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became attached to the needle during the "training" period and created an acid zone which persisted when the needle was withdrawn and which influenced behavior of the paramecia during the later test.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

newly reported by Gelber. Experiments less amenable to alternative interpretation are needed to justify recourse to the concept of learning.

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9 October 1957

Enhancement of Goitrogenic Action of Propylthiouracil by Thyroxin

Previous studies in this laboratory (*1*, *2*) have shown consistently that the goitrogenic effect of propylthiouracil (PTU) could be enhanced by dried thyroid powder, administered concurrently over a specific range of dosage for long periods (8 to 18 months). This treatment also resulted in greatly enlarged pituitary glands. The antigoitrogenic effect of thyroid hormones, when administered over short periods, is of course well known and has been observed many times in this laboratory. Therefore, the increased goitrogenesis was unexpected. It was not clear whether the substance causing increased goitrogenesis which was present in the dried thyroid powder was thyroxin or some other material (*3*). It was also possible that a goitrogenic substance was present in Fox chow (*4*), our commercial laboratory ration. Iodide added by the supplier to the commercial ration could be expected to affect goitrogenesis.

Therefore an experiment was designed (*5*) to examine the effects of iodide and crystalline L-thyroxin (Na salt) (*6*) on the thyroids and pituitaries of PTU-treated rats. Male Wistar rats were fed either a standard chow diet or a semisynthetic diet of low iodide content (whole-wheat flour, 52 percent; soya bean flour, 22 percent; skim milk powder, 10 percent; beef fat, 10 percent; corn oil, 5 percent; cod liver oil, 1 percent) with or without a supplement of potassium iodide (1 mg/100 g of diet) and with or without a dietary supplement of crystalline L-thyroxin (2.5×10^{-2} mg/100 g of diet). Propylthiouracil (20 mg/100 g of diet) was added to all diets except the control. Sacrifice of the animals was begun 16 months after the start of the experiment. Table 1, experiment 1, shows the weights of the thyroid and pituitary glands, the statistical significance of the difference between average weights, and estimates of the thyrotrophin content of the pituitaries. The pituitaries were frozen on Dry Ice and stored in a Deep-Freeze for approximately 3 months (*7*).

The enhanced goitrogenesis occurred when crystalline L-thyroxin rather than dried thyroid was used as a supplement to the PTU diet. It also occurred when a semisynthetic diet of low iodine content rather than chow formed the basal diet, although the enhancement was less than it was with the "chow" diet. In this comparison, the relative importance of the diet itself and the supplement of thyroxin can only be assumed because no control group that was fed the low iodine diet and PTU was included in the experiment. The pituitaries of rats that had received the semisynthetic diet supplemented with PTU and thyroxin were also slightly smaller than those of the corresponding group that had been fed chow. The thyrotrophin content was less than that of the chow-PTU-thyroxin group, but it was greater than that of the chow-PTU group. When iodide was added to the semisynthetic diet (in an amount estimated to equal that in the chow), intermediate values were found for the weights of the thyroid glands, although the weights and thyrotrophin content of the pituitary glands were less than they were with either the chow or low-iodide diet. The presence of excess iodide apparently enhanced the goitrogenic effect of thyroxin in the dosage used here. The smaller size and lesser thyrotrophin content of the pituitary glands and the larger thyroids suggest an action directly on the thyroid gland. Because the amount of iodide added to the semisynthetic diet was similar to that in the laboratory ration, the results also indicate that laboratory chow may contain other substances which interfere with the thyroid-pituitary interrelationship.

Although the pituitaries were enlarged, in this experiment only one tumor of the pituitary was found in a rat which succumbed relatively soon after the experiment started. Previous experience had led us to expect a high incidence.

Previously, experiments using the same low-iodide diet only, the diet supplemented with thyroxin (5×10^{-2} mg/100 g), and the diet with thyroxin plus PTU (20 mg/100 g of diet) had been carried on over a period of 10 to 14 months (Table 1, experiment 2) (*8*). While the weights of glands are not comparable statistically to those of the first experiment, it is seen that the average weight of thyroids from the group fed the low-iodine diet only (group 5) is higher than that of thyroids from the control group fed chow (group 0). Animals fed the diet supplemented with thyroxin only

Table 1. Enhancement of the goitrogenic action of propylthiouracil by thyroxin. All values represent mean values. Organ weights and pituitary thyrotrophin (TSH) contents are expressed in milligrams per 100 grams of rat weight. The semisynthetic diet was low in iodine.

Group and diet	Initial No. of rats	*N*	Rat wt.	Pituitary	Thyroid	Pituitary TSH units
Experiment 1; duration, 16 months						
0; Controls, on chow	30	26	539	2.2	5.8	0.59
1; Chow, PTU	50	41	320	3.2	19.8	0.24
2; Chow, PTU, thyroxin	50	40	418	4.7	112.2	1.29
3; Semisynthetic diet, PTU, thyroxin	25	19	396	4.0	54.3	0.88
4; Semisynthetic diet, PTU, thyroxin, KI	25	19	463	3.2	85.3	0.43
Experiment 2; duration, 10 to 14 months						
5; Semisynthetic diet	15	8	424	2.5	11.3	—
6; Semisynthetic diet, thyroxin	15	8	427	2.5	5.7	—
7; Semisynthetic diet, PTU, thyroxin	20	11	445	2.8	14.8	—
*Experiment 1; t values**						
0 versus 1			16.01	4.99	8.09	
0 versus 2			7.76	5.79	6.60	
1 versus 2			8.09	4.16	7.18	
1 versus 3			4.04	2.59	6.70	
2 versus 3			(1.06)	(1.24)	2.97	
2 versus 4			5.38	2.90	(1.27)	
3 versus 4			4.86	(1.91)	(2.00)	

* $t = (\bar{x}_1 - \bar{x}_2)\left\{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2)\Sigma x^2}\right\}^{\frac{1}{2}}$; *t*-values for which *P* is greater than 0.02 are in parentheses.