochim. biophys. Acta (Amst.)*, **15**, 332-339.
- (1955). Zellmodelle und die beiden Hauptmechanismen der Zellbewegung. *Ber. ges. Physiol.*, **172**, 149-150.
- (1961). Über die verschiedenen molekularen Mechanismen der Bewegungen von Zellen. *Erg. Physiol.*, **51**, 98-130.
- HOFMEIER, G., & GRUNDMANN, E. (1962). Interferenzmikroskopische Trockenmassenbestimmungen an Rattenleberzellkernen nach partieller Hepatektomie. *Beitr. path. Anat.*, **126**, 413-425.
- HOGEBOOM, G. H., & SCHNEIDER, W. C. (1952). Intracellular distribution of enzymes. XI. Glutamic dehydrogenase. *J. biol. Chem.*, **204**, 233-238.
- HOGEBOOM, G. H., SCHNEIDER, W. C., & STRIEBICH, M. J. (1952). Cytochemical studies. V. On the isolation and biochemical properties of liver cell nuclei. *J. biol. Chem.*, **196**, 111-120.
- HOGEBOOM, G. H., & SCHNEIDER, W. C. (1953). Intracellular distribution of enzymes. XI. Glutamic dehydrogenase. *J. biol. Chem.*, **204**, 233-238.
- HOGEBOOM, G. H., KUFF, E. L., & SCHNEIDER, W. C. (1957). Recent approaches to the cytochemical study of mammalian tissues. *Int. Rev. Cytol.*, **6**, 425-467.
- HOGNESS, D. S., COHN, M., & MONOD, J. (1955). Studies on the induced synthesis of β -galactosidase in *Escherichia coli*: The kinetics and mechanism of sulfur incorporation. *Biochim. biophys. Acta (Amst.)*, **16**, 99-116.
- HOHL, K. (1949). *Experimentelle Untersuchungen über Röntgeneffekte und chemische Effekte auf die pflanzliche Mitose*. Thieme, Stuttgart.
- HOLLEY, R. W. (1957). An alanine-dependent, ribonuclease-inhibited conversion of AMP to ATP, and its possible relationship to protein synthesis. *J. Amer. chem. Soc.*, **79**, 658-662.
- HOLLMANN, K.-H. (1959). L'ultrastructure de la glande mammaire normale de la souris en lactation. Étude au microscope électronique. *J. Ultrastruct. Res.*, **2**, 423-443.
- HOLMGREN, E. (1899). Zur Kenntnis der Spinalganglienzellen von *Lophius piscatorius Lin.* Bergmann, Wiesbaden.
- HOLT, C. M. (1917). Multiple complexes in the salivary tract of *Culex pipiens*. *J. Morph.*, **29**, 607-618.
- HOLT, S. J., & HICKS, R. M. (1961). The localization of acid phosphatase in rat liver cells as revealed by combined cytochemical staining and electron microscopy. *J. biophys. biochem. Cytol.*, **11**, 47-66.
- HOLTER, H. (1959). Pinocytosis. *Int. Rev. Cytol.*, **8**, 481-504.
- (1961). Pinocytosis. *Proc. I. IUB/IUBS*, Vol. I. Acad Press, London.
- HOLTFRETER, J. (1954). Observations on the physico-chemical properties of isolated nuclei. *Exp. Cell Res.*, **7**, 95-102.
- HOLTZER, L., ABBOT, J., & CAVANAUGH, M. W. (1959). Some properties of embryonic cardiac myoblasts. *Exp. Cell Res.*, **16**, 595-601.
- HOLZER, H., & KRÖGER, H. (1958a). Zum Mechanismus der Wirkung von B 518 (Endoxan-Asta) auf das Jensen-Sarkom und zur Hemmung der Chemotherapie durch das Vitamin Nicotinsäureamid. *Klin. Wschr.*, **36**, 677-678.
- (1958b). Zum carcinostatischen Wirkungsmechanismus von Athylenimin-Verbindungen. *Biochem. Z.*, **330**, 579-590.
- HOMANN, W. (1952). Zur Biologie des Mäuseascitescarcinoms. I. Über die Morphologie der Zellteilungsvorgänge beim Mäuseascitescarcinom. *Z. Krebsforsch.*, **58**, 511-523.

- (1954). Endomitotische Teilungsvorgänge in bösartigen Geschwülsten. *Ärztl. Forsch.*, **8**, 161-164.
 — (1955). Die Amitose als Zellteilungsform in bösartigen Geschwülsten. *Z. Krebsforsch.*, **60**, 283-290.
 HOKE, R. (1665). *Micrographia*. Martyn and Allestry, London.
 HORNING, E. S. (1951). Micro-incineration and the inorganic constituents of cells. In: *Cytology and Cell physiology*, 2nd edition, edited by BOURNE. Oxford Univ. Press.
 HOROWITZ, N. H., & URS, L. (1951). Some recent studies on the one-gene-one-enzyme hypothesis. *Cold Spr. Harb. Symp. quant. Biol.*, **16**, 65-74.
 HORSTMANN, E. (1961). Elektronenmikroskopische Untersuchungen zur Spermiohistogenese beim Menschen. *Z. Zellforsch.*, **54**, 68-89.
 HORSTMANN, E., & KNOOP, A. (1957). Zur Struktur des Nucleolus und des Kernes. *Z. Zellforsch.*, **46**, 100-107.
 HORT, W. (1953). Quantitative histologische Untersuchungen an wachsenden Herzen. *Virchows Arch. path. Anat.*, **323**, 223-242.
 HOSHINO, M. (1961). The deep invagination of the inner nuclear membrane into the nucleoplasm in the ascites hepatoma cells. *Exp. Cell Res.*, **24**, 606-609.
 HOWARD, A., & PELC, S. R. (1951). Nuclear incorporation of P^{32} as demonstrated by autoradiographs. *Exp. Cell Res.*, **2**, 178-187.
 HOWARD, A., & DEWEY, D. L. (1960). Variation in the period preceding deoxyribonucleic acid synthesis in bean root cells. In: *The Cell Nucleus*. Butterworths, London.
 HOWATSON, A. F., & HAM, A. W. (1955). Electron microscope study of sections of two rat liver tumors. *Cancer Res.*, **15**, 62-69.
 — (1957). The fine structure of cells. *Canad. J. Biochem.*, **35**, 549-564.
 HSU, T. C. (1955). Mammalian chromosomes in vitro. VI. Observations on mitosis with phase cinematography. *J. Nat. Cancer Inst. (Bethesda)*, **16**, 691-707.
 — (1955). Observations on mitosis and chromosomes. *Tex. Rep. Biol. Med.*, **12**, 833-846.
 — (1962). Differential rate in RNA synthesis between euchromatin and heterochromatin. *Exp. Cell Res.*, **27**, 332-334.
 HSU, T. C., & MOORHEAD, P. S. (1956). Chromosome anomalies in human neoplasms with special reference to the mechanisms of polyploidization and aneuploidization in the HeLa strain. *Ann. N. Y. Acad. Sci.*, **63**, 1083-1094.
 HSU, T. C., & SOMERS, C. E. (1961). Effect of 5-bromodeoxyuridine in mammalian chromosomes. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 396-403.
 HUANS, R. C., & MAHESHWARI, N. (1961). RNA synthesis by an enzyme preparation isolated from young pea seedlings. *Fed. Proc.*, **20** I, 362.
 HUBER, P. (1945). Histophysiological Untersuchungen am Dickdarmepithel der weißen Maus mit Delphinin. *Viertelj.-Schr. Naturforsch. Ges. Zürich*, Beih. 4, 90, 1-88.
 HUDSON, G., & HARTMANN, J. F. (1961). The relationship between dense bodies and mitochondria in motor neurones. *Z. Zellforsch.*, **54**, 147-157.
 HUDSON, G., LAZAROW, A., & HARTMANN, J. F. (1961). A quantitative electron microscopic study of mitochondria in motor neurones following axonal section. *Exp. Cell Res.*, **24**, 440-456.
 HÜBNER, G., & BERNHARD, W. (1961). Das submikroskopische Bild der Leberzelle nach temporärer Durchblutungsperre. *Beitr. path. Anat.*, **125**, 1-30.
 HUECK, W. (1926). Die Synthesiologie von Martin Heidenhain als Versuch einer allgemeinen Theorie der Organisation. *Naturwissenschaften*, **14**, 149-157.
 HUETTNER, A. F. (1933). Continuity of the centrioles in *Drosophila melanogaster*. *Z. Zellforsch.*, **19**, 119-134.
 HUETTNER, A. F., & RABINOWITZ, M. (1933). Demonstration of the central body in the living cell. *Science*, **78**, 367-368.
 HUGHES, A. F. (1952). *The Mitotic cycle*. Butterworth, London.
 HUGHES, A. F., & SWANN, M. M. (1948). Anaphase movements in the living cell. A study with phase contrast and polarised light on chick tissue cultures. *J. exp. Biol.*, **25**, 45-70.
 HUGHES, A. F., & PRESTON, M. M. (1949). Mitosis in living cell of amphibian tissue cultures. *J. roy. micr. Soc.*, **69**, 121-131.
 HUGHES, W. L. (1959). The metabolic stability of deoxyribonucleic acid. In: *The kinetics of cellular proliferation*. Grune & Stratton, New York.
 HUGHES-SCHRADER, S. (1924). Reproduction in *Acroschismus wheeleri* Pierce. *J. Morph.*, **39**, 157-205.
 — (1931). A study of the chromosome cycle and the meiotic division-figure in *Llaveia bouvari*—a primitive coccid. *Z. Zellforsch.*, **13**, 742-770.
 — (1942). The chromosomes of *Nautococcus schraderae* Vays., and the meiotic division figure of male *Llaveiina* coccids. *J. Morph.*, **70**, 261-299.
 — (1943). Polarization, kinetochore movements, and bivalent structure in the meiosis of male mantids. *Biol. Bull.*, **85**, 265-300.

- (1947). The "pre-metaphase stretch" and kinetochore orientation in phasmids. *Chromosoma (Berl.)*, **3**, 1-21.
- (1955). The chromosomes of the giant scale *Apidoproctus maximus* Lounsbury (Coccoidea marginaridae) with special reference to synapsis and sperm formation. *Chromosoma (Berl.)*, **7**, 420-438.
- (1959). On the cytobotany of Phasmids (Phasmatodea). *Chromosoma (Berl.)*, **10**, 268-277.
- HUGHES-SCHRADER, S., & RIS, H. (1941). The diffuse spindle attachment of coccids verified by the mitotic behaviour of induced chromosome fragments. *J. exp. Zool.*, **87**, 429-456.
- HUGHES-SCHRADER, S., & SCHRADER, F. (1961). The kinetochore of the Hemiptera. *Chromosoma (Berl.)*, **12**, 327-350.
- HUNGERFELD, D. A. (1961). Chromosome studies in human leukemia. I. Acute leukemia in children. *J. nat. Cancer Inst.*, **27**, 983-1011.
- HUNTER, F. E., & FORD, L. (1955). Inactivation of oxidative and phosphorylative systems in mitochondria by preincubation with phosphate and other ions. *J. biol. Chem.*, **216**, 357-369.
- HUNTER, F. E., DAVIS, J., & CARLAT, L. (1956). The stability of oxidative and phosphorylative systems in mitochondria under anaerobic conditions. *Biochim. biophys. Acta (Amst.)*, **20**, 237-242.
- HUSKINS, C. L. (1941). The coiling of chromonemata. *Cold Spr. Harb. Symp. quant. Biol.*, **9**, 13-17.
- (1948). Chromosome multiplication and reduction in somatic tissues. *Nature (Lond.)*, **161**, 80-83.
- HUSKINS, C. L., & WILSON, G. B. (1938). Probable causes of the changes in direction of the major spiral in *Trillium erectum* L. *Ann. Bot.*, **2**, 281-292.
- HUSKINS, C. L., & STEINITZ, L. N. (1948). The nucleus in differentiation and development. I. and II. *J. Hered.*, **39**, 35-43, 67-77.
- HYDÉN, H. (1943). Protein metabolism in the nerve cell during growth and function. *Acta physiol. scand.*, **6**, Suppl. **17**, 1-136.
- IHM, P. (1954). Experimentelle Untersuchungen an isolierten Wurzelspitzen. *Z. Bot.*, **42**, 191-124.
- IMMERS, J. (1957). Cytochemical studies of fertilization and first mitosis of the sea urchin egg. *Exp. Cell Res.*, **12**, 145-153.
- INAMDAR, N. B. (1958). Development of polyploidy in mouse liver. *J. Morph.*, **103**, 65-86.
- INKER, G., PALKOVITZ, M., GYÁRFÁS, I., & BAJTAI, A. (1958). Über methodische Fragen der Kernvariationsstatistik. IV. Wirkung der verschiedenen Fixier- und Einbettungsmethoden auf das Kernvolumen. *Acta morph. Acad. Sci. hung.*, **8**, 233-251.
- INOUE, S. (1952). Effect of temperature on the birefringence of the mitotic spindle. *Biol. Bull.*, **103**, 316.
- (1953). Polarization optical studies of the mitotic spindle. I. The demonstration of spindle fibers in living cells. *Chromosoma (Berl.)*, **5**, 487-500.
- (1962). Effect of nerve fibers on mitotic activity in regenerating limb of adult newt, *Triturus pyrrhogaster*. *Gunma J. med. Sci.*, **10**, 192-200.
- INOUE, S., & BAJER, A. (1961). Birefringence in endosperm mitosis. *Chromosoma (Berl.)*, **12**, 48-63.
- IRVIN, J. L., & IRVIN, E. M. (1954). The interaction of a 9-amino-acridine derivative with nucleic acids and nucleoproteins. *J. biol. Chem.*, **206**, 39-49.
- ISHIKAWA, T. (1961). *Ph.D. Thesis (Botany Dept.)*, Yale University.
- ISHIZAKI, M., & KOSIN, I. L. (1960). Sex chromatin in early chick embryos. *Exp. Cell Res.*, **21**, 197-200.
- ISOLATO, A., & TEIR, H. (1953). Influence of cortisone on mitosis. II. Effects of simultaneously applied cortisone and colchicine. *Ann. Med. Exptl. et Biol. Fenniae, Helsinki*, **31**, 301-304.
- ITERSON, W. VAN, & ROBINOW, C. F. (1961). Observations with the electron microscope on the fine structure of the nuclei of two spherical bacteria. *J. biophys. biochem. Cytol.*, **9**, 171-181.
- ITO, S. (1960). The lamellar systems of cytoplasmic membranes in dividing spermatogenic cells of *Drosophila virilis*. *J. biophys. biochem. Cytol.*, **7**, 433-442.
- IVERSEN, S. (1960a). Visual observations on the localisation of DNA and nucleoli in somatic cell nuclei. *Acta anat. (Basel)*, **41**, 160-172.
- (1960b). Absorbance of Feulgen-stained somatic cell nuclei. *Nature (Lond.)*, **187**, 96.
- (1961). The Feulgen-stained somatic cell nucleus as a homogeneously absorbing object. *Nature (Lond.)*, **191**, 150-152.
- IVERSON, R. M. (1962). Passage of material containing uracil-¹⁴C between the nucleus and cytoplasm of *Amoeba proteus*. *Exp. Cell Res.*, **27**, 125-131.
- IWAMURA, T. (1955). Change of nucleic acid content in Chlorella cells during the course of their life-cycle. *J. Biochim. (Tokyo)*, **42**, 575-589.
- IWATA, J. (1940). Studies on chromosome structure. II. *Jap. J. Bot.*, **10**, 375-382.
- IZUTSU, H. (1958). Phase-contrast cinematographic studies on meiosis in Orthopteran spermatocytes. I. Development of the chromosomal spindle fibers and the kinetic behaviour of the chromosomes before metaphase. *Cytologia (Tokyo)*, **23**, 485-495.
- (1960). Phase-contrast cinematographic studies on meiosis in Orthopteran spermatocytes. II. Chromosomal movement in the first meiotic anaphase. *Cytologia (Tokyo)*, **25**, 293-304.

- JACKSON, B. (1959). Time-associated variations of mitotic activity in livers of young rats. *Anat. Rec.*, **134**, 365-378.
- JACOB, J., & SIRLIN, J. L. (1959). Cell function in the ovary of *Drosophila*. I. DNA classes in nurse cell nuclei as determined by autoradiography. *Chromosoma (Berl.)*, **10**, 210-228.
- JACOB, V. (1959). Transfer and expression of genetic information in *Escherichia coli* K 12. *Exp. Cell Res.*, Suppl. **6**, 51-68.
- JACOBI, W. (1925). Über das rhythmische Wachstum der Zellen durch Verdoppelung ihres Volumens. Beitrag X zur synthetischen Morphologie. *Arch. Entwickl.-Mech. Org.*, **106**, 124-192.
- (1926). Die Kerngrößen der männlichen Geschlechtszellen beim Säugetier in bezug auf Wachstum und Reduktion. Beitrag XI zur synthetischen Morphologie. *Z. Anat. Entwickl.-Gesch.*, **81**, 563-600.
- (1942). Die verschiedenen Arten des gesetzmäßigen Zellwachstums und ihre Beziehungen zu Zellfunktion, Umwelt, Krankheit, maligner Geschwulstbildung und innerem Bauplan. *Arch. Entwickl.-Mech. Org.*, **141**, 584-692.
- JACOBS, P. A., & STRONG, J. A. (1959). A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature (Lond.)*, **183**, 302-303.
- JACOBS, P. A., BAIKIE, A. G., COURT BROWN, W. M., MACGREGOR, T. N., MACLEAN, N., & HARDEN, D. G. (1959). Evidence for the existence of human "super female". *Lancet*, **II**, 423-425.
- JACOBSON, W., & WEBB, M. (1952). Nukleoproteine und Zellteilung. *Endeavour*, **II**, 200-207.
- JACQUEZ, J. A., & BIESELE, J. J. (1954). A study of Michel's film on meiosis in *Psophus stridulus* L. *Exp. Cell Res.*, **6**, 17-29.
- JÄRNEFELT, J. (1961). Mechanism of sodium transport in cellular membranes. *Nature (Lond.)*, **190**, 694-697.
- JAGENDORF, A. T. (1955). Purification of chloroplasts by a density technique. *Plant Physiol.*, **30**, 138-143.
- JAHN, T. L., RINALDI, R. A. (1959). Protoplasmic movement in the foraminifera. *Allogromia laticollaris*, and a theory of its mechanism. *Biol. Bull.*, **117**, 100-118.
- JAMES, J. (1960a). The "sex chromatin" and the nucleic acids. *Exp. Cell Res.*, **21**, 205-208.
- (1960b). Observations on the so-called sex chromatin. *Z. Zellforsch.*, **51**, 597-616.
- JAMESON, A. P. (1920). The chromosome cycle of Gregarines, with special reference to *Diplocystus schneideri* Kunstler. *Quart. J. micr. Sci.*, **64**, 207-266.
- JANSSENS, F. A. (1909). Supermatogénèse dans les Batraciens V. La théorie de la chismatotypie, nouvelle interpretation de cincèses de maturation. *Cellule*, **25**, 387-411.
- JENSEN, W. A., & KAVALJIAN, L. G. (1958). An analysis of cell morphology and the periodicity of division in the root tip of *Allium cepa*. *Amer. J. Bot.*, **45**, 365-372.
- JÖRGENSEN, M. (1913). Zellstudien II. Die Ei- und Nährzellen von *Piscicola*. *Arch. Zellforsch.*, **10**, 127-160.
- JOHANSEN, D. A., & FLINT, F. F. (1959). Some observations on the nucleolus of the megasporocyte of *Lilium*. *Cytologia (Tokyo)*, **24**, 19-28.
- JOHNSON, H. A. (1961). Some problems associated with the histological study of cell proliferation kinetics. *Cytologia (Tokyo)*, **26**, 32-41.
- JOHNSON, H. A., & BOND, V. P. (1961). A method of labeling tissues with triated thymidine in vitro and its use in comparing rates of cell proliferation in duct epithelium fibroadenoma and carcinoma of human breast. *Cancer (Philad.)*, **14**, 639-643.
- JOHNSON, H. H. (1931). Centrioles and other cytoplasmic components of the male germ cells of the Gryllidae. *Z. wiss. Zool.*, **140**, 115-166.
- JOHNSON, L. P. (1956). Observations on *Euglena fracta* sp. nov., with special reference to the locomotor apparatus. *Trans. Amer. Microsc. Soc.*, **75**, 271-281.
- JOHNSTON, A. W., FERGUSON-SMITH, M. A., HANDMAKER, S. D., JONES, H. W., & JONES, G. S. (1961). The triple-X-syndrome. Clinical, pathological, and chromosomal studies in three mentally retarded cases. *Brit. med. J.*, **ii**, 1146-1152.
- JOHNSTON, R. B., & BLOCH, K. (1951). Enzymatic synthesis of glutathione. *J. biol. Chem.*, **188**, 221-240.
- JONES, O. P. (1960). De novo origin of the nuclear membrane. *Nature (Lond.)*, **188**, 239-240.
- JONES, R. F., & LEWIN, R. A. (1960). The chemical nature of the flagella of *Chlamydomonas Moewusii*. *Exp. Cell Res.*, **19**, 408-410.
- JUDAH, J. D., & REES, K. R. (1959). Changes in cellular components in liver injury. *Biochem. Soc. Symp. London*, **16**, 95-100.
- JUDAH, J. D. (1960). Phosphoproteins and mitochondrial and cell water. *Nature (Lond.)*, **187**, 506-507.
- JÜHLING, L. (1954). In Vitro-Versuch über den Einfluß von Chinonen und Chinon-Äthylenium-Derivate auf Atmung und Gärung verschiedener Zellen und Gewebe. *Verh. dtsch. path. Ges.*, **38**, 349-361.
- JÜNGLING, D., & LANGENDORFF, H. (1930). Über die Wirkung verschieden hoher Röntgendifosen auf den Kernteilungsablauf bei *Vicia faba equina*. *Strahlentherapie*, **38**, 1-10.

- KABELITZ, J. H. (1958). Die Zytologie der Defensivreaktionen im menschlichen Knochenmark. *Med. Theorie u. Klinik*, Vol. VIII. Huthig, Heidelberg.
- KÄPPNER, W. (1961a). Bewegungsphysiologische Untersuchungen an der Amoibe Chaos chaos L. I. Der Einfluß des pH des Mediums auf das bewegungsphysiologische Verhalten von Chaos chaos L. *Protoplasma (Wien)*, **53**, 81–105.
- (1961b). Bewegungsphysiologische Untersuchungen an der Amoibe Chaos chaos L. II. Die Wirkung von Salyrgan, Cystein und ATP. *Protoplasma (Wien)*, **53**, 504–529.
- KAMIYA, N. (1959). Protoplasmic streaming. *Protoplasmatologia*, Vol. VIII/3a. Springer, Wien.
- (1961). Protoplasmastörung. *Protoplasma (Wien)*, **53**, 600–614.
- KAMIYA, N., & KURODA, K. (1956). Velocity distribution of the protoplasmic streaming in Nitella cells. *Bot. Mag. (Tokyo)*, **69**, 544–554.
- (1958). Studies on the velocity distribution of the protoplasmic streaming in the myxomycete plasmodium. *Protoplasma (Wien)*, **49**, 1–4.
- KANE, R. E. (1962). The mitotic apparatus: Isolation by controlled pH. *J. Cell Biol.*, **12**, 47–55.
- KANEDA, M. (1960). The structure and reorganization of the macronucleus during the binary fission of Chlamydomon pedarius. *Jap. J. Zool.*, **12**, 477–491.
- (1961). On the division of macronucleus in the living gymnosome ciliate, Chlamydomon pedarius, with special reference to the behaviors of chromonemata, nucleoli and endosome. *Cytologia (Tokyo)*, **26**, 89–104.
- (1961). Fine structure of the macronucleus of the gymnosome ciliate, Chlamydomon pedarius. *Jap. J. Genet.*, **36**, 223–234.
- KARLSON, P. (1962). Kurzes Lehrbuch der Biochemie für Mediziner und Naturwissenschaftler, 3rd edition. Thieme, Stuttgart.
- KARRER, H. E., & COX, J. (1960). Electron microscopic study of the phagocytosis process in lung. *J. biophys. biochem. Cytol.*, **7**, 357–366.
- KARSTEN, G. (1918). Über die Tagesperiode der Kern- und Zellteilungen. *Z. Bot.*, **10**, 1–20.
- KASTEN, F. H. (1960). Recent studies of the Feulgen reaction for deoxyribonucleic acid. *Biochem. Pharmacol.*, **4**, 86–98.
- KAUDEWITZ, F. (1958). *Transduktion*. 9. Coll. Ges. Physiol. Chem. Springer, Berlin.
- KAUFMAN, B. T., & KAPLAN, N. O. (1960). Mechanism of depletion of mitochondrial pyridine nucleotides. *Biochim. biophys. Acta (Amst.)*, **39**, 332–342.
- KAUFMANN, B. P. (1934). Somatic mitoses in Drosophila melanogaster. *J. Morph.*, **56**, 125–155.
- (1948). Chromosome structure in relation to the chromosome cycle. II. *Bot. Review*, **14**, 57–126.
- (1960). Varying patterns of chromosomal fine structure. In: *The Cell Nucleus*, Butterworths, London.
- KAUFMANN, B. P., & DAS, N. K. (1955). The role of ribonucleoproteins in the production of mitotic abnormalities. *Chromosoma (Heidelberg)*, **7**, 19–38.
- KAUFMANN, B. P., & DE, D. N. (1956). Fine structure of chromosomes. *J. biophys. biochem. Cytol.*, **2**, Suppl., 419–424.
- KAUFMANN, B. P., GAY, H., & McDONALD, M. R. (1960). Organizational patterns within chromosomes. *Int. Rev. Cytol.*, **9**, 77–127.
- KAUSHIVA, B. S. (1961). Structural basis of nucleolar function. *Experientia (Basel)*, **17**, 455–456.
- KAUTZ, J., & MARSH, Q. B. (1955). Fine structure of the nuclear membrane in cells from the chick embryo: On the nature of the so-called "pores" in the nuclear membrane. *Exp. Cell Res.*, **8**, 394–396.
- KAWAMATU, S. (1961). Electronmicrographs on the plastids in the root of Azolla imbricata. *Experientia (Basel)*, **17**, 313–315.
- KAWAMURA, K. (1960b). Studies on cytokinesis in neuroblasts of the grasshopper Chortophaga viridifasciata (De Geer). I. Formation and behavior of the mitotic apparatus. *Exp. Cell Res.*, **21**, 1–8.
- (1960c). Studies on cytokinesis in neuroblasts of the grasshopper, Chortophaga viridifasciata (De Geer). II. The role of mitotic apparatus in cytokinesis. *Exp. Cell Res.*, **21**, 9–18.
- KAWAMURA, K., & CARLSON, J. G. (1962). Studies on cytokinesis in neuroblasts of the grasshopper, Chortophaga viridifasciata (De Geer). III. Factors determining the location of the cleavage furrow. *Exp. Cell Res.*, **26**, 411–423.
- KAWAMURA, N. (1957). Studies on mitotic apparatus and cytokinesis of spermatocytes of nine species of grasshopper. *Cytologia (Tokyo)*, **22**, 337–345.
- (1960a). Cytochemical and quantitative study of protein-bound sulphydryl and disulfide groups in eggs of Arbacia during the first cleavage. *Exp. Cell Res.*, **20**, 127–138.
- KAWAMURA, N., & DAN, K. (1958) A cytochemical study of the sulphydryl groups of sea urchin eggs during the first cleavage. *J. biophys. biochem. Cytol.*, **4**, 615–620.
- KAYE, J. S. (1958). Changes in the fine structure of nuclei during spermiogenesis. *J. Morph.*, **103**, 311–321.
- KELLENBERGER, E., RYTER, A., & SÉCHAUD, J. (1958). Electron microscope study of DNA-containing

- plasmas. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. *J. biophys. biochem. Cytol.*, **4**, 671-678.
- KELSELL, P. J. (1961). Non-disjunction of the sex-chromosomes in the male of *Drosophila melanogaster*. *Nature (Lond.)*, **190**, 1035-1036.
- KERCKHOVE, D. VAN DE (1960). La teneur en acide désoxyribonucléique des cellules de la granulosa et du corps jaune dans l'ovaire du rat blanc et ses modifications expérimentales. *Exp. Cell Res.*, **19**, 399-401.
- KERR, D. N. S., & MUIR, A. R. (1960). A demonstration of the structure and disposition of ferritin in the human liver cell. *J. Ultrastruct. Res.*, **3**, 313.
- KETTLER, L. H. (1954). Parenchymsschädigungen der Leber. *Ergebn. allg. Path. path. Anat.*, **37**, 1-153.
- KEVORKIAN, J., & WESSEL, W. (1959). So-called "nuclear pellets" ("Kernkugeln") of pineocytes. *Arch. Path.*, **68**, 513-524.
- KEYL, H.-G. (1958). Untersuchungen am Karyotypus von *Chironomus thummi*. II. Mitteilung. Strukturveränderungen an den Speicheldrüsen-Chromosomen nach Röntgenbestrahlung von Embryonen und Larven. *Chromosoma (Berl.)*, **9**, 441-483.
- (1960). Erhöhung der chromosomalen Replikationsrate durch Mikrosporodieninfektion in Speicheldrüsenzellen von *Chironomus*. *Naturwissenschaften*, **47**, 212.
- KIHLMAN, B. A. (1955). Studies on the effect of oxygen on chromosome breakage induced by 8-ethoxy-caffein. *Exp. Cell Res.*, **8**, 404-407.
- (1956). Factors affecting the production of chromosome aberrations by chemicals. *J. biophys. biochem. Cytol.*, **2**, 543-555.
- KIHLMAN, B. A., & LEVAN, A. (1949). The cytological effect of caffein. *Hereditas (Lund)*, **35**, 109-111.
- KIMBALL, R. F., CASPERSSON, T. O., SVENSON, G., & CARLSON, L. (1959). Quantitative cytochemical studies on *Paramecium aurelia*. I. Growth in total dry weight measured by the scanning interference microscope and x-ray absorption methods. *Exp. Cell Res.*, **17**, 160-172.
- KIMBALL, R. F., & BARKA, T. (1959). Quantitative cytochemical studies on *Paramecium aurelia*. II. Feulgen microspectrophotometry of the macronucleus during exponential growth. *Exp. Cell Res.*, **17**, 173-182.
- KIMOTO, Y. (1958a). An attachment of the salivary gland chromosomes to the nuclear membrane found in *Chironomus dorsalis*. *Cytologia (Tokyo)*, **23**, 478-484.
- (1958b). On the vesicle formation in the salivary gland chromosomes of *Chironomus*. *Kromosomo*, **34-36**, 1225-1231.
- (1959). On the vesiculation of bands in salivary gland chromosomes induced by physiological treatments. I. On the heavy bands of *Chironomus* salivary gland chromosomes. *Jap. J. Genet.*, **34**, 55-60.
- KING, T. J., & BRIGGS, R. (1956). Serial transplantation of embryonic nuclei. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 271-289.
- KIRBY-SMITH, J. S., & DANIELS, D. S. (1953). The relative effects of x-rays, gamma rays, and beta rays on chromosomal breakage in *Tradescantia*. *Genetics*, **38**, 375-388.
- KLEIN, S. (1960). The effect of low temperatures on the development of the lamellar systems in chloroplasts. *J. biophys. biochem. Cytol.*, **8**, 529-538.
- KLEINFELD, R., & CHU, E. H. Y. (1958). DNA determination of kidney cell cultures of three species of monkeys. *Cytologia (Tokyo)*, **23**, 452-459.
- KLEINFELD, R. G., & v. HAAM, E. (1959). Effect of thioacetamide on rat liver regeneration. II. Nuclear RNA in mitosis. *J. biophys. biochem. Cytol.*, **6**, 393-398.
- KLEMM, P. (1895). Desorganisationerscheinungen der Zelle. *Jahrb. wiss. Bot.*, **28**, 627-700.
- KLINEFELTER, H. F., REIFENSTEIN, E. C., & ALBRIGHT, F. (1942). Syndrome characterized by gynecomastia, aspermatogenesis, without aleydigism, and increased excretion of follicle-stimulating hormone. *J. clin. Endocr.*, **2**, 615.
- KLINGENBERG, M. (1963). Struktur und funktionelle Biochemie der Mitochondrien. II. Die funktionelle Biochemie der Mitochondrien. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- KLINGER, H. P. (1958). The fine structure of the sex chromatin body. *Exp. Cell Res.*, **14**, 207-211.
- KLINGER, H. P., & SCHWARZACHER, H. G. (1960). The sex chromatin and heterochromatic bodies in human diploid and polyploid nuclei. *J. biophys. biochem. Cytol.*, **8**, 345-364.
- KLINGMÜLLER, W. (1962). Molekulargenetik. *Naturwissenschaften*, **15**, 363-372.
- KLINGSTEDT, H. (1928). Eine Beobachtung über die Beschaffenheit der Kernmembran. *Mem. Soc. Fauna Flora Fenn.*, **4**, 32-37.
- KLUG, H. (1960). Über funktionelle Bedeutung der Feinstrukturen der exokrinen Drüsenzellen (Untersuchungen an Euplanaria). *Z. Zellforsch.*, **51**, 617-632.
- KOBURG, E. (1960). Autoradiographische Untersuchungen zum Nukleinsäurestoffwechsel einzelner Zellarten der Lunge. *Verh. disch. path. Ges.*, **44**, 160-165.
- KOBURG, E., & SCHULTZE, B. (1961). Autoradiographische Untersuchungen mit H^3 -Thymidin über

- die Dauer der DNS-Synthese, der Ruhephase und der Mitose bei proliferierenden Systemen wie den Epithelen des Darmes, des Oesophagus und der Cornea der Maus. *Verh. dtsh. path. Ges.*, **45**, 103-107.
- KOCH, G. (1961). Metabolisch und chromosomal bedingte Schwachsinnzustände. *Ärztl. Forsch.*, **15**, 1/354-I 366.
- KODANI, M., & STERN, C. (1946). An "invisible" chromosome. *Science*, **104**, 620-621.
- KÖLLIKER, A. (1844). *Entwickelungsgeschichte der Cephalopoden*. Meyer und Zeller, Zürich.
- KÖRNIGER, F. (1935). Über die direkte Teilung der Herzmuskelkerne. *Z. mikr.-anat. Forsch.*, **38**, 441-470.
- KOERNICKE, M. (1905). Über die Wirkung der Röntgen- und Radiumstrahlen auf pflanzliche Gewebe und Zellen. *Ber. dtsh. bot. Ges.*, **23**, 404-415.
- KOLB, I. (1959). Chromosomenuntersuchungen in Beziehung zur Zweikernigkeit und zur Amitose am Ehrlichschen Mäuseascitescarcinom. *Z. mikr.-anat. Forsch.*, **65**, 495-528.
- KOLLER, P. C. (1934). The movements of chromosomes within the cell and their dynamic interpretation. *Genetica*, **16**, 447-472.
- (1946). The response of Tradescantia pollen grains to radiation of different dosage-rates. *Brit. J. Radiol.*, **19**, 393-404.
- (1947). Abnormal mitosis in tumors. *Brit. J. Cancer*, **1**, 38-47.
- (1947). The experimental modification of nucleic acid systems in the cell. *Symposia Soc. exp. Biol.*, **1**, 270-290.
- KOLLER, P. C., & CASARINI, A. (1952). Comparison of cytological effects induced by x-rays and nitrogen mustard. *Brit. J. Cancer*, **6**, 173-185.
- KOLMER, W., & FLEISCHMANN, W. (1928). Beobachtungen an den Speicheldrüsen von Chironomus-Larven. *Protoplasma (Wien)*, **4**, 358-366.
- KONOPACKI, M. (1936). Le rôle de l'epithélium folliculaire et des cellules du test pendant l'oogenèse des Ascides (Clavelina lepadiformis Müll.). *C. R. Soc. Biol. (Paris)*, **122**, 139-142.
- KOPAC, M. J. (1951). Probable ultrastructures involved in cell division. *Ann. N. Y. Acad. Sci.*, **51**, 1541-1546.
- KORNBERG, A. (1960). Die biologische Synthese von Desoxyribonucleinsäure (DNS). *Angew. Chem.*, **72**, 231-236.
- KOSENOW, W. (1959). Leukocytenkernanhänge und chromosomalale Geschlechtsdiagnose. In: *Physiologie und Physiopathologie der weißen Blutzellen*, edited by BRAUNSTEINER, H. Thieme, Stuttgart.
- (1960). Neue Ergebnisse der Chromosomenforschung und ihre Bedeutung für klinische Pathologie. *Arch. Kinderheilk.*, **162**, 219-251.
- KOSENOW, W., & NIERMANN, H. (1960). Die Bedeutung der Kerngeschlechtsbestimmung bei Fertilitätsstörungen des Mannes. *Münch. med. Wschr.*, **102**, 2332-2334.
- KOSENOW, W. R., & PFEIFFER, R. A. (1962). Chromosomen-Aberrationen und ihre Bedeutung für die Klinik. *Dtsch. med. Wschr.*, **87**, 1413-1419.
- KOSLOV, V. E. (1937). Observations on the kinetochore of mitotic chromosomes. *Biologicheskiy Zhurnal*, **6**, 759-766.
- KOSSEL, A. (1882). Zur Chemie des Zellkernes. *Z. phys. Chem.*, **7**, 7-22.
- (1884). Über einen peptontartigen Bestandteil des Zellkerns. *Z. phys. Chem.*, **8**, 511-515.
- (1911). Über die chemische Beschaffenheit des Zellkerns. Nobelvortrag. *Münch. med. Wschr.*, **58**, 65-69.
- KRACHT, J. (1958). Funktionszustände des Inselzellensystems. *Verh. dtsh. path. Ges.*, **42**, 116-121.
- KRAFT, H. (1960). Über das Geschlechtschromatin am Ruhekern von Leukozyten der Säugetiere. *Blut*, **6**, 18-25.
- KRAHL, M. E. (1950). Metabolic activities and cleavage of eggs of the sea urchin, Arbacia punctulata. A review, 1932-1949. *Biol. Bull.*, **98**, 175-217.
- KRAHL, M. E., & CLOWES, G. H. A. (1936). Studies on cell metabolism and cell division. VI. Stimulation of cellular oxidation and reversible inhibition of cell division by dihalo- and trihalophenols. *J. gen. Physiol.*, **20**, 173-184.
- KRAMER, P. J. (1949). *Plant and soil water relationships*. McGraw-Hill, New York.
- (1955). Water content and water turnover in plant cells. In: *Handbuch der Pflanzenphysiologie*, Vol. I. Springer, Berlin.
- KRANTZ, H. (1947). Reaktion der Zellkerne auf Narcotica. *Z. Naturforsch.*, **2b**, 428-433.
- KRIMSKY, I., & RACKER, E. (1952). Glutathione, a prosthetic group of glyceraldehyde-3-phosphate dehydrogenase. *J. biol. Chem.*, **198**, 721-729.
- KRISZAT, G. (1954). *Die Wirkung von Adenosintriphosphat auf die Teilung und Entwicklung des Seeigeleies bei Anwendung von Hemmungsfaktoren*. Akademisk Avhandling, Stockholm.
- KROPOTKIN, P. (1958). In: Zur Entstehung des Lebens auf der Erde; Symposium, Moscow 1957; cited after A. WACKER: *Angew. Chemie*, **70**, 519-526.
- KRUEGER, W., & DIHLMANN, W. (1957). Über das Verhalten geschlechtsspezifischer Kehranhänge nach Kastration. *Klin. Wschr.*, **35**, 1047-1048.

