Aging of the Human Adrenal Cortex

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The most striking age-related change in the human adrenal cortex is the decline in secretion of dehydroepiandrosterone and its sulfate, steroids synthesized by the inner zone of the cortex, the zona reticularis. Because these steroids are of essentially unknown function, the importance of this age-related change is the subject of considerable debate. It is likely that the age-related change in these steroids results from loss of zona reticularis cells or impairment of their function. During aging, cumulative damage to the zona reticularis could occur through ischemia-related infarcts and other causes of cell death. Cellular senescence could contribute to a loss of the ability of the tissue to replace lost cells. In contrast, feedback mechanisms that regulate adrenocortical growth cause compensatory local tissue hyperplasias called nodules. The effect of imperfect repair of damage combined with compensatory overgrowth in the form of nodules leads to an increasingly abnormal tissue architecture.

Introduction: Structure and Function of the Adrenal Cortex

The adrenal glands are paired organs that overlie the cranial poles of the kidneys. Embryologically, the glands arise from two distinct precursor cell types: mesodermal cells that become the outer part of the gland, the cortex, and ectodermal cells that become the inner part of the gland, the medulla. The cortex secretes steroids, whereas the medulla secretes catecholamines. This Review concerns only the cortex and its changes during aging.

The adult human adrenal cortex, as in other mammals, has three morphologically defined zones. In most species, these zones are an outer zona glomerulosa (ZG), a middle zona fasciculata (ZF), and an inner zona reticularis (ZR) (Fig. 1). These traditional divisions have been recognized in mammals generally since the middle of the 19th century. In all mammals, the ZG synthesizes mineralocorticoids (corticosteroids such as aldosterone that primarily function in the regulation of electrolyte balance in the kidney) and the ZF synthesizes glucocorticoids (corticosteroids such as cortisol that function in carbohydrate metabolism and have a variety of other roles). However, it is likely that the ZR has a unique function in humans and a few other primates (1, 2). The morphologically recognizable ZR in other species does not functionally resemble the zone in the human cortex. In humans, this zone secretes the unusual steroid dehydroepiandrosterone and its sulfate [DHEA and DHEAS, referred to here collectively as DHEA(S)]. The function of these steroids is essentially unknown, despite much speculation.

Adrenocortical cells divide at a slow rate throughout the life span. The generally accepted model for cell renewal is that cells in the outer part of the adrenal cortex, the ZG and the outer ZF, divide at a higher rate than those located in the more central areas of the cortex, the inner ZF and the ZR. Cells are thereby pushed by the pressure of cell division from the outer part of the cortex to the inner part. A ZG cell may therefore find itself pushed down into the ZF, and it is assumed that, once past the boundary between the zones, cells that were originally ZG cells now become ZF cells. Similarly, a ZF cell that is pushed into the ZR is assumed to become a ZR cell. There is evidence for these transformations from both cell culture and in vivo experiments (3). The inner ZR appears to be a site of a higher rate of cell death than the rest of the cortex (3). Thus, over time there is cell turnover without a net change in tissue size.

The question of whether there is a stem cell compartment in the adrenal cortex has not yet received a definitive answer. Typically, stem cells are defined by their ability to repopulate an organ and generate various differentiated cells. By this definition, many or most adrenocortical cells might be described as stem cells; the adrenal cortex can regenerate from very small numbers of cells, after most of the organ has been destroyed (3), and cell transplantation experiments have shown that ~25% of clones of bovine adrenocortical cells can regenerate functional tissue (4). However, genes involved in Wnt signaling (http://stke.sciencemag.org/cgi/cm/stkecm%3BCMP_5533)—WNT4 and DKK3—are expressed at higher levels in the ZG than in the rest of the cortex (5, 6). By analogy with stem cell systems in other organs, the higher levels of Wnt signaling molecules in the ZG might suggest that it is a stem-cell zone (7), but more studies are needed to decide these issues.

Age-Related Changes in the Structure and Function of the Human Adrenal Cortex

Changes in zonation

As shown in Fig. 2, the major age-related change in the human adrenal cortex is a striking decrease in the biosynthesis of DHEA(S) (8-11). In contrast, cortisol biosynthesis is relatively unaffected by aging, but there may be a subtle hypocortisolism associated with nodules and adenomas of the adrenal cortex. The simplest hypothesis for the decline in DHEA(S) is that it results from a decline in the number of functional ZR cells, but this has not been firmly established. Direct measurements of the width of the zones show a decrease in the size of the ZR during aging (12, 13), but this is a relatively modest decrease and does not account quantitatively for the decline in DHEA(S).

