PHYSIOLOGICAL BASIS OF GERIATRICS

PAOLA S. TIMIRAS M.D., PH.D., EDITOR

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Paola S. Timiras, M.D., Ph.D.
Professor of Physiology
Department of Physiology-Anatomy
University of California, Berkeley
Berkeley, California

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CHAPTER

4 THEORIES OF AGING

Ramesh Sharma

From even a cursory review, it emerges clearly that there are many theories of aging. The maximum lifespan potential is a constitutional feature of speciation subject to polygenic controls and to environmental influences. The enormous genetic heterogeneity that characterizes many species, particularly humans, and the complexity of environmental experiences create quantitative and qualitative variations of the senescent phenotype. Until now, no single theory has accounted for all phenotypes, although many have attempted to explain at least some of the major and most frequent aging phenomena. Inasmuch as all phenotypes result from an interaction between nature and nurture, an integrating view of these interactions may help in our endeavors toward a more fundamental understanding of aging. Thus, a most productive route to understanding the biology and pathology of aging (including the major aging-related diseases of humans) will be one that derives from a dissection of molecular, cellular, and systemic events. It is with this rationale in mind that the present chapter will examine the major theories of aging categorized as molecular, cellular, and systemic.

Molecular theories mainly assume that the lifespan of any species is governed by the genes interacting with environmental factors. Genetic information is stored in the genes (segments of nucleotides in DNA), is transcribed into RNA, and is subsequently translated into proteins. These proteins, either structural (e.g., collagen and keratin) or functional (e.g., enzymes and receptors), govern the form and function of organisms. Aging may result from changes in DNA template activity, which regulates the formation of the final cellular products.

Cellular theories include changes in cellular proteins (structural and functional) and other macromolecules that may occur as a function of age. These changes are produced with the passage of time under the influence of environmental factors (e.g., nutrition and stress) they may be chemical and/or morphologic and involve enzymes, hormones, age pigments, membrane permeability, macromolecule cross-linking, and changes in various cell organelles such as lysosomes and mitochondria.

Systemic theories ascribe aging of the entire organism to decrements in the function of a key system, such as the nervous, endocrine, and immune systems. Such decrements could be genetically programmed, as are the early developmental phases of the lifespan, or be the consequence of environmental insults. Alterations in the key system will generate changes throughout the entire organism.

Presented here is only a rapid survey of the major theories of aging some of which are discussed more completely in other chapters (see Chapters 7–9, 12, and 15).

MOLECULAR THEORIES

These theories originate with the concept that all individuals within a species have a similar length of life and that different species have different lifespans. For example, mayflies live only 1 day, houseflies 30 days, rats 3 years, dogs 12 years, horses 25 years, and humans 70 years (Comfort, 1979). It is presumed that there is some genetic program that determines the maximum lifespan for each species. Another argument for a genetic basis of aging is that (as well demonstrated in humans) the offspring of long-lived parents have a longer lifespan than those born from average-lived parents (Dublin, 1949). The average lifespan for females (approximately 78 years) is generally longer than for males (approximately 71 years) in most developed countries, like the United States, Sweden, and

Japan. This sex difference in lifespan is also observed in other groups of animals (Rockstein, 1974). An equally significant contribution to a genetic basis of aging is derived from the duration of the three phases of the lifespan—developmental, reproductive, and senescent. In most animals, the reproductive phase occupies a significant period in the lifespan followed by a postreproductive phase. In mammals, the time taken to reach reproductive maturity is correlated with maximum lifespan (Asdell, 1946; Kanungo, 1975) (see also Chapter 2). *Homo sapiens* and long-lived mammals take longer to reach reproductive maturity compared to other animals and continue to live after reproduction has ceased. Conversely, certain lower vertebrates (Pacific salmon, Atlantic eel, and lamprey) and invertebrates (octopus) die soon after their first reproduction as if reproduction might involve depletion of certain essential factors necessary for maintenance of later life. Expression of the genetic program that regulates the lifespan may be altered by various environmental factors. Some of the major molecular theories involve errors in this genetic program.

CODON RESTRICTION

All the genetic information stored in DNA directs the structure and function of the organism, although only part of the total DNA information is utilized by the cell at a given time. The information is transferred from DNA to messenger RNA (mRNA) by the process of transcription. The functional mRNA in eukaryotic cells is derived from excision of intervening sequences (introns) that are transcribed along and between information sequences (exons) by splicing. This mRNA is then translated into protein. The codon restriction theory of aging is based on the hypothesis that the fidelity or accuracy of translation, which depends on the cell ability to decode the triplet codons (three bases) in mRNA molecules, is impaired with aging (Strehler, 1977). The accurate reading of the codons is done by two main biomolecules: transfer RNAs (tRNAs) and aminoacyl-tRNA synthetases. Any change in these tRNAs and aminoacyl-tRNA synthetases may alter the rate of translation (decoding of the message).

There is experimental evidence for quantitative changes in the tRNAs and synthetases during development and aging. Ilan *et al.* (1970) have reported alterations in $tRNA^{tyr}$, $tRNA^{leu}$, and corresponding synthetases during the developmental period of the insect *Tenebrio molitor.* These quantitative alterations also occur in the isoacceptors of $tRNA^{arg}$ and $tRNA^{tyr}$ during aging of the free living nematode *Turbatrix aceti* (Reitz and Sanadi, 1972). Support for this theory has come from the findings of Hosbach and Kubli (1979), who demonstrated that tRNA isolated from 35-day-old *Drosophila melanogaster* cannot be aminoacylated as well as that of 5-day-old flies. The aminoacylating ability of some synthetases of old flies is only 50 percent that of the young flies. The fetal rat liver contains six isoacceptors for $tRNA^{tyr}$ compared to the adult liver, which has only three (Yang, 1971). A lower ability of tRNA aminoacetylation has been reported in hepatic parenchymal cells of old rats (Mays *et al.*, 1979).