- KRUH, J., SCHAPIRA, G., & DREYFUS, J. C. (1960). Incorporation de la (¹⁴C) valine dans les protéines des fractions subcellulaires de réticulocytes de lapin. *Biochim. biophys. Acta (Amst.)*, **39**, 157-159.
- KRUSZYNSKI, J., & OSTROWSKI, K. (1959). Golgi structure of mouse intestinal epithelium examined by refractometry and interferometry. *Exp. Cell Res.*, **16**, 358-363.
- KUDO, R. R. (1947). *Pelomyxa carolinensis* Wilson. II. Nuclear division and plasmotomy. *J. Morph.*, **80**, 93-143.
- KÜHN, A. (1955). *Vorlesungen über Entwicklungsphysiologie*. Springer, Berlin.
- KÜHNE, W. (1958). Zur Cytologie des Ascitescarcinos und des Ehrlich-Carcinoms der Maus. Morphologische und statistische Untersuchungen. *Arch. Geschw. forsch.*, **12**, 338-351.
- KUFF, E. L., HOGEBOOM, G. H., & DALTON, A. J. (1956). Centrifugal, biochemical, and electron microscopic analysis of cytoplasmic particulates in liver homogenates. *J. biophys. biochem. Cytol.*, **2**, 33-54.
- KUFF, E. L., & DALTON, A. J. (1958). Biochemical studies of isolated Golgi membranes. In: *Subcellular particles*, edited by HAYASHI, T. Ronald Press, New York.
- KULENKAMPFF, H. (1961). Der 24-Stunden-Mitoserhythmus im Spinalependym der weißen Maus und seine experimentelle Beeinflussung. *Z. Anat. Entwickl.-Gesch.*, **122**, 518-533.
- KULENKAMPFF, H., & WÜSTENFELD, E. (1954). Funktionsbedingte Veränderungen der Kerngröße von Gliazellen im Grau des Rückenmarkes der weißen Maus. *Z. Anat. Entwickl.-Gesch.*, **118**, 97-101.
- KULENKAMPFF, H., & KOLB, W. (1960). Die Tageszeit von Tierversuchen und ihre Bedeutung für karyometrische Untersuchungen. Zugleich ein Beispiel für Anwendung und Leistungsfähigkeit von Großzahlenmethodik und Häufigkeitsanalyse nach Daeves und Beckel. *Z. Anat. Entwickl.-Gesch.*, **122**, 121-136.
- KULENKAMPFF, H., & KÖHLER, G. (1961). Über geschlechtsabhängige Kerkgrößenunterschiede somatischer Zellen der weißen Maus und ihre statistische Sicherung. *Z. Anat. Entwickl.-Gesch.*, **122**, 534-538.
- KUPKA, E., & SEELICH, F. (1948). Die anaphasische Chromosomenbewegung. Ein Beitrag zur Theorie der Mitose. *Chromosoma (Berl.)*, **3**, 302-327.
- KURNICK, N. B. (1950). Methyl-green-pyronin. I. Basis of selective staining of nucleic acids. *J. gen. Physiol.*, **33**, 243-264.
- KURNICK, N. B., & FOSTER, M. (1950). Methyl green. III. Reaction with desoxyribonucleic acid, stoichiometry, and behavior of the reaction product. *J. gen. Physiol.*, **34**, 147-159.
- KUROSUMI, K. (1958). Electron microscope studies on mitosis in sea-urchin blastomeres. *Protoplasma (Wien)*, **49**, 116-139.
- (1961). Electron microscopical analysis of the secretion mechanism. *Int. Rev. Cytol.*, **11**, 1-124.
- KUROSUMI, K., YAMAGISHI, M., & SEKINE, M. (1961). Mitochondrial deformation and apocrine secretory mechanism in the rabbit submandibular organ as revealed by electron microscopy. *Z. Zellforsch.*, **55**, 297-312.
- KUWADA, Y. (1939). Chromosome structure. A critical review. *Cytologia (Tokyo)*, **10**, 213-256.
- KUWADA, Y., & SAKAMURA, T. (1927). A contribution to the colloidchemical and morphological study of chromosomes. *Protoplasma (Wien)*, **1**, 239-254.
- KUWADA, Y., SHINKE, N., & OURA, G. (1938). Artificial uncoiling of the chromonema spirals as a method of investigation of the chromosome structure. *Z. wiss. Mikr.*, **55**, 8-16.
- LA COUR, L. F., DEELEY, E. M., & CHAYEN, J. (1956). Variations in the amount of Feulgen stain in nuclei of plants grown at different temperatures. *Nature (Lond.)*, **177**, 272-273.
- LA COUR, L. F., & CHAYEN, J. (1958). A cyclic staining behaviour of the chromosomes during mitosis and meiosis. *Exp. Cell Res.*, (1958), **13**, 462-468.
- LA COUR, L. F., CHAYEN, J., & GAHAN, P. S. (1958). Evidence for lipid material in chromosomes. *Exp. Cell. Res.*, **14**, 469-485.
- LA COUR, L. F., & PELC, S. R. (1958). Effect of colchicine on the utilization of labelled thymidine during chromosomal reproduction. *Nature. (Lond.)*, **182**, 506-508.
- LACY, D., & CHALLICE, C. E. (1957). The structure of the Golgi apparatus in vertebrate cells examined by light and electron microscopy. *Symp. Soc. exp. Biol.*, **10**, 62-91.
- LAFONTAINE, J. G. (1958). Structure and mode of formation of the nucleolus in meristematic cells of *Vicia faba* and *Allium cepa*. *J. biophys. biochem. Cytol.*, **4**, 777-784.
- LAFONTAINE, J., & RIS, H. (1955). A study of lampbrush chromosomes with the electron microscope. *Rec. Genet. Soc. Amer.*, **24**, 579-580.
- LAIRD, A. K. (1953). Nuclear changes induced in rat liver cells by thioacetamide. *Arch. Biochem.*, **46**, 119-127.
- LAIRD, E. (1933). Peritoneale Resorption mit besonderer Berücksichtigung der Wirkung auf die Leber und die Resorptionswege zur Leber. *Virchows Arch. path., Anat.*, **291**, 440-460.
- LAMB, A. B. (1907). A new explanation of the mechanics of mitosis. *J. exp. Zool.*, **5**, 27-33.
- LAMPRECHT, I. (1961a). Die Feinstruktur der Plastiden von *Tradescantia albiflora* (Kth) bei Eisenmangelchlorose. I. Lichtmikroskopische Untersuchungen. *Protoplasma (Wien)*, **53**, 118-147.

- (1961b). Die Feinstruktur der Plastiden von *Tradescantia albiflora* (Kth) bei Eisenmangelchlorose. II. Elektronenmikroskopische Untersuchungen. *Protoplasma (Wien)*, **53**, 162–199.
- LANDAU, E. (1910). Einige Worte zur karyokinetischen Zellteilung. *Biol. Zbl.*, **30**, 646–650.
- LANDAU, J. V., ZIMMERMAN, A. M., & MARSLAND, D. A. (1955). The energetics of division; effects of adenosine triphosphate and related compounds on the furrowing capacity of marine eggs. *J. cell. comp. Physiol.*, **45**, 309–327.
- LANG, K., & SIEBERT, G. (1954). Die chemischen Leistungen der morphologischen Zellelemente. In: *Handbuch der physiologischen Chemie*, Vol. II/1b, edited by FLASCHENTRAEGER u. LEHNATZ. Springer, Berlin.
- LANGER, E. (1942). Kernveränderungen an Rattenlebern unter Einwirkung des cancerogenen Stoffes 4-Di-methylaminoazobenzol (Buttergelb). *Z. Krebsforsch.*, **52**, 443–454.
- LARK, C., & LARK, K. G. (1961). Studies on the mechanism by which d-amino acids block cell wall synthesis. *Biochim. Biophys. Acta (Amst.)*, **49**, 308–322.
- LASER, H. (1933). Der Stoffwechsel von Gewebekulturen und ihr Verhalten in der Anaerobiose. *Biochem. Z.*, **264**, 72–86.
- LASNITZKI, I., & WILKINSON, J. H. 1948, cited after BIESELE, J. J.: *Mitotic Poisons and the cancer problem*. Elsevier, Amsterdam.
- LASSEK, A. M. (1955). A study of the precipitating effects of basic fixing solutions. *Anat. Rec.*, **107**, 409–414.
- LAUF, P., SEEMAYER, N., & OEHLERT, W. (1962). Die Größe und der zeitliche Verlauf der RNS-Synthese in den Ehrlich-Ascites-Tumorzellen der weißen Maus. *Z. Krebsforsch.*, **64**, 490–498.
- LAUTERBORN, R. (1896). *Untersuchungen über Bau, Kernteilung und Bewegung der Diatomeen*. Engelmann, Leipzig.
- LA VELLE, A., & LA VELLE, F. W. (1958). The nucleolar apparatus and neuronal reactivity to injury during development. *J. exp. Zool.*, **137**, 285–315.
- LAWLEY, P. D. (1957). The relative reactivities of deoxyribonucleotides and the bases of DNA towards alkylating agents. *Biochim. biophys. Acta (Amst.)*, **26**, 450–451.
- LAWS, J. O. (1952). The relationship of carbohydrate metabolism and mitotic activity in the mouse. *J. exp. Biol.*, **29**, 328–335.
- LAZAROW, A., & COOPERSTEIN, S. J. (1953). Studies on the mechanism of Janus green B staining of mitochondria. I. Review of the literature. *Exp. Cell Res.*, **5**, 56–69.
- LAZARUS, S. S., & BARDEN, H. (1962). Histochemistry and electron microscopy of mitochondrial adenosinetriphosphatase. *J. Histochem. Cytochem.*, **10**, 285–293.
- LEA, D. B. (1947). *Actions of radiations on living cells*. Macmillan, New York.
- LEBLOND, C. P., & AMANO, M. (1962). Symposium: Synthetic processes in the cell nucleus. IV. Synthetic activity in the nucleolus as compared to that in the rest of the cell. *J. Histochem. Cytochem.*, **10**, 162–174.
- LEDOUX, L., LECLERC, J., & VANDERHAEGHE, F. (1954). Influence of ribonuclease on the division of amphibian eggs. *Nature (Lond.)*, **174**, 793–794.
- LEDUC, E. H., & WILSON, J. W. (1958). Injury to liver cells in carbon tetrachloride poisoning. *Arch. Path.*, **65**, 147–157.
- (1959a). An electron microscope study of intranuclear inclusions in mouse liver and hepatoma. *J. biophys. biochem. Cytol.*, **6**, 427–430.
- (1959b). A histochemical study of intranuclear inclusions in mouse liver and hepatoma. *J. Histochem. Cytochem.*, **7**, 8–16.
- LEDUC, E., & BERNHARD, W. (1960). Essais de cytochimie ultrastructurale. Action sur la chromatine. *C. R. Acad. Sci. (Paris)*, **250**, 2948–2950.
- LEDUC, E. H., & BERNHARD, W. (1961). Ultrastructural cytochemistry. Enzyme and acid hydrolysis of nucleic acids and protein. *J. biophys. biochem. Cytol.*, **10**, 437–455.
- LEEDALE, G. F. (1959a). Periodicity of mitosis and cell division in the Eugleniae. *Biol. Bull.*, **116**, 162–174.
- (1959b). Amitose in three species of Euglena. *Cytologia (Tokyo)*, **24**, 213–219.
- LEEUWENHOEK, A. v. (1674). *Phil. Trans. roy. Soc. London*, **9**, 23.
- (1679). *Phil. Trans. roy. Soc. London*, **12**, 1040.
- (1702). *Phil. Trans. roy. Soc. London*, **22**, 552.
- LEFÈVRE, P. G. (1954). The evidence for active transport of monosaccharides across the red cell membrane. *Symp. Soc. exp. Biol.*, **8**, 118–135.
- LEHMAN, I. R., BESSMAN, M. J., SIMMS, E. S., & KORNBERG, A. (1958a). Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*. *J. biol. Chem.*, **233**, 163–170.
- LEHMANN, F. E. (1952). Mikroskopische und submikroskopische Bauelemente der Zelle. *Coll. dtsh. Ges. physiol. Chem.*, **2**, 1–18.

- (1958). Der Feinbau der Organide von Amoeba proteus und seine Beeinflussung durch verschiedene Fixierstoffe. *Ergebn. Biol.*, **21**, 88–127.
- LEHMANN, F. E., WEBER, R., AEBI, H., BÄUMLER, J., & ERLENMEYER, H. (1954.) Versuche zur Kennzeichnung regenerationshemmender Lösungen von α -Aminoketonen. *Helv. physiol. pharmacol. Acta*, **12**, 147–180.
- LEHNINGER, A. L. (1960). The enzymic and morphologic organization of the mitochondria. *Pediatrics*, **26**, 466–475.
- (1961). Components of energy-coupling mechanism and mitochondrial structure. *Proc. I. IUB/ IUBS Symp.*, Vol. II. Acad. Press, London.
- LEHNINGER, A. L., RAY, B. L., & SCHNEIDER, M. (1959). The swelling of rat liver mitochondria by thyroxine and its reversal. *J. biophys. biochem. Cytol.*, **5**, 97–108.
- LEJEUNE, J., TURPIN, T., & GAUTIER, M. (1959). Le mongolisme, maladie chromosomique (trisomie). *Bull. Acad. nat. Méd. (Paris)*, **143**, 256–265.
- LEMBERG, R., & LEGGE, J. W. (1949). *Hematin compounds and bile pigments*. Intersci. Publ., New York.
- LENHOSSEK, M. v. (1898) Über Flimmerzellen. *Anat. Anz.*, **14** Erg. Heft.
- LENZ, W. (1961a). "Superfemales" (XXX-Zustand, Triplo-X-Zustand). *Dtsch. med. Wschr.*, **1267**–1268.
- (1961b). *Medizinische Genetik*. Thieme, Stuttgart.
- LEONE, V., HSU, T. C., & POMERAT, C. M. (1955). Cytological studies on HeLa, a strain of human cervical carcinoma. II. On rotatory movements of the nuclei. *Z. Zellforsch.*, **41**, 481–492.
- LEPESCHINSKAJA, O. B. (1951). Über die Entstehung von Zellen. *Sowjetwissenschaft, Beih.* **12**. Kultur und Fortschr., Berlin.
- LEPESCHKIN, W. (1924). *Kolloidchemie des Protoplasmas*. Springer, Berlin.
- LETHAM, D. S., & BOLLARD, E. G. (1961). Stimulants of cell division in developing fruits. *Nature (Lond.)*, **191**, 1119–1120.
- LETTERER, E., CAESAR, R., & VOGT, A. (1960). Studien zur elektronenoptischen und immunmorphologischen Struktur des Amyloids. *Dtsch. med. Wschr.*, **85**, 1909–1910.
- LETTRE, H. (1946). Ergebnisse und Probleme der Mitosegiftforschung. *Naturwissenschaften*, **33**, 75–86.
- (1948). Antagonisten der Mitosegifte. *Angew. Chem.*, **60**, 57.
- (1950). Über Mitosegifte. *Ergebn. Physiol.*, **46**, 379–452.
- (1951). Zellstoffwechsel und Zellteilung. *Naturwissenschaften*, **38**, 490–496.
- (1952a). Zellstoffwechsel und Zellteilung. *Z. Krebsforsch.*, **58**, 621–631.
- (1952b). Weitere Beobachtungen über die Abhängigkeit der Zellform und Bewegung von der Adenosintriphosphorsäure. *Naturwissenschaften*, **39**, 266.
- (1952c). Some investigations on cell behaviour under various conditions: A review. *Cancer Res.*, **12**, 847–860.
- (1961). Mitose und Dissoziabilität einzelner Mitoseschritte. *Fortsch. Fortschr. dtsch. Wiss.*, **35**, 39–44.
- LETTRE, H., & ALBRECHT, M. (1941). Zur Wirkung von β -Phenyläthylaminen auf in vitro gezüchtete Zellen. *Z. Physiol. Chem.*, **271**, 200–207.
- LETTRE, H., & LETTRÉ, R. (1946). Aufhebung der Wirkung von Mitosegiften durch chemische Faktoren. *Naturwissenschaften*, **33**, 283–284.
- LETTRE, H., & ALBRECHT, M. (1951). Über die Abhängigkeit der Colchicinwirkung von der Adenosintriphosphorsäure. *Naturwissenschaften*, **38**, 547–548.
- LETTRE, H., LETTRÉ, R., & RIEMENSCHNEIDER, W. (1951). Zur mitosehemmenden Wirkung des Adrenalin. *Naturwissenschaften*, **38**, 282–283.
- LETTRE, H., LETTRÉ, R., & PFLAM, CH. (1951). Über Synergisten von Mitosegiften. VII. Mittl. Weitere Alkaloide und Colchicin oder N-Methyl-Colchicanid. *Z. Physiol. Chem.*, **287**, 150–157.
- LETTRE, H., & LETTRÉ, R. (1954). Einige Beobachtungen über die experimentelle Erzeugung multipolarer Mitosen. *Z. Krebsforsch.*, **60**, 1–8.
- LETTRE, H., & SCHLEICH, A. (1955). Zur Bedeutung der Adenosintriphosphorsäure für Formkonstanz und Formänderungen von Zellen. *Protoplasma (Wien)*, **44**, 314–321.
- LETTRE, H., & SIEBS, W. (1955). Zur chromatinfreien Zytoplasmateilung. *Naturwissenschaften*, **42**, 465.
- LETTRE, H., & ENDO, H. (1956). Zur Wirkung von Kinetin und Analogen auf tierische Zellen. *Naturwissenschaften*, **43**, 84–85.
- LETTRE, H., & SIEBS, W. (1956). Zur Entstehung chromatinfreier Cytoplasmen durch Teilung. *Protoplasma (Wien)*, **46**, 523–527.
- LETTRE, H., & LETTRÉ, R. (1958). Un problème cytologique: la persistance des structures de fusée dans l'intervalle des mitoses. *Rev. Hémat.*, **13**, 337–362.
- LETTRE, H., & SIEBS, W. (1962). Abortive Mitosen. *Acta Anat. (Basel)*, **48**, 173.
- LETTRE, R. (1954). Observations on the behaviour of the nucleolus of cells in vitro. In: *Fine Structure of cells*. Symp. 8, Congr. Cell. Biol., Leiden.

- LETRÉ, R., & SIEBS, W. (1961a). Some studies of the nucleolus of cells cultivated "in vitro". *Path. et Biol.*, **9**, 819-823.
- LETRÉ, R., & SIEBS, W. (1961b). Film sur les études du nucléole de cellules cultivées in vitro. *Chemothérapie (Basel)*, **2**, 223-225.
- LEUCHTENBERGER, C. (1950). A cytochemical study of pyknotic nuclear degenerations. *Chromosoma (Berl.)*, **3**, 449-473.
- (1958). Quantitative determination of DNA in cells by Feulgen microspectrophotometry. In: *General Cytochemical Methods*, Vol. I, edited by DANIELLI, J. F. Acad. Press, New York.
- LEUCHTENBERGER, C., SCHRADER, F., WEIR, D. R., & GENTILE, D. P. (1953). The desoxyribonucleic acid (DNA) content in spermatozoa of fertile and infertile human males. *Chromosoma (Berl.)*, **6**, 61-78.
- LEUCHTENBERGER, C., LEUCHTENBERGER, R., & DAVIS, A. M. (1954). A microspectrophotometric study of the desoxyribosid nucleic acid (DNA) content in cells of normal and malignant human tissues. *Amer. J. Path.*, **30**, 65-85.
- LEUCHTENBERGER, C., & BOYER, G. S. (1957). The occurrence of intranuclear crystals in living HeLa cells infected with adenovirus. *J. biophys. biochem. Cytol.*, **3**, 323-324.
- LEVAN, A., & HAUSCHKA, T. S. (1953). Endomitotic reduplication mechanisms in ascites tumors of the mouse. *J. nat. Cancer Inst.*, **14**, 1-43.
- LEVAN, A., & HSU, T. C. (1960). The chromosomes of two cell strains from mammary carcinomas of the mouse. *Hereditas*, **46**, 231-240.
- LEVAN, A., & HSU, T. C. (1961). Repeated endoreduplication in a mouse cell. *Hereditas*, **47**, 69-71.
- LEVER, J. D. (1956). Physiologically induced changes in adrenocortical mitochondria. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 313-318.
- LEVER, J. D., & CHAPPELL, J. B. (1958). Mitochondria isolated from rat brown adipose tissue and liver. *J. biophys. biochem. Cytol.*, **4**, 287-290.
- LEVI, G. (1934). Explantation, besonders die Struktur und die biologischen Eigenschaften der in vitro gezüchteten Gewebe. *Ergebn. Anat. Entwickl.-Gesch.*, **31**, 125-707.
- (1954). *Trattato di istologia*, 4th edition. Turin.
- LEVY, H. B. (1961). Intracellular sites of poliovirus reproduction. *Virology*, **15**, 173-184.
- LEWIS, M. R. (1934). Reversible solation of the mitotic spindle of living chick embryo cells studied in vitro. *Arch. exp. Zellforsch.*, **16**, 159-166.
- LEWIS, W. H. (1927). Binucleate cells and giant cells in tissue cultures and the similarity of the latter to the giant cells of tuberculous lesions. *Tubercle (Lond.)*, **8**, 317-330.
- (1931). Pinocytosis. *Bull. Johns Hopkins Hosp.*, **49**, 17-27.
- (1937). Pinocytosis by malignant cells. *Amer. J. Cancer*, **29**, 666-679.
- (1951). Cell division with special reference to cells in tissue cultures. *Ann. N. Y. Acad. Sci.*, **51**, 1287-1294.
- LEWIS, W. H., & LEWIS, M. R. (1917). The duration of the various phases of mitosis in the mesenchyme cells of tissue cultures. *Anat. Rec.*, **13**, 359-368.
- LEYBOLD, K., & STAUDINGER, HJ. (1962). Zur Funktion der Mikrosomen. *Dtsch. med. Wschr.*, **87**, 1989-1995.
- LEYDIG, F. (1857). *Lehrbuch der Histologie des Menschen und der Tiere*. Diesterweg, Frankfurt.
- LEYON, H. (1954). The structure of chloroplasts. IV. The development and structure of the Aspidistra chloroplast. *Exp. Cell. Res.*, **7**, 265-273.
- LILLIE, R. S. (1905). On the conditions determining the dispositions of the chromatic filaments and chromosomes in mitosis. *Biol. Bull.*, **8**, 193-204.
- LIMA-DE-FARIA, A. (1950). The Feulgen test applied to centromeric chromomeres. *Hereditas*, **36**, 60-74.
- (1955). Structure, division and delimitation of the kinetochore in Tradescantia. *Hereditas*, **41**, 209-226.
- (1958). Recent advances in the study of the kinetochore. *Int. Rev. Cytol.*, **7**, 123-157.
- (1959a). Matrix and kinetochore in living material. *Hereditas*, **45**, 463-465.
- (1959b). Incorporation of tritiated thymidine into meiotic chromosomes. *Science*, **130**, 503-504.
- LIMA-DE-FARIA, A., REITALU, J., & BERGMAN, S. (1961). The pattern of DNA synthesis in the chromosomes of man. *Hereditas*, **47**, 695-704.
- LIN, M. (1955). Chromosomal control of nucleolar composition in maize. *Chromosoma (Berl.)*, **7**, 340-370.
- LINDNER, E. (1958). Der elektronenmikroskopische Nachweis von Eisen im Gewebe. *Ergebn. allg. Path. path. Anat.*, **38**, 46-91.
- LINDNER, H. (1959). Die lichtabhängige Vakuolisierung der Chloroplasten in Nikotinlösungen. I. Lichtmikroskopische Untersuchungen zur Frage der Reversibilität. *Protoplasma (Wien)*, **51**, 91-111.
- LING, G. N. (1952). The role of phosphate in the maintenance of the resting potential and selective ion accumulation in frog muscle cells. In: *Phosphorus and Metabolism*. Johns Hopkins Press, Baltimore.
- (1955). Muscle electrolytes. *Amer. J. phys. Med.*, **34**, 89.

- LINK, D. H. F. (1807). *Grundlehrn der Anatomie und Physiologie der Pflanzen*. Danckwerts, Göttingen.
- LINZBACH, A. J. (1947). Mikrometrische und histologische Analyse hypertropher menschlicher Herzen. *Virchows Arch. path. Anat.*, **314**, 534-594.
- (1955). Quantitative Biologie und Morphologie des Wachstums einschließlich Hypertrophie und Riesenzellen. In: *Handbuch der allgemeinen Pathologie*, Vol. VI, edited by BÜCHNER, F., LETTERER, E., & ROULET, F. Springer, Berlin.
- LIPP, CH. (1953). Über Kernwachstum, Endomitosen und Funktionszyklen in den trichogenen Zellen von *Cortex punctata Illig.* *Chromosoma (Berl.)*, **5**, 454-486.
- LIPP, W. (1952). Die frühe Entwicklung der Architektur des Leberparenchyms beim Meerschweinchen. *Z. mikr.-anat. Forsch.*, **58**, 289-319.
- LISON, L., & FAUTREZ-FIRLEFFYN, N. (1950). Desoxyribonucleic acid content of ovarian cells in *Artemia salina*. *Nature (Lond.)*, **166**, 610-611.
- LISON, L., & VALERI, V. (1955). Effect of hypophysectomy on the desoxyribonucleic acid content and on the volume of rat liver nuclei. *Acta endocr. (Kbh.)*, **20**, 257-267.
- (1958). On the constancy of the desoxyribose nucleic acid (DNA) in the individual nuclei. Observations on the binucleate cells of the rat liver. *Acta histochem. (Jena)*, **5**, 337-350.
- LITARDIÈRE, R. DE. (1921). Recherches sur l'élément chromosomique dans la caryocinèse somatique des filicines. *Cellule*, **31**, 255-473.
- LITTLEFIELD, J. W., KELLER, E. B., GROSS, J., & ZAMECNIK, P. C. (1955). Studies on cytoplasmic ribonucleoprotein particles from the liver of the rat. *J. biol. Chem.*, **217**, 111-123.
- LOEB, J. (1899). Warum ist die Regeneration kernloser Protoplaststücke unmöglich oder erschwert? *Arch. Entwickl.-Mech. Org.*, **8**, 689-693.
- LONG, M. E., DOKO, F., & TAYLOR, H. C., JR. (1958). Nucleoli and nucleolar ribonucleic acid in non-malignant human endometria. *Amer. J. Obstet. Gynec.*, **75**, 1002-1014.
- LONGWELL, A. C., & SVIHLA, G. (1960). Specific chromosomal control of the nucleolus and of the cytoplasm in wheat. *Exp. Cell Res.*, **20**, 294-312.
- LOONEY, W. B. (1960). The replication of desoxyribonucleic acid in hepatocytes. *Proc. nat. Acad. Sci. (Wash.)*, **46**, 690-698.
- LORBEER, G. (1934). Die Zytologie der Lebermoose mit besonderer Berücksichtigung allgemeiner Chromosomenfragen. *Abh. wiss. Bot.*, **80**, 567-818.
- LORCH, I. J., DANIELLI, J. F., & HÖRSTADIUS, S. (1953). The effect of enucleation on the development of sea urchin eggs. I. Enucleation of one cell at the 2, 4 or 8 cell stage. *Exp. Cell Res.*, **4**, 253-274.
- LOVE, R. (1957). Distribution of ribonucleic acid in tumour cells during mitosis. *Nature (Lond.)*, **180**, 1338-1339.
- (1961). Further observations on the ribonucleoproteins of mitotically dividing mammalian cells. *Exp. Cell Res.*, **22**, 193-207.
- LOVE, R., & LILES, R. H. (1959). Differentiation of nucleoproteins by inactivation of protein-bound amino groups and staining with toluidine blue and ammonium molybdate. *J. Histochem. Cytochem.*, **7**, 164-181.
- LOVE, R., & BHARADWAJ, T. P. (1959). Two types of ribonucleoprotein in the nucleolus of mammalian cells. *Nature (Lond.)*, **183**, 1453-1454.
- LOVE, R., & RABSON, A. S. (1961). Nucleoproteins in murine lymphoma cells infected with polyoma virus in vitro. *Path. et Biol.*, **9**, 694-698.
- LOVE, R., & SUSKIND, R. G. (1961). Further observations on the ribonucleoproteins of mitotically dividing mammalian cells. *Exp. Cell Res.*, **22**, 193-207.
- LOW, F. N. (1956). Mitochondrial structure. *J. biophys. biochem. Cytol. Suppl.*, **2**, 337-339.
- (1960). Electron microscopy of the lymphocyte. In: *The Lymphocyte and Lymphocytic Tissue*, edited by V. REBUCK. Hoeber, New York.
- LOW, F. N., & FREEMANN, J. A. (1958). *Electron microscopic atlas of normal and leukemic human blood*. McGraw-Hill, New York.
- LOWE, C. U., BOX, H., VENKATARAMAN, P. R., & SARKARIA, D. S. (1959). Cytophotometric study of deoxyribonucleic acid in cortisone-treated rat hepatocytes. *J. biophys. biochem. Cytol.*, **5**, 251-255.
- LUDEWIG, S. (1959). Hemosiderin. Iron extraction studies. *Proc. Soc. exp. Biol. (N. Y.)*, **100**, 299-301.
- LUDFORD, R. J. (1925). The cytology of tar tumors. *Proc. roy. Soc. B.*, **98**, 557-577.
- (1936). The action of toxic substances upon the division of normal and malignant cells in vitro and in vivo. *Arch. exp. Zellforsch.*, **18**, 411-441.
- (1954). Nuclear structure and its modifications in tumours. *J. Canc.*, **8**, 112-131.
- LUDFORD, R. J., SMILES, J., & WELCH, F. V. (1948). The study of living malignant cells by phase-contrast and ultraviolet microscopy. *J. roy. micr. Soc.*, **68**, 1-9.
- LÜERS, TH. (1955). Zur Frage eines Geschlechtsunterschiedes in den Nervenzellkernen von *Drosophila*. *Z. Naturforsch.*, **10b**, 166-168.
- (1956). Vergleichende Untersuchungen über morphologische Geschlechtsunterschiede der neutrophilen Leukocytenkerne bei Mensch und Kaninchen. *Blut*, **2**, 81-88.

- LUFT, J., & HECHTER, O. (1957). An electron microscopic correlation of structure with function in the isolated perfused cow adrenal, preliminary observations. *J. biophys. biochem. Cytol.*, **3**, 615-620.
- LUNDEGÅRDH, H. (1912). Chromosomen, Nucleolen und die Veränderungen im Protoplasma bei der Karyokinese. *Beitr. Biol. Pflanz.*, **11**, 373-542.
- (1922). Ein Beitrag zur quantitativen Analyse des Photoprismus. *Ark. Bot. (Stockh.)*, **18**, 1-62.
- LUSE, S. A. (1956). Electron microscopic observation of the central nervous system. *J. biophys. biochem. Cytol.*, **2**, 531-542.
- LUSE, S. A., & SMITH, M. G. (1958). Electron microscopy of salivary gland viruses. *J. exp. Med.*, **107**, 623-632.
- LYTTLETON, J. W. (1962). Isolation of ribosomes from spinach chloroplasts. *Exp. cell Res.*, **26**, 312-317.
- MACFARLAND (1897). Celluläre Studien an Mollusken-Eiern. *Zool. Jb., Abt. Anat.*, **10**.
- MACLEAN, N. (1962). The drumsticks of polymorphonuclear leukocytes in sex-chromosome abnormalities. *Lancet*, **i**, 1154-1158.
- MACLEAN, N., HARNDEN, D. G., & BROWN, W. M. C. (1961). Abnormalities of sex chromosome constitution in newborn babies. *Lancet*, **ii**, 406-408.
- MAKAROV, P. (1957). Über ungelöste Probleme der gegenwärtigen Zytologie. *Wiss. Z. Univ. Halle, Math.-nat. Reihe*, **6**, 549-567.
- (1960). Über den Mechanismus der Anaphasebewegung der Chromosomen. *Biol. Zbl.*, **79**, 413-422.
- MAKINO, S. (1957). The chromosome cytology of the ascites tumors of rats, with special reference to the concept of stemline cell. *Int. Rev. Cytol.*, **6**, 26-84.
- MAKINO, S., & TANAKA, T. (1953). The cytological effect of chemicals on ascites sarcomas. I. Partial damage in tumor cells by podophyllin, followed by temporary regression and prolongation of life of tumor-bearing rats. *J. nat. Cancer Inst.*, **13**, 1185-1198.
- MAKINO, S., & NAKAHARA, H. (1955). Behaviour of the mitochondria in relation to the division of the anuclear cytoplasmic bud in grasshopper spermatocytes. *Chromosoma (Berl.)*, **7**, 14-18.
- MAKINO, S., & NAKANISHI, Y. N. (1955). A quantitative study on anaphase movement of chromosomes in living grasshopper spermatocytes. *Chromosoma (Berl.)*, **7**, 439-450.
- MALHEIRO, N., DE CASTRO, D., & CAMARA, A. (1947). Chromosomas sem centrómero localizado. O caso de *Luzula purpurea* Link. *Agron. Lusitana*, **9**, 51-71.
- MALHOTRA, S. K. (1959). What is the "Golgi apparatus" in its classical site within the neurones of vertebrates? *Quart. J. micr. Sci.*, **100**, 339-367.
- MALKIN, A., & DENSTEDT, O. P. (1956). The metabolism of the erythrocyte. XI. Synthesis of diphosphopyridine nucleotide in the erythrocyte. *Canad. J. Biochem.*, **34**, 130-140.
- MALPIIGHI, M. (1675). *Anatome plantarum* (abridged translation by M. Möbus). Ostwalds Klassiker Nr. 120.
- (1687). *Opera omnia*. Vander Lugduni Batav.
- DE MAN, J. C. H., DAEMS, W. TH., WILLIGHAGEN, R. G. J., & VAN RIJSSEL, T. G. (1960). Electron-dense bodies in liver tissue of the mouse in relation to the activity of acid phosphatase. *J. Ultrastruct. Res.*, **43**, 43-57.
- MANDELSTAM, J. (1957). Turnover of protein in starved bacteria and its relationship to the induced synthesis of enzyme. *Nature (Lond.)*, **179**, 1179-1181.
- MANTON, I. (1935). Some new evidence on the physical nature of plant nuclei from intraspecific polyploids. *Proc. Roy. Soc. Med. Lond. B*, **118**, 522-547.
- MANUELIDIS, E. E. (1958). Pathological swelling and vacuolization of cells. In: *Frontiers in Cytology*, Yale Univ. Press, New Haven.
- MARBERGER, E., & NELSON, W. (1955). Geschlechtsbestimmung in der menschlichen Haut. *Bruns' Beitr. klin. Chir.*, **190**, 103-112.
- MARINESCO, M. G. (1896). Les lésions primitives et les lésions secondaires de la cellule nerveuse. *C. R. Soc. Biol. (Paris)*, **48**, 106-108.
- MARINOS, N. G. (1960). The nuclear envelope of plant cells. *J. Ultrastruct. Res.*, **3**, 328-333.
- MARKS, G. E. (1957a). The cytology of *Oxalis dispar* (Brown). *Chromosoma (Berl.)*, **8**, 650-670.
- (1957b). Telocentric chromosomes. *Amer. Naturalist*, **91**, 223-232.
- MARQUARDT, H. (1937). Neuere karyologische Probleme und Ergebnisse III. Die Röntgenpathologie der Mitose. *Z. Bot.*, **31**, 572-593.
- (1938). Die Röntgenpathologie der Mitose I und II. *Z. Bot.*, **32**, 401-480.
- (1941). Untersuchungen über den Formwechsel der Chromosomen im generativen Kern des Pollens und Pollenschläuches von *Allium* und *Lilium*. *Planta (Berl.)*, **31**, 670-725.
- (1950). Neuere Auffassungen über einige Probleme aus der Pathologie der Kernteilungen. *Naturwissenschaften*, **37**, 416-424 & 433-438.
- (1952). Über die spontanen Aberrationen in der Anaphase der Meiosis von *Paeonia tenuifolia*. *Chromosoma (Berl.)*, **5**, 81-112.

- (1957). Strahlenschädigungen des Erbgutes durch energiereiche Strahlen. *Dtsch. med. J.*, **8**, 345-350.
- (1958). Die somatischen Mutationen. *Dtsch. med. Wschr.*, **83**, 1721-1725.
- (1959). Eränderungen somatischer Zellen und ihr Anteil bei experimenteller Krebsauslösung. *Naturwissenschaften*, **46**, 217-223.
- (1962). Der Feinbau von Hefezellen im Elektronenmikroskop. I. Mitt. *Rhodotorula rubra*. *Z. Naturforsch.*, **17b**, 42-48.
- MARQUARDT, H., LIESE, W., & HASSENKAMP, G. (1956). Die elektronenoptische Feinstruktur pflanzlicher Zellkerne. *Naturwissenschaften*, **43**, 540-541.
- MARQUARDT, H., & GLÄSS, E. (1957). Die Chromosomenzahlen in den Leberzellen von Ratten verschiedenem Alters. *Chromosoma (Berl.)*, **8**, 617-636.
- MARSLAND, D. (1951). The action of hydrostatic pressure on cell division. *Ann. N. Y. Acad. Sci.*, **51**, 1327-1335.
- (1957). In: *Influence of temperature on biological systems*. Amer. Physiol. Soc., Washington.
- MARSLAND, D., ZIMMERMAN, A. M., & AUCLAIR, W. (1960). Cell division: Experimental induction of cleavage furrows in the eggs of *Arbacia punctata*. *Exper. Cell Res.*, **21**, 179-196.
- MARTENS, P. (1927). Observation vitale de la caryocinèse. *C. R. Acad. Sci. (Paris)*, **184**, 758-760.
- MARTIN, B. A. (1953). Temporary elimination of the autosomes from the meiotic spindle in a halyimid pentatomid. *J. Morph.*, **92**, 207-239.
- MARTIN, P. G. (1961). Evidence for the continuity of nucleolar material in mitosis. *Nature (Lond.)*, **190**, 1078-1079.
- MARTIUS, C. & HESS, B. (1951). Über den Wirkungsmechanismus des Schilddrüsenhormons. *Arch. exp. Path. Pharmak.*, **216**, 45-46.
- MASCHLANKA, H. (1946). Kerneinwirkungen in artgleichen und artverschiedenen Acetabularia-Transplantaten. *Biol. Zbl.*, **65**, 167-176.
- MAST, S. O. (1926). Structure, movement, locomotion, and stimulation in amoeba. *J. Morph.*, **41**, 347-425.
- (1931). Locomotion in amoeba proteus (Leidy). *Protoplasma (Wien)*, **14**, 321-330.
- MAST, S. O., & DOYLE, W. L. (1934). Ingestion of fluid by amoeba. *Protoplasma (Wien)*, **20**, 555-560.
- MATHER, K. (1944). The genetical activity of heterochromatin. *Proc. roy. Soc. B.*, **132**, 308-332.
- MATTISON, A. G. M., & BIRCH-ANDERSEN, A. (1962). On the fine structure of the mitochondria and its relation to oxidative capacity in muscles in various invertebrates. *J. Ultrastruct. Res.*, **6**, 205-228.
- MAURER, C. (1962). Kerneinschlüsse bei normal differenzierten Knochenmarkplasmazellen. *Blut*, **7**, 34-36.
- MAURER, W., & KOBURG, E. (1961). Autoradiographische Untersuchungen mit H-3-Thymidin über den zeitlichen Verlauf der DNS-Synthese bei den Epithelien des Darms und bei anderen Zellarten der Maus. *Verh. dtsch. path. Ges.*, **45**, 108-112.
- MAXIMOW, A. (1908). Über Amitose in den embryonalen Geweben bei Säugetieren. *Anat. Anz.*, **33**, 89-98.
- MEYERSBACH, H., & SCHLAGER, F. (1960). Leberzellkernreaktionen nach parenteraler Eiweißzufuhr. *Anat. Anz.*, **108**, 129-147.
- MAZIA, D. (1955). The organization of the mitotic apparatus. *Symp. Soc. exper. Biol.*, **9**, 335-357.
- (1956). The life history of the cell. *Amer. Scientist*, **44**, 1-32.
- (1960). The analysis of cell reproduction. *Ann. N. Y. Acad. Sci.*, **90**, 455-469.
- (1961a). Mitosis and the physiology of cell division. In: *The Cell*, Vol. III, Acad. Press, New York.
- (1961b). The central problems of the biochemistry of cell division. *Proc. I. IUB/IUBS Symp.*, Acad. Press, London, Vol. II.
- MAZIA, D., & HIRSHFIELD, H. (1950). The nucleus-dependence of P³² uptake by the cell. *Science*, **112**, 297-299.
- MAZIA, D., & DAN, K. (1952). The isolation and biochemical characterization of the mitotic apparatus of dividing cells. *Proc. nat. Acad. Sci. (Wash.)*, **38**, 826-838.
- MAZIA, D., & PRESCOTT, D. M. (1954). Nuclear function and mitosis. *Science*, **120**, 120-122.
- MAZIA, D., HARRIS, J., & BIBRING, T. (1960). The multiplicity of the mitotic centers and the time-course of their duplication and separation. *J. biophys. biochem. Cytol.*, **7**, 1-20.
- MCCLENDON, J. F. (1907). Experiments on the eggs of *Chaetopterus* and *Asterias* in which the chromatin was removed. *Biol. Bull.*, **12**, 141-145.
- MCCLINTOCK, B. (1934). The relation of a particular chromosomal element to the development of the nucleoli in *Zea Mays*. *Z. Zellforsch.*, **21**, 294-328.
- (1941). Spontaneous alterations in chromosome size and form in *Zea Mays*. *Cold Spr. Harb. Symp. quant. Biol.*, **9**, 72-81.
- (1942). The fusion of broken ends of chromosomes following nuclear fusion. *Proc. nat. Acad. Sci. (Wash.)*, **28**, 458-463.

