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THYROXIN AS A DEPRESSANT OF THE DIVISION RATE OF PARAMECIUM.

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I.

It is now 16 years since Nowikoff (1908) accelerated the division rate of *Paramecium* by adding thyroid gland to his cultures. Later, Shumway (1914) reached the same conclusion after a series of more critical and painstaking experiments, and in the following year Budington and Harvey (1915) showed that thyroids from various vertebrates would produce similar results. Still later, Shumway (1917) observed the effect of total thyroid on other processes of *Paramecium*, notably excretion and gastric vacuole formation. From these observations it appeared that thyroid was augmenting processes of dissimilation in so far as these might be indicated by effects on excretion and at the same time processes of assimilation as indicated by effects on the division rate. This striking combination of contrasting functions suggested the possibility that two distinct causes might be operating, both associated with the thyroid substance.

With this suggestion in mind, a series of experiments was begun some 3 years ago by Miss Dora E. Birchard, a student in this laboratory. Her objective was a comparison of the effects of total thyroid and its crystalline derivative thyroxin on *Paramecium*. With her kind permission the following facts are taken from her unpublished manuscript. The culture medium was stock hay infusion containing a mixed infection of bacteria. Thyroid and thyroxin were added as required, the former (Armour and Company's desiccated, containing 0.2 per cent iodine) in suspension (1:600), the latter (Squibb's) in solution (1:20,000) in approximately N/400 NaOH.

The dosage of thyroxin to which *Paramecium* was exposed was thus in terms of iodine about ten times as large as that of thyroid. The difference, however, was not reflected in the behavior of *Paramecium*, except perhaps in connection with the

division rate. The latter was markedly accelerated in the thyroid lines and greatly depressed in the thyroxin lines. The latter results, however, were not satisfactory, owing to an excessive death rate indicating toxic effects demanding further investigation.

In other respects, as summarized below, the effects of thyroid and thyroxin were similar.

1. The individuals from thyroid and thyroxin cultures were on the average shorter and more slender than control individuals. The following figures represent the average length and width obtained from random samples of 100 individuals in each case: from thyroid culture, 0.196 × 0.068 mm., and control, 0.220 × 0.086 mm.; from thyroxin culture, 0.191 × 0.062 mm., and control (in N/400 NaOH) 0.218 × 0.079 mm.

2. Gastric vacuoles were formed more rapidly in both thyroid and thyroxin cultures than in their controls. Using the method devised by Shumway and basing the figures on a count of 100 individuals in each case, as before, the average number of gastric vacuoles formed in 5 minutes was, for the thyroid cultures, 3.87, an increase of 76 per cent over the control figure of 2.19; for the thyroxin culture, 3.88, an increase of 62 per cent over the control figure of 2.39.

Shumway, whose figures do not show so marked an acceleration of gastric vacuole formation, did not regard the effect of thyroid of much significance in this connection.

3. The contractile vacuoles pulsated at rates 75 per cent greater in thyroid and thyroxin cultures than in their controls. The average rates per minute for groups of 100 individuals were: thyroid cultures, 8.11, control, 4.61; thyroxin cultures, 7.19, control, 4.10.

4. Among two groups of 100 individuals each from thyroid and thyroxin lines three contractile vacuoles were found in two of the former, three of the latter.

5. The canals tributary to the contractile vacuoles were observed to be longer and more numerous in both thyroid and thyroxin lines than in the controls. No detailed counts were made.

6. Non-contractile vacuoles were usually present in the individuals from both thyroid and thyroxin lines. In the controls they were only exceptionally present.

II.

With these results before us, the present authors took up the investigation with a modified technique. The use of sodium hydrate as a solvent of thyroxin was temporarily abandoned. Crystals of thyroxin were added directly to the culture medium, in this case 0.025 per cent beef extract, as recommended by Woodruff and Baitsell (1911).

Pure lines were cultured on the slide in 0.2 cc. drops. Experimental lines and their controls were begun with sister individuals in the customary way. The cultures were examined daily and one individual

from each drop was transferred to a similar drop of culture medium freshly prepared, containing a plentiful supply of a mixed bacterial flora and a green monad, both of which were ingested. All slides were kept in similar moist chambers under similar temperature conditions.