An older study emphasized that the boundary between the ZF and the ZR becomes increasingly irregular with age (14). The working hypothesis that I adopt here is that measurements of
ZR- 

width underestimate the decline in the number of functional ZR cells and that the increasing irregularity of the boundary between the ZF and the ZR makes it difficult to use morphological criteria alone to estimate the number of ZR cells. A more accurate count of ZR cells at different ages requires the use of molecular markers to enable recognition of ZR cells. Such markers include the lack of expression of type II 3\beta-hydroxysteroid dehydrogenase and higher levels of expression of DHEA sulfotransferase, cytochrome b5, and major histocompatibility complex (MHC) class II molecules (15-17). Direct proof that the decline in DHEA(S) abundance results from a decrease in the number of ZR cells will be difficult to obtain, because it depends on observations on postmortem material with little opportunity to study functional/morphological correlations.

Neoplastic and preneoplastic changes
Apart from disorders of zonation, disruption of the normal architecture of the adrenal cortex also occurs because of an age-
related increase in the frequency of neoplastic and nonneoplastic lesions. These most commonly comprise nodules, somewhat less commonly adenomas, and more rarely carcinomas. The age-related accumulation of small focal hyperplasias termed nodules is so frequent as to make it more or less a part of normal aging (18-21) (Fig. 3). In one study, mild to distinct nodularity was observed in 65% of adrenal glands from 113 consecutive autopsies in adults (18). Multiple small nodules are usually present, but relatively frequently one nodule enlarges, concurrently with atrophy of the rest of the adrenal cortex (22).

Benign tumors (adenomas) are larger than nodules (in terms of weight, up to ~100 grams) and also occur relatively often. The increasingly routine use of patient imaging [computed tomography (CT, in which multiple x-ray images are incorporated into a cross-sectional image) and magnetic resonance imaging (http://sageke.sciencemag.org/cgi/content/full/2003/4/pe2) (MRI)] in diagnostic procedures has made the discovery of suspicious findings in the adrenal glands a very common occurrence. The great majority of the suspicious lesions are in the cortex, rather than the medulla, and of these the great majority are adenomas or nodules (23-26). Over the past 20 years, the common designation for these incidentally discovered adenomas has been “adrenal incidentalomas.” In one autopsy series, 8.7% of the population over 65 was shown to have lesions of the adrenal cortex >1 cm in diameter (27). In a recent review, the authors comment, “With the widespread escalation in the use of these imaging modalities and marked improvements in image resolution, the probability of finding an incidentaloma on cross-section imaging is rapidly approaching the 6% prevalence of previously reported autopsy studies” (26). Grumbach et al., reporting on the conclusions of a National Institutes of Health consensus conference, state “Improvements in abdominal imaging techniques have increased detection of adrenal incidentalomas, and because the prevalence of these masses increases with age, appropriate management of adrenal tumors will be a growing challenge in our aging society” (25). Continued improvements in technology will make the discovery of adrenal incidentalomas an even greater problem for the future, but even at present the adrenal incidentaloma has been termed a major public health problem (23-26).

Conventionally, most incidentalomas have been thought to be “non-functioning adenomas.” This term is a misnomer, in that in vitro studies have consistently shown that isolated cells from these tumors synthesize glucocorticoids and/or mineralocorticoids (20, 28, 29). The term refers to the concept that the circulating plasma concentrations of adrenocortical steroids were not thought to be elevated above normal in individuals with incidentalomas. However, recent studies have challenged this idea. Subtle elevations in the concentrations of circulating adrenocortical steroids, specifically cortisol, might be a cause of the metabolic syndrome (http://sageke.sciencemag.org/cgi/content/full/2003/41/ns6) (syndrome X), an increasingly prevalent age-related disorder characterized by obesity, dyslipidemia (altered levels of lipoproteins and triglycerides), insulin resistance, impaired glucose tolerance, and hypertension (30, 31).