Changes in tRNAs and aminoacyl-tRNA synthetases with aging occur in plant systems as well. Young and old tissues of soybean cotyledons differ from each other in the kinds of completely chargeable tRNA that are present. Moreover, old tissue extracts are not only deficient in certain aminoacylating abilities but possess factors that inhibit the charging of some tRNAs by extracts of young cotyledons (Bick and Strehler, 1972). Gene sequencing of rabbit β-globin shows a highly restricted use of the synonymous codons for various amino acids; only 39 of the 61 usable codons are used in the framing of the message (Efstradiadis *et al.*, 1977). Comparison of the isoaccepting species of $tRNA^{lys}$ from early and late human fibroblasts shows a smaller proportion of these species in senescent cells than in those from early passage cultures (Agris *et al.*, 1985).

As a result of differentiation, cells would lose their ability to translate genetic information. Despite a number of supportive observations, this theory, based on the view that sequential changes in the tRNAs and aminocyl-tRNA synthetases during lifespan may lead to the aging of an organism, needs

further validation. It is still difficult to explain the basic cause(s) for the alterations with aging in these message-reading molecules and the implications of such changes in aging phenomena.

SOMATIC MUTATION

Alteration of the structure of the DNA molecule changes the genetic message and results in differences in protein structure that lead to physiologic deficits. This proposed theory was based on the report that rats exposed to limited irradiation died at a younger age than nonirradiated controls (Szilard, 1959). These considerations were extended to humans (Warren, 1956; Henshaw, 1957; Failla, 1960; Sacher, 1977) and included a higher incidence of neoplasia in irradiated individuals, suggesting that irradiation accelerates the aging process. According to this theory, exposure to radiation damages DNA with subsequent induction of mutations, which, in turn, lead to progressive loss of genes is postmitotic cells throughout the lifespan. The increased rate of mutation and loss of functional genes decrease the production of functional proteins and cause cell death at a critical level.

Support for this theory was provided by the observation that increased exposure to x-rays shortens life expectancy and increases chromosomal aberrations in parallel with increasing doses of x-rays (Stevenson and Curtis, 1961; Curtis, 1964). Older animals have a greater number of chromosomal abnormalities than younger, and in short-lived mice, the rate of development of abnormalities is more rapid than in long-lived ones. These data suggest that natural radiation also affects the aging process. However, contrasting evidence negates a causative role for somatic mutation in aging.

In some species, such as humans, the sex chromosomes of females are similar (XX) but those of males are different (XY), while in others the reverse is true. If radiation is a cause for aging, then one might expect a longer life for individuals with identical sex chromosomes. However, in most species, females generally live longer than males, irrespective of the chromosomal composition. Another example is the wasp, *Habrobracon,* in which males have either two sex chromosomes (diploid) or one (haploid). If both types of males are exposed to x-rays, the haploid male should die earlier than the diploid, but, in fact, this is not the case: both males have similar lifespans despite the greater resistance of the diploid male to ionizing radiation because of the larger number of repairable chromosomes (Clark and Rubin, 1961). Chemical substances that change DNA structure have no effect on lifespan (Curtis, 1966). Colchicine exposure of human fetal lung fibroblasts produces 60 percent tetraploid cells, which continue to divide (Thompson and Holliday, 1978) and have growth rate and lifespan similar to those of diploid cells. Diploid as well as tetraploid human skin fibroblasts likewise have similar lifespans (Hoehn *et al.*, 1975). The somatic mutation theory is further contradicted by studies of the effects of low-dose ionizing radiation on the lifespan of human fibroblasts *in vitro*; irradiation of early embryonic as well as postnatal cells may shorten, prolong, or have no effect on doubling potential and lifespan (Macieira Coelho *et al.*, 1977, 1978; Azzarone *et al.*, 1980).

Somatic mutations are no longer regarded as a probable cause of aging because the rate at which they occur in the absence of ionizing radiation is too low to account for overall age changes (Maynard-Smith, 1966). Furthermore, the primary lesions of aging are different from those of radiation (Walburg, 1975). Radiation acts primarily on dividing cell lines such as bone marrow stem cells, leukocytes, and gut epithelial cells, and its effects are observed after cell division. In contrast, effects of age are centered in cell lines that no longer divide, such as nerve and muscle cells.

Most cells have mechanisms for the repair of damaged DNA molecules (Hart and Setlow, 1974; Wheeler and Lett, 1974) and there is little evidence that DNA repair mechanisms decline in senescent animals (Tice, 1978); rather, these repair mechanisms appear more effective in long-lived species as compared to short-lived (Figure 4–1) (Hart and Setlow, 1974). The species-specific differences in the lifespan of animals could be attributed to the ability of animals to tolerate DNA damage rather than

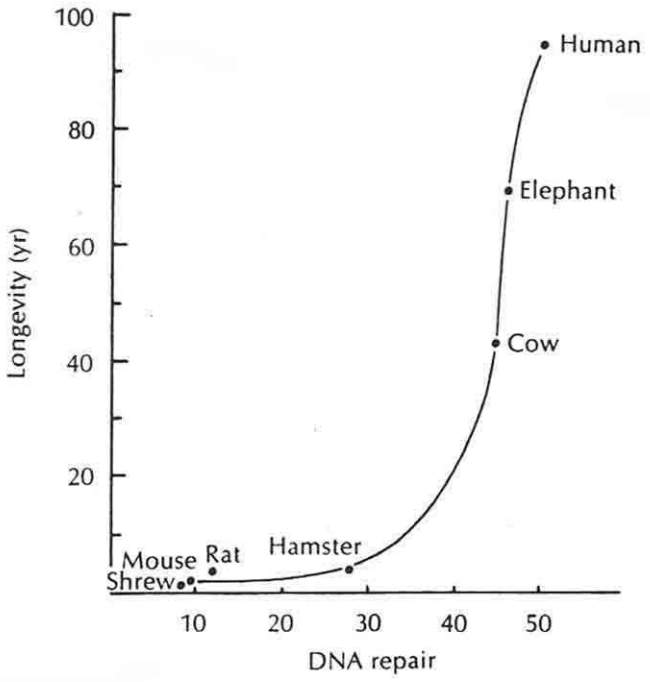

Figure 4–1. *Correlation between longevity and ability to repair DNA by replacement of thymine dimers following ultraviolet radiation in different mammals.* (From Sinex, F.M., In Finch, C.E.; Hayflick, L. (eds.): *Handbook of the Biology of Aging*, Van Nostrand Reinhold Co., New York, 1977.)

to repair. The presence of multiple copies of the same message coded within the DNA would offer protection to DNA damage. The number of repetitive genes for the major rRNA is 5 to 10 in bacteria, 100 to 130 in *Drosophila*, and 250 to 600 in vertebrates (Medvedev, 1972), suggesting a correlation between the number of repetitive genes and the lifespan of the species. Cutler (1973) reported that the average redundancy of transcribing mRNA in the brain was greater in humans than cows and greater in cows than mice. The higher the redundancy of transcribing mRNA, the longer is the lifespan. Based on available experimental evidence, radiation does not seem to play a major role in accelerating the aging process or in causing aging.

