tend to concentrate awards below the senior academic levels. This is the case because the prevailing practice is to give first priority in the use of firm institutional funds to the payment of salaries to the most able senior faculty members. If the needs for firm funds for payment of salaries to outstanding persons progressing to senior positions expand more rapidly than the firm institutional funds available for salaries, the federal funds for career support will become progressively more important at the senior levels.

In terms of money, $4 million is available in the year that began 1 July 1961 for the Research Career Award Program. It is anticipated that this will finance about 275 awards.

As a long-range possibility, amalgamation of parts or all of this program with the new General Research Support Grant program will be considered. The General Research Support Grant provides broad aid for medical and related research, not support in the form of aid to specified projects or programs. The General Research Support Grant is a single grant to an institution, allowing it to meet those direct costs of research not covered by other forms of research support which are, in the judgment of the institution, most urgent. For these grants, $20 million will be available in calendar year 1962 to schools of medicine, dentistry, osteopathy, and public health. The grant will be increased and extended to other institutions engaged in medical and related research in subsequent years.

To view federal support for research in universities in perspective, the Research Career Award Program represents a shift towards emphasis upon the long-term support of highly qualified people for research and teaching, as constrasted with support of current research. The General Research Support Grant represents a trend, evident in the actions of a number of federal agencies and most explicitly in the institutional grant of the National Science Foundation, toward aid to research and education on a broad basis, detailed determinations being left to the institutions. Accordingly, the long-range relationships between the programs must be taken into account in considering the evolution of the grant programs of the National Institutes of Health.

Biochemistry of Aging

The mechanism of aging presents a challenge to modern biochemistry and biology.

F. Marott Sinex

The present development of biochemistry and biology suggests that the question, "Why do we get old?" may be answered in the foreseeable future. There are now several ways of investigating the mechanisms of aging in the laboratory, and new insights are bound to come from work in associated areas. I shall attempt to review in this article some trends in research on the biochemistry of aging.

Mortality data provide one approach to the aging problem. Gompertz (*1*) observed that a plot of the logarithm of the death rate in the surviving human population against age is a straight line after maturity. A similar relationship has been found in other captive populations, such as rodents and *Drosophila* (*2*). In human beings, the death rate doubles every 7 to 8.5 years (*3*). This implies that there is a 100-fold increase in the probability of death between ages 35 and 85 (*4*).

If separate physiological variables are measured, such as maximal breathing capacity, renal plasma flow (*5*), integration of complex mental skills, and speed of voluntary responses (*6*), there is a definite decrease with age, although this decrease seldom exceeds 30 to 50 percent (*4, 5, 7*).

When an explanation of this impaired function is sought in tissue pathology, a number of changes are observed. In certain areas of the brain there is a decrease in total numbers of viable cells, amounting in some areas to 25 to 30 percent, together with a decrease in the total amount of brain tissue—a decrease which may be of the order of 9 to 17 percent. At the same time, aberrations appear in the cytoplasm and nucleus of nerve cells (*6*).

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Decrease in strength may result from a decrease in the functioning mass of muscle as well as from an impairment in innervation. Evidence for the replacement of muscle fibers by connective and adipose tissue in older animals has been reviewed by Andrew (*8*), who attempts the difficult task of correlating what is known of the changes with age in skeletal, smooth, and cardiac muscle.

The age decrement in discrete renal functions can be attributed to a loss of functioning nephrons. The relationship between number of functioning units and functional capacity in kidney and other tissues is reviewed by Shock in the AAAS publication on aging (*7*).

In spite of the great current interest in hormones and the aging process, the exact relationships between endocrine function and aging is not well understood. Pincus (*9*) has reviewed much of the literature on this subject and attaches particular importance to the function of the pituitary.

Enzymes

To the biochemist the subject of enzymatic activity of aging tissue is of great interest. It is not always easy to distinguish between the amount of an enzyme present in a tissue and the activity of the enzyme. It is particularly difficult to measure the amount of inactivated enzyme which might be present in tissue as a consequence of

the passage of time. Very little is really known about the rate of replacement of enzyme in resting cells. Barrows, Yiengst, and Shock (*10*) have stressed the importance of expressing enzymatic activity on a per-cell basis, using deoxyribonucleic acid (DNA) content as an indicator of the number of cells in making comparisons between young and old animals in situations where cells are being replaced by elements of connective tissue.