- McDONALD, B. (1958). Quantitative aspects of deoxyribose nucleic acid (DNA) metabolism in an amicronucleate strain of Tetrahymena. *Biol. Bull.*, **114**, 71-94.
- (1962). Synthesis of deoxyribonucleic acid by micro- and macronuclei of Tetrahymena pyriformis. *J. Cell Biol.*, **13**, 193-203.
- MCLEISH, J. (1953). The action of maleic hydrazide in Vicia. *Heredity*, **6 Suppl.**, 125-148.
- (1960). Photometric measurements of Feulgen and Sakaguchi stain in nuclei of diploid, triploid and tetraploid plants. In: *The Cell Nucleus*. Butterworth, London.
- MCLEISH, J., & SUNDERLAND, N. (1961). Measurements of deoxyribosenucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods. *Exp. Cell Res.*, **24**, 527-540.
- MCMASTER-KAYE, R. (1962). Symposium: Synthetic processes in the cell nucleus. III. Metabolism of nuclear ribonucleic acid in salivary glands of Drosophila repleta. *J. Histochem. Cytochem.*, **10**, 154-161.
- MCINDOE, W. M., DAVIDSON, J. N. (1952). The phosphorus compounds of the cell nucleus. *Brit. J. Cancer*, **6**, 200-214.
- McMURRAY, W. C., STRICKLAND, K. P., BERRY, J. F., & ROSSITER, R. J. (1957). Incorporation of ^{32}P -labelled intermediates into the phospholipids of cell-free preparations of rat brain. *Biochem. J.*, **66**, 634-644.
- MECHELKE, F. (1952). Die Entstehung der polyploiden Zellkerne des Antherentapetums bei Antirrhinum majus L. *Chromosoma (Berl.)*, **5**, 246-295.
- (1953). Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von Acricotopus lucidus. *Chromosoma (Berl.)*, **5**, 511-543.
- (1960). Strukturmodifikationen in Speicheldrüsenchromosomen von Acricotopus als Manifestation eines Positionseffektes. *Naturwissenschaften*, **47**, 334-335.
- (1963). Spezielle Funktionszustände des genetischen Materials. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- MELANDER, Y. (1950). Studies on the chromosomes of Ulophysina öresundense. *Hereditas (Lund)*, **36**, 233-255.
- MELLORS, R. C. (1953). The quantitative analysis of the cell by interference and ultraviolet microscopy. *Tex. Rep. Biol. Med.*, **11**, 693-708.
- MELLORS, R. C., STOHLISKI, A., & BEYER, H. (1954). Quantitative cytology and cytopathology. III. Measurement of the organic mass of sets of chromosomes in germinal cells of the mouse. *Cancer*, **7**, 873-883.
- MENDEL, G. (1865). Versuche über Pflanzenhybriden. *Verh. Naturforsch. Verein, Brünn*, **4**, 3.
- MENDELSON, M. L. (1958). The two-wavelength method of microspectrophotometry. I. A microspectrophotometer and tests on model systems. *J. biophys. biochem. Cytol.*, **4**, 407-414.
- MENG, K., & POHLE, K. (1961). Die 24-Std.-Mitoserhythmie beim Ehrlich'schen Mäuse-Ascites-Carcinom. nach Ovarektomie und Adrenalektomie. *Z. Krebsforsch.*, **64**, 219-223.
- MENKE, W. (1938). Untersuchungen der einzelnen Zellorgane in Spinatblättern auf Grund präparativ-chemischer Methodik. *Z. Bot.*, **32**, 273-295.
- (1961). Über das Lamellarsystem des Chromatoplastas von Cyanophyceen. *Z. Naturforsch.*, **16b**, 543-546.
- MENKE, W., & JORDAN, E. (1959). Über das lamelläre Strukturprotein der Chloroplasten. *Naturforsch.*, **14b**, 234-240.
- MENKIN, V. (1959). Nature of factors regulating the rate of cell division. *J. exp. Zool.*, **140**, 441-470.
- MENKIN, V., MENKIN, L., & MENKIN, G. (1959). Accelerator and retarding cell division factors of one species on ova of an unrelated form. *J. exp. Zool.*, **140**, 471-491.
- MENON, M. (1962). Effect of cortisone and related compounds on growth and cell division in the chick embryo. *Z. Zellforsch.*, **56**, 619-624.
- MERCER, E. H., & WOLPERT, L. (1958). Electron microscopy of cleaving sea urchin eggs. *Exp. Cell Res.*, **14**, 629-632.
- MERKLE, U. (1961). Volumenmessungen an amitotisch geteilten Muskelkernen der menschlichen Harnblase. *Z. mikr. anat. Forsch.*, **67**, 303-312.
- MERRIAM, R. W. (1959). The origin and fate of annulate lamellae in maturing sand dollar eggs. *J. biophys. biochem. Cytol.*, **5**, 117-122.
- (1961). On the fine structure and composition of the nuclear envelope. *J. biophys. biochem. Cytol.*, **11**, 559-570.
- (1962). Some dynamic aspects of the nuclear envelope. *J. cell Biol.*, **12**, 79-90.
- MERRIAM, R. W., & KOCH, W. E. (1960). The relative concentration of solids in the nucleolus, nucleus, and cytoplasm of the developing nerve cell of the chick. *J. biophys. biochem. Cytol.*, **7**, 151-160.
- MERTENS, P. (1929). Nouvelles recherches expérimentales sur la cinèse dans les cellules vivantes. *Cellule*, **39**, 169-215.
- METZ, C. W. (1933). Monocentric mitosis with segregation of chromosomes in Sciara and its bearing on the mechanism of mitosis. *Biol. Bull.*, **64**, 333-347.

- METZ, C. W., MOSES, M. S., & HOPPE, E. N. (1926). Chromosome behaviour and genetic behaviour in *Sciaria* (Diptera). I. Chromosome behaviour in the spermatocyte divisions. *Z. indukt. Abstamm.-u. Vererb.-L.*, **42**, 237-270.
- METZNER, R. (1894). Beiträge für Granulalehre I. *Arch. Anat. Physiol.*, **309**-348.
- MEVES, F. (1897). Über die Entwicklung der männlichen Geschlechtszellen von *Salamandra maculosa*. *Arch. mikr. Anat.*, **48**, 1-83.
- (1898). Zellteilung. *Ergebn. Anat. Entwickl.-Gesch.*, **8**, 430-542.
- (1907). Über die Mitochondrien bzw. Chondriokonten in den Zellen junger Embryonen. *Anat. Anz.*, **31**, 399-407.
- (1918). Über Umwandlung von Plastosomen in Sekretkugelchen, nach Beobachtungen an Pflanzenzellen. *Arch. mikr. Anat.*, **90**, 445-462.
- MEYER, A. (1883). *Das Chlorophyllkorn in chemischer, morphologischer und biologischer Beziehung*. Felix, Leipzig.
- MEYER, G. F. (1960). The fine structure of spermatocyte nuclei of *Drosophila melanogaster*. *Proc. Eur. Reg. Conf. on Electron Microsc.*, Vol. II.
- MEYER, G. F., HESS, O., & BEERMANN, W. (1961). Phasenspezifische Funktionsstrukturen in Spermatozytenkernen von *Drosophila melanogaster* und ihre Abhängigkeit vom Y-Chromosom. *Chromosoma (Berl.)*, **12**, 676-716.
- MEYER, M. (1954). Über den Tagesrhythmus und die relative Dauer der Zellteilungen im Epithel der spätlarvalen äußeren Cornea von *Rana temporaria* L. *Z. Zellforsch.*, **40**, 228-256.
- MEYERHOF, O., & RANDALL, L. O. (1948). The inhibitory effects of adrenochrome on cell metabolism. *Arch. Biochem.*, **17**, 171-182.
- MICHAELIS, L. (1900). Die vitale Färbung, eine Darstellungsmethode der Zellgranula. *Arch. mikr. Anat.*, **55**, 558-575.
- MICHEL, K. (1943). Die Kern- und Zellteilung im Zeitrafferfilm. Die meiotischen Teilungen bei der Spermatogenese der Schnarrheuschrecke *Piophus strichulus* L. *Zeiss-Nachr.*, **4**, 236-251.
- (1950). Das Phasenkontrastverfahren und seine Eignung für cytologische Untersuchungen. *Naturwissenschaften*, **37**, 52-57.
- MICKEY, G. H. (1946). The presence of multiple strands in chromosomes of Romalea (Orthoptera). *Amer. Nat.*, **80**, 446-452.
- MIESCHER, F. (1871). Chemische Zusammensetzung der Eiterzelle. *Hoppe-Seylers med. chem. Untersuchungen*, **441**-460.
- (1897). *Die histochemischen und physiologischen Arbeiten*. Vogel, Leipzig.
- MIESCHER, P. (1959). Gegen Zellkerne gerichtete Antikörper. In: *Physiologie und Physiopathologie d. weißen Blutzellen*, edited by BRAUNSTEINER, H. Thieme, Stuttgart.
- MILES, CH. P. (1959). Sex chromatin in cultured normal and cancerous human tissues. *Cancer*, **12**, 299-305.
- MILIĆIĆ, D. (1960). Sind verschiedene Eiweißkristalle der Kakteen Viruskörper? *Acta Bot. Croatica*, **18/19**, 37-63.
- MILLER, C. O., SKOOG, F., v. SALTZA, M. H., & STRONG, F. M. (1955). Kinetin, a cell division factor from deoxyribonucleic acid. *J. Amer. chem. Soc.*, **77**, 1392.
- MILLER, C. S. (1953). Reversible inhibition of cell division and enlargement in plant tissues by 2,6-diaminopurine. *Proc. Soc. exp. Biol.*, **83**, 561-565.
- MILLER, C. S., GURIN, S., & WILSON, D. W. (1950). C^{14} -labeled 4(5)-amino-5(4)-imidazolecarboxamide in the biosynthesis of purines. *Science*, **112**, 654-655.
- (1958). Orthologie und Pathologie der Zelle im elektronenmikroskopischen Bild. *Verh. dtsch. Ges. Path.*, **42**, 261-332.
- (1960). Hemoglobin absorption by the cells of the proximal convoluted tubule in mouse kidney. *J. biophys. biochem. Cytol.*, **8**, 689-718.
- MILLER, O. J., BREG, W. R., SCHMICKEL, R. D., & TRETTER, W. (1961). A family with an XXXXY male, a leukaemic male, and two 21-trisomic mongoloid females. *Lancet*, **ii**, 78-79.
- MILLER, S. L. (1955). Production of some organic compounds under possible primitive earth conditions. *J. Amer. chem. Soc.*, **77**, 2351-2361.
- MILLER, S. L., & UREY, H. (1959). Organic compound synthesis on the primitive earth. *Science*, **130**, 245-251.
- MILLINGTON, P. F., & FINEAN, J. B. (1962). Electron microscope studies of the structure of the microvilli on principal epithelial cells of rat jejunum after treatment in hypo- and hypertonic saline. *J. cell Biol.*, **14**, 125-139.
- MILNE-EDWARDS, H. (1823). *Mémoire sur la structure élémentaire des principaux tissus des animaux*. Crochard, Paris.
- MILovidov, P. (1938). Bibliographie der Nucleal- und Plasmareaktion. *Protoplasma (Wien)*, **31**, 246-266.
- (1960). Über die Definition der Zelle. *Acta anat. (Basel)* **295**-304.

- MILovidov, P. F. (1949). *Physik und Chemie des Zellkernes*. Borntraeger, Berlin.
- MIRSKY, A. E., & POLLISTER, A. W. (1946). Chromosin, a deoxyribose nucleoprotein complex of the cell nucleus. *J. gen. Physiol.*, **30**, 117-148.
- MIRSKY, A. E., & RIS, H. (1949). Variable and constant components of chromosomes. *Nature (Lond.)*, **163**, 666-667.
- (1951). The deoxyribonucleic acid content of animal cells and its evolutionary significance. *J. gen. Physiol.*, **34**, 451-462.
- MIRSKY, A. E., OSAWA, S., & ALLFREY, V. G. (1956). The nucleus as a site of biochemical activity. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 49-73.
- MIRSKY, A. E., & OSAWA, S. (1961). The interphase nucleus. In: *The Cell*, Vol. II. Acad. Press, New York.
- MITCHELL, J. S. (1942). Disturbance of nucleic acid metabolism produced by therapeutic doses of X and gamma radiations. Part I. Methods of investigation. *Brit. J. exp. Path.*, **23**, 285-295.
- MITCHELL, J. S., & SIMON-REUSS, I. (1952). Experiments on the mechanism of action of tetra-sodium-2-methyl-1:4-naphthohydroquinone diphosphate as a mitotic inhibitor and radiosensitiser, using the technique of tissue culture: relation between cytological effects and chemical constitution. *Brit. J. Cancer*, **6**, 317-338.
- MITCHISON, J. M. (1952). Cell membranes and cell division. *Symp. Soc. exp. Biol.*, **6**, 105-127.
- (1957). The growth of single cells. I. Schizosaccharomyces pombe. *Exp. Cell Res.*, **13**, 244-262.
- MITCHISON, J. M., & SWANN, M. M. (1952). Optical changes in the membranes of the sea-urchin egg at fertilization, mitosis and cleavage. *J. exp. Biol.*, **29**, 357-362.
- (1955). The mechanical properties of the cell surface. III. The sea-urchin egg from fertilization to cleavage. *J. exp. Biol.*, **32**, 734-750.
- MITRAKOS, K. (1959). Tagesperiodische Schwankungen der Fähigkeit zur Chlorophyllbildung. *Planta (Berl.)*, **52**, 583-586.
- MIURA, T., & UTAKOJI, T. (1961). Studies on synchronous division of FL cells by chilling. *Exp. Cell Res.*, **23**, 452-459.
- MÖBIUS, M. (1920). Über die Größe der Chloroplasten: *Ber. dtsch. bot. Ges.*, **38**, 224-232.
- MÖLBERT, E. (1957a). Das elektronenmikroskopische Bild der Leberparenchymzelle nach histotoxischer Hypoxidose. *Beitr. path. Anat.*, **118**, 203-227.
- (1957b). Die Herzmuskelzelle nach akuter Oxydationshemmung im elektronenmikroskopischen Bild. *Beitr. path. Anat.*, **118**, 421-435.
- MÖLBERT, E., & GUERRITORE, D. (1957). Elektronenmikroskopische Untersuchungen am Leberparenchym bei akuter Hypoxie. *Beitr. path. Anat.*, **117**, 32-49.
- MÖLBERT, E., IJIJIMA, S. (1958). Beitrag zur experimentellen Hypertrophie und Insuffizienz des Herzmuskels im elektronenmikroskopischen Bild. *Naturwissenschaften*, **45**, 322-323.
- MÖLBERT, E., & ARNESEN, K. (1960). Elektronenmikroskopische Untersuchungen zur Ultrastruktur der Nebennierenrinde der weißen Maus. Zugleich ein Beitrag zur Struktur und Funktion der Mitochondrien. *Beitr. path. Anat.*, **122**, 31-56.
- MÖLBERT, E., DUSPIVA, F., & v. DEIMLING, O. (1960). The demonstration of alkaline phosphatase in the electron microscope. *J. biophys. biochem. Cytol.*, **7**, 387-390.
- MÖLBERT, E., HILL, K., & BÜCHNER, F. (1962). Die Kanzerisierung der Leberparenchymzelle durch Diäthylnitrosamin im elektronenmikroskopischen Bild. *Beitr. path. Anat.*, **126**, 218-242.
- v. MÖLLENDORF, M. (1929). Bindegewebsstudien VIII. Über die Potenzen der Fibrocyten des erwachsenen Bindegewebes in vitro. *Z. Zellforsch.*, **9**, 183-228.
- (1931). Beobachtungen bei der Dauerzüchtung von Bindegewebe erwachsener Kaninchen. *Z. Zellforsch.*, **12**, 274-283.
- MOERICKE, V., & WOHLFARTH-BOTTERMANN, K. E. (1960). Zur funktionellen Morphologie der Speicheldrüsen von Homopteren. I. Mitteilung. Die Hauptzellen der Hauptdrüse von *Myzus persicae* (SULZ). *Aphididae. Z. Zellforsch.*, **51**, 157-184.
- v. MOHL, H. (1958). *Botan. Ztg.*, **13**, 89 and 105.
- MOŁE-BAJER, J. (1958). Cine-micrographic analysis of c-mitosis in endosperm. *Chromosoma (Berlin)*, **9**, 332-358.
- MOLISCH, H. (1885). Über merkwürdig geformte Proteinkörper in den Zweigen von Epiphyllum. *Ber. dtsch. bot. Ges.*, **3**, 195-202.
- MOLLENHAUER, H. H., ZEBRUN, W. (1960). Permanganate fixation of the Golgi complex and other cytoplasmic structures of mammalian testes. *J. biophys. biochem. Cytol.*, **8**, 761-775.
- MOLLENHAUER, H. H., WHALEY, W. G., & LEECH, J. H. (1961). A function of the Golgi apparatus in outer rootcap cells. *J. Ultrastruct. Res.*, **5**, 193-200.
- MONTGOMERY, P. O'B., & BONNER, W. A. (1959). Ultraviolet motion picture observation of mitosis in the presence of ribonuclease. *Tex. Rep. Biol. Med.*, **17**, 224-228.
- MONTY, K. J., LITT, M., KAY, E. R. M., & DOUNCE, A. L. (1956). Isolation and properties of liver cell nucleoli. *J. biophys. biochem. Cytol.*, **2**, 127-145.

- MOORE, A. E., SOUTHAM, C. M., & STERNBERG, S. S. (1956). Neoplastic changes developing in epithelial cell lines derived from normal persons. *Science*, **124**, 127-129.
- MOORE, D. H., & RUSKA, H. (1957). The fine structure of capillaries and small arteries. *J. biophys. biochem. Cytol.*, **3**, 457-462.
- MOORE, K. L., & BARR, M. L. (1954). Nuclear morphology, according to sex in human tissues. *Acta anat. (Basel)*, **21**, 197-208.
- (1955). Smears from the oral mucosa in the detection of chromosomal sex. *Lancet*, **ii**, 57-58.
- MOORE, R. D., MUMAW, V. R., & SCHOENBERG, M. D. (1961). The transport and distribution of colloidal iron and its relation to the ultrastructure of the cell. *J. Ultrastruct. Res.*, **5**, 244-256.
- MOORE, S. M. (1888). Studies in vegetable biology. *J. Linn. Soc. Bot.*, **24**, 200-251, 351-389.
- MOORHEAD, P. S., & HSU, T. C. (1956). Cytologic studies of HeLa, a strain of human cervical carcinoma. III. Durations and characteristics of the mitotic phases. *J. nat. Cancer Inst.*, **16**, 1047-1066.
- MORGAN, C., ROSE, H. M., & MOORE, D. H. (1957). An evaluation of host cell changes accompanying viral multiplication as observed in the electron microscope. *Ann. N. Y. Acad. Sci.*, **68**, 302-323.
- MORGAN, C., ROSE, H. M., HOLDEN, M., & JONES, E. P. (1959). Electron microscopic observations on the development of herpes simplex virus. *J. exp. Med.*, **110**, 643-656.
- MORGAN, C., GODMAN, G. C., BREITENFELD, P. M., & ROSE, H. M. (1960). A correlative study by electron and light microscopy of the development of type 5 adenovirus. I. Electron microscopy. *J. exp. Med.*, **112**, 373-382.
- MORGAN, L. V. (1933). A closed x-chromosome in *Drosophila melanogaster*. *Genetics*, **18**, 250-283.
- MORGAN, T. H. (1896). The production of artificial astrospheres. *Arch. Entwickl.-Mech. Org.*, **3**, 339-361.
- (1900). Further studies in the action of salt-solutions and other agents on the eggs of *Arbacia*. *Arch. Entwickl.-Mech. Org.*, **10**, 489-524.
- MORIYAMA, H. (1959). Cell-like bodies prepared from mixtures of egg white and oil. *Jap. J. exp. Med.*, **29**, 9-14.
- MORRISON, J. W., & CH. LIN, S. (1955). Chromosomes and Nucleoli in *Pisum sativum*. *Nature (Lond.)*, **175**, 343-344.
- MORTHLAND, F. W., DEBRUYN, P. P. H., & SMITH, N. H. (1954). Spectrophotometric studies on the interaction of nucleic acids with aminoacridines and other basic dyes. *Exp. Cell Res.*, **7**, 201-214.
- MOSES, M. J. (1956). Studies on nuclei using correlated cytochemical, light, and electron microscope techniques. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 397-406.
- (1958). The relation between the axial complex of meiotic prophase chromosomes and chromosome pairing in Salamander (*Plethodon cinereus*). *J. biophys. biochem. Cytol.*, **4**, 633-638.
- (1960). Patterns of organization in the fine structure of chromosomes. *Verh. 4 Int. Kongr. Elektr. Mikr.*, Berlin **1958**, 199-211.
- MOSES, M. J., & TAYLOR, J. H. (1955). Desoxypentose nucleic acid synthesis during microsporogenesis in *Tradescantia*. *Exp. Cell Res.*, **9**, 474-488.
- MOTA, M. (1950). Karyokinesis without cytokinesis in the grasshopper. *Exp. Cell Res.*, **17**, 76-83.
- MOTTIER, D. M. (1899). The effect of centrifugal force upon the cell. *Ann. Bot.*, **13**, 325-361.
- MOULÉ, Y., ROUILLER, C., & CHAUVEAU, J. (1960). A biochemical and morphological study of rat liver microsomes. *J. biophys. biochem. Cytol.*, **7**, 547-558.
- MÜHLDORF, A. (1951). *Die Zellteilung als Plasmateilung*. Springer, Wien.
- MÜHLETHALER, K. (1955). The structure of chloroplasts. *Int. Rev. Cytol.*, **4**, 197-220.
- (1960). Die Struktur und Entwicklung der Plastiden. *Dtsch. med. Wschr.*, **85**, 1063-1065.
- MÜLLER, E. (1955). *Der Zelltod. Handbuch der allg. Path.*, Vol. **II**i. Springer, Berlin.
- MÜLLER, H.-A. (1961a). Karyologische Studien an den Purkinje-Zellen des menschlichen Kleinhirns. I. Ein Beitrag zum Funktionsformwechsel des Ganglionzellkerns. *Beitr. path. Anat.*, **124**, 19-45.
- (1961b). Karyologische Studien an den Purkinje-Zellen des menschlichen Kleinhirns. II. Über mehrkernige Purkinje-Zellen. *Beitr. path. Anat.*, **124**, 46-56.
- MÜLLER, M., & TÖRÖ, I. (1962). Studies on feeding and digestion in Protozoa. III. Acid phosphatase activity and food vacuoles of *Paramecium multimicronucleatum*. *J. Protozool.*, **9**, 98-102.
- MÜLLER, M., TÓTH, J., & TÖRÖ, I. (1962). Studies on feeding and digestion in Protozoa. IV. Acid phosphatase and nonspecific esterase activity of food vacuoles in *Amoeba proteus*. *Acta biol. Acad. Sci. hung.*, **13**, 105-116.
- MÜNZER, F. T. (1923). Über die Zweikernigkeit der Leberzelle. *Arch. mikr. Anat.*, **98**, 249-282.
- (1925). Experimentelle Studien über die Zweikernigkeit der Leberzellen. *Arch. mikr. Anat.*, **104**, 138-184.
- MUGELE, F., & HAUPP, W. (1961). Die Temperaturabhängigkeit der Chloroplastenbewegung. *Naturwissenschaften*, **48**, 531-532.
- MULLER, H. J. (1938). The remaking of chromosomes. *Collect. Net.*, **13**, 181-195.
- MULNARD, J. (1956). Présence d'inclusions Feulgen-positives dans les nucléoles larvaires de quelques Diptères. *Arch. Biol. (Liège)*, **67**, 485-498.

- MUMME, C., & BUDDE, H. (1959). Die Adenovirusgruppe. *Ergebn. inn. Med. NF.*, **11**, 264-298.
- MUNDRY, K. W., & GIERER, A. (1958). Die Erzeugung von Mutationen des Tabakmosaikvirus durch chemische Behandlung seiner Nucleinsäure in vitro. *Z. indukt. Abstamm.- u. Vererb.-L.*, **89**, 614-630.
- MUNGER, B. L. (1958). A light and electron microscopic study of cellular differentiation in the pancreatic islets of the mouse. *Amer. J. Anat.*, **103**, 275-297.
- (1961). The ultrastructure and histophysiology of human eccrine sweat glands. *J. biophys. biochem. Cytol.*, **11**, 385-402.
- MURAKAMI, M. (1962). Elektronenmikroskopische Untersuchungen der neurosekretorischen Zellen im Hypothalamus der Maus. *Z. Zellforsch.*, **56**, 277-299.
- MURAKAMI, S., & UEDA, R. (1960). Electron microscope studies on the fine structure of plastids in normal and variegated tissues in *Liriope* plant. *Cytologia (Tokyo)*, **25**, 59-68.
- NACHLAS, M. M., TSOU, K.-C., DE SOUZA, E., CHENG, C. S., & SELIGMAN, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.*, **5**, 420-436.
- NACHLAS, M. M., WALKER, G. D., & SELIGMAN, A. M. (1958). The histochemical localization of triphosphopyridine nucleotide diaphorase. *J. biophys. biochem. Cytol.*, **4**, 467-474.
- NACHMIAS, V. T., & MARSHALL, J. M. (1961). Protein uptake by pinocytosis in amoebae: Studies on ferritin and methylated ferritin. *Proc. I. IUB/IUBS Symp.*, Acad. Press, London, Vol. II.
- NACHTSHEIM, H. (1959). Betrachtungen zur Ätiologie und Prophylaxe angeborener Anomalien. *Dtsch. med. Wschr.*, **84**, 1845-1851.
- (1960). Chromosomenaberrationen beim Menschen und ihre Bedeutung für die Entstehung von Mißbildungen. *II. Naturwissenschaften*, **47**, 361-371.
- NÄGELI, C. (1855). *Pflanzenphysiologische Untersuchungen. I.* Schulthess, Zürich.
- NAGANO, T. (1961). The structure of cytoplasmic bridges in dividing spermatocytes of the rooster. *Anat. Rec.*, **141**, 73-79.
- NAGATA, T. (1961). A quantitative study of the DNA contents in rat hepatic cell nuclei by means of microspectrophotometry, with special reference to binucleate cells. *Med. J. Shinshu Univ.*, **6**, 143-153.
- NAKAI, T., SHUBIK, P., & FELDMANN, R. (1962). An electronmicroscopic study of skin carcinogenesis in the mouse with special reference to the intramitochondrial body. *Exp. Cell Res.*, **27**, 608-611.
- NANNEY, D. L., & RUDZINSKA, M. A. (1960). Protozoa. In: *The Cell*, Vol. IV. Acad. Press., New York.
- NAORA, H. (1957). Microspectrophotometry of cell nuclei stained with Feulgen reaction. III. The DNA content of individual nuclei of rat tissues in the postnatal growth. *Exp. Cell Res.*, **12**, 1-14.
- (1958). *Microspectrophotometry in visible light range*. Hdbch. d. Histochemie Bl. I I. Fischer, Stuttgart.
- NAORA, H., NAORA, H., & BRACHET, J. (1960). Studies on independent synthesis of cytoplasmic ribonucleic acids in *Actinobacteria mediterranea*. *J. gen. Physiol.*, **43**, 1083-1102.
- NASSONOW, D. (1924). Das Golgische Binnennetz und seine Beziehungen zu der Sekretion. Morphologische und experimentelle Untersuchungen an einigen Säugetierdrüsen. *Arch. mikr. Anat.*, **100**, 433-472.
- NATH, V., BAWA, S. D. R., & BHIMBER, B. S. (1954). Are spindle fibres and mid-body granules artifacts? *Nature (Lond.)*, **173**, 312.
- NAWASCHIN, M. (1927). Über die Veränderung von Zahl und Form der Chromosomen infolge der Hybridisation. *Z. Zellforsch.*, **6**, 195-233.
- NEARY, G. J., EVANS, H. J., & TONKINSON, S. M. (1959). A quantitative determination of the mitotic delay induced by gamma radiation in broad bean root meristems. *J. Genet.*, **56**, 363-394.
- NEBEL, B. R. (1935). Chromosomenstruktur VI. Ein Auschnitt. *Züchter*, **7**, 132-136, 155-156.
- (1939). Chromosome structure. *Bot. Rev.*, **5**, 563-626.
- (1957). Chromosomal and cytoplasmic microfibrillae in sperm of an Iceryine Coccid. *J. Hered.*, **48**, 51-56.
- NEBEL, B. R., & RUTTLE, M. L. (1936). Chromosome structure IX. *Tradescantia reflexa* and *Trillium erectum*. *Amer. J. Bot.*, **23**, 652-663.
- NEBEL, B. R., & HACKETT, E. M. (1961). Lampbrush fine structure of vertebrate male chromosomes in meiotic prophase. *Naturwissenschaften*, **48**, 655.
- NELSON, E., BLINZINGER, K., & HAGER, H. (1962). Ultrastructural observations on phagocytosis of bacteria in experimental (*E. coli*) meningitis. *J. Neuropath. exp. Neurol.*, **21**, 155-169.
- NEMEC, B. (1910). *Das Problem der Befruchtungsvorgänge und andere zytologische Fragen*. Borntraeger, Berlin.
- (1927). Über die Beschaffenheit der achromatischen Teilungsfigur. *Arch. exp. Zellforsch.*, **5**, 77-82.
- NEMETSCHKE, TH., WIDUCH, M., & SCHLIPKÖTER, H.-W. (1961). Die intracelluläre Quarzspeicherung in den tracheobronchialen Lymphknoten. *Z. Naturforsch.*, **16b**, 806-810.
- NETTER, H. (1959). *Theoretische Biochemie*. Springer, Berlin.

- (1961). Mögliche Mechanismen und Modelle für aktive Transportvorgänge. *Colloquium. Ges. Physiol. Chem.*, **12**,
- NICKELL, L. G., & RHOADS, C. P. (1955). Antimetabolites and cancer. *Amer. Ass. Advancement Science, Washington*.
- NICKLÄS, R. B. (1961). Recurrent pole-to-pole movements of the sex chromosomes during prometaphase I in *Melanoplus differentialis* spermatocytes. *Chromosoma (Berl.)*, **12**, 97–115.
- NIETH, H. (1949). Histologische und cytologische Untersuchungen am menschlichen Herzmuskel nach Hypertrophie und Insuffizienz. *Beitr. path. Anat.*, **110**, 618–634.
- NIKLOWITZ, W. (1962). Elektronenmikroskopische Untersuchungen zur Struktur der normalen und kollapsgeschädigten Purkinje-Zelle. *Beitr. path. Anat.*, **127**, 424–449.
- NIRENBERG, M. W., & MATTHAEI, J. H. (1961). The dependence of cell-free protein synthesis on *E. coli* upon naturally occurring or synthetic polyribonucleotides. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 1588–1602.
- NISSL, F. (1894). Über die sogenannten Granula der Nervenzellen. *Neurol. Zbl.*, **13**, 676–685, 781–789, 810–814.
- (1903). *Die Neuronentheorie und ihre Anhänger*. Fischer, Jena.
- NOËL, R. (1923). Sur l'état binucléé des cellules hépatiques. *C. r. Soc. Biol. (Paris)*, **88**, 212–213.
- NOETHLING, W., & ROCHLIN, E. (1931). Über Photodinse im kurzweligen Ultraviolet. *Planta*, **14**, 112–131.
- NOLL (1883) cited after M. Hartmann, *Allgemeine Biologie*, 4th edition. Fischer, Stuttgart 1953.
- NOLTENIUS, H. (1960). Karyologische und karyometrische Untersuchungen an den Endothel- und Deckzellenkernen der Glomerulusschlingen bei experimenteller Glomerulonephritis des Kaninchens. *Beitr. path. Anat.*, **122**, 80–105.
- NOTTHMANN-ZUCKERKANDL, H. (1915). Über die Erregung der Protoplasmastreuung durch verschiedene Strahlenarten. *Ber. disch. bot. Ges.*, **33**, 301–313.
- NOVICK, A., & SPARROW, A. H. (1949). The effects of nitrogen mustard on mitosis in onion root tips. *Heredity*, **40**, 13–17.
- NOVIKOFF, A. B. (1956). Preservation of the fine structure of isolated liver cell particulates with polyvinylpyrrolidone-sucrose. *J. biophys. biochem. Cytol.*, **2**, Suppl., 65–66.
- (1961a). Mitochondria (Chondriosomes). In: *The Cell*, 2. Acad. Press, New York.
- (1961b). Lysosomes and related particles. In: *The Cell*, 2. Acad. Press, New York.
- NOVIKOFF, A. B., & ESSNER, E. (1960). The liver cell. Some new approaches to its study. *Amer. J. Med.*, **29**, 102–131.
- NOVIKOFF, A. B., & GOLDFISCHER, S. (1961). Nucleosidediphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 802–810.
- NOVIKOFF, A. B., SHIN, W.-Y., & DRUCKER, J. (1961a). Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. *J. biophys. biochem. Cytol.*, **9**, 47–61.
- NOVIKOFF, A. B., GOLDFISCHER, S., ESSNER, E., & IACIOFANO, P. (1961b). The relations between acid phosphatase lysosomes and the Golgi apparatus. *J. histochem. Cytochem.*, **9**, 630.
- NOVIKOFF, A. B., & ESSNER, E. (1962). Cytolysomes and mitochondrial degeneration. *J. cell Biol.*, **15**, 140–146.
- NOWELL, P. C. (1959). Stimulation of mitosis in rat marrow cultures by serum from infected rats. *Proc. Soc. exp. Biol. (N. Y.)*, **101**, 347–350.
- (1962). The minute chromosome (Ph^1) in chronic granulocytic leukemia. *Blut*, **8**, 65–66.
- NOWELL, P. C., & HUNGERFORD, D. A. (1960). Chromosome studies on normal and leukemic human leukocytes. *J. nat. Cancer Inst.*, **25**, 85–109.
- NÚÑES-MONTIEL, O., WEIBEL, J., & VITELLI-FLORES, J. (1961). Electron microscopic study of the cytopathology of ECHO virus infection in cultivated cells. *J. biophys. biochem. Cytol.*, **11**, 457–467.
- NURNBERGER, J., ENGSTRÖM, A., & LINDSTRÖM, B. (1952). A study of the ventral horn cells of the adult cat by two independent cytochemical microabsorption techniques. *J. cell. comp. Physiol.*, **39**, 215–254.
- NUSSBAUM, M. (1882). Über den Bau und die Tätigkeit der Drüsen. *Arch. mikr. Anat.*, **21**, 296–351.
- NYGAARD, O. F., GÜTTES, S., & RUSCH, H. P. (1960). Nucleic acid metabolism in a slime mold with synchronous mitosis. *Biochim. biophys. Acta (Amst.)*, **38**, 298–306.
- OBERLING, C., & ROUILLER, CH. (1956). Les effets de l'intoxication aigue au tetrachlorure de carbone sur le foie du rat. *Ann. Anat. path.*, **1**, 401–427.
- OBERLING, C., & BERNHARD, W. (1961). The morphology of the cancer cells. In: *The Cell*, 5/2. Acad. Press, New York.
- O'CONNOR, R. J. (1949). The effect of acridine compounds on mitosis and oxygen consumption in the isolated brain of the embryonic chick. *Brit. J. exp. Path.*, **30**, 30–34.
- O'DONELL, E. H. J. (1961). Deoxyribonucleic acid structures in the nucleolus. *Nature (Lond.)*, **191**, 1325–1326.

- OEHLERT, W. (1961). Autoradiographische Untersuchungen zur Ribonukleinsäure-Synthese in den verschiedenen Strukturen der Zelle. *Beitr. path. Anat.*, **124**, 311-350.
- OEHLERT, W., & SCHULTZE, B. (1960). Die Kerngröße als Ausdruck der synthetischen Aktivität des Kerns. Autoradiographische Untersuchungen mit radioaktiv markierten Aminosäuren zur Größe der Proteinsynthese im Kern verschiedener Zellarten der Ratte. *Beitr. path. Anat.*, **123**, 101-113.
- OEHLERT, W., SCHULTZE, B., & MAURER, W. (1960). Autoradiographische Untersuchungen zur Frage der Eiweißsynthese innerhalb des Kerns und des Cytoplasmas der Zelle. *Beitr. path. Anat.*, **122**, 289-312.
- OEHLERT, W., & BÜCHNER, TH. (1961). Mechanismus und zeitlicher Ablauf der physiologischen Regeneration im mehrschichtigen Plattenepithel und in der Schleimhaut des Magen-Darmtraktes der weißen Maus. *Beitr. path. Anat.*, **125**, 374-402.
- OEHLERT, W., & BLOCK, P. (1962). Der Mechanismus und zeitliche Ablauf der reparativen Regeneration in Geweben mit post- und intermitotischem Zellbestand. *Verh. dtsch. Ges. Path.*, **46**, 333-340.
- OEHLERT, W., SEEMAYER, N., & LAUF, P. (1962a). Autoradiographische Untersuchungen über den Generationszyklus der Zellen des Ehrlich-Ascitescarcinoms der weißen Maus. *Beitr. path. Anat.*, **127**, 63-78.
- OEHLERT, W., HÄMMERLING, W., & BÜCHNER, F. (1962b). Der zeitliche Ablauf und das Ausmaß der Desoxyribonukleinsäure-Synthese in der regenerierenden Leber der Ratte nach Teilhepatektomie. *Beitr. path. Anat.*, **126**, 91-112.
- OEHLKERS, F. (1943). Die Auslösung von Chromosomenmutationen in der Meiosis durch Einwirkung von Chemikalien. *Z. Vererbungsdl.*, **81**, 313-341.
- (1953). Chromosome breaks influenced by chemicals. *Heredity*, **6**, Suppl., 95-105.
- (1956). *Das Leben der Gewächse*, I. Springer, Berlin.
- OEHLKERS, F., & LINNERT, G. (1949). Neue Versuche über die Wirkungsweise von Chemikalien bei der Auslösung von Chromosomenmutationen. *Z. indukt. Abstamm.-u. Vererb.-L.*, **83**, 136-156.
- OEHLKERS, F., & EBERLE, P. (1957). Spiralen und Chromomeren in der Meiosis von *Bellevalia romana*. *Chromosoma (Berl.)*, **8**, 351-363.
- ÖSTERGREN, G. (1945). Transverse equilibria on the spindle. *Bot. Not.*, **467-468**.
- (1948). Equilibria and movements of chromosomes. *Proc. 8th Int. Congr. of Gen. (Hereditas Suppl. Vol.)*
- (1949). A survey of factors marking at mitosis. *Hereditas*, **35**, 525-528.
- (1950a). Considerations on some elementary features of mitosis. *Hereditas*, **36**, 1-18.
- (1950b). Isopyknosis and isopyknotic, two new terms for use in chromosome studies. *Hereditas*, **36**, 511-513.
- (1951). The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling. *Hereditas*, **37**, 85-156.
- ÖSTERGREN, G., & WAKONIG, T. (1954). True or apparent subchromatid breakage and the induction of labile states in cytological chromosome loci. *Botan. Not.*, **357-375**.
- OEXLE, J. (1954). *Morphologische Untersuchungen über die Kernstruktur während der Furchung und der Organendifferenzierung beim Alpenmolch (Triton alpestris Laur.)*. Med. Diss. Freiburg/Br.
- OGUR, M., MINCKLER, S., LINDEGREN, G., & LINDEGREN, C. C. (1952). The nucleic acids in a polyploid series of Saccharomyces. *Arch. Biochem.*, **40**, 175-184.
- OHNO, S., & KINOSITA, R. (1956). Three-dimensional observations on the intranuclear structure. *Exper. Cell Res.*, **10**, 569-574.
- OHNO, S., KAPLAN, W. D., & KINOSITA, R. (1959). Formation of the sex chromatin by a single X-chromosome in liver cells of *Rattus norvegicus*. *Exp. Cell Res.*, **18**, 415-418.
- OHNO, S., & HAUSCHKA, T. S. (1960). Allocyly of the X-chromosome in tumors and normal tissues. *Cancer Res.*, **20**, 541-545.
- OHNO, S., & MAKINO, S. (1961). The single-X nature of sex chromatin in man. *Lancet*, **i**, 78-79.
- OKEN, L. (1809). *Naturphilosophie*. Frommann, Jena.
- OLSZEWSKA, M. J. (1960a). Recherches sur le caractère chimique de la plaque cellulaire. *Acta Soc. Bot. Pol.*, **29**, 249-261.
- (1960b). Comparaison de l'incorporation de la méthionine-³⁵S et de la méthionine-méthyle-¹⁴C au cours de la formation du phragmoplaste. *Acta Soc. Bot. Pol.*, **29**, 743-747.
- OLSZEWSKA, M. J., & BRACHET, J. (1961). Incorporation de la DL-Méthionine-³⁵S dans les fragments nucléés et anucléés d'*Acetabularia mediterranea*. *Exp. Cell Res.*, **22**, 370-380.
- OLSZEWSKA, M., DE VITRQ, F., & BRACHET, J. (1961). Influence d'irradiations UV localisées sur l'incorporation de l'adenine-8-¹⁴C, de l'uridine-³H et de la DL-Méthionine-³⁵S dans l'algue *Acetabularia mediterranea*. *Exp. Cell Res.*, **24**, 58-63.
- OLSZEWSKI, J. (1947). Zur Morphologie und Entwicklung des Arbeitskernes unter besonderer Berücksichtigung des Nervenzellkerns. *Biol. Zbl.*, **66**, 265-304.
- OPARIN, A. (1938). *The origin of life*. MacMillan, New York.
- (1957). *Die Entstehung des Lebens auf der Erde*. Dtsch. Verl. d. Wiss., Berlin.