The reaction of the beef extract medium after sterilization in test-tubes was distinctly acid. After infection and incubation for 24 hours it acquired a reaction represented by pH 7.0 to 7.6. The slide culture drops were made from these tubes after the latter had been incubated 24 to 48 hours. It was to these alkaline drops that thyroxin crystals were added.

TABLE I.

Pulsations per Minute of the Contractile Vacuoles.

Group.	Diet.	No. of animals measured.	Average rate of pulsation.	Increase in thyroxin cultures.
				per cent
1	Thyroxin.	1	6	50
	Control.	3	4	
2	Thyroxin.	1	9	38.5
	Control.	3	6.5	
3	Thyroxin.	7	11.7	25.8
	Control.	2	9.3	
4	Thyroxin.	4	10.75	22.9
	Control.	4	8.75	
5	Thyroxin.	4	10	0
	Control.	2	10	
6	Thyroxin.	6	5.83	11
	Control.	8	5.25	

That thyroxin thus administered actually affected the behavior of *Paramecium* was clear from the following facts: that in the thyroxin cultures as compared with the controls, the rate of pulsation of the contractile vacuoles was higher (Table I), the number of canals leading into each vacuole greater (Table II), the average number of food vacuoles less (Table III), and the crystals in the endoplasm resembling those that Schewiakoff (1893) regarded as calcium phosphate, fewer (Table IV).

In these tables the individuals in each group were observed within a few minutes of each other under what appeared to be constant

conditions. Different groups were observed at different times under conditions that could not be regarded as constant for all groups. For this reason and because of the variable number of individuals in the several groups, average figures for all groups in each table would not satisfactorily express the facts, even for larger numbers of individuals than those actually observed. Despite the relatively small number of the latter, and the manner of recording them, the differences between experimental and control individuals are sufficiently marked to justify the conclusions drawn.

In these respects, and under a modified technique, thyroxin again resembled total thyroid in its effect on certain metabolic processes. In its effect upon the division rate, however, thyroxin differed unequivocally from the latter. The average daily division rate calculated from observations on eighteen experimental cultures under observation for periods ranging from 7 to 71 days, was 0.8687 per cent in the controls, 0.8205 per cent in the thyroxin cultures. These figures represent a decrease of 5.54 per cent in the division rate in the latter. This difference is too small in itself to establish conclusively a depressing action of thyroxin on the division rate. But it does make clear that the marked accelerations of the division rate customarily observed following the administration of total thyroid can hardly be due to the thyroxin contained therein.

Attention was called to this conclusion and the facts on which it was based in a preliminary report by Riddle and Torrey (1922–23). This conclusion is cited and supported by Woodruff and Swingle (1923), who are convinced from their experiments, that

> ". . . . neither thyroxin (Squibb's) nor commercial desiccated thyroid, or fresh desiccated thyroid of the turtle produce any significant acceleration of the division rate of *Paramecium*.
>
> "Data to the contrary published by previous investigators apparently are attributable chiefly to variations in the bacterial food supply which the different media afforded the *Paramecia*.
>
> "Accordingly, all the evidence from studies on *Paramecium* to the effect that thyroid products accelerate cell anabolism is, we believe, erroneous."

About the same time, Cori (1923) concluded, from experiments on *Paramecium*, that thyroxin accelerates the division rate though to a much smaller degree than thyroid extract, which thus presumably

contained "another active substance besides thyroxin, which accelerates the division rate."[1]

Prior to the appearance of Woodruff and Swingle's paper we had concluded another series of experiments to test further the difference

TABLE II.

Number of Canals Tributary to Each Contractile Vacuole.

Group.	Diet.	No. of animals measured.	Average No. of canals.		Increase in thyroxin cultures.	
			Anterior vacuole.	Posterior vacuole.	Anterior.	Posterior.
					per cent	*per cent*
2	Thyroxin.	1	11	12	37.5	50
	Control.	3	8	8		
3	Thyroxin.	7	10	10	25	25
	Control.	2	8	8		
4	Thyroxin.	4	9.25	9.25	2.8	13.9
	Control.	4	9	8		
5	Thyroxin.	4	9	9	12.6	20
	Control.	2	8	7.5		
6	Thyroxin.	6	9.83	9.66	17.3	17.1
	Control.	8	8.38	8.25		

TABLE III.

