Aging Research (I): Cellular Theories of Senescence

Age carries all things, even the mind, away. [VIRGIL, *Eclogues IX*, line 51]

Remarkably little has changed since Virgil wrote these words almost 2000 years ago. The average human life expectancy has indeed increased, largely because the infectious diseases that once killed so many people have themselves succumbed to improved sanitation, vaccination, and antibiotics. But the maximum life-span has not increased significantly, and the physical and mental deterioration that afflicted the aged in Virgil's time afflict them in ours.

Identification of the cause or causes of that deterioration is the goal of research on the biology of aging. While a unifying hypothesis that explains the aging process has not yet emerged (in fact, a one researcher–one theory portrayal of aging research would be a more accurate approximation of the current situation), it is clear that there is a trend away from simple description of changes in the physical and mental characteristics of the aged to an effort to identify the cellular and molecular mechanisms that underlie those changes. Investigators hope that identifying the cause or causes of aging may permit not just an extension of the maximum human life-span, but also—and more importantly—prevention of the declining vigor that accompanies increasing years.

For a long time, conventional wisdom held that aging was a property of the whole, complex organism, and that individual cells, if properly cultured, would be immortal; that is, there would be no limit to the number of times they could divide. A series of experiments begun in the early 1960's by Leonard Hayflick, now at Stanford University, Stanford, California, and his colleagues, has invalidated this notion in the views of many investigators.

Hayflick found that cultured human fibroblasts double only a limited number of times before they deteriorate, become senescent, lose their capacity to divide, and finally die. The number of cell doublings is roughly related to the age of the cell donors and to the longevity of the species. Fibroblasts from human embryos divide about 50 times; those taken from persons after birth divide 20 to 30 times. Work that has been done in Hayflick's laboratory and in those of several other investigators indicates that, in general, the longer the life-span of the species the greater the number of times its cells will divide in culture.

Experiments on cultured cells may be criticized on the grounds that the conditions are unphysiological and that the "right" conditions would permit unlimited proliferation; however, when Hayflick cultured young and old cells (distinguishable by chromosome markers) in the same flasks, the old cells died out and the young cells divided the expected number of times.

Some cells do have the capacity to divide indefinitely in culture. They are cancer cells. Thus, a better understanding of why normal cells have a finite lifetime may contribute to the solution of the problem of what causes cancer.

In vivo Experiments

Cells transplanted in vivo also have a limited life-span. Charles Daniel and his colleagues at the University of California in Santa Cruz used mouse mammary epithelial cells that they transplanted serially into the mammary fat pads of female mice. The recipients had the same genetic makeup as the donors, and the fat pad is the site where these cells normally grow. The investigators found that, even in this highly physiological situation, the cells showed a characteristic decline in proliferative capacity with repeated transplantation.

These observations raise two questions. The first concerns whether they are related to aging in vivo. Hayflick thinks that they are in fact an expression of human aging at the cellular level, as do a large number of other investigators using cultured cells to study aging. He does not think that people age because some of their cell types lose the capacity to divide. Rather, Hayflick attributes senescence to losses of cell function that occur before cells reach their maximum division limit. As cells malfunction, organs or whole systems suffer adverse effects, and eventually the individual dies.

The second question is a critical one. What causes cells to become senescent and lose their capacity to divide? It is here that the one researcher–one theory approach to aging research enters the picture. Most investigators think that an organism's genes determine, at least partially, how long it will live. Different species, after all, have characteristic and inheritable life-spans. So some investigators think that senescence is part of the cell's genetic program, just as are other changes during development. Their theories could be considered intrinsic or programmed theories of aging. Nevertheless, a role for extrinsic or environmental forces, including those that damage the cell's DNA, cannot be ruled out. An accumulation of environmental insults, however small, may result in death. Some theories have both intrinsic and extrinsic components. The relative contributions of heredity and environment to sensecence remain a central issue of aging research.

A number of investigators, such as F. Marott Sinex of Boston University School of Medicine in Boston, Massachusetts, think that mutations may cause aging. If the damage to DNA is too subtle for the DNA repair system to detect, or if it accumulates faster than the repair system functions, the cell will gradually become defective in essential control systems or enzymes. This situation would be particularly serious for cells that do not divide after they have differentiated to their mature forms. These include brain and muscle cells. If they function poorly or die, they are not replaced. It might be less serious for dividing cells such as those of the liver or the lining of the gastrointestinal track.

Free radicals are highly reactive entities that can damage both DNA and other cell structures. They may act either directly or indirectly by generating strong oxidizing agents. Free radicals may either be produced by cells as a result of their metabolic processes or they may come from the environment.

Support for the involvement of free radicals in aging has recently come from Lester Packer of the University of California, Berkeley, and James R. Smith of the Veterans Administration Hospital in Martinez, California. They added vitamin E to cultured WI-38 cells. These cells, which they obtained from Hayflick, are the same human embryonic cells that normally have an in vitro life-span encompassing only about 50 divisions. But in the presence of vitamin E, an antioxidant that can interfere with reactions mediated by free radicals, the cells continued to

divide and to have youthful characteristics for about 120 population doublings; after that they too became senescent and died out. Packer and Smith estimate that the concentration of the vitamin in the enriched culture medium is approximately the same as that in serum in vivo.