Error Theory

The form and function of organisms are governed by specific structural and functional proteins. Certain protein molecules, such as RNA polymerase and tRNA synthetases, are involved in the production of other proteins. Medvedev (1961) first advanced the concept that errors in the transmission of information through RNA to proteins may be responsible for cellular aging. This theory was extended to investigate which errors occurring in information transfer steps, such as transcription and translation, may cause accumulation of defective proteins and cause aging (Orgel, 1963; Medvedev, 1964). Errors such as the incorporation of wrong nucleotides into mRNA during transcription may change the triplet codons, or incorporation of wrong amino acids into protein during translation may change the amino acid sequence. Orgel (1973) further argued that production of functional proteins such as enzymes depends not only on the genetic information stored in DNA, but also on the protein synthetic machinery, and he pointed out that inaccuracy occurs in both protein and DNA syntheses (Figure 4–2). The initial error in proteins may be low, but it increases exponentially with the passage of time and may lead to an "error catastrophe" and ultimately death of the cell. The error accumulation can be expressed mathematically as $Et = Eo_{e\alpha t}$, where Eo is the initial error frequency, Et is the error frequency at time t, and α is a proportionality constant.

Evidence for the error theory is primar-

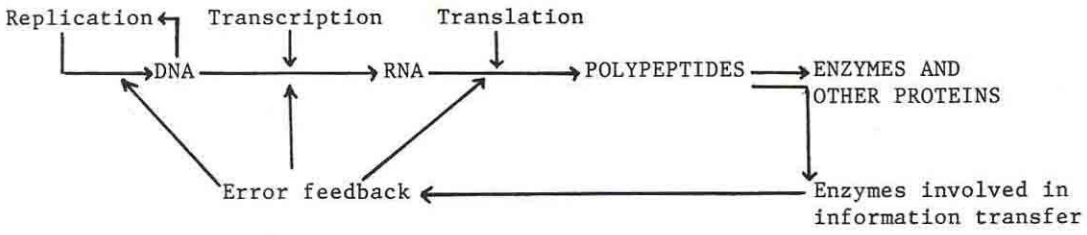

Figure 4–2. *Feedback of errors in protein synthesis during information transfer in biological systems.*

ily based on an experimentally-induced error in fruitflies produced by feeding them with amino acid analogs. These flies have a shorter lifespan than normal (Harrison and Holliday, 1967). Much of the support for this theory comes from the work of Holliday and Tarrant (1972), who reported an increased accumulation of heat-labile glucose-6-phosphate dehydrogenase in old fibroblasts. These heat-labile enzymes in old fibroblasts also show an altered substrate specificity, indicating the presence of possible errors. Using immunologic techniques, the proportions of inactive lactate dehydrogenase (LDH) in human fibroblasts, isocitrate lyase in the nematode, and aldolase in mouse liver increase in old age (Lamb, 1977). Functionally altered enzymes are known to accumulate in various animal tissues with age (Gershon, 1979; Rothstein, 1979). The decrease in the functional activity of tissues with increasing age would be due to accumulation of such altered proteins. Some reports suggest that altered enzymes in old tissues are generated by conformational changes (Sharma and Rothstein, 1978; Sharma *et al.*, 1980).

Many reports contradict the occurrence of errors in protein that may cause aging. Kanungo and Gandhi (1972) could not detect any age-related differences in liver malate dehydrogenase (MDH) using immunologic preparations. Kinetic properties (K_m and K_i) and electrophoretic mobilities of rat hepatic cytoplasmic alanine aminotransferase (Patnaik and Kanungo, 1976) and aspartate aminotransferase (Sharma and Patnaik, 1982) do not reveal age-related differences. Studies of aldolase from mouse liver (Gershon and Gershon, 1973) and cytoplasmic superoxide dismutase (SOD) from liver, brain, and heart of rats and mice have not detected differences in antigenicity, K_m, K_i, and electrophoretic mobility between young and old animals (Reiss and Gershon, 1976a,b). The fidelity of protein synthesis, measured in human diploid skin fibroblasts *in vitro*, remains unchanged as a function of age (Goldstein *et al.*, 1985). Mitochondrial proteins do not reveal changes in molecular weight or isoelectric point in young and old *Drosophila* (Fleming *et al.*, 1986), even though significant quantitative changes do occur with age. The fidelity of synthesis of mitochondrial proteins would then be preserved throughout the lifespan of *Drosophila*. Thus, there are sufficient data to show that errors in fidelity of protein synthetic machinery do not occur with increasing age and, therefore, cannot be responsible for aging.

GENE REGULATION THEORY

This theory was proposed to explain the two important characteristics of the aging process: (1) the gradual decline in adaptability to the environment after attaining reproductive maturity; and (2) the approximately fixed lifespan for a species (Kanungo, 1975, 1980). According to this model (Figure 4–3), senescence may result from changes in the expression of genes after reproductive maturity is reached. Differentiation and growth would follow sequential activation and repression of certain genes that are unique for these phases. Sequential activation and repression of genes occur for various hemoglobin chains during the gestational period in humans (Zuckerlandl, 1965). The hemoglobin, a tetramer protein, consists of $\alpha_2\varepsilon_2$ chains in the fetus at the age of 1 to 2 months of gestation. The α chain remains the same, but the ε chain is re-

