Letters

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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

the feeder or feeders, silhouetted against the sky over the side of the boat, on every trial which was made. Having one feeder hold a fish while a second held nothing fails to solve this problem. Unknown and unsuspected optical stimuli may always have been present. The only adequate solution is complete screening to eliminate entirely the human observers as sources of uncontrolled visual cues. Research in comparative psychology has many times demonstrated this fundamental principle (*2*).

The porpoise in the *Breviora* study—even with poor eyesight—may therefore have located its fish (i) by watching the experimenters from beneath the water and noting the position of their bodies, arms, and hands; (ii) by orienting (in some cases) to the sound of the surface splash; or (iii) by echolocation; or by a combination of these methods. The methods of investigation employed, it would appear, do not permit us to say in just what way the animal discovered the fish. When possibilities other than echolocation remain uncontrolled, then echolocation has not been demonstrated to exist as a primary causal factor.

Perhaps the best that can be said of the *Breviora* evidence is that it is inferential. Other inferential evidence, which may be less questionable, was furnished by Kohler, Morris, and me in 1953 (*3*), when we obtained recordings of the actual echoes from porpoise noises and measured the duration of some of these echoes. In fact, I discussed echolocation in the porpoise as a possibility as far back as 1952 (*4*). It should be noted in this connection that two separate papers by Schevill and Lawrence concerning their earlier work with a different porpoise state clearly that "we never heard any sound from her [the porpoise] that could be in any way related to her navigation or food-getting" (*5*), and another states that there was "a complete lack of any evidence that she was using echolocation" (*6*). The most recent of these statements appeared in print in 1954.

It is gratifying to observe that these authors have finally conquered their long-time determination to ride the wrong horse and are now trying to get up on the right one.

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