- (1963). *Das Leben, Seine Natur, Herkunft und Entwicklung*. Fischer, Stuttgart.
- OPIE, E. L. (1946). Mobilization of basophile substance (ribonucleic acid) in the cytoplasm of liver cells with the production of tumours by butter yellow. *J. exp. Med.*, **84**, 91–106.
- (1947). Normal structure and degenerative changes of the cytoplasm of liver cells and tumor cells derived from them. *J. exp. Med.*, **85**, 339–346.
- (1954). Osmotic activity of liver cells and melting point of liver. *J. exp. Med.*, **99**, 29–41.
- OPITZ, E. (1935). Herzmuskelveränderungen durch Störung der Sauerstoffzufuhr. *Z. Kreisl.-Forsch.*, **27**, 227.
- ORO, J. (1961a). Comets and the formation of biochemical compounds on the primitive earth. *Nature (Lond.)*, **190**, 389–390.
- (1961b). Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive earth conditions. *Nature (Lond.)*, **191**, 1193–1194.
- ORR, M. F., & MCSWAIN, B. (1957). The effects of kinetin upon epithelium and fibroblasts in tissue culture. *Cancer*, **10**, 617–624.
- ORTEGA, L. G., & MELLORS, R. C. (1957). Cellular sites of formation of gamma globulin. *J. exp. Med.*, **106**, 627–640.
- ORTMANN, R. (1949). Über Kernsekretion, Kolloid- und Vakuolenbildung in Beziehung zum Nukleinsäuregehalt in Trophoblast-Riesenzellen der menschlichen Placenta. *Z. Zellforsch.*, **34**, 562–583.
- (1952). Über die Einformigkeit morphologischer Reaktionen der Ganglienzellen nach experimentellen Eingriffen. *Dtsch. Z. Nervenheilk.*, **167**, 431–441.
- OURA, G. (1953). On the mitosis of the Spirogyra with special reference to the nucleolar organization and nucleolar organizing chromosome. *Cytologia (Tokyo)*, **18**, 297–304.
- PAINTER, R. B. (1961). Asynchronous replication of HeLa S 3 chromosomal deoxyribonucleic acid. *J. biophys. biochem. Cytol.*, **11**, 485–488.
- PAINTER, R. B., & DREW, R. M. (1959). Studies on deoxyribonucleic acid metabolism in human cancer cell cultures (HeLa). I. The temporal relationships of deoxyribonucleic acid synthesis to mitosis and turnover time. *Lab. Invest.*, **8**, 278–285.
- PAINTER, T., & REINDORF, E. C. (1939). Endomitosis in the nurse cells of the ovary of *Drosophila melanogaster*. *Chromosoma (Berl.)*, **1**, 276–283.
- PAINTER, T. S. (1955). Do nuclei of living cells contain more DNA than is revealed by the Feulgen stain? *Tex. Rep. Biol. Med.*, **13**, 659–666.
- PAINTER, T. S., & STONE, W. (1935). Chromosome fusion and speciation in *Drosophila*. *Genetics*, **20**, 327–342.
- PALADE, G. E. (1952). The fine structure of mitochondria. *Anat. Rec.*, **114**, 427–451.
- (1953). An electron microscope study of the mitochondrial structure. *J. Histochem. Cytochem.*, **1**, 188–211.
- (1955). A small particulate component of the cytoplasm. *J. biophys. biochem. Cytol.*, **1**, 59–68.
- (1956). The endoplasmic reticulum. *J. biophys. biochem. Cytol.*, **2**, Suppl., 85–98.
- (1958). A small particulate component of the cytoplasm. In *Frontiers on Cytology*, Yale Univ. Press, New Haven.
- PALADE, G. E., & SIEKEVITZ, P. (1955). A correlated structural and chemical analysis of microsomes. *Anat. Rec.*, **121**, 347–348.
- (1956). Liver microsomes. An integrated morphological and biochemical study. *J. biophys. biochem. Cytol.*, **2**, 171–200.
- PALAY, S. L. (1956). Synapses in the central nervous system. *J. biophys. biochem. Cytol.*, **2** Suppl., 193–202.
- (1958a). The morphology of secretion. In *Frontiers in Cytology*. Yale Univ. Press, New Haven.
- (1958b). The morphology of synapses in the central nervous system. *Exp. Cell Res.*, Suppl. **5**, 275–293.
- (1960a). On the appearance of absorbed fat droplets in the nuclear envelope. *J. biophys. biochem. Cytol.*, **7**, 391–392.
- (1960b). The fine structure of secretory neurons in the preoptic nucleus of the goldfish (*Carassius auratus*). *Anat. Rec.*, **138**, 417–443.
- PALAY, S. L., & KARLIN, L. J. (1959). An electron microscopic study of the intestinal villus. II. The pathway of fat absorption. *J. biophys. biochem. Cytol.*, **5**, 373–384.
- PALMADE, C., CHEVALLIER, C. M. R., KNOBLOCH, A., & VENDRELY, R. (1958). Isolement d'une desoxyribonucléohistone à partir d'*Escherichia coli*. *C. R. Acad. Sci. (Paris)*, **246**, 2534–2537.
- PALME, G. (1961). Über das Cytocentrum in Glomen und gemästeten Gliazellen. *Virchows Arch. path. Anat.*, **334**, 160–172.
- PALMER, C. G., LIVENGOOD, D., WARREN, A. K., SIMPSON, P. J., & JOHNSON, I. S. (1960). The action of vincazoleukoblastine on mitosis in vitro. *Exp. Cell Res.*, **20**, 198–201.

- PANITZ, R. (1960). Innersekretorische Wirkung auf Structurmödifikationen der Speicheldrüsenchromosomen von *Aricotopus lucidus* (Chironomide). *Naturwissenschaften*, **47**, 383.
- PAPPAS, G. D. (1956). The fine structure of the nuclear envelope of *Amoeba proteus*. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 431-434.
- PARAT, M., & PAINLEVÉ, J. (1924). Observation vitale d'une cellule glandulaire en activité. Nature et rôle de l'appareil reticulaire interne de Golgi et de l'appareil de Holmgren. *C. R. Acad. Sci. (Paris)*, **179**, 612-614.
- PARDEE, A. B. (1959). Experiments on the transfer of information from DNA to enzymes. *Exp. Cell Res. Suppl.*, **6**, 142-151.
- PARKER, J., & PHILPOTT, D. E. (1961). An electron microscopic study of chloroplast condition in summer and winter in *Pinus strobus*. *Protoplasma (Wien)*, **53**, 575-583.
- PARKER, R. C. (1932). The races that constitute the group of common fibroblasts. I. The effect of blood plasma. *J. exp. Med.*, **55**, 713-734.
- PARMENTIER, R., & DUSTIN, P. (1951). Reproduction expérimentale d'une anomalie particulière de la métaphase des cellules malignes (métaphase "à trois groupes"). *Caryologia*, **4**, 98-109.
- PASTEELS, J. (1953). La teneur des noyaux en acide désoxyribonucléique (DNA) au cours du développement, de la différenciation et de la croissance. *J. Embryol. exp. Morph.*, **1**, 227-233.
- (1954). Structure des noyaux intercinétiques des vertébrés après fixation à l'acide osmique. *Symp., 8, Congr. of Cell Biol.*, Leiden.
- PASTEELS, J., & LISON, L. (1950). Teneur des noyaux au repos en acide désoxyribonucléique dans différents tissus chez le rat. *C. R. Acad. Sci. (Paris)*, **230**, 780-782.
- PATAU, K. (1952). Absorption microphotometry of irregular shaped objects. *Chromosoma (Berl.)*, **5**, 341-362.
- PATAU, K., & SWIFT, H. (1953). The DNA-content (Feulgen) of nuclei during mitosis in a root tip of onion. *Chromosoma (Berl.)*, **6**, 149-169.
- PATAU, K., & SRINIVASACHAR, D. (1959). The DNA content of nuclei in the meristem of onion roots. *Chromosoma (Berl.)*, **10**, 407-429.
- PATAU, K., THERMAN, E., SMITH, D. S., & INHORN, S. L. (1961). Two new cases of D1-trisomy in man. *Hereditas*, **47**, 239-242.
- PAULING, L. (1939). *The Nature of the chemical bond*. Cornell Univ. Press, Ithaca, New York.
- PEASE, D. C. (1941). Hydrostatic pressure effects upon the spindle figure and chromosome movement. I. Experiments on the first mitotic division of urechis eggs. *J. Morph.*, **69**, 405-441.
- (1946). Hydrostatic pressure effects upon the spindle figure and chromosome movement. II. Experiments on the meiotic divisions of *Tradescantia* pollen mother cells. *Biol. Bull.*, **91**, 145-165.
- PELC, S. R., & LA COUR, F. L. (1960). Some aspects of replication in chromosomes. In: *The Cell Nucleus*. Butterworths, London.
- PELLING, G. (1959). Chromosomal synthesis of ribonucleic acid as shown by incorporation of uridine labelled with tritium. *Nature (Lond.)*, **184 Suppl.**, 9, 655-656.
- PERLMANN, P., & MORGAN, W. S. (1961). Immunological studies of microsomal structure and function. *Proc. I. IUB/IUBS Symp.*, Acad. Press, London, Vol. I.
- PERRY, R. P. (1960a). On the nucleolar and nuclear dependence of cytoplasmic RNA synthesis in HeLa cells. *Exp. Cell Res.*, **20**, 216-220.
- (1960b). Cytoplasmic ribonucleic acid which is nucleolar and nuclear dependant and its relation to amino acid incorporation. *Science*, **132**, 1497.
- PERRY, R. P., & ERRERA, M. (1960). The influence of nucleolar ribonucleic acid metabolism on that of the nucleus and cytoplasm. In: *The Cell Nucleus*, Butterworth, London.
- PERRY, R. P., HELL, A., & ERRERA, M. (1961). The role of the nucleolus in ribonucleic acid- and protein synthesis. I. Incorporation of cytidine into normal and nucleolar inactivated HeLa cells. *Biochem. biophys. Acta (Amst.)*, **49**, 47-57.
- PERUGINI, S., TORELLI, U., & SOLDATI, M. (1956). Ricerche citofotometriche sulla componente proteica delle cellule ematiche. 2. Il contenuto nucleare di proteina istonica e di ac. desossiribonucleico nei linfociti e negli epatociti del ratto. *Riv. ist. Norm Patol.*, **2**, 449-460.
- (1957). Ricerche citofotometriche sulla componente proteica delle cellule ematiche. 4. Il contenuto nucleare di proteina istonica e di acido desossiribonucleico nei linfociti e nei mielociti dell'uomo normale. *Riv. ist. Norm Patol.*, **3**, 3-14.
- PERUTZ, M. F., ROSSMANN, M. G., CULLIS, A. F., MUIRHEAD, H., WILL, G., & NORTH, A. C. (1960). Structure of haemoglobin. A three-dimensional fourier synthesis at 5.5-Å resolution, obtained by x-ray analysis. *Nature (Lond.)*, **185**, 416-422.
- PETER, K. (1940). Die indirekte Teilung der Zelle in ihren Beziehungen zu Tätigkeit, Differenzierung und Wachstum. Rückblick und Ausblick. *Z. Zellforsch.*, **30**, 721-750.
- (1947). Differenzierung und Mitose. Ein experimenteller Beitrag zu dem Problem des Verhältnisses der indirekten Zellteilung zur spezifischen Zelltätigkeit. *Arch. Entwickl.-Mech. Org.*, **143**, 1-18.

- PÉTERFI, T., & KOJIMA, H. (1936). Die Wirkung mikrurgischer Eingriffe auf den Ruhekern. I. Anstichversuche. *Protoplasma (Wien)*, **25**, 489-500.
- PETRY, G., OVERBECK, L., & VOGELL, W. (1961). Vergleichende elektronen- und lichtmikroskopische Untersuchungen am Vaginalepithel in der Schwangerschaft. *Z. Zellforsch.*, **54**, 382-401.
- PEVELING, E. (1961). Elektronenmikroskopische Untersuchungen an Zellkernen von *Cucumis sativus L.* *Planta (Berl.)*, **56**, 530-554.
- PFAUNDLER, M. (1935). Studien über Frühtod, Geschlechtsverhältnis und Selektion. I. Mitt. zur intrauterinen Absterbeordnung. *Zbl. ges. Kinderheilk.*, **57**, 185-227.
- PFEIFFER, H. H. (1939). Experimentelle Beiträge zur Mitosephysiologie. *Arch. exp. Zellforsch.*, **22**, 263-267.
- (1951). Polarisationsoptische Untersuchungen am Spindelapparat mitotischer Zellen. *Cytologia (Tokyo)*, **16**, 194-200.
- (1954). Vorläufige Versuche über die Elastizität des Atraktoplasmas. *Protoplasma (Wien)*, **44**, 47-51.
- (1956). Neue Fortschritte bei der Strukturanalyse der Zentromeren während der Meiose. *Naturwissenschaften*, **43**, 229.
- (1959). Über die Ermittlung der kinetischen Energie von Chromosomen in der Anaphase. *Protoplasma (Wien)*, **51**, 390-398.
- PFUHL, W. (1938). Die mitotischen Teilungen der Leberzellen im Zusammenhang mit den allgemeinen Fragen über Mitose und Amitose. *Z. Anat. Entwickl.-Gesch.*, **109**, 99-133.
- PHAN, N. VAN, & DAVID, H. (1958). Zur Frage der Genese und Zahl groß- und zweikerniger Leberzellen bei Mäusen in und nach absolutem Hunger. *Z. Zellforsch.*, **48**, 653-660.
- PHILPOT, J. S. L., & STANIER, J. E. (1957). Comparison of interphase and prophase in isolated rat liver nuclei. *Nature (Lond.)*, **179**, 102-103.
- PICHOTKA, J. (1942). Tierexperimentelle Untersuchungen zur pathologischen Histologie des akuten Höhentodes. *Beitr. path. Anat.*, **107**, 117-155.
- PICHOTKA, J., HÖFLER, W., & REISSNER, J. (1954). Untersuchungen über die Wasserbindung in organischen Systemen. *Arch. exp. Path. u. Pharmak.*, **223**, 217-233.
- PIEKARSKI, G. (1939a). Lichtoptische und übermikroskopische Untersuchungen zum Problem des Bakterienzellkernes. *Zbl. Bakter. I. Orig.*, **144**, 140-148.
- (1939b). Cytologische Untersuchungen an einem normalen und einem micronucleuslosen Stamm von *Colpoda steini* MAUPAS. *Arch. Protistenk.*, **92**, 117-130.
- (1941). Endomitose beim Großkern der Ciliaten? Versuch einer Synthese. *Biol. Zbl.*, **61**, 416-426.
- (1949). Zum Problem des Bakterienzellkerns. *Ergeb. Hyg. Bakt.*, **26**, 333-364.
- PIERCE, W. P. (1937). The effect of phosphorus on chromosome and nuclear volume in a violet species. *Bull. Torrey Bot. Club*, **64**, 345-356.
- PLESNER, P. E. (1958). The nucleoside triphosphate content of *Tetrahymena pyriformis* during the division cycle in synchronously dividing mass cultures. *Biochem. biophys. Acta (Amst.)*, **29**, 462-463.
- PLUMMER, J. I., WRIGHT, L. T., ANTIKAJIAN, G., WEINTRAUB, S. (1952). Triethylene melamine in vitro studies. I. Mitotic alterations produced in chick fibroblast tissue cultures. *Cancer Res.*, **12**, 796-800.
- POCHE, R. (1957). Das submikroskopische Bild der Herzmuskelveränderungen nach Überdosierung von Schilddrüsenhormon. *Beitr. path. Anat.*, **188**, 407-420.
- (1958). Submikroskopische Beiträge zur Pathologie der Herzmuskelzelle bei Phosphorvergiftung, Hypertrophie, Atrophie und Kaliummangel. *Virchows Arch. path. Anat.*, **331**, 165-248.
- PODWYSSOZKI, W. V. (1886). Experimentelle Untersuchungen über die Regeneration der Drüsengewölbe. I. Untersuchungen über die Regeneration des Lebergewebes. *Beitr. path. Anat.*, **1**, 259-360.
- POGO, A. O., POGO, B. G. T., & FUNES, J. R. C. (1960). Correlation between deoxyribonucleic acid and volume in liver cell nuclei during post-natal growth. *Exp. Cell Res.*, **20**, 208-211.
- POLICARD, A., & BESSIS, M. (1956). Sur l'espace périnucléaire. *Exp. Cell Res.*, **11**, 490-492.
- (1958). Sur un mode d'incorporation des macromolécules par la cellule, visible au microscope électronique: la rhophéocytose. *C. R. Acad. Sci. (Paris)*, **246**, 3194-3197.
- POLICARD, A., BESSIS, M., BRETON, J., & THIERY, J. P. (1958). Polarité de la centrosphère et des corps de Golgi dans les leucocytes des mammifères. *Exp. Cell Res.*, **14**, 221-223.
- POLICARD, A., & BESSIS, M. (1959). La pinocytose. (Le phénomène de Lewis et son histoire.) *Rev. Hémat.*, **14**, 487-495.
- POLICARD, A., COLLET, A., & PREGERMAIN, S. (1959). Aspects de la phagocytose de la silice observés au microscope électronique. *Path. et Biol.*, **7**, 2041-2062.
- POLICARD, A., COLLET, A., DANIEL-MOUSSARD, H., PREGERMAIN, S., & REUET, C. (1961). Deposition of silica in mitochondria: An electron microscopic study. *J. biophys. biochem. Cytol.*, **9**, 236-238.
- POLLISTER, A. W. (1929). Notes on cell division in the pancreas of the dogfish. *Anat. Rec.*, **44**, 29-51.
- (1933). Notes on the centrioles of amphibian tissue cells. *Biol. Bull.*, **65**, 529-545.
- (1941). Mitochondrial orientations and molecular patterns. *Physiol. Zool.*, **14**, 268-280.

- POLLISTER, A. W., & POLLISTER, P. F. (1943). The relation between centriole and centromere in atypical spermatogenesis of Viviparid snails. *Ann. N. Y. Acad. Sci.*, **45**, 1-48.
- POLLISTER, A. W., & RIS, H. (1947). Nucleoprotein determinations in cytological preparations. *Cold Spr. Harb. Symp. quant. Biol.*, **12**, 147-157.
- POLLISTER, A. W., GETTNER, M., & WARD, R. (1954). Nucleocytoplasmic interchange in oocytes. *Science*, **120**, 789.
- POLLISTER, A. W., & ORNSTEIN, L. (1955). Cytophotometric analysis in the visible spectrum. In: *Analytical Cytology. Methods for studying cellular form and function*. McGraw-Hill Book Comp., London.
- POLSTER, CH. (1959). Karyometrische und karyologische Untersuchungen an den Hauptstückepithelien der Ratteniere bei experimenteller Eiweißnephrose. *Virchows Arch. path. Anat.*, **332**, 420-430.
- POMERAT, C. M. (1953). Rotating nuclei in tissue cultures of adult human nasal mucosa. *Exp. Cell Res.*, **5**, 191-196.
- (1961). Cinematography, indispensable tool for cytology. *Int. Rev. Cytol.*, **11**, 307-334.
- POORT, C. (1961). Electrophoretic comparison of nuclear and nucleolar proteins. I. Beef pancreas. *Biochim. biophys. Acta (Amst.)*, **46**, 373-380.
- PORTER, K. R. (1954). Changes in cell fine structure accompanying mitosis. *Symp. 8, Cong. of Cell Biol., Leiden*.
- (1961). The endoplasmatic reticulum: Some current interpretations of its forms and functions. *Proc. I. IUB/IUBS Symp.*, Acad. Press, London, Vol. I.
- PORTER, K. R., CLAUDE, A., & FULLAM, E. (1945). A study of tissue culture cells by electron microscopy. Methods and preliminary observations. *J. exp. Med.*, **81**, 233-246.
- PORTER, K. R., & THOMPSON, H. P. (1947). Some morphological features of cultured rat sarcoma cells as revealed by the electron microscope. *Cancer Res.*, **7**, 431-438.
- PORTER, K. R., & PALADE, G. E. (1957). Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. *J. Biophys. biochem. Cytol.*, **3**, 269-300.
- PORTER, K., & BRUNI, C. (1959). An electron microscope study of the early effects of 3'-Me-DAB on rat liver cells. *Cancer Res.*, **19**, 997-1009.
- PORTER, K. R., & BRUNI, C. (1960). Fine structural changes in rat liver cells associated with glycogenesis and glycogenolysis. *Anat. Rec.*, **136**, 260-261.
- PORTER, K. R., & MACHADO, R. D. (1960). Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip. *J. biophys. biochem. Cytol.*, **7**, 167-180.
- PORTER, K. R., & YAMADA, E. (1960). Studies on the endoplasmic reticulum. V. Its form and differentiation in pigment epithelial cells of the frog retina. *J. biophys. biochem. Cytol.*, **8**, 181-205.
- PORTUGALOV, V. V., DOVEDOVA, E. L., & SKREBITSKY, V. G. (1962). On some mechanisms regulating the chemical activity of the neuron mitochondria. *J. Histochem. Cytochem.*, **10**, 213-221.
- PORTER, J. S., & WARD, E. N. (1940). The development of the megacaryocyte in adult mice. *Anat. Rec.*, **77**, 77.
- POULSEN, D. F., & BOWEN, V. T. (1952). Organization and function of the inorganic constituents of nuclei. *Exp. Cell Res. Suppl.*, **2**, 161-174.
- POWERS, E. L., EHRET, C. F., ROTH, L. E., & MINICK, O. T. (1956). The internal organization of mitochondria. *J. biophys. biochem. Cytol.*, **2**, 341-346.
- PRATJE, A. (1952). Beiträge zur Kernteilung von Noctiluca miliaris. *Verh. Dtsch. Ges. Zool.*, 300-311.
- PRESCOTT, D. M. (1955). Relations between cell growth and cell division. I. Reduced weight, cell volume, protein content, and nuclear volume of Amoeba proteus from division to division. *Exp. Cell Res.*, **9**, 328-337.
- (1956). Relation between cell growth and cell division. II. The effect of cell size on cell growth rate and generation time in Amoeba proteus. *Exp. Cell Res.*, **11**, 86-98.
- (1959a). Nuclear synthesis of cytoplasmic ribonucleic acid in Amoeba proteus. *J. biophys. biochem. Cytol.*, **6**, 202-206.
- (1959b). Microtechniques in amoeba studies. *Ann. N. Y. Acad. Sci.*, **78**, 655-661.
- (1960). Relation between cell growth and cell division. IV. The synthesis of DNA, RNA and protein from division to division in Tetrahymena. *Exp. Cell Res.*, **19**, 228-238.
- (1961a). RNA synthesis in the nucleus and RNA transfer to the cytoplasm in Tetrahymena pyriformis. *Proc. I. IUB/IUBS Symp.*, Acad. Press, London, Vol. II.
- (1961b). The growth-duplication cycle of the ccell. *Int. Rev. Cytol.*, **11**, 255-282.
- (1962). Symposium: Synthetic processes in the cell nucleus. II. Nucleic acid and protein metabolism in the macronuclei of two ciliated protozoa. *J. Histochem. Cytochem.*, **10**, 145-153.
- PRESCOTT, D. M., & KIMBALL, R. F. (1961). Relation between RNA, DNA, and protein syntheses in the replicating nucleus of Euplotes. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 686-693.
- PRESCOTT, D. M., & BENDER, M. A. (1962). Synthesis of RNA and protein during mitosis in mammalian tissue culture cells. *Exp. Cell Res.*, **26**, 260-268.

- PROPACH, H. (1940). Die Centromeren in der Pollenkornmitose von *Tradescantia gigantea* Rose. *Chromosoma (Berl.)*, **1**, 521-525.
- PUFF, A. (1951). Neue morphologische Befunde an der Netzhaut zur Bestätigung der Duplizitätstheorie. *Verh. anat. Ges.*, **48**, 124-125.
- PURKINJE, J. E. (1825). *Symbolae ad ovi avium historiam ante incubationem*. Schulz, Breslau.
- (1837). Über den Bau der Magendrüsen und über die Natur des Verdauungsprozesses. *Ber. Ver. dtsch. Ges. Naturforsch. u. Ärzte*, **174**-175.
- QUASTLER, H. (1959). Storage and actuation of genetic information. The information theory approach. *Lab. Invest.*, **8**, 480.
- QUASTLER, H., & SHERMAN, F. G. (1959). Cell population kinetics in the intestinal epithelium of the mouse. *Exp. Cell Res.*, **17**, 420-438.
- RABINOVITZ, M., & OLSON, M. E. (1959). Protein synthesis by rabbit reticulocytes. I. Kinetics of amino acid incorporation in vitro into protein fractions of intact cells. *J. biol. Chem.*, **234**, 2085-2090.
- RABINOWITCH, E. I. (1945). *Photosynthesis*. Interscience, New York.
- RABL, C. (1885). Über Zellteilung. *Morph. Jb.*, **10**, 214-330.
- RABSON, A. S., KILHAM, L., & KIRSCHSTEIN, R. L. (1961). Intranuclear inclusion in *rattus* (*Mastomys natalensis*) infected with rat virus. *J. nat. Cancer Inst.*, **27**, 1217-1223.
- RACKER, E. (1954). Alternative pathways of glucose and fructose metabolism. *Advanc. Enzymol.*, **15**, 141-182.
- RAHMANN, Y. E. (1962). Electron microscopy of lysosome-rich fractions from rat thymus isolated by density-gradient centrifugation before and after whole-body x-irradiation. *J. Cell Biol.*, **13**, 253-260.
- RAPKINE, L. (1931). Sur les processus chimiques au cours de la division cellulaire. *Ann. Physiol.*, **7**, 382-418.
- RASCH, E., SWIFT, H., & KLEIN, R. M. (1959). Nucleoprotein changes in plant tumor growth. *J. biophys. biochem. Cytol.*, **6**, 11-34.
- RASCH, E., WOODWARD, J. W. (1959). Basic proteins of plant nuclei during normal and pathological cell growth. *J. biophys. biochem. Cytol.*, **6**, 263-276.
- RASCH, E., & SWIFT, H. (1960). Microphotometric analysis of the cytochemical millon reaction. *J. Histochem. Cytochem.*, **8**, 4-17.
- RATHER, L. J. (1958). The significance of nuclear size in physiological and pathological processes. *Ergebn. alg. Path., path. Anat.*, **38**, 127-199.
- REALE, E., & BUCHER, O. (1962). Quelques observations ultrastructurales sur le rein. *Z. Anat. Entwickl.-Gesch.*, **123**, 106.
- REBHUN, L. I. (1956). Electron microscopy of basophilic structures of some intervertebrate oocytes. I. Periodic lamellae and the nuclear envelope. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 93-104.
- (1957). Nuclear changes during spermatogenesis in a pulmonate snail. *J. biophys. biochem. Cytol.*, **3**, 509-524.
- REGEMORTER, D. VAN (1926). Les troubles cinétiques dans les racines chloralisées et leur portée pour l'interprétation des phénomènes normaux. *Cellule*, **37**, 43-73.
- REITBERGER, A. (1940). Die Cytologie des pädogenetischen Entwicklungscyklus der Gallmücke Oligarces paradoxus. *Chromosoma (Berl.)*, **1**, 391-473.
- (1949). Über polyploide Ruhekerne bei Cruziferen. *Naturwissenschaften*, **36**, 380.
- REMAK, R. (1958). Über die Teilung der Blutzellen beim Embryo. *Arch. Anat. Physiol. wiss. Med.*, **178**-188.
- REMY, R., & TERBRÜGGEN, A. (1950). Der Einfluß von Monosacchariden bei experimenteller Leberschädigung. *Z. ges. inn. Med.*, **5**, 645-649.
- RESCH, A., & PEVELING, E. (1962). Carminessigsäure zur Darstellung des Zellkerns im Electronenmikroskop. *Planta (Berl.)*, **59**, 85-90.
- RESENDE, F., LETTRÉ, H., & LETTRÉ, R. (1959). Zur Persistenz der Chromosomenspindelfasern. *Naturwissenschaften*, **46**, 117.
- REUTER, J. P. (1959). Über Einschlußkörperchen und ihr Vorkommen bei Poliomyelitis. *Fortschr. Neurol. Psychiat.*, **27**, 573-595.
- REVELL, S. H. (1953). Chromosome breakage by x-rays and radiomimetic substances in *Vicia*. *Heredity*, **6 Suppl.**, 107-124.
- RHO, J. H., & BONNER, J. (1961). The site of ribonucleic acid synthesis in the isolated nucleus. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 1611-1619.
- RHOADES, M. M. (1936). A cytological study of a chromosome fragment in maize. *Genetics*, **21**, 491-502.
- (1943). Genic induction of an inherited cytoplasmic difference. *Proc. Acad. Sci. (Wash.)*, **29**, 327-329.
- RHOADES, M. M. (1946). Plastid mutations. *Cold Spr. Harb. Symp. quant. Biol.*, **11**, 202-207.
- (1961). Meiosis. In: *The Cell*, Vol. III. Acad. Press, New York.

- RHODADES, M. M., & MCCLINTOCK, B. (1935). The cytogenics of maize. *Bot. Rev.*, **1**, 292-325.
- RHODIN, J. (1954). Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney. *Karolinska Institute, Stockholm*.
- (1958). Electron microscopy of the kidney. *Amer. J. Med.*, **24**, 661-675.
- RHUMBLER, L. (1897). Stemmen die Strahlen der Astroosphäre oder ziehen sie? *Arch. Entw.-Mech. Org.*, **4**, 659-730.
- (1898). Allgemeine Zellmechanik. *Ergebn. Anat. Entw.-Gesch.*, **8**, 543-625.
- RIBBANDS, C. R. (1941). Meiosis in Diptera. I. Prophase association of non-homologous chromosomes and their relation to mutual attraction between centromeres, centrosomes, and chromosome ends. *J. Genet.*, **41**, 411-442.
- RICHARDS, B. M. (1960). Redistribution of nuclear proteins during mitosis. In: *The Cell Nucleus*, Butterworths, London.
- RICHARDS, B. M., & BAIER A. (1961). Mitosis in endosperm. Changes in nuclear and chromosome mass during mitosis. *Exp. Cell Res.*, **22**, 503-508.
- RICHTER, G. (1959). Die Auswirkungen der Zellkern-Entfernung auf die Synthese von Ribonucleinsäure und Cytoplasma-Proteinen bei Acetabularia mediterranea. *Biochim. biophys. Acta (Amst.)*, **34**, 407-419.
- RICHTER, G. W. (1958). Electron microscopy of hemosiderin: Presence of ferritin and occurrence of crystalline lattices in hemosiderin deposits. *J. biophys. biochem. Cytol.*, **4**, 55-58.
- RICHTER, G. W. (1959). Internal structure of apoferritin as revealed by the "negative staining technique". *J. biophys. biochem. Cytol.*, **6**, 531-534.
- RICKER, G. (1924). *Pathologie als Naturwissenschaft. Relationspathologie*. Springer, Berlin.
- RIEGER, R. (1957). Inhomogenenpaarung und Meioseablauf bei haploiden Formen von Antirrhinum majus L. *Chromosoma (Berl.)*, **9**, 1-38.
- RIES, E. (1932). Die Prozesse der Eibildung und des Eiwachstums bei Pediculiden und Mallophagen. *Z. Zellforsch.*, **16**, 314-388.
- (1935). Zur Histophysiologie des Mäusepankreas nach Lebendbeobachtungen, Vitalfärbung und Stufenuntersuchung. *Z. Zellforsch.*, **22**, 523-585.
- RIES, E., & VAN WEEL, P. B. (1934). Die Eibildung der Kleiderlaus, untersucht an lebenden, vitalgefärbten und fixierten Präparaten. *Z. Zellforsch.*, **20**, 565-618.
- RIFKIND, R. A., GODMAN, G. C., HOWE, C., MORGAN, C., & ROSE, H. M. (1961). Structure and development of viruses as observed in the electron microscope. *J. exp. Med.*, **114**, 1-12.
- RIGLER, R., ROSENKRANZ, W., & BOUVIER, I. (1960). Polyploidie und Milz. *Wien. med. Wschr.*, **110**, 498-500.
- RIS, H. (1942). A cytological and experimental analysis of the meiotic behaviour of the univalent X-chromosome in the bearberry aphid Tamalia (Phylaphis) coweni (Cklo). *J. exp. Zool.*, **90**, 267-330.
- (1943). A quantitative study of anaphase movement in the aphid Tamalia. *Biol. Bull.*, **85**, 164-178.
- (1947). The composition of chromosomes during mitosis and meiosis. *Cold Spr. Harb. Symp. quant. Biol.*, **12**, 158-160.
- (1949). The anaphase movement of chromosomes in the spermatocytes of the grasshopper. *Biol. Bull.*, **96**, 90-106.
- (1954). The submicroscopic structure of chromosomes. *Symp. 8, Cong. of Cell Biol.*, Leiden.
- (1956). A study of chromosomes with the electron microscope. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 385-392.
- (1957). Chromosome structure. In: *Chemical basis of heredity*. Johns Hopkins Univ. Press, Baltimore.
- (1958). Die Feinstruktur des Kerns während der Spermiogenese. *9. Coll. Ges. Physiol. Chem.* Springer, Berlin.
- (1963). Ultrastructure of the cell nucleus. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- RIS, H., & MIRSKY, A. E. (1949). The state of the chromosomes in the interphase nucleus. *J. gen. Physiol.*, **32**, 489-502.
- RIS, H., & KLEINFELD, R. (1952). Cytochemical studies on the chromatin elimination in Solenobia (Lepidoptera). *Chromosoma (Berl.)*, **5**, 363-371.
- RIS, H., & PLAUT, W. (1962). Ultrastructure of DNA-containing areas in the chloroplast of Chlamydomonas. *J. Cell. Biol.*, **13**, 383-391.
- RISLER, H. H. (1950). Kernvolumenänderungen in der Larvenentwicklung von Ptychopoda seriata Schrk. *Biol. Zbl.*, **69**, 11-28.
- RISLER, H. (1959). Polyploidie und somatische Reduktion in der Larvenepidermis von Aedes Aegypti L. (Culicidae). *Chromosoma (Berl.)*, **10**, 184-209.
- RIXON, R. H., & STEVENSON, J. A. F. (1958). Movements of sodium, potassium and water in rat diaphragm in vitro. *Amer. J. Physiol.*, **194**, 363-368.

- ROBERTIS, E. DE. (1956). Electron microscopic observations on the submicroscopic morphology of the meiotic nucleus and chromosomes. *J. biophys. biochem. Cytol.*, **2**, 785-795.
- ROBERTIS, E. DE, & SABATINI, D. D. (1960). Submicroscopic analysis of the secretory process in the adrenal medulla. (*Symposium*). *Fed. Proc.*, **19**, Nr. 4, Part II, 70-78.
- ROBERTS, H. S. (1961). Mechanism of cytokinesis: A critical review. *Quart. Rev. Biol.*, **36**, 155-177.
- ROBERTS, K. B., FLOREY, H. W., & JOKLIK, W. K. (1952). The influence of cortisone on cell division. *Quart. J. exp. Physiol.*, **37**, 239-257.
- ROBERTSON, J. D. (1959). The ultrastructure of cell membranes and their derivatives. *Biochem. Soc. Symp.*, **16**, 3-43.
- ROBINOW, C. F. (1957). The structure and behaviour of the nuclei in spores and growing hyphae of *Mudorales*. I. *Mucor hiemalis* and *Mucor fragilis*. *Canad. J. Microbiol.*, **3**, 771-789.
- (1961). Mitosis in the yeast *Liomyces lipofer*. *J. biophys. biochem. Cytol.*, **9**, 879, 892.
- ROBINSON, J. R. (1953). The active transport of water in living systems. *Biol. Rev.*, **28**, 158-192.
- ROELS, H. (1954). Mitosis and deoxyribonucleic acid content of the nucleus. *Nature (Lond.)*, **173**, 1039-1040.
- (1956). La teneur en acide désoxyribonucléique du noyau de la cellule thyroïdienne du rat blanc dans diverses conditions expérimentales. *Arch. Biol. (Liège)*, **67**, 211-268.
- (1957). Activité cellulaire et teneur en DNA des noyaux de la corticosurrénale chez le rat blanc. *C. R. Soc. Biol. (Paris)*, **150**, 2273-2275.
- ROELS-DE SCHRIJVER, M. P. (1961). Influence de l'activité cellulaire sur la teneur en ADN et le volume des noyaux dans la médullosurrénale du cobaye. *Arch. Biol. (Liège)*, **72**, 173-184.
- RÖSSLE, R. (1926). Wachstum der Zellen und Organe, Hypertrophie und Atrophie. In: *Handbuch der normalen und pathologischen Physiologie* **14**. Springer, Berlin.
- ROIZMAN, B., & SCHLUEDER-BERG, A. E. (1962). Virus infection of cells in mitosis. III. Cytology of mitotic and amitotic HEp-2 cells infected with measles virus. *J. nat. Cancer Inst.*, **28**, 35-53.
- ROLLHÄUSER, H., & VOGELL, W. (1957). Elektronenmikroskopische Untersuchungen über die aktive Stoffausscheidung in der Niere. *Z. Zellforsch.*, **47**, 53-76.
- ROSE, G. G. (1957). Mikrokinetospheres and VP satellites of pinocytic cells observed in tissue cultures of Gey's strain HeLa with phase contrast cinematographic techniques. *J. biophys. biochem. Cytol.*, **3**, 697-704.
- (1961). The Golgi complex in living osteoblasts. *J. biophys. biochem. Cytol.*, **9**, 463-478.
- ROSE, G. G., POMERAT, C. M. (1960). Phase contrast observations of the endoplasmic reticulum in living tissue cultures. *J. biophys. biochem. Cytol.*, **8**, 423-430.
- ROSE, G. G., & STEHLIN, J. S. (1961). The Golgi complex and melanin elaboration of human melanomas in tissue culture. *Cancer Res.*, **21**, 1455-1460.
- ROSENBAUM, R. M., & ROLON, C. I. (1960). Intracellular digestion and hydrolytic enzymes in the phagocytes of planarians. *Biol. Bull.*, **118**, 315-323.
- ROSENBERG, O. (1899). *Physiologisch-cytologische Untersuchungen über Drosera rotundifolia*. Diss., Bonn.
- ROSENFIELD, I., & TOBIAS, C. A. (1951). Distribution of Co⁶⁰, Cu⁶⁴, and Zn⁶⁵ in the cytoplasm and nuclei of tissues. *J. biol. Chem.*, **191**, 339-349.
- ROSIN, A., & DOLJANSKI, L. (1946). Pyroninophilic structures of liver cells in carbon tetrachloride poisoning. *Proc. Soc. exp. Biol. (N. Y.)*, **62**, 62-64.
- ROSS, B. (1960). Die submikroskopische Struktur der Rattenschilddrüse. Ihre Beeinflussung durch hohe Dosen von thyreotropem Hormon. Ein Beitrag zum Studium der Sekretionsvorgänge in der Schilddrüse. *Path. et Microbiol.*, **23**, 129-157.
- ROSS, K. F. A. (1954). The changes of water distribution in cytoplasm and nuclear sap during division as indicated by changes in their refractive indices. *Quart. J. micr. Sci.*, **95**, 425-432.
- ROSSNER, W. (1961). Elektronenmikroskopische Untersuchungen an den Chromozentren von *Urtica pilulifera*. *Chromosoma (Berl.)*, **12**, 717-727.
- ROTH, L. E., OBETZ, S. W., & DANIELS, E. W. (1960). Electron microscopic studies of mitosis in amoebae. I. *Amoeba proteus*. *J. biophys. biochem. Cytol.*, **8**, 207-220.
- ROTH, L. E., & DANIELS, E. W. (1962). Electron microscopic studies of mitosis in amoebae. II. The giant amoeba *Pelomyxa carolinensis*. *J. Cell Biol.*, **12**, 57-78.
- ROTMAN, B., & SPIEGELMAN, S. (1954). On the origin of the carbon in the induced synthesis β -galactosidase in *Escherichia coli*. *J. Bact.*, **68**, 419-429.
- ROTTER, W., LAPP, H., & ZIMMERMANN, H. (1962). Pathogenese und morphologisches Substrat des "akuten Nierenversagens" und seine Erholungszeit. *Dtsch. med. Wschr.*, **87**, 669-677.
- ROUILLER, C. (1956). Les canalicles biliaires. *Acta Anat. (Basel)*, **26**, 94-109.
- (1960). Physiological and pathological changes in mitochondrial morphology. *Int. Rev. Cytol.*, **9**, 227-292.
- ROUILLER, C., & BERNHARD, W. (1956). "Microparticles" and the problem of mitochondrial regeneration in liver cells. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 355-359.

