the unequal θ conditions are some distance from their asymptotes, but their relative positions are the same under both conditions of reinforcement.

Different schedules of reinforcement thus produce large differences in rate and temporal patterning of response. It seems safe to conclude, however, that despite these effects, rate of response is a sensitive datum for the evaluation of probabilistic predictions. This is of importance, for it makes possible direct extensions of current learning models to more general experimental conditions than have hitherto been employed.

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## Food or Training in Paramecium?

From behavioral and biological studies of the protozoan *Paramecium aurelia,* we hope to get generalizable information about relations between intracellular dynamisms and behavior.

In a series of experiments I have investigated a response by which food-deprived *P. aurelia* can be induced to cling to the sides of a clean and sterile platinum wire after having been exposed to the wire when it was baited with food. It has been suggested (*1*) that the organisms' approach to the clean wire after training is a response to bacterial material that was previously left in the culture and nothing more. This would seem to mean that exposure of paramecia to food with wire would not have any very different effect from exposure to food alone.

To test this notion, two experiments were performed to investigate the effects of various amounts of wire presentation (*2*). In one experiment, a microdrop of bacterial suspension (food) was introduced at the *edge* of a depression containing a "hungry" culture of paramecia, while the clean wire was simultaneously lowered into the middle of it. After 8 minutes, the wire was removed. Control cultures received the food but not the wire. After 30 minutes, the clean and sterile wire was introduced into both kinds of cultures. The experimental culture, which had had food and wire simultaneously, ringed the wire significantly more than did the controls ($p < 0.02$).

In another experiment, two wires were used, one 3 times the diameter of the other. On the larger wire, 3 times as many wipes of bacteria were applied as on the smaller, but the smaller wire was immersed in the paramecium culture 3 times as often, with shorter time intervals between immersions. Total duration of training period, amount of food, and area of wire exposed were equated for both groups, but the time of exposure to wire was 3 times as long in one group as in the other. The cultures which had longer exposure gave the wire-clinging response on tests, while the large-wire, shorter-exposure group did not noticeably exceed zero. For the difference between the groups, $p$ was less than 0.01.

In all experiments, "trained" cultures have been routinely stirred up by rotation of the slide before placement on the microscope stage for final tests. Yet, when the wire is lowered, paramecia come to it. The response, in a good culture, is a slow and direct swoop toward the wire, different from any other behavior we have observed.

A response of lying motionless at the bottom seems to be built into the organism. When isolations are being made with a micropipette, many paramecia settle motionless to the bottom of the depression when the pipette is reintroduced. In "training" experiments, this lying down usually appears by the fifth descent of the wire and can be elicited as readily by a clean wire as by one which is baited with food. The response of actually clinging to the side of a clean wire, or remaining motionless in a limited area, is quite unusual.

If modification of behavior is due to presence of carbon dioxide or of bacterial food, and only to this, then change in training schedules (*3*) or in life-history (*4*), or from light to darkness (*5*), with food reinforcement administered similarly throughout, should affect strength of response only to the extent of chance variability, but the differences were found to be highly significant statistically.

We have tried to repeat Jensen's experiment with paramecia in the following way. We used media and bacteria on which paramecia were being satisfactorily maintained at the time, since a strain of, say, *Aerobacter areogenes* on which satisfactory cultures of *P. aurelia* have been bred for some time may suddenly become inadequate or even lethal (*6*). One drop of a suspension of bacteria in medium was added to a moderately food-deprived culture of paramecia such as we usually use in training experiments. To a matched culture, a drop of distilled water was added. A drop from each culture, was placed on a bacteriological slide, with a space of about 1 mm between the two drops. The two drops of paramecium culture were joined by drawing a narrow bridge of fluid between them. In one case, the bridge was drawn from the clear to the bacteria-clouded drop. In the other case, the bridge was drawn in the opposite direction. Fluid from each drop diffused into the other, forming clearly discernible phases of bacterial dilution.