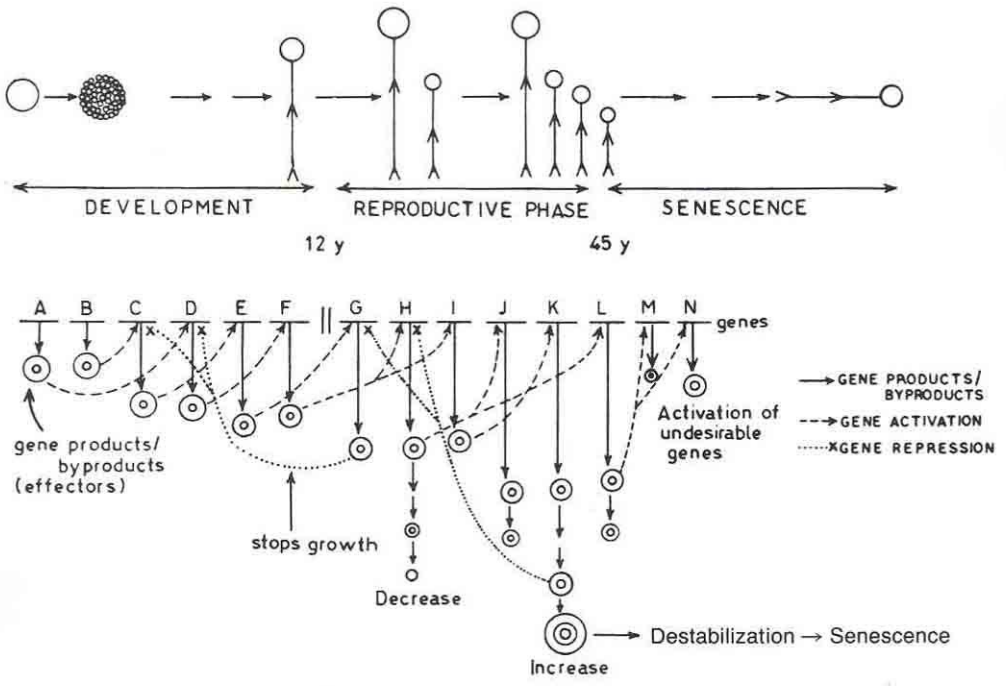

Figure 4–3. *Model for aging.*

(*Upper part*:) Various phases of the lifespan as development, reproduction and senescence. (*Lower part.*) The number of active genes for development (A to F) and reproductive (G to L) phases. No specific genes for senescence are visualized in this model. Development occurs by the sequential activation of genes (A to F); the product of gene A switching on gene B and so on. Some of the late development genes (E and F) switch on some unique genes (G and F) of early reproductive phase. The organism attains reproductive ability when required amounts of gene products are formed. Continued reproduction may cause depletion of certain factors necessary for the activity of certain essential genes. Switching off these genes may cause deterioration of some functions. It may also lead to accumulation of certain gene products beyond a certain level resulting in the activation of some undesirable genes (M and N), whose products may cause diseases, e.g., autoimmune diseases. The decline in physiological functions after certain periods of the reproductive phase may be due to destabilization of the function of the genes of the reproductive phase or adulthood. (From Kanungo, M.S.: *Biochemistry of Ageing*. Academic Press, London, 1980.)

placed by the γ chain in later phases of gestation. Just before birth, the γ chain is further replaced by the β chain, which gives rise to the adult hemoglobin ($\alpha_2\beta_2$). The synthesis of these chains is governed by different sets of genes. These genes are sequentially activated and repressed by certain factors during the development of the human fetus. Another example of the differential activation is lactate dehydrogenase (LDH) isoenzymes in different tissues during development (Markert and Ursprung, 1962). The proportion of M_4-LDH is significantly lower in the heart, brain, and skeletal muscle of old rats, and the gene responsible for synthesis of the M subunit would be somehow repressed in old age (Singh and Kanungo, 1968). Studies on rat hepatic alanine aminotransferase (AAT), a dimer having A and B subunits, have shown that the gene for A is more active in the early period of the lifespan and subsequently repressed, whereas the B subunit is activated in old age and the dimer is formed only by the B subunit (Patnaik and Kanungo, 1976). According to these stud-

ies, the sequential activation and repression of genes would not be restricted to development (Caplan and Ordahl, 1978), but would extend into adulthood and aging (Kanungo, 1980).

The genes responsible for the synthesis of various enzymes do not appear to undergo any change in their basic sequences during the lifespan. Rather, the observed changes in levels of enzymes may be due to alterations in the template activity of corresponding genes induced by various extrinsic and intrinsic factors. For example, the level and inducibility of many enzymes by hormones change in different tissues as a function of age without any sign of error incorporation into these molecules (Kanungo, 1980; Sharma and Patnaik, 1982, 1984, 1985). Modulating factors may either appear or disappear and/or their levels may change at different phases of the lifespan (Kanungo, 1980). The products or by-products of the genes responsible for differentiation and growth, on reaching critical levels, stimulate certain unique genes responsible for the reproductive phase. However, as a result of continued reproduction, certain factors may be depleted as they may not be replenished as fast as they disappear. Such factors may be of crucial importance for keeping certain genes expressed or repressed. They may also cause activation of undesirable genes and, thereby, lead to destabilization of expression of the unique genes that are required for reproduction—hence a gradual decline in reproductive rate with age. This model also predicts that should the organism be able to replenish the factors that become depleted owing to continued reproduction, the reproductive period and lifespan would be lengthened. This theory is supported by the data on the lifespan of mammals, particularly the reproductive phase, which has continuously lengthened with the progress of evolution (Cutler, 1975).

The lifespan of a species may be divided into three phases: developmental, reproductive, and senescent. Each phase has a characteristic duration, rate and regulatory mechanisms. The initiation, rate, and duration of developmental and reproductive phases depend on unique sets of genes that are sequentially activated and repressed. Human genetic diseases, such as progeria and progeroid syndromes, are in agreement with this sequence (Kanungo, 1980). Progeria is caused by the mutation of an autosomal gene. In this case, the newborn child appears normal and grows normally up to about 6 years; then the signs of aging, such as atherosclerosis, accumulation of lipofuscin, greying of hairs, and so forth, appear. Fibroblasts taken from a 10-year-old progeria patient do not show as many population doublings as those of a normal child of the same age. It appears that some genes responsible for normal development are altered to induce this condition. Perhaps the production of essential factors necessary for further development and growth is prevented by this mutation. The reproductive phase is not initiated owing to lack of switching on of the necessary genes during the later phases of development and growth. The lifespan is shortened following expression of the mutated gene. Another example is the sudden death of the female octopus, which lays eggs only once, broods them, reduces food intake, and dies soon after the hatching of the young (Wodinski, 1977). Removal of the two optic glands after spawning prevents brooding, and the octopus continues to eat and to grow and increases longevity. It appears quite obvious that certain factors are produced in the optic gland that are responsible for brooding and cessation of feeding followed by senescence and death. Egg laying may deplete certain factors, which may in turn cause the optic gland to produce a hormone that causes behavioral change. A similar phenomenon is observed in salmon and certain insects. Each species has a unique set of genes for development and reproduction. Their sequential activation or repression determines the duration of development and the onset of reproduction and is governed by the proper balance between various factors that are essential for maintenance of the reproductive phase. According to this model, no unique gene would be responsible for aging, nor is aging programmed, as are development and reproduction. Rather, normal aging would merely be a consequence of the organism attaining reproductive ability (irrespective of whether it reproduced).

