Cellular Aging—Postreplicative Cells

A Review (Part II)

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THERE IS WIDESPREAD BELIEF that the alterations which are crucial in limiting the life-spans of mammals are those which involve cells no longer capable of mitotic replication and hence no longer capable of compensating—via increased numbers of progeny—for deficiencies in cell numbers, structure, or function. While this is intuitively appealing, I know of no compelling evidence that the phenomenon of clonal senescence, discussed in Part I of this review, should not turn out to be of at least equivalent importance to the pathobiology of aging. Clearly, a great deal more research is required on both subjects.

Since it is manifestly impossible, in this comparatively brief review, to critically discuss the major observations on age-associated changes in all or even most postreplicative cell types, I have chosen to confine myself (except for minor digressions) to three such cell types: a) oocytes, b) neurons, and c) myocardial cells.

Oocytes are of special interest because of the availability of the ultimate bioassay for functional defects—namely, the effects of age on the viability and phenotype of their zygotic progeny. Aging of this cell type is also of great evolutionary consequence, since in some mammalian species, including man and at least one inbred strain of mice (CBA),[1] oocytes are depleted long before the somatic death of the individual and therefore are among those factors which limit the reproductive performance of the population. Finally, there is the important possibility, in view of the enormous attrition of oocytes during growth, development, and senescence, that a vital genetic selectional process may be at work that involves one or a whole variety of quality control and variably successful repair mechanisms; some of these may be highly specialized and some (such as various modalities of DNA repair) may be comparable to those which take place in somatic cells. In this connection, it is of great interest that in ataxia telangiectasia, now considered to result from a hereditary defect in a γ-ray–responsive endonuclease,[2] there could be a profound and pre-

Supported in part by Grants AM-04826, AG-00257, and GM-15253 from the National Institutes of Health.

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mature atresia of oocytes, although the conventional interpretation is that of agenesis.[3]

The reasons for the interest in neurons should be obvious. They are classic postreplicative cells, and their functional decline is of the greatest clinical importance. Moreover, there seems to be widespread belief that, if there exist special "pacemaker" cells which determine the onset and rate of senescence, they are likely to be central nervous system neurons.[4]

Myocardium rather than skeletal muscle was chosen (despite the much larger body of literature on the latter), because, it seems to me, one is less likely to be misled by changes associated with atrophy of disuse. (In this respect, I am surprised at how rarely diaphragmatic skeletal muscle has been chosen as a model system.)

Many excellent gerontologic studies have been carried out with mammalian hepatic parenchymal cells and erythrocytes. However, in the former, there is some degree of cell turnover and in the latter, one already knows what the primary lesion is and that it is quite a special case (namely the loss of the entire nucleus). Furthermore, there are ample supplies of erythropoietic stem cells, so that aging of that postreplicative cell type is not likely to be of clinical significance. This is not to say that important phenomena could not be uncovered by such research. For example, the apparently selective attachment of IgG to the surfaces of senescent red cells (possibly to exposed glycoprotein receptors) as a marker for phagocytosis by isologous macrophages [5] might serve as a model system for the study of a variety of cell surface alterations in other postreplicative cells.

The three cell types which have been chosen for more detailed consideration are obviously widely diverse expressions of cellular differentiation, and it would be surprising if they shared a single common mechanism or a few communal mechanisms as the basis for senescent alterations, especially in view of our conclusion that the senescent phenotype in mammals is under polygenic controls.[6] Nevertheless, gerontologists should be alert to any data that may lead to unifying generalizations at the cellular and molecular levels. As we shall see, there is just not enough information available to permit even tentative answers to that question, nor is such information likely to be available in the near future. The basic dilemma is that it is extremely difficult to sort out what might be primary, rate-determining effects closely coupled to the host's genotype from a wide array of secondary, tertiary, and more remote pleiotropic effects. It seems probable that only a genetic analysis will permit one to identify such primary phenomena with any degree of confidence. Recently, some remarkable developments in mammalian genetics have permitted, *in principle*, an experimental approach to such problems. We shall review certain

