Reports

Experiments on "Learning" in Paramecia

One of the major conclusions from Jennings' classical studies of lower organisms (*1, 2*) was that the behavior of paramecia is based almost entirely on the avoiding reaction to stimuli and involves no approach tendencies. A recent study by Gelber (*3*) appears to be contrary to Jennings' findings: "The question was whether *P[aramecium] aurelia* would acquire an approach reaction to a bare platinum needle as a result of training trials in which the needle was baited with non-motile *Aerobacter aerogenes*" (*3*, pp. 58–59). "With reinforcement, the animals moved into the area and remained almost immobile at the end of the wire, the number of such animals increasing with almost every count" (*3*, p. 61).

The process of reinforcement used by Gelber consisted of repeatedly placing a small amount of bacterial suspension on a platinum needle and lowering the needle briefly into a pool containing paramecia. Test trials made immediately before and after involved lowering the sterilized, unbaited needle into the pool for 3 minutes and then recording the number of subjects that adhered to the sterile needle ("approach score"). Gelber's apparatus apparently insured that the locus of the needle was constant during the test and training trials.

Gelber refrained from generalizing broadly regarding the occurrence of learning in paramecia, restricting her conclusions to the observations that approach scores of the reinforced group were significantly higher than scores of control groups, that training without reinforcement had no significant effect, and that control groups showed no significant change. Her caution, however, has not always been adopted by authors who cite her studies. For example, Thorpe (*4*) wrote, "Gelber (1952) seems to have provided satisfactory evidence of learning in food presentation tests with *Paramecium aurelia.*"

The purpose of this report (*5*) is to suggest for Gelber's data an explanation which is more consistent with Jennings' findings and to report certain experiments supporting this explanation. The explanation is that Gelber's reinforcement procedure created a localized, bacteria-rich area into which the needle was lowered during both training trials and tests. This area is believed to have influenced the behavior of the subjects.

Gelber discussed the possibility that the nutritive value of the bacteria introduced into the cultures of paramecia might have influenced the behavior of the subjects and dismissed it as unlikely. She did not discuss the possibility that the bacteria introduced were concentrated about the needle locus or the possible effects of such bacterial concentrations on the behavior of paramecia.

To discover whether the reinforcement procedure described by Gelber did concentrate bacteria about the needle locus, the reinforcement procedure was carried out on a pool of 0.3 ml of distilled water according to the description given by Gelber. Reinforcement fluid was prepared according to her instructions, except that distilled water instead of exhausted culture fluid was used to wash a slant of *Aerobacter aerogenes*. Gelber's reinforcement procedure was followed in detail until 40 trials had been completed. Then 0.02-ml samples of fluid were drawn from immediately around the lowered, but now unbaited, needle (near samples) and from points several millimeters from the needle (far samples) and placed in pairs on glass slides. The slides were stained with crystal violet and examined under high magnification (1350×) for the presence of bacteria. In three of the four pairs of samples taken, a large number of bacteria were observed in the near samples, none or very few in the far samples. In the fourth pair of samples, few, if any, bacteria were identifiable in either the far or the near sample.

The conclusion was reached that Gelber's reinforcement procedure did indeed introduce *Aerobacter aerogenes* into the experimental cultures and that the bacteria so introduced were concentrated in an area around the needle.

To discover whether *Paramecium aurelia* tend to accumulate in bacteria-rich culture fluid, a 1-ml sample was taken from a culture of *P. aurelia,* VII, sensitive, stock 51, which had cleared—that is, from which turbidity owing to food organisms was absent. The sample was divided; into one half (experimental), one drop of reinforcement fluid, prepared as in the first experiment, was added. Into the other half (control), one drop of distilled water was added. Each half was then agitated thoroughly. Samples of both halves were transferred to a glass slide, forming two small, separate pools, which were then united at one margin. The culture fluid of the joined pools remained visibly different, and the distribution of subjects in the bacteria-rich portion (experimental) and in the bacteria-poor portion (control) of the pool was observed.

Before the pools were united, 40 subjects were in the experimental pool and 35 in the control pool. Eight minutes after union, 62 subjects were in the experimental portion and 13 in the control portion. This difference is highly significant statistically if tested by chi-square. This experiment was performed four times, each time with a similar result. It was concluded that *P. aurelia* accumulate in bacteria-rich portions of pools of culture fluid.

It is of special significance that individual paramecia freely entered the bacteria-rich portions of the united pools but avoided the bacteria-poor portions.

To discover whether the addition of bacteria to pools of cleared culture fluid increases the attachment tendencies or positive thigmotropic responses of *P. aurelia,* a sample of the culture as in the second experiment was divided into six pools of about equal size, which were placed in depression slides and observed under 38× magnification. The criterion measure of attachment was the number of subjects, of a sample of 25 subjects, that were motionless on the bottom of a depression slide, rather than swimming freely about the pool.

Two drops of reinforcement fluid, prepared as in the first experiment, were added to each of three pools constituting the experimental group; two drops of distilled water were added to each of the remaining three pools, which constituted the control group. All pools were agitated, and criterion measures were recorded after various intervals.

The difference between the scores of the experimental and control groups was not significant until the experimental manipulation was made; thereafter it was

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highly statistically significant. The mean score for the experimental group increased from 4.7 before the experimental manipulation to 19.7 12 minutes later and remained significantly higher than that of the control group until the experiment was halted 1 hour later. It was concluded that addition of bacteria increases the attachment tendencies (positive thigmotropism) of *P. aurelia* (*6*).