Gastric Vacuoles in Endoplasm.

Group.	Diet.	No. of animals examined.	Average No. of food vacuoles.
3	Thyroxin.	7	17.1
	Control.	2	24.5
4	Thyroxin.	4	8.25
	Control.	4	19.25
5	Thyroxin.	4	19.3
	Control.	2	16
6	Thyroxin.	6	10.5
	Control.	8	16.9

shown in our previous work between the effects of thyroid and thyroxin on the division rate of *Paramecium*, and a preliminary report of the results was published by Torrey (1923).

For these experiments, *Paramecium* was cultured on the slide in

[1] Cori (1923), p. 299.

0.2 cc. drops of 0.025 per cent beef extract medium infected with a mixed culture of bacteria containing a minute flagellate. Four experimental lines with four control lines each containing two files, were established in the usual way, each file and its control beginning with sister individuals, and all sixteen files descended from a single individual four generations back. To experimental line I, thyroxin in crystalline form was given; to experimental line II, NaOH strong enough to give an initial concentration of 0.005 N in the culture drops; to experimental line III, 0.01 per cent thyroxin in the solution of NaOH used for II; to experimental line IV, desiccated thyroid in

TABLE IV.

Crystals in Endoplasm.

Group.	Diet.	No. of animals measured.	Average No. of crystals.	Decrease in thyroxin cultures.
				per cent
1	Thyroxin.	1	51	30.4
	Control.	3	73.3	
2	Thyroxin.	1	38	75.1
	Control.	3	153	
3	Thyroxin.	7	55.2	30.6
	Control.	2	72	
4	Thyroxin.	4	64.4	24.3
	Control.	4	85.25	
5	Thyroxin.	4	89.25	10.8
	Control.	2	100	
6	Thyroxin.	6	57	27.3
	Control.	8	78.4	

suspension (1:1000). Estimated on the basis of iodine content, the thyroxin culture drops contained about thirty times as much thyroxin as the thyroid culture drops.

The results of this series of experiments are shown in the accompanying graphs made from original records subsequently lost through an unfortunate accident.

Each graph represents the division rate of the two files of an experimental line (broken line) and the two files of its control (solid line) averaged for 10 day periods. Experimental conditions continued through four such periods for each file. The graphs also show the average division rate for the period immediately preceding the intro-

