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REVIEW ARTICLE

Experimental Modification of the Chemistry and Biology of the Aging Process

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INTRODUCTION

Throughout his history, man has sought answers to why he grows old, and he has continually advanced theories and tested treatments designed to extend his life (1). According to recent technological forecasts, it is possible that, within the next 20 to 30 years, the average human lifespan will be extended significantly through an alteration of the basic aging process (2, 3). Thus, the ancient wish of man to attain longevity appears about to move from fantasy into reality. This review will consider the basic question: Is there any sound scientific evidence to support such bold optimism? Perhaps it is too early to propose rational or productive research which would retard the aging process and prolong life through pharmacological control because of the lack of experimental evidence indicating the causes of aging *per se*. What seems likely, however, is that, in the near future, progress in age research will lead to therapies and procedures that will permit a more comfortable and productive life for man in his later years. It is our hope that the material presented here will: (*a*) enable those considering future research in this field to draw their own conclusions regarding anticipated research progress, and (*b*) help them design the experiments they feel are most meaningful and relevant.

We believe that this review is particularly timely. For many years, age research has suffered from unfortunate circumstances created, in part, by Brown Sequard's ill-reputed experiment, youth doctors, and self-proclaimed rejuvenators (4). In recent years, however, the tide has turned; age research is becoming a respectable discipline and has attracted national and international attention, with the result that many significant and important contributions have been made. This situation is evident from the fact that one-third of the papers cited in this review appeared in 1969 and 1970. Verzár (5) recently described this change in interest and expansion of age research.

A major stimulus to this increasing interest in experimental gerontology has come from social as well as economic considerations. Actuarial statistics place the current average life expectancy in the United States at nearly 75 years, while the lifespan (that is, the maximum attainable age) is estimated to be around 110 years (1). If we achieve no further increase in lifespan,

the number of individuals in this country over 65 years of age will grow from the current 20 million figure to 30 million in the year 2000. The ability to improve the quality of life in later years for this growing segment of the population could do much to relieve many financial burdens and free resources for other social problems. Thus, the conservation or restoration of vigor in old age presents a practical challenge to those engaged in age research.

As noted, we shall summarize those experiments that demonstrate an ability to alter expressions of aging or to extend the lifespan itself. No attempt has been made to review extensively, highlight, or list the various theories that have been proposed to explain why aging occurs. This subject has been dealt with in a more extensive review (6). We have also excluded clinical studies, such as those with procaine and hormones, since they belong more appropriately to geriatrics. A recent review may fill the gap for the interested reader (6). Nor shall we discuss the vast literature on intermediary metabolism, since it could add little to our objectives; a recently published book (7) provides detailed information on this subject. Likewise, the following related subjects have been adequately covered elsewhere: environmental factors (8), nutritional aspects (9, 10), and modification of drug activity with aging (11–14).

AGING *IN VITRO*

With the advancement of tissue culture techniques, it has become possible to study the aging process of mammalian cells *in vitro*. A clonal aging phenomenon, discovered by Hayflick and Moorhead (15), in a human diploid cell line has received considerable attention and has provided age research with a convenient experimental model (16–20). When trypsinized human embryonic fibroblasts are cultivated serially in a ratio of 2:1 in a basal medium supplemented with 10% calf serum and standard antibiotics, the cells survive only 50 ± 10 doublings (15). At this point the cells rapidly lose their dividing ability and ultimately die. Cellular proliferation occurs in a three-phase pattern: adaptation (Phase I), logarithmic growth (Phase II), and mitotic arrest followed by death (Phase III). An important feature of the system is that the cells maintain their normal diploid karyotype throughout the entire growth cycle. Subsequently, Hayflick (21) showed that the doubling capacity correlates with the age of the donor; fibroblasts from adults have a significantly shorter lifetime than those of embryos.[1] Furthermore, cell populations of different doubling levels when mixed together maintain their individual clonal doubling capacity (21).

Both intrinsic and extrinsic factors have been implicated in limiting the number of cell doublings. However, Hayflick's (21, 22) experience suggests that neither medium artifacts nor infection nor depletion of some nonreplicating component from the cells is responsible for cell death. He attributed the finite number of doublings to a manifestation of cellular aging. This suggestion is consistent with the results obtained by others employing human fibroblasts (23–25).

Several intrinsic factors have been suggested as responsible for limiting the number of cell divisions; however, each suggestion lacks sufficient experimental verification. These possibilities range from the accumulation of errors in DNA during Phase III (21) to possibly an age-dependent increase in cell volume which could conceivably compensate for cellular disorganization (26). In addition, defects in cellular regulatory mechanisms are also prime suspects and will be discussed later.

Certain, yet unknown, cytoplasmic elements have also been cited as potential factors causing mitotic arrest. For instance, Muggleton and Danielli (27) transformed "immortal" clones of *amoebae* to "mortal" (spanned) ones by injecting them with the cytoplasm of spanned cells. A puzzling question is whether the Szent-Györgyi *et al.* (28) retine, believed to be a glyoxal-containing mitotic inhibitor and present in the cells, plays a role in the events leading to cellular senescence. Recently, it was suggested that retine might be identical with dehydroascorbic acid, a substance structurally related to glyoxal (29).

Although, as mentioned earlier, Hayflick (21, 22) ruled out certain extrinsic factors as possible causes of cell death, general observations (30) and specific experimental results of others (20, 26, 31) indicate that culturing techniques and the composition of the media may have a direct bearing on the number of generations produced in normal diploid cells. Thus, it could be dangerous to overinterpret tissue culture data and attach a dogmatic meaning to the number of doublings obtained with a particular cell type in an artificial milieu. It would be equally erroneous to extrapolate from the properties of one cell type to that of another, since the proliferative behavior depends on the level of differentiation. This point is perhaps best illustrated by LeBlond's (32) classification of normal postnatal cells on the basis of their mitotic capacity: *static cells* do not divide and compose the nervous system and retina; *expanding cell populations* such as the parenchymal and supporting cells of the kidney, pancreas, adrenal, liver, thyroid, skeletal, and cardiac muscle fibers show only scattered mitosis; *renewing cells*, which include the epithelial, alveolar, and red-blood cells and the lymphocytes, proliferate abundantly through the entire lifespan. From the point of view of age research, it would be specially important to investigate static cells, particularly those of the nervous system, which are likely to suffer most from age-inflicted damage (33). The techniques of culturing nervous and muscle tissues are in fact available (34–36). Postmitotic cells, which are believed to approximate a senescent state, have been suggested as a suitable tool for age research (17, 37). In this regard the works of Chang (38) and Yuan *et al.* (39, 40), who used postmitotic human amnion cultures, are noteworthy. Their results will be discussed later.

An inherent limitation of tissue culture ought to be

[1] *Note added in proof:* Extending Hayflick's (21) observations, a recently published study [G. M. Martin, C. A. Sprague, and C. J. Epstein, *Lab. Invest.*, 23, 86(1970)] established that the replicative capacity of cultured human skin fibroblast cells inversely correlates with the age of the donor.

