swim bladder increases linearly with hydrostatic pressure (*5*), whereas the volume of gas deposited by the swim bladder decreases hyperbolically relative to increasing hydrostatic pressure (*16*). The maximum hydrostatic pressure for maintenance of swim bladder volume may be derived by integration of linear and hyperbolic models (Fig. 2). The hydrostatic pressure at which a 545-mm yellow eel can just maintain its swim bladder volume is 6.8 atm (58 m). A 545-mm silver eel can maintain its swim bladder volume to a pressure of 15.9 atm (149 m). Thus the elevated rate of gas deposition and the reduced rate of diffusive gas loss in silver eels may extend the depth at which the volume of their swim bladders is maintained by at least 91 m.

The silver eels examined in this study would not be able to maintain an inflated swim bladder to a depth of 2000 m. However, silver eels collected during downstream migration close to the coast in Rhode Island are not fully mature (*17*). There probably is further modification in swim bladder morphology and physiology during the oceanic phase of the spawning migration.

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8. A submersible top limited the depth range in the aquarium to between 7.5 and 35.0 cm beneath the surface. Dissolved oxygen was maintained at 7 parts per million; temperature, at 17° ± 0.5°C; and salinity, at 30 to 31 per mil. Buoyant mass was determined before and after the test period by weighing the reanesthetized eel submerged in fresh water. The test period was 90 minutes for the silver eels and 240 minutes for the yellow eels. (The period was shorter for the silver eels because of the rapid rate at which their swim bladders inflated.)
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18. Linear equations for the calculated rates of oxygen loss (V, in cubic centimeters per hour) versus hydrostatic pressure (p, in atmospheres) of 545-mm eels are as follows: for the yellow eel, $V = -0.26 \times 10^{-2} + 1.32 \times 10^{-2}\ p$; for the silver eel, $V = -0.21 \times 10^{-2} + 1.10 \times 10^{-2}\ p$. These equations are based on gas diffusion measurements reported in (*5*). The hyperbolic equations follow the form $V_2 = V_1/P_2$, where the rate of gas deposition at depth (V_2) is a function of the deposition rate at 1 atm (V_1) divided by the hydrostatic pressure at depth (p_2); V_1 may be calculated for 545-mm yellow and silver eels from regression equations listed in Fig. 1.
19. This work was submitted in partial fulfillment of a dissertation requirement at the University of Rhode Island. I thank my adviser, W. H. Krueger, for his helpful suggestions and criticism of the manuscript.

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Loss of Division Potential in Culture: Aging or Differentiation?

Bell *et al.* (*1*) have suggested that fibroblasts that exhibit a finite life-span in culture stop dividing as a process of further differentiation (*1*). We do not dispute Bell *et al.*'s cell lineage data but we do disagree with their interpretation. The cause of the cessation of division of cultured fibroblasts is most probably not further differentiation.

The life history of human fibroblasts in culture closely resembles that of both bovine fibroblasts and bovine adrenocortical cells. All demonstrate a phase 2 of exponential growth followed by a phase 3 of slowed growth with total life-spans in the range of 40 to 100 population doublings (*2, 3*). Adrenocortical cells, however, have the advantage that their differentiated function, the synthesis and secretion of adrenocortical steroids, is specific and readily quantifiable. Adrenocortical cells show no evidence that their eventual cessation of proliferation is caused by further differentiation. Steroidogenesis may be stimulated at any stage up to final cessation of proliferation (*3*). In the case of one stimulating hormone, angiotensin II, the stimulation of steroid production is accompanied by stimulation of growth (*3*). The expression of full differentiated features stimulated by this hormone does not result in cessation of proliferation. The reverse is also true; when adrenocortical cells ultimately stop proliferating in culture, they show no change in their differentiated function (*3*). These observations in bovine adrenocortical cells suggest, in the absence of any positive evidence that fibroblasts acquire new differentiated features in phase 3, that the cause of the cessation of proliferation of human and bovine fibroblasts is not further differentiation.

Such positive evidence for further differentiation is lacking. Several functional declines may be observed in phase 3 fibroblasts, but among those mentioned by Bell *et al.* the only one involving a differentiated function is a loss of the dependence of collagen proline hydroxylation on the presence of ascorbic acid (*4*). The other changes mainly involve the catabolic system of the cell (lysosomes, for example). Although other explanations are certainly possible, such changes may result from adaptation of the cell to accumulated senescent damage or errors but are not normally associated with differentiation. The fact that cells that have stopped dividing are large and less motile probably reflects the cessation of nuclear replication which defines phase 3, since cells hypertrophy when DNA synthesis ceases. Changes in the pattern of proteins synthesized by terminal fibroblasts are very minor (*5*). When certain cell types—both normal and neoplastic—are induced to undergo terminal differentiation (as opposed to reaching phase 3 in culture), the change in the pattern of synthesized proteins is clear and dramatic.

