Thus, when food or water have been used as unconditioned stimuli in similar experiments, approach and contact of a lighted key (conditioned stimulus) could be considered part of normal eating or drinking behavior, redirected toward a new stimulus.

Observations of a broody hen with young chicks in my laboratory suggest, however, that approaching, pecking, and snuggling are part of the normal heat-seeking behavior of young chicks. I have kept a mother hen with small groups of 3- to 8-day-old chicks in a large observation cage (1.2 by 2.4 m) for classroom demonstrations. On many occasions I have observed that one or more of the chicks will approach the hen—which may be feeding or standing quietly—and begin pecking the feathers on the underpart of her body. Such behavior is usually followed by snuggling, in which the chick rubs and pushes its head up into the hen's feathers. These behaviors appear to stimulate the hen to sit. The sitting hen makes sounds and movements that then attract the other chicks to be brooded.

The hen initiates brooding on many occasions, but she is less likely than normal to sit and initiate brooding in the observation cage when she is slightly disturbed by the presence of an audience. Under these circumstances, the chicks become cool and frequently show the pecking and snuggling behaviors described above.

In light of these observations, one can interpret the behavior of Wasserman's chicks toward the lighted key as part of normal heat-seeking behavior redirected toward a new stimulus. Thus, it seems premature to postulate any new determinants of the form and direction of the conditioned responses in conditioning studies.

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Hogan describes the behavior of young chicks in the presence of a broody hen as frequently involving approach, pecking, and snuggling behaviors. He further suggests that these observations bear upon the conclusions I drew from an investigation employing heat stimulation (*1*). In that study, chicks in a cooled chamber were irradiated from an overhead heat lamp at random intervals. Experimental subjects had each heat presentation signaled by the lighting of a small key, whereas control subjects received random presentations of key-light and heat stimuli. The results were that only the experimental subjects learned to approach and to peck at or snuggle with the lighted key. These conditioned responses arose despite the fact that diffuse warming of the chick's chamber elicited reduced locomotion, extension of the wings, twittering, and eye closure.

I interpreted these results as supportive of the view that, in addition to the behavior-eliciting properties of unconditioned stimuli, such physical characteristics of conditioned stimuli as their accessibility and localizability may participate in determining the form and direction of responses conditioned with Pavlov's procedure (*2*). This proposal represents an elaboration of the principle of stimulus substitution, which holds that the topography of the conditioned response ought to be a "replica" of the unconditioned response (*3*). The conditioned responses that I observed seemed to be more directly related to the physical properties of the small lighted key than to the increase in ambient temperature. Woodruff and Williams (*4*) have also made observations that call for a modification of the stimulus-substitution hypothesis. These researchers paired the lighting of a small key with the delivery of water directly into the mandibles of thirsty pigeons. Although the introduction of water into the bill did not elicit directed skeletal behavior, but rather swallowing, subjects learned to approach and contact the lighted key. Both of these studies clearly indicate that directed skeletal behaviors may be conditioned to localized conditioned stimuli even when they are not elicited by the reinforcing stimulus.

Hogan's comments do not distinguish between the behaviors that precede the reinforcing stimulus and those that follow its reception. At issue is how to explain the control of appetitive "heat-seeking" behaviors as distinct from the factors that control consummatory "heat-elicited" behaviors (*5*). With this distinction in mind, it may be that Hogan's observations do not refute my earlier conclusions—they may even support them. If we assume that the broody hen serves the dual functions of a localized visual stimulus and a heat source, and that the sight and warmth of the hen have been repeatedly paired during the chick's first week of life, then approach and contact may come to be controlled by the former stimulus property while body lowering and wing extension are evoked by the latter (*6*). Here, laboratory findings permit us to unconfound and elucidate naturalistic observations.

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References and Notes

1. E. A. Wasserman, *Science* **181**, 875 (1973).
2. When the lighting of a nickel-sized key is repeatedly followed by food, hungry pigeons learn to approach and peck the lighted key [P. L. Brown and H. M. Jenkins, *J. Exp. Anal. Behav.* **11**, 1 (1968)]. However, when auditory stimuli are paired with grain, subjects do not frequently engage in pecking at the speaker or any other environmental features [J. Bilbrey and S. Winokur, *ibid.* **20**, 323 (1973); B. Schwartz, *ibid.*, p. 17; G. W. Farthing, *Psychonom. Sci.* **23**, 343 (1971); E. A. Wasserman, thesis, Indiana University (1972)]. Presumably, the different physical characteristics of punctate visual stimuli compared to diffuse auditory stimuli are responsible for the failure of appreciable pecking to develop with auditory signals. Further evidence and discussion bearing on this issue have been presented by D. Bindra [*Psychol. Rev.* **81**, 199 (1974)]; E. A. Wasserman [*Anim. Learn. Behav.* **1**, 198 (1973)]; E. A. Wasserman, S. R. Franklin, E. Hearst [*J. Comp. Physiol. Psychol.* **86**, 616 (1974)]; E. A. Wasserman and S. B. McCracken [*J. Exp. Anal. Behav.* **22**, 39 (1974)].
3. A discussion of various interpretations of the stimulus-substitution notion was presented by H. M. Jenkins and B. R. Moore [*J. Exp. Anal. Behav.* **20**, 163 (1973)].
4. G. Woodruff and D. R. Williams, paper presented at the 1974 meeting of the Eastern Psychological Association, Philadelphia, Pa.
5. The distinction between appetitive and consummatory responses was discussed by W. Craig [*Biol. Bull.* **34**, 91 (1918)].
6. A similar analysis of imprinting has recently been proposed by H. S. Hoffman and A. M. Ratner [*Psychol. Rev.* **80**, 527 (1973)].