Table I—Biological Expressions of Aging

Target (Cell Component)	Observed Change	Possible Consequence
DNA	Covalent (?) binding of histones	
Chromatin	Increased nucleoprotein binding; decreased template activity	
RNA	Decreased *m*-RNA content (?); decreased ribosomal (polysomal) content	Impaired protein synthesis
Protein	Decreased protein synthesis; delayed response to enzyme induction	
Lysosomes ↕?	Increased enzyme activity (*e.g.*, RNase)	Breakdown of essential cellular components due to enzyme leakage
Lipofuscin	Increased accumulation in postmitotic cells (*e.g.*, brain and heart)	Impaired cellular function
Amyloid ↕?	Increased accumulation; "senile plaques"	Product of autoimmune response
Immune system ↕?	Decreased number of antigen-sensitive and antibody-forming cells; increased autoimmune factors	Reduced immunocompetence; Progressive autoimmunity
Collagen	Increased stability *via* crosslinks; increased Ca-binding	Loss of plasticity; autoantigenic
Hormonal regulation	Decreased tissue sensitivity to hormones	Impaired homeostatic control

considered, namely, that the cells are in an artificial environment detached from the intricate physiological regulation. Consequently, a tissue culture approach cannot substitute, but only complement, whole organ or *in vivo* experiments. There is no agreement, however, concerning which species or strains are most appropriate for gerontological research. A recent symposium (41) dealt with this problem in considerable detail.

EXPRESSIONS OF AGING

Changes associated with age may occur in a number of cellular systems and may be expressed in DNA, RNA, protein synthesis and metabolism, lysosomes, waste-product accumulation, structural substances such as collagen, hormonal function, and immunological competence. These expressions of aging are summarized in Table I, along with the possible biological consequences of these changes. The subsequent discussion deals with a further consideration of these biochemical and chemical aspects of aging, with particular emphasis on those studies that have dealt with experimental approaches to modifying these expressions of advancing age.

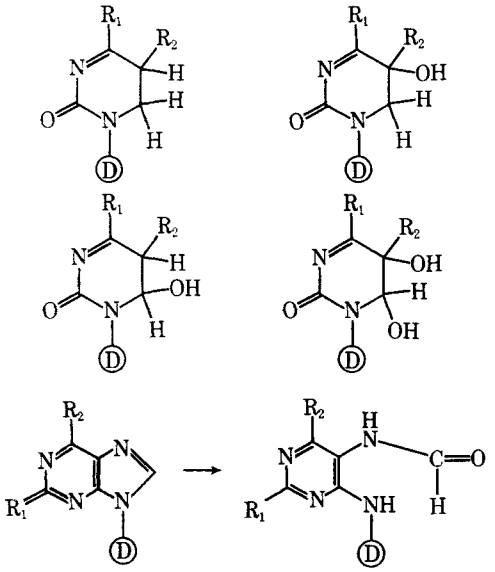
DNA—Today many regard aging as some sort of information loss on the molecular level (42). Hence, DNA, the key matrix of the genetic and regulatory information, could be a potential locus for such a functional loss. Specifically, a programmed sequential mechanism, random somatic mutation, and aging of DNA through protein binding have been considered as possibilities.

The theory of programmed aging (43) proposes an epigenetic switching-on and switching-off of certain regulatory genes which determine the rate of aging of a given species. Such mutational alterations would introduce error factors in DNA, RNA, and protein synthesis leading to impaired cellular function and eventual death. If such a mechanism would prove to be correct, then the elucidation of the genetic program could provide the basis for a rational approach to alter the aging process. However, with the rare exception of the so-called premature aging syndromes (44, 45) such as progeria and Werner's syndrome, and perhaps Alzheimer's disease, a type of presenile dementia (45), there is no convincing evidence to support a gene-programmed aging.

Considerable experimentation and debate have surrounded the question of somatic mutations as a primary cause of aging (44, 45). In essence, it was assumed that gradual accumulation of random mutations in DNA impairs an increasing number of somatic cells through faulty RNA and protein synthesis. The mutagenic agents could be extrinsic factors, *e.g.*, low grade radiation, or intrinsic factors, such as free radicals or destructive enzymes. In an attempt to test the validity of this hypothesis, the relationship between radiation and aging has been investigated (44–48). Animals, who in early life receive sublethal doses of X-irradiation, become prematurely senile and develop fatal diseases. Mutations increase steadily with age, as indicated by chromosomal aberrations in liver cells.

From a chemical point of view, radiation damage of DNA arises from free radical reactions (49–51) and from strand breaks (50, 52). The known mechanisms involve the addition of $H^{\cdot}$ and $OH^{\cdot}$ radicals to the base components (Scheme I), breaks of the water–hydrogen bonds in the secondary structure leading to local separation of the double helix, and "direct hits" (Scheme II), which can result in complete scission of the twin strands. It should be noted here that besides radiation, normal metabolic pathways also can produce free radicals. According to Harman (53), free-radical reactions, regardless of the source of the free radicals, promote the aging process. To probe this hypothesis, he tested a variety of free-radical scavengers, including antiradiation agents, for their effects on lifespan in mice. These results will be discussed in the section dealing with waste products.

Not only ionizing but also UV radiation can catalyze transformations in the DNA structure (50). Photochemical attack takes place along the 5,6-double bond of pyrimidine bases, yielding water adducts (*a*) and cyclobutane-bridged dimers (*b*) as the UV radiation products (50).

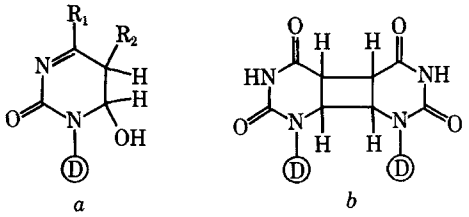


Scheme I—Ionizing radiation products of pyrimidine and purine bases. D = deoxyribophosphate; uridine: R_1 = OH, R_2 = H; thymidine: R_1 = OH, R_2 = CH_3; cytidine: R_1 = NH_2, R_2 = H; adenosine: R_1 = H, R_2 = NH_2; and guanosine: R_1 = NH_2, R_2 = OH.

In lower organisms, as well as in mammalian cells, direct and enzymatic photoreactivation restores the UV-damaged DNA (54). While there are no experimental data about the effects of age on the efficiency of the repair mechanism in mammals, the degree of photoreactivation in old macroconidia of *Neurospora crassa* does not differ from that of young ones (55).

In addition to radiation, DNase, a lysosomal enzyme, could conceivably produce DNA strand breaks. Allison and Paton (56) demonstrated experimentally in human diploid cell cultures that photosensitized lysosomes readily release DNase which can enter the intact nucleus. It remains to be seen whether actual strand scission can occur under these conditions.