One alternative concept is that the differences between early and late passage fibroblasts result from cellular senescence (sublethal damage or errors progressively accumulated during the life-span which lead first to a cessation of nuclear replication with lesser effects on other cellular processes). There may be an element of randomness in the occurrence of or sensitivity to such damage, leading to variability in the time required for individual cells to acquire sufficient damage to inhibit replication. Sister cells may receive unequal burdens of damaged cellular components, leading to variability in subsequent interdivision times as shown by Bell *et al.* (*1*).

We agree with Bell *et al.* that when cultured cells leave the cell cycle permanently they may have passed through widely varying numbers of divisions even though the population as a whole does demonstrate a reproducible limit of cell doublings (*2*). We agree also that the relationship between life-span of the ani-

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mal, the maximal proliferative potential of tissues repeatedly transplanted in vivo, and proliferative potential in culture, remains complex. The hypothesis that they are related—in that the kinds of cellular damage that accumulate rapidly in cell culture are similar to the kinds of damage that accumulate over a longer period in vivo—should be thoroughly evaluated. Cell cultures with finite life-spans do therefore form a valid system for the study of aging and, in the case of human aging, the only practical experimental system.

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The adrenocortical cell model system described by Hornsby *et al.* (*1*) for studying regulation of synthesis and secretion of adrenocortical steroids can maintain itself in vitro whether or not the cells are dividing. O'Hare and Neville (*2*) have maintained human and rat adrenocortical cells as mitotically quiescent cultures for long periods (up to 4 months) with no loss of functional competence. The 4-month limit does not reflect cell aging or functional diminution but is due only to technical difficulties such as peeling of cell sheets and multilayering of stromatous elements, and these difficulties can be eliminated (*3*). In fact there is no evidence that rat, bovine, or human adrenocortical cells cannot be kept alive and functioning for very long periods (years) without pressuring them to divide.

Neither adrenocortical cells nor fibroblasts are stem line cells; rather, they are *out* of cycle, so that creating a system in vitro that keeps them *in* cycle is not a model of anything found normally in the organism.

In examining the fitness of the cell aging model in vitro, a model whose chronometer counts cell divisions, we observe that there are trivial causes for the departure of cells from cycle. Periodically new "fixes" are discovered that extend the proliferative life-spans of cultures—fibroblast growth factor, for example, lengthens that for adrenocortical cells by an order of magnitude (*4*). It could be supposed, if the right "cocktail" were formulated, that normal diploid cells in vitro might join the ranks of transformed cells as perpetual proliferators. It seems to us that the only way to measure the age of a cell is to use a conventional clock rather than the population-doubling clock of models in vitro. Nonetheless, such so-called models, particularly the fibroblast model, have been widely used to study cell aging.

The question at hand is why, in such models that impose abnormal—nonphysiological—conditions, cells leave cycle.

We have proposed that departure from cell cycle is a consequence of a particular constellation of culture conditions that causes cells, at least fibroblasts, to drift into an alternative state of differentiation. In so doing, the cells escape the abnormal pressure to divide. We have not said, as Hornsby *et al.* imply, that differentiation is the cause of departure from cell cycle. We have tried, by means of cell lineage analysis, to pinpoint the subpopulation of mitotically quiescent cells present at any population-doubling level and to describe the features that distinguish it from the cycling population. We have recently discussed (*5*) some of the features that may describe a specifically differentiated class of fibroblasts that appears after cells are stimulated mitotically; these features are also characteristic of fibroblasts of granulation tissue (*6*).

We think that Hornsby *et al.* have confused the issue by assuming that the well-defined and quantifiable function of adrenocortical cells in vitro assists in deciding whether cells that leave cycle are further differentiated. They cite steroidogenesis as being unchanged in nonproliferating cells, but they have not determined whether this class of cells is or is not further differentiated; in fact they have not distinguished experimentally between the proliferating and nonproliferating subpopulations present throughout the life history of the culture.

Even after cessation of mitosis the adrenocortical cells in the system of Hornsby *et al.* function as well as before. Cell divisions seem not to compromise their cell type—specific synthetic responses. Nor is there evidence that a cell that leaves cycle is older or closer to death than one which does not. In fact, it would be of interest to know how long the adrenocortical cells that have left cycle can continue to function. But why force a cell population to divide when it can be maintained as a mitotically quiescent functional culture for long periods? In that state the culture constitutes a more appropriate cell aging model because it reproduces conditions like those in the organism.

We do not think that a causal relation between cell divisions and loss of function or decrease of cell life-span has been shown by Hornsby *et al.* or by others; for that reason we reject the idea that cell aging is encoded in the genome. We do think that factors extrinsic to cells, in or out of the organism, can damage cells.

It is the effect of these factors that should be tested in model systems. A severe stumbling block has been the failure to distinguish between the proliferative span of cultures and the life-span of cells. While under certain conditions the latter has been shown to be finite, still little is known about the former.

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