When the bridge was drawn from the clear drop to the cloudy one there were some 48 paramecia in each drop. As time passed, the feeding paramecia slowed down, but at no time were any entirely motionless. After 1 hour, there were 21 animals in the cloudy side; 74 in the originally clear side, which by now showed a large infusion of bacteria; and some three paramecia in the bridge between the drops. This difference, which is opposite to what Jensen found, is significant beyond the 0.001 level of confidence.

When the bridge was drawn from the cloudy drop to the clear one, 25 paramecia were in each drop, exactly as Jensen reported. Again, no animals were motionless, but activity decreased with feeding. At the end of 1 hour, there were 31 paramecia (one in fission) in the cloudy side and 19 in the originally clear side. This difference is not statistically significant. Other, previous efforts to repeat Jensen's experiment had also yielded differences either not significant or in the direction opposite to that of his report. No doubt this can be explained by differences in procedure or in the condition of paramecia or bacteria, or both, that were used.

Our results conform well with the known fact that sufficiently dilute acid such as carbon dioxide or acetic acid will induce congregation of paramecia, while higher concentrations will repel them (*7*), response being made to monovalent, but apparently not to divalent or trivalent, cations (*8*). A rich suspension of bacteria lowers $p$H. The paramecia probably collected in those areas which offered the most nearly optimal $p$H conditions, always near the bridge between the drops.

Of course, in Jensen's experiments, many thousand times as many bacteria were used as in our behavioral work. Such a large quantitative difference has qualitatively different effects. Introduction of distilled water into a culture also has effects. Even the addition of a very small amount of water (as from condensation) into a culture growing rapidly in a depression slide will delay fission for hours.

In Jensen's experiment No. 1, bacteria were apparently introduced into distilled water from a platinum wire. The location and number of bacteria found after introduction into clear water cannot be compared with the location and number of bacteria similarly introduced into a thick culture of actively moving and feeding paramecia; (the amount of steak found in a dish placed on the floor in an

empty room would be very different from the contents of the same dish with a hungry dog present). We even find differences in the location of different strains of our *Aerobacter aerogenes*. Some strains tend to drop to the lowest part of the depression, while others remain rather evenly dispersed in a growing spot culture of paramecia.

From all of the foregoing work, I conclude that Jensen, by briefly investigating the dispersion in distilled water of a single strain of the bacterium *Aerobacter aerogenes,* cannot account for results I have been able to achieve with the protozoan *Paramecium aurelia* by use of techniques and controls developed during a number of years of careful study.

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## More on "Learning" in Paramecia

In a previous report (*1*) I suggested a mechanism, other than learning, to explain the results reported in 1952 by Gelber (*2*). In a current report (*3*), Gelber describes some additional experiments. The question is whether these and certain other results (*4, 5*) are explicable in terms of the effects of bacterial concentrations. I do not subscribe to the view that the sole influence on the behavior of paramecia in Gelber's experiments is the number and distribution of bacteria introduced into the cultures by the reinforcement procedure. Instead, I suggest that in one instance (*2*) differential introduction of bacteria into cultures influenced behavior; that in certain other instances (*4*) changes in life-history and in light intensities probably influenced the reactivity of paramecia to equal bacterial concentrations; and that in one instance (*5*) both differential introduction of bacteria and differential reactivity were involved.