The relevancy of somatic mutations in general and nonlethal radiation effects specifically to aging is debatable on a number of points. Mammalian cells and, in particular, the postmitotic types have a remarkable ability to rejoin enzymatically the broken strands, to replenish the altered base units in DNA (33, 50, 52, 57), or to excise unphysiological inclusions in the chain. Electron-spin resonance studies (51) also showed that the mitochondrial electron-transport system is capable of inactivating radiation-induced free radicals. When CBA mice, a strain known to have a low incidence of neoplasia, were X-irradiated, their rate of aging as measured by the tail tendon test (*cf.*, collagen) remained unchanged although their average lifespan decreased (33). If radiation-induced life shortening is, indeed, a result of somatic mutations, then powerful mutagens, such as monofunctional alkylating agents, would be expected to decrease the average lifespan of treated animals. However, the results with ethyl methanesulfonate (EMS) are conflicting. In one set of experiments (33), even very high doses of EMS did not alter the lifespan of mice. In another experiment (58), EMS significantly increased the rate of mortality in mice.



a *b*

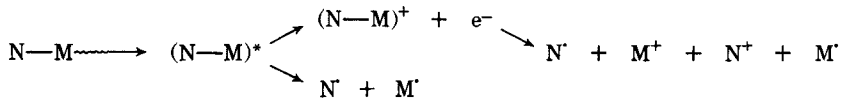
While somatic mutation may occur and increase with time in mammalian cells, it is not possible now to correlate it directly with aging. Since the shortening of the lifespan does not necessarily indicate an acceleration of aging, the observed effects of radiation do not seem to support a mutational theory.

Cellular aging was also attributed to an irreversible nuclear protein–DNA interaction (59). It was found that DNA isolated from bovine thymus (59) and rat liver (60) of old animals contains strongly bound residual histones, and that this DNA–protein complex is denatured at higher temperatures than comparable DNA preparations from young animals. Similarly, whole nucleoprotein (chromatin) isolated from the brain of older mice (61) and from adult bovine thymus (62) showed an increase in the mean temperatures of denaturation.

Examination of histone patterns indicates a distinct loss of the arginine-rich fraction from "old" nucleoprotein (63–65). The nature of the histone–DNA bond and how it differs from young to old have yet to be determined. It is believed (59) that in "young" nucleoprotein, histones are attached to DNA mainly by loose ionic bonds, while in "old" nucleoprotein, covalent bonds are formed between histones and phosphate end-groups or base substituents of DNA. Recent observations suggest that perhaps divalent cations, such as Mg(II) or Mn(II), mediate the ionic bonds; the depletion of these cations, for instance by treatment with EDTA, a strong chelating agent, renders the "young" nucleoprotein preparation similar to an "old" one (65).

The firm binding of histones to DNA, if it accumulates progressively with age, could permanently repress an increasing number of genes which normally code for specific proteins; hence, cellular function could be irreversibly impaired (59).

The priming ability of lung and prostatic chromatin decreases with age (66), which may reflect an age-dependent deficiency in DNA-directed RNA synthesis. There is no age-associated change in template activity of bovine thymus chromatin (62). The observed template activities of "old" liver chromatin are conflicting. Mainwaring (66) and Samis and Wulff (67) did not detect age variations at all. On the other hand, Devi



Scheme II—"Direct hit" of ionizing radiation on the nucleic acid strand

et al. (68) reported a significant decrease in RNA synthesis when primed with liver chromatin from old rats. They attributed this finding to a failure of the DNA double helix to unwind. For instance, a tight DNA–histone complex as suggested (59) could hinder unwinding. However, other possibilities that do not involve DNA, such as RNA polymerase inactivation by arginine-rich histones (69), should also be considered.

The metabolic fate of DNA during aging has been monitored *via* radioactive thymidine incorporation. Chang (38) found a decreased incorporation in postmitotic human amnion cells. On the other hand, cultured human fibroblasts in their senescent state (Phase III) continued to synthesize DNA, even though most cells ceased to divide (22). The turnover of liver DNA has been shown to increase in aging rats (70). The active DNA synthesis in senescent fibroblasts and in the liver of old rats might be interpreted as a compensatory mechanism for age-inflicted cellular damage. However, Chang's (38) findings do not fit this interpretation.

RNA and Protein Synthesis—The cellular events that control protein synthesis, such as the synthesis of enzymes, are transcription and translation. The former involves DNA-directed RNA synthesis; in the latter, messenger RNA directs the assembly of polypeptides which requires ribosomal and transfer RNA. Thus, theoretically, it is conceivable that aging may affect the protein-synthesizing machinery either at the DNA level, due to mutation or structural changes, or at subsequent stages. Such an impairment could cause a serious deficit in vital proteins (59) or generate errors in the protein structure (71).

Cultured diploid fibroblasts in their senescent stage (Phase III) seem to continue to synthesize RNA and protein at normal or near normal rates (72, 73). When fibroblast cells are near death, degradation exceeds the biosynthesis of RNA (74). In postmitotic cells, such as in human amniotic cells, RNA and protein synthesis do not change appreciably with age (38).

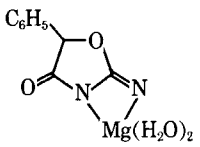
Wulff *et al.* (75) reviewed the metabolism of RNA of aging rodents and concluded that the muscle, liver, and kidney of old animals incorporate more cytidine in nuclear and cytoplasmic RNA and have a more rapid turnover in nuclear RNA than the same tissues of young animals. From the characteristics of liver nuclear RNA, Wulff *et al.* (75) suggested that perhaps the increased RNA turnover during aging is associated primarily with *m*-RNA. However, sedimentation patterns of ribonucleoprotein particles from the prostate and liver of aging mice seem to indicate an age-associated depletion of *m*-RNA (66, 76). According to recent reports, total RNA, polysomal content (77), and uridine incorporation into RNA (78) decrease with increasing age in the mouse skeletal muscle.

The metabolism of brain RNA is different from that of the other tissues investigated, inasmuch as total RNA levels remain fairly constant during the adult and senescent state of rodents (75, 79). On the other hand, polysome-bound ribosomal RNA decreases in aging rats (80–83). These findings, together with the finding that an *r*-RNA fraction may mediate learning and memory in the rat brain (84), might help to uncover the biochemical processes involved in the mental function and its deterioration in senility.

The search for agents that enhance learning and memory received considerable attention in recent years. Weissman's (85) critical review covered this subject adequately; therefore, we shall mention here only those drugs that supposedly act on brain RNA or are derivatives of RNA.

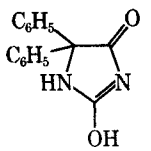
Yeast RNA—Cook *et al.* (86) demonstrated that yeast RNA administered chronically to young rats potentiates the acquisition of conditioned response and enhances its retention. Subsequently, other investigators also studied the psychopharmacological properties of yeast RNA by various test methods and obtained mixed results. Cook and Davidson (87) extended their studies and discussed them in detail along with the results of others. Gardner (88) reviewed the effects of yeast RNA on aging. Mice and rats fed daily with yeast RNA showed an increase in lifespan of 7–8.4 and 25%, respectively. It is not known how yeast RNA exerts its beneficial effect on performance or lifespan.

5-Phenylpseudohydantoin [*Pemoline* (*I*)]—This mild CNS stimulant, complexed with magnesium hydroxide, was reported to enhance the conditioned avoidance response in young rats (89). According to Glasky and Simon (90), magnesium pemoline stimulates brain RNA polymerase and the synthesis of brain nuclear RNA (91). However, the results of independent investigators support neither the claimed memory and learning-enhancement activity (85) nor the biochemical properties (85, 92). Whatever effect magnesium pemoline may have on performance is probably attributable to its CNS-stimulant activity (85). Similar conclusions were drawn from avoidance acquisition experiments with old rats (93).



I

5,5-Diphenylhydantoin (*II*)—This known antiepileptic drug was shown to improve the avoidance response and retention in old and, to a lesser extent, in middle-aged rats (82, 94). Others observed similar performance-enhancing effects (95). This drug supposedly stimulates polyribosome formation in the aging brain tissue (83).