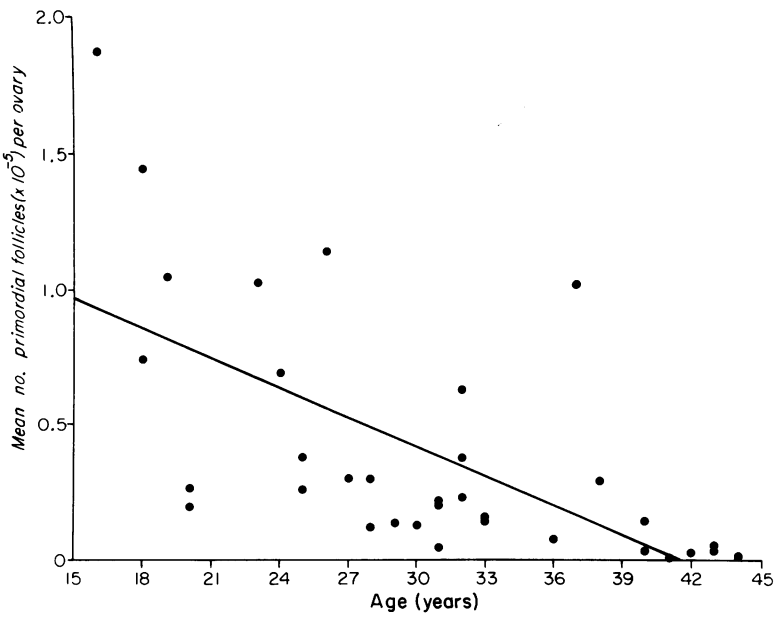
of these developments, pointing out their great potential not only for gerontologic research but also for all of experimental pathology.

Oocytes

In mammals, oogonial proliferation ceases at about the time of birth, or in most cases (including the human), mitotic amplification of oogonia has already been completed within the fetal ovary considerably before birth.[7] Therefore, there are no residual stem cell pools to provide a continuing supply of oocytes in the adult and it it reasonable to consider that cell type as postreplicative. The terms *postmitotic* or *terminally differentiated* would not be entirely appropriate, as a small proportion of the oocytes are destined to undergo further maturation into gametes via meiosis, which includes a division (meiosis II) very much like mitosis except that there is no semiconservative DNA synthesis between meiosis I and II. The vast majority of oocytes (and oogonia) undergo atresia. Those that escape are arrested, for periods of up to 50 years, in a late stage (diplotene) of the first meiotic prophase. Semiconservative DNA synthesis should have already occurred before the earliest stage of prophase. Synaptonemal complexes will have already been well developed at pachytene, the prior stage of meiotic I prophase. Judging from fine structure studies on lower eukaryotes,[8] at the transition from pachytene to diplotene, portions of the synaptonemal complex are shed from the bivalents. The residual short regions of retained (or transformed) synaptonal complexes, the *chiasmata*, hold the repulsing homologous chromosomes together. The importance of chiasmata is that they are almost certainly related to the process of crossing over, one of the two primary mechanisms of genetic recombination which characterizes meiosis, the two resulting in the incredible array of genetic heterogeneity observed within randomly bred populations.

It seems likely that during the prolonged arrested diplotene of prophase I, the chromosomes are in a lampbrush configuration [9] entirely analogous to that which has been extensively studied in amphibian oocytes.[10,11] In primordial follicles (oocytes with a single layer of surrounding follicular cells), they appear to be extremely active in RNA synthesis, including ribosomal RNA synthesis, at least judging from uridine incorporation studies.[12] There seem to be species differences in the size of the lampbrush loops and in the thickness of the RNA sheaths around them. In rats and mice, the appearance is such as to warrant a special terminology for this arrested stage of diplotene (called the *dictyate* or *dictyotene stage*). There seems to be less evidence for such a special category of diplotene for the human species.[13,14]