- ROUILLER, C., & FAURÉ-FREMIET, E. (1958). Ultrastructure des cinétosomes à l'état de repos et à l'état cilifère chez un Cilié péritrichie. *J. Ultrastruct. Res.*, **1**, 289-294.
- RUDOLPH, G. (1960). Die Enzymhistochemie der DPN-Diaphorase, der TPN-Diaphorase und der Succinodehydrogenase. *Acta histochem. (Jena)*, **10**, 293-325.
- RUDZINSKA, M. A., & PORTER, K. R. (1953). An electron microscope study of a protozoan, Tokophyra infusionum. *Anat. Rec.*, **115**, 363-364.
- RÜCKERT, I. (1892). Zur Entwicklungsgeschichte des Ovarialeies bei Selachiern. *Anat. Anz.*, **7**, 107-158.
- RÜTTNER, J. R., RONDEZ, R., & GAASSMANN, R. (1959). Histochemische Untersuchungen an der toxisch geschädigten Rattenleber. *Schweiz. Z. allg. Path.*, **22**, 294-300.
- RUNNSTRÖM, J. (1928). Die Veränderungen der Plasmakolloide bei der Entwicklungserregung des Säugetiers. *Protoplasma (Wien)*, **4**, 388-514.
- (1961). A gelating factor involved in fertilization and cytoplasmic cleavage of the sea urchin egg. *Exp. Cell Res.*, **23**, 145-158.
- RUSKA, C. (1962). Die Zellstrukturen des Dünndarmepithels in ihrer Abhängigkeit von der physikalisch-chemischen Beschaffenheit des Darminhalts. V. Lipoidlösungsmitel verschiedener Wasserlöslichkeit. *Z. Zellforsch.*, **56**, 762-788.
- RUSKA, H. (1959). Das System der Zelle. *Stud. gen. (Heidelberg)*, **12**, 133-142.
- RUSKA, H., STUART, D. C., & WINSSEY, J. (1955). Electron microscopic visualization of intranuclear virus-like bodies in epithelial cells infected with poliomyelitis virus. *Arch. ges. Virusforsch.*, **6**, 379-387.
- RUSKA, H., EDWARDS, G. A., & CAESAR, R. (1958). A concept of intracellular transmission of excitation by means of the endoplasmic reticulum. *Experientia (Basel)*, **14**, 117-120.
- RUSTAD, R. C. (1959). An interference microscopical and cytochemical analysis of local mass changes in the mitotic apparatus during mitosis. *Exp. Cell Res.*, **16**, 575-583.
- RUTHMANN, A. (1958). The fine structure of the meiotic spindle of the crayfish. *J. biophys. biochem. Cytol.*, **5**, 177-180.
- RUTISHAUSER, A. (1956). Chromosome distribution and spontaneous chromosome breakage in Trillium grandiflorum. *Heredity*, **10**, 367-407.
- RYTER, A., KELLENBERGER, E., BIRCH-ANDERSEN, A., & MAALØE, O. (1958). Étude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoids des bactéries en croissance active. *Z. Naturforsch.*, **13b**, 597-605.
- SABATINI, D. D., & DE ROBERTIS, E. D. P. (1961). Ultrastructural zonation of adrenocortex in the rat. *J. biophys. biochem. Cytol.*, **9**, 105-119.
- SAGER, R., & PALADE, G. E. (1957). Structure and development of the chloroplast in Chlamydomonas. I. The normal green cell. *J. biophys. biochem. Cytol.*, **3**, 463-488.
- SAKAGUCHI, S. (1925). Über eine neue Farbenreaktion von Protein und Arginin. *J. Biochem. (Tokyo)*, **5**, 25-31.
- SAKAI, H. (1960a). Studies on sulfhydryl groups during cell division of sea urchin egg. II. Mass isolation of the egg cortex and change in its SH-groups during cell division. *J. biophys. biochem. Cytol.*, **8**, 603-607.
- (1960b). Studies on sulfhydryl groups during cell division of sea urchin egg. III.—SH groups of KCl-soluble proteins and their change during cleavage. *J. biophys. biochem. Cytol.*, **8**, 609-615.
- SAKAI, H., & DAN, K. (1959). Studies on sulfhydryl groups during cell division of sea urchin egg. (*Exp. Cell Res.*, **16**, 24-41).
- SAKAMURA, T. (1927). Chromosomenforschung am frischen Material. *Protoplasma (Wien)*, **1**, 537-565.
- SANDERSON, A. R., & STEWART, J. S. S. (1961). Nuclear sexing with aceto-orcein. *Brit. med. J.*, **1**, 1065-1067.
- SANDRITTER, W. (1958). Ultravioletmikroskopie. *Hdbch. d. Histochemie*, Vol. I. Fischer, Stuttgart.
- (1961). Methoden und Ergebnisse der quantitativen Histochemie. *Dtsch. med. Wschr.*, **86**, 2177-2183.
- SANDRITTER, W., & HÜBOTTER, F. (1954). Über die Bedeutung des Nucleolus in der Nebennierenrinde. *Frankfurt. Z. Path.*, **65**, 219-229.
- SANDRITTER, W., & SCHIEMER, H. G. (1958). Histochemische Untersuchungen an HeLa-Zellen. *Verh. disch. path. Ges.*, **42**, 449-458.
- SANDRITTER, W., BECKER, U., MÜLLER, D., & PFEIFFER, E. F. (1959). Histochemische Untersuchungen zur Frage der Funktion der B-Zellen der Langerhanschen Inseln nach Stimulierung mit D 860. *Endocrinologie*, **37**, 193-217.
- SANDRITTER, W., & KRYGIER, A. (1959). Cytophotometrische Bestimmungen von proteingebundenen Thiolen in der Mitose und Interphase von HeLa-Zellen. *Z. Krebsforsch.*, **62**, 596-610.
- SANDRITTER, W., SCHIEMER, H. G., & ALT, W. (1960a). Das Interferenzmikroskop im Dienste der Cytologie und Krebsforschung. *Klin. Wschr.*, **38**, 590-595.
- SANDRITTER, W., SCHIEMER, H. G., KRAUS, H., & DÖRRRIEN, U. (1960b). Interferenzmikroskopische

- Untersuchungen über das Wachstum von Einzelzellen (HeLa-Zellen) in der Gewebekultur. Frankfurt. *Z. Path.*, **70**, 271-299.
- SANDRITTER, W., MÜLLER, C., & MANTZ, O. (1960c). Zur Histochemie der Cytomegalie. *Frankfurt. Z. Path.*, **70**, 589-597.
- SANO, S., INOUÉ, S., TANABE, Y., SUMIYA, C., & KOIKE, S. (1959). Significance of mitochondria for porphyrin and heme biosynthesis. *Science*, **129**, 275-276.
- SARAM, M. (1957). Über die azelluläre Entstehung von Narben bei Durchblutungsstörungen im Herzmuskel. *Beitr. path. Anat.*, **118**, 275-291.
- SATŌ, S. (1958). Electron microscope studies on the mitotic figure. I. Fine structure of the metaphase spindle. *Cytologia (Tokyo)*, **23**, 383-393.
- (1959). Electron microscope studies on the mitotic figure. II. Phragmoplast and cell plate. *Cytologia (Tokyo)*, **24**, 98-106.
- (1960). Electron microscope studies on the mitotic figure III. Process of the spindle formation. *Cytologia (Tokyo)*, **25**, 119-131.
- SAUAIA, H., & MAZIA, D. (1961). Action of colchicine on the mitotic apparatus. *Path. et Biol.*, **9**, 473-476.
- SAUSER, G. (1936). Die Größe der Zellkerne in verschiedenen Tierklassen unter Berücksichtigung des Geschlechtes, der Domestikation und Kastration. *Z. Zellforsch.*, **23**, 677-700.
- SAX, H. J., & SAX, K. (1935). Chromosome structure and behaviour in mitosis and meiosis. *J. Arn. Arboret.*, **16**, 423-439.
- SAX, K., & HUMPHREY, L. M. (1934). Structure of meiotic chromosomes in microsporogenesis of *Tradescantia*. *Bot. Gaz.*, **96**, 353-362.
- SAX, K., & O'MARA, G. (1941). Mechanism of mitosis in pollen tubes. *Bot. Gaz.*, **102**, 629-636.
- SAX, K., & SWANSON, C. P. (1941). Differential sensitivity of cells to x-rays. *Amer. J. Bot.*, **28**, 52-59.
- SAX, K., KING, E. D., & LUIPPOLD, H. (1955). The effect of fractionated x-ray dosage on the frequency of chromatid and chromosome aberrations. *Rad. Res.*, **2**, 171-179.
- SCARASCIA, G.-T. (1953). Sviluppo del tappeto dell'antera in *Nicotiana tabacum* L. var. Bright. *Caryologia*, **5**, 25-42.
- SCHACHMANN, H. K., PARDEE, A. B., & STANIER, R. Y. (1952). Studies on the macromolecular organization of microbial cells. *Arch. Biochem. Biophys.*, **38**, 245-260.
- SCHAEDE, R. (1927). Vergleichende Untersuchungen über Cytoplasma, Kern und Kernteilung im lebenden und im fixierten Zustand. *Protoplasma (Wien)*, **3**, 145-190.
- (1928). Über das Verhalten während der Kernteilung. *Protoplasma (Wien)*, **5**, 41-54.
- (1929). Die Kolloidchemie des pflanzlichen Zellkerns in der Ruhe und in der Teilung. *Ergebn. Biol.*, **5**, 1-28.
- (1937). Anordnung und Gestalt der Chromosomen von *Galtonia candicans*. *Ber. dtsch. bot. Ges.*, **55**, 485-492.
- SCHARRER, E., & SCHARRER, B. (1954). Neurosekretion. *Hdbch. mikr. Anat. d. Mensch.*, Vol. 6/V. Springer, Berlin.
- SCHARRER, E., & BROWN, S. (1961). Neurosecretion. XII. The formation of neurosecretory granules in the earthworm, *Lumbricus terrestris* L. *Z. Zellforsch.*, **54**, 530-540.
- SCHAUMKELL, K. W., STANGE, H. H., & RUMPHORST, K. (1957). Über das Vorkommen von Desoxy-Ribonucleinsäure in den drumsticks polymorphkerniger Granulocyten. *Klin. Wschr.*, **35**, 1029-1030.
- SCHERBAUM, O. H. (1960). Possible sites of metabolic control during the induction of synchronous cell division. *Ann. N. Y. Acad. Sci.*, **90**, 565-579.
- SCHERBAUM, O., & ZEUTHEN, E. (1954). Induction of synchronous cell division in mass cultures of *Tetrahymena pyriformis*. *Exp. Cell Res.*, **6**, 221-227.
- SCHERBAUM, O. H., LOUDERBACK, A. L., & JAHN, T. L. (1959). DNA-synthesis, phosphate content and growth in mass and volume in synchronously dividing cells. *Exp. Cell Res.*, **18**, 150-166.
- SCHEVING, L. E. (1959). Mitotic activity in the human epidermis. *Anat. Rec.*, **135**, 7-20.
- SCHIEBLER, T. H., & KNOOP, A. (1959). Korrelation zwischen elektronenmikroskopischen und histochemicalen Befunden, erläutert am Beispiel der Placentarriesenzelle. *Verh. anat. Ges.*, **1958** (*Anat. Anz.*), 206-211.
- SCHIEMER, H. G. (1961). Über den Wassergehalt von normalen und geschädigten Zellen sowie von Karzinomzellen. *Verh. dtsch. path. Ges.*, **45**, 135-142.
- SCHIEMER, H. G., ALT, W., & SANDRITTER, W. (1957). Zur Methodik der Trockengewichtsbestimmungen mit dem Bakterschen Interferenzmikroskop. *Acta histochem. (Jena)*, **4**, 325-360.
- SCHILLER, A. A., SCHAYER, R. W., & HESS, E. L. (1952). Fluorescein-conjugated bovine albumin. Physical and biological properties. *J. gen. Physiol.*, **36**, 489-505.
- SCHILLER, E. (1944). Über Kernsekretion in der Nebennierenrinde. *Z. mikr.-anat. Forsch.*, **54**, 598-603.
- (1949). Variationsstatistische Untersuchungen über Kerneinschlüsse und -krystalle in der menschlichen Leber. *Z. Zellforsch.*, **34**, 337-355.
- SCHIMPER, A. (1885). Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. *Jb. wiss. Bot.*, **16**, 1-247.

- SCHLAGER, F. (1960). Nukleolenveränderungen an der Leber der Maus nach parenteraler Eiweißzufuhr. *Anat. Anz.*, **108**, 26-37.
- SCHLEICHER, W. (1878). Die Knorpel-Zellteilung. *Arch. mikr. Anat.*, **16**, 248-300.
- SCHLEIDEN, M. (1838). Beiträge zur Phylogenesis. *Müller's Arch.*, 137-176.
- SCHMID, R. (1958). Über die Struktur und Teilung der Kerne von *Geotrichum candidum* und *Geotrichum magnusi*. *Planta (Ber.)*, **52**, 320-333.
- SCHMIDT, F.-C. (1961). Ergastoplasma. *Dtsch. med. Wschr.*, **86**, 1823-1830.
- SCHMIDT, R. R. (1961). Nitrogen and phosphorus metabolism during synchronous growth of *Chlorella pyrenoidosa*. *Exp. Cell Res.*, **23**, 209-217.
- SCHMIDT, W. J. (1936). Die Doppelbrechung der Kernspindel und ihre Feinstruktur, ein Argument für die Zugfasertheorie und die Fadenmolekültheorie. *Biodynamica*, **1**, 1-5.
- (1937). *Die Doppelbrechung von Karyoplasma, Cytoplasma und Metaplasma*. Borntraeger, Berlin.
- (1939a). Über Doppelbrechung und Feinbau der Kernmembran. *Protoplasma (Wien)*, **32**, 193-198.
- (1939b). Doppelbrechung der Kernspindel und Zugfasertheorie der Chromosomenbewegung. *Chromosoma (Berl.)*, **1**, 255-264.
- (1941). Einiges über optische Anisotropie und Feinbau von Chromatin und Chromosomen. *Chromosoma (Berl.)*, **2**, 86-111.
- (1942). Polarisationsmikroskopische Beobachtungen an *Amoeba proteus*. *Protoplasma (Wien)*, **36**, 370-380.
- SCHNEIDER, B. (1933). Über die Umordnung der Chromosomen während der Mitose. *Z. Zellforsch.*, **17**, 255-312.
- (1938). Die Zellteilung der Pflanzenzelle im Reihenbild (Beobachtungen an *Tradescantia virginica*). *Z. Zellforsch.*, **28**, 829-860.
- (1939). Die Plasmaveränderungen bei der Pflanzenzellteilung. *Arch. exp. Zellforsch.*, **22**, 298-303.
- SCHNEIDER, L. (1959). Neue Befunde über den Feinbau des Cytoplasmas von *Paramecium* nach Einbettung in Vestopal. *W. Z. Zellforsch.*, **50**, 61-77.
- SCHNEIDER, R. M. (1955). The effect of anions on the optical properties of rat liver nuclei isolated in glycerol solutions. *Exp. Cell Res.*, **8**, 24-34.
- SCHNEIDER, W. C. (1948). Intracellular distribution of enzymes. III. The oxidation of octanoic acid by rat liver fractions. *J. biol. Chem.*, **176**, 259-266.
- (1959). Mitochondrial metabolism. *Advanc. Enzymol.*, **21**, 1-72.
- SCHNEIDER, W. C., & HOGEBOOM, G. H. (1956). Biochemistry of cellular particles. *Ann. Rev. Biochem.*, **25**, 201-224.
- SCHNEPF, E. (1961). Quantitative Zusammenhänge zwischen der Sekretion des Fangschleimes und den Golgi-Strukturen bei *Drosophyllum lusitanicum*. *Z. Naturforsch.*, **16b**, 605-610.
- SCHÖBERL, A. (1962). Moderne Eiweißforschung. *Naturw. Rdsch.*, **15**, 9-13.
- SCHOLTISSEK, CH. (1962). An unstable ribonucleic acid in rat liver nuclei. *Nature (Lond.)*, **194**, 353-355.
- SCHOLTISSEK, CH., & POTTER, V. R. (1960). Austritt von Ribonucleinsäure aus isolierten Rattenleber-Zellkernen während der Inkubation in vitro. *Z. Zellforsch.*, **15b**, 453-460.
- SCHRADER, F. (1931). The Chromosome cycle of *Prototonia primitiva* (Coccidae) and a consideration of the meiotic division apparatus in the male. *Z. wiss. Zool.*, **138**, 386-409.
- (1932). Recent hypotheses on the structure of spindles in the light of certain observations in hemiptera. *Z. wiss. Zool.*, **142**, 520-539.
- (1934). On the reality of spindle fibers. *Biol. Bull.*, **67**, 519-533.
- (1935). Notes on the mitotic behaviour of long chromosomes. *Cytologia (Tokyo)*, **6**, 422-430.
- (1936). The kinetochore or spindle fibre locus in *Amphiuma tridacrylum*. *Biol. Bull.*, **70**, 484-498.
- (1939). The structure of the kinetochore at meiosis. *Chromosoma (Berl.)*, **1**, 230-237.
- (1941). The spermatogenesis of the earwig *Anisolabis maritima* Bon. with reference to the mechanism of chromosomal movement. *J. Morph.*, **68**, 123-148.
- (1947). Data contributing to analysis of metaphase mechanism. *Chromosoma (Berl.)*, **3**, 22-47.
- (1954). Mitose. *Die Bewegungen der Chromosomen bei der Zellteilung*. Deuticke, Wien.
- SCHRADER, F., & LEUCHTENBERGER, C. (1950). A cytochemical analysis of the functional interrelations of various cell structures in *Arvelius albopunctatus* (de Geer). *Exp. Cell Res.*, **1**, 421-452.
- SCHRADER, F., & HUGHES-SCHRADER, S. (1952). Polyploidy and fragmentation in the chromosomal evolution of various species of *Thyanta* (Hemiptera). *Chromosoma (Berl.)*, **7**, 469-496.
- SCHRADER, F., & LEUCHTENBERGER, C. (1952). The origin of certain nutritive substances in the eggs of hemiptera. *Exp. Cell Res.*, **3**, 136-146.
- SCHRADER, F., & HUGHES-SCHRADER, S. (1958). Chromatid autonomy in *Banasa* (Hemiptera: Pentatomidae). *Chromosoma (Berl.)*, **9**, 193-215.
- SCHREIBER, G. (1949). Statistical and physiological studies on the interphasic growth of the nucleus. *Biol. Bull.*, **97**, 187-205.

- SCHREIBER, M., SANDRITTER, W., & GEDIGK, P. (1960). Ultravioletmikrospektrophotometrische Untersuchungen am Eisenpigment. *Virchows Arch. path. Anat.*, **333**, 288-293.
- SCHREK, R. (1960). Radiation effects on lymphocytes. In: *The Lymphocyte and Lymphocytic Tissue*. Hoeber, New York.
- SCHRÖTER, G. (1937). Variationsstatistische Untersuchungen über die Kerngrößen in den Leberzellen der weißen Maus bei verschiedener Fütterung. *Z. Zellforsch.*, **26**, 481-506.
- SCHUBOTHE, H., & ALTMANN, H. W. (1950). Kältchämaggglutinine als Ursache chronischer hämolytischer Anämien. *Z. klin. Med.*, **146**, 428-479.
- SCHÜMMELFEDER, N. (1959). Der Verlauf der experimentellen Strahlenschädigung des Hirngewebes. *Verh. dtsch. path. Ges.*, **42**, 244-250.
- SCHÜMMELFEDER, N., KROGH, E., & EBSCHNER, K. J. (1958). Färbungsanalysen zur Acridinorange-Fluorochromierung. Vergleichende histochemische und fluoreszenzmikroskopische Untersuchungen am Kleinhirn der Maus mit Acridinorange- und Gallocyanin-Chromalaun-Färbungen. *Z. Zellf. Abt. Histochemie*, **1**, 1-28.
- SCHÜMMELFEDER, N., WESSEL, W., & NESSEL, E. (1959). Die Wirkung von 3,6-Diaminoacridinen auf Wachstum und Zellteilung im Ehrlich-Ascitestumor. *Z. Krebsforsch.*, **63**, 129-141.
- SCHULTZ, J. (1941). The evidence of the nucleoprotein nature of the gene. *Cold Spr. Harb. Symp. quant. Biol.*, **9**, 55-65.
- (1947). The nature of heterochromatin. *Cold Spr. Harb. Symp. quant. Biol.*, **12**, 179-191.
- (1956). The relation of the heterochromatic chromosome regions to the nucleic acids of the cell. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 307-328.
- SCHULTZE, B., OEHLMER, W., & MAURER, W. (1959). Autoradiographische Untersuchung zum Mechanismus der Eiweißbildung in Ganglienzellen. *Beitr. path. Anat.*, **120**, (1959), 58-84.
- SCHULTZE, B., & OEHLMER, W. (1960). Autoradiographic investigation of incorporation of H^3 -thymidine into cells of the rat and mouse. *Science (Wash.)*, **131**, 737-738.
- SCHULTZE, K. W. (1958). Zur Bedeutung der Abortiveier und über ihre zellkernmorphologische Geschlechtsbestimmung. *Dtsch. med. Wschr.*, **83**, 1818-1823.
- SCHULTZE, M. (1861). Über Muskelkörperchen und das, was man eine Zelle zu nennen habe. *Arch. Anat. u. Physiol.*, 1-27.
- SCHULTZ-LARSEN, J. (1953). On the structure of the nuclear spindle. An electron microscopic study. *Acta path. microbiol. scand.*, **32**, 567-573.
- SCHULZ, H. (1958a). Die Pathologie der Mitochondrien im Alveolarepithel der Lunge. *Beitr. path. Anat.*, **119**, 45-70.
- (1958b). Die submikroskopische Pathologie der Cytosomen in den Alveolarmakrophagen der Lunge. *Beitr. path. Anat.*, **119**, 71-91.
- (1959). *Die submikroskopische Anatomie und Pathologie der Lunge*. Springer, Berlin.
- SCHULTZ, H., LÖW, H., ERNSTER, L., & SJÖSTRAND, F. J. (1956). Elektronenmikroskopische Studien an Leberschnitten von thyroxinbehandelten Ratten. *Proc. Stockholm Conf.*, 134-137.
- SCHULZ, H., & DE PAOLA, D. (1958). Delta-Cytomembranen und lamelläre Cytosomen. Ultrastruktur, Histochemie und ihre Beziehungen zur Schleimsekretion. *Z. Zellforsch.*, **49**, 125-141.
- SCHULZ, H., & WEDELL, J. (1962). Elektronenmikroskopische Untersuchungen zur Frage der Fettphagocytose und des Fettransportes durch Thrombocyten. *Klin. Wschr.*, **40**, 1114-1120.
- SCHULZE, K. L. (1939). Cytological Untersuchungen an *Acetabularia mediterranea* und *Acetabularia wettsteinii*. *Arch. Protistenk.*, **92**, 179-225.
- SCHUMAKER, V. N. (1958). Uptake of protein from solution by *Amoeba proteus*. *Exp. Cell Res.*, **15**, 314-331.
- SCHUSTER, H., & SCHRAMM, G. (1958). Bestimmung der biologisch wirksamen Einheit in der Ribonucleinsäure des Tabakmosaikvirus auf chemischem Wege. *Z. Naturforsch.*, **13b**, 697-704.
- SCHWANITZ, F., & PIRSON, H. (1955). Chromosomengröße, Zellgröße und Zellzahl bei einigen diploiden Gigaspflanzen. *Züchter*, **25**, 221-229.
- SCHWANN, T. (1839). *Mikroskopische Untersuchungen über die Übereinstimmungen in der Struktur und dem Wachstum der Tiere und Pflanzen*. Sander, Berlin.
- SCHWARTZ, D. (1952). The effect of oxygen concentration on x-ray-induced chromosome breakage in maize. *Proc. nat. Acad. Sci. (Wash.)*, **38**, 490-494.
- (1953). The behaviour of an x-ray-induced ring chromosome in maize. *Amer. Nat.*, **87**, 19-28.
- (1960). Deoxyribonucleic acid and chromosome structure. In: *The Cell Nucleus*. Butterworths, London.
- SCHWARTZ, V. (1957). Über den Formwechsel achromatischer Substanz in der Teilung des Makronukleus von *Paramecium bursaria*. *Biol. Zbl.*, **76**, 1-23.
- (1958). Chromosomen im Makronukleus von *Paramecium bursaria*. *Biol. Zbl.*, **77**, 347-364.
- SCHWARZACHER, H. G. (1962). Die Beziehungen zwischen Geschlechtschromatin und Geschlechtschromosomen. *Wien klin. Wschr.*, **74**, 481-484.

- SCHWEIGER, H. G., & BREMER, H. J. (1960). Nachweis cytoplasmitischer Ribonucleinsäuresynthese in kernlosen Acetabularien. *Exp. Cell Res.*, **20**, 617-618.
- (1961). Cytoplasmatische RNS-Synthese in kernlosen Acetabularien. *Biochim. biophys. Acta (Amst.)*, **51**, 50-59.
- SCHWEMMLE, J. (1927). Der Bastard Oenothera Berteriana x Onagra (muricata) und seine Cytologie. *Jb. wiss. Bot.*, **66**, 579-595.
- SCOTT, A. (1960a). Furrowing in flattened sea urchin eggs. *Biol. Bull.*, **119**, 246-259.
- (1960b). Surface changes during cell division. *Biol. Bull.*, **119**, 260-272.
- (1961). Surface changes during cytokinesis in flattened eggs. *Biol. Bull.*, **121**, 407.
- SCOTT, D. B. M., & CHU, E. (1958). Synchronized division of growing cultures of Escherichia coli. *Exp. Cell Res.*, **14**, 166-174.
- SEDAR, A. W., & ROSA, CH. G. (1961). Cytochemical demonstration of the succinic dehydrogenase system with the electron microscope using nitro-blue tetrazolium. *J. Ultrastruct. Res.*, **5**, 226-243.
- SEED, J. (1960). X-irradiation of the nucleolus and its effect on nucleic acid synthesis. In: *The Cell Nucleus*. Butterworths, London.
- SEIFERT, G. (1952). Über Kerneinschlüsse im menschlichen Pankreas. *Zbl. Path.*, **88**, 203-208.
- SEIFERT, G., & GIESEKING, R. (1961). Elektronenmikroskopische Befunde am Rattenpankreas nach experimenteller Äthioninschädigung. *Beitr. path. Anat.*, **124**, 81-107.
- SEIFERZ, W. (1936). *The protoplasm*. McGraw-Hill, New York.
- SELMAN, G. G., & WADDINGTON, C. H. (1955). The mechanism of cell division in the cleavage of the newt's egg. *J. exp. Biol.*, **32**, 700-733.
- SENN, G. (1908). *Die Gestalt- und Lageveränderung der Pflanzenchromatophoren*. Engelmann, Leipzig.
- SENTEIN, P. (1951). Sur les déviations de l'axe mitotique au cours de la segmentation de l'œuf traité par la colchicine et leur signification. *C. R. Soc. Biol. (Paris)*, **145**, 97-99.
- (1961). L'action des antimitotiques pendant la segmentation de l'œuf et le mécanisme de cette action. *Path. et Biol.*, **9**, 445-466.
- SERBER, B. J. (1961). Large nuclear inclusions in pituitary gland basophils of the golden hamster. *Anat. Rec.*, **139**, 345-355.
- SERR, M. D., SACHS, L., & DANON, M. (1955). The diagnosis of sex before birth using cells from the amniotic fluid. *Bull. Res. Coun. Israel*, **5B**, 137.
- SERRA, J. A. (1947). Composition of chromonemata and matrix and the role of nucleoproteins in mitosis and meiosis. *Cold Spr. Harb. Symp. quant. Biol.*, **12**, 192-210.
- (1958). Interpretation of nuclear inclusions. *Nature (Lond.)*, **181**, 1544-1545.
- SETTERFIELD, G., & DUNCAN, R. E. (1955). Cytological studies on the antimetabolite action of 2,6-diaminopurine in Vicia faba roots. *J. biophys. biochem. Cytol.*, **1**, 399-419.
- SHAH, P. N., NAIK, S. N., MAHAJAN, D. K., PAYMASTER, J. C., DAVE, M. J., & TIWARI, R. (1961). Male pseudohermaphroditism with female chromosomal complement. *J. clin. Endocrin.*, **21**, 727-731.
- SHAPIRO, H. (1935). The respiration of fragments obtained by centrifuging the egg of the sea urchin, Arbacia punctulata. *J. cell. comp. Physiol.*, **6**, 101-116.
- SHAPIRO, I. M. (1961). On the role of nucleus in the mechanism of cell division (based on the data obtained in irradiation experiments). *Path. et biol.*, **9**, 490-492.
- SHARMA, A. K., & SHARMA, A. (1958). Recent advances in the study of chromosome structure. *Bot. Rev.*, **24**, 511-549.
- SHARMA, A. K., & BHATTACHARYYA, N. K. (1959). Cytology of several members of Zingiberaceae and a study of the inconstancy of their chromosome complements. *La Cellule*, **59**, 299-346.
- SHARMA, A. K., & SHARMA, A. (1960). Spontaneous and chemically induced chromosome breaks. *Int. Rev. Cytol.*, **10**, 101-136.
- SHARMA, S. P., & MANOCHA, S. L. (1962). The classical Golgi apparatus in the vertebrate nerve cells. *Experientia (Basel)*, **18**, 135-136.
- SHARP, L. W. (1934). *An introduction to cytology*. 3rd edition. McGraw-Hill, New York.
- SHELDON, H., & KIMBALL, F. B. (1962). Studies on cartilage. III. The occurrence of collagen within vacuoles of the Golgi apparatus. *J. Cell Biol.*, **12**, 599-613.
- SHELDON, H., SILVERBERG, M., & KERNER, I. (1962). On the differing appearance of intranuclear and cytoplasmic glycogen in liver cells in glycogen storage disease. *J. Cell Biol.*, **13**, 468-473.
- SHETTLES, L. B. (1959). Die lebende menschliche Eizelle. *Arch. Gynäk.*, **193**, 278-284.
- (1960). Nuclear morphology of human spermatozoa. *Nature (Lond.)*, **186**, 648-649.
- SHIMAMURA, T. (1940). Studies on the effects of the centrifugal force upon nuclear division. *Cytologia (Tokyo)*, **11**, 186-216.
- SHIMAMURA, T., & OTA, T. (1956). Cytochemical studies on the mitotic spindle and the phragmoplast of plant cells. *Exp. Cell Res.*, **11**, 346-361.
- SHINKE, N. (1937). An experimental study on the structure of living nuclei in the resting stage. *Cytologia Fujii Jub.*, Vol. 449-463.

- SHINKE, N., & SHIGENA, M. (1933). A histochemical study of plant nuclei in rest and mitosis. *Cytologia (Tokyo)*, **4**, 189-221.
- SHINKE, N., & UEDA, K. (1956). A cytomorphological and cytochemical study of Cyanophyta I. An electron microscope study of Oscillatoria princeps. *Mem. Coll. of Sci. Univ. Kyoto, Ser. B*, **23**, 101-104.
- SHORT, R. B. (1946). Observations on the giant amoeba, Amoeba carolinensis (Wilson, 1900). *Biol. Bull.*, **90**, 8-18.
- SIEBERT, G. (1958a). Der Zellkern der somatischen Zelle. **9. Coll. Ges. physiol. Chem.** Springer, Berlin.
- (1958b). Bestimmung von Glykolyse-Metaboliten in isolierten Zellkernen. *Experientia (Basel)*, **14**, 449.
- (1961). Gewebsproliferation und Zellstrukturen in biochemischer Sicht. *Verh. dtsh. path. Ges.*, **45**, 59-74.
- SIEBERT, G., & SMELLIE, R. M. S. (1957). Enzymatic and metabolic studies on isolated nuclei. *Int. Rev. Cytol.*, **6**, 383-424.
- SIEBS, W. (1960). Mitoseablaufstörungen. I. Polchromosomen- und Dreigruppen-Metaphasen, (Chromosomenspindelfaserstörung). *Z. Zellforsch.*, **51**, 497-524.
- SIEGERT, R. (1960). Elektronenoptische Untersuchungen über die Kernveränderungen herpesinfizierter Zellen. *Wien. Z. Nervenheilk.*, **18**, 159-178.
- SIEKERVITZ, P. (1952). Uptake of radioactive alanine in vitro into the proteins of rat liver fractions. *J. biol. Chem.*, **195**, 549-565.
- SIEKERVITZ, P., & PALADE, G. F. (1958). A cytochemical study on the pancreas of the guinea pig. II. Functional variations in the enzymatic activity of microsomes. *J. biophys. biochem. Cytol.*, **4**, 309-318.
- SIEKERVITZ, P., & PALADE, G. E. (1960). A cytochemical study on the pancreas of the guinea pig. V. In vivo incorporation of leucine- I-C^{14} into the chymotrypsinogen of various cell fractions. *J. biophys. biochem. Cytol.*, **7**, 619-630.
- SIMONET, M., & GUINOCHET, M. (1939). Anomalies morphologiques et caryologiques provoquées, sur les jeunes plantules, par les dérivés halogènes des carbures cycliques. *C. R. Soc. Biol. (Paris)*, **131**, 222-224.
- SINAPIUS, D. (1961). Zur Darstellung maskierter Fette der Leber. *Verh. dtsh. path. Ges.*, **45**, 297-298.
- SINHA, S. K. (1960). *Med. Diss. Indiana Univ.* Bloomington, Indiana.
- SINNOTT, E. W., & BLOCH, R. (1941). Division in vacuolate plant cells. *Amer. J. Bot.*, **28**, 225-232.
- SINSHEIMER, R. L. (1959). A single-stranded deoxyribonucleic acid from bacteriophage ΦX 174. *J. molec. Biol.*, **1**, 43-53.
- SIRLIN, J. L. (1958). Autoradiography of nucleolar contents. *Exp. Cell Res.*, **14**, 447-453.
- (1960a). Facts and speculation on the function of nuclear components. In: *The Cell Nucleus*. Butterworths, London.
- (1960b). Cell sites of RNA and protein synthesis in the salivary gland of *Smittia* (Chironomidae). *Exp. Cell. Res.*, **19**, 177-180.
- (1961). Der Nucleolus. *Endeavour*, **20**, 146-153.
- SIRLIN, J. L., KATO, K., & JONES, K. W. (1961). Synthesis of ribonucleic acid in the nucleolus. *Biochim. biophys. Acta (Amst.)*, **48**, 421-423.
- SIRLIN, J. L., & SCHOR, N. A. (1962a). Macromolecular syntheses in isolated polytene nuclei. *Exp. Cell Res.*, **27**, 165-167.
- (1962b). Further observations on isolated polytene nuclei. *Exp. Cell Res.*, **27**, 363-366.
- SISKEN, J. E. (1959). The synthesis of nucleic acids and proteins in the nuclei of *Tradescantia* root tips. *Exp. Cell Res.*, **16**, 602-614.
- SISKEN, J. E., & KINOSITA, R. (1961). Intranuclear incorporation of tritiated cytidine. *Exp. Cell Res.*, **24**, 168-170.
- (1961). Timing of DNA synthesis in the mitotic cycle in vitro. *J. biophys. biochem. Cytol.*, **9**, 509-618.
- SISSAKIAN, N. M. (1958). Enzymology of the plastids. *Advanc. Enzymol.*, **20**, 201-236.
- SITTE, P. (1958). Die Ultrastruktur von Wurzelmeristemzellen der Erbsa (*Pisum sativum*). *Protoplasma (Wien)*, **49**, 447-522.
- SJÖSTRAND, F. S. (1954a). The ultrastructure of mitochondria. *Symp. 8 Congr. of Cell Biol., Leiden*.
- (1954b). The ultrastructure of the ground substance of the cytoplasm. *Symp. 8 Congr. of Cell Biol., Leiden*.
- (1956). The ultrastructure of cells as revealed by the electron microscope. *Int. Rev. Cytol.*, **5**, 455-533.
- SJÖSTRAND, F. S., & RHODIN, J. (1953). The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy. *Exp. Cell Res.*, **4**, 426-456.
- SJÖSTRAND, F. S., & HANZON, V. (1954). Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas. *Exp. Cell. Res.*, **7**, 415-429.
- SLUITER, J. W. (1944). Das Restitutionsproblem in der Pankreaszelle. II. Vitamin C und Golgi-Körper. *Z. Zellforsch.*, **33**, 299-310.