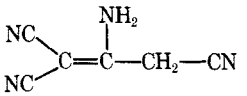
II

Procaine Amide (*III*)—This drug is used in the treatment of cardiac arrhythmias. Gordon *et al.* (82) tested it for learning-enhancement activity in rats of different ages. While it improved acquisition and retention in old animals, it did not affect young adults nor was it as active as diphenylhydantoin. Procaine amide was shown to increase brain RNA content in the microsome-plus-cell-sap fraction and to redistribute the intracellular

amino acids from the free pool to an RNA-bound form (94). However, lysine incorporation into brain protein decreased, which is inconsistent with the observed RNA-bound amino acid profile.

III

Tricyanoaminopropene (IV)—This drug is reported to enhance retention of an avoidance response and "consolidation" of memory in the rat (85). Biochemically, it increases RNA content of the nerve cell and it also seems to alter the RNA-base makeup (85).



IV

Ribaminol—This complex of RNA and diethylaminoethanol (96) was claimed to enhance learning and to stimulate protein synthesis in the brain (85). Despite the wide publicity this agent received, more definitive scientific data are needed for its objective evaluation.

Of course, the ultimate test of these agents must come from objective clinical studies in senile conditions. Since the discussion of clinical results is beyond the scope of this paper, the interested reader is referred to recent reviews that deal with this subject (6, 85). However, the clinical trials conducted so far prove, at best, marginal and perhaps only subjective improvement in the elderly suffering from senility.

Protein Synthesis—One fashionable theory of aging is currently that of Orgel's (71), which proposes a cytoplasmic "error catastrophe" in protein synthesis. More precisely, Orgel suggested that, while low-level errors introduced into certain metabolic enzymes can be washed out by rapid degradation and *de novo* synthesis, errors in the synthesis of information-handling enzymes, such as RNA polymerase or *t*-RNA acylating enzymes, can accumulate progressively. Hence, the accuracy of the entire protein-synthesizing machinery might ultimately be jeopardized.

Recent findings may be taken as conjectural evidence. Earlier, we mentioned the experiments of Muggleton and Danielli (27) in which they transferred an unspecified cytoplasmic factor from "mortal" *amoebae* to "immortal" ones and thus transformed them to a "mortal" state. This transformation might have involved the transfer of an error-producing component such as a misspecified regulatory enzyme. Harrison and Holliday (97) were able to shorten the lifespan of adult fruit flies by administering nontoxic amounts of amino acid or RNA-base analogs. They attributed the life-shortening effect of such analogs to the synthesis of faulty proteins. However, more recently Dingley and Smith (98) found that the lifespan of adult *Drosophila subobscura* fed with amino acid analogs was not reduced. Holliday (99) reported an irreversible clonal senescence of certain fungi, which he ascribed to errors in protein synthesis. Finally, it was shown that error frequency in *poly-U* transcription increases when RNA polymerase is X-irradiated (100).

These results, however, do not prove the validity of Orgel's (71) theory, nor can one extrapolate from insects and fungi to a mammalian system. Until direct and unambiguous evidence is presented such as the isolation and characterization of a regulatory enzyme (*e.g.*, RNA polymerase) which embodies age-dependent errors, Orgel's concept remains in doubt.

Protein synthesis, as demonstrated by incorporation studies, declines with increasing age. Hrachovec (101) compared the incorporation of amino acids of microsomes isolated from the livers of young and old rats by incubating the microsomes with a uniformly labeled yeast protein hydrolysate. Microsomes from old animals incorporated significantly less radioactivity into protein than microsomes from young animals, but maximum incorporation occurred at about the same time in both age groups. Guanosine triphosphate (GTP) stimulated amino acid uptake which, in the case of "old" microsomes, approached the level of the GTP-free "young" microsomes. Liver cell-sap, on the other hand, inhibited the incorporation of amino acids, but the cell-sap prepared from the liver of old animals had an increased inhibitory effect. In Hrachovec's (101) view, these results indicate an age-dependent increase of two inhibitory factors of protein synthesis; one is antagonized by an excess of GTP, while the other is not.

Leucine incorporation also decreases in the homogenate and cellular fractions of mouse skeletal muscle during aging (78). This decrease was attributed to a loss of polysomes (77, 78). Liver microsomes from older mice exhibited a decreased ability to synthesize proteins, probably because of reduced *m*-RNA content (76).

Enzyme concentration and activity are known to fluctuate with environmental stress. Recently, several examples appeared in the literature suggesting that age, too, may have an effect. Singhal (102) demonstrated an age-dependent decrease in the activity of glucose-6-phosphatase and fructose-1,6-diphosphatase in rat liver following hormonal induction. He obtained similar results with testosterone-induced phosphofructokinase in the prostate and seminal vesicle of the rat (103). However, in both cases, enzyme activities were measured only at one time point after induction; therefore, possible changes in enzyme levels cannot be ascertained as a function of time. More recently, the kinetics of several enzymes were analyzed in relation to aging. Haining and Correll (104) determined the rate of synthesis and degradation for liver tryptophan pyrrolase stimulated by tryptophan in rats ranging in age from 1 month to 2 years. The rate constants for synthesis and degradation corresponded in each age group, decreasing between 1 and 6 months of age, then increasing around 1 year, and, finally, decreasing again in the 2-year-old group.

These observations indicate that age-dependent correlations between enzyme (protein) depletion and repletion are important to consider for further investigation. Finch *et al.* (105) and Adelman (106, 107) carried out kinetic analyses of enzyme inductions in stressed young and old animals. Finch *et al.* (105) induced liver tyrosine aminotransferase (TAT) in fasted young and old mice with exposure to cold and measured the rate of TAT activity at different time intervals. Adelman (106)

studied the kinetics of liver glucokinase as a function of age in rats after fasting the animals for 72 hr. and subsequently refeeding them with glucose. The common element of these experiments is that, while in young animals the respective enzyme activities begin to rise shortly after induction, the activities in old animals start to rise only after a significant lag period of several hours but eventually reach levels comparable to those of the young animals. Adelman (108) demonstrated a similar age-dependent pattern in the phenobarbital-induced NADPH/cytochrome-c reductase system.

The additional finding that newly regenerated liver cells in aging animals respond to glucokinase induction like old cells (107) raises some interesting questions. For instance, since the response of old animals to exogenous hormones parallels that of the young animals in liver TAT (109) and liver glucokinase activities (106), an age-associated somatic mutation of liver cells is not likely the cause of the delayed adaptive response (106, 108). Other possible explanations that have been considered are humoral or neural factors, alterations in membranes, and deficiencies in protein synthesis and degradation (105, 106, 108).