Kohn has expressed the opinion [15] that since the rate of oocyte atresia is probably greater earlier in life, the death of oocytes cannot be considered as an intrinsic aging process. This seems to me to be an unnecessarily restrictive definition of *cellular* aging (as opposed to aging of populations) in view of our ignorance of underlying mechanisms and their varying kinetics; the latter could be modulated in different ways in different cell types as a result of complex interactions with the cellular and extracellular environment. In the case of oocyte atresia, there appear to be three or four temporally, quantitatively, and probably cytologically distinct waves of germ cell atresia,[13,16] only one of which involves oocytes in the diplotene stage of arrested prophase I. It seems likely that there are different mechanisms underlying these separate and discrete patterns of cell degeneration. The degeneration of diplotene-arrested oocytes continues beyond sexual maturity. Although every pathologist is aware that comparatively few primordial follicles can be detected in postmenopausal ovaries, I can only find a single methodologically valid study in the literature which attempts to quantitate this decline beyond puberty.[17,18] Text-figure 1 is derived from Block's data,[18] beginning with his 16-year-old patient, which gave figures of 192,000 primordial follicles in the left ovary and 182,000



TEXT-FIGURE 1—The average number of primordial follicles per ovary for each of 34 women plotted as a function of donor age. The data is taken from Table 1 of Block; a calculated linear regression line has been fitted to the data by the method of least squares; it gives a correlation coefficient of −0.64.

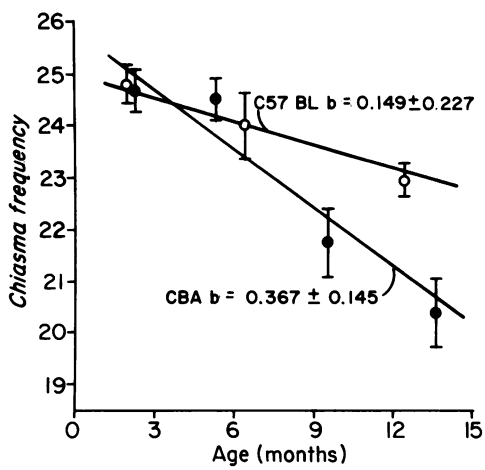
primordial follicles in the right ovary, and ending with the specimen from his oldest patient (age 44), which yielded 310 in the left and 40 in the right ovary. On the linear regression model depicted in Text-figure 1 (which may not be the optimum model), essentially all oocytes should be depleted by the middle of the fifth decade. This conclusion is strengthened by the fact that Block did not differentiate between atretic and nonatretic follicles. Judging from the work of Baker [13] with younger specimens, it is probable that substantially more than half were atretic. Thus, one vital cause of reproductive failure in the human female is the loss of essentially all of her stock of potential gametes. In view of the fundamental significance of such cellular degeneration, I am astonished at the virtual complete absence of cytopathologic research on this subject. Virtually all that exists in the literature are brief descriptions, at the light microscopic level, of vacuolization, chromatin alterations, eosinophilia, and pyknosis.[16,13,19] We know that these oocytes have mitochondria, lysosomes, endoplasmic reticulum, ribosomes, nuclear and plasma cell membranes, lampbrush chromosomes, annulate lamellae, etc.,[20] yet we know virtually nothing about the nature and sequence of fine structural changes which precede and accompany atresia. We know that the cells are actively synthesizing RNA and protein, yet there are no investigations as to how such macromolecular synthesis (and turnover) may be quantitatively and qualitatively changed during atresia. There is one excellent electron microscopic study of mammalian eggs aging within the fallopian tubes,[21] but while aging of ovulated and fertilized gametes may prove to be clinically important and can be defended by the purist as representing a more kinetically valid model of cellular aging,[15] in my opinion, aberrations within the preovulated oocytes are of greater significance and hence deserving of careful investigation.

Although there is very little morphologic or biochemical information on aging oocytes, the situation is brighter with respect to functional changes, and this is really the primary rationale for their consideration in this review. In addition to the obvious decline in fertility, there is unequivocal evidence that the frequency of nondisjunction increases in human female oocytes as a function of age.[22] Advanced maternal age is the major indication for amniocentesis, permitting, via cell culture, the prenatal diagnosis of the Down syndrome and other trisomies. There is also evidence of a similar increased risk of such meiotic errors in aging female mice.[23-26] A relationship between a decline in the frequency of genetic recombination and chiasmata and an increase in the frequency of nondisjunction has been known for many years.[27] In mice, a decline in the frequency of gene recombination has been shown as a function of mater-