In certain instances, changes in enzymatic activity can be attributed to a decrease in the number of intracellular elements. Barrows, Falzone, and Shock (*11*) reported that the decrease which they had observed in the succinoxidase of rat kidney was associated with a decrease in the number of mitochondria per cell. On the other hand, Weinbach and Garbus (*12*) found that hydroxybutyrate metabolism by liver and kidney mitochondria decreases with age. Of particular interest are the observations of Barrows (*13*) that the catheptic activity of the liver increases markedly with age. A phenomenon commonly associated with aging, the greying of hair, may be due to a loss of tyrosinase activity of the melanocytes of the hair bulb (*14*).

Within recent years there has been a better appreciation of the necessity of correlating morphological changes, both within the cell and in the total cell population, with observed levels of enzymatic activity.

Ionizing Radiation

Ionizing radiation produces many changes analogous to those observed during normal aging. It decreases life expectancy; there is a shifting of the Gompertz function on the time axis. That is to say, the death rate of animals in any particular age group is greater after radiation. Or, to put it another way, after radiation the observed death rate corresponds to the death rate in an older age group. The slope of the function does not change in animals exposed to a single dose of radiation. As a consequence of radiation injury there is a decrease in the number of viable cells in many tissues, and these cells are often replaced by elements of connective tissue. In many cases the pathological changes which occur resemble those found in normal aging. But this is not to say that the injury sustained from ionizing radiation is identical to aging; the reader interested in more exact comparisons is referred to the reviews by Upton (*15*), Strehler (*16*), and Handler (*17*).

Pigmentation

There is an increase in pigmentation of a number of tissues associated with aging. Some of these pigments are extracellular, some intracellular. Many exhibit blue or yellow fluorescence in ultraviolet light. The pigments of heart muscle (*18, 19*), neurons (*20*), atheromatous plaques (*21*), and elastin (*22*) have attracted particular attention. Histological terms such as *lipofuscin* and *ceroid* are used to describe these pigments as they are observed in tissue sections. Pearse (*23*) gives an excellent discussion of the ways in which liquid peroxides, lipofuscin, and ceroid are distinguished histochemically and morphologically. The most generally held view is that these pigments result from the auto-oxidation of lipid.

Harman (*24*) was among the first to implicate auto-oxidation of lipid and the interaction of auto-oxidized lipid with protein as a factor in aging. Auto-oxidation of lipid in vitro is characterized by an induction period in which oxidation is initiated and antioxidants are destroyed (*25*). Peroxides form, and systems of double bonds conjugate. Carbonyl compounds, particularly aldehydes, appear (*26*). Many of these products are capable of condensation and polymerization. The products of auto-oxidation are pigmented, fluorescent, tough, insoluble films that precipitate with many of the properties of ceroid (*27*) and lipofuscin (*18, 19*).

Attempts to isolate pigment from the tissues of senescent animals have been successful only in the case of the lipofuscin of heart muscle. The cytoplasmic granules of age pigment of heart muscle were isolated by Heidenreich and Seibert (*18*), using density-gradient techniques. Mildvan and Strehler (*28*) have reported that these intracellular granules may be identified with tissue particulates known as lysosomes. They found that lipid extracts of such granules chromatographed on silicic acid columns revealed a pale blue fluorescence in the cholesterol ester fraction and a yellow orange band in the cephalin fraction. Chromatography of peroxidized cephalin on paper gave a pattern similar to that obtained from the column fraction.

The yellow age pigment of nerve cells has never been isolated. Heyden and Lindstrom (*20*) have studied its spectra in tissue sections. Sulkin (*29*), who is currently investigating the nerve-cell pigment, feels that the pigment is lipoidal in origin and resembles ceroid. Duncan, Noll, and Morales (*30*) feel that the pigment arises in mitochondria.