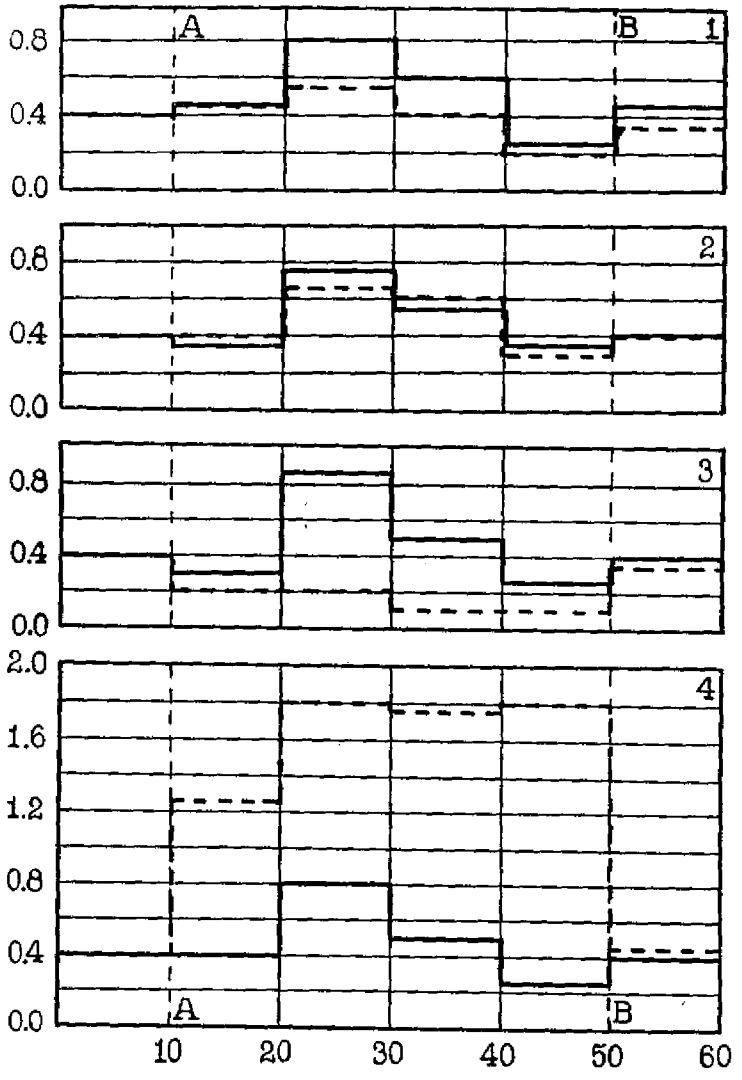

FIG. 1. The division rate of *Paramecium* as affected by total thyroid (marked acceleration) and thyroxin (marked depression). Each graph shows the daily division rate of an experimental culture (broken line) and its control (solid line) averaged for 10 day periods. *A*—*B* marks the limits of experimental feeding. Graph 1, effect of thyroxin in crystalline form; Graph 2, control for Graph 3; Graph 3, effect of thyroxin in solution; Graph 4, effect of desiccated thyroid.

duction of experimental conditions and the period immediately following their removal.

According to Graph 1, the addition of thyroxin crystals was followed by a depression of the normal curve averaging 20 per cent in the two files for the 40 experimental days.

According to Graph 2, the amount of NaOH contained in experimental line III, had a negligible influence on the division rate.

According to Graph 3, thyroxin in 0.01 per cent solution very markedly depressed the division rate, to a point in fact less than one-third its value in the control line.

According to Graph 4, in striking contrast with the last case, the division rate following the addition of desiccated thyroid was three times greater than in the control.

The very large degrees of acceleration and depression of the division rate following thyroid and thyroxin, respectively, are unique in our experience, and as yet not adequately accounted for. There is no question, however, about the facts themselves.

Up to this time thyroxin had been used by us in concentrations that in terms of iodine content corresponded with doses of desiccated thyroid eighteen to thirty times larger than those of the latter actually given. We are speaking, of course, of the doses to which *Paramecium* was exposed. Just how much thyroid and thyroxin were taken has not been determined. That thyroid is ingested by *Paramecium* has already been directly observed by Shumway. And that it modifies certain metabolic processes in the same way that thyroxin does has already been established. Farther than this we cannot now go except to say that the thyroid was always present in quantities greatly in excess of what the organism obtained and a similar statement is probably true for thyroxin.

In solution, thyroxin was presumably more available than when given in combined form in the thyroid substance itself. This fact would serve to diminish the relative amount of thyroxin administered as thyroid, in comparison with that available in soluble form.

Even though it is probable that thyroxin was present in our drop cultures in amounts far in excess of what *Paramecium* actually absorbed, the degree of its concentration in solution played an appreciable rôle in the determination of the division rate. This was clearly shown in the following experiment.

Four groups of drop cultures, each consisting of twelve files, were begun with *Paramecia* descended from a single individual. Corresponding files in the several groups were begun with granddaughters of the same individual. The culture medium was 0.025 per cent beef extract[2] infected with a mixed culture of bacteria from the air, containing as before the small flagellate previously mentioned. To this control medium were added, for the three experimental lines, 0.01 per cent, 0.001 per cent, and 0.0001 per cent thyroxin, respectively, dissolved in N/2,500 NaOH. The pH figure was taken at the beginning and end of each 24 hour period.[3] The diurnal variation was between 7.4 and 7.6 or 7.7 for all files. On only one day was this latter figure exceeded, when the control medium with an initial pH of 7.7 reached pH 8.0 by the end of the 24 hour day. Each

TABLE V.