nal but not paternal age.[28,29] Direct cytologic evidence of a decline in chiasma frequency in aging mice was first reported by Henderson and Edwards.[28] Text-figure 2, from their paper, illustrates that the rate of this decline is greater for the CBA strain than the C57 strain of mice. This is of interest in that Jones and Krohn [1] have found that CBA mice lose their oocytes at a rate substantially greater than that of several other strains which were investigated. Thus, there is a concordance between the rate of loss of chiasmata and the rate of atresia of oocytes. Henderson and Edwards [28] and Edwards [30] discuss various possible mechanisms for the loss of chiasmata, including their own "production line" theory, which suggests that oocytes with low chiasma frequencies are ovulated toward the end of the reproductive cycle because they were formed later during fetal life. I see no hard evidence for or against this or other theories. Of special interest is the observation of Franchi and Mandl [31] that the rapid elimination of degenerating oocytes in the rat is mediated by the phagocytic activities of neighboring somatic cells. As with the case of the effete erythrocytes mentioned in the introduction,[5] could there be deposits of immunoglobulins on the surfaces of cells marked for atresia? If so, what are the antecedent changes?

Neurons

The picture which is emerging with respect to age-related neuronal cell loss in the human brain is that it is a function of the precise regional localization. Thus, while there is no evidence of cell loss within the ventral cochlear nucleus,[32,33] the abducens nucleus,[34] or the trochlear nucleus [35] in specimens of locus ceruleus from subjects 65 years of age and older, there



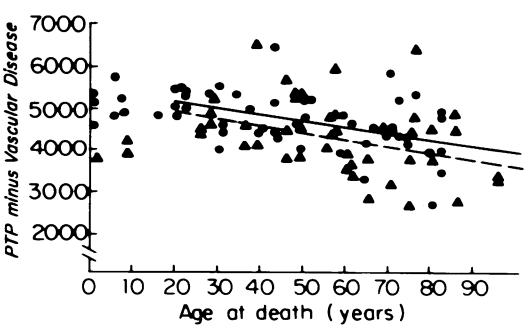
TEXT-FIGURE 2—The frequency of chiasmata found in meiotic I prophase of oocytes of C57B and CBA inbred strains of mice plotted as a function of donor age. (Reproduced from Henderson and Edwards with permission.)

is a loss of at least 30% [36] and probably closer to 40%.[37] This latter finding if of considerable interest in view of the remarkably extensive ramifying connections of the locus ceruleus and its consequent influence on a wide range of behavior [38] including that of sleep patterns,[39] aberrations of which are a major cause of morbidity in aging humans.[40]

Much has been made of the accumulation of lipofuscin pigment as a marker of cellular aging. (This will be more thoroughly discussed under the section on myocardial cells.) However, as Brody has emphasized,[36] the largest accumulation of this pigment is found in the neurons of the inferior olivary nucleus, one of the several brain stem nuclei which do *not* show age-related loss; [41] in contrast, there is substantially less lipofuscin in regions of the cerebral cortex characterized by significant cell loss, such as the superior temporal gyrus, the superior frontal gyrus, and the precentral gyrus.[36]

One of the best documented examples of age-related neuronal cell loss is that of cerebellar Purkinje cells.[42] The calculations took into account variations in the fresh cerebellum volume from subject to subject. In Text-figure 3, the parameter "proportion of the total population" (*PTP*) is plotted as a function of subject age. $PTP = NV^{2/3}$, where N = the number of cells per square millimeter of fresh Purkinje cell sheet and V = the fresh volume of the cerebellum, $V^{2/3}$ being proportional to the surface area. In Text-figure 3, all cases of moderate or severe cerebral vascular disease were excluded. Statistically significant negative regressions were calculated for both males ($r = -0.443$) and females ($r = -0.347$). Therefore, one can expect about a 25% reduction in the Purkinje cell population during the maximum life-span of humans.