Lysosomes—Lately, the possible role of lysosomal enzymes in cellular aging has received considerable attention. Lysosomes are membrane-encased cytoplasmic organelles which store within their lumen acid hydrolytic enzymes such as acid phosphatase, ribonuclease, DNase, collagenase, and esterases. Normally, lysosomes dispose of intracellular waste and debris and phagocytose alien particles, but they can also cause or contribute to certain pathological conditions. DeDuve and Wattiaux (110) reviewed the functions of lysosomes in detail. Specific agents or stresses, such as free radicals (111); vitamin A, diethylstilbestrol, testosterone, endotoxins (112); viruses (113); ischemia, starvation, anoxia (112); and UV or gamma radiation (111) can labilize the lysosomal membrane which engenders the leakage of lytic enzymes into the cytoplasm. Rampant lysosomal enzyme activity was suggested as a cause of cellular death in aging (37). Increased lysosomal enzyme activity was demonstrated in old human leukocytes (114), in senescent human fibroblasts (Phase III generation) (73), in skeletal muscle of old mice (115), and in the liver of aged mice, hamsters, and guinea pigs (116). Goto *et al.* (116) specifically identified a rise in acid ribonuclease with increasing age which might account, at least in part, for the age-associated loss of cellular RNA. As yet, it is not known whether lysosomal DNase activity (56) increases with age. Collagen, which manifests aging in its greater stability due to crosslink formation, is unaffected by collagenase from rat liver lysosomes; other types of collagen, presumably having fewer crosslinks, are hydrolyzed (117). A positive, age-dependent correlation was established between the increase of acid phosphatase activity and collagen denaturation (118). Although lysosomal enzyme activity is apparently aggravated during aging, a causal relationship cannot be corroborated at this time.

Certain agents [*e.g.*, corticosteroids, chloroquine, aspirin (119), chlorpromazine (120), and stilbamidines (121, 122)] are known to stabilize lysosomal membranes. Treatment with hydrocortisone (39), prednisolone, and other 11-hydrocorticosteroids (123) increased cell density and decreased disintegration in X-irradiated human amniotic cells. Hydrocortisone was also shown to be beneficial to the recovery of amnion cells from their degenerative phase (124). According to a subsequent study (123), hydrocortisone seems to interfere in amnion cells with the age-associated increase of the lipid/CO_2 ratio and the incorporation of cholesterol into lipids. On the other hand, ketocorticosteroids such as cortisone, prednisone, and sex hormones, like testosterone and estradiol (123), had no effect on primary human amnion cultures with or without X-irradiation.

Cortisone, however, prolonged the mitotic capacity of human embryonic fibroblasts by about 15 doublings, if treatment commenced at the 21st passage (125); this finding was confirmed by others (123). Similarly, hydrocortisone added to the culture medium of diploid fibroblasts extended the number of generations significantly, provided treatment began before Phase III (73). Contrary to expectations, hydrocortisone did not suppress lysosomal enzyme activity which increases in fibroblast cells of late passages. Cristofalo (73) suggested that hydrocortisone might mediate the mitotic recovery of Phase III cells by inducing protein synthesis. Such a mode of action agrees with the idea that corticosteroids might stimulate transcription of RNA *via* the activation of regulatory genes which are thought to become strongly repressed in the process of aging (126).

The question whether corticosteroids in aging systems stimulate protein synthesis or stabilize lysosomal membranes, or perhaps both, merits thorough investigation.

Accumulation of Waste Products—A discernible concomitant of aging is the progressive accumulation of lipofuscin pigments in the cytoplasm and deposition of amyloids in the interstitial space of tissues. Both lipofuscin and amyloids are believed to be biologically degenerate substances—hence their name waste products.

Lipofuscin, also known as age-pigment or ceroid, tends to accumulate with advancing age, especially in postmitotic cells such as those of the brain, heart muscle, and adrenals. Even though its origin and role are poorly understood, lipofuscin, based on circumstantial evidence, might be an important indicator of the degree of cellular insufficiency in aging. Earlier reviews discussed in considerable detail the histochemistry and ultrastructure (127, 128), isolation and chemical composition (18, 129), and origin (18, 130, 131) of lipofuscin.

Lipofuscin is a basophilic autofluorescent, chemically heterogeneous substance. It consists of 30–40% lipids, a substantial amount of protein, and traces of inorganic elements. The protein component seems to be relatively abundant in glycine and valine, whereas the lipids resemble those of the source tissue (18, 129).

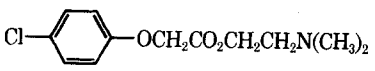
The possible origin of lipofuscin is still a much debated question. The lipid component is most likely derived from the peroxidation of phospholipids and unsaturated fatty acids (132, 133). Growing evidence suggests (133, 134) that irreversible autoxidation of lipids can generate free radicals, which are not inactivated by the mitochondrial electron-transport chain. Hence, these free radicals can attack membranes (*e.g.*, mitochondrial

and lysosomal) as well as proteins, yielding eventually the lipofuscin conglomerate of degraded and polymerized cellular debris.

Cytochemical assays indicated the presence of acid hydrolytic enzymes, which would support a lysosomal origin of lipofuscin (130). Membranous fragments also were found in the pigment (131). Some investigators detected mitochondrial enzymes such as DPN-diaphorase and succinic dehydrogenase around, but not inside, the lipofuscin granules, which suggested a mitochondrial origin (130). Others proposed the endoplasmic reticulum of the Golgi apparatus as possible loci of lipofuscin formation (130). Although lipofuscin can occur early in life (135, 136), aging elicits a significant increase in number, size, and distribution. Such an increase was clearly demonstrated in the different topological areas of brain neurons of rodents (136–138), guinea pigs (139–141), dogs, pigs (142), rhesus monkeys (143), and humans (144); in the heart muscle of rats (145), dogs (146), and humans (147); and in the kidneys, adrenals, testes, and ovaries of rats (145). Excessive lipofuscin deposits were also observed in certain mental disorders of children (148–150) and in a case of presenile dementia (151).

However, the relationship between lipofuscin accumulation and age-associated dysfunction, such as in presenile and senile CNS deficiencies, needs to be investigated under well-controlled experimental conditions. In this regard, experimental lipofuscinosis induced in young animals by various means of stress (152, 153) or chronic vitamin E deficiency (152, 154, 155) could provide useful methods to study this problem in greater detail.

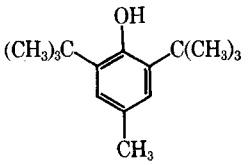
Meclofenoxate [centrophenoxine (V)], a drug of reported benefit in presenile and senile mental disorders, was shown to reverse the accumulation of lipofuscin pigments in old guinea pigs (140, 141).



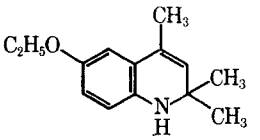
V

Nandy and Bourne (140) observed that lipofuscin, although present in neurons of young and old guinea pigs, begins to increase steadily at the age of 4 years until it occupies a considerable segment of the cytoplasm. Guinea pigs, 4–6 years of age, treated with meclofenoxate daily for 4–8 weeks showed a significant decrease in pigmentation in most areas of the CNS. The degree of activity depends upon the duration of the treatment, giving rise to the greatest reduction after 10 or more weeks (141). At the same time, meclofenoxate reduced the activities of succinic and lactic dehydrogenase and cytochrome oxidase, all predominantly present in the pigmented zone. The decrease in succinic acid dehydrogenase activity seems to indicate a drug-induced reduction in glucose metabolism *via* the Krebs cycle, while the decrease in lactic dehydrogenase activity reflects a reduction in glycolysis. On the other hand, glucose-6-phosphate dehydrogenase activity increased, which, according to Nandy (141), may suggest the diversion of glucose into the pentose cycle. The lipofuscin-depleting activity of meclofenoxate, however, has not been correlated with its claimed efficacy in confusional and demented states.