CELLULAR THEORIES

These theories relate to the changes that occur in structural and functional elements of cells with the passage of time. They also concern the biomolecules after their synthesis is completed, suggesting that these changes impair the effectiveness of these molecules as a function of age.

WEAR AND TEAR

According to this theory, living organisms are like machines (Sacher, 1977); i.e., with repeated use, parts wear out, become defective, and the machinery finally fails to function. This assumption is not entirely appropriate: organisms have a mechanism by which they can repair their damages, whereas machines do not. The premise of this theory originates from the observation that the lifespan of poikilotherms is shortened by increasing the environmental temperature and prolonged by decreasing it; indeed, the metabolic rate of chemical reactions is increased by increasing temperatures, and the reverse is true for low temperatures. This phenomenon has been reported for fruitflies (Loeb and Northrup, 1917; Strehler, 1962) and rotifers (Fanestil and Barrows, 1965). The increased metabolic rate may shorten the lifespan by accelerating wear and tear. The lifespans of different animal species are inversely proportional to the basal metabolic rate (Sohal, 1976). Basal oxygen consumption rates of short-lived animals such as rats and mice are much higher than those of long-lived animals such as elephants and humans. Within the same species, however, it is difficult to correlate individual differences in lifespan with the metabolic rate.

AGE PIGMENTS

Accumulation of lipofuscin or age pigment is the most prominent age-associated change present in a variety of cell types from many organisms. It is predominantly deposited in nondividing cells such as neurons and cardiac myocytes (Figure 4–4) as a function of age (Strehler *et al.*, 1959; Brody and Vijayashanker, 1977; Miquel *et al.*, 1978). Lipofuscin accumulation has been reported in the cortex and hippocampus of humans (Friede, 1966), rhesus monkey (Brizzee *et al.*, 1974), and rat (Brizzee *et al.*, 1969; Brizzee and Ordy, 1979) as one of the common morphologic features associated with aging and has been correlated with the loss of neurons in old age. Lipofuscin is also deposited in dividing cells like liver (Essner and Novikoff, 1960), adrenal cortex (Szabo *et al.*, 1970), and testis (Miquel *et al.*, 1978). Its accumulation is also associated with the loss of cytoplasmic mass, mitrochondrial number, rough endoplasmic reticulum, and vacuolation of cytoplasm (Tonna, 1973). Indeed, lipofuscin accumulation may be a basic feature of cellular aging. The existence of a specific relationship between the rate of aging and lipofuscin accumulation was demonstrated in the housefly (Sohal and Donato, 1978). For example, the rate of lipofuscin deposition has been inversely correlated with the lifespan of many animals. The faster the rate of lipofuscin accumulation, the shorter will be the lifespan. The rate of lipofuscin deposition in the dog heart has been found to be approximately 5 times faster than in the human heart, a difference that roughly corresponds to the lifespan of these two species (Munnel and Getty, 1968).

The origin of age pigments is not clear, although it has been ascribed to morphologic and chemical causes. Lipofuscin would arise by a process of autophagocytosis involving lysosomes (Strehler, 1964; Samorajski *et al.*, 1965). A chemical hypothesis concerning the origin of lipofuscin suggests that it is an end-product of lipid peroxidation (Tappel, 1975). The cause for accumulation of age pigment remains to be explored. It is discussed in more detail in Chapter 5.

FREE-RADICAL THEORIES

The free-radical theory of aging postulates that free-radical reactions (modified by genetic and environmental factors) are involved in aging and age-related disorders (Harman, 1983, 1986). Free-radical reactions are ubiquitous in living organisms. These reactions arise upon exposure to ionizing radiations, from nonenzymatic and enzymatic reactions, particularly those of the energy-gaining processes such as reduc-