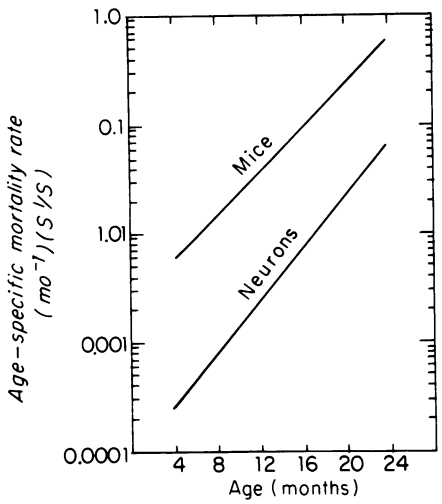
The situation with respect to neuronal loss in animals is less clear.[43] A particularly impressive positive result was reported by Johnson and Erner [44] in aging inbred Swiss albino mice (Brookhaven National Laboratory strain). After formalin fixation, the brains were sonicated in water (after



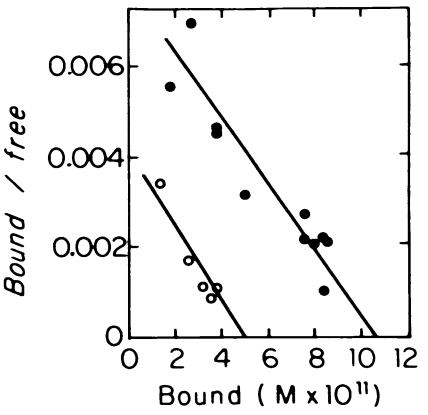
TEXT-FIGURE 3—The numbers of cerebellar Purkinje cells corrected for variations in cerebellar volume (see text for discussion of the parameter PTP) plotted as a function of donor age. All cases with moderate or severe cerebral vascular disease were excluded. (Male, *solid circles* and *line* [$r = -0.443$, N = 59]; female, *solid triangles* and *broken line* ($r = -0.347$, N = 58). (Reproduced from Hall *et al.* with permission.)

mashing with a glass rod), resulting in a suspension consisting of finely dispersed fibrillar debris and cells which could be classified as large neurons (greater than 15 μ in diameter), glia, endothelial cells, and erythrocytes. The cytoplasm of neurons remained intact except for the axons and dendrites, which appeared to have broken off close to the perikaryon. Text-figure 4, taken from their paper, shows a joint plot of the age-specific mortality rates for both the mice and their large neurons. The curves are nearly parallel, suggesting that the neurons are aging at about the same rate as the mice. It is important that efforts be made to duplicate these interesting results using other strains of mice, especially those with longer life-spans, as well as other species. The fact that DNA determinations of aging mouse brains showed no change [45] could be attributed to an associated proliferation of glial cells. Such proliferation appears to take place in the aging primate brain [46] and is in keeping with our general principle of paradoxical age-associated cellular proliferation discussed in Part I of this review. A more interesting explanation for the discordance between the DNA result and the sonication enumeration results, however, is the possibility that there is an increase in the fragility of neurons with age, so that increasing numbers are lost to sonication.[47]

Quite recently, several laboratories have been searching for alterations in aging neurons, using assays for specific plasma cell membrane or cytosol receptors. Such preparations from the cerebral cortices of 24- to 26-month-old male Sprague-Dawley rats exhibited a 55 to 65% reduction in the concentration of specific cytosol glucocorticoid binding sites as compared to the concentration in 12- to 13-month-old animals.[48] Text-figure 5 is a Scatchard plot documenting differential binding in mature



TEXT-FIGURE 4—Gompertz plots showing age-specific mortality rates for a Brookhaven National Laboratory strain of Swiss albino mice and a class of their large central nervous system neurons which survived postfixation sonication. (Reproduced from Johnson and Erner with permission.)



TEXT-FIGURE 5—Scatchard plots of specific glucocorticoid binding in isolated cerebral cortical perikarya of mature (12 to 13 months, *solid circles*) versus senescent (24 to 26 months, *open circles*) male Sprague-Dawley rats (CD strain). (Reproduced from Roth with permission.)

versus senescent rats shown by that study. There is no reason to believe that these results were attributable to differential survival of a heterogeneous population, although this will have to be eventually ruled out. Whether the results are attributable to a loss of receptors or to a masking of receptors remains to be determined.

