Letters

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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References

1. T. Mittwer, *Science* 128, 1213 (1958).
2. J. F. Barbaro and E. R. Kennedy, *J. Bacteriol.* 67, 603 (1954).
3. F. Wensinck and J. Boevé, *J. Gen. Microbiol.* 17, 401 (1957).
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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