of RNA secondary structure. It has been shown that changing the secondary structure of bacteriophage f2 RNA by treatment with formaldehyde increases its initiation efficiency. This was assayed by measuring the binding of formylmethionine (fmet)-tRNA to *Escherichia coli* ribosomes (*8*). In order to study the effect of changes in RNA secondary structure on the rate and extent of protein synthesis and the possible relation of these changes to drug treatment, both myeloma mRNA and TMV RNA were heated before translation. When the RNA was heated for 3 minutes at 60°C in 28 m*M* Hepes, *p*H 7.1, or distilled water and chilled immediately, the rate and extent of protein synthesis produced by heat-treated RNA's were increased up to twofold as compared to the controls which contained RNA that had not been heated. Neither drug stimulated the translation of heat-treated RNA further. Heating mRNA changes its secondary structure, possibly making it more available for translation. The rapid chilling of the samples prevents the reforming of those structures. The fact that actinomycin D and cordycepin do not further stimulate translation once the RNA has been heated suggests that both drugs may be altering the secondary structure of RNA.

These in vitro studies show that actinomycin D and cordycepin do affect the incorporation of [^{35}S]methionine into protein. Although we do not understand the basis for this phenomenon, it is not restricted to one mRNA nor to one in vitro protein synthesis system. Before it is possible to extrapolate these findings to in vivo translation, studies should be done on intact cells and on other in vitro systems. However, our experiments demonstrate a significant effect of actinomycin D and cordycepin on in vitro translation which should be taken into consideration in the use of these drugs.

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Overlapping Platelets: A Diffusion Barrier in a Teleost Swimbladder

Abstract. *Overlapping platelets are layered within the connective tissue of the wall of a closed (physoclistous) swimbladder. The close, staggered arrangement of the platelets is viewed as a physical barrier that can interfere with the diffusion pathway of gas molecules. The result is a more efficient retention of gas pressures within the swimbladder.*

Fishes with swimbladders demonstrate remarkable variation in their abilities to actively secrete free gases into the organ. Physostomous fishes retain a functional connection—the pneumatic duct—between the swimbladder and the alimentary tract. Physoclistous fishes lose the connection during development. Gas secretion has been demonstrated in many physostomes; however, it is among the physoclists that gas-secreting abilities are most highly developed. The gas-filled luminal space, important for hydrostatic equilibrium, is maintained against the pressure of water at the depth in which a fish may live. This pressure increases approximately 1 atm per 10 m of depth. Fishes that secrete oxygen against great pressures, particularly those that inhabit a depth of 1000 m or more, are known to have as much as 85 to 95 percent oxygen within the swimbladder lumen (*1, 2*). Thus, oxygen pressure approaches 100 atm and oxygen transport is against a considerable gradient (*1, 3–5*). Fishes die quickly when exposed to water in which dissolved oxygen is equivalent to elevated oxygen pressures found within their own swimbladders (*6*). Oxygen pressures in excess of 5 atm are generally toxic to living tissues (*7*). Many fishes—particularly physoclists—are therefore faced to a varying degree with two physical necessities: (i) the secretion of free gases against pressure and (ii) the retention of those gases within the swimbladder lumen in order to minimize the load on the secreting mechanism and, in many cases, to prevent diffusion pressures that may reach toxic levels. The former problem has received considerable attention (*3, 8, 9*), the latter, relatively little. It has been shown, however, that the swimbladder wall can be 100 times less permeable to gaseous diffusion than normal connective tissues (*10*). Crystalline material, composed principally of guanine, in an outer layer of the swimbladder wall has been described as a barrier against diffusion in a few species (*5, 10, 11*). In other species, dense collagen is believed to be a barrier (*12*). We now describe another structure that may provide a physical barrier to gaseous diffusion in the swimbladder walls of the mummichog (*Fundulus heteroclitus*), gulf killifish (*Fundulus grandis*), sheepshead minnow (*Cyprinodon variegatus*), and sailfin molly (*Poecilia latipinna*).

The luminal surface of a typical swimbladder wall is bounded by a layer of epithelial cells. Anteriorly, the epithelium is thickened, highly vascularized, and secretory in nature. The rete mirabile, a countercurrent exchange multiplier of blood gases (*13*), is most anterior. These two tissues are implicated in the functional release of gases into the lumen (*8, 14*).

Within the submucosal region of the swimbladder wall (*15*) in those species we have investigated, can be seen round, flattened platelets (Figs. 1 and 2) dispersed throughout the loose fibroelastic connective tissue. The platelets vary in diameter from approximately 10 to 80 μm, although most are in the range between 40 to 60 μm. Many, but not all, exhibit a single nucleus that is most often centrally located. A minute central hillock is seen on the surface of many of the platelets. Since adjacent platelets consistently overlap one another by one-half of their diameters, the hillock may have the function of preventing complete superimposition. Such an orderliness would increase the efficiency of any given number of platelets as diffusion barriers.

The entire swimbladder wall thickens and the relative abundance of platelets is greatest about the gas-secreting mechanisms. Posteriorly, the wall is relatively thin, vascularization is much reduced, and the platelets are less concentrated. Light-microscope observations of whole tissue can be made most easily in this region, and the platelets can be clearly seen in face view (Figs. 1 and 2). Phase-

contrast or interference-contrast microscopy reveals them in fresh, unfixed, unstained tissue. For photography, the phase-contrast image was improved by fixation in buffered formaldehyde (4 percent) for 20 to 30 minutes, followed by brief rinsing and treatment for 3 to 5 minutes with aqueous potassium permanganate (1 percent). Treated tissues were mounted in glycerin.