Packer said that these results do not necessarily conflict with Hayflick's hypothesis that the cells have a built-in "biological clock" that determines the number of population doublings. He thinks that they may have such a programmed potential but that it is not always attained. Addition of antioxidants to the environment may allow the cells to reach their full potential for dividing and thus achieve an apparently lengthened life-span.

If mutations in somatic cells are involved in senescence, then the efficiency of DNA repair mechanisms may help to determine susceptibility to aging. There is evidence that the efficacy of DNA repair correlates with longevity. Ronald Hart of Ohio State University in Columbus and Richard Setlow of Oak Ridge (Tennessee) National Laboratory measured the extent of DNA repair in cultured fibroblasts from seven species. The cells were first exposed to ultraviolet radiation, a known inducer of damage to DNA. Human, elephant, and bovine fibroblasts were almost five times more active in repairing their DNA than were fibroblasts from rats, mice, and shrews. The repair system of hamster cells had an intermediate efficiency even though the life-span of the hamster is similar to that of the rat.

Zhores Medvedev, now at the National Institute for Medical Research in London, England, has suggested that redundancy in genetic information caused by repetition of genes may be an intrinsic mechanism that determines the potential life-span of species. Repetitive genes are known to occur, especially in the cells of higher (eukaryotic) organisms. According to this theory, as mutations and, thus, errors accumulate in functioning genes, reserve sequences containing the same information take over until the redundancy in the system is exhausted and senescence results. The differences in species' life-spans would then be a reflection of the degree of gene repetition. Mutations in genes that are both unique and essential for cell function or maintenanace would be especially harmful.

Loss of genetic material would be another way of exhausting the redundancy. Bernard Strehler of the University of Southern California in Los Angeles has found that certain repetitive gene sequences—those coding for ribosomal RNA (rRNA)—may be lost by the aging animal. He observed that DNA extracted from the brains of 10-year-old beagles bound 30 percent less labeled rRNA than did DNA from young animals. This indicates that the former DNA contained fewer sequences complementary to the RNA than did the latter.

Strehler is among those who think that the time patterns of senescent deterioration are ultimately genetically determined. His rather complex theory places the proximal events, however, at the level of translation or protein synthesis. The theory is based on the well-known degeneracy of the genetic code in which almost all of the amino acids have more than one code word.

There is evidence that only some of the code words are used at any given time in the cell's lifetime. Thus, for protein synthesis to occur, the synthetic machinery corresponding to the current code words must also be available. All of this is genetically controlled so that during the course of development, including aging, the genes for the protein-synthesizing machinery are switched on and off in proper sequence. As they are turned off, the production

Aging Institute: Current Status

The recent establishment of the National Institute on Aging (NIA) within the National Institutes of Health (NIH) should give increased impetus to all facets of aging research. Congress passed the Research on Aging Act of 1974 authorizing the creation of NIA in May 1974, and President Nixon signed the bill into law on 31 May 1974.

The new institute is to conduct and support "biomedical, social, and behavioral research and training related to the aging process and the diseases and other special problems and needs of the aged." The act requires NIA to coordinate its efforts with those of existing agencies that deal with the aged.

Since May, officials at NIH have been taking the legal and administrative steps involved in setting up a new institute. The NIA was officially established on 7 October with Norman Kretchmer, director of the National Institute of Child Health and Human Development (NICHHD), serving as acting director. The Gerontology Research Center in Baltimore, Maryland, and the Adult Development and Aging Branch, both formerly in NICHHD, will constitute the nucleus of the new institute.

The federal budget for fiscal year 1975 was established before NIA was created. Initial funding for NIA must therefore come from the $14.4 million allocated for aging research at NICHHD. The budget for fiscal year 1976 is now in the planning stages.

Substantive questions of the scientific scope and content of the NIA program will be the province of the director and the National Advisory Council on Aging. The NIA director has not yet been selected but a search committee, chaired by Ronald Lamont-Havers, deputy director of NIH, has narrowed the list of candidates to about six. The committee expects to submit their recommendation to the director of NIH for approval before the first of the year. Officials at NIH submitted a list of 24 nominees for the 12 positions on the Advisory Council to the Department of Health, Education, and Welfare in September. The department has not yet acted on the nominations.

One of the major jobs that the new director and council must tackle is the development of a comprehensive plan designed to coordinate and promote research into all aspects of aging—from basic biology to the economics of growing old. The Research on Aging Act specifies that this plan be completed and submitted to Congress and the President by 31 May 1975, but no one really thinks that this massive undertaking can be finished by then. A "plan for the plan" appears to be the current goal.

—J.L.M.

of all products specified in messages using the corresponding code words stops and the products eventually deteriorate. According to Strehler, aging occurs as a result of such programmed loss of necessary cell constituents.