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Viscosity of Cellular Protoplasm: What Do Spin Probes Tell Us?

Keith and Snipes (*1*) recently studied the electron spin resonance (ESR) line shape of a spin probe (tempone) dissolved in cellular protoplasm. By comparing the line shapes with those from the spin probe in glycerol-water mixtures of known viscosity, they concluded that protoplasmic viscosity in some cells is many times that of pure water.

According to the Stokes-Einstein hydrodynamic approach, viscosity should

be inversely proportional to the translational diffusion coefficient and directly proportional to the rotational correlation time. Early measurements of nuclear magnetic resonance (NMR) line widths in muscle (*2*) suggested that the rotational correlation time for protoplasm water was at least an order of magnitude greater than for ordinary water. However, it was subsequently demonstrated that the diffusion constant of at least 95 percent of this water was no less than about one-half that of ordinary water (*3*). Recent studies (*4*) suggest that the correlation time for most muscle water is very close to that of ordinary water and that the NMR line widths are greatly influenced by a small fraction (perhaps 5 percent) of muscle water with increased correlation times.

We suggest that ESR line widths of spin labels dissolved in protoplasm may also be subject to ambiguous interpretation. If spin labels rapidly diffuse in and out of local environments with different viscosities, an observed line width may reflect motional information that is the time average in the various environments. Considering a simple two-state model, the measured line width, W, could be given by the sum $(W_1x_1 + W_2x_2)$, where W_1 and W_2 are the line widths in the two environments and x_1 and x_2 represent the fractional time weighting factors for the environments. The line widths are assumed to be proportional to the spin label rotational correlation time, τ, which would be given by the sum $(\tau_1x_1 + \tau_2x_2)$.

If environment 1 represents the water adjacent to macromolecules and membranes, τ_1 could be of the order of 10^{-7} second (*4*). We will assume that τ_2 has a value close to that of ordinary water, $\sim 0.5 \times 10^{-10}$ second. Thus, even if the spin labels spend only 5 percent of the time in environment 1 (that is, $x_1 = 0.05$ and $x_2 = 0.95$) the correlation time obtained from ESR line widths would be of the order of 0.5×10^{-8} second. This demonstrates, we believed, that the correlation time obtained from the ESR line width of spin labels dissolved in protoplasm may not reflect the true viscosity of protoplasm.

Sachs and Latorre (*5*) have recently studied the solvent structure of barnacle muscle cytoplasm by using the spin label technique. They found that ESR line widths depended on water content. Their findings are consistent with exchange averaging if the water content in one environment changes while that in the other remains relatively constant.

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In the analysis by Finch and Harmon, where the rotational correlation time, τ, is given by the sum $(\tau_1x_1 + \tau_2x_2)$, it is assumed that the line widths are linearly proportional to the measured rotational correlation time, τ_c, for values of τ_c from 0.5×10^{-10} to 10^{-7} second. This is not the case. For example, the high-field tempone hyperfine line has a width of 0.6 gauss when $\tau_c = 0.5 \times 10^{-10}$ second. The assumed proportionality would predict that, for $\tau_c = 10^{-7}$ second, the high-field line width would be 1200 gauss. The overall spectral breadth for tempone, even in the completely immobilized state, is about 78 gauss. Nitroxide ESR lines are inhomogeneously broadened by anisotropies in the hyperfine coupling and g-factor. The line widths are proportional to τ_c only in the range of motion where the dominant effect on line width is the degree to which the spectral anisotropies are averaged away.

A second consideration is what constitutes "rapid" diffusion in and out of local environments with different viscosities. For the observed line width to reflect a time average of motional information in the various environments, this diffusion must be rapid on the time scale of ESR measurements. Quantitatively, the spin label must diffuse in and out of the separate environments in a time of the order of that given by the anisotropic hyperfine coupling energy, expressed in frequency units. For tempone, this is about 25 gauss (*1*), or 70 Mhz. For rotational narrowing to reflect the average of two environments, the spin label must therefore diffuse in and out of these two environments in approximately 1.4×10^{-8} second. However, one of the two environments is assumed to be highly viscous ($\tau_c = 10^{-7}$ second) and will severely restrict translational diffusion of the spin label.

For a given molecule, the Stokes-Einstein equations for the relation of viscosity to τ_c and to the translational diffusion coefficient, D, can be combined to give $D = 0.22\ r^2/\tau_c$, where r is the molecular radius. This assumes that the effective viscosity for rotation and translational diffusion are the same. This makes it possible to calculate D for tempone in any environment of assumed τ_c and therefore provides an estimate of the linear distance diffused in 1.4×10^{-8} second. Even though diffusion must, at some time, occur in an environment where $\tau_c = 10^{-7}$ second in the example discussed by Finch and Harmon, we take a liberal value of $\tau_c = 10^{-9}$ second for our present purpose. With $r = 3$ Å for tempone, $D = 2 \times 10^{-7}$ cm^2/sec, and the linear distance diffused in 1.4×10^{-8} second is 5.3 Å. This illustrates that, within the time interval relevant to the averaging of hyperfine anisotropies, the spin label diffuses translationally a distance that is less than its own diameter.

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Viscosity of Cellular Protoplasm: What Do Spin Probes Tell Us?

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