is correlated with a parallel rise in corpuscular volume as indicated in the table.

The concentration of sugar falls steadily throughout the work period. It should be noted, however, that the variations during the first period in work and those during the same period in rest are almost identical, but from this point on there is a divergence which becomes quite pronounced in the later stages of the experiment.

It is perhaps worthy of notice that there is a rather distinct change in the magnitude of the effects of work after the first period. This we have interpreted as indicating that the day's work falls into at least two phases; first, one in which certain augmenting effects of exercise apparently predominate, second, one in which fatigue phenomena are relatively more prominent.

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Further studies in the measurement of vitamine content.

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During the past year two papers have appeared in the *Journal of Biological Chemistry*, one by Dr. Bachman[1] and the other by Dr. Williams[2], which postulate the requirement of the water-soluble B vitamine in yeast growth. Both these papers suggest the use of yeast cells as a means for the measurement of vitamine content and present a technique that may be used to that end.

In an earlier number of the PROCEEDINGS[3] we reported on a study of the suitability of the Bachman test for vitamine measurement and some of the difficulties encountered. Since that time we have carried out similar studies of the Williams technique and as a result have devised a method which employs features of both

[1] Bachman F., Vitamine requirements of certain yeasts. *Journ. Biol. Chemistry* 1919, xxxix, 235.

[2] Williams, Roger J., The vitamine requirement for yeast. A simple biological test for vitamine. *Journ. Biol. Chem.*, 1919, xxxviii, 465.

[3] PROCEEDINGS SOC. EXPER. BIOL. AND MED., 1919, xvii, 52.

authors but seems to permit of better control and to be at the same time more easily handled than the Williams method. This method is presented herewith, not as a finished product for we are still experimenting with certain details of standardization, but with a view to stimulating criticism and suggestion.

The Bachman test measures the vitamine activity in terms of gas generated; it is really a measure of enzyme activity. This fact makes it difficult to be sure that the stimulus is a growth stimulus or merely an enzyme control in any given test. The Williams test measures the effect of the vitamine in growth of yeast cells and this seems to us a more reliable indicator than the gas production. On the other hand the hanging drop method of Williams makes difficult the control of concentration during incubation and we found the preparation of drops containing single yeast cells far from easy to prepare. We believe that our method obviates both these difficulties while retaining the yeast cell count as an indicator.

The Method.—The illustration shows the tools used, being essentially those of the opsonin technique. The first step is the preparation of two capillary pipettes by drawing out in the flame a 5 mm. glass tube as shown in *A*. This tube is marked at the center with a pen point (1) and with the aid of a drop of mercury accurately calibrated into units of equal volume on each side of the center point (2) and (3). The two halves are then separated at the point (1) into two pipettes. Each pipette is then heated at the large end and a constriction made to permit a flame seal later, the end plugged with cotton and the pipette sterilized. After sterilization and by fitting a rubber bulb to the end as in *B* the pipette is ready for use. After filling as described below the tip and constriction point are sealed in the flame and the tube is ready for incubation, *C*.

The materials necessary for the test are the pipette described above, a dilute suspension of yeast cells in Nageli solution, and the vitamine extract to be tested. These are prepared as follows: A pure culture of Fleischman round yeast is maintained on an agar slant. Two days before the test a transfer is made to a fresh slant and in all our tests, cells of 48 hour growth are used. From such a slant the smallest amount of yeast that can be taken up on a

needle point is transferred to 10 c.c. of Nageli solution in a sterile test tube and the tube shaken from 1¾ to 2 hours on a shaking machine. At the end of that time the uniformity of the emulsion is tested by removing ten units of the suspension with the pipette, blowing them out on a microscope slide, staining and counting. If the counts are uniform the suspension is used, if not it is shaken further. This uniformity of the emulsion is extremely important in comparing results. Having secured a uniform emulsion the pipettes are prepared as follows: Two units of a sterile extract of vitamine are first drawn up into the pipette (the unit we have used is about 1/800 c.c.). We have prepared our extracts in various ways but in all cases they have been sterilized for two 30-minute intervals in the Arnold Sterilizer with a 24-hour interval. Next two units of yeast suspension are drawn up. By manipulating the bulb a bubble of air separates the yeast and vitamine in the tube and permits accurate measurement. When both materials are in the tube they are drawn up to the large part of the tube and mixed. The tube is then sealed as described above. The control tube contains the yeast cells in the Nageli solution (two units) but no vitamine. Our Nageli solution is the same as used by Dr. Bachman, being a sterilized solution of the following components: 100 c.c. distilled water; 10 gms. dextrose; 1 gm. ammonium nitrate 0.05 gms. calcium phosphate, 0.5 gms. potassium acid phosphate; 0.25 gms. magnesium sulfate.