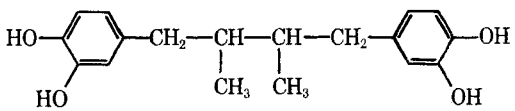
Harman (53, 156) suggested that free-radical production is responsible for many degenerative changes associated with the pathogenesis of aging. While the actual mechanism by which free radicals may produce their effects is as yet undetermined, there is sufficient support for the observation that they tend to react with various cellular materials and produce bonding of adjacent macromolecules, resulting in degradation of the involved substances. Support for Harman's hypothesis comes primarily from a series of experiments (157–160) which he conducted using various inhibitors of free-radical reactions. The results of these studies indicate that many antioxidants and free-radical scavengers, such as cysteine, 2-mercaptoethylamine, 2,2′-diaminodiethyldisulfide, hydroxylamine, butylated hydroxytoluene (VI), ethoxyquin (VII), and ammonium diethyldithiocarbamate, when added to the daily diet, produce an increase in the mean lifespan of mice. Beneficial effects of free-radical inhibitors have been shown for female C3H mice, male AKR mice, and male LAF_1 mice. The first two strains died prematurely of lymphatic leukemia and mammary carcinoma, respectively, while the LAF_1 mouse is a low tumor incidence strain. No effect was found when antioxidants were added to the diet of male Swiss mice. Another study, conducted in Wistar rats, showed that an increase in lifespan may be produced with the antioxidant nordihydroguaiaretic acid (VIII) (161).



VI

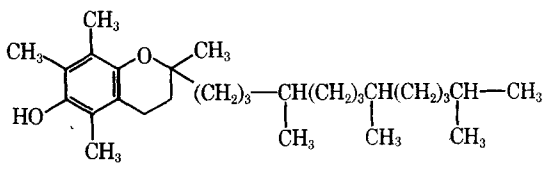


VII



VIII

To counteract antioxidant decay, Tappel (133) suggested vitamin E, glutathione, cysteine, and selenium compounds as potential free-radical scavengers. Among these agents, vitamin E (IX) might have particular significance since its deficiency is known to enhance the production of lipofuscin in rodents (152, 154, 155).



IX

Amyloids, in contrast to lipofuscin, deposit extracellularly in various organs of old mice, hamsters, rabbits, dogs, apes, birds, horses, and humans (162, 163). While the exact chemical composition of amyloid has not yet been established, its major constituent is protein, consisting in part of γ-globulins and collagen, which is complexed with a mucopolysaccharide (132, 164). Amyloidosis can be induced experimentally, for example, with Freund's adjuvant, casein-rich diet fed to mice (132), hyperimmunization (163), *Leishmania* injection, and parabiosis (162). These inducing conditions, as well as the presence of serum globulin components in amyloids, strongly suggest that an autoimmune mechanism might be involved in the genesis of amyloidosis (162, 163, 165).

Amyloids accumulate most severely with increasing age in the brain, heart, aorta, and pancreatic islets (163). The occurrence of cerebral amyloids and "senile plaques," a form of amyloidosis, seems to correlate with senile and Alzheimer's presenile dementia (163, 164, 166, 167). Thus far, any attempt to prevent or mitigate senile amyloidosis has not been reported in the literature.

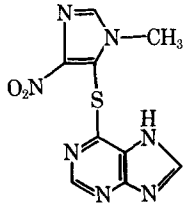
Changes in the Immune System—Age-associated deterioration of immunocompetence is manifested in two ways: (*a*) the responsiveness to extrinsic antigens decreases, and (*b*) several autoimmune factors increase with age.

The primary antibody response of the mouse to heterologous erythrocytes declines significantly with increasing age (168, 169). This deficiency, however, is not caused by a decrease in the amount of antibody produced by the individual antibody-forming cells (169) but rather by the progressive loss of the number of antigen-sensitive cells (168, 169). According to a recent report (170), mixed polynucleotide complexes such as *poly A:U*, *poly C:G*, and *poly I:C* can restore the impaired antigen–antibody response of aging mice.

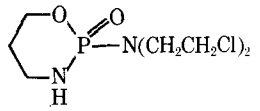
Walford (165, 171–173) elaborated on the idea of an autoimmune mechanism of aging. He regards it as a mild but chronic autorejection phenomenon involving the progressive failure of the organism's own cells to distinguish self from foreign cells. Observed rises of several indicators which may reflect autoimmunity are serum γ-globulins; rheumatoid, antinuclear, antithyroid, and antiparietal factors; and renal lysozyme, which usually increases after immunization and transplantation. According to Walford (165, 171–173), senile amyloidosis is, to date, one of the most specific expressions of an age-associated autoimmune reaction in which the amyloids are the visible end-products. This, however, does not exclude the possibility that amyloids themselves could further act as antigens.

Recently, Albright *et al.* (174) reported that the spleen of old mice harbors life-shortening factors, since splenectomy almost doubled their survival, while spleen cells from old animals injected into younger ones reduced lifespan. Although the factor(s) has not been identified, it is tempting to speculate on its immunologic nature.

Based on the autoimmune theory of aging, Walford (165) tested the effect of azathioprine (X), an immunosuppressive agent, on the lifespan of aging mice. A daily dose of 100 mg./kg. extended the 50% survival score of the treated group by 10 weeks as compared to controls. However, the lifespan of the oldest survivors in the control and treated groups was alike, which Walford (165) attributed to the high toxicity of the drug. Similar results were obtained with cyclophosphamide (XI), another toxic immunosuppressant (173).



X

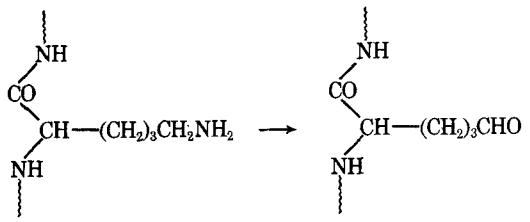


XI

From the foregoing, it is evident that attrition of the immunosurveillance mechanism does accompany aging, but the principal elements involved in cause and effect have yet to be identified.

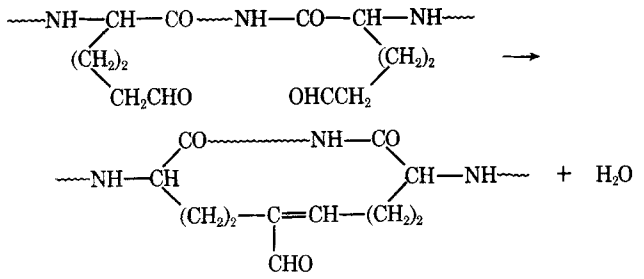
Collagen—A considerable amount of research has been devoted to describing the chemical and physical changes that occur in collagen with age (175, 176) and, in more general terms, has contributed to the understanding of the role of molecular crosslinking as it determines both the structure and plasticity of connective tissue (177). The changes noted in collagen with age include a decreased susceptibility to enzymatic digestion, a decrease in solubility and swelling capacity, and an increase in stability of rat tail tendon subjected to a breaking load. These changes have been attributed to an increase in the formation of covalent crosslinkages which, in a sense, immobilize these large macromolecules. Evidence cited by Bailey (175) confirms that the proportion of intermolecular and intramolecular crosslinking increases with age. Current evidence, however, suggests that the physical changes in collagen with age are mainly a reflection of increased intermolecular crosslinking.

