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**The action of pancreatic vitamine upon the metabolic activity of paramecium.**

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The following experiments were undertaken to see if vitamins extracted from sheep's pancreas and adsorbed in fuller's earth by the method employed by Dr. W. H. Eddy have any effect on the metabolic activities of free cells.

The free living cells used in the experiments were the descendants of a single individual *Paramecium aurelia*. These were cultivated on half and half pure spring water and 24-hour-old boiled flour water used fresh each day. On this diet the average division rate varied from 1.5 to 2.0 divisions per day at the time the vitamine tests were started.

The vitamine-bearing fuller's earth was kept in capsules prepared by Dr. Eddy. A small dose of this was obtained by moistening the point of a needle in the *Paramecium* culture water and dipping this in the capsule. The adhering granules were then shaken off in the culture water.

It was necessary at first to determine whether or not the fine granules of the fuller's earth would be ingested by the *Paramecium*. For this purpose sterile spring water was used with the earth. It is well known that similar granules of carmine or indigo will be ingested and a few preliminary tests showed that the granules of fuller's earth are similarly taken in and stored in the gastric vacuoles. To be doubly sure of this a quantity of the fuller's earth was stained with neutral red, washed and then supplied as before to *Paramecium*. The bright pink granules in the resulting gastric vacuoles left no doubt that the *Paramecium* will ingest the vitamine-bearing granules.

The individual *Paramecium* to be studied was isolated in a watch glass containing one drop of Great Bear water, one drop of boiled flour water which has been exposed for twenty-four hours, and the usual dose of vitamine-bearing fuller's earth. It was then stored away in a moist chamber and left for twenty-

four hours when the culture was again examined and the number of individuals counted to get the number of divisions. The control individual was isolated in an identical medium but without the fuller's earth. Five double sets prepared and examined in this way were carried on for a period of 10 weeks.

#### EXPERIMENTS.

1. In the first series of experiments the organisms were kept in their usual medium of hay infusion and the vitamine-bearing fuller's earth was added to this. Five lines of experimental animals and five of controls were carried on for 5 days giving 27 divisions in the control series and 26 in the vitamine set. The results were therefore entirely negative.

2. In the second series boiled flour was substituted for boiled hay. Five sets of control *Paramecium* and five sets treated with vitamine were carried along for 15 days. The results may be best considered in three groups covering 5 consecutive days each, as follows:

	Controls.		Vitamine-treated.	
	Divisions.	Rate.	Divisions.	Rate.
Group A. First 5 days . . . . .	27	1.08	32	1.28
Group B. Second 5 days . . . . .	12	0.48	22	0.88
Group C. Third 5 days . . . . .	21	0.84	24	0.96
Totals . . . . .	60	0.80	78	1.08

Here with organisms of different grades of vitality there is a very slight increase in the division rate of the vitamine group, but not enough to prove that the vitamins were effective in stimulating nutrition.

3. In both of the preceding series, individuals were taken at random from a culture dish. While all came from the same original individual it was impossible to tell whether young or old individuals were isolated. In this third series of tests this objection was overcome by using sister cells, one for control, the other for vitamine treatment. In this experiment, as in the others, five lines of control animals and five of vitamine-treated animals were carried on, this time for a period of thirty days,

sister cells being isolated every day. The results may be summarized in six groups including 5 consecutive days each, as follows:

	Controls..		Vitamine-treated.	
	Divisions.	Rate.	Divisions.	Rate.
Group A. First 5 days.....	45	1.80	42	1.68
Group B. Second 5 days.....	28	1.12	26	1.04
Group C. Third 5 days.....	16	0.64	24	0.96
Group D. Fourth 5 days.....	30	1.20	25	1.00
Group E. Fifth 5 days.....	19	0.76	24	0.96
Group F. Sixth 5 days.....	15	0.60	19	0.76
Totals.....	153	1.02	160	1.06

4. The final experiment was introduced to see if the vitamine treatment will stimulate subsequent metabolic activities. Individuals that had been treated for twenty-four hours were isolated in the usual medium without vitamine. Here again 5 lines with controls were carried on for 15 days. The control animals divided 64 times, the post-vitamine animals 63 times with division rates of 0.85 and 0.84 respectively. The minute differences in all these results fall within the limits of experimental error and show that the vitamines as used have no effect on the nutrition of *Paramecium* cells.

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#### Further observations on pancreatic vitamine.

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In connection with the use of pancreatic vitamine two problems have arisen which must be solved if the treatment is to be more than empirical. One is the establishment of a satisfactory method of dosage determination and the other is the explanation of the method of action of the vitamine upon the organism. In the previous paper is reported an attempt to solve the latter problem. In this paper I wish to present certain collated observations bearing upon the dosage problem.