After the tubes are filled and sealed they are incubated at 35° Cent. for the time necessary, usually 18–24 hours. At the end of that time the ends are broken off, the rubber bulb adjusted and the contents blown out on a specially prepared counting slide, fixed and stained and can then be counted at leisure. For this purpose we prepare common microscope slides by etching 7 mm. squares on them, experience having shown that such a square will hold the contents of a tube. In this way it is possible to get a series of the contents of ten or twelve pipettes on one slide. Counting is done under the high power with the aid of a mechanical stage.

Results with the Method.—The methodology reported above has been arrived at by experiment. The necessity for accurate calibration, the need for uniform suspensions of yeast, the size of

the units etc. are the result of preliminary trials and tests that need not be reported here in detail. Certain questions however arise at once and some of our results to date are presented here as partial answers to these questions pending the accumulation of more complete data. The first question that arises is naturally as to the specificity of the test. Bachman and Williams have gone into this matter somewhat but we believe that our results with the above technique demonstrate additional evidence that this test is specific for the so-called antineuritic vitamine or water-soluble B. Dr. Funk kindly supplied us with a specimen of his purified antineuritic vitamine prepared after his well-known method in 1913. A water solution of this material sterilized in the Arnold sterilizer was tested with the results given below. We also made comparative studies of an extract of navy bean made after the McCollum method[4] before and after shaking with Lloyd's reagent. This reagent is supposed to remove quantitatively the B and apparently with little effect upon the other constituents of the extract. The results follow:

TABLE I.

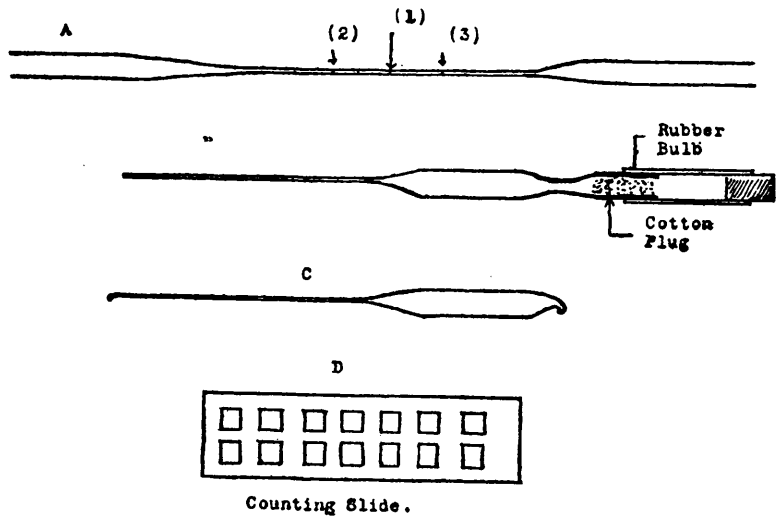
Vitamine Preparation Tested.	No. Yeast Cells in Unit.	Incubation Period.	Cell Count of Incubated Tubes.	
			Vitamine Tube.	Control Tube.
Water sol. Navy bean (*a*).	7–8	20 hours	11,237	113
Water sol. Navy bean (*b*).	2	20	672	15
Water sol. Navy bean (*c*).	2	20	633	15
Same after shaking with Lloyd reagent (*a*).....	7–8	20	100	113
Same after shaking with Lloyd reagent (*b*).....	2	20	2	15
Same after shaking with Lloyd reagent (*c*).....	2	20	0	15
Funk 1913 preparation...	7–8	20 hours	404	11
	2	20	31	2
	2	20	68	2

These results seem to demonstrate that what Funk calls the antineuritic vitamine as prepared by him and what we call water-soluble B as prepared by the McCollum method both respond to the test whether they are identical in character or not. The absence of salts and other substances present in the crude navy

[4] McCollum and Simmonds, A study of the dietary essential, water-soluble B. *Journ. Biol. Chem.* 1918, xxxiii, 55.

bean extract resulting from the Funk method seems to indicate that it is the vitamine and not the impurities that is responsible for the action. Many more experiments are of course necessary to settle this point fully but these preliminary ones justify faith that we are here dealing with a specific test for vitamine. We hope to confirm them in much greater detail later.