Are there abnormal proteins detectable in aging neurons? The best current lead involves the peculiar paired helically wound filaments which are the ultrastructural hallmarks of neurofibrillary degeneration, the classic lesion of aged brains, senile dementia, and Alzheimers disease.[49] Although chemical characterization of isolated material is still in progress,[50] the constituent polypeptides could prove to be distinct from both tubulin and neurofilamentous proteins. Such data will be essential for the construction of hypotheses to explain their origin. An intriguing possibility is that one pathogenetic component involves chromatin binding with aluminum.[51]

A pathogenesis of senile dementia based upon slow virus infection is now a major consideration in view of the demonstration that certain susceptible strains of mice, when inoculated with certain strains of the scrapie agent, develop numerous neuritic (senile) plaques and associated amyloid after extremely long latent periods.[52] It is not inconceivable that ubiquitous endogenous agents, including agents capable of vertical transmission, could explain the neuritic plques detectable in the brains of many clinically normal aging humans.[53] Recently, there has been considerable interest in the findings, due principally to the painstaking Golgi staining studies by Scheibel and Scheibel [54] and by Feldman [55] in loss of cortical dendritic spines in aged brains. However, it remains to be seen to what extent such alterations may be secondary to the diminished sensory input which characterizes aging populations.

Is there any evidence of damage to the DNA of aging neurons which

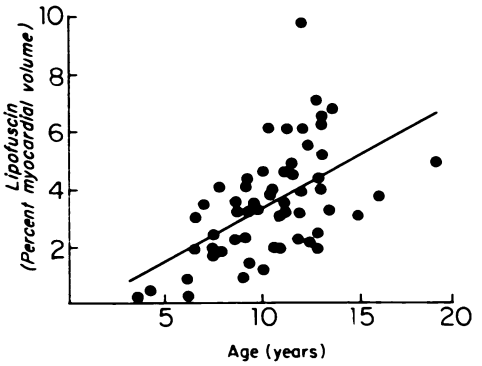
might be consistent with the intrinsic mutagenesis hypothesis of aging as outlined in Part I of this review? Price, Modak, and Makinodan [56] studied the template activity of the DNA of fixed sections of brain in young versus senescent mice using calf thymus DNA polymerase. This enzyme catalyzed greater incorporation of deoxyribonucleotide monophosphates by the nuclei of old neurons, suggesting an accumulation of DNA strand breaks with age. Evidence of structural alterations in senescent brain DNA have also been observed by Wheeler and Lett [57] and by Chetsanga *et al.*[58] It is conceivable that differential rates of repair could be responsible for differential rates of accumulation of such lesions among various species. In this respect, there is considerable need for an evaluation of the CNS pathology in patients with inborn errors of metabolism associated with various DNA repair defects, such as the different complementation groups of xeroderma pigmentosum [59] and ataxia telangiectasia;[60,2] there are virtually no published reports on the neuropathology of such patients.

Evidence for selective loss of specific regions of DNA (ribosomal RNA cistrons) in senescent canine brain tissue has been presented by Strehler and co-workers [61,62] using molecular hybridization methods. However, this could not be confirmed in similar investigations by Cutler's laboratory, in which C57BL/6J mice were studied.[63] This important line of research will be mentioned again in the following section on myocardial cell aging.

Hybridization techniques have also indicated a decrease in the number of a variety of RNA species detectable in aging whole brain.[64] Such results can be interpreted in many ways. One of the currently most popular interpretations is that it is the result of the progressive cross-linking of DNA, especially via chromosomal proteins.[65,66]