There is extracellular pigmentation associated with aging in blood vessels. Atheromatous plaques are reported to contain ceroid (*21*) and lipid peroxides (*31*). Preparations of acid-solubilized elastin from older animals appear more yellow. This yellow fluorescent pigmentation associated with elastin is also found in ligumentum-nuchae elastin. Partial hydrolyzates of elastin prepared with either elastase or dilute acid are yellow and fluorescent. Fluorescent pigments can be prepared from both partial and complete hydrolyzates (*32, 33*). Loomeijer (*33*) believes that lipid-soluble pigments derived from elastase hydrolyzates are derived from auto-oxidized lipid. Work in our own laboratory, as yet unpublished, also causes us to believe that the water-soluble pigments of both elastase and acid hydrolyzates are derived from auto-oxidized lipid.

The degree of functional impairment from the accumulation of such pigment is difficult to evaluate. Strehler, Mark, Mildvan, and Gee (*19*) find that lipofuscin can account for 3 percent or more of the wet weight of cardiac muscle. Nishida and Kummerow (*34*) report that linoleic peroxide interacts with beta-lipoprotein in such a way as to alter its electrophoretic pattern, and they suggest that lipid peroxides may play a role in the accumulation of lipid in intima. Interpretation of the relationship between auto-oxidation and aging is complicated by the observation that accumulation of age-associated pigments in neurons is accelerated by deficiency in vitamin E, administration of acetanilid, and stress (*29*).

Connective Tissue

Interest in the role of connective tissue in aging arose from the fact that unquestionably there are differences between the connective tissue of young

and of old animals (*35*). Both the amount and the character of connective tissue may change. In some tissues, the disappearance of cells is accompanied by replacement of the cells by elements of connective tissue.

Changes in connective tissue can arise from a variety of causes, including alterations in endocrine function (*36*) that stem from changes in the types of cells represented in the total population, or from chemical changes within the extracellular phase. Gross (*37*) has suggested that chemical changes similar to the extracellular changes occur within cells during aging, for which the aggregation of collagen might serve as a model.

In connective tissue there are changes in the mucopolysaccharides present. Davidson, Woodhall, and Baxley (*38*) report a gradual accumulation of keratosulfate with age in cartilage, nucleus pulposus, and other tissues.

With advancing age collagen becomes tougher, more crystalline, and more difficult to dissolve. Elastin in human blood vessels appears less elastic and fragments with age. This fragmentation is associated with calcification and pigmentation (*39*).

In the ground substance there may be an increase in density and aggregation. The significance of such changes is difficult to evaluate, but they may influence the nutrition of cells. Gersh and Catchpole (*40*) postulate that all interchanges between ground substance and epithelium must occur through two basement membranes, consisting of aggregated ground substance, the permeability of which probably decreases with age. However, if a dispersed colloid aggregates into a more aggregated and a more aqueous phase, diffusion through the aqueous phase may increase. In one of the few attempts that have been made to measure diffusion in young and old tissue, Kirk and Laursen (*41*) actually found increased diffusion coefficients for nitrogen, oxygen, carbon dioxide, lactate iodide, and glucose in intima and media of older subjects. In some instances aggregation may decrease in senescence. Banfield and Brindley (*42*) report that the extractability of abdominal skin collagen in 0.1-percent acetic acid increased in subjects between 40 and 80 years of age.

The question of decreased vascularization and arteriocapillary fibrosis of aging tissue is another aspect of the problem of diffusion of essential nutrient. Changes occur with age in the reserve supply of blood and in the distribution of blood to tissue. The diminution in cardiac output, with age, of approximately 1 percent a year (*15–17*) is in part a reflection of increased peripheral resistance. It is important that all the factors responsible for this increased resistance be recognized, and that morphological changes in the barriers between capillaries and cells be analyzed both in terms of physical chemical changes in mucopolysaccharide, collagen, and elastin and in terms of the properties of the living cells of the vessels. The question of the vascularity of aging tissue is reviewed by Landowne and Stanley (*43*), and by Handler in the recent AAAS symposium on aging (*17*).

Lability of Macromolecules

It is possible that aging results from chemical changes in irreplaceable macromolecules (*44*). Altered molecules may accumulate in postmitotic cells and in elements of connective tissue with limited rates of replacement.