- SMITH, D. W., PATAU, K., & THERMAN, E. (1962). The Nr. 18 trisomy syndrome. *J. Pediat.*, **60**, 513-527.
- SMITH, K. M. (1958a). Virus inclusions in plant cells. *Protoplasmatologia*, **IV/4a**, Springer, Wien.
- (1958b). Virus inclusions in insect cells. *Protoplasmatologia*, **IV/4b**, Springer, Wien.
- SMOLLICH, A. (1959). Zur Problematik der Karyometrie unter besonderer Berücksichtigung des Fixierungseinflusses auf die Kerngröße der Nebenniere des Schweines. *Arch. exp. Vet.-Med.*, **13**, 26-30.
- SOTELO, J. R., & TRUJILLO-CENÓZ, O. (1958). Submicroscopic structure of meiotic chromosomes during prophase. *Exp. Cell Res.*, **14**, 1-8.
- (1960). Electron microscopy study on spermatogenesis. Chromosome morphogenesis at the onset of meiosis (cyte I) and nuclear structure of early and late spermatids. *Z. Zellforsch.*, **51**, 243-277.
- SPARROW, A. H. (1942). The structure and development of the chromosome spirals in microspores of Trillium. *Canad. J. Res.*, **D 20**, 257-266.
- SPARROW, A. H., & HAMMOND, M. R. (1947). Cytological evidence for the transfer of desoxyribose nucleic acid from nucleus to cytoplasm in certain plant cells. *Amer. J. Bot.*, **34**, 439-445.
- SPATZ, H. (1952). Neuronenlehre und Zellenlehre. *Münch. med. Wschr.*, **94**, 1154-1163, 1210-1218, 1255-1262.
- (1959). *Franz Nissl. Große Nervenärzte*, Bd. II. Thieme, Stuttgart.
- SPECHT, W. (1959). Zur Spezifität der Methylgrün-Pyronin-Färbung. *Acta histochem. (Jena) Suppl.*, **II**, 200-206.
- (1961). Bildung, Bau und Funktion des sog. achromatischen Teilungsapparates der Zelle, erläutert am Beispiel der Reifungsspindele im Ei von *Tubifex*. *Z. Anat. Entwickl.-Gesch.*, **122**, 266-288.
- SPECHTER, H. J., RUFF, O., & BARTELHEIMER, A. (1962). Untersuchungen über das Vorkommen von Barrischen Chromatinkörperchen in Ovarialtumoren. *Gebursh. u. Frauenheilk.*, **22**, 61-67.
- SPER, J. (1918). Oberflächenspannungsdifferenzen als eine Ursache der Zellteilung. *Arch. Entwickl.-Mech.-Org.*, **44**, 5-113.
- (1925). Die Protoplasmabewegung, ihre Haupttypen, ihre experimentelle Beeinflussung und ihre theoretische Erklärung. *Hdbch. normal u. path. Physiol.* VIII. Springer, Berlin.
- SPERANSKY, A. D. (1950). *Grundlagen der Theorie der Medizin*. Saenger, Berlin.
- SRINIVASACHAR, D. (1958). A case of sub-haploid cells in mitosis. *Cytologia (Tokyo)*, **23**, 419-421.
- STAMMLER, A. (1950). Histochemische Untersuchungen des "lipoiden Pigmente" in den Ganglien-zellen des Gehirns. *Virchows Arch. path. Anat.*, **332**, 347-357.
- STAUBESAND, J. (1960). Experimentelle elektronenmikroskopische Untersuchungen zum Phänomen der Membranvesikelation (Pinocytose). *Klin. Wschr.*, **38**, 1248-1249.
- STAUBESAND, J., & SCHMIDT, W. (1960). Zur Histophysiologie des Herzbeutels. I. Mitteilung. Elektronenmikroskopische Beobachtungen an den Deckzellen des Peri- und Epikards. *Z. Zellforsch.*, **53**, 55-68.
- STAUDINGER, H., & STAUDINGER, M. (1954). Die makromolekulare Chemie und ihre Bedeutung für die Protoplasmaforschung. *Protoplasmatologia*, Vol. I. Springer, Wien.
- STEDMANN, E., & STEDMANN, E. (1950). Cell specificity of histones. *Nature (Lond.)*, **166**, 780-781.
- STEFANI, R. (1955). Divisioni amitotiche e modificazioni durante l'oogenesi nell'ovario degli Embiotteri. *Boll. Zool.*, **22**, 79-91.
- STEFFENSEN, D. (1953). Induction of chromosome breakage at meiosis by a magnesium deficiency in *Tradescantia*. *Proc. nat. Acad. Sci. (Wash.)*, **39**, 613-620.
- (1961). Chromosome structure with special reference to the role of metal ions. *Int. Rev. Cytol.*, **12**, 163-197.
- STEINERT, M. (1960). Mitochondria associated with the kinetonucleus of *Trypanosoma mega*. *J. biophys. biochem. Cytol.*, **8**, 542-546.
- STENRAM, U. (1952). The effect of vitamin B₁₂ and the animal protein factor on thyroid fed rats, with special reference to liver cytology. *Exp. Cell Res.*, **3**, 147-153.
- (1958a). Interferometric determinations of the ribose nucleic acid concentration in liver nucleoli of protein-fed, and protein-deprived rats. *Exp. Cell Res.*, **15**, 174-183.
- (1958b). The volume and ribose nucleic acid concentration of nucleoli in liver and hepatoma cells of rats fed on high and non protein diets. *Acta path. microbiol. scand.*, **44**, 239-246.
- (1961). Interferometric and refractometric determinations of the dry matter concentration of nuclei and nucleoli. *Exp. Cell Res.*, **22**, 545-558.
- (1962a). On protein deficiency in mice with special reference to liver nucleolar size. *Z. Zellforsch.*, **56**, 125-129.
- (1962b). Radioautographic studies with methionine-³H and cytidine-³H on protein deficiency in mice and rats with special reference to liver cells. *Exp. Cell Res.*, **26**, 485-492.
- STERN, C. (1936). Somatic crossing over and segregation in *Drosophila melanogaster*. *Genetics*, **21**, 625-730.
- (1958). The nucleus and somatic cell variation. *J. cell comp. Physiol.*, **52 Suppl. 1**, 1-34.
- STERN, H. (1958). Variations in sulfhydryl concentration during microsporocyte meiosis in the anthers of *Lilium* and *Trillium*. *J. biophys. biochem. Cytol.*, **4**, 157-161.

- STERN, H., & KIRK, P. L. (1948). The oxygen consumption of the microspores of Trillium in relation to the mitotic cycle. *J. gen. Physiol.*, **31**, 243-248.
- STERN, H., ALLFREY, V. G., MIRSKY, A. E., & SAETREN, H. (1952). Some enzymes of isolated nuclei. *J. gen. Physiol.*, **35**, 559-578.
- STERN, H., & MIRSKY, A. E. (1952). The isolation of wheat germ nuclei and some aspects of their glycolytic metabolism. *J. gen. Physiol.*, **36**, 181-200.
- STERN, H., JOHNSTON, F. B., & SETTERFIELD, G. (1959). Some chemical properties of isolated pea nucleoli. *J. biophys. biochem. Cytol.*, **6**, 57-60.
- STERN, H., & FRIEDMANN, J. (1960). Intranuclear formation of cytomegalic inclusion disease virus. *Nature (Lond.)*, **188**, 769-770.
- STEWART, J. S. S. (1960). Genetic mechanism in human intersexes. *Lancet*, **i**, 825-826.
- STEWART, R. N., & BAMFORD, R. (1942). The chromosomes and nucleoli of *Medeola virginiana*. *Amer. J. Bot.*, **29**, 301-303.
- STICH, H. (1951a). Das Vorkommen von Ribonucleinsäure in Kernsaft und Spindel sich teilender Kerne von Cyclops strenuus. *Z. Naturforsch.*, **6b**, 259-261.
- (1951b). Experimentelle karyologische und cytochemische Untersuchungen an Acetabularia mediterranea. (Ein Beitrag zur Beziehung zwischen Kerngröße und Eiweißsynthese.) *Z. Naturforsch.*, **6b**, 319-326.
- (1954). Stoffe und Strömungen in der Spindel von Cyclops strenuus. Ein Beitrag zur Mechanik der Mitose. *Chromosoma (Berl.)*, **6**, 199-236.
- (1956). Bau und Funktion der Nukleolen. *Experientia (Basel)*, **12**, 7-14.
- (1960). Regulation of mitotic rate in mammalian organisms. *Ann. N. Y. Acad. Sci.*, **90**, 603-609.
- STICH, H., & HÄMMERLING, J. (1953). Der Einbau von ^{32}P in die Nukleolarsubstanz des Zellkerns von Acetabularia mediterranea. *Z. Naturforsch.*, **8b**, 329-333.
- STICH, H., & MCINTYRE, J. (1958). X-ray absorption studies on the nuclear protein and RNA content during the development of the mitotic apparatus. *Exp. Cell Res.*, **14**, 635-638.
- STICH, H., FLORIAN, S. F., & EMSON, H. E. (1960). The DNA content of tumor cells. I. Polyps and adenocarcinomas of the large intestine of man. *J. nat. Cancer Inst.*, **24**, 471-482.
- STOBBE, H. (1958). Der "Golgi-Apparat" vitaler Blut- und Knochenmarkzellen. *Z. ges. inn. Med.*, **13**, 772-776.
- STOCKING, C. R. (1956). Histology and development of the root. *Hdb. Pflanzenphysiol.*, **3**. Springer, Berlin.
- STOCKINGER, L. (1953). Das Kernkörperchen. *Protoplasma (Wien)*, **42**, 365-413.
- STOCKINGER, L., & KELLNER, G. (1952). Der Lymphocytennukleolus. I. Mittl. Die Darstellung und Bedeutung des Nukleolus. *Wien. Z. inn. Med.*, **33**, 135-141.
- STOECKENIUS, W. (1957a). Golgi-Apparat und Centriol menschlicher Plasmazellen. *Frankfurt. Z. Path.*, **68**, 404-409.
- (1957b). Zur Feinstruktur der Granula menschlicher Gewebsmastzellen. *Exp. Cell Res.*, **11**, 656-658.
- STÖCKER, E. (1962a). Autoradiographische Untersuchungen zur Deutung der funktionellen Kernschwellung am exokrinen Pankreas. *Z. Zellforsch.*, **57**, 47-62.
- (1962b). Autoradiographische Untersuchungen zur Ribonucleinsäure- und Eiweiß-Synthese im nuklearen Funktionsformwechsel der exokrinen Pankreaszelle. *Z. Zellforsch.*, **57**, 145-171.
- STÖCKER, E., MAURER, W., & ALTMANN, H. W. (1961a). Autoradiographische Untersuchungen mit H^3 -Cytidin über die RNS-Synthese in Nukleolus und Kern und die Migration der RNS zum Cytoplasma während des Funktionsformenwechsels im exokrinen Pankreas. *Naturwissenschaften*, **48**, 582-583.
- (1961b). Autoradiographische Untersuchungen der Eiweiß- und RNS-Synthese mit H^3 -Leucin und H^3 -Cytidin zur Deutung der funktionellen Kernschwellung während des Funktionsformwechsels im exokrinen Pankreas. *Klin. Wschr.*, **39**, 926-927.
- STÖHR, PH. JR. (1941). Zusammenfassende Ergebnisse über die normale und pathologische Histologie der sympathischen Ganglionzellen und der Endapparate im vegetativen Nervensystem. *Ergebn. Anat. Entwickl.-Gesch.*, **33**, 135-284.
- (1951). *Lehrbuch der Histologie und der mikroskopischen Anatomie des Menschen*. Springer, Berlin.
- STOLK, A. (1960). The role of the Golgi apparatus in the formation of melanin granules in the malignant cutaneous melanoma of killifish hybrids. *Naturwissenschaften*, **47**, 448-449.
- (1961). Two types of ribonucleoprotein in the nucleolus of intestinal carcinoma of the newt following injection of herring-sperm deoxyribonucleic acid. *Nature (Lond.)*, **192**, 1215-1216.
- STOMPS, T. J. (1910). Kernteilung und Synapsis bei *Spinacia cleracea*. *Biol. Zbl.*, **31**, 257-308.
- STONE, R. S., SHOPE, R. E., & MOORE, D. H. (1959). Electron microscope study of the development of the papilloma virus in the skin of the rabbit. *J. exp. Med.*, **110**, 543-546.
- STOWELL, R. E. (1949). Alterations in nucleic acids during hepatoma formation in rats fed p-dimethyl-aminoazobenzene. *Cancer*, **2**, 121-131.

- STOWELL, R. E., & LEE, C. S. (1950). Histochemical studies of mouse liver after single feeding of carbon tetrachloride. *Arch. Pathol.*, **50**, 519-537.
- STRAIN, H. H. (1949). Functions and properties of the chloroplast pigments. In: *Photosynthesis in plant*. Iowa State College Press.
- STRANGEWAYS, T. S. P., & OAKLEY, H. E. H. (1924). The immediate changes observed in tissue cells after exposure to soft x-rays while growing in vitro. *Proc. Roy. Soc. B.*, **95**, 373-381.
- STRASBURGER, E. (1875). *Zellbildung und Zellteilung*. 1st edition. Gustav Fischer, Jena.
- (1880). *Zellbildung und Zellteilung*. 3rd edition. Gustav Fischer, Jena.
- (1900). *Über Reduktionsteilung, Spindelbildung, Centrosomen und Ciliensbildner im Pflanzenreich*. Fischer, Jena.
- STRAUB, J. (1938). Neuere karyologische Probleme und Ergebnisse. Sammelreferat. IV. Die Spiral-kultur der Chromosomen. *Z. Bot.*, **33**, 65-126.
- (1943). Chromosomenstruktur. *Naturwissenschaften*, **31**, 97-108.
- STRAUS, W. (1954). Properties of isolated carrot chromatophores. *Exp. Cell Res.*, **6**, 392-402.
- (1959). Rapid cytochemical identification of phagosomes in various tissues of the rat and their differentiation from mitochondria by the peroxidase method. *J. biophys. biochem. Cytol.*, **5**, 193-204.
- (1961a). Cytochemical observations on the transport of intravenously injected horseradish peroxidase and the development of phagosomes in the cells of the kidney of the rat. *Exp. Cell Res.*, **22**, 282-291.
- (1961b). Studies on the chromatophores of carrots. *Protoplasma (Wien)*, **53**, 405-421.
- STRELZOFF, E. (1961). Identification of base pairs involved in mutations induced by base analogues. *Biochem. biophys. Res. Commun.*, **5**, 384-388.
- STRITTMATTER, C. F., & BALL, E. G. (1952). A hemochromogen component of liver microsoma. *Proc. nat. Acad. Sci. (Wash.)*, **38**, 19-25.
- STRUGGER, S. (1930). Beitrag zur Kolloidstruktur des pflanzlichen Ruhekerns. *Protoplasma (Wien)*, **10**, 363-378.
- (1954). Die Proplastiden in den jungen Blättern von Agapanthus umbellatus L'Hérit. *Protoplasma (Wien)*, **43**, 120-173.
- (1956). Schraubig gewundene Fäden als sublichtmikroskopische Bauelemente des Zytoplasmas. *Naturwissenschaften*, **43**, 451-452.
- (1957). Die sublichtmikroskopische Struktur des Cytoplasmas bei verschiedener Fixation. *Naturwissenschaften*, **44**, 543-544.
- STUBBE, W., & v. WETTSTEIN, D. (1956). Zur Struktur erblich verschiedener Chloroplasten von Oenothera. *Protoplasma (Wien)*, **45**, 241-250.
- STUDNICKA, F. K. (1926). *Die Organisation der lebendigen Masse*. Hdb. mikr. Anat. d. Mensch., I/1. Springer, Berlin.
- STURTEVANT, A. H. (1925). The effects of unequal crossing over at the Bar locus in *Drosophila*. *Genetics*, **10**, 117-147.
- SULLIVAN, B. J., & WECHSLER, H. I. (1957). The cytological effects of podophyllin. *Science*, **105**, 433.
- SUN, C. N. (1960). The fine structure of plant mitochondria. *Cytologica (Tokyo)*, **25**, 493-501.
- SUTTER, L.-A. (1960). Über das Geschlechtschromatin des Mundhöhlenepithels unter Berücksichtigung von Lebensalter und Menstruationszyklus. *Acta anat. (Basel)*, **43**, 204-218.
- SWAN, A. G., & MILLER, A. T. (1960). Osmotic regulation in isolated liver and kidney slices. *Amer. J. Physiol.*, **199**, 1227-1231.
- SWANN, M. M. (1951a). Protoplasmic structure and mitosis. I. The birefringence of the metaphase spindle and asters of the living sea-urchin egg. *J. exp. Biol.*, **28**, 417-433.
- (1951b). Protoplasmic structure and mitosis. II. The nature and cause of birefringence changes in the sea-urchin egg at anaphase. *J. exp. Biol.*, **28**, 434-444.
- (1952). Structural agents in mitosis. *Int. Rev. Cytol.*, **1**, 195-210.
- (1954). The mechanism of cell division. Experiments with ether on the sea urchin egg. *Exp. Cell Res.*, **7**, 505-517.
- (1957). The control of cell division: A review. I. General mechanisms. *Cancer Res.*, **17**, 727-757.
- (1958). The control of cell division: a review. II. Special mechanisms. *Cancer Res.*, **18**, 1118-1160.
- SWANN, M. M., & MITCHISON, J. M. (1953). Cleavage of sea urchin eggs in colchicine. *J. exp. Biol.*, **30**, 506-514.
- SWANSON, C. P. (1943). The behaviour of meiotic prophase chromosomes as revealed through the use of high temperatures. *Amer. J. Bot.*, **30**, 422-428.
- (1947). X-ray ultraviolet studies on pollen tube chromosomes. II. The quadripartite structure of the prophase chromosomes of *Tradescantia*. *Proc. nat. Acad. Sci. (Wash.)*, **33**, 229-232.
- (1955). Relative effects of qualitatively different radiations on the production of chromatid aberrations in air and in nitrogen. *Genetics*, **40**, 193-203.
- (1960). *Cytologie und Cytogenetik*. Fischer, Stuttgart.

- SWARTZ, F., SAMS, B. F., & BARTON, A. G. (1960). Polyploidization of rat liver following castration of males and females. *Exp. Cell Res.*, **20**, 438-446.
- SWIFT, H. H. (1950a). The desoxyribose nucleic acid content of animal nuclei. *Physiol. Zool.*, **23**, 169-198.
- (1950b). The constancy of desoxyribose nucleic acid in plant nuclei. *Proc. nat. Acad. Sci. (Wash.)*, **36**, 643-654.
- SWIFT, H. (1953). Quantitative aspects of nuclear nucleoproteins. *Int. Rev. Cytol.*, **2**, 1-76.
- (1956). The fine structure of annulate lamellae. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 415-418.
- SWIFT, H., & KLEINFELD, R. (1953). DNA in grasshopper spermatogenesis, oogenesis, and cleavage. *Physiol. Zool.*, **26**, 301-311.
- SWIFT, H., & RASCH, E. M. (1955). Nucleoproteins in Drosophila polytene chromosomes. *J. Histochem. Cytochem.*, **2**, 456-458.
- SWIFT, H., & RASCH, E. (1956). Microphotometry with visible light. In: *Physical Techniques in Biol. Res.*, Vol. 3. Acad. Press, New York.
- SYVERTON, J. T. (1961). Cellular response to viral infection. *Path. et Biol.*, **9**, 685-689.
- SZARKOWSKI, J. W., & GOLASZEWSKI, T. (1961). RNS-Gehalt der Plastiden von grünen und etiolierten Pflanzen. *Naturwissenschaften*, **48**, 457-458.
- SZEJNMAN, A. (1933). Observations sur la formation des pyrénoïdes chez Spirogyra. *Acta Soc. Bot. Pol.*, **10**, 331-359.
- SZONTÁGH, F. E., JAKOBIVITS, A., & MÉHES, CH. (1961). Primary embryonal sex ratio in normal pregnancies determined by the nuclear chromation. *Nature (Lond.)*, **192**, 476.
- TÄCKHOLM, G. (1922). Zytologische Studien über die Gattung Rosa. *Acta Hort Berg.*, **7**, 97-381.
- TAHMISIAN, T. N. (1957). Mechanics of cell division. I. The living spindle. *Proc. Soc. exp. Biol. (N. Y.)*, **78**, 444-447.
- (1952). Binucleate cell formation in Melanoplus differentialis spermatocytes. *Science*, **116**, 603.
- TAKATS, S. T. (1959). Chromatid extrusion and DNA transfer during microsporogenesis. *Chromosoma (Berl.)*, **10**, 430-453.
- TANAKA, H. (1958). Comparative cytologic studies by means of an electron microscope on monocytes, subcutaneous histiocytes, reticulum cells in the lymph nodes and peritoneal macrophages. *Ann. Rep. Inst. Virus Res., Kyoto Univ.*, Ser. A1, 87-149.
- TANAKA, H., HANAOKA, M., & AMANO, S. (1957). Observations on the centriole of interkinetic blood cells under the electron microscope by ultra-thin sections. Relationship between the centrioles and golgi canaliculi. *Acta haemat. jap.*, **20**, 85-98.
- TANAKA, K. (1955). Central body in the male reproductive cells of the silkworm with special reference to a peculiarity of centriole division in meiosis. *Cytologia (Tokyo)*, **20**, 307-414.
- TANAKA, T. (1953). A study of the somatic chromosomes of rats. *Cytologia (Tokyo)*, **18**, 343-355.
- TANAKA, T., OHNO, S., KINOSHITA, R., & BIERMAN, H. (1955). Effect of myleran on normal and malignant tissues. *Proc. Amer. Ass. Cancer Res.*, **2**, 51.
- TANDLER, C. J. (1959). The silver-reducing property of the nucleolus and the formation of prenucleolar material during mitosis. *Exp. Cell Res.*, **17**, 560-564.
- TARTAR, V. (1956). *Cellular Mechanism in Differentiation and Growth*. Princeton Univ. Press.
- TATUNO, S. (1960a). Zytologische Untersuchungen über die Gattung Frullania. *J. Hattori Bot. Labor.*, **99**-114.
- (1960b). Chromosomen der Gattung Atrichum von Japan. *J. Hattori Bot. Labor.*, **115**-121.
- (1960c). Weitere Untersuchungen über die Vergleichung der Heterochromasie bei einigen europäischen und amerikanischen Arten der Marchantiaceae. *Cytologia (Tokyo)*, **25**, 214-228.
- TAYLOR, J. H. (1953). Intracellular localization of labelled nucleic acid determined with autoradiographs. *Science*, **118**, 555-557.
- (1958a). The mode of chromosome duplication in Crepis capillaris. *Exp. Cell Res.*, **15**, 350-357.
- (1958b). Incorporation of phosphorus-32 into nucleic acids and proteins during microgametogenesis of Tulbaghia. *Amer. J. Bot.*, **45**, 123-131.
- (1960a). Nucleic acid synthesis in relation to the cell division cycle. *Ann. N. Y. Acad. Sci.*, **90**, 409-421.
- (1960b). Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. biophys. biochem. Cytol.*, **7**, 455-464.
- (1962). Chromosome reproduction. *Int. Rev. Cytol.*, **13**, 39-73.
- TAYLOR, J. H., & McMASTER, R. D. (1954). Autoradiographic and microphotometric studies of desoxyribose nucleic acid during microgametogenesis in Lilium longiflorum. *Chromosoma (Berl.)*, **6**, 489-521.
- TAYLOR, J. H., WOODS, P. S., & HUGHES, W. L. (1957). The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium labelled thymidine. *Proc. nat. Acad. Sci. (Wash.)*, **43**, 122-128.

- TEDESCHI, H. (1961). Osmotic reversal of mitochondrial swelling. *Biochim. biophys. Acta (Amst.)*, **46**, 159-169.
- TEIR, H., & LAHTIHARJU, A. (1961). Effect of necrotic liver tissue on regeneration in hepatectomized rats. *Exp. Cell Res.*, **24**, 424-428.
- TELLYESNICZKY, K. (1902). Zur Kritik der Kernstrukturen. *Arch. mikr. Anat.*, **60**, 681-706.
- TEORELL, T. (1953). Transport processes and electrical phenomena in ionic membranes. *Progr. Biophys.*, **3**, 305-319.
- TERRA, N. DE (1960a). The effect of enucleation on restoration of the interphase rate of ^{32}P uptake after cell division in Stentor coeruleus. *Exp. Cell Res.*, **21**, 34-40.
- (1960b). A study of nucleo-cytoplasmic interactions during cell division in Stentor coeruleus. *Exp. Cell Res.*, **21**, 41-48.
- THALER, I. (1961). Virus-Eiweißkristalle in Phajus grandifolius. *Protoplasma (Wien)*, **53**, 106-112.
- THEMANN, H. (1960). Zur elektronenmikroskopischen Darstellung von Glykogen mit Best's Carmin. *J. Ultrastruct. Res.*, **4**, 401-412.
- (1961). Zur elektronenmikroskopischen Darstellung von Glykogen und die Beziehungen der Zellorganellen bei der Glykogensynthese und der Glykogenolyse. *Verh. dtsch. path. Ges.*, **45**, 291-296.
- THÉRET, C., & JÉZEQUEL, A. M. (1961). Identification en microscopie électronique des systèmes déshydrogénasiques dans la cellule hépatique. *Ann. Histochem. (Paris)*, **4**, 131-136.
- THERMAN-SUOMALEINEN, E. (1949). Investigation on secondary constrictions in Polygonatum. *Hereditas (Lund)*, **35**, 86-108.
- THIEL, A. (1959). Mitochondrien. Ergebnisse und Probleme. *Dtsch. med. Wschr.*, **84**, 2038-2045.
- THODAY, J. M. (1953). Sister-union isolocus breaks in irradiated Vicia faba: the target theory and physiological variation. *Heredity*, **6 Suppl.**, 299-309.
- THODAY, J. M., & READ, J. (1947). Effect of oxygen on the frequency of chromosome aberrations produced by x-rays. *Nature (Lond.)*, **160**, 608.
- THOENES, G. (1961). Die submikroskopische Struktur des Barrschen Zellkernkörpers (des sog. Geschlechtschromatins). *Naturwissenschaften*, **48**, 388-389.
- THOENES, W. (1962). Fine structure of lipid granules in proximal tubule cells of mouse kidney. *J. Cell Biol.*, **12**, 433-437.
- THOENES, W., & BANNASCH, P. (1962). Elektronen- und licht-mikroskopische Untersuchungen am Cytoplasma der Leberzellen nach akuter und chronischer Thioacetamid-Vergiftung. *Virchows Arch. path. Anat.*, **335**, 556-583.
- THOMAS, J. A. (1935). Un mode nouveau de multiplication cellulaire directe: la méroamitose. *C. R. Acad. Sci. (Paris)*, **201**, 988-990.
- (1938). Au sujet de considérations qui viennent d'être publiées sur le mode de multiplication cellulaire nommé: méroamitose. *Arch. exp. Zellforsch.*, **21**, 281-285.
- THOMAS, O. L. (1960). Electron microscopy of the Golgi apparatus. *Nature (Lond.)*, **185**, 703-704.
- THORELL, B. (1947). *Studies on the formation of cellular substance during blood cell production*. Kimpton, London.
- TISCHLER, G. (1934-1951). *Allgemeine Pflanzenkaryologie. Hdb. der Pflanzenanatomie*. Borntraeger, Berlin.
- TIJO, J. H., & LEVAN, A. (1950). Quadruple structure of the centromere. *Nature (Lond.)*, **165**, 368.
- TOBIOKA, M., BIESELE, J. J. (1956). Mitochondria in living cells: An analysis of movements. *J. biophys. biochem. Cytol.*, **2 Suppl.**, 319-324.
- TÖRÖ, I. (1955). Contribution à l'histophysiologie du thymus. *C. R. Ass. Anat.*, **1312-1325**.
- (1962). Amitotische Kernteilung und Endocytogenese. *Acta anat. (Basel)*, **48**, 177-178.
- TONMLIN, S. G., & CALLAN, H. G. (1951). Preliminary account of an electron microscope study of chromosomes from newt oocytes. *Quart. J. micr. Sci.*, **92**, 221-224.
- TORREY, J. G. (1961). Kinetin as trigger for mitosis in mature endomitotic plant cells. *Exp. Cell Res.*, **23**, 281-299.
- TOURNIER, P., CATHALA, F., & BERNHARD, W. (1957). Ultrastructure et développement intracellulaire du virus de la varicelle observé au microscope électronique. *Presse méd.*, **52**, 1229-1234.
- TRANKOWSKY, D. A. (1930). "Leitkörperchen" der Chromosomen bei einigen Angiospermen. *Z. Zellforsch.*, **10**, 736-743.
- TREVIRANUS, L. C. (1806). *Vom inwendigen Bau der Gewächse*. Dieterich, Göttingen.
- TRUHAUT, R., & DEYSSON, G. (1954). Étude des effets, sur les mitoses des cellules végétales, du 1,4-diméthylsulfonybutane. Essais de protection par la β -mercaptoprothylamine. *C. R. Soc. Biol. (Paris)*, **238**, 1833-1835.
- TRUMP, B. F. (1961). An electron microscope study of the uptake, transport, and storage of colloidal materials by the cells of the vertebrate nephron. *J. Ultrastruct. Res.*, **5**, 291-310.
- TSCHERMAK-WOESS, E. (1954). Über die Phasen der Endomitose, Herkunft und Verhalten der "nuklealen Körper" und Beobachtungen zur karyologischen Anatomie von Sauromatum guttatum. *Planta (Berl.)*, **44**, 509-531.

- (1956). Notizen über die Riesenkerne und "Riesenchromosomen" in den Antipoden von Aconitum. *Chromosoma (Berl.)*, **8**, 114–134.
- (1959). Die DNS-Reproduktion in ihrer Beziehung zum endomitotischen Strukturwechsel. *Chromosoma (Berl.)*, **10**, 497–503.
- TSCHERMAK-WOESS, E., & HASITSCHKA, G. (1953). Veränderungen der Kernstruktur während der Endomitose, rhythmisches Kernwachstum und verschiedenes Heterochromatin bei Angiospermen. *Chromosoma (Berl.)*, **5**, 574–614.
- TURNER, B., & JENNINGS, A. N. (1961). Trisomy of chromosome 22. *Lancet*, **ii**, 49–50.
- TURNER, H. H. (1938). A syndrome of infantilism congenital, webbed neck and cubitus valgus. *Endocrinology*, **23**, 566–574.
- UEDA, K. (1960). Structure of plant cells with special reference to lower plants. IV. Structure of Tracheolomas sp. *Cytologia (Tokyo)*, **25**, 8–16.
- ULE, G., & ROSSNER, J. A. (1960). Elektronenmikroskopische Studien zur akuten Körnerzellnekrose im Kleinhirn. *Verh. dtsch. path. Ges.*, **44**, 210–214.
- UNDRITZ, E. (1942). Über das Vorkommen von Abbauformen der Leukocyten im Blut. *Folia haemat. (Lpz.)*, **65**, 195–202.
- UPCOTT, M. (1936). The mechanics of mitosis in the pollentube of tulipa. *Proc. roy. Soc. B. (Lond.)*, **121**, 207–220.
- URETZ, R. B., BLOOM, W., & ZIRKLE, R. E. (1954). Irradiation of parts of individual cells. II. Effects of an ultraviolet microbeam focused on parts of chromosomes. *Science*, **120**, 197–199.
- USSING, H. H. (1961). Experimental evidence and biological significance of active transport. *Coll. ges. physiol. Chem.*, **12**, 1–11.
- VALENTIN, G. (1836). Über den Verlauf und die letzten Enden der Nerven. *Nov. Acta. phys. med. Acad. Leopoldiana*, **18/1**, 51–240.
- VEJDOVSKY, F. (1911–1912). Zum Problem der Vererbungsträger. *Verlag d. Kgl. böhm. Ges. Wiss., Prag.*
- VEJDovsky, F., & MRAZEK, A. (1903). Umbildung des Cytoplasmas während der Befruchtung und Zellteilung. *Arch. mikr. Anat.*, **62**, 431–579.
- VENDRELY, C. (1952). L'acide désoxyribonucléique du noyau des cellules animales. Son rôle possible dans la biochimie de l'hérédité. *Bull. biol. France et Belg.*, **86**, 1–87.
- VENDRELY, R., & VENDRELY, C. (1948). La teneur du noyau cellulaire en acide désoxyribonucléique à travers les organes, les individus et les espèces animales. *Experientia (Basel)*, **4**, 434–436.
- (1956). The results of cytophotometry in the study of the deoxyribonucleic acid (DNA) content of the nucleus. *Int. Rev. Cytol.*, **5**, 171–197.
- VENDRELY, R., KNOBLOCH-MAZEN, A., & VENDRELY, C. (1960). A comparative biochemical study of nucleohistones and nucleoprotamines in the cell nucleus. In: *The Cell Nucleus*. Butterworths, London.
- VINCENT, W. S. (1952). The isolation and chemical properties of the nucleoli of starfish oocytes. *Proc. nat. Acad. Sci. (Wash.)*, **38**, 139–145.
- (1955). Structure and chemistry of nucleoli. *Int. Rev. Cytol.*, **4**, 269–298.
- (1960). A biochemical approach to cell morphology. In: *New Approaches in Cell Biology*. Acad. Press, London.
- VINCENT, W. S., & HUXLEY, A. H. (1954). The dry matter content of starfish oocytic nucleoli. *Biol. Bull.*, **107**, 290.
- VINCENT, W. S., & BALTUS, E. (1960). The ribonucleic acids of nucleoli. In: *The Cell Nucleus*. Butterworths, London.
- VIRCHOW, R. (1851). Die endogene Zellenbildung beim Krebs. *Arch. path. Anat.*, **3**, 197–227.
- (1855). Cellular-Pathologie. *Arch. path. Anat.*, **8**, 3–39.
- (1858a). *Die Cellularpathologie*. Hirschwald, Berlin.
- (1858b). Eine Antwort an Herrn Spieß. *Arch. path. Anat.*, **13**, 481–490.
- (1880). Krankheitswesen und Krankheitsursachen. *Arch. path. Anat.*, **79**, 1–19, 185–228.
- VISHNIAC, W. (1955). Biochemical aspects of photosynthesis. *Ann. Rev. Plant Physiol.*, **6**, 115–134.
- VOGEL, F. (1961). *Lehrbuch der allgemeinen Humangenetik*. Springer, Berlin.
- VOGEL, W. (1963). Struktur und Funktionelle Biochemie der Mitochondrien. I. Die Morphologie der Mitochondrien. In: *Funktionelle und morphologische Organisation der Zelle*. Springer, Berlin.
- VOGT, C., & VOGT, O. (1946). Eine neurohistologische Beleuchtung der Nucleusfunktion. *Biol. Zbl.*, **65**, 61–69.
- (1947). Lebensgeschichte, Funktion und Tätigkeitsregulierung des Nucleolus. *Ärztl. Forsch.*, **1**, 8, 43.
- VOGT, P. K. (1960). Die Immunologie der Lebermikrosomen. III. Die Lokalisation gewebsspezifischer Antigene innerhalb der Strukturkomponenten des endoplasmatischen Retikulums. *Z. Naturforsch.*, **15b**, 221–225.

- VOGT-KÖHNE, L. (1961). Quantitative cytochemische Untersuchungen an Nukleolen aus Speicheldrüsenernen von Chironomus thummi. *Chromosoma (Berl.)*, **12**, 382-397.
- VOLKIN, E., & ASTRACHAN, L. (1956). Intracellular distribution of labelled ribonucleic acid after phage infection of Escherichia coli. *Virology*, **2**, 433-437.
- VOLKIN, E., ASTRACHAN, L., & COUNTRYMAN, J. L. (1958). Metabolism of RNA phosphorus in Escherichia coli infected with bacteriophage T 7. *Virology*, **6**, 545-555.
- VRIES, H. DE (1885). Plasmolytische Studien über die Wand der Vakuolen. *Jb. wiss. Bot.*, **16**, 465-598.
- WACKER, A. (1958). Bakterien-Transformation. *Coll. Ges. Physiol. Chem.* Springer, Berlin.
- WADA, B. (1935). Mikrurgische Untersuchung lebender Zellen in der Teilung. II. Das Verhalten der Spindelfigur und einige ihrer physikalischen Eigenschaften in den somatischen Zellen. *Cytologia (Tokyo)*, **6**, 381-406.
- (1941). Über die Spindelfigur bei der somatischen Mitose der Prothalliumzellen von Osmunda japonica Thunb. *In vivo*. *Cytologia (Tokyo)*, **11**, 353-368.
- (1950). The mechanism of mitosis based on studies of the submicroscopic structure and of the living state of the Tradescantia cell. *Cytologia (Tokyo)*, **16**, 1-26.
- WAGNER, E. (1953). Überdauernde Nucleoli und tripolare Spindeln in der Pollenmeiose eines Vitis-Artbastardes. *Naturwissenschaften*, **40**, 488.
- WAKONIG, R. (1960). Further evidence of diploid neoplasmas. *Canad. J. Genet. Cytol.*, **2**, 325-330.
- WALKER, B. E. (1959). Radioautographic observations on regeneration of transitional epithelium. *Tex. Rep. Biol. Med.*, **17**, 375-384.
- WALKER, D. L. (1960). In vitro cell-virus relationships resulting in cell death. *Ann. Rev. Microbiol.*, **14**, 177-196.
- WALKER, P. M. B., & YATES, H. B. (1952). Some nuclear components of dividing cells. *Proc. roy. Soc. B.*, **140**, 274-289.
- WALKER, P. M. B., & MITCHISON, J. M. (1957) DNA synthesis in two ciliates. *Exp. Cell Res.*, **13**, 167-170.
- WALLACE, B. J. (1960). The relation of mitochondrial morphology to succinoxidase activity as observed in the rat kidney after protein injection. *J. Histochem. Cytochem.*, **8**, 105-112.
- WALTERS, M. S. (1951). Spontaneous chromosome breakage and atypical chromosome movement in meiosis of the hybrid Bromus marginatus B. pseudolaevis. *Genetics*, **37**, 8-25.
- (1958). Aberrant chromosome movement and spindle formation in meiosis of Bromus hybrids: An interpretation of spindle organization. *Amer. J. Bot.*, **45**, 271-289.
- WARBURG, O. (1926). *Über den Stoffwechsel der Tumoren*. Springer, Berlin.
- (1955). Über die Entstehung der Krebszellen. *Naturwissenschaften*, **42**, 401-406.
- WARBURG, O., KRIPPAHL, G., GEWITZ, H.-S., & VÖLKER, W. (1959). Über den chemischen Mechanismus der Photosynthese. *Z. Naturforsch.*, **14b**, 712-724.
- WASIELEWSKI, W. v. (1903). Theoretische und experimentelle Beiträge zur Kenntnis der Amitose. I. *Jb. wiss. Bot.*, **38**, 377-420.
- (1904). Theoretische und experimentelle Beiträge zur Kenntnis der Amitose. II. *Jb. wiss. Bot.*, **39**, 581-606.
- WASSERMANN, F. (1926). Zur Analyse der mitotischen Kern- und Zellteilung. *Z. Anat. Entwickl.-Gesch.*, **80**, 344-432.
- (1929). Wachstum und Vermehrung der lebendigen Masse. *Hdb. mikr. Anat. d. Mensch.*, **I/2**. Springer, Berlin.
- (1939). Mechanismus der Mitose. *Arch. exp. Zellforsch.*, **22**, 238-251.
- (1962). Schlußbemerkungen zum Problem der Amitose. *Acta anat. (Basel)*, **48**, 181.
- WASSILEWA-DRANOWSKA, O. (1961). Cytochemische Untersuchungen bei Pflanzen—ein Gegenbeweis gegen die Theorie der Konstanz der Desoxyribonukleinsäure im Zellkern. *Acta histochem. (Jena)*, **II**, 42-57.
- WATKINS, M. (1961). Interferometric measurements of the chromosomal mass in a grasshopper. *Exp. Cell Res.*, **23**, 595-602.
- WATSON, J. D., & CRICK, F. H. C. (1953). Molecular structure of nucleic acids. *Nature (Lond.)*, **171**, 737-738.
- WATSON, M. L. (1955). The nuclear envelope. *J. biophys. biochem. Cytol.*, **1**, 257-270.
- (1959). Further observations on the nuclear envelope of the animal cell. *J. biophys. biochem. Cytol.*, **6**, 147-156.
- WATTS, A. H. G. (1952). Spermatogenesis in the slug, Arion subfuscus. *J. Morph.*, **91**, 53-77.
- WAUGH, D. F. (1954). Protein-protein interactions. *Advanc. Protein Chem.*, **9**, 325-437.
- WEAVER, M. E. (1959). The effect of sodium malonate on mitotic rate in the germinal epithelium of rat ovaries grown in vitro. *Anat. Rec.*, **135**, 303-311.
- WEBER, H. H. (1958). *The motility of muscle and cells*. Harvard Univ. Press, Cambridge, Mass.