In contrast, their presence is almost impossible to ascertain in cross section with the light microscope. We find the explanation at the magnification of the electron microscope. Our studies indicate that each platelet is composed of numerous circular membranes superimposed on each other (*16*). With the light microscope, optical summation of 10 to 20 membranes whose edges are in register results in a platelet easily seen in face view. In cross section, however, the individual membranes are difficult to resolve, in contrast to the other connective tissue components. This may explain, in part, why the platelets have escaped previous notice.

The overlapping nature and the regional distribution of the platelets in a tissue known to retain free gases leads to the interpretation that they could act as physical barriers to gas diffusion.

The fine structural nature and the cytoplasmic origin of the platelet membranes have not been completely described as yet.

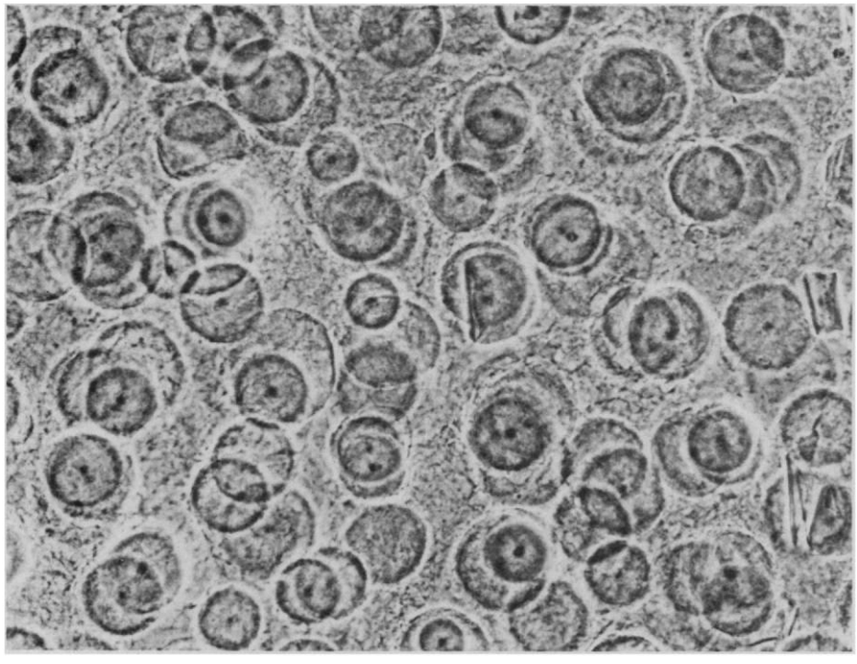

Fig. 1. Phase-contrast photomicrograph of posterior portion of swimbladder wall (*Fundulus grandis*). Tissue was fixed in buffered formaldehyde (4 percent), and the peritoneum was stripped away. Treatment after fixation with potassium permanganate (1 percent) improves detail. Frontal whole mount (× 200).

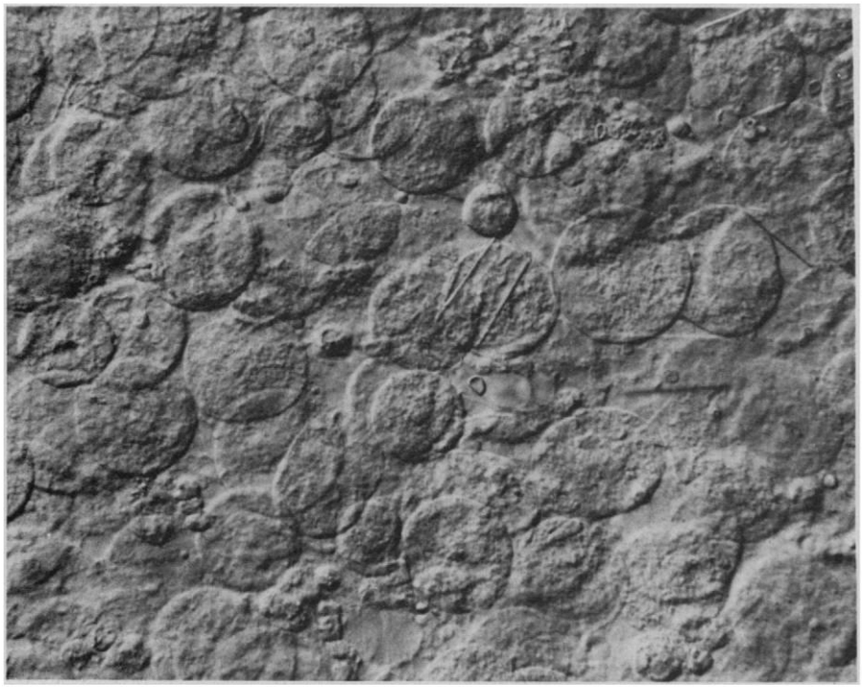

Fig. 2. Overlapping platelets as seen by interference-contrast microscopy upon stripping away the peritoneum overlying the swimbladder wall (*Poecilia latipinna*). Tissue is unfixed and unstained (× 250).

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