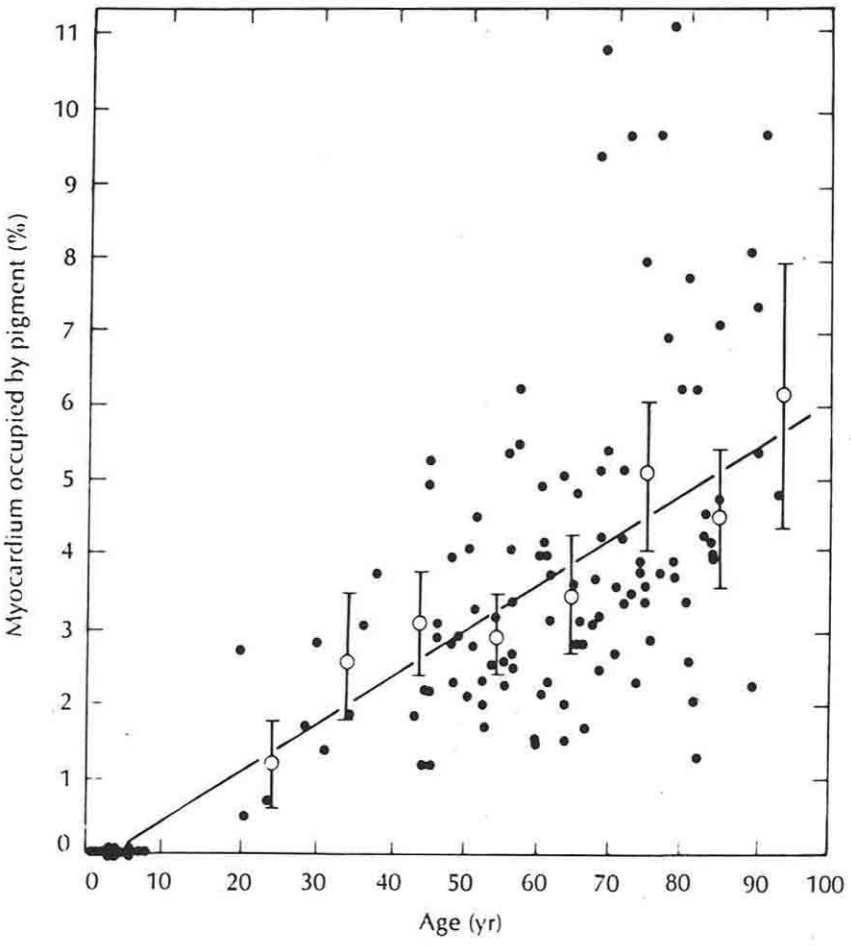

Figure 4–4. *Accumlation of lipofuscin as a function of age in human cardiac muscle.*

●, percentage of the myocardium occupied by pigment in individual cases; ○, means for 10-year periods; vertical bars represent standard errors of the means. Individual values are scattered but means represent a definite linear increase with age (From Strehler, B.L.; Mark, D.D.; Mildvan, A.S.; and Gee, M.V.: Rate and magnitude of age pigment accumulation in the human myocardium. *J. Gerontol.*, 14:430–39, 1959).

tion of O_2 to water (Harman, 1986). Free radicals are chemical compounds highly reactive owing to the presence of extra electrons in the outer orbit. These radicals include $O_2^{\cdot}$, $HO^{\cdot}$, $R^{\cdot}$ (any organic radical), $RO^{\cdot}$, and $RO_2^{\cdot}$ and participate in an interacting network of free-radical reactions going on continuously throughout the cells and tissues.

The deleterious effects of free-radical reactions can be repaired by the presence of antioxidants such as tocopherols (Harman, 1986), glutathione peroxidase (Flohe *et al.*, 1976), superoxide dismutases (Fridovich, 1977), elevated serum uric acid levels (Ames *et al.*, 1981), carotenes (Klebanoff, 1980), and DNA repair mechanisms (Hart *et al.*, 1979). Antioxidants that are known to inhibit free-radical reactions have been reported to prolong the lifespan of rotifers (Enesco and Verdone-Smith, 1980), *Turbatrix* (Epstein and Gershon, 1972), fruitflies (Miquel and Economos, 1979), and mice (Miquel and Economos, 1979). This theory predicts that overproduction of free radicals and/or reduction of their removal

causes cell and molecular damage and ultimately aging of the organisms (Figure 4–5). This theory is presented in more detail in Chapter 7.

CROSS-LINKING THEORY

Many of the biologic macromolecules develop cross-linkages or bonds between identical molecules or with different molecules with the passage of time. These linkages alter the physical and chemical properties of these molecules (Bjorksten, 1968). Major support for this theory was provided by the work of Verzar (1963) on the extracellular fibrous protein collagen. Collagen is synthesized in all types of cells and is deposited extracellularly in all tissues. The structural unit of collagen is tropocollagen. It is a long (300 nm) and thin (1.5 nm in diameter) protein that consists of three coiled polypeptide subunits called α chains. Each α chain contains 1050 amino acid residues. The three chains wind around each other in a right-handed triple helix, which is held together by hydrogen bonds. The helix is rich in glycine, proline, and hydroxyproline amino acids. In collagen fibers, tropocollagen molecules pack together side by side and are stabilized by chemical cross-links between the chains. The mode of packing creates periodic striations in the structure of collagen fibers. The number of striations in the collagen of rat tail tendon and its thermal stability increase whereas solubility decreases with age (Figure 4–6). The increased cross-links in collagen would make it more insoluble with aging (Verzar, 1964). Cross-linking agents are produced during normal metabolism as charged groups. Such ionized groups are replaced in early life by normal metabolic processes, but accumulate in increasingly larger amounts in old age (Bjorksten, 1977). These groups react irreversibly with biomolecules like DNA and proteins and inactivate them, possibly reducing physiologic competence with aging (Verzar, 1964; Kohn, 1978). Increased cross-linking of aged collagen has been correlated with an increased rigidity of the cell membrane, a probable cause of the decreased potassium conductance of the membrane (Nagy, 1978) (Chapter 23). The higher intracellular potassium content

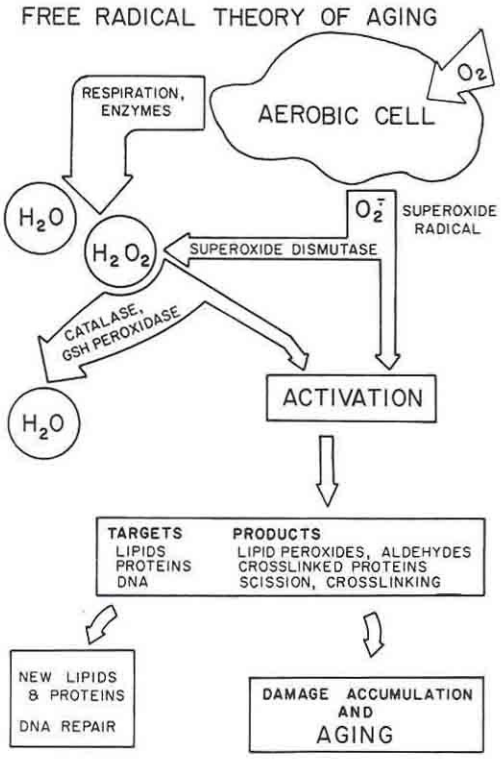

Figure 4–5. *Viewing this figure from top to bottom, oxygen, in the course of cell metabolism and via cell respiration and enzymes, gives rise to superoxide radicals and to peroxide molecules.*

Most free radicals are intercepted by three protective enzymes: superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH). The common by-product is water. Despite these protective mechanisms, products of free radical damage accumulate with time and such accumulation eventually results in impaired cell and organ function.

