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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References and Notes
7. The eel was anesthetized with a continuous flow of o2 saturated 0.01 percent ethyl m-amino benzoate methanesulfonate (MS-222) over the gills. The swim bladder was exposed by a midventral incision (10-12 cm) and was evacuated with a small incision into the pneumatic duct. The gas-generating region was isolated by a ligature secured around the pneumatic duct—swim bladder junction beneath the overlying retia mirabilia. Atmospheric gas was eliminated from the peritoneal cavity with a small syringe filled with 0.9% saline, and the overlying retia were ligated. The eel was then cut into 4 to 5 cm lengths and the gas content of the swim bladders was determined from the volume of gas released into the headspace of the chamber during the course of the experiment. The volume of gas released was measured with a glass graduated pipette.
8. The temperature in the aquatic medium was 15°C, and the pH was 8.3. The concentration of dissolved oxygen was 9.0 mg/l. The oxygen concentration was determined by the Winkler method and the pH was determined by the glass electrode.
9. The results were consistent with the findings of previous studies (16-18). The authors observed that the swim bladder volume of eels increased linearly with the pressure, while the volume of gas deposited by the swim bladder decreased hyperbolically relative to the pressure. The authors also observed that the rate of gas deposition and the rate of diffusive gas loss in silver eels were lower than in yellow eels. Therefore, the volume of the swim bladder of silver eels can be maintained at a depth greater than that of yellow eels.

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 not 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 fully 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 hydroxylase 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 life span 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-
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 chromometer 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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