Whereas benign lesions (nodules and adenomas) are a frequent occurrence in the elderly, adrenocortical carcinomas are rare. Carcinomas are larger (50 to 400 grams) than the benign lesions and most often discovered because of the symptoms caused by increased growth (22, 32). The most straightforward interpretation of the available clinical data is that adenomas are the precursors of carcinomas but that the transition of adenoma to carcinoma is rare. This interpretation is consistent with the concept of multistep tumorigenesis as it occurs in other organs, such as the colon. Few adenomatous polyps in the colon progress to colon cancer (33). However, as better diagnostic tests are developed, increasingly smaller, earlier premalignant lesions will be detected (as observed with colonoscopy and CT/MRI scanning, for the colon and the adrenal cortex respectively), thereby contributing to the clinical dilemma of whether further tests and treatment are necessary.

![Diagrammatic representation of the plasma concentrations of DHEAS over the life span in humans. Before birth (A) the fetal zone of the adrenal cortex secretes large amounts of DHEA(S); following birth (B), the fetal zone rapidly involutes (C). By about 6 to 7 years of age, ZR cells have developed in the adrenal cortex and plasma DHEAS concentrations begin to rise (adrenarche) (D). The achievement of the peak concentration of plasma DHEAS in young adulthood (E) is followed by a progressive decline in adrenal secretion of DHEA(S), so that plasma DHEAS concentrations are often very low by age 70 and beyond (F). The ordinate represents age in years; the scale is expanded before 10 years of age. Possible Molecular Mechanisms for Age-Related Changes](http://sageke.sciencemag.org/cgi/content/full/2004/35/re6)

To summarize the age-related changes in the human adrenal cortex, there is a reduction in the size of the ZR and a loss of normal zonation, which may account for the steep decline in DHEA(S) synthesis and an increase in disruptions of the normal architecture—nodules, adenomas, and carcinomas, in order of decreasing frequency. Here, I propose that all or some of the following pathological processes may contribute to these age-related changes: (i) infarcts (areas of tissue death) caused by temporary ischemia, and other causes of cell death, leading to disruption of tissue architecture; (ii) cellular senescence (telomere shortening), leading to failure of replacement of cells; (iii) DNA mutations or chromosome aberrations, initiating and promoting neoplastic processes; and (iv) possibly, the production of senescence-related enzymes and cytokines by adrenocortical cells or by fibroblasts within the tissue, leading to disruption of architecture and/or the promotion of neoplasia.
Ischemia/reperfusion

A role for temporary ischemia (abnormally low blood flow) in age-associated changes in the adrenal cortex has been suspected for many years (18, 21), although direct evidence is still lacking. In this laboratory, we first became interested in this question when we assessed expression of the cell-cycle inhibitor p21 in the human adrenal cortex. In adrenocortical cells in glands from some organ donors (trauma victims), evidence of DNA damage was accompanied by the presence of immunoreactive p53 and p21 (34), which normally would not be present in these cells. DNA damage, present as single-strand breaks, caused activation of the tumor suppressor p53, which transcriptionally induced p21 (35). This process was accompanied by some apoptosis, presumably also mediated by p53 (36).

We hypothesized that damage to the adrenal glands in human trauma victims would involve periods of low blood flow alternating with periods of higher blood flow, as observed in clinical practice (21, 37-39). We observed that DNA damage and increases in immunoreactive p53 and p21 could be induced experimentally in the rat adrenal gland by ischemia/reperfusion and by sepsis (high levels of bacteria in the blood) (34, 40). A likely common element in injury of the adrenal gland is damage to the microvasculature. The function of capillary endothelial cells is altered by cytokines (http://sageke.sciencemag.org/cgi/content/full/2002/29/re3#SEC5) that circulate at high concentrations during shock and sepsis. As a result, the endothelium (the layer of cells that lines the blood vessels) becomes leaky, and red blood cells and leukocytes escape from the vessels into the tissue (41). Although there is no direct evidence for this mechanism, it would account for DNA damage by exposure of the cells in the tissues to oxygen-radical generating systems that are normally sequestered in the blood, such as granulocytes (http://sageke.sciencemag.org/cgi/content/full/2002/29/re3#SEC7) or transition metal/protein complexes (42-44). Oxygen radicals (http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/oa5) and their products cause extensive strand breaks in target cell DNA (45). The hypothesis I present here is that over the life span of an individual many such episodes of damage could occur, caused by temporary ischemia or by other kinds of damage to the capillary bed in the adrenal gland. Each such episode may result in a small infarct, the death of a few cells or perhaps a segment of the cortex. In fact, many years ago pathologists had already noted evidence for such infarcts and their relation to ischemia (18, 21). For several reasons, direct evidence for this hypothesis is difficult to obtain. The rate of cell death in the human adrenal cortex under “normal” circumstances is quite uncertain. Adrenal glands available for study are almost never normal; either they have been surgically removed from patients with serious illnesses or they have been obtained from cadaver organ donors, who have often been maintained for several days on life-support systems before death.

