

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.

I. A COMPARISON OF PANCREATIC VITAMINE AND YEAST VITAMINE.

In a recent paper Seidell<sup>1</sup> has outlined the result of his studies of autolyzed yeast filtrate. Making use of similar methods and using an arbitrary standard of 2.7 gm. pancreas to each cubic cm. of water extract as my solution I obtain the following results:

TABLE.

	Autolyzed Yeast Filtrate (Seidell).	Pancreatic Prep. I.	Extract Prep. II.
1. Nitrogen in 100 c.c. . . . .	2.0 gm.	0.754 gm.	0.644 gm.
2. 5 gm. Lloyd reagent shaken with 100 c.c. extract cont. . . . .	0.09 gm. N.	0.150 gm. N.	0.159 gm. N.
3. 5 gm. Lloyd reagent shaken with filtrate from 2. cont. . . . .	0.073 gm. N.	0.088 gm. N.	0.102 gm. N.

From experiments conducted by Williams and Seidell<sup>2</sup> they conclude that vitamine probably contains approximately 50 per cent. nitrogen. Other experiments indicate that practically all the vitamine is removed in the first shaking with the Lloyd reagent. My own experiments confirm their view on the latter point. In regard to the first conclusion I have no data. Accepting their assumptions in both particulars it follows that:

	Yeast Filtrate.	Pancreatic Ext.
100 c.c. of extract contains . . . . .	0.18 gm. Vitamine	0.30 gm. Vitamine.

Both experiments show that the Lloyd reagent adsorbs nitrogenous substances that are not vitamins. Furthermore it is reasonable to suppose that some of the N of the first shaking is not vitamine N. Hence the vitamine content given above must be taken not as the absolute figure but as the maximum content possible.

Seidell has also found that 1 c.c. of yeast filtrate given every other day protects pigeons from polyneuritis, and that these birds consume 30 gm. of food per day. One cubic cm. of yeast filtrate would therefore contain not more than 0.0018 gm. of vitamine, hence he concludes that 0.0018 gm. vitamine to each 60 gm. of food is a proper vitamine ratio for a pigeon, *i. e.*, one containing 0.003 per cent. vitamine. In experiments with rats and babies my aim has been growth rather than antineuritic tests. In my experi-

<sup>1</sup> Atherton Seidell, "The Vitamine Content of Brewer's Yeast," *Journ. Biol. Chem.*, XXIX, 145.

<sup>2</sup> Williams, R. R., and Seidell, A., *J. Biol. Chem.*, XXVI, 431.

ments no minimum amount has been determined as yet. The data at hand, however, figured on the same basis as follows

*Rat No. 4.* In 10 days received 38 gm. Lloyd and 116 gm. casein diet, *i. e.*, 2 per cent. vitamine. The gain in weight was 13 gm.

*Rat No. 5.* In 10 days received 20 gm. Lloyd and 94 gm. casein diet, *i. e.*, 1.2 per cent. vitamine. The gain was 15 gm.

*Rat No. 20.* In 13 days received 13 gm. Lloyd, 433 gm. farina and 240 gr. condensed milk, *i. e.*, 0.11 per cent vitamine. The gain was 20 gm.

*Rat No. 31.* In 14 days received 7 gm. of Lloyd, 450 gm. farina and 210 gm. boiled milk, *i. e.*, 0.063 per cent. vitamine. The gain was 15 gm.

*Rat No. 39.* In 14 days received 3½ gm. Lloyd, 450 gm. farina and 210 gm. boiled milk, *i. e.*, 0.031 per cent. vitamine. The gain was 21 gm.

*John G.* In 21 days received 42 gm. Lloyd, 630 gm. farina and 1,280 gm. condensed milk, *i. e.*, 0.13 per cent. vitamine. The gain was 588 gm.

10 other babies gained, but in no case on less than 0.13 per cent. vitamine. From these results however it seems hardly a desirable method of figuring vitamine needs. Per cent. of vitamine nitrogen would probably be a better basis.

## II. POSSIBILITY OF A COLORIMETRIC METHOD OF DETERMINING DOSAGE.

In one of his papers Funk noted that the fraction containing the vitamine gives the blue color reaction with the Folin-Macallum uric acid reagent. This fact suggested another possible method of determining dosage. Experiments to date with pancreatic extract have given the following facts:

1. The water extract gives the color reaction.
2. The extract after treatment with the Lloyd reagent (50 gm. to the liter) and removal of the Lloyd by filtration, still gives the reaction but with a marked reduction in intensity.
3. The Lloyd reagent from the first shaking gives the reaction.
4. The Lloyd reagent shaken with the filtrate from 3 gives the reaction but with marked reduction in intensity.
5. An alkaline and an acetic acid extract of the activated

Lloyd reagent both give the reaction and there is a marked lessening in color intensity when the extract is made of the second shaking of Lloyd reagent.

6. That the color is due in part to the vitamine is shown by the fact that a portion of the extract carried through the complete Funk preparation process gives the color strongly.

7. The color can be obtained with the aid of the centrifuge in a clear form usable against a uric acid standard.

These results show that the substance adsorbed by the Lloyd has the power to give the color reaction and indicates that there is also present some substance other than vitamine which has this power. Granting this fact, however, the results offer a method of standardizing dosage that seems to give promise of practical value.

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**Lipoid-free immune serum does not produce passive immunity to transplanted tumors.**

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[From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.]

Although it has been repeatedly attempted, no one has succeeded in transferring immunity to transplantable tumor in animals by injecting the serum of immune animals. Our experiments on passive immunity, duplicating in part the work of others, have also led to negative results, and are recorded simply to add to the existing data.

Serum was obtained from normal rats, from rats immune to inoculations of the Flexner-Jobling rat carcinoma, and from guinea pigs sensitized by three injections of the Flexner-Jobling tumor in doses of 1 gm. These sera were injected daily in doses of 1 c.c. for nine days previous to an inoculation of 0.003 gm. of the Flexner-Jobling tumor, and a control series of non-injected animals was inoculated at the same time, twenty-five animals being used for each series. Four weeks after inoculation, no immunity was demonstrable in any of the treated groups, which showed from 88 to 92 per cent. takes, as compared with 92 per cent. takes in the control groups.