Myocardial Cells

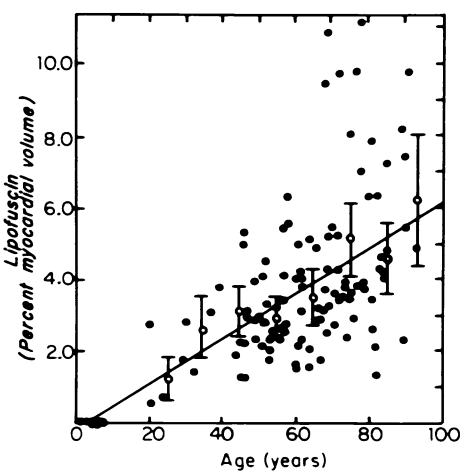
Every pathologist is familiar with the yellow-brown granular pigment which accumulates in the perinuclear cytoplasm of the myocardium of aged individuals and which is responsible for the gross appearance of "brown atrophy." Perhaps the best evidence implicating this lipofuscin pigment as a valid marker for aging comes from comparative studies of its rate of accumulation as a function of age within the myocardium of dogs (purebred beagles) [67] and man.[68] Beagles accumulate aging pigment at the rate of 3.6% of myocardial volume per decade (Text-figure 6). The equivalent figure for man was reported to be 0.67% (Text-figure 7). Therefore, beagles accumulate their pigment at a rate 5.4 times that of man. Judging from the 50% survival figure of 12 to 13 years,[69] the maximum longevity of beagles is likely to be of the order of 25 years. The comparable figure for man is likely to be about 120 years; claims of much greater longevities for



TEXT-FIGURE 6—The percent of myocardial volume occupied by lipofuscin pigment plotted as a function of donor age. Beagle dogs of both sexes. (Reproduced from Munnell and Getty with permission.)

man have never been well documented.[70] The ratio of human/beagle maximum longevities, about 4.8, is in rather good agreement with the figure of 5.4 calculated above. It would be of interest to extend such an analysis to other mammalian species.

Lipofuscin is of considerable theoretical importance to aging research in that its accumulation is predicted by the free radical theory of aging.[71-74] Definitive analyses of various lipofuscins have yet to be achieved, but it seems likely that they consist of polymers of lipid and phospholipid with amino acids either bound to the lipids and/or as included proteins.[75] Unsaturated fatty acids of membrane phospholipids are thought to react with oxygen-containing free radicals to form unstable lipid hydroperoxides. The latter yield additional oxygen-containing free radicals, leading to a chain reaction of lipid or lipoprotein autoxidation. Membrane damage is further accelerated by cross-linking attributable to malon-



TEXT-FIGURE 7—The percent of myocardial volume occupied by lipofuscin pigment plotted as a function of donor age in humans of both sexes. (Reproduced from Strehler *et al.* with permission.)

aldehyde, an end product of the lipid autoxidation. Of the various defense mechansims which organisms have evolved to protect themselves from such oxidative catastrophes, superoxide dismutases are likely to be of paramount importance.[76] The set of loci coding for such enzymes probably should be added to our list of genes (Part I of this review) relevant to the intrinsic mutagenesis hypothesis, as chromosomes are likely to be among the numerous targets for such oxidative attacks.

Is there evidence for alterations in the DNA of aging myocardial cells? Work from two of the groups referred to above in connection with neurons have also presented evidence consistent with an accumulation of DNA single strand breaks in myocardial cells of senescent mice.[77,78] In the latter study, sedimentation profiles also were interpreted as indicative of an age-associated preferential loss of segments of DNA rich in guanine and cytosine. This observation would be consistent with RNA-DNA hydridization studies indicative of a selective loss of ribosomal RNA genes of myocardium during aging.[62,79] The latter authors considered the possibility that their results might be attributable to asynchronous DNA synthesis associated with the increase in myocardial nuclear ploidy and the proliferation of mesenchymal components which characterize cardiac hypertrophy, a common feature of aging hearts. However, the extent of loss of ribosomal cistrons from what was judged to be a nonhypertrophic aged heart was comparable to the loss within hypertrophic myocardium of comparable aged individuals. Although it seems unlikely that the extent of the loss (about 30%) would be rate limiting in protein synthesis, the authors pointed out that some degree of functional failure might occur under conditions of increased workloads and stress, especially when associated with cardiac pathology. There is, in fact, evidence for a decline in protein synthesis in aged myocardium, but this is a complex methodologic and physiologic problem subject to a variety of interpretations.[80] One of the most interesting interpretations is that the observations could result from genetically programmed variations in the activities or concentrations of selected species of aminoacylated transfer RNAs.[80,81]

Although there have been many attempts to detect abnormal proteins in mammalian liver and skeletal muscle, especially in connection with tests of Orgel's error catastrophe theory,[82,83] I am unaware of any study which has investigated aging myocardium from this point of view. Ultrastructural findings comparable to the peculiar paired helical filaments of neurofibrillar degeneration have not been reported, although I rather doubt if anyone has made a serious search for such structures in tissues other than brain.