It seems unlikely that cell death per se, in the absence of other changes, could result in permanent alterations in the structure of the adrenal cortex. In the absence of other changes, cell loss would be expected to be balanced by cell division to restore the original size of the tissue. Indeed, this is what is assumed to occur under normal conditions in the young healthy adult, where cell death in the ZR is balanced by cell division in the outer cortex. Although the normal mode by which the size of the adrenal cortex is controlled is only partly understood, it is known to involve feedback via the hypothalamus and pituitary (3). Loss of adrenocortical cells (ZF cells) leads to some decrease in secreted steroids (cortisol). By feedback at the level of the hypothalamus and pituitary, this decrease leads to increased secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland, which stimulates the growth of the adrenal cortex (3). A dramatic example of the operation of this feedback cycle is seen when one of the enzymes of steroidogenesis is missing because of a genetic defect; this absence causes congenital adrenal hyperplasia, a greatly enlarged adrenal cortex (46). Suppression of ACTH secretion leads to a reversal of the
excess growth. Therefore, in adult life the loss of some adrenocortical cells would be expected to be compensated by feedback. For cell death to create a permanent defect in structure and function, there must be some interference with the normal mode of cell replacement.

Several factors could lead to imperfect feedback and thereby contribute to making the defect permanent. First, loss of ZR cells, as opposed to ZF cells, does not lead to a decrease in cortisol secretion and therefore does not increase ACTH secretion by the hypothalamus and pituitary; so far as is known, a decrease in DHEA(S) levels does not exert a feedback action. Second, the cells could lose the ability to divide, perhaps as a result of cellular senescence (see below). Third, it may be that infarcts caused by ischemia cannot be repaired with complete efficiency. The loss of an entire capillary (or one segment of it), together with the adrenocortical cells that are dependent on the vessel, might be permanent. The loss of such a unit may well increase the stimulus to growth acting on the rest of the cortex, but regeneration of the local deficit in the vicinity of an infarct might not occur. Over time, this process may result in the loss of segments of the cortex, together with compensatory hyperproliferation of other parts of the cortex.

Specific susceptibility of ZR cells to apoptosis
Given these potential mechanisms for the loss of adrenocortical cells, why are ZR cells especially vulnerable to being lost and not replaced? One possibility is that, because the ZR is on the venous end of the adrenocortical capillary bed (3), it is more liable to be damaged by ischemia. That possibility would also be consistent with the increasing irregularity of the ZF/ZR border—infarcts occurring on the venous end of the capillary bed could produce small areas of damage in the ZR. Additionally, it is possible that differences in gene expression between ZF cells and ZR cells cause ZR cells to be primed to undergo apoptosis and/or to be eliminated by the immune system. ZR cells, but not ZF cells, express MHC class II antigens on their cell surface (47, 48). MHC class II molecules can be expressed by a wide variety of normal tissues in vivo, as well as in tumors and tissues affected by abnormal conditions such as autoimmune diseases (49). Cells expressing class II genes do not generally act as antigen-presenting cells because they lack the costimulatory molecules (49). MHC class II antigen presentation on ZR cells may result from the effects of cytokines released by macrophages or other immune cells within the adrenal gland (50). However, unexpectedly, MHC class II expression was reported to persist in ZR cells in culture, even though they were mixed with ZF cells that did not become class II-positive (47). Whatever the cause, expression of MHC class II molecules could make ZR cells susceptible to immune attack. Fas protein (a cell-surface protein that mediates apoptosis, also called CD95) is also expressed at a greater level on ZR cells (51, 52). Cells expressing Fas protein may be killed by Fas ligand or by anti-Fas antibodies (53).

