produced irregularity and sporadicity in the acquisition of the salivary conditioning, interfered with its progressive extinction in non-reinforced trials, and in general masked a number of otherwise established conditioning attributes. While attitudes as variables are in themselves of considerable interest and while it may very well be that "conditioning with attitudes" parallels human life-situations, it still is, of course, apparent that they must be ruled out when the properties of conditioning *as such* are to be studied.

In the last three years the writer has, therefore, used a method which is comparatively free from this incubus of subjective interferences. The method may be called the "seriate-continuous-and-misinform" technique. It consists of presenting a *series* of conditioned stimuli rather than a single stimulus during comparatively long continuous eating periods and misinforming the subjects about the purpose of the experiment. Thus, in one study the eating periods—small pretzels, tea-sandwiches, lollipops or chewing gum—were of 2-minute duration, in the course of which 40 intermittent flashes of colored lights were presented. The subjects were told that the purpose of the experiment was to "find out the effect of eye-fatigue upon digestion." In another investigation in which musical chords were the stimuli-to-be-conditioned the eating period lasted for 4 minutes and the influence of music on digestion was offered as the experimenter's real objective. In a third experiment with nonsense syllables a pure conditioned salivation set-up was disguised as a seemingly *bona fide* memory problem, while in a fourth study eating and conditioning were successfully combined with hypothetical solutions of electrical mazes. Thirty-six subjects were used in these experiments and 5 to 10 experimental sessions of 1 to 3 hours' duration were made with each subject. However, so far not a single subject has shown any evidence of any *conscious* associative set between the feedings and the stimuli given during their action in the sense that he thought that he was *expected* to secrete or not to secrete saliva when the stimuli were on; the conscious task-set was merely directed elsewhere—digestion, memorization, maze solution—and was non-functional for the particular association. At any rate, the conditionings were in all these cases highly successful and strikingly conforming with Pavlov's main behavioral findings in acquistion, extinction, spontaneous recovery, generalization, differentiation, and the like.

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proximity and continuity in configural conditioning. (In press, *Jour. Exp. Psychol.*) IV. Gestalt organization and configural conditioning, *Jour. Psychol.*, 7: 3–16, 1939. V. Generalization and transposition. (In press. *Jour. Genet. Psychol.*) VI. Comparative extinction and forgetting of pattern and single stimulus conditioning. (In press, *Jour. Exp. Psychol.*)

THE CULTIVATION OF TRITRICHOMONAS FOETUS IN DEVELOPING CHICKEN EGGS

RECENTLY a considerable number of viruses have been propagated in developing chicken embryos and in their extra-embryonic membranes; Zia (1934)[1] has reported similar cultivation of a *Rickettsia*; and the chorio-allantoic membrane of avian eggs is a favorite position for tissue grafts in experimental embryology. However, there is apparently no record of protozoa ever having been cultivated in developing eggs. The investigation here reported was designed to ascertain the possibility of propagating certain protozoa in this environment.

The strain of *Tritrichomonas foetus* which was used was obtained in bacteria-free culture on Ringer's-egg-serum medium[2] by one of us (N. D. L.) from a case of bovine abortion in an Illinois herd of dairy cattle. The eggs were obtained from a breeding flock of White Rock chickens free from infectious diseases.

Eggs which after incubation for periods of 8 to 16 days showed strong vigorous embryos were used. Then the technique described by Brandly[3] for propagation of laryngotracheitis virus was employed with the necessary modification of introducing the inoculum into the allantoic sac instead of upon or within the chorio-allantoic membrane. Following inoculation, the opening is closed with Cellophane or cellulose tape and the eggs are returned to the incubator.

In examining the eggs, the shell over the air-sac and the shell membranes are removed aseptically, and some allantoic fluid drawn off with a sterile pipette and examined under a microscope. Trichomonads may not be demonstrated in all eggs inoculated with suitable cultures. Unsatisfactory introduction of inoculum is considered to account only in part for this variation. Furthermore, trichomonads, when present, may vary in numbers from less than one to several hundred per field under the 16 mm objective. Usually, transfers were made only from those eggs in which the trichomonads were abundant.

