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14. We thank H. J. Ralston for reviewing this paper and E. Calaustro, G. Thoren, and D. Akers for preparing histological and photographic material. This work was supported by grants NS 14627, NSF BNS78-24762, DA 01949, and research career development award NS 00364 to A.I.B. A.I.B. is a Sloan Foundation Fellow.

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Swim Bladder Volume Maintenance Related to Initial Oceanic Migratory Depth in Silver-Phase *Anguilla rostrata*

Abstract. *Gas deposition rates in the swim bladders of postmetamorphic (silver)* Anguilla rostrata *eels are about five times greater than those of premetamorphic (yellow) individuals. This extends the maximum depth at which silver eels can maintain swim bladder volume and prepares them for their spawning migration to the Sargasso Sea.*

The migration of the American eel *Anguilla rostrata* from shallow freshwater and estuarine environments to its spawning ground in the southern Sargasso Sea may occur at depths exceeding 2000 m (*1*). Before its migration offshore, *A. rostrata* undergoes a metamorphosis in which there are alterations in integument pigmentation (*2*), the visual sensory system (*3*), and the swim bladder (*4, 5*). Of these, the transformations in swim bladder morphology are most clearly related to the pronounced increase in depth during migration. The swim bladder retia mirabilia capillaries undergo a 2- to 3-fold increase in length and a 1.5-fold increase in luminal diameter, enhancing their calculated countercurrent exchange efficiency by over 300 percent (*4*). A 1.5-fold increase in the crystalline guanine content of the swim bladder wall reduces diffusive gas loss from the swim bladder by at least 15 percent (*5*). Even with this reduction, diffusive gas loss increases with depth because of the increase in the partial-pressure gradient of gas across the swim bladder wall. This volume must be replaced in order to maintain the energetic advantage for horizontal swimming imparted by neutral buoyancy (*6*).

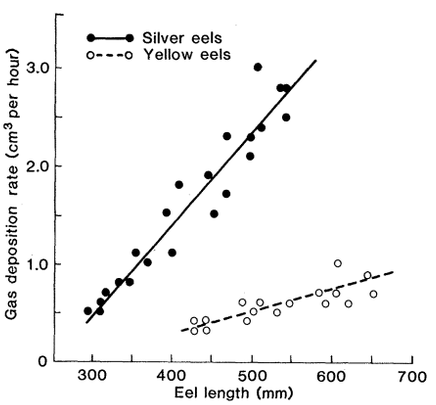

Fig. 1. Rate of swim bladder gas deposition compared with length of yellow and silver eels. Linear regression equations for the plotted lines are as follows: for yellow eels, $y = -0.7370 + 0.00246x$ ($r = .847$); for silver eels, $y = -2.3781 + 0.00942x$ ($r = .957$).

I measured gas deposition rates in yellow (premetamorphic) and silver (postmetamorphic) eel swim bladders and calculated the maximum depths at which their volume can be maintained. Eighteen yellow eels and 22 migrating silver eels, trapped in fresh and brackish water in Rhode Island during the fall of 1978, were gradually adapted to seawater and kept for 2 to 8 weeks before analysis. Determination of metamorphic phase was based on integument pigmentation and retial capillary length (in yellow eels < 2.5 mm; in silver eels > 4.5 mm).

Methodology included preliminary surgical procedures to evacuate gas from the swim bladder and pneumatic duct, followed by isolation of the gas-generating region from the gas-reabsorbing pneumatic duct (*7*). Evacuation stimulated the eel to initiate gas deposition. The rate of deposition was determined indirectly as the change in buoyant mass of the eel over a test period during which it was allowed to swim freely in a blacked-out 150-liter aquarium (*8*). For every cubic centimeter of gas generated, buoyant mass decreased 1.07 g (*9*).

Swim bladder gas deposition rates of silver eels average about five times those of equivalently sized yellow eels (Fig. 1). The linear relation between gas deposition rate and eel length is due to the elongated configuration of the body and swim bladder. Yellow eels less than 430 mm long were not tested because it was impossible to pass a ligature beneath their short retia mirabilia. Gas deposition rates of yellow *A. rostrata* are within the range reported for *A. anguilla* (*10*).

Swim bladder gases collected from ten silver eels immediately after the test period were analyzed (*11*) and found to contain 83 to 90 percent oxygen (mean, 86 percent), 7 to 13 percent carbon dioxide (mean, 10 percent), and 3 to 5 percent nitrogen (mean, 4 percent). The carbon dioxide fraction is slightly higher than the average value (7 percent) reported for *A. anguilla* (*12*).

The higher gas deposition rate in silver eels may result from a number of morphological and physiological transformations during metamorphosis. Gases deposited into the swim bladder are released from blood circulating through the vascular loop between the retia mirabilia and the acid secretory cells of the swim bladder parenchyma (*13*). An increase in the volume of blood flowing into this system increases the amount of gas available for deposition. Comparative measurements are not available for blood flow rates through the retia mirabilia of yellow and silver eels during gas deposition. However, the increase in luminal diameter of the retial capillaries during metamorphosis should permit an increase in total blood flow, making more gas available for deposition. Metamorphic increase in retial capillary length should contribute to maintenance of countercurrent function at higher rates of blood flow. Also, the amount of gas available for deposition can be increased by an elevation in hematocrit. In *A. anguilla*, hematocrit increases from 26.5 percent in yellow eels to 36.4 percent in silver eels (*14*). Because both species undergo nearly identical transformations during metamorphosis (*2–4, 15*), it is likely that *A. rostrata* also develops an elevated hematocrit.

The rate of diffusive gas loss by the

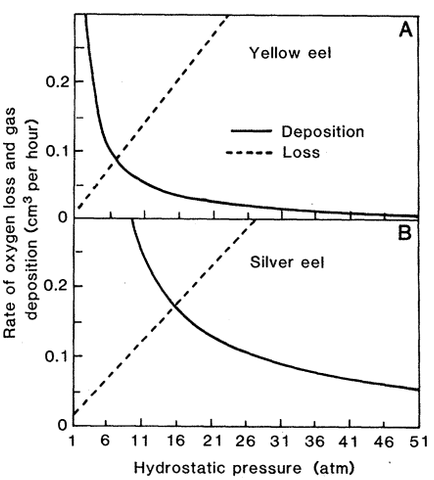

Fig. 2. Rates of swim bladder oxygen loss and gas deposition relative to hydrostatic pressure for 545-mm yellow (A) and silver (B) eels (*18*). The intersection of the two curves represents the maximum pressure at which swim bladder volume can be maintained. It is assumed that the number of moles of gas generated per unit of time remains constant with increasing depth.

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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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7. The eel was anesthetized with a continuous flow of cooled, aerated 0.01 percent ethyl *m*-aminobenzoate methanesulfonate (MS-222) over the gills. The swim bladder was exposed by a midventral incision (35 to 50 mm) 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 coelom with a marine-fish Ringer solution as the midventral incision was sutured. A 2-hour recovery period after revival allowed dissolution of gas remaining in the coelom and the initiation of gas deposition.
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.)
9. This was determined by weighing ten moribund eels in fresh water before and after adding known volumes of gas to the swim bladder through a cannula.
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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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