1625

- WEBER, P. (1959). Artsspezifische Unterschiede zwischen lamellären Strukturproteiden aus Chloroplasten. (3. Mitteilung über lamelläre Strukturproteide). *Z. Naturforsch.*, **14b**, 691-692.
- WEBER, W., & KRIESTEN, K. (1960). Über die Ausschleusung nukleolärer Substanz im Milchdrüsenepithel der weißen Maus. *Experientia (Basel)*, **16**, 540-542.
- WEBSTER, G. C. (1953). Peptide-bond synthesis in higher plants. I. The synthesis of glutathione. *Arch. Biochem. Biophys.*, **47**, 241-250.
- (1960). Specificity of acceptor RNA for alanine activation. *Biochim. biophys. Res. Commun.*, **2**, 56-58.
- WEBSTER, L. T., & DAVIE, E. W. (1959). Enzymic formation of adenylyserine and an unknown carboxyl-activated compound. *Biochim. biophys. Acta (Amst.)*, **35**, 559-560.
- WEED, I. G. (1937). Cytological studies of developing muscle with special reference to myofibrils, mitochondria, Golgi material and nuclei. *Z. Zellforsch.*, **25**, 516-540.
- WEGNER, G., STUTZ, E., & BÜCHNER, F. (1961). Tumoren und Mißbildungen bei der Wistar-Ratte in der ersten Generation nach Ganzbestrahlung des Muttertieres mit 270 am 18. Graviditätstag. *Beitr. path. Anat.*, **124**, 396-414.
- WEHRMEYER, W. (1961). Neue Befunde über die Ausbildung des "lamellaren Musters" im Chloroplasten höherer Pflanzen. *Z. Naturforsch.*, **16b**, 627-628.
- WEICKER, H., & NÖLLER, H. G. (1951). Morphologische Beobachtungen über den Vermehrungs- und Kernteilungsmechanismus der Knochenmarkriesenzellen. *Klin. Wschr.*, **29**, 184-190.
- WEIDEL, W. (1958). Einige Probleme der Phagogenetik. *Coll. Ges. Physiol. Chem.* Springer, Berlin.
- WEIER, T. E., & THOMSON, W. W. (1962). The grana of starch-free chloroplasts of *Nicotiana rustica*. *J. Cell Biol.*, **13**, 89-108.
- WEILING, F. (1961). Elektronenmikroskopische Beobachtungen über Pinocytose-Vorgänge bei pflanzlichen Zellen. *Ber. dtsch. bot. Ges.*, **74**, 246-254.
- WEINHOUSE, S. (1955). Oxidative metabolism of neoplastic tissues. *Advanc. Cancer Res.*, **3**, 270-326.
- WEISBLUM, B., HERMAN, L., & FITZGERALD, P. J. (1962). Changes in pancreatic acinar cells during protein deprivation. *J. Cell Biol.*, **12**, 313-327.
- WEISS, J. M. (1955). Mitochondrial changes induced by potassium and sodium in the duodenal absorptive cell as studies with the electron microscope. *J. exp. Med.*, **102**, 783-788.
- WEISS, S. B. (1960). Enzymatic incorporation of ribonucleoside triphosphates into the interpoly nucleotide linkages of ribonucleic acid. *Proc. nat. Acad. Sci. (Wash.)*, **46**, 1020-1030.
- (1962). Biosynthesis of ribopolynucleotides. *Fed. Proc.*, **21**, 120-126.
- WEISS, S. B., & GLADSTONE, L. (1959). A mammalian system for the incorporation of cytidine triphosphate into ribonucleic acid. *J. Amer. chem. Soc.*, **81**, 4118-4119.
- WEISS, S. B., & NAKAMOTO, T. (1961). On the participation of DNA in RNA biosynthesis. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 694-697.
- WEISSE, K., & KRÜCKE, W. (1959). Die Einschlußkörper-Enzephalitiden. Neue Enzephalitisformen. *Dtsch. med. Wschr.*, **84**, 777-781, 793-794, 799.
- WEISSENFELS, N. (1958a). Über die Entleerung und Entwicklung der Mitochondrien und den Feinbau des Cytoplasmas von embryonalen Zellen. *Z. Naturforsch.*, **13b**, 182-186.
- (1958b). Über die Entstehung der Promitochondrien und ihre Entwicklung zu funktionstüchtigen Mitochondrien in den Zellen von Embryonal- und Tumorgewebe. *Z. Naturforsch.*, **13b**, 203-205.
- (1961). Der Einfluß der Gewebezüchtung auf die Morphologie der Hühnerherzmyoblasten. I. Die Transformation der Mitochondrien. *Protoplasma (Wien)*, **54**, 229-240.
- (1962a). Der Einfluß der Gewebezüchtung auf die Morphologie der Hühnerherzmyoblasten. II. Herkunft und Entwicklung der Cytosomen. *Protoplasma (Wien)*, **54**, 328-344.
- (1962b). Der Einfluß der Gewebezüchtung auf die Morphologie der Hühnerherzmyoblasten. III. Der Einfluß der Ernährung auf die Entstehung der Cytosomen. *Protoplasma (Wien)*, **54**, 540-554.
- WELCH, R. M., HANLY, E. W., & GUEST, W. (1961). The deoxyribonucleic acid (DNA) deviation in the semen spermatozoa of bulls of unknown fertility under two years of age and its relationship to motility, count and morphology. *J. Morph.*, **108**, 145-163.
- WELLENSIECK, H. J. (1957). Zur submikroskopischen Morphologie von Plasmazellen mit Russelschen Körperchen und Eiweißkristallen. *Beitr. path. Anat.*, **118**, 173-202.
- WELLINGS, S. R., & SIEGEL, B. V. (1959). Role of Golgi apparatus in the formation of melanin granules in human malignant melanoma. *J. Ultrastruct. Res.*, **3**, 147-154.
- WELLINGS, S. R., & DEOME, K. B. (1961). Milk protein droplet formation in the Golgi apparatus of the C₃H/Crgl mouse mammary epithelial cells. *J. biophys. biochem. Cytol.*, **9**, 479-485.
- WELSH, R. S. (1960). Water soluble, non-fibrous deoxyribonucleoprotein from calf thymus nuclei. *Nature (Lond.)*, **187**, 943-945.
- WENDLER-DEANE, H., & PORTER, K. R. (1960). A comparative study of cytoplasmic basophilia and the population density of ribosomes in the secretory cells of mouse seminal vesicle. *Z. Zellforsch.*, **52**, 697-711.

- WENDT, E. (1959). Lebendbeobachtungen an bestrahlten Interphasenkernen. *Z. Zellforsch.*, **49**, 677-689.
- (1960). Strahlenbedingte amitotische Kernteilungen. (Lebendbeobachtungen.) *Zool. Anz., Suppl. 23*, 495-600.
- WENT, F. A. F. (1887). Beobachtungen über Kern- und Zellteilung. *Ber. dtsch. bot. Ges.*, **5**, 247.
- WENT, H. A. (1959). Some immunochemical studies on the mitotic apparatus of the sea urchin. *J. biophys. biochem. Cytol.*, **5**, 353-356.
- WENT, H. A., & MAZIA, D. (1959). Immunochemical study of the origin of the mitotic apparatus. *Exp. Cell Res., Suppl.*, **7**, 200-218.
- WERMEL, E. M., & IGNATIEWA, Z. P. (1933). Studien über Zellengröße und Zellenwachstum. III. Über die Veränderungen der Kerngröße bei Vergiftungen. *Z. Zellforsch.*, **17**, 476-504.
- (1934). Studien über Zellengröße und Zellenwachstum. VI. Weitere Beobachtungen über den Einfluß der Gifte auf die Kerngröße der Leberzellen. *Z. Zellforsch.*, **20**, 43-53.
- WERMEL, E. M., & SZINEWA, M. W. (1934). Studien über Zellengröße und Zellenwachstum. X. Veränderung der Zellgröße bei Stickstoffhunger. *Z. Zellforsch.*, **21**, 749-756.
- WERZ, G. (1960). Über Strukturierungen der Wuchszenen von Acetabularia mediterranea. *Planta (Berl.)*, **55**, 38-56.
- (1961). Zur Frage der Herkunft und Verteilung cytoplasmatischer Ribonucleinsäure und ihre Beziehung zu "morphogenetischen Substanzen" bei Acetabularia mediterranea. *Z. Naturforsch.*, **16b**, 126-129.
- WESSEL, W. (1958). Elektronenmikroskopische Untersuchungen von intranukleären Einschlußkörpern. *Virchows Arch. path. Anat.*, **331**, 314-328.
- (1960a). Das elektronenmikroskopische Bild menschlicher endometrialer Drüsenzellen während des menstruellen Zyklus. *Z. Zellforsch.*, **51**, 633-657.
- (1960b). Über die Form der Mitochondrien in elektronenmikroskopischen Bildern. *Z. Zellforsch.*, **52**, 712-714.
- WESSEL, W., & GEDIGK, P. (1959). Die Verarbeitung und Speicherung von phagocytiertem Eisen im elektronenmikroskopischen Bild. *Virchows Arch. path. Anat.*, **332**, 508-532.
- WETTSTEIN, D. v., & KAHN, A. (1960). Macromolecular physiology of plastids. *Proc. Europ. Conf. Electr. Microsc., Vol. II*. Delft.
- WHALEY, W. G., MOLLENHAUER, H. H., & KEPHART, J. E. (1959). The endoplasmatic reticulum and the Golgi structures in maize root cells. *J. biophys. biochem. Cytol.*, **5**, 501-506.
- WHALEY, W. G., MOLLENHAUER, H. H., & LEECH, J. H. (1960). Some observations on the nuclear envelope. *J. biophys. biochem. Cytol.*, **8**, 233-245.
- WHITE, M. J. D. (1936). Chromosome cycle of Ascaris megalocephala. *Nature (Lond.)*, **137**, 783.
- WIESNER, J. (1892). *Die Elementarstruktur und das Wachstum der lebenden Substanz*. Hölder, Wien.
- WILKINSON, J. F., & DUGUID, J. P. (1960). The influence of cultural conditions on bacterial cytology. *Int. Rev. Cytol.*, **9**, 1-76.
- WILLIAMSON, D. H., & SCOPES, A. W. (1962). A rapid method for synchronizing division in the yeast, *Saccharomyces cerevisiae*. *Nature (Lond.)*, **193**, 256-257.
- WILSON, C. C., BOGESS, W. R., & KRAMER, P. J. (1953). Diurnal fluctuations in the moisture content of some herbaceous plants. *Amer. J. Bot.*, **40**, 97-100.
- WILSON, E. B. (1899). On protoplasmic structure in the eggs of echinoderms and some other animals. *J. Morph.*, **15 Suppl.**, 1-28.
- (1901). Experimental studies in cytology. I. A cytological study of artificial parthenogenesis in sea-urchin eggs. *Arch. Entwickl.-Mech. Org.*, **12**, 529-596.
- (1904). Experimental studies in germinal localization. II. Experiments on the cleavage mosaic in *Patella* and *Dentalium*. *J. exp. Zool.*, **1**, 197-268.
- (1928) *The cell in development and heredity*. 3rd edition. MacMillan, New York.
- WILSON, G. B. (1959). Fourth Huskins memorial lecture: Studies on the control of mitotic activity. *Canad. J. Genet. Cytol.*, **1**, 1-9.
- WILSON, G. B., & HYYPPIO, P. A. (1955). Some factors concerned in the mechanism of mitosis. *Cytologia (Tokyo)*, **20**, 177-184.
- WILSON, J. W., & LEDUC, E. H. (1948). The occurrence and formation of binucleate and multinucleate cells and polyploid nuclei in the mouse liver. *Amer. J. Anat.*, **82**, 353-391.
- WIMBER, D. E. (1961). Asynchronous replication of deoxyribonucleic acid in root tip chromosomes of *Tradescantia paludosa*. *Exp. Cell Res.*, **23**, 402-407.
- WINCKLER, G. (1960). Caractéristiques des noyaux vacuolisés des cellules adipeuses chez l'homme (Lochkerne). *Z. Anat. Entwickl.-Gesch.*, **122**, 241-246.
- WISCHNITZER, S. (1958). An electron microscope study of the nuclear envelope of amphibian oocytes. *J. Ultrastruct. Res.*, **1**, 201-222.
- (1959). Three dimensional visualization of the nuclear membrane in vertebrate oocytes. *Cytologia (Tokyo)*, **24**, 478-486.

- (1960). The ultrastructure of the nucleus and nucleocytoplasmic relations. *Int. Rev. Cytol.*, **10**, 137-162.
- WISSIG, S. L. (1960). The anatomy of secretion in the follicular cells of the thyroid gland. *J. biophys. biochem. Cytol.*, **7**, 419-432.
- WITTEKIND, D. (1960). Untersuchungen zur Frage der Eiweißaufnahme in kernhaltigen Zellen des peripheren Blutes und in Ergußhistiocytien (Pinocytose). *Schweiz. med. Wschr.*, **90**, 1264-1265.
- WITTMANN, H. G., (1961). Ansätze zur Entschlüsselung des genetischen Codes. *Naturwissenschaften*, **48**, 729-734.
- WOHLER, F. (1828). *Über künstliche Bildung des Harnstoffes*. Poggendorfs Annalen.
- WOHLFAHRT-BOTTERMANN, K. E. (1957). Cytological Studies IV. Die Entstehung, Vermehrung und Sekretabgabe der Mitochondrien von Paramecium. *Z. Naturforsch.*, **12b**, 164-167.
- (1959). Gestaltet das elektronenmikroskopische Bild Aussagen zur Dynamik in der Zelle? Cytological Studies VI. *Z. Zellforsch.*, **50**, 1-27.
- (1960). Protistenstudien X. Licht- und elektronenmikroskopische Untersuchungen in der Amöbe *Hyalodiscus simplex* n. sp. *Protoplasma (Wien)*, **52**, 58-107.
- (1961). Cytological Studies VIII. Zum Mechanismus der Cytoplasmaströmung in dünnen Fäden. *Protoplasma (Wien)*, **54**, 1-26.
- WOJTCZAK, L., & LEHNINGER, A. L. (1961). Formation and disappearance of an endogenous uncoupling factor during swelling and contraction of mitochondria. *Biochim. biophys. Acta (Amst.)*, **51**, 442-456.
- WOLF, B. E. (1960). Zur Karyologie der Eireifung und Furchung bei Cloeon dipterium L. *Biol. Zbl.*, **79**, 153-198.
- WOLF, E. (1940). Die Anordnung der Chromosomen in Spermienkern von *Dicranomyia trinotata* Meig. *Chromosoma (Berl.)*, **1**, 336-342.
- WOLFF, C. F. (1896). *Theoria generationis*. 1759. German edition. *Ostwalds Klassiker d. exakt. Naturwiss.* Nr. 84.
- WOLFF, S. (1957). Recent studies on chromosome breakage and rejoicing. *Advances in Radiobiology*, 403-479.
- WOLFF, S., & LUIPPOLD, H. E. (1955). Metabolism and chromosome-break rejoicing. *Science*, **122**, 231-232.
- WOLKEN, J. J. (1961). The photoreceptor structures. *Int. Rev. Cytol.*, **11**, 195-218.
- WOLKEN, J. J., & SCHWERTZ, F. A. (1954). Chlorophyll monolayers in chloroplasts. *J. gen. Physiol.*, **37**, 111-120.
- WOLL, E., (1954). Untersuchungen über die cytologische Differenzierung einiger Pflanzengallen. *Planta (Berl.)*, **43**, 477-494.
- WOLMAN, M. (1955). Problems of fixation in cytology, histology and histochemistry. *Int. Rev. Cytol.*, **4**, 79-102.
- WOLPERT, L. (1960). The mechanics and mechanism of cleavage. *Int. Rev. Cytol.*, **10**, 163-216.
- WOODARD, J., RASCH, E., & SWIFT, H. (1961a). Nucleic acid and protein metabolism during the mitotic cycle in *Vicia faba*. *J. biophys. biochem. Cytol.*, **9**, 445-462.
- WOODARD, J., GELBER, B., & SWIFT, H. (1961b). Nucleoprotein changes during the mitotic cycle in *Paramecium aurelia*. *Exp. Cell Res.*, **23**, 258-264.
- WOODRUFF, M. F. A., & LENNOX, B. (1959). Reciprocal skin grafts in a pair of twins showing blood chimaerism. *Lancet*, **II**, 476-478.
- WOODS, P. S. (1960). Autoradiographic studies of ribonucleic acid metabolism with tritium-labelled cytidine. In *The Cell Nucleus*. Butterworths. London.
- WOODS, P. S., & TAYLOR, J. H. (1959). Studies of ribonucleic acid metabolism with tritium-labeled cytidine. *Lab. Invest.*, **8**, 309-318.
- WOODS, P. S., GAY, H., & SENGÜN, A., (1961). Organization of the salivary-gland chromosome as revealed by the pattern of incorporation of H^3 -thymidine. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 1486-1493.
- WORLEY, L. G., & MORIBER, L. G. (1961). The origin of protein yolk from the Golgi apparatus in gastropods. *Trans. N. Y. Acad. Sci.*, Ser. **2**, **23**, 352-356.
- WOTTON, R. M., & LEVIN, S. S. (1957). The direct absorption of previously stained lipid by intact nuclei in liver cells of the rabbit. *Anat. Rec.*, **129**, 155-166.
- WRBA, H., RIPOLI-GÓMEZ, M., & RANZ, H. (1960a). Zum Nachweis eines humoralen "Regenerationsfaktors" im Serum partiell hepatektomierter Ratten. *Naturwissenschaften*, **47**, 182-183.
- WRBA, H., RANZ, H., & RIPOLL-GÓMEZ, M. (1960b). Zur Spezifität des "Regenerationsfaktors" im Blutserum nach Hepatektomie. *Naturwissenschaften*, **47**, 306.
- WULFF, H. D. (1935). Ein Vergleich zwischen Kultur- und Griffelpräparaten von Pollenschläuchen von *Narthecium orcuttigum*. *Beth. bot. Zbl.*, Abt. A, **54**, 83-98.
- YAMADA, E. (1957). The fine structure of the megakaryocyte in the mouse spleen. *Acta Anat.*, **29**, 267-290.

- (1958). A peculiar lamellated body observed in the cells of the pigment epithelium of the retina of the bat, *Pipistrellus abramus*. *J. biophys. biochem. Cytol.*, **4**, 329-330.
- YAMADA, M., & PUCK, T. T. (1961). Action of radiation on mammalian cells. IV. Reversible mitotic lag in the S3 HeLa cell produced by low doses of x-rays. *Proc. nat. Anat. Sci. (Wash.)*, **47**, 1181-1191.
- YASUZUMI, G. (1959). Electron microscopy of the nuclear membrane in prophase and telophase in Yoshida sarcoma cells. *Z. Zellforsch.*, **50**, 110-120.
- (1960). Licht- und elektronenmikroskopische Studien an kernhaltigen Erythrocyten. *Z. Zellforsch.*, **51**, 325-335.
- YASUZUMI, G., & KONDO, A. (1951). The physico-chemical properties of the sex chromosome. A study by means of ultrasonic waves. *Heredity*, **42**, 219-223.
- YASUZUMI, G., & HIGASHIZAWA, S. (1955). Submicroscopic structure of the carp erythrocyte as revealed by electron microscopy. *Cytologia (Tokyo)*, **20**, 280-290.
- YASUZUMI, G., SAWADA, T., SUGIHARA, R., KIRIYAMA, M., & SUGIOKA, M. (1958). Electron microscope researches on the ultrastructure of nucleoli in animal tissues. *Z. Zellforsch.*, **48**, 10-23.
- YASUZUMI, G., & TANAKA, H. (1958). Spermatogenesis in animals as revealed by electron microscopy. VI. Researches on the spermatozoon-dimorphism in a pond snail, *Cipangopaludina malleata*. *J. biophys. biochem. Cytol.*, **4**, 621-632.
- YATSU, N. (1904). Experiments on the development of egg fragments in *Cerebratulus*. *Biol. Bull.*, **6**, 123-136.
- (1905). The formation of centrosomes in enucleated egg-fragments. *J. exp. Zool.*, **2**, 287-313.
- YEAR, M. (1962). The coding hypothesis. *Int. Rev. Cytol.*, **13**, 1-37.
- YOKOYAMA, H. O., TSUBOI, K. K., WILSON, M. E., & STOWELL, R. E. (1953). Histological studies on regenerating mouse liver. *Lab. Invest.*, **2**, 91-108.
- ZALOKAR, M. (1960). Sites of protein and ribonucleic acid synthesis in the cell. *Exp. Cell Res.*, **19**, 559-576.
- ZANELLA, E., PERACCHIA, A., & CHIAMPO, L. (1961). Über die Bedeutungslosigkeit des Geschlechtschromatins für die Behandlung von Tumoren hormonabhängiger Organe. *Z. Krebsforsch.*, **64**, 83-87.
- ZEIGEL, R. F., & DALTON, A. J. (1962). Speculations based on the morphology of the Golgi systems in several types of protein-secreting cells. *J. Cell Biol.*, **15**, 45-54.
- ZEIGER, K. (1935). Zum Problem der vitalen Struktur des Zellkernes. *Z. Zellforsch.*, **22**, 607-632.
- (1938). *Physikochemische Grundlagen der histologischen Methodik*. Theodor Steinkopff, Dresden und Leipzig.
- (1955). Morphologie des Cytoplasmas. *Hdb. allg. Pathol.*, **II/1**. Springer, Berlin.
- ZETTERQUIST, H. (1956). *The ultrastructural organization of the columnar absorbing cells of the mouse jejunum*. Karolinska, Inst. Stockholm.
- ZEUTHEN, E. (1947). Respiration and cell division in eggs of the sea urchin *Psammechinus miliaris*. *Nature (Lond.)*, **160**, 577-578.
- (1955). Mitotic respiratory rhythm in single eggs of *Psammechinus miliaris* and of *Ciona intestinalis*. *Biol. Bull.*, **108**, 366-385.
- (1961). Cell division and protein synthesis. *Proc. I. IUB/IUBS Symp. Acad. Press, London. Vol. II.*
- ZHINKIN, L. N., & MIKHAILOV, V. P. (1958). On "the new cell theory". Two Soviet authors critically review recent Soviet work on the origin of the cell. *Science*, **128**, 182-186.
- ZIEGLER, H. E. (1891). Die biologische Bedeutung der amitotischen (direkten) Kernteilung im Tierreich. *Biol. Zbl.*, **11**, 372-389.
- (1895). Untersuchungen über die ersten Entwicklungsvorgänge der Nematoden. *Z. wiss. Zool.*, **60**, 351-410.
- ZILLIG, W. (1958). Die Synthese der Proteine in der Zelle. *Dtsch. med. Wschr.*, **83**, 980-982.
- ZIMMERMAN, A. M. (1960). Physico-chemical analysis of the isolated mitotic apparatus. *Exp. Cell Res.*, **20**, 529-547.
- ZIMMERMANN, (1898). Beiträge zur Kenntnis einiger Drüsen und Epithelien. *Arch. mikr. Anat.*, **52**, 552.
- ZIMMERMANN, W. (1960). Unser heutiges Wissen von der Evolution. *Med. Grundlagenforsch.*, **3**, 651-704.
- ZINCK, K. H. (1940). Pathologische Anatomie der Verbrennung, zugleich ein Beitrag zur Frage der Blutgewebschranke und zur Morphologie der Eiweißzerfallsvergiftung. *Veröffentl. Konstit.-u. Wehrpath.*, **10**, H. 4/5.
- ZIRKLE, R. E. (1957). Partial-cell irradiation. *Advanc. biol. med. Phys.*, **5**, 104-146.
- ZOLLINGER, H. U. (1948a). Cytologic studies with the phase microscope. III. Alterations in the nuclei of "resting" and dividing cells induced by means of fixatives, anisotonic solutions, acids, and alkali. *Amer. J. Path.*, **24**, 797-811.
- (1948b). Cytologic studies with the phase microscope. IV. Morphologic changes associated with the death of cells in vitro and in vivo. *Amer. J. Path.*, **24**, 1039-1053.

- (1948c). Trübe Schwellung und Mitochondrien. (Phasenmikroskopische Untersuchungen.) *Schweiz. Z. allg. Path.*, **11**, 617-634.
- ZUBAY, G. (1958). A template model for the synthesis of ribonucleic acid from deoxyribonucleic acid. *Nature (Lond.)*, **182**, 1290-1292.
- ZUBAY, G., & DOTY, P. (1959). The isolation and properties of deoxyribonucleoprotein particles containing single nucleic acid molecules. *J. molec. Biol.*, **1**, 1-20.
- ZWEIBAUM, J., & SZEJNMAN, M. (1936). Recherches sur les cellules binucléées dans le tissu cultivé in vitro. *Arch. exp. Zellforsch.*, **18**, 102-126.

Subject Index

- Acetabularia*, 31, 33, 36ff, 42, 43, 50, 53, 55
Acetic acid, 69, 73
Acetylcoenzyme A, 14
Acetocarmine stain, 47, 73, 74, 78
Acetylene, 19
Achromatin, 139, 151
Aconitum, 127
Acridine, 202
Acridine orange, 97
Acrosome, 259
Actin, 148
Adenine, 7, 20, 39, 50, 51, 99, 113, 202, 208, 211
Adenosine diphosphate, 250, 309, 327
Adenosine monophosphate, 250
Adenosine triphosphate, 21, 33ff, 40, 41, 52, 309
and chloroplasts, 328
and colchicine effect, 209
and contraction of mitochondria, 316
in cell cleavage, 173
in cytoplasmic streaming, 275, 276
in flagella movements, 158
in intercellular water transport, 277
in mitosis, 158, 159, 163, 184
in protein synthesis, 21, 40, 250ff
Adenosine triphosphatase, 308
Adenoviruses, 83ff
Adenylic acid, 117
ADP. (*See Adenosine diphosphate.*)
Adherences, chromosomal. (*See Chromosomal stickiness.*)
Adrenal cells, 91, 93
Adrenaline, 202
Adrenochrome, 202
Aedes aegypti, 226
Alanine, 94, 96, 251
Aldolase, 34
Aleurone plastids, 322, 332, 334
Algae, 70
Allium, 45, 142
Allopolyploidy, 227
Allocycle, 74, 107
Aloe, 45
 α -cytomembranes, 10, 246
Ambystoma, 243
Amino-acids, 7, 19ff, 32, 40, 51, 56, 63, 86, 91, 94ff, 115ff, 181, 250ff
activated, 117, 250
formation of, in mitochondria, 309
oxidation of, 309
sequence of, 20, 115ff, 251
Aminoacyladenylate, 250
Aminopeptidase, 290
Amitosis. (*See Nuclear division, amitotic.*)
Ammonia, 19
Ammonium chloride, 20
Amoebae, 32, 33, 38ff, 59, 64, 158, 170, 183, 266ff, 274ff, 282, 284, 288
AMP. (*See Adenosine monophosphate.*)
Amyloplasts, 322, 334
Anaphase, 101, 107, 108, 113, 125, 127, 129, 137, 138, 141, 145, 153, 155–165, 169, 170, 174, 175, 183, 190, 196, 197, 200, 209, 212, 221, 234
Anaphase bridges, 205
Anareplication, 221
Anaemia, siderochrestic, 293
Aneuploidy, 99, 213
Aneura, 45
Annuli, of nuclear pores, 59, 61
Anorthospiral, 113
Anoxia, 207
Antephase, 184, 202
Antibody, 20, 40
Antigen, 20, 40
Antimony sulphide, 58
Antirrhinum majus, 225
Apparatus, achromatic, 139
mitotic, 148
Arbacia, 320
Archiplasm, 133, 151
Arginine, 94, 181
Arhylsulfatase A and B, 290
Arm ratio, chromosomal, 105
Artifact, *in vivo*, 68, 191
Arvelius albopunctatus, 90
Ascaris megalcephala, 29, 72, 73, 102, 132, 133, 137, 142
Aspartic acid, 20, 94
Astrosphere, 133, 135
Atmosphere, at the origin of the earth, 19
ATP. (*See Adenosine triphosphate.*)
ATP–ADP exchange enzyme, 316
Atrophy of the cell, 274
Attachment points, 271
Attachment regions, 271

- Autocatalysis, 20
 Autolysis vacuoles, 280
 Autopolyploidy, 227
 Autosome, 102, 105, 160, 216, 223
 Axial current, in spindle, 165
 Axon, bisection of, 91, 247
 Bacteria, 6, 25, 51, 95, 115
 Bacteriophages, 5, 115
 Balbiani rings, 50, 75, 118, 123, 225
 Banding pattern, in giant chromosomes, 118
 Bands, transverse chromosomal, 45, 47, 118, 119, 123
Barbulanympha, 140
 Basal granules, 137, 138, 144, 149
 Basement membrane, 3, 4, 285, 287
 Bases, sequential arrangement of, in DNA, 115, 178
 in RNA, 51, 56
 Basophilic, cytoplasmic, 12
 nucleolar, 48
Bellevalia, 192, 194, 196
 Benzoquinone, 203
 Berlin blue reaction, 291
 β-cytomembranes, 246
 β-galactosidase, 290
 β-glucuronidase, 290
 β-N-acetylglucosaminidase, 290
 Birefringence, 57, 167
 Bivalents, 160, 193
 Blastomeres, 72
 Blepharoblast, 137
 Blood cells, maturation of, 53
 Blood pigment. (*See Haemoglobin.*)
 Blue algae, 6, 71
 Bone marrow, giant cells of, 219
 Bone marrow cells, DNA content of, 98
 Bouquet stage, in meiosis, 191
 Breakage, of chromosomes. (*See Fragmentation.*)
 Brown algae, 323
 5-bromo-uracil, 116
 Brushborder, 16, 282, 283
Bufo bufo, 287
 Caffeine, 207, 210
 Calcium, 34, 291
 pectate, 16
Calliphora, 225
Caltha palustris, 221
 Carbide, 19
 Carbohydrate cycle, in chloroplasts, 328
 Carbohydrates, 6, 8, 33, 291, 295, 301, 321, 322, 328
 Carbon dioxide, assimilation of, 321
 Carbon tetrachloride, 247, 248, 280, 315, 320
 Carcinogenesis, 52, 213, 239, 247–248, 318
 Carcinoma cells, 48, 53, 54, 70, 77, 79, 92, 95, 99, 177, 181, 219, 223, 231, 258, 282, 313
 Carotene, 14, 322
 Carotinoid crystals, 323
 Catalase, 280
 Catalysts, 19
 Cathepsin A and B, 290
 Cation exchange, intracellular, 305
 Cell, energetic insufficiency of, 33
 Cell cleavage, 3, 170, 171, 174, 236
 Cell death, 273, 300
 Cell divisions, synchronous, 185
 Cell mass, critical, 183
 Cell membrane. (*See Cell wall.*)
 Cell plate, 162, 169, 174
 formation of, successive, 170
 ring, 169
 Cell pores, 254
 Cells, fusion of, 229
 Cells, origin of, 17–21
 Cell theory, 3, 4, 23, 24, 166
 Cellular pathology, 26, 286
 Cellulose, 16
 Cell wall, 8, 16, 115, 167, 168, 173, 243, 264, 286, 300
 Central fibres, 141, 144ff, 149, 158, 165
 Central spindle, 139–141, 154
 Central vacuole, of plant cells, 276
 Centriole 8, 132–133, 135, 136, 137, 141, 242, 257, 258
 Centrodesmose, 139
 Centromere, 104, 105, 108, 114, 122, 141
 diffuse, 142
 Centroplasm, 132
 Centrosome, 16, 17, 123, 132, 135, 137, 138ff, 144, 147
 intranuclear mechanism of, 233
 Centrosphere, 135, 242
 Ceroid pigment, 295
Chaos chaos, 276
 Charge, electrostatic, 69
 Chemotherapy of malignant tumours, 201, 207
 Chiasmata, 193, 194, 197, 198–200
 Chicken osteoblasts, 159
 Chimaeras, chromosomal, 333
Chironomus, 43, 47, 50, 51, 53, 55, 56, 72, 118
Chlamydomon pedarius, 234
 Chloracetophenone, 210
Chlorella, 185
 Chloroform intoxication, 280
 Chlorophyll, 14, 15, 19, 321, 323, 327–329, 333, 334
 mutants, 333
 Chloropicrine, 210
 Chloroplasts, 14, 15, 33, 36, 38, 41, 321, 322, 323–330, 332, 333
 movements of, 329

- Cholesterol, droplets of, deposited in cytoplasm, 296
- Chondriocytes, 262
- Chondriome, 301
- Chondriosomes, 301
- Chromatid halves, 108, 112
- Chromatid quarters, 108
- Chromatids, 108ff, 131, 141, 175, 190, 193, 195, 197, 199, 200
exchange of, 110, 193, 197, 200
separation of, 156
- Chromatin, 40, 44, 50, 67, 73-75, 81, 87, 107, 139, 150
behaviour in amitosis, 231
elimination, 129
- Chromatogenesis, 249, 256
- Chromatolysis, 247, 256
- Chromatophores, 321, 322
- Chromocentres, 9, 46, 57, 74ff, 79, 89, 106, 222ff
- Chromomeres, 107, 113, 122, 191, 199
- Chromonemata, 108, 114, 118, 130, 199, 225, 234
primary, 71
secondary, 71
- Chromoplasts, 14, 321ff, 332, 334
- Chromosomal injury, 201
- Chromosomal spindle, 144-145, 149
- Chromosomal spindle fibres, 141, 143ff, 149, 151, 154, 156, 158, 159, 161, 165, 167
- Chromosomal substance, 107
- Chromosomal stickiness, 106, 156, 205, 207
- Chromosome arms, 110, 152, 163
- Chromosome breaks, potential, 208
- Chromosome ends. (*See* Chromosome arms.)
- Chromosome fragments, 142, 143
acentric, 142
centric, 142
- Chromosome models, 110ff
- Chromosome mutations, spontaneous, 208
- Chromosome sets, 90, 92, 99, 114, 218, 227, 233, 235
defect in, 156
- Chromosomes, 5, 9, 21, 28, 41, 44-47, 56, 57, 64, 66, 67, 72, 73, 75ff, 89, 90, 92, 94, 100, 101-123, 160, 165, 175, 178, 181, 211-218
acentric, 206
acrocentric, 104, 105
bicentric, 206, 209
bivalent, 154
ejectcd, 211
invisible, 102
mass of, 90
number of, 90, 101-103, 188, 209, 213, 218
size of, 101-103
splitting of, 175
- Chymotrypsinogen, 253
- Cilia, 16, 158, 183
- Cinematographic observations. (*See* Film observations.)
- Circulation, of cytoplasmic streaming in plants, 275
- Circumvallation, form of phagocytosis, 288
- Cisterns, perinuclear. (*See* Perinuclear space.)
- Cis-aconitic acid, 14
- Citric acid, 14
- Cleavage divisions, 3, 24, 73, 77, 132, 133, 137, 144, 166, 170, 171, 174, 319
- Clivia*, 153
- C-mitosis, 209, 211, 223
- Coacervates, 20, 21
- Coagulation, 87, 89
- Coagulative necrosis, 273-274, 301
- Cobalt, 19, 100
- Coenzyme Q, 310
- Cohesion forces, intermolecular, 271
- Coiling, change in orientation of, 113
- Coiling, degree of, 199
- Coiling, of chromosomes, 75, 107, 113, 123, 128, 130, 191, 223, 225
paranematic, 113
plectonematic, 113
relational, 193, 197, 200
- Colcemide, 210
- Colchicine, 110, 111, 172, 200, 209, 221
derivatives, 209
- Collagen, 262, 264
- Collective chromocentres, 223
- Collective chromosomes, 72, 102
- Collective nucleolus, 42, 56
- Colloid chemistry, 66
- Colloids, hydrophilic, 272
- Colpoda steinii*, 233
- Concentration gradient, osmotic, 277
- Condensation, chromosomal, 107, 191, 223ff
- Constriction, elastic, 105
primary, 104, 107, 123, 141, 191, 197, 208
secondary, 105, 107, 123, 191, 197, 208
- Contraction, of chromosomes, 64
- Contraction ring, 173
- Contraction stage, at prophase, 151, 153
- Copper, 100, 291
- Corixa*, 224
- Coronary insufficiency, 315
- Cortex, in egg cells, 274
- Cortisone, 202, 209
- Cosmopolitan hypothesis, 18
- Cover cells, glomerular, 91
- Crepis*, 45
- Cristae mitochondriales, 12, 305, 306, 313, 315ff, 318, 321
- Crossing over, 200
- Cryptendomitoses, 225
- Crystal lattice, intranuclear, 82, 83ff

- Crystals, cytoplasmic, 12, 296
 CTP = Cytidine triphosphate, 252
Culex, 220
 Cyanide, 316
 Cyanogen compounds, 34
Cyanophycea. (*See Blue algae.*)
Cyclops, 151
 Cystine, 94, 253
 Cytaster, artificial, 137
 Cytidine, 39, 50
 Cytidyllic acid, 117
Cytoblastema, 24, 41
 Cytochrome, 12, 254, 302, 309, 310, 311, 320
 oxidase, 202, 303, 309
 Cytochrome C oxidase, 34
 Cytochrome C reductase, 303
 Cytogenetics, 25
 Cytokinesis, 166-174, 197
 impairments of, 201, 211
 Cytolysomes, 318
 Cytomegaly, 86
 Cytonemata, 266
 Cytopempsis, 286
 Cytophotometry, 25, 93, 95, 97, 99, 175, 176, 213,
 225
 Cytoplasmic movement, 158
 Cytoplasmic fragments, non-nucleate, 211
 Cytoplasmic spindle, 141
 Cytoplasmic streaming, 158, 274-275, 301
 Cytoplasmic structures, denseness of, reversible
 increase in, 274
 Cytosinc, 7, 51, 113
 Cytosomes, 294, 295

Dactylophryga stage, 235
Datura, 158
 Daughter cells, 6, 166, 174, 188
 Daughter chromosome, 156, 165, 175
 Daughter nuclei, 129, 205, 219
 Defaecation vacuole, 284
 Degeneration,
 vacuolar, 277-280
 vesicular, 280-281, 301
 Dehydration, 68, 71, 89, 128, 132, 191, 273
 Dehydrogenase, 34
 Deletion, chromosomal, 205, 206, 213
 Demococcine, 210
 Denver convention, 102, 104, 213
 Depolymerization, 100
 Deposits, cytoplasmic, 266, 291ff
 in mitochondria, 318
 Desoxycholate, 249
 Desoxyribonuclease, 34, 97, 320
 acid, 290
 Desoxyribonucleic acid. (*See DNA.*)
 Desoxyribose, 8, 116

 Dextran drops, phagocytized, 289
 Dextran vacuoles, 290
 Diakinesis, 193, 196
 Diatoms, 323
Diaulula, 139
 Diethylnitrosamine, 49, 248, 281
 Dictyosome, 257, 259
 Dicumerol, 34
 Differentiation, 19, 29, 30, 52, 77, 95, 118, 126,
 238
 loss of, 52
 Digressing movement, of cytoplasm, in plants, 275
 Dimethylaminoazobenzol, 248, 291
 Dimethylnitrosamine, 248
 Dinitrophenol, 34, 36, 316
 Diphosphopyridine nucleotide. (*See Nicotinamide adenine dinucleotide.*)
 Diplosomes, 137, 138, 151
 Diplotene, 193, 194, 197ff
Diptera, 45, 102, 118, 119
 Directional body, 197
 Dissection, of the nucleus, 231, 233
 Distraction, of the nucleus, 231, 233
 Diurnal rhythm, of nuclear volume, 91
 Division amitosis, 237, 238
 DNA, 5, 7ff, 28, 34, 44-45, 47-48, 51-52, 57, 73,
 77, 79, 82, 83, 86, 87, 94-95, 96-100, 105,
 113, 114-123, 130, 141, 164, 195, 203, 225
 and mitochondria, 320
 chains, 111
 classes, 98-99, 219, 225
 code, 115-117
 content, of nucleus, 90, 93, 97, 114, 118
 constancy in, 98, 99
 in amitosis, 231, 236
 double helix, 109, 111ff, 116, 123, 132, 178
 extranuclear, 97-98
 primer, 52, 116, 178
 single stranded, 116
 soluble, 100, 101
 synthesis, 108, 110, 115, 175-180, 182, 188, 190
 cytoplasmic, 98
 disturbances in, 202, 205, 207
 synthesis time, 178, 182
 DOCA = desoxycorticosterone acetate, 209
 Double chromocentres, 223
 Double chromosomes, 223
 Double nuclei, 236
 Doubling rhythm, 81
 of nuclear DNA, 99
 of nuclear volume, 30, 90
 DPN. (*See Nicotinamide adenine dinucleotide.*)
 DPNH-oxidase, 309
 Drops, hyaline, 280, 295, 301
Drosera, 143
Drosophila, 48, 50, 71, 78, 137, 199, 216, 227