Interpretation of Gelber's results is now clearly possible in terms other than those of learning. The reinforcement procedure used is seen as causing a local concentration of bacteria. Bacteria-rich areas tend to retain subjects entering them and to increase the attachment tendencies of subjects within them. For these reasons, when the unbaited needle was replaced into this concentration for tests following training, increased approach scores resulted.

The mechanism by which the local concentration of bacteria so influenced the behavior of subjects can be suggested. *Aerobacter aerogenes* produces acid and carbon dioxide in the course of normal metabolism (*7*). Carbon dioxide increases attachment tendencies (*2*, p. 51) and dissolves in water to form weak carbonic acid. Weak concentrations of acid are known to affect paramecia in precisely the manner observed in the second experiment (*2*, p. 93).

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References and Notes

1. H. S. Jennings, *Am. J. Psychol.* 10, 503 (1897).
2. H. S. Jennings, *Behavior of Lower Organisms* (Columbia Univ. Press, New York, 1906).
3. B. Gelber, *J. Comp. and Physiol. Psychol.* 45, 58 (1952).
4. W. H. Thorpe, *Learning and Instinct in Animals* (Methuen, London, 1956), p. 165.
5. I am indebted to Earl D. Hanson, department of zoology, Yale University, for extensive technical assistance.
6. Richard Duryea and Austin Phelps, University of Texas, have carried out experiments demonstrating such increases in thigmotropic tendencies but have not published their results (personal correspondence from Duryea, 1956).
7. R. S. Breed *et al.*, *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, Baltimore, Md., 1948), p. 454.

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Critical Preparation of Plant Material for Autoradiography

Autoradiography was first used in tracer studies with phosphorus-32 in plants by Arnon, Stout, and Sipos (*1*). Colwell studied translocation in plants, using sections as well as whole plant organs (*2*). He produced satisfactory autoradiograms of stem and petiole sections of squash and was able to study the images produced in a known spatial relation to the tissues containing the tracer.

Fig. 1. Relationship of method of preparation to distribution of tracer (2,4-D*) in bean plants. Autoradiograms were superimposed upon traced outlines of the treated plants. (Left) Blotter-dried plant; (right) freeze-dried plant. Treatment time in each case was zero—that is, killing and drying followed immediately the application of the tracer solution to the bean leaves. The actual treated area within the lanolin ring was punched out of the leaf at the end of the drying period.

In the many uses of the autoradiographic technique since these initial studies, there are indications that certain treatments of plants or plant parts before or during autographing may make accurate interpretation of experimental results difficult. Movement of a mobile radioactive tracer during manipulation and after the death of the plant makes possible an artifact that is not always easily identifiable. Particularly is this true if movement via a specific, discrete tissue system is being studied.

Millikan (*3*) observed such a movement of radioactive manganese from the interveinal tissues into the veins of pea leaves during the autographing of fresh plant parts. He interpreted this to be a result of enclosing the plant part between two sheets of glass during exposure to the films, thus limiting evaporation and allowing movement of sap in the veins. Rice and Rohrbaugh (*4*) studied the movement of radioactive 2,4-dichlorophenoxyacetic acid (2,4-D*) in kerosene. They found that unsectioned bean plants that were harvested ½ hour after application, placed in a plant press, and dried in an oven at 60°C showed 2,4-D* throughout upon autographing, whereas a sectioned plant harvested after 1 hour showed that 2,4-D* had reached only the stem and terminal bud. It was pointed out that movement occurred while the plants were drying, or afterward. The authors thought that the artifact of movement could be explained on the basis of the ease of movement of kerosene through the intercellular spaces by capillarity.

Crafts failed to recognize the importance of the artifact of movement during drying in work on translocation of 2,4-D* (*5*, Fig. 8). This error has been pointed out in a subsequent paper (*6*). The present work (*7*) was done in an effort to measure and delimit this artifact and to find methods for avoiding it.

Red kidney beans were planted (four seeds per pot in 4-inch pots), germinated, and grown under artificial light of 800 ft-ca. The plants were thinned to one per pot shortly after emergence. In all experiments, pots with uniform plants were selected at the time the first trifoliate leaves were beginning to expand.

The treatment consisted in applying a 0.01-ml droplet containing 5 μg of radioactive 2,4-D in 50-percent alcohol solution with 0.10-percent Nonic 218 to the upper surface of one of the primary leaves about 1 cm above the base of the blade. The droplet was confined by a lanolin ring 5 mm in diameter.

At intervals of 0, ½, 1, 2, 4, and 8 hours, plants were harvested in groups of eight by washing out the root systems and quick-freezing between blocks of Dry Ice. Four replications of plants were then dried between newspaper and warm blotters in a plant press, and the other four replications were kept frozen and dried in the continuous frozen state (lyophylized). The treated areas of the leaves were removed after drying by means of a cork borer. All plants were autographed under refrigeration on Kodak No-Screen x-ray film for 15 days. The film was developed in Kodak liquid x-ray developer.

A comparison of the autoradiograms (Fig. 1) illustrates the artifact of movement of 2,4-D* in the drying process. These plants represent zero time treatment, for they were frozen immediately upon application of the drop, and dried. The lyophilized plant (Fig. 1, right) shows a localization of the radioactive material at the point of application. The image produced in Fig. 1 (right) is the result of spreading of the droplet on the leaf surface during quick freezing. The blotter-dried plant (Fig. 1, left) shows a movement throughout the leaves and stem; it illustrates the artifact. A comparison of all autoradiograms shows that, with increasing time, the difference between blotter-dried and freeze-dried plants is less evident.

Because the lyophilization method necessitates the use of special equipment and is time-consuming, a more suitable method of drying was sought. It was found that breaking the frozen plant into sections and then drying by conventional methods approximates lyophilization. The possible loss of radioactive materials from the severed ends, how-