As shown in the lower center of this figure, lipids undergo peroxidation, proteins undergo crosslinking and DNA undergoes scission or crosslinking. All of these products impair normal cell function. Yet, at a young age and in the absence of disease or environmental noxious influences, repair enzymes are capable of containing this damage. In aging and under toxic conditions, damage is not repaired and may accumulate to compromise cell integrity, inducing aging and such diseases as cancer, atherosclerosis, and senile dementia. (Drawing by S. Oklund.)

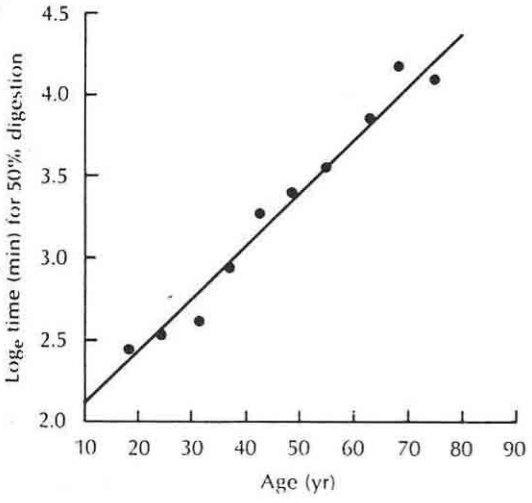

Figure 4–6. *Crosslinking of collagen.*

Collagenase digestion of human tendon collagen as a function of age. Straight line indicates increase in digestion time due to more crosslinks with age. (From Kohn, R.R.: *Principles of Mammalian Aging*, 2nd ed., Prentice-Hall, N.J., 1978.)

would in turn increase the intracellular ionic strength and lead to a decreased rate of transcription by chromatin and slowing down of protein synthesis (Nagy, 1978). Cross-links are present not only in extracellular collagen, but also in intracellular proteins (enzymes) as well as nucleic acids (DNA) (Bjorksten, 1977). The decrease in the extractability of chromosomal proteins from chromatin may be regarded as increased cross-linking with DNA (Hahn, 1970).

SYSTEM LEVEL THEORIES

Neuroendocrine Control Theory

According to Shock (1979), the overall performance of an animal is closely related to the effectiveness of a variety of control mechanisms that regulate the interplay between different organs and tissues. As discussed in Chapter 2, with aging, the effectiveness of homeostatic adjustments declines with consequent failure of adaptive mechanisms, and aging and death may be viewed as the result of this failure (Frolkis, 1982). Adaptation to stress, either external (from environmental stimuli) or internal (from emotional, hormonal, immunologic, and metabolic stimuli) depends on control mechanisms orchestrated by the nervous and endocrine system. The activity of several endocrine glands, thyroid, adrenals, gonads, is controlled directly by the pituitary gland and indirectly by the signals this endocrine gland receives from nervous centers, primarily the hypothalamus. For efficient adaptation, nervous and endocrine signals must be synchronized and responsive to the needs of the many functions they regulate (Timiras, 1978, 1980, 1983, 1985; Timiras *et al.*, 1982). However, with aging, some of the efficiency of the hypothalamopituitary signals is lost or altered (Everitt and Burgess, 1976), and this results in decreased function and increased pathology of most organs and tissue systems.

The neuroendocrine theory views aging as part of a lifespan program regulated by neural and hormonal signals (Figure 4–7). The program unfolds from fertilization through birth, childhood, adulthood, and finally, old age and death. According to this theory, command neurons in higher brain centers would act as "pacemakers" that regulate the "biologic clock" that governs development and aging. With the passage of time, aging changes may result from programmed deterioration or cessation of the programming that regulates homeostasis (Walker and Timiras, 1982). In either case, aging would be manifested through a slowing down or imbalance in the activity of the pacemaker neurons with consequent neurotransmitter and hormonal alterations and their repercussion on neural, muscular, and secretary functions. Such functional decrements are exemplified by involution of reproductive organs, loss of fertility, diminished muscular strength, lesser ability to recover from stress, and impairment of cardiovascular and respiratory activity.

Immunologic Theory

The immune system protects the individual from a variety of potentially harmful substances and organisms. Several organs, such as bone marrow, thymus, lymph

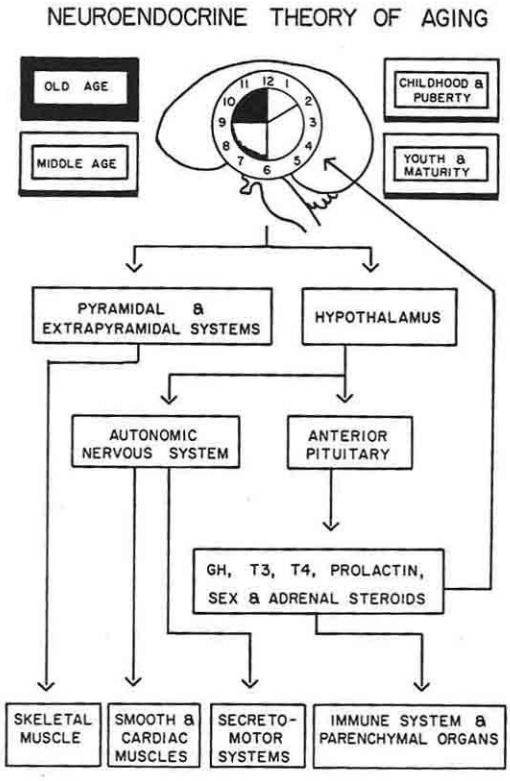

Figure 4–7. *The neuroendocrine theory views aging as part of a lifespan program regulated by neural and hormonal signals.*

The program unfolds, as shown at the top of the diagram, from fertilization through birth, childhood and puberty, youth and maturity, middle age and, finally, old age and death. "Command" neurons in higher brain centers would act as "pacemakers" that regulate the "biologic clock" governing development and aging.

As shown in the middle portion of the diagram, signals from these neurons would be relayed by neurotransmitters—acetylcholine, monamines and peptides—directly through the pyramidal and extrapyramidal systems to the skeletal muscles to regulate motor activity; or they would be relayed to the hypothalamus to stimulate autonomic and neuroendocrine neurons. Autonomic stimulation mediates the peripheral sympathetic and parasympathetic responses of the heart, smooth muscle, and exocrine glands. Hypothalamic hormones regulate the secretion of the anterior pituitary hormones and these, in turn, stimulate the peripheral endocrines to secret their respective hormones. As shown at the bottom of the diagram, several hormones—thyroid hormones, T4 and T3, adrenal and sex steroids, growth hormone (GH), and prolactin—act on specific receptors in target tissues and regulate metabolic, immunologic, reproductive, and adaptive functions necessary for the survival of the individual and the preservation of the species.