Explanation of the first of Gelber's newly reported experiments does not seem possible in terms of bacterial concentrations but requires consideration of a related influence on the behavior of paramecia. Jennings (*6*) has pointed out that products of the animal's respiratory metabolism, secreted while the paramecia remain in a certain area, may create an acid zone which will trap paramecia. Animals enter that zone freely but do not leave it. It seems quite possible that, in this experiment by Gelber, the animals in the "food plus needle" cultures became attached to the needle during the "training" period and created an acid zone which persisted when the needle was withdrawn and which influenced behavior of the paramecia during the later test. This phenomenon of paramecium-produced, movement-restricting acid zones has been encountered both with aggregates (*6*) and with individual paramecia (*7*). For this explanation to be plausible, a manipulation newly reported by Gelber must be considered. Gelber reports having routinely stirred cultures "by rotating the slide." She has said that the rotating motion involved is a movement most easily described as that used in twirling ice cubes in a tumbler. To determine the efficacy of this movement for mixing 0.3-ml droplets of culture fluid in depression slides, such droplets were manipulated and observed, attention being directed to the pattern of paramecia and sedimentation in the droplets. Vigorous movement was required to mix the fluid appreciably, and the fluid nearest the center and the bottom of the hemispherical depression—the very area into which Gelber's needle was placed—was particularly difficult to mix by this manipulation, even though angular rotation of the fluid occurred.

The results of the second experiment, newly reported, appear to be explicable in terms of the original mechanism—the effects of differential bacterial concentrations on behavior of paramecia. The possibility exists that the number of bacteria deposited in the cultures by the large needle, inserted a few times, and the number deposited by the small needle, inserted a larger number of times, are unequal. To test this possibility, Gelber's reinforcement procedures were carried out on two 0.3-ml pools of distilled water, the diameters of needles, number of wipes of bacteria, and time intervals being as described by her. The pools were individually homogenized—that is, they were expelled from sterile micropipettes a number of times—and then equal-sized samples were taken from the two pools and stained with crystal violet. Four counts of bacteria along the margin of each sample were made at a magnification of 970. Seventy percent more bacteria (totals of 560 versus 326) were counted in the sample from the pool that had been reinforced with the smaller needle, inserted the greater number of times. It seems likely that the two reinforcement procedures introduced different numbers of bacteria into the cultures and that this produced differential bacterial concentrations, thus producing the observed differences in behavior.

The third experiment reported by Gelber (*3*) is a modification of one performed by me (*1*). The fluid added to the experimental pool, however, was culture fluid rather than reinforcement fluid and was much less rich in bacteria. It is certainly true that the addition of a drop of reinforcement fluid introduces many times the number of bacteria that are introduced by adding a drop of culture fluid or by the swabbed-needle reinforcement procedure. However, it is suggested that the density of bacteria is the variable that influences the behavior of paramecia. There is no evidence that the pool to which rich reinforcement fluid is added, and which is then homogenized, and the small portion of a pool into which portion a needle, smeared with reinforcement fluid, is repeatedly inserted, do not have comparable densities of bacteria.

Curiously, Gelber accepts the principle upon which the experiment she repeated was based: "that sufficiently dilute acid . . . will induce congregation of paramecia" (*3*). The difference of opinion appears to be simply one of what density of bacteria will produce enough acid. It is my view that Gelber's baited-needle reinforcement procedure produces a density of bacteria sufficient to influence the behavior of paramecia. Gelber may feel otherwise, but she has presented no evidence in support of the contrary view.

Gelber (*3*) asserts that introduction of bacteria into clear water cannot be compared with their introduction into a thick culture of actively moving and feeding paramecia, and she suggests an analogy between bacteria and paramecia and a bowl of food and a hungry dog. The use of this analogy symbolizes what is perhaps the most basic difference of opinion between Gelber and me. Gelber freely applies to Protozoa concepts (reinforcement and approach response) and situations (food presentation) developed with higher metazoan animals. I feel that such application overestimates the sensory and motor capabilities of this organism. As Jennings has pointed out (*6*), a paramecium is not a voracious predator which sights and stalks its prey and food; it is a filter feeder which blunders into its food by chance. If analogies are necessary, a more apt one might be that of an earthworm which crawls and eats its way through the earth, blundering onto food-rich soil and avoiding light, heat, and dryness. Gelber's assertion loses its force when the blind, filter-feeding mode of life of paramecia is considered.

In summary, one can conclude that, by the presence of bacterial concentrations resulting from reinforcement procedures, the effect of bacterial concentrations on the behavior of paramecia, and the influence of paramecium-produced, movement-restricting acid zones, it is possible to account for the results