Group.		pH diurnal variation.	No. of divisions.	Average daily division rate.	Ratio of experimental division rate to control.
I.	Control (0.025 per cent beef extract).	7.4–7.7 (On 1 day 7.7–8.0)	244	2.033	1.00
II.	Thyroxin 0.0001 per cent.	7.4–7.7	230	1.91	0.942
III.	" 0.001 " "	7.4–7.7	215	1.79	0.881
IV.	" 0.01 " "	7.4–7.7	178	1.483	0.729

[2] Woodruff and Baitsell (1911) reached their standard concentration of beef extract by a process of dilution. This procedure was necessary, as it appears, in order to reduce the acidity of the medium which in higher concentrations is too great for *Paramecium*. At the same time, however, dilution lessened the nutritive value of the medium for the bacteria which serve as food for *Paramecium*. After the data recorded in the text were obtained, we found that the acidity of the medium could be readily controlled by sodium hydroxide without interfering with the superior nutritive values of higher concentrations. *Paramecium* thrives in 0.05 and 0.075 per cent beef extract adjusted to a pH of 7.4 to 7.8.

[3] This was obtained by the drop method described by Felton (1921). Buffer mixtures were made of Sörensen's primary potassium phosphate and secondary sodium phosphate as recommended by Clark (1922). Phenol red, brom-cresol purple, and brom-phenol blue were used as indicators, made up in sodium hydroxide according to his directions. The tests were made commonly on a clear sheet of glass, with neutral surface over a white ground, instead of on a porcelain plate. The indicator was also added directly to the culture drop on the culture side.

file was observed for 10 days, morning and evening, when all save one individual were removed from each drop. The culture medium was changed each morning. All cultures were kept at the same temperature, which varied during the course of the experiment from 24–27°C.

Table V summarizes the results for the 10 day period.

These figures show that thyroxin in solution in a concentration as low as 1 part in 1,000,000 (Group II) depresses the division rate slightly but distinctly, and that thyroxin in the higher concentrations shows more marked effects in the same direction. The magnitude of the effect, however, does not increase with the same rapidity as the concentration of thyroxin. Indeed, the curve of depression flattens rapidly as the concentration of thyroxin increases, reaching a maximum value of 27 per cent as the solution of thyroxin, at 1 part in 10,000, approaches saturation in the concentration of NaOH used. Thus while the division rate is unmistakably depressed in these experiments by this maximum concentration of thyroxin, it is far from completely inhibited.

That the action of thyroxin on *Paramecium* is direct and not by way of an effect on the food supply, as in the case of total thyroid according to Woodruff and Swingle (1923), is supported by the effect of thyroxin on the food organisms in both drop and mass cultures.

Many culture drops containing a superabundance of organisms were stained at the end of 24 hours and compared with respect to the abundance of the organisms as a whole, and the relative abundance of the several kinds involved. Inspection revealed no appreciable differences in either case between control drops and drops containing the several concentrations of thyroxin used in the experiments.

The evidence from mass cultures was obtained by infecting equal amounts of 0.05 per cent beef extract medium corrected to pH 7.4 and containing the concentration of thyroxin (in NaOH) desired, in test-tubes of the same diameter, with equal amounts of a suspension of a given species or mixture of species, incubating for 24 hours, and determining the relative turbidity of the medium at the end of that time. The tubes were infected, in lots of several each, (1) with the mixed culture just mentioned, (2) with a pure culture of *Bacillus subtilis*, and (3) with a pure culture of *Bacillus fluorescens*. Thyroxin was used in concentrations of 1:10,000, 1:100,000, and 1:1,000,000.

From the first no satisfactory test was obtained owing to the tendency of thyroxin at this concentration to precipitate out of solution, increasing the turbidity thereby beyond the degree reached in the other tubes.

At the end of 24 hours, good growths were observed in all the tubes. The mixed cultures tended to flock, owing to the presence of a non-motile form which grew in sheets. The cultures of both pure strains were free of flocks, *Bacillus subtilis* forming its characteristic pellicle in our experience only in more concentrated beef extract medium than was here used.