The time-dependent chemical changes postulated may be of a variety of types, and may include thermal denaturation involving unfolding of tertiary structures (*45*), hydrolysis of amide and peptide bonds (*46*), and oxidation (*47*). Among the proteins which might not survive a lifetime of incubation at 38°C is the extracellular protein elastin. Since it is less crystalline than collagen, it does not have the added protection of extensive hydrogen bonding to protect it against thermal denaturation and other deleterious chemical changes.

Aging and the Gene

There is evidence to support the belief that many of the changes which accompany aging occur in the nucleus. Such evidence includes the observation of abnormal nuclei and abnormal cell division in senescent animals (*8*), as well as the difficulty which adult tissue has in initiating the first mitotic events in tissue culture or after stimulation.

Adherents of the theory that aging is centered in the nucleus generally believe either that aging is an extension of normal differentiation or that it is due to accidental genetic noise.

The first group points out that, while we as individuals may view aging as a catastrophe, it probably serves a useful evolutionary purpose in insuring succession of generations. Insect physiologists and plant physiologists are particularly likely to hold this view. Many insects, in the adult form, have a relatively short life expectancy and may even be born without mouth parts. In such insects differentiation produces a phenotype with a limited life expectancy. The death of an annual plant often appears to be the final step in an orderly development. One may therefore argue that aging is a deliberate event, consisting of differentiation to a point where the interdependence of tissue and cells is incompatible with the indefinite life of the total organism. The deaths of individuals, could however, contribute to the survival of the species by insuring a progression of generations and reducing competition for the food supply between young and old. Dobzhansky has ably presented aging as an adaption of evolution (*48*).

A second group holds the view that aging arises from genetic noise or random somatic mutations. Henshaw (*49*), Failla (*50*), Szilard (*51*), and Strehler (*16*) have all discussed theories of aging based on somatic mutation. These are reviewed by Glass (*52*) in the recent AAAS publication on aging.

The rate constant for somatic mutations viewed as chemical reactions would be very small, possibly of the order of 10^{-13}. If genetic material had the thermal stability of purified DNA (*53*), there would be little probability of thermal mutation, because of the great stability of the hydrogen-bonded DNA helix. On the other hand, in certain cells, genes may be considerably less stable than purified DNA. The rate constant for thermal mutation of *Escherichia coli* and *Bacillus subtilis* is of the order of 1×10^{-6} at temperatures between 55° and 60°C (*54*). Human genes of this order of stability might undergo considerable spontaneous somatic change at 38°C. Such deductions, however, must remain speculative until they can be made to rest on firmer experimental evidence. It will be difficult to demonstrate that random somatic mutations do occur in aging tissue, particularly if such mutations are truly random. However, an effort should be made to ascertain whether clones of cells from aging individuals have altered biochemical properties. The greying of hair might be an ex-

ample of a somatic mutation in aging melanocytes (*14*).

A particular aspect of genetic interest concerns the instructive theory of antibody formation. Is there an impairment in self-recognition in aging animals due to alteration in either antigen or antibody?

Somatic theories of aging have appeal to those who feel that ionizing radiation also produces somatic mutations, for such theories would explain the similarity between aging and radiation injury.

Free Radicals

The similarity between certain aspects of radiation injury and aging may reflect common physiological and cellular impairments or, as suggested by Harman (*55*), may be due to analogous chemical events, such as free-radical reactions (*56*), occurring in both radiation-induced and normal aging. Concepts based on the chemistry of the free radicals produced by ionizing radiation have proved very helpful in explaining the biological effects of such radiation (*57*).

There is a growing body of evidence, based on findings of an accumulation of pigment believed to arise from auto-oxidized lipid, that auto-oxidation occurs in senescent tissues. It is thought that auto-oxidation proceeds by a free-radical mechanism, with formation of peroxides and of both carbon and oxygen radicals. More attention should be given to the substances which might initiate such reactions in tissue, such as trace metals, hematin, hydrogen peroxide, or oxygen itself. Free-radical hypotheses have the attractive feature of suggesting that preventive therapy with specific antioxidants is a possibility.

Summary

It should be apparent that while no one really understands all the fundamental mechanisms underlying the aging process, progress is being made, and theories are being advanced which may be tested in the laboratory.

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