There is no doubt that the protozoa actually multiply in the eggs, rather than merely survive. Twenty-two serial transfers from egg to egg of bacteria-free protozoa have been made over a period of about three months, with a multiplication factor of about 30 at each transfer. The eggs are opened and examined two or three days after inoculation, as much allantoic fluid as possible being saved in sterile test-tubes. Five or more cc may be obtained from one egg, although usually about 2 cc are readily recoverable. If bacteriologically sterile, the fluid is used for the inoculation of another series of eggs. The trichomonads will survive in allantoic fluid placed in cotton-plugged test-

[1] S. Zia, *Am. Jour. Path.*, 10: 211, 1934.
[2] C. W. Rees, *Am. Jour. Hyg.*, 26: 283, 1937.
[3] C. A. Brandly, *Jour. Infect. Diseases*, 57: 201, 1935.

tubes for several days at room temperatures, but in the egg, death of the embryo may be followed very shortly by mortality of the protozoa.

No pathogenic action by these protozoa on the chick embryo has ever been noted. If left in the incubator for the full incubation period, normal chicks hatch out. Trichomonads are no longer present, since the allantoic fluid has disappeared.

It is apparently not necessary for the trichomonads to be entirely free of bacteria in order to multiply in the allantoic fluid, since the presence of saprophytic air-contaminants has been found not to prevent their development. If, however, the bacteria kill the chick embryo, the trichomonads die in a day or two.

Preliminary series of experiments indicate that eggs which have been incubated about 12 days are more satisfactory for cultivation of *Tritrichomonas foetus* than eggs incubated for shorter or longer periods. An incubation temperature of 37° C. is better than 34.5° C. or 39° C.

Tritrichomonas muris from the rat has also been cultivated in developing eggs, but no sub-cultures have been attempted to date.

Since protozoa have already been cultivated in artificial culture media and in tissue culture, the use of developing chicken eggs offers a third type of medium which may prove of value in experimental propagation studies.

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THE PRODUCTION OF GOITER IN CHICKENS[1]

ALTHOUGH goiter in poultry has been noted in iodine-deficient areas,[2] no report of its experimental production has been found. We have obtained goiter in chickens with a ration containing .145 mg per kilo of iodine, and have prevented goiter by the addition of 5 mg per kilo of iodine to the same ration.

Five hundred White Leghorn chicks were used in the two lots. The basal ration contained 71.5 per cent. yellow corn meal, 25 per cent. soybean oil meal, 1 per cent. casein, 0.5 per cent. salt, 2 per cent. bone ash, 0.001 per cent. activated animal provitamin (50,000 units of D per gram), 0.015 per cent. manganous sulfate, and 0.03 per cent. of a salt mixture. The vitamin G requirements for growth were supplied by liquid skim milk.[3]

Thyroid weights of chickens fed the basal ration were 130 per cent. of the control thyroid weights at 6 weeks, 240 per cent. at 12 weeks and 294 per cent. at 18 weeks. In extreme cases, thyroids have been found to approximate twenty times normal weight.

Histopathological examinations of the enlarged thyroids at these intervals during growth showed an absence of colloid and a hyperplasia of the living cells of the follicles. These changes were apparent at 6 weeks and increased in severity with age. Thyroid glands from the control group remained normal.

Work on the iodine requirements of poultry is being continued.

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* Assigned by the State Department of Agriculture to assist in diagnostic and research work.

[1] This work is being conducted through an investigatorship established by the Iodine Educational Bureau, Inc., New York. The Vitamin D solution was generously supplied by Dr. J. J. Waddell, Biological Laboratory, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

[2] H. Welch, *Montana Agr. Exp. Station Bulletin*, 214, 1928.

[3] G. F. Heuser, H. S. Wilgus, Jr., and L. C. Norris. *Poultry Science*, 11: 105, 1938.

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