Virtually all of the biochemical and molecular aging studies, not only

on myocardium but also on other tissues, have been deficient in not including careful (and especially quantitative) histologic controls to evaluate the problem of a changing pattern of cellular heterogeneity, an increase in fibrosis, inflammatory cell infiltrates, etc. On the other hand, the few excellent light microscopic studies [84] or electron microscope studies [85] have not included correlative biochemical research. The study of Sulkin and Sulkin,[85] for example, revealed striking morphologic alterations in the mitochondria of the myocardium of aging rats subjected to chronic hypoxia. Biochemical studies by other workers have demonstrated some degree of functional impairment.[86,87] It is possible that more substantial biochemical abnormalities could be detected with *in vitro* studies of isolated mitochondria were it not for the fact that the mitochondria from senile myocardium seem to be more fragile and hence are probably preferentially lost during most isolation techniques.[88] I am increasingly impressed with the mounting evidence for age-related alterations in the membranes of cells and organelles.

Prospects for a Genetic Analysis

Although this review has been very selective, it must be already apparent to the reader that there are numerous phenotypic aberrations in old animals involving both replicating and nonreplicating somatic cells as well as germ cells. It is extremely difficult to sort out those changes and underlying mechanisms which are of primary importance in the determination of the varying life-spans of mammals and of the varying rates of development of geriatric pathology. The most powerful and direct approach to this problem is via a genetic analysis. For obvious reasons, this is extremely difficult to do via conventional meiotic analysis, even with the shortest-lived mammals. It would be extraordinarily inefficient to simply examine a wide array of mutations which shorten life-span, since this end result is so nonspecific. What we need is a method to test specific hypotheses regarding which genes were involved in the evolution of enhanced life-span. One would wish to transfer such a test gene from a long-lived mammal to a closely related short-lived mammal, preferably after mutational inactivation or deletion of the resident locus. (An excellent choice of such an experimental pair would be *Mus musculus*, the conventional laboratory mouse, which has a maximum life-span of about 4 years, and *Peromyscus leukopus*, the white-footed mouse, which has a maximum life-span of about 8 years.[89]) Incredibly, all the methodologic steps necessary to carry out such a genetic analysis have become potentially available within the past few years, although there are formidable technical obstacles for their realization in even the simplest case.

In 1974, Brinster published a landmark paper [90] in which he produced a limited chimerism in a mouse by injecting blastocysts with mouse teratocarcinoma cells of a different genotype. In a series of brilliant and definitive experiments by Mintz and Illmensee,[91,92] this research strategy was able to demonstrate multipotency of such teratocarcinoma cells. One chimeric male sired apparently normal progeny carrying multiple genetic markers of the input teratocarcinoma cells. At about the same time, Papaioannou *et al.*[93] also produced multiple somatic cell chimerism, including gonadal tissues, by the inoculation of *cultured* teratocarcinoma cells. Although several neoplasms developed in these chimeras, the cultures are likely to have sustained subtle, but significant, chromosomal aberrations during their long-term culture.[94] Meanwhile, substantial advances have been made in the tissue culture of various lines of multipotent mouse terotacarcinoma cells, including the use of feeder layers, which maintain the stability of the phenotype [95,96] and the karyotype.[97] The availability of such cultured lines opens the door to a variety of somatic cell genetic experiments involving the selection of stable mutants and deletions and the interspecific transfer of genetic material. Although it has yet to be demonstrated that gonadal chimerism produced by the inoculation of blastocysts with *cultivated* teratocarcinoma cells will result in the differentiation of normal gametes bearing the input teratocarcinoma genes, it seems likely that this will eventually be achieved, especially with short-term cultures or with cultures which have been cycled between an *in vivo* ascites form and *in vitro* culture.

Techniques for gene transfer are progressing with great speed.[98-103] With sufficient imagination, these could be applied to a great many genetic loci, including those which might play crucial roles in modulating the rates of cellular aging in various organ systems.

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Acknowledgments

I am grateful to Dr. Roger Donahue for helpful comments on the section dealing with oocytes.