DIAGRAM OF APPARATUS.



A (2) (1) (3) B Rubber Bulb Cotton Plug C D Counting Slide

The second question that arises is as to whether it is possible to compare solutions by this test and actually measure vitamine content quantitatively. Experiments in this direction have consumed most of our time to date and have led to the technique presented, since the earlier tests showed the necessity for rigorous control in each feature of the test. We have not yet attained satisfactory results in this respect but believe it is attainable by improvement in technique. In this connection it must be borne in mind that aside from uniform yeast suspensions, accurate calibration and other mechanical details the test is biological. Yeast cells like rats vary in metabolic activity, the vitamine necessary to stimulate growth does not vary directly with concentration but exhibits an optimum and minimum effect which must be

established in the comparative standard used. These factors complicate the problem. The following results show some of these difficulties and are given rather to suggest the problems than as conclusions. All these features are under study and we hope soon to present a report of progress toward their solution:

TABLE II.

Vitamine Preparation Used.	Concentration.	Period of Incubation.	Count of Incubated Tubes.	
			Vitamine Tube.	Control Tube.
Water sol. vitamine from Navy bean, F24.....	c/1	24 hours	5312	5
F24.....	c/2	24	3192	28
F24.....	c/1	18	6161	14
	c/2	18	472	58
	c/8	18	59	20
F24.....	c/1	18	9724	123
	c/2	18	362	60
	c/8	18	85	9
F24.....	c/1	18	6248	48
	c/2	18	729	60
F24.....	c/1	18	8531	97
F24.....	c/1	6	24	2
	c/2	6	147	6
	c/4	6	119	3

The above were made with the first pipettes before accurate calibration or standardized suspensions were made. The following were results with calibrated pipettes but the suspensions were not entirely uniform as shown by a count of 25 units of the suspension used. These units were taken after an hour shaking and varied as follows: 18–37–12–1–3–2–2–0–15–35–46–78–57–6–27–31–33–12–11–17–34–21–60–29 cells.

F24.....	c/1	6	27	15
	c/2	6	9	one control
	c/4	6	14	for series
	c/1	6	20	6
	c/2	6	16	one control
	c/4	6	60	for series
	c/8	6	12	

The following were made with calibrated pipettes and a suspension for which eight counted units resulted as follows (2 hours shaking): 4–2–3–1–3–5–2–3 cells.

F24.....	c/1	22 hours	492	1
	c/2	22	660	one control
	c/4	22	102	for series
	c/8+	22	11	

It is unnecessary to suggest the various fields of application for the test in testing the effect of heat, alkali, quantity, etc. The above represents merely a preliminary communication and we hope soon to report such modifications as will meet the defects that have shown in the preliminary study outlined here.

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A study of local anesthetics in respect to their antiseptic properties.

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An inquiry into the antiseptic properties of local anesthetics is interesting for two reasons: Firstly, in relation to the healing of post-operative wounds and secondly in connection with genito-urinary practice, where these drugs are introduced into the bladder cavity and may thus directly influence its bacterial flora. The following local anesthetics were studied by the authors: cocain, novocain, alpha-eucain, beta-eucain, stovain, holocain, alypin, apothesine and benzyl alcohol. Bacteriological tests were made in three ways. Firstly, the growth of bacteria in bouillon containing small amounts of the anesthetics was studied. Secondly, bacteria were suspended in weak solutions of the anesthetics for definite periods of time, then washed, and planted in various media in order to determine whether they will grow or not. Thirdly, bacteria were planted on agar impregnated with the various drugs and their growth in this medium was noted.

It was found that some local anesthetics possess definite antiseptic properties, while others are entirely devoid of such. It was interesting to find that the chief local anesthetics in use, namely, cocain and novocain, possess no antiseptic properties. On the other hand, some of the other bodies studied and in particular, benzyl alcohol, beta-eucain and holocain, showed distinct antiseptic properties. The fuller data will appear in due time in the *Journal of Pharmacology and Experimental Therapeutics.*