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Vertebrate Metamorphosis

Biological teaching in its manifold aspects emphasizes cyclical development in nature. In his article George Wald gives interesting details, supported by chemical evidence, of two opposed metamorphoses in fishes, one bringing a fish to maturity, the second one returning it to its natal environment [*Science* **128**, 1481 (1958)]. He admits that in land vertebrates physiological changes of the second type do not take place but contends that the entry of a single representative cell, the spermatozoon, into the womb is an analogous event, leading to the completion of the life cycle of such animals in water.

This appears to be open to question. What I find far more objectionable, however, is his use of a Biblical quotation, which is cut short, obviously to lend support to his thesis. To Nicodemus' question as to how a man could be reborn, "can he enter the second time into his mother's womb, and be born? Jesus answered, Verily, verily, I say unto thee, Except a man be born of water . . ." (this is where Wald leaves off). However, the sentence continues: ". . . and of the Spirit, he cannot enter into the kingdom of God." Nobody who knows anything at all about Christian teaching would believe that Jesus is talking about physical rebirth. He is solely concerned with spiritual conversion.

To prevent misrepresentation of this kind in the future, I believe it would be wise for the editors of *Science* to check carefully on authors' use of references from fields other than their own.

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Quantitative Gram Reaction

The semiquantitative evaluation of the Gram reaction reported by T. Mittwer (*1*) is based on applying small amounts of stained and iodinated suspensions of bacterial cells as spots to filter paper, as in paper chromatographic techniques. The resulting streaks of crystal violet are compared for length, as it is assumed that variation in length depends upon the degree of Gram staining behavior, the most Gram-positive species showing the longest streak.

In principle Mittwer's method appears to be a suitable one. However, his staining of bacteria with an overdiluted crystal violet solution (0.1 percent) reduces the relevance and reliability of his evaluation of the degree of Gram-positive behavior. This is especially so since, as Barbaro and Kennedy have conclusively demonstrated, an increase in dye concentration is accompanied by a *differential* dye uptake between Gram-positive and Gram-negative bacteria (*2*). These authors used 10-percent solutions of crystal violet in accordance with the recommended range (1 to 10 percent) of the Gram staining procedure (*2*). My model experiments also illustrate the fact that uptake of crystal violet by proteins can be substantially increased by raising the concentration of the dye. For example, in 10 minutes 1 mg of casein and 1 mg of iodinated casein take up 1.7 and 2.6 μg, respectively, of crystal violet from a Tris-buffered (*p*H 7.2) $10^{-5}M$ dye solution at 20°C; a sixfold increase in dye concentration raises the previous values to 4.2 and 7.2 μg, respectively. The data were obtained with the aid of a Perkin-Elmer Spectracord spectrophotometer at $H_2O = 595$ mμ. If, however, we deal with bacteria rather than proteins, a suboptimal range of 0.1-percent in dye concentration must be considered. In and below that critical range the differential in crystal violet uptake between a Gram-positive and a Gram-negative organism ceases to exist (*3*).

Finally, it should be pointed out that a semimicro method for measuring the degree of Gram-positive staining behavior of bacteria and other biological material has been published (*4*). We used a concentration of 1-percent crystal violet and a chromatographic technique, separating the total amount of dye taken up by bacteria into compact spots; then the spots were eluted, and their dye content was quantitatively determined with a spectrophotometer.

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4. Society of American Bacteriologists, *Manual of Methods for Pure Culture Study of Bacteria* (Geneva, N.Y., 1946), vol. 4, pp. 51–58; R. Fischer, *Naturwissenschaften* 45, 287 (1958).

Roland Fischer's comment appears to be based upon my use of an "overdiluted" solution of crystal violet. It is true that an increase in dye concentration can be correlated with an increase in "dye uptake" by a constant amount of bacterial cell material, with the time of contact constant (*1*). However, no reduction in the reliability of my method should result from the use of 0.1-percent dye solution, since numerous tests have established the validity of this concentration in routine qualitative Gram differentiation. Such tests can be confirmed by anyone in a few minutes. In fact, a rather

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wide range of concentrations may be used, and some popular modifications of the Gram stain employ crystal violet near this concentration—for example, Nicolle's (0.33 percent) (*2*). At relatively higher concentrations (1 to 2 percent), the bacteria clump and the dye polymerizes, causing uncertainties in the interpretation of work of this and similar types.

The work of Fischer (*3*) and of Fischer and Zaleschuk (*4*) deals with a method of measurement of crystal violet taken up by various biological materials. This is applicable to the Gram reaction only if one accepts Fischer's statement that "gram positiveness is related to the amount of primary dye absorbed" (*3*). This is not necessarily "conclusively demonstrated," since other studies have shown that crystal violet uptake by bacterial cells is not correlated with their Gram character (*5*). As a matter of fact, the precise and extensive data presented by Wensinck and Boevé (*6*), as cited above by Fischer, indicate that at a dye concentration of about 0.1 percent, a "differential in crystal violet uptake between a Gram-positive and a Gram-negative organism ceases to exist." Since I have shown that Gram differentiation readily occurs when dye of this concentration is used, it appears that measurement of dye uptake does not suffice as a measure of Gram positiveness. The Gram differentiation seems to depend more upon the integrity of the cell membrane or membranes and the relative permeabilities of these membranes to the decolorizing solvent (*6, 7*).

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Echo Ranging in the Porpoise

W. N. Kellogg's paper "Echo ranging in the porpoise," which appeared in a recent issue of *Science* [**128**, 982 (1958)], causes me to wonder about the efficacy of the journal's referee system (or that part of the editorial procedure used instead).

To my knowledge, echolocation in a marine animal was experimentally "demonstrated for the first time" by W. E. Schevill and B. Lawrence of Harvard University and the Woods Hole Oceanographic Institution. Their paper, "Food-finding by a captive porpoise (*Tursiops truncatus*)," appeared as No. 53 of *Breviora* in April 1956 and is discussed at some length in one piece of literature Kellogg cites (Donald R. Griffin's *Listening in the Dark*).

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I am sorry about the omission of a reference to the Schevill and Lawrence paper (*1*) from my recent article in *Science*. The omission was entirely my responsibility and not that of the editors of *Science*. It would have been better to have included it. However, whether the *Breviora* article actually "demonstrates" anything is a matter of opinion. In a way it is regrettable that Backus has raised the issue, for this leaves me no alternative but to point out why the *Breviora* paper fails to prove echolocation, and consequently why the omission was not really a serious one.

It is a basic rule of good research that the variables involved must be properly controlled. This becomes particularly important in a difficult field involving a unique perceptual avenue like echolocation. Under these circumstances we are quite unable to see how leaning over the side of a small boat and feeding fishes to a porpoise by hand can be construed as "demonstrating echolocation." Of course Schevill's porpoise found the fish which he offered, as the title of his article indicates, but from the descriptions given it is impossible to tell what method the porpoise employed to locate the bait. Almost any animal—marine or otherwise—will seek and find food which is near it.

To determine whether a porpoise reacts to the echoes of its own noises, one should certainly not introduce extraneous auditory signals which might help guide the animal to its goal. Slapping the water upon the insertion of the fish—a practice followed in a good deal of the work reported in the *Breviora* article—is the very thing not to do. It simply confuses the issue by telling the animal where (or where not) to go.

Even more serious is the failure to eliminate crucial visual stimuli. Not only can porpoises see through the water and in the air, but they also view objects in the air from a swimming position beneath the surface of the water. Since no visual screening was employed in the *Breviora* study, there would seem to be no reason why the animal could not have observed the movements and postures of