The turbidity was determined by three observers who were unable to distinguish control and thyroxin tubes on the basis of this criterion.

TABLE VI.

(1)	0.075 per cent beef extract + 0.1 per cent desiccated thyroid.	151
(2)	(1) + 0.0001 per cent thyroxin.	149
(3)	(1) + 0.001 " " "	134
(4)	(1) + 0.005 " " "	132
(5)	0.075 per cent beef extract.	104
(6)	(5) + 0.0001 per cent thyroxin.	100
(7)	(5) + 0.001 " " "	103
(8)	(5) + 0.005 " " "	73

The small flagellate present in the mixed cultures was abundant in controls and all three concentrations of thyroxin. *Bacillus subtilis* and *Bacillus fluorescens* were selected to test in pure culture, because it was suspected that if, as Hargitt and Fray (1917) had shown, the former was superior to the latter as a food organism for *Paramecium*, thyroxin might tend to depress the division rate of the former relatively to the latter, and, as both of these bacteria were present in the mixed culture, such a differential action of thyroxin would change the nutritive balance, with a possible effect upon the division rate of *Paramecium*. This suspicion was not supported by the facts.

Further evidence that thyroxin affects *Paramecium* directly, and not indirectly through the food supply is set forth in Table VI which records the total number of divisions of ten lines of *Paramecium* during 10 days, in each of the media listed. The pH of all cultures was adjusted to 7.4 each day, as in the series previously noted.

In both beef extract plus thyroid, and beef extract alone, the depressant action of thyroxin is again apparent in spite of the exceptionally high figure in Table VI under (7).

SUMMARY.

In concentrations of $1:10^4$, $1:10^5$, $1:10^6$, in alkaline solution with a hydrogen ion concentration approximating that characteristic of normal human blood, thyroxin acts like total thyroid in *accelerating certain metabolic processes* in *Paramecium*, notably those connected with the phenomena of excretion.

Unlike total thyroid, thyroxin *depresses the division rate* of *Paramecium*, the degree of depression varying directly with its concentration.

This depression of the division rate is due to the action of the thyroxin directly on *Paramecium*, not to a modification of the food supply.

BIBLIOGRAPHY.

Bodine, J. H., *Biol. Bull.*, 1921, xl, 73.
Boothby, W. M., *J. Am. Med. Assn.*, 1921, lxxvii, 252.
Budington, R. A., and Harvey, H. F., *Biol. Bull.*, 1915, xxviii, 304.
Champy, C., *Arch. morphol. gén. et exp.*, 1922, No. 4.
Clark, W. M., The determination of hydrogen ions, Baltimore, 2nd edition, 1922.
Cori, G. T., *Am. J. Physiol.*, 1923, lxv, 295.
Felton, L. D., *J. Biol. Chem.*, 1921, xlvi, 299.
Hargitt, G. T., and Fray, W. W., *J. Exp. Zool.*, 1917, xxii, 421.
Horning, B., and Torrey, H. B., *Anat. Rec.*, 1922–23, xxiv, 395, 399.
Kendall, E. C., *Papers from the Mayo Foundation for Medical Education and Research and the Medical School*, 1916, viii, 513; 1917, ix, 309; 1919, iii, 156; *Am. J. Physiol.*, 1919, xlix, 136.
McLean, A. J., *Puget Sound Marine Station Pub.*, 1921, iii, 93.
Nowikoff, M., *Arch. Protistenk.*, 1908, xi, 309.
Plummer, H. S., and Boothby, W. M., *Am. J. Physiol.*, 1921, lv, 295.
Riddle, M. C., and Torrey, H. B., *Anat. Rec.*, 1922–23, xxiv, 396.
Schowiakoff, W., *Z. wissensch. Zool.*, 1893, lvii, 32.
Shumway, W., *J. Exp. Zool.*, 1917, xxii, 529.
Torrey, H. B., *Anat. Rec.*, 1923, xxvi, 367.
Woodruff, L. L., and Baitsell, G. A., *J. Exp. Zool.*, 1911, xi, 135.
Woodruff, L. L., and Swingle, W. W., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 386.