Cellular senescence
A second factor contributing to the age-related loss of ZR cells may be loss of replicative capacity. In culture, normal human cells exhibit a limited proliferative capacity that results from shortening of telomeres, which in turn results from lack of telomerase activity (54) (see Hornsby Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/30/pe21 and “More Than a Sum of Our Cells” at http://sageke.sciencemag.org/cgi/content/full/2001/1/oa4). We investigated the relationship between telomere biology and replicative senescence in human adrenocortical cells by measuring replicative capacity and telomere length as a function of donor age (55). Cells cultured from adrenal tissue from donors of different ages were found to show a strong age-related decline in total replicative capacity, falling from ~50 population doublings for fetal cells to an almost total lack of division in culture for cells from older donors (>70 years). Telomere restriction fragment (TRF) length was analyzed in the same sets of cells and was found to decrease from a value of ~12 kb in fetal cells to ~7 kb in cells from older donors. The latter value is consistent with that in fibroblasts that have reached replicative senescence. Furthermore, there was a good correlation in individual donor samples between TRF length and replicative capacity in culture. These data show that adrenocortical cells undergo continuous telomere shortening over the life span and that cells in glands from older donors have relatively short telomeres.

Telomere shortening has been observed in a variety of human tissues as a function of donor age (56). Studies on various nonfibroblast cell lines have often shown striking decreases in proliferative capacity as donor age increases (56). However, whether telomere shortening in vivo actually results in cells that can no longer divide in situ has never been directly determined. This complex question has been discussed in detail elsewhere (57).

Age-Related Disruptions of Tissue Architecture
These factors—ischemia, apoptosis, and deficits in the capacity for cellular replacement—may also contribute to the development of nodules and neoplastic lesions of the cortex. As suggested by George Martin, one consequence of loss of replicative capacity may be compensatory local hyperplasia (58). This scenario certainly fits the observed age-related changes in the adrenal cortex. The cumulative effect of cell loss in the adrenal cortex is an increase in circulating ACTH concentrations, mediated by feedback to the hypothalamus and pituitary. When part of the cortex is damaged and is unable to undergo compensatory growth, there may be an overcompensation of growth of other parts of the cortex, perhaps leading to nodule formation. The pattern of capillaries in nodules is in agreement with this possibility (59); the normal capillary pattern is discernible both in nodules and in the adjacent tissue but is distorted by expansion of the tissue within the nodule and by compression of the tissue adjacent to the nodule (59). Evidence that ACTH can drive nodule formation comes from a rare cause of Cushing’s disease, in which excess cortisol is secreted by a hyperplastic nodular cortex that results from the action of abnormally high concentrations of ACTH on adrenocortical cell proliferation (60). Moreover, the hyperplastic adrenal glands in congenital adrenal hyperplasia often show nodules, and these diminish in size when the excess ACTH that occurs in this syndrome is normalized (61).

Nodules appear to be distortions of the architecture of the cortex rather than clonal neoplastic growths. On the other hand, there is substantial evidence that adrenomas and carcinomas are clonal and that their chromosomes have been altered by mutations and rearrangements (32, 62), but a detailed consideration of the genetic and other factors that lead to adrenomas and carcinomas of the adrenal cortex is beyond the scope of this Review (32, 62).

Possible effects of senescent cells
Senescent cells may be formed by telomere shortening and also by a variety of other forms of cellular stress and damage (54). If
cells within the adrenal cortex do become senescent during aging, and if they persist in the tissue rather than dying or being eliminated by the immune system, they could have substantial effects on neighboring cells (63). Senescent cells undergo a wealth of changes in gene expression. This finding has been well documented in fibroblasts but is less well established for other cell types, including adrenocortical cells. Enzymes, extracellular matrix proteins, and cytokines produced by senescent cells could affect the surrounding tissue by (i) promoting the growth of neoplastic cells (adenomas or carcinomas), (ii) disrupting tissue architecture, and (iii) causing alterations in capillary maintenance and growth, for example (63). However, the frequency of the occurrence of senescent cells within aging tissues is still not well established, and therefore the relative importance of this mechanism for age-related changes in tissues is uncertain (57).