With time, aging changes may result from programmed deterioration or, alternatively, cessation of the programming that regulates homeostasis. In either case, aging would be manifested through a slowing down or imbalance in the activity of the pacemaker neurons with consequent neurotransmitter and hormonal alterations and their repercussion on neural, muscular, and secretory functions, e.g., involution of reproductive organs, loss of fertility, diminished muscular strength, lessened ability to recover from stress and impairment of secretory, cardiovascular, and respiratory activity. It is possible that programmed changes in the activity of genes are not confined to fertilization but may continue at late ages and are triggered by neural and hormonal influences. (Drawing by S. Oklund.)

nodes, and spleen, are involved in the production of immunologic responses directed toward the invading foreign (nonself) substances, called antigens. An antigen must either be a macromolecule or built up from macromolecules (i.e., viral particle). Most proteins and some polysaccharides and nucleic acids act as antigens. Two major types of cells are vital to an efficient immune response. One type is represented by the T-cell lymphocytes, matured in the thymus, and the other, the B-cell lymphocytes, stored in lymph nodes and spleen. T cells are responsible for cell-mediated immune responses that protect the body from pathogenic microorganisms, and also for rejecting foreign tissue grafts. The cytotoxic (killer) T cells destroy the antigen by direct attack on antigens. Subsets of T cells such as T helpers, T amplifiers, and T suppressors also influence immunologic cascade reactions by interacting with cytotoxic T cells and B lymphocytes. The B cells secrete antibodies (immunoglobulins) that bind to antigens and thereby help to destroy them. Competence of the immune system declines with aging (Figure 4–8). This decline has been attributed primarily to a reduced function of the thymus and T cells. The thymus begins to involute at adolescence and continues to atrophy throughout

the lifespan. The active thymic epithelial cells produce a specific protein, called thymosin, which promotes T-cell maturation. With thymic involution, thymosin level declines, and this, in turn, diminishes the T cells' ability to destroy foreign substances (Kay, 1979, 1984). The ability of B lymphocytes to produce specific antibodies against antigens depends on T-cell function, which declines with the thymic involution and T-cell impairment. For these many reasons it has been proposed that the thymus gland may be the "clock" for immunologic aging (Kay, 1979). The probable cause of thymic involution is still not known. Another consequence of thymic involution in aging is the loss of immune tolerance, i.e., the ability to distinguish self from nonself. The autoimmune theory of aging (Blumenthal and Berns, 1964; Walford, 1969) proposes that aging results from the production of antibodies that react with normal cells of the body and destroy them. An age-related increase in autoimmune antibodies has been reported (Walford, 1969, 1974). Thus, aging would be the consequence of reduced immunologic surveillance and of increased autoantibody production (for more information see Chapter 8).

Hormonal influences on the immune system have long been known; for example, the involutionary actions of glucocorticoid hormones on the thymus and lymphatic system have been exploited successfully for the treatment of allergies and as immunosuppressant in organ transplantation. In addition, nervous influences also have been shown to regulate some aspects of the immune response. Thus, neuroendocrine and immunologic theories of aging may converge, or alterations in their function may articulate with each other to lead to aging changes. A neuroendocrine-immunomodulation of thymic aging has been demonstrated in experiments in which tumor (GH3) pituitary adenoma cells, which secrete both growth hormone and prolactin, can reconstitute thymic structure and improve T-cell production and function when implanted in old rats (Kelley *et al.*, 1986). The possibility of thymic rehabilitation in old age suggests that lymphoid cells in aged animals are not inherently defective, but, given the proper stimulus, can return to normal function.

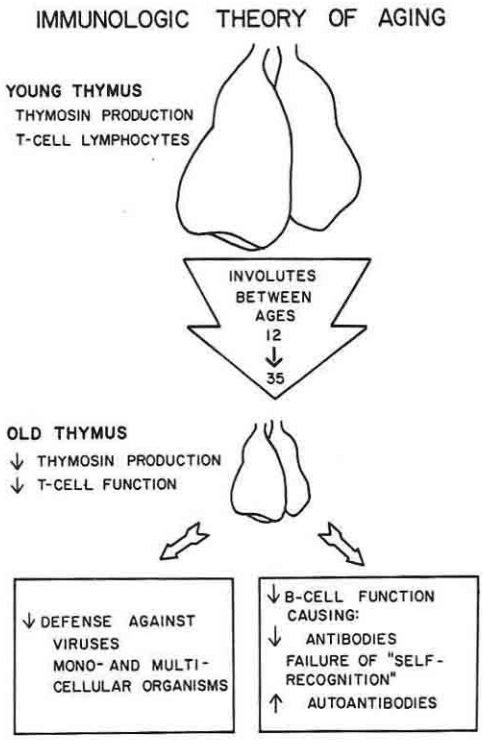

Figure 4–8. *Competence of the immune system declines with aging and immunologic involution may play an important role in the aging process and the induction of age-associated diseases.*

Decline in immunologic competence with aging has been attributed primarily to a reduced function of the thymus and T-cells. As shown in the middle portion of the diagram, the thymus begins to involute at adolescence and continues to atrophy throughout life; with thymic involution and declining thymosin production, T-cell activity and ability to fight foreign organisms diminishes. The ability of B-cell lymphocytes to produce specific antibodies against antigens depends also on T-cell function. This ability declines with thymic involution and T-cell impairment. Thus, as shown in the lower portion of the diagram, thymic insufficiency affects both T- and B-cell function and immunologic surveillance of foreign invaders is diminished. Another consequence of thymic involution with aging is the loss of immune tolerance, that is, the ability of distinguishing self from nonself. With aging, decreased tolerance and increased production of abnormal molecules induce the production of autoantibodies. These are directed against the individual's own tissues and cells, causing deterioration and destruction of the attacked organ, tissue, or cell, a condition that characterizes the so-called autoimmune diseases. (Drawings by S. Oklund.)

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