The collagen fiber has a triple helical structure composed of three similar polypeptide chains. While intramolecular crosslinking occurs within the same polypeptide strand, intermolecular networks are formed between the three chains. The initial step in both intramolecular and intermolecular crosslink formation seems to involve the same enzymatic deamination of free ε-amino groups of lysyl residues yielding the corresponding aldehyde (Scheme III) (177, 178). Intra-



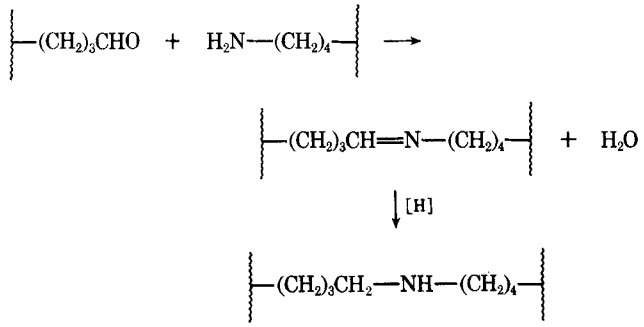
Scheme III

molecular crosslinks arise from aldol condensation of the spacially close aldehydes (Scheme IV). Less certain



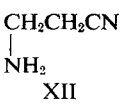
Scheme IV

is the nature of the intermolecular crosslinks; but according to cursory evidence (177, 178), most likely the aldehyde residues couple with free ε-amino groups of lysine and hydroxylysine to a Schiff base, which is further stabilized by reduction to the secondary amine (Scheme V). However, there is no direct proof that such a reductive step takes place *in vivo*.



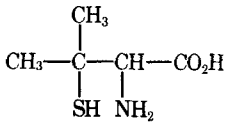
Scheme V

A number of studies have been directly concerned with the inhibition or reversal of crosslink formation within collagenous tissue. The most dramatic of these situations deals with the condition known as lathyrism. This condition is characterized by increased tissue fragility and extractability of collagen and is the result of an inhibition in the formation of normal crosslinkages. Lathyrism was first observed in animals that had ingested sweet pea, *Lathyrus odoratus;* the toxic principle has been identified as β-aminopropionitrile (BAPN) (XII). This substance, when fed to animals, produces a distinct pathological syndrome characterized by a defective maturation of collagen. Levene (179) noted that other substances, in addition to the nitriles, possess lathyrogenic properties. Some urea derivatives such as semicarbazide and certain hydrazides and hydrazines will produce effects similar to that seen with BAPN, but they are not as potent as BAPN. Kohn and Leash (180) failed to show any influence of BAPN on the survival of pathogen-free rats, despite the appearance of characteristic lathyrism at high doses.



XII

Inhibition of intermolecular crosslinks was also demonstrated for penicillamine (XIII) (175, 177). It has been shown that D-penicillamine, when fed to rats, tends to produce a reversal in the degree of stability exhibited by rat tail tendons in older animals. This observation apparently occurs because penicillamine is capable of inhibiting the formation of crosslinkages. Other studies showed that when penicillamine is removed from the diet, the inhibition of crosslinkage is reversed (177). Recently, Ruiz-Torres (181) indicated that penicillamine disturbs the collagen maturation process.



XIII

Several other studies on the reversibility of crosslink formation in collagen are of interest. Wynn (117) showed that rat liver lysosomes are capable of degrading undenatured insoluble collagen (*cf.*, lysosomes). Spichtin and Verzár (182) showed that extraction of calcium from skin collagen leads to a decrease in the stability of collagen as measured by its solubility. According to some studies (183–185), collagen also has antigenic properties. However, it appears that antigenicity decreases with increased structural stability which characterizes aging (184).

Hormones—From the early days of gerontological research, probably no other area has received as much attention as the endocrine system. The literature has recorded countless experiments with glandular extracts and hormones, ranging from naive rejuvenation attempts to palliative replacement therapies of the elderly. Despite the long-standing interest in the relationship of hormonal function and aging, the problem is relatively little understood; a serious deficiency is the lack of comprehensive biochemical studies.

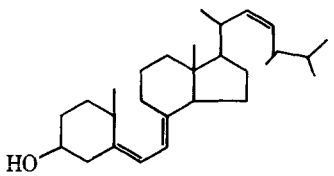
Bellamy (186) reviewed in detail the recently available information on the hormonal states of the aging mammal. Therefore, we shall summarize here only the general observations. Hormone output as a function of age cannot be measured adequately, since there are no techniques available to assay plasma hormone levels accurately. Age-associated sex hormone deficiencies have only been inferred from the decreased urinary excretion of 17-ketosteroids. Thyroid and growth hormone, adrenocorticotrophin, and thyrotrophin levels do not seem to change with age. Generally, tissue sensitivity to hormones declines with increasing age, as in the case of insulin, the antidiuretic activity of exogenous vasopressin, and the renal activity of aldosterone and hydrocortisone. In contrast, exogenous thyroid hormone elicits greater response in older than in young animals. Hormonal regulation in age-related tissue involution has not been clearly demonstrated.

The electrolyte distribution undergoes marked changes during aging, whereby water shifts from the intracellular to the extracellular milieu and is accompanied by potassium depletion and sodium retention. With this in mind, Friedman and his coworkers (187–189) studied the effect of whole posterior hypophysal extract, vasopressin, and their combination with corticosteroids on aging rats. The pituitary extract alone and in combination with hydrocortisone extended the lifespan significantly of the chronically treated group. Adrenocorticotrophin did not potentiate the effect of the pituitary preparation, nor did aldosterone. Vasopressin, alone and together with aldosterone (190),

ameliorated the electrolyte balance and the function of the *gastrocnemius* muscle of old rats without affecting the lifespan. However, both hormones proved to be toxic to old animals (187, 188). Independent investigators (191) related the life-prolonging effect of the posterior pituitary extract to oxytocin. Further experimentation is needed to ascertain whether the posterior pituitary has a *de facto* control over the rate of aging.

Corticosteroids are known to enhance the viability of cells cultured *in vitro* (*cf.*, lysosomes); furthermore, chronic dietary administration of prednisolone to a short-lived, highly inbred strain of mice sharply increased their lifespan (192). At the same time, prednisolone treatment minimized the age-associated rise in the calcium–collagen ratio and the loss of liver nucleic acids. This activity of prednisolone might be related to its immunosuppressive properties, since the short life expectancy of the strain used in these experiments might be due to an autoimmune condition.

Dihydrotachysterol (XIV) administered chronically to rats induces a progerialike syndrome, a form of premature aging (193, 194). The syndrome is accom-



XIV

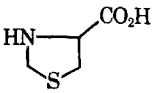
panied by arteriosclerosis, loss of weight and skin elasticity (193, 195), deposition of calcium (195, 196), and loss of collagen (197). Anabolic steroids, such as testosterone and methyltestosterone (194), conjugated equine estrogens (198), and ferric dextran (193) prevent the adverse effects of dihydrotachysterol. Adminstration of pituitary growth hormone (199), thyroxine (200), and the plant growth hormone, indole-3-acetic acid (201), did not influence the lifespan of the rat.