- Drosophyllum*, 45
 Drum stick, 79
 Dry mass, of nucleus, 92, 101, 151, 179
 of nucleolus, 43, 48
 Dry weight. (*See* Dry Mass.)
 Dysionicity, 277
Dytiscus, 97
- Ecdysone, 189
 ECHO viruses, 296ff
 Eclipse, 300
 Ectoplasm, 268, 269, 271, 275, 276
 Egg albumin, 63
 Egg cell. (*See* Oocyte.)
 Elaioplasts, 322, 332, 334
 Electron transport system, 12, 303, 306, 310,
 327
 Elementary fibrils, 108
 Elimination chromatin, 129
Elodea, 331
 Embden-Meyerhof cycle, 12
 Embryogenesis, 29, 77
 Embryo sac, in seed plants, 3
 Endocytogenesis, 24, 240
 Endometaphase, 222, 223
 Endomitosis, 90, 124, 156, 218-227, 239, 240
 Endoplasm, 267, 269ff, 276
 axial, in amoebae, 276
 Endoprophase, 222
 Endoreplication, 219, 220
 Endoschisis, 228
 Endotelophase, 222, 223
 Engulfing, form of phagocytosis, 288
 Enolase, 34
 Enzymes
 amino-acid activating, 51, 250
 hydrolytic, 290, 301
 isolation of, by electron microscopy, 306-308
 Ependyma cells of spinal cord, 90-91
 Equatorial plane, 125, 129, 132, 152, 154, 157, 160,
 164, 170ff, 195, 197, 209
 Equatorial plate, 125, 132, 149, 153, 154, 163, 166,
 183, 195, 208
 Equation division, 189
 Equilibrium, transversal, at metaphase, 155
 Equivalents, 241, 268, 272
 Equivalents, nuclear, 6
 Ergastoplasm, 8, 12, 29, 51, 54, 56, 61, 169, 247-
 249, 253, 256, 257, 280, 300
Erantis hemalis, 224
 Erythroblasts, 89, 292-294
 Erythrocytes, 22, 23, 28, 29, 32, 35, 57, 71, 96, 108,
 126, 242
 Esterase, non-specific, 290
 Ethionine, 249
 Ethylenimine, 207
- Euchromatin, 74, 75, 79, 89, 105-108, 123, 165,
 197, 223
Euglena, 231, 322
Euploës, 179, 182
 Evolution, biological, 18
 chemical, 18
 structural, 21
 Expulsion vacuole, 273
 Extracellular space, 59, 242
- Fast green stain, 95, 176, 181
 Fasting. (*See* Hunger.)
 Fat. (*See* Lipid.)
 Fat deposits, 12
 Fat drops, transformation of mitochondria into,
 311
 Fat metabolism and mitochondria, 311
 Fat resorption, 264
 Fat storage, 264
 Fattening diet, 295
 Fat tissue, necroses, 295
 Fertilization, 29, 218
 Ferric hydroxide phosphate, 291
 Ferritin, 284, 291, 292, 294
 Feulgen stain, 9, 28, 44, 57, 72, 73, 77, 80, 97, 98,
 100, 176, 225
 Fibres, continuous, 141
 Fibrilolysis, 280
 Fibroblasts, 98
 Film observations, 59
 Fixation, 66, 68, 71
 Fixation, representation of nucleus after, 27, 42,
 44, 57, 68-71
 Flagella, 16, 158
 Flavoprotein, 48, 302
 Fluorescence microscopy, 97
 Foam cells, 296
 F₁ generation, uniformity of, 333
 Formazan, precipitations of, 303
 Formic acid, 19
 Fountain zone, in amoebae, 276
 Fragmentation, of chromosomes, 205ff, 208
 Fumaric acid, 14
 Functional cycle, of the nuclear shape, 30, 54, 55
 6-furfurylaminopurine, 187
- Gametes, 188, 189, 217
 Gametic type, of meiosis, 189
 Gametophyte, 189
 γ -cytomembranes, 246
 Gamma globulins, fluorescent, 63
 Ganglion cell. (*See* Nerve cell.)
 Gastric epithelium cell, 74
 Gastrula, 77, 320
 Gel, 67, 173, 274

- Gene, 5, 9, 17, 21, 25, 28-30, 31, 41, 48, 50, 97, 107, 114-118, 119, 123, 198, 199, 213
 Gene function, 115-118
 "Generatio spontanea", 17
 Gene substance, 5, 9, 28, 99, 114, 175, 236
 Genetic map, 117, 119
 Genome, 188, 195, 197, 220, 233, 235, 333
 rearrangement, 195
 segregation, 233, 235
 Germ cells, 101, 114
 Germinal vesicle, 23
Gerris lateralis, 222
 Giant amoeba, 146, 147
 Giant cells, multinuclear, 211, 212
 Giant chromosomes, 45, 47, 50-51, 72, 75, 114, 118-123, 127, 197, 224, 225
 Giant lysosomes, 290
 Gierke's disease, 295
 Globulin, 63
 Glomerulonephritis, extracapillary, 91
 Glucose, 6, 14, 322
 and mitosis, 184
 Glucose-1-phosphate, 306
 Glucose-6-phosphatase, 254
 Glutamic acid, 20, 94, 310
 dehydrogenase, 310
 Glyceraldehyde phosphate dehydrogenase, 34
 Glycine, 19, 94, 96, 252
 Glycogen, 33, 35, 80, 91, 295
 production of, 254
 Glycogen nuclei, 80
 Glycolysis, 34, 35, 41, 266, 300
 anaerobic, 317
 and mitosis, 184
 Glycoside bond, 8
 Gold, colloidal, 63
 Golgi apparatus, 8, 15, 16, 136, 183, 241, 242, 246, 253, 256-266, 291, 294, 318, 320
 Gonia, 197
 Grana, primary, in plastids, 332
 Grana lamellae, in plastids, 323, 325, 328, 332
 Granules, intramitochondrial, 305, 318
 pericanalicular, in the liver, 290
 Granule theory, 5
 Granulocytes, 57, 79, 88, 98, 126, 239, 286, 287, 290, 305
 Ground substance, cytoplasmic, 11, 12, 17, 136, 241, 247, 254, 264, 266-301, 310, 322, 330
 Growth, 4, 6, 90, 127, 175, 186
 Growth amitosis, 238
 GTP = guanosine triphosphate, 251
 Guanine, 7, 51, 113, 202
 Guanylic acid, 117

Haemanthus, 153, 157, 162
 Haemofuscin, 294
 Haemoglobin, 19, 32
 Haemosiderin, 291
 Heat shock, 185
 Heliozoon, 150
 Hemicelluloses, 16
 Hepatectomy. (*See* Partial hepatectomy.)
 Hepatitis, 86
 Hepaton, 3
 Heredity, extrakaryotic, 333
 maternal, 333
 plasmatic, 334
 Heredity, science of, 25
 Herpes virus, 82, 83
 Heterochromasia, 75
 Heterochromatin, 44, 45, 74, 75, 89, 105-108, 122, 123, 128, 165, 191, 198, 208, 223
 Heteropycnosis, 78, 105
 Heterosome, 102, 216, 217
 Hexokinase, 202
 Hexose, 6
 Hexose phosphate, 328
 Histamine, 278
 Histidine, 94, 181
 Histion, 4
 Histone, 9, 48, 52, 63, 86, 88, 93ff, 101, 176, 181, 188
 Holoschisis, 228
Hordeum, 45
 Humoral pathology, 26
 Hunger, 30, 32, 35, 229, 244, 254, 264, 265, 311, 312, 317
Hyalodiscus simplex, 267, 268, 275
 Hyaloplasm, 12, 266, 267, 270
 Hydration, 68, 75, 89, 92, 101, 130, 132, 165, 181
 Hydrocyanic acid, 20
 Hydroxylases, 254
Hypermastigina, 134
 Hypertonicity, intracellular, 277
 Hypoglossus nerve cells, nuclei of, 77
 Hypophysectomy, 91
 Hypoxia, 11, 277, 278, 280, 296, 315
 Idiogram, 108
 Incorporation, form of phagocytosis, 288
 Incineration, of nuclei, 100
 Inclusion body encephalitis, 82
 Information, genetic, 9, 25, 41, 51, 116
 Intercalated discs, 4
 Intercellular substance, 3, 5
 Interference microscope, 92, 179
 Interkaryokinesis, 132, 135, 138, 145, 150, 165
 Interkaryokinetic time, 126, 127
 Interkinesis, 195, 196
 Intermediary body, 125, 164, 167, 183
 Intermitosis, 126, 127, 177, 178, 181, 201, 205, 223, 224, 236

- Interphase, 44, 46, 75, 106, 107, 109, 113, 114, 125ff, 133, 137, 174-188, 192, 201, 209, 221, 223, 234
interference with, 202-204
- Interreplication, 221
- Intersexes, 78
- Interval, mitosis-free, 203, 205
 prekaryokinetic, 177, 178, 184, 185, 204
- Interzonal fibres, 163, 167
- Interzonal region, 129, 165
- Interzone structure, 166ff
- Intestinal epithelia, 59, 73, 74, 91
- Invagination, form of phagocytosis, 288
- Inversion, chromosomal, 206
- Ionization density, linear, 208
- Iron, catalytic properties of, 19
- Iron pigment, 80, 301
- Iron sulphide, 20
- Ischaemia, 91
- Isochromatid breaks, 206
- Isochromosome, 105
- Isocitric acid, 14
- Iso-ionicity, 277
- Isolation of nuclei, 92
- Isoleucine, 94, 96
- Janus green, 34, 301ff, 306, 320
- Jensen's sarcoma, 55, 204, 207, 212
- Kalymmauxosis, 228
- Karyogamy, 188
- Karyokineses, abnormal, 221
 monopolar, 156
 multicentric, 210
- Karyokinesis, 64, 124, 125, 129, 132, 134, 137, 139, 144, 145, 150ff, 154, 155, 160, 166, 167
- Karyology, 27
- Karyolymph. (*See* Nuclear sap.)
- Karyolysis, 87
- Karyomeres, 72
- Karyonomy, 240
- Karyoplasm, 9, 43, 48, 58, 59, 61, 63, 66-89, 101, 114
- Karyorrhexis, 87
- Karyotine droplets, 66
- Karyotype, 108, 214, 215
- Kidney cell, 73, 74, 81, 91, 95, 98, 282, 283, 285, 286, 295, 296, 304, 311, 318
- Kidney tubules, failure of, role of mitochondria in, 315
- Kinetin, 187
- Kinetocentres, 132-139, 141, 144, 145, 147, 149, 151, 154, 157ff, 163, 165ff, 170-172, 173, 174, 191, 198, 200, 209
disturbance in division of, 210
invisible, 135
- Kinetochore, 104, 107, 131, 137, 138, 141-144, 147, 151, 152ff, 156, 159, 163, 165ff, 179, 191, 195, 197, 198, 200, 205, 209
 diffuse, 142
- Kinetoplast, 98, 320
- Kinetosomes. (*See* Basal granules.)
- Kinoplasm, 133, 151
- Klinefelter's syndrome, 79, 216, 217
- Kornberg system, 116
- Krebs citric acid cycle, 14, 321
- Lampbrush chromosomes, 55, 108, 118, 119, 121ff, 197
- Langerhans islet cells, 90
- Lead poisoning, 81
- LE-cell phenomenon, 88
- Lepidosomes, 256, 260
- Leptotene, 108, 191, 192, 199
- Leucine, 40, 94, 252, 261
- Leucocyte. (*See* Granulocyte.)
- Leucocyte test, 79
- Leucoplasts, 14, 321, 322, 328, 332
- Leukaemia, 95, 213, 215
- Lever, in chromosome movement, 160
- Light cells, in *Chlorella*, 185
- Lignin, 16
- Lilium*, 128, 140, 169, 184, 190, 191
- Lipidosis, 296
- Lipids, 6, 8, 35, 58, 80, 100, 147, 291, 295-296, 301, 303, 320, 322
 storage of, in plastids, 329
- Lipochondria, 256, 260, 264
- Lipofuscin, 290, 294, 303
- Lipoproteins, 8, 86, 310
- Liver cell, 4, 8, 9, 11, 30, 31, 34, 39, 42ff, 48, 51, 53, 54, 69, 73, 74, 76, 77, 80, 87, 90ff, 94, 99, 219, 236, 242, 246ff, 254, 273, 278, 279, 281, 288, 290, 291, 303, 311, 312, 317, 318
- Liver cells, dark, 274
- Living units, subcellular, 5
- Lochkerne*. (*See* Nuclei, vacuolized.)
- Locomotion, of nucleoli, 54
- Locus, chromosomal, 46, 51, 56, 117, 119
- Locust spermatocytes, 64
- Longitudinal split, in chromosomes, 108, 130-131, 190, 223
- Long range forces, 138, 198
- Loose coiling, regions of, in chromosomes, 105, 208
- Lophius piscatorius*, 54
- Lupus erythematosus, 88
- Lymphocyte, 42, 57, 73, 74, 81, 89, 95, 99, 258
- Lysine, 94, 95
- Lysosomes, 260, 290-291, 294, 295, 301, 305
- Macroglobulinaemia Waldenström, 41

- Macronucleus, 68, 71, 72, 134, 151, 176, 179, 181, 182, 220, 230, 233, 234
 Macrophages, 282, 287, 288
 Magnesium, catalytic properties of, 19
 Magnesium pectate, 16
Makinoa, 45
 Maleic hydrazide, 208
 Malformations, in humans, 227
 Malic acid, 14, 20
 Mantle fibres, 140, 141, 149
 Matrix, chromosomal, 107, 113, 122, 130
 in mitochondria, 14, 304, 305, 312, 315, 318
 Maturation division, 142, 189
 Mciosis, 64, 112, 114, 124, 127, 154, 188-200
 bouquet stage, 114
 disturbances of, 200-218
 Melanin, 262, 294
 Melanoma cell, 263
 Membrane potentials, electrostatic, 255
 Membrane transport, active, 286, 301
 Membranes, osmophilic, 58, 59
 Mengo virus, 296
 Mercaptoethanol, 134, 145
 Meristem cells, 331, 332, 334
 Microamitosis, 240
 Merotomy, 32-33, 38, 39
 Mesothelium cell, 72
 Metachromasia, 49
 Metakinesis, 152
 Metaphase, 73, 101, 102, 104, 105, 107, 112, 125, 127ff, 131, 132, 137, 138, 141, 145, 146, 154-155, 158, 163, 171, 183, 195, 197, 198, 200, 212, 220, 234
 arrest, 209
 chromosome, 108
 plate, 101, 103, 129, 154, 160, 165, 233
 Metareplication, 221
 Methane, 19, 20
 Methionine, 40, 94, 250
 Methylene blue, 34, 48, 257
 Methyl green, 97, 100
 Micelles ferrugineuses, 294
 Microbody, 11, 294, 320
 Micro-electrophoresis, 25
 Microfibrils, 108, 109, 112
 Micronuclei, 47, 64, 205
 Microsomes, 12, 241, 249, 250, 253, 266
 Microsporocytes, 46
 Microvilli, 10, 282, 283
 Migration substance of anaphase, 163
 Millon reaction, 93
Mirabilis jalapa, 333
 Mitochondria, 8, 12ff, 17, 26, 34, 59, 64, 80, 135, 163, 183, 218, 241, 250, 254, 259, 262, 263, 268, 278, 294, 300, 301-321.
 contraction, 316
- motility, 303, 311
 regeneration rate, 320
 swelling, 312-315
 Mitosis, 9, 16, 28, 44, 64, 71, 75, 90, 101, 109, 110, 124-188
 abnormal, 124
 balance theory of, 163
 onset, impairment of, 204
 Mitotic disturbances, 47, 200-218
 Mitotic poisons, 201, 218
 Mitotic rhythm, 185-188
 Mongolism, 213, 214
 Monocytes, 70
 Mono-iodacetamide, 210
 Mono-iodacetic acid, 210
 Mononucleotides, 116
 Mosaic eggs, 29, 30
 Mouth epithelium test, 78, 79
 Movement, amoeboid, 32, 79, 157, 174, 268, 275-276, 301
 Multicellularity, stage of, 4
 Multidivision, simultaneous, 166
 successive, 166, 235
 Multi-enzyme systems, 308-310, 321
 Multiple strand hypothesis, of chromosome, 109ff, 112, 131
 Muscle fibres, 5, 13, 29, 77
 Muscle phosphorylase, 306
 Mutagens, 115, 206, 211
 Mutation, 111, 117, 213
 Mutation theory of cancer, 213
 Muton, 118
 Myelin structures, 294
 Myeloid structures, 246
 Myleran, 207
Mytilus, 158
- NAD. (See Nicotinamide adenine dinucleotide.)
 NAD diaphorase, 312
 Nadi reaction, 303 306
 Natural philosophy, 23
 Necrosis, 230, 291, 294
 Nephron, 3, 91
 Nerve cell, 3, 4, 30, 39, 42, 77, 87, 89, 91, 126, 171, 233, 242, 247
 "Neuralpathologie", 3
 Neuroblasts, 150
 Neurofibrils, 29
 Neurosecretion, 253, 262, 264, 311
Neurospora, 117
 Neutral fats, 7
 Neutral red, 257
 Nicotinamide adenine dinucleotide, 35, 41, 48, 203, 280, 316
 Nile blue sulphate, 257
 Nissl bodies, 247, 257

- Nitrates, 19
 Nitrogen mustard, 88, 203, 207
 Nondisjunction, 216, 217
 Non-histone protein, 93
 Nuclear
 barrel, 139
 buds, 235, 239, 240
 corpuscle. (*See* Nucleolus.)
 degeneration, 86
 division, amitotic or direct, 124, 228-240
 envelope, 8, 17, 34, 41, 42, 53ff, 57-66, 72, 73,
 75, 77, 82, 89, 101, 114, 124, 125, 144, 147,
 150, 165, 183, 193, 195, 221, 246, 247, 320
 hyperchromatosis of, 86
 infolding in, 300
 evaginations, 42, 61
 folds, 54
 framework, 66, 67
 growth, rhythmic, 90, 92, 101, 223
 inclusion bodies, 80-87
 indentations, 53
 invaginations, 54
 material, extrachromosomal, 151
 membrane. (*See* Nuclear envelope.)
 oedema, functional, 30, 91, 92, 101
 pathological, 91, 92, 101
 pellets, 53, 80
 polymorphism, 232, 239
 pores, 9, 55, 58-61, 66
 processes, 55, 79
 sap, 67, 71, 89, 189
 spindle, 139
 substance, non-chromosomal, 151
 volume. (*See* Nucleus, size of.)
 volume reaction, 91
 volume test. (*See* Nuclear volume reaction.)
 wall. (*See* Nuclear envelope.)
 Nuclei, classes of. (*See* Nuclear growth, rhythmic.)
 fusion of, 221
 vacuolized (*Lochkerne*), 80
 Nucleic acid, maximal light absorption, 114
 Nucleic acid load, theory of, 106
 Nucleocytoplasmic relationship, 183
 Nucleohistone, 9, 95, 100
 Nucleolar
 functions, 49-56
 matrix, 9
 membrane, artificial, 44
 organizer, 44, 46, 48, 50ff, 56, 106, 129, 193
 processes, 54
 Nucleoli, number of, 42, 56
 Nucleoli, persisting, 129
 Nucleolini, 43
 Nucleolonema, 9, 10, 43, 45, 51, 53, 56, 129
 primary, 43
 secondary, 43
 Nucleolus, 8, 9, 17, 24, 30, 31, 35, 39, 41-57, 66
 67, 69, 71ff, 75ff, 80ff, 86, 89, 100, 101,
 106, 114, 122, 125, 129, 132, 150ff, 165,
 191, 193, 210, 222, 302
 amitotic division of, 231
 enlargement of, 31, 52, 53, 86, 203, 204, 300
 formation of, 44, 105
 main, 55, 69
 shape of, 42
 shrinking of, 31, 53
 size of, 42, 45, 46, 52, 53
 special, 42
 Nucleomalacia, 88
 Nucleoplasm. (*See* Karyoplasm.)
 Nucleoside diphosphatase, 260
 Nucleosides, 8, 97, 113, 178
 Nucleotides, 8, 49, 116ff
 Nucleus,
 death of, 87-88
 enlargement of, 30, 31, 63, 67, 68, 90, 203, 204
 fractionation of, 51
 fragmentation of, 228, 239, 240
 functions of, 28-41, 93ff, 100, 101
 membrane nucleotides of, 55
 rotation of, 59
 segmentation of, 228, 239
 shrinking of, 90
 size of, 63, 89ff, 101, 181, 182, 229, 231
 in amitosis, 235, 236
 volume of. (*See* Nucleus, size of.)
 swelling of. (*See* Nucleus, enlargement of.)
 Nutritional cell nuclei, in ovaries, 62, 97
 Nutrition vacuole, 183
Oenothera, 208
 Oöcyte, 3, 29, 35, 42-43, 48, 49, 51, 52, 55, 57, 63,
 69, 72, 89, 97, 118, 119, 121, 139, 144, 151,
 170, 174, 184, 189, 197, 212, 216, 217, 274,
 319.
 Oögonia, 97
 Opossum, 246, 305
 Orientation birefringence, 138
 Orthospiral, 113
 Oscillations, of chromosomes, 155
 Osmium tetroxide, 69, 75, 76, 147, 256, 258, 264
 Osteoblasts, 262
 Oxalic acid, 19
 Oxaloacetic acid, 14
 Oxalosuccinic acid, 14
 Oxidations, biological, 302
 Pachytene, 109, 191, 197ff
 Pairing, meiotic, 102
 procentric, 191, 197
 proterminal, 191, 197
 somatic, 199

- Pale granules. (*See* Ribosomes.)
- Pancreas cells, exocrine, 39, 41, 54, 55, 67, 91, 98, 240, 244, 258, 260, 261, 303, 308, 311
- Papaver*, 226
- Papilloma, in rabbit, 86
- Para-erythroblasts, 291
- Paragenoplastin, 151
- Paramecium*, 151, 158, 176, 181, 187, 220, 268
- Paranuclei, endoplasmic, 239, 245, 246, 305
- Paraplastin, 266, 291
- Partial hepatectomy, 31, 53, 91, 187, 202, 226, 229, 237, 311, 320
- Pectins, 16
- Pellicula, of chromosomes, 16, 155, 167
- Pentose, 6ff
- Pepsin, 97
- Peptide bond, 7, 20
- Peptide chains, synthesis of, 250
- Perinuclear space, 10, 55, 59, 246, 255
- Periodic acid leucofuchsin reaction, 135
- Permeability, of nuclear envelope, 63, 66
- Permeability, pathology of, 286
- Phagocytosis, 87, 88, 282, 286-290, 292, 295, 300
- Phagosomes, 294
- Phase contrast, 9, 25, 27, 47, 66, 68, 72, 74, 87, 122, 124, 135, 140, 145, 154, 160, 170, 179, 181, 247, 257, 262, 267, 312, 320
- Phaseolus vulgaris*, 329
- Phenoschisis, 228
- Phenylalanine, 40, 94, 117, 252
- Phenylurethane, 212
- Pheoplasts, 323
- ^{1}Ph chromosome, 213
- Phosphatase, alkaline, 48, 285, 286
acid, 48, 260, 288, 290
- Phosphatides, deposits in cytoplasm, 296
- Phosphoglyceric acid, 328
- Phospholipids, 107, 249, 260, 272
- Phosphoprotein phosphatase, 290
- Phosphorus intoxication, 280
- Phosphorylation, oxidative, 12, 14, 33-34, 309, 310, 315, 316, 321
- Photolysis, of water, 328, 334
- Photophosphorylation, 328, 334
- Photosynthesis, 14, 37, 321, 323, 328, 329, 334
and protein synthesis, 326, 328
- Phototaxis, negative, 330
positive, 330
- Phragmoplast, 162, 165, 166-170
- Phragmoplast division, 174
- Phragmosomes, 169
- Phragmosphere, 169
- Phyco-erythrine, 323
- Phytochrome, 330
- Pigment, old age, 295
- Pigments, 12, 266, 291
iron, rich in, 291-294
lipogenic, 294
- Pilocarpine, 30, 258
- Pinocytosis, 16, 242, 254, 268, 281-286, 287, 290, 292, 300
- Plant galls, 53, 185
- Plasma cap, hyaline, in amoebae, 275
- Plasma cell, 69, 71, 81, 242, 243, 254, 257ff, 295
- Plasma gel, 267
- Plasmalemma, 284
- Plasmasol, 267
- Plasmodium, 4
- Plasmolysis, 277
- Plasmon, 334
heredity, 334
- Plastidome, 333-334
heredity, 334
- Plastids, 5, 8, 14, 17, 163, 241, 305, 321-334
formation of, 331-333
grana, 15, 323, 325, 329, 334
- Platelets, osmophilic, 256
- Ploidy, degree of, 56, 78, 90, 95, 99, 100, 222, 224, 239
- Ploidy mutations, 227
- Podophyllin, 172, 210
- Polar body, 197
- Polarization microscope, 57, 138, 145, 149, 161, 162, 167
- Polar radiation, 132, 133, 135, 139, 140, 141, 144, 147, 158, 165, 167, 171, 173, 209, 218
- Pole caps, 183
- Pole region, 151
- Poliomyelitis, 86
- Pollen mother cell, 140
- Pollen tube, 128
- Polyacrylic acid, 34
- Polyanions, 34
- Polycations, 34
- Polyenergids, concept of, 219, 220
- Polygene, 107
- Polyhedron disease, of insects, 83
- Polymerase, 116
- Polymer mitoses, 225
- Polymer, 225
- Polynucleotides, 116
- Polyoma virus, 86
- Polypeptides, 20, 63, 115, 117
- Polyplloidization 95, 114, 209, 213, 238
and differentiation, 226
and nuclear function, 226
- Polypliody, 42, 78, 90, 92, 99, 102, 124, 210, 218-227, 239
- Polysaccharides. (*See* Carbohydrates.)
- Polysomaty, 226
- Polyteny, 118, 225-226

- Polyuria, tubular**, 281
Polyuridylic acid, 117
Polyvinyl pyrrolidone, 63
Pore complex, in nuclear envelope, 61
Pores, in cell wall, 242
Porphyrin, 19, 294
Position effect, 107
Postmitosis, 126, 137
Potassium, 277
 deficiency, 281
Pre-amyloid, 264
Precocity theory of meiosis, 190
Pressure, colloid osmotic, 63
 osmotic, 276-277
Primary effect of X-rays, 205
Principal nucleolus. (*See Nucleolus, main.*)
Probionts, hypothesis of, 18, 20
Prochromosomes, 106
Pro-enzyme granules, 261
Prolamellar body, of plastids, 332
Proline, 94, 96, 252
Prometaphase, 125, 129, 150, 151-154, 193, 197
Prometaphase stretching, 154ff, 193
Prophase, 64, 72, 105, 106, 108, 112, 113, 124ff,
 132, 137, 138, 140, 150ff, 166, 175, 176,
 183, 184, 188, 205, 221, 235
 meiotic, 113, 189ff, 208
Prophase entanglement, 132, 149
Proplastids, 59, 331, 332, 334
Propylthiouracil, 91
Proreplication, 221
Prosecretion granules, 253
Protamine, 63, 96
Protection colloid, 291
Protein, 6ff, 12, 20, 21, 26, 30, 37, 40-41, 47, 52,
 53, 58, 66, 69, 82, 84, 85, 89, 90, 93-96,
 114, 115, 122, 129, 135, 147ff, 249, 291,
 295, 320, 322
 content in the nucleus, 90, 93-96, 100
 deficiency, dietary, 53, 92, 244, 249
 drops, intracytoplasmic, 261
 nephrosis, experimental, 91
 storage of, 91
Proteinoid, 20
Proteins, contractile, 158, 173, 275, 316
Protein synthesis, 9, 12, 31, 32, 36-41, 49, 52, 56,
 91, 100, 117, 181-182, 242, 246, 249-253,
 260, 280, 309
 and photosynthesis, 326, 328
Protoplasts, 322
Protochlorophyll, 329
Protomeres, 5, 90
Protoplasm, 8, 17
Pseudoamitoses, 205, 229, 239
Pseudoendomitoses, 224
Pseudohermaphrodisim, 78
Pseudopodia, 32
Pseudo-uridine, 51
Pseudoxanthoma cells, 296
Puff, chromosomal, 50, 118, 119, 123
Pulverization phase, 223, 224
Purine analogues, 202
Purine derivatives, 7
Purkinje cells, 249
Pycnomitoses, 239
Pycnosis, 87, 88, 207, 273
Pyrenoids, 322
Pyrimidine derivatives, 7
Pyruvic acid, 19
Quiescent phase, premitotic, 177, 185
Quinone, derivatives of, 203
Rabí orientation, 151
Radioautography, 39-40, 45, 50-51, 55, 75, 91,
 110, 175-176, 179, 250, 261, 320
Rays, cosmic, 19
 ionizing, 88, 106, 108, 156, 201, 203, 206, 208,
 248
 ultraviolet, 19, 50
Reaction amitosis, 238
Rearrangement, at prometaphase, 149, 151, 154,
 163, 166, 200, 209
Reduction, somatic, 220
Reduction division, 189, 200
Recombination, of chromosomes, 205, 208
Recombination unit, 117
Recombinations, intragenetic, 117
Reconstruction, at post-telophase, 74, 105, 125,
 130, 165, 212
Regeneration, 31, 36, 187, 188, 237
"Relationspathologie", 3
Remainder protein, 94, 101
Remainder spiral, 128
Reorganization bands, 179, 181, 182
Replications, interphase, 91, 126, 174ff
Respiration processes and mitosis, 184
Respiratory chain, 12, 14, 33, 48, 310, 315, 321
Respiratory enzymes, 306
Respiratory inhibitors, 316
Resting nucleus, 9, 101, 126, 127
Reticulocytes, 32, 38
Reticuloendothelial system, 286
Reticulum, endoplasmic, 8, 10, 11, 15, 55, 59, 60,
 64, 80, 138, 147, 168, 183, 241-256, 257,
 260, 261, 264, 266ff, 274, 278ff, 285, 296,
 306, 309, 320
 rough-surfaced, 245, 247, 249, 256
 smooth-surfaced, 246, 249, 256, 260
Reticulum cells, 292
Retina, pigment epithelium of, 246, 325
Rhizoid, 31, 36, 37

SUBJECT INDEX

- Rhodnius*, 62
Rhodoplasts, 323
Rhoeo discolor, 223
Rhopheocytosis, 285, 292
Ribonuclease, 48, 63, 210
 acid, 290
Ribonucleic acid. (See RNA.)
Ribose, 8, 116
Ribosome RNA, 51, 251, 255
Ribosomes, 11, 12, 51, 53ff, 61, 71, 129, 135, 146, 147, 242, 244, 248ff, 256, 260, 306, 309
Ribulose diphosphate, 328
Ribulose-5-phosphate, 328
Ring chromosome, 104, 112, 205
Ring bodies, DNA-containing, 97
RNA, 7, 9, 10, 12, 32, 36-41, 46, 48, 49-52, 61ff, 75, 78, 89, 93, 97, 100, 115, 116, 118, 129, 130, 132, 135, 147, 151, 165, 181, 190, 202, 210, 249-253
 cycle, chromosomal, 129
 messenger, 40, 41, 51, 52, 56, 116, 123, 251, 252, 255
 polymerase, 52, 116
 soluble, 40, 49, 51, 56, 116, 117, 251, 255
 synthesis, 38ff, 49-52, 77, 91, 94, 119, 123, 181ff, 188
 disturbances in, 202
Rods, of retina, 325
Roentgen irradiation, 102, 142, 315
Rotating movement, of cytoplasmic streaming, in plants, 275
Rotation, of preprophase nucleus, 152
Russell's corpuscles, 295

Sakaguchi reaction, 95
Salivary gland viruses, 83
Sarcoma, 150
SAT-chromosomes, 45, 105
 most frequent fragmentation of, 206, 207
SAT segments, 44, 45, 106, 123
Satellite, 107, 191, 197
Sauvornatum guttatum, 223
Sciaria, 161
Sea urchin, 34, 56, 108, 134, 148, 156, 170, 173, 174, 187, 210
Secondary effect of X-rays, 205
Secretion, apocrine, 262
 eccrine, 262
 holocrine, 262
 merocrine, 262
Secretion granules, 258, 260, 264, 301
Secretion process, 16, 29, 53, 81, 253, 258, 260-263
 intramitochondrial alterations in, 311
Serine, 94, 96, 250
Sertoli cells, 99
Scrum albumin, 63

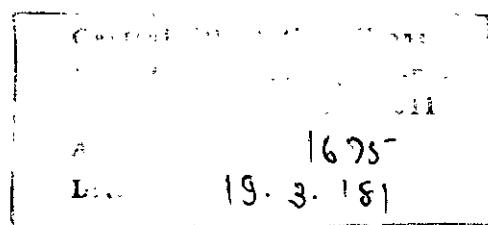
Sex, nucleomorphological determination of, 78-79
Sex, prenatal determination of, 78
Sex chromatin, 77-80, 89, 216
Sex chromosomes, 78, 79, 102, 106, 160, 163, 217, 223
SH cycle, mitotic, 148
SH groups, 148, 203
Shock cells, 274
Side chains, hydrophylic, of amino-acids, 272
Siderosomes, 293, 294
Silicates, 294
Silver impregnation, of Golgi apparatus, 256
Single strand hypothesis, of the chromosome, 110
Sites, genetic, 51, 117, 123
Skin papilloma, in humans, 83
Sliding movement, of cytoplasm, in plants, 275
Sodium, 40, 277
Sol 67, 71, 173, 274
Sol-gel transformations, 173, 275, 276
Solidistic pathology, 26
Spermatids, 109, 114, 305
Spermatocytes, 22, 28, 71, 90, 92, 96, 99, 108, 152, 161, 163, 183, 190, 191, 197, 259, 305
Spermatogenesis, 64, 95, 134, 144, 258
Spermatozoa. (See Spermatocytes.)
 head piece, 92
 middle piece, sheath of, 305
Spheroplasm, 133
Sphero-proteins, 268
Spindle, 125, 139-149, 157, 158, 164, 167, 170-172, 183, 193, 209, 210, 218
 centrosomeless, 144
 multicentric, 143
 multipolar, 210, 211
 with blunt poles, 144
Spindle contraction, 154
Spindle corpuscle, 141, 142, 156
Spindle current, 163-165, 166, 183
Spindle disturbances, 200, 209-211
Spindle fibres, 143ff, 147, 154, 165, 209
Spindle fusion, 221, 227
Spindle granule, 141
Spindle insertion, 114, 123, 125, 131, 137, 141, 199, 223
 subterminal, 105
 telocentric, 105
 terminal, 105
Spindle pole, 108, 125, 129, 130, 144, 149, 151, 152, 158, 166, 193, 210
Spindle, stretching of, 159, 165, 166
Spiralization. (See Coiling.)
Spiral prophase, 128, 151
Spirme, 222
Spleen cells, DNA content of, 98
Sporangia, 189
Spores, 189

- Sporophytes, 189
 Starch, 322, 328
 storage of, in plastids, 329
 Standard spiral, chromosomal, 128, 131
 Starvation. (*See Hunger.*)
 Stem lines, of tumour cells, 99, 213
Stentor, 29, 32
 Steroid hormones, 254
 Steroid secretion, 305
 Stickiness. (*See Chromosomal stickiness.*)
 Stroma, of chloroplasts, 15, 328
 Stroma lamellae, 323, 325, 331, 333
 Structure protein of chromosomes, 94
 Structures, photoreceptor, 325
 Subannuli, of nuclear pores, 59
 Subepithelial membrane, in lungs. (*See Basement membrane.*)
 Subfibrils, 109, 112
 Substrate chains, phosphorylation via, 33, 34
 Succinic acid, 14, 19
 Succinic dehydrogenase, 34, 48, 202, 280, 303,
 306, 309, 312
 Superfemales, 216
 Supravital representation, 41, 44, 53, 57, 312
 Surface tension, of chromosomes, 155
 Sweat gland cells, 70, 71
 Swelling, cloudy, 312
 Symplasts, multinuclear, 166
 Synapsis, 4, 197-198
 Syncytium. (*See Trophoblast.*)
 Syndesis, 191, 197-198
 Synergists, of mitotic poisons, 209
 Synizesis, 191

Tachyblaston ephelotensis, 237
 Tapetum cells, 97
 Tellurium, 294
 Telomeres, 106, 114, 156, 191, 208
 Telophase, 64, 72, 125, 132, 147, 162, 165, 169,
 170, 175, 183, 195ff, 221, 234, 236
 Telophase model, 173
 Teloreplication, 221
 Terminalization, of chiasmata, 193, 198, 200
Tetrahymena, 39, 176, 181, 182, 184, 185, 186, 236
 Thallus, 189
 Thioacetamide, 53, 129, 246, 248, 254
 Thioamino-acids, 40
 Thorium dioxide, 284
 Thorotраст, 284
 Thread metamorphosis, 124
 Three group metaphase, 208, 211
 Threonine, 94, 96
 Thrombocytes, 242, 288
 Thylacoids, 325
 Thymidine, 98, 110, 111, 114, 116, 175, 176, 179,
 181, 187, 208, 320
 Thymine, 7, 113, 116, 207
 Thymonucleic acid. (*See Desoxyribonucleic acid.*)
 Thymus cells, 28, 70, 93, 98
 Thyroxine, 248, 315, 316
 Tigroid bodies, 247
 Tipulid, 152
 Tissue cultures, 42, 50, 59, 98, 176, 177, 187, 207,
 213, 219, 231, 236, 262, 263, 294, 295, 305,
 318
 Tobacco mosaic virus, 118
 Toluhydroquinone, 203
 Toluidine blue molybdate, 49
 Toluquinone, 203
 Torres' inclusion bodies, 86
 Tracheoles, 311
 Traction fibres, 141, 157
 Traction mechanism, 156-158, 161
Tradescantia, 67, 108, 157, 158, 181, 190, 208
 Transaminases, 310
 Transfer RNA, 251, 252, 255
 Transformation, of DNA, 115
 lamellar, of mitochondria, 318
 Translocation, chromosomal, 198, 206, 208, 213
 Transpiration, physiological, 272
 Transplantation, 36, 37
 Transport, intracellular, 254
 Trihydroxy-N-methylindole, 202
 Triose phosphate, 328
 Triplets, of the DNA bases, 115, 251
 Triploidy, in humans, 216, 227
 Trisomy, 214, 216
 Triton, 158
Triturus, 123, 158, 187
 Trophoblast, syncytium of, in man, 4
 Trophoblast cells, 55
 Trypaflavine, 36, 202
 Trypan blue, 91, 238
 Trypanosomes, 98, 166
 Trypsin, digestion with, 94
 Tryptophane, 93ff
 Tubuli, intramitochondrial, 305
 Tumour cells. (*See Carcinoma cells.*)
 Tyrosine, 93, 94, 252

 Ullrich Turner's syndrome, 79, 215, 216
 Ultracentrifugation-sedimentation, 25, 249, 266,
 302, 306
 Uncoiling, of chromosomes, 74, 132, 165
 Uracil, 7, 20, 51, 117, 187, 207
 Urea, 20
 Urethane, 207, 208, 210
 Uricase, 280
 Uridine, 50, 118, 181, 182, 208
 UTP = Uridine triphosphate, 252
 UV rays, 114, 154, 275, 330

- Vacuoles, in nucleoli, 43, 46, 53, 70
contractile, 264
in vicinity of nucleus, 278ff
- Vacuolation, of cytoplasmic ground substance, 300
- Vacuome hypothesis, of Golgi apparatus, 257
- Valine, 94, 96
- Varicella virus, 86
- Vascular endothelia, 126
- Vesicles, nuclear, 54
nucleolar, 53, 54
- Vicia faba*, 39, 44, 45, 110, 176, 202, 203, 206, 208, 209
- Villi, intramitochondrial, 305
- Viral toxins, 87
- Virology, 26
- Virus, 5, 17, 26, 53, 61, 63, 80-87, 115, 118, 266, 273, 296-300
- Virus antigen, 83
- Virus replication, 82, 296-300
- Viscosity, 32, 94, 127, 274, 276
- Vital structure, of nucleus, 66-68
- Vitamin E deficiency pigment, 295
- Walker carcinoma, 147, 207
- Water content, daily variations of, in plant cells, 272
- Water content, of nucleus, 92
- Water hull, of molecules, 69
- Water reabsorption, tubular, 91
- Water transport, active, 277
intercellular, 274, 276-286, 300
intracellular, 264, 266, 272-276, 300
- Wound hormones, 187
- Xanthophyll, 14
- X chromosome, 78, 102, 216, 217, 222
- X-rays. (See Roentgen irradiation.)
- Y chromosome, 78, 102, 105, 217
- Yellow fever, 86, 296
- Zea mays*, 46, 329
- Zinc, 100, 130
- Zygote, 29, 56, 188, 217, 218
- Zygotene, 192, 198, 199
- Zygotic, type of meiosis, 189
- Zymogen granules, 261



**C. P. C. R. I. LIBRARY
CALICUT**

Acc. No. **1675**....

Cl. No. **5765 (023)** Date of release for loan.....
K6

This book should be returned on or before the date last stamped below.

--	--	--

Call No. 576.3 (023) kb

Accession No. 1675

Auth

Title

Acc. No. 16775

Call No. 576.3 (023)

End manna
End
+ cytop

CPCRI LIBRARY

CALICUT

THE MECHANISM OF HEREDITY

H. L. K. Whitehouse, M.A., Ph.D., Fellow of Darwin College, Cambridge,
and University Lecturer in Botany

55s. net

THE BIOLOGICAL ROLE OF THE NUCLEIC ACIDS

David Cohen, M.A., Ph.D., Lecturer in the Department of Chemistry,
University of Keele

10s. 6d. net (Paper); 21s. net (Boards)

THE CHROMOSOMES OF THE ALGAE

Edited by Maud B. E. Godward, M.Sc., Ph.D., Professor, Department of
Botany, Queen Mary College, University of London

55s. net

AN OUTLINE AND ATLAS OF GYNAECOLOGICAL CYTODIAGNOSIS

H. Smolka, Director of the Department of Obstetrics and Gynaecology,
Robert Koch Hospital, Gehrden/Hannover; and H.-J. Soost, Research
Assistant and Director of the Cytology Laboratory, Hospital for Women,
University of Munich

£6 net