**Functional Consequences of Age-Related Changes**

In humans, ZR cells are responsible for the synthesis of DHEA(S). The function of DHEA(S) is not known. As I have discussed elsewhere (1, 2), these steroids are usually and correctly termed “adrenal androgens,” because, although they lack direct androgen activity, they are readily converted into active androgens in many tissues, but whether this is their major function is not clear. Low serum concentrations of DHEAS have been associated with frailty (see Walston Perspective at http://sageke.sciencemag.org/cgi/content/full/2004/4/pe4), whereas higher than normal serum concentrations have been observed in rhesus monkeys undergoing calorie restriction (http://sageke.sciencemag.org/cgi/content/full/2003/8/2e2) and in long-lived human males (see “Monkey in the Middle” at http://sageke.sciencemag.org/cgi/content/abstract/2002/31/nw108). DHEA has been reported to have a variety of effects when administered to human subjects (64, 65), but these studies have not shed light on the normal functions of these steroids. The lack of suitable animal models has severely impeded progress in this area.

The human adrenal cortex has three functional zones only in the adult, from prepuberty to old age (66). ZR cells do not appear in substantial numbers in the adrenal cortex until about 6 to 7 years of age, when plasma DHEAS concentrations begin to rise (67-69). This process is termed “adrenarche.” Adrenarche is observed only in species closely related to humans, such as the gorilla and chimpanzee (67, 70). Primate species outside this group, such as the rhesus and cromomolgus monkeys, have measurable concentrations of DHEAS in plasma but do not undergo adrenarche and do not maintain the high adult concentrations of DHEAS found in humans (71-74). Evidently, therefore, the biosynthesis of DHEA(S) by the adult human adrenal cortex has evolved recently. It is an inescapable conclusion that these steroids must have some important function, despite our ignorance of its nature. That function might not be evident under the present-day living conditions of the human species, however (1, 2).

What factors at the time of adrenarche cause the differentiation of a new cell type within the adrenal cortex? Presumably, although this is not unequivocally established, ZR cells derive from existing ZF cells. As the adrenal cortex grows in thickness, ZR cells may differentiate in response to signals existing on the venous end of the capillary bed, perhaps as a result of the presence of a certain concentration of a morphogen originating in the outer cortex. The signal for the differentiation of ZR cells appears to be of adrenal origin, but its biosynthesis might be influenced by putatory factors. Adrenarche does not take place in individuals with congenital unresponsiveness to ACTH (75). Some cases of precocious adrenarche are associated with growth hormone and prolactin excess (76). A dependence on adrenal growth for the occurrence of adrenarche can be inferred from data on patients treated with glucocorticoids for congenital adrenal hyperplasia resulting from genetic deficiency of 21-hydroxylase, one of the enzymes of steroid biosynthesis (77, 78). This treatment was associated in adult life with extremely low levels of DHEAS, even when the patients received no steroids for a period of 6 weeks (79). The authors suggested that steroid treatment interferes with adrenarche by suppressing growth of the cortex so that it does not achieve a width appropriate for the development of the ZR.

At adrenarche, the development of a certain number of ZR cells sets a certain level of DHEA(S) biosynthesis. The surge of DHEA(S) biosynthesis at adrenarche can be viewed as fulfilling an undetermined biological requirement for DHEA(S) at this phase of the life span, where crossing some threshold for the plasma concentrations of these steroids is needed but precise regulation is not required (1, 2). The high variability of peak adrenal androgen levels in young adulthood and the variability in the age-related decline is consistent with this concept. Solving the problem of the importance of the age-related decline in DHEA(S) biosynthesis requires an understanding of the function of these steroids in young adult life.

**Summary and Future Questions**

An attractive model for the age-related changes in the human adrenal cortex can be made that fits together the consequences of cellular senescence resulting from telomere shortening, temporary ischemia leading to small infarcts, and compensatory growth resulting in increased nodularity and zonal irregularity. A consequence of these changes might be the gradual loss of ZR cells and the loss of DHEA(S) biosynthesis. However, this model is speculative and needs experimental verification. A large problem here is that animal models may be unreliable for several reasons. First, most mammalian species that are available for experimental study lack a functional DHEA(S)-secretion ZR. Second, age-related cellular processes in rodents could differ substantially from those in humans and other long-lived mammals (see Hornsby Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/30/p2e1). In my laboratory, we have used cell transplantation techniques to approach these questions (4), but evidently a variety of creative approaches is needed to solve the difficult problems of human aging.

**References**