Miscellaneous Agents—This category includes substances and preparations that have been claimed to hinder the aging process, but their mode of action is either unknown or different from those described earlier.

Panax Ginseng—A crude aqueous extract of this plant was shown to mitigate cellular degeneration of X-irradiated and unirradiated cultures of postmitotic human amnion cells (40). The chemical composition of the active principle has not been identified.

Thiazolidinecarboxylic Acid (*XV*)—Experimental evidence indicates (202–204) that disulfide groups tend to accumulate in proteins during aging. Furthermore, serum proteins of old rats were shown to contain a decreased amount of free carboxylic and amino groups (205).

The relative number of the free carboxyl and amino groups increased when aged rats were treated with a mixture of folic acid and 4-thiazolidinecarboxylic acid (XV), a precursor of *N*-formylcysteine (205). This mixture, as well as cysteine, also extended the lifespan of male mice and female guinea pigs but not of female rats (206).

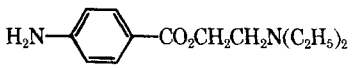


XV

Pantothenic Acid (*XVI*)—Given daily as a dietary supplement to mice, this drug increased their mean lifespan from about 550 to 653 days and increased the body weight (207).

XVI

Procaine (*XVII*)—As a rejuvenating agent, this drug has created one of the greatest controversies in gerontology. Here, it will suffice to say that other controlled human trials (6, 208, 209) could not substantiate clinical claims made by Aslan (210). Hence the gerontological usefulness of this drug is in great doubt. From subsequent animal studies, Aslan *et al.* (211) reported that chronic parenteral administration of procaine to rats increased the lifespan of the males but not of females, improved maze-running efficiency, and mitigated age-associated morphological changes in various organs. In contrast, Verzár (212) obtained only negative results in rats treated with procaine and its hydrolytic components, *p*-aminobenzoic acid and diethylaminoethanol.



XVII

There is widespread belief among the laity that the Rumanian preparation, which in addition to procaine contains *p*-aminobenzoic acid, benzoic acid, diethylaminoethanol, and potassium salts of dibasic phosphate and metabisulfite, is effective, and despite the dubious value of procaine, it might merit a stringently controlled, double-blind clinical battery test such as Comfort (213) outlined in his experimental model.

CONCLUSIONS

In this review we have attempted to focus the attention on a relatively new science, experimental gerontology, which set out to learn the causes of aging and, hopefully, to mitigate them on the basis of a sound rationale. The problem is by no means near to a solution since we do not know what causes us to age. With the current state of the art, we can only define *expressions* of aging which do not necessarily equate with *causes*. Agents that were shown to subdue one or another expression of aging or that prolonged the lifespan of experimental animals should not be looked at as cures of senility but rather as useful models for future research. At the present, one vexing problem of gerontology is the lack of sound measures of biological aging as opposed to chronological age. Lifespan alone has serious liabilities as a measure of aging inasmuch as the rate of mortality is its only critical parameter and does not take into account

the gradual loss of function with the increase of age. Until we learn more about the intricate mechanisms of the aging process, the short-range objectives of age research might be best geared toward ways of improving the quality of life of the elderly, that is, improving their general mental and physical conditions by pharmacological measures.

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RESEARCH ARTICLES

Absolute Configuration of *cis*- and *trans*-2-(*o*-Bromophenyl)cyclohexanols and (+)-*cis*-2-(*o*-Bromophenyl)cyclohexylamine

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Abstract □ The absolute configurations of (−)-*trans*-2-(*o*-bromophenyl)cyclohexanol (V), (+)-*cis*-2-(*o*-bromophenyl)cyclohexanol (VII), and (+)-*cis*-2-(*o*-bromophenyl)cyclohexylamine (X) have been chemically related to that of (2S)-(−)-2-(*o*-bromophenyl)-cyclohexanone (VI), whose absolute configuration can be reliably assigned from its optical rotatory dispersion and circular dichroism spectra.

Keyphrases □ *cis*- and *trans*-2-(*o*-Bromophenyl)cyclohexanols and (+)-*cis*-2-(*o*-bromophenyl)cyclohexylamine—synthesis, absolute configuration □ Absolute configuration—cyclohexanol and cyclohexylamine derivatives □ IR spectrophotometry—identity □ NMR spectroscopy—identity □ Optical rotatory dispersion—structure □ Circular dichroism—structure

Concurrent with the study of the optical rotatory dispersion (ORD) and circular dichroism (CD) of the aromatic chromophore, it became necessary to synthesize, resolve, and assign the absolute configuration to a number of substituted 2-arylcyclohexanols and cyclohexylamines. Galpin and Huitric discussed the resolution (1) and absolute configurations (2) of (1S,2R)-(+)-*trans*-2-*o*-tolylcyclohexanol and (1R,2R)-(−)-*cis*-2-*o*-tolylcyclohexanol. The assignment of absolute configuration was based on the sign of the Cotton effect of the (2R)-(+)-2-*o*-tolylcylcohexanone obtained upon oxidation of the *cis*- and *trans*-alcohols, and it has recently been confirmed by single-crystal X-ray diffraction analysis of the 3-nitro-4-bromobenzoate of the (+)-*trans*-alcohol (3). In this paper, the authors report on the synthesis and optical resolution of (−)-*trans*-2-(*o*-bromophenyl)cyclohexanol (V) and its conversion to (−)-2-(*o*-bromophenyl)cyclohexanone (VI), (+)-*cis*-2-(*o*-bromophenyl)cyclohexanol (VII), and (+)-*cis*-2-(*o*-bromophenyl)cyclohexylamine (X). The absolute configuration of this series of compounds can be related to that of the ketone VI, whose absolute configuration is assigned from its ORD and CD spectra.

The synthesis and optical resolution of *trans*-2-(*o*-bromophenyl)cyclohexanol (V) are depicted in Scheme I. Racemic 2-(*o*-bromophenyl)cyclohexanone (II) was obtained from the permanganate oxidation (4, 5) of the potassium aci-nitro salt of *trans*-2-(*o*-bromophenyl)-nitrocyclohexane (I), which was previously prepared as an intermediate in the synthesis of the *cis*- and *trans*-2-(*o*-bromophenyl)cyclohexylamines (6). Reduction of II with lithium aluminum hydride gave *trans*-2-(*o*-bromophenyl)cyclohexanol (III) as the predominant isomer. The racemic mixture of alcohols III was resolved by fractional crystallization of its diastereomeric (−)-menthoxyacetate esters, in the manner described by Galpin and Huitric for the resolution of *trans*-2-*o*-tolylcyclohexanol (1). The resolution was conveniently followed by NMR spectroscopy, utilizing the magnetic nonequivalence of the geminal methylene protons (hydrogens a and a′) on the acetate portion of the esters.[1] This nonequivalence is sufficiently different in the two diastereomeric esters to allow the progression of the separation of the two diastereomers to be readily followed by NMR.

Figure 1 shows the pertinent portions of the NMR spectra of a 50:50 mixture of the diastereomers (A) and

[1] For a general discussion of the use of NMR spectroscopy as a monitor for optical purity, see Raban and Mislow (7). For the specific application to (−)-menthoxyacetates, see Galpin and Huitric (1).