Not all theories of aging involve DNA, either as the source of programmed aging or as the target of somatic mutations. One of these is the "error catastrophe" theory suggested by Leslie Orgel of the Salk Institute in La Jolla, California. According to his hypothesis, an accumulation of errors in amino acid sequence in proteins, especially errors that affect the specificity of enzymes needed for protein synthesis, will result in further mistakes in protein synthesis and, consequently, will lead to cell deterioration and death.

The mistakes could occur during transcription, that is, during synthesis of the messenger RNA that specifies the amino acid sequences of proteins. Alternatively, they could happen during protein synthesis. At the level of practical experimentation, distinguishing between errors in proteins caused by mutation and those arising during transcription or translation may be difficult, if not impossible.

There is evidence that errors in proteins, however they originate, may be involved in aging. Robin Holliday and his colleagues at the National Institute for Medical Research in London, shortened the life-span of adult fruit flies by feeding a variety of amino acid analogs to the larvae. Amino acid analogs such as *p*-fluorophenylalanine and ethionine are incorporated into proteins in place of the normal amino acids (phenylalanine and methionine, respectively) but interfere with the functions of the proteins.

Holliday has also found abnormal forms of enzymes in aging human fibroblasts. Up to 25 percent of two enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate, had increased sensitivity to heat when isolated from old cells whereas the enzymes from young fibroblasts were completely normal.

Other investigators have observed decreases in the specific activities (activity per unit of enzyme protein) in enzymes isolated from aged organisms. David Gershon and Harriet Gershon of the Technion-Israel Institute of Technology in Haifa found such a decrease in the specific activity of isocitrate lyase from nematodes (roundworms). They attributed it to the presence of inactive enzyme molecules in addition to the active ones in old nematodes.

Morris Rothstein of the State University of New York at Buffalo confirmed this observation but his interpretation of it differs from that of the Gershons. Rothstein thinks that the enzyme isolated from old nematodes consists of partially active molecules rather than a mixture of totally active and totally inactive ones. He expected that completely inactive enzyme molecules would not be capable of binding to the substrate but, in binding experiments, he did not detect any unbound fraction of old enzyme, and thus concluded that all the enzyme molecules were partially active. The mechanism by which enzyme from aged nematodes becomes partially inactivated is unknown, but Rothstein said that early experiments have indicated no change in amino acid composition.

Evidence against Error Catastrophe

There is also evidence against error catastrophe as a basis for aging. A number of investigators, including John Holland of the University of California at San Diego and Hayflick, have compared virus production in young and old cultured cells and observed no differences. Since viruses use the cells' own machinery for synthesizing proteins, this implies that the accuracy of that machinery is maintained in old cells.

Amino acid analogs do not appear to impair the proliferative capacity of cultured WI-38 cells. According to Vincent Cristofalo of the Wistar Institute in Philadelphia, Pennsylvania, nontoxic concentrations of *p*-fluorophenylalanine and ethionine had no effect on the number of cell doublings even though the analogs were incorporated into cell proteins. Higher concentrations did inhibit proliferation, but if the cells were placed in fresh, analog-free medium, they recovered and achieved essentially the same number of population doublings as the controls. Cristofalo concludes that cells with errors serious enough to compromise their survival are overgrown by healthy cells so that the overall life-span of the population is not affected. Errors in cells that do not proliferate may still be a cause of aging.

Cristofalo and others have compared the concentrations of a number of cell constituents in old and young cells. No generalizations can be made about the results because some constituents increased, some decreased, and others did not change in concentration. Cristofalo did observe increases in two enzymes in WI-38 cells that he thinks may be related to the aging process. The enzymes, acid phosphatase and β-glucuronidase, are found in lysosomes and are considered markers for the presence of these organelles. Lysosomes are small sacs that contain a variety of hydrolytic enzymes. They are thought to be involved in cell destruction occurring both in the course of normal development and in certain disease processes. Electron microscopy has confirmed an increase in the number of lysosomes with aging.

Because hydrocortisone is thought to stabilize lysosomal membranes, Cristofalo added it to the cultured cells. It increased the life-span of WI-38 cells by approximately 40 percent, but he thinks that the explanation of this phenomenon is more complicated than simple lysosomal stabilization. Cristofalo is a proponent of a programmed senescence. He has found that the percentage of cells unable to proliferate increases with the number of population doublings. He hypothesizes that, with each division, a certain proportion of the daughter cells undergoes a type of terminal differentiation and loses its capacity to divide. If this happens because the nondividing cells can no longer synthesize a protein necessary for division (loss of this capacity would be genetically programmed), hydrocortisone might reverse the process by inducing synthesis of the protein. Glucocorticoids such as hydrocortisone can induce synthesis of some proteins in cultured cells.

Although identifying a single underlying cause of senescence would be beneficial from the viewpoint of intervening in—and halting—the aging process, the possibility exists that several mechanisms are at work in the same cell type or that different cells age for different reasons. The current crop of theories on cellular aging is an abundant one, but most are now testable, and investigators think that the mechanism of aging will be elucidated.

A second article in this series will consider some recent findings in immunology, endocrinology, and neurobiology that are related to aging. A higher level of biological organization is involved in these studies than in the ones described here, but they share a common attempt to trace observed changes to their cellular origin.

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