

TABLE III.

	CO <sub>2</sub> gm.	O <sub>2</sub> gm.	Calories.
In diabetes 1 gm. urinary N =	4.00	4.56	13.04
In periods 4-5, 0.637 gm. N =	2.55	2.90	8.31
Period 4. Total . . . . .	23.02	24.38	R.Q.
Deduct for N . . . . .	2.55	2.90	.687
Non-protein . . . . .	20.47	21.48	.693
Period 5. Total . . . . .	23.73	25.36	.681
Deduct for N . . . . .	2.55	2.90	
Non-protein . . . . .	21.18	22.46	.686
Compare with	D : N	R.Q.	Non-protein R.Q.
Phlorhizinized dog . . . . .	3.54	.687	.704
Diabetic man G. S. . . . .	3.5	.697	.700
Diabetic man C.K. . . . .	3.97	.687	.699

93 (1553).

### Experiments on a quantitative and qualitative test for anti-beriberi vitamine.

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In 1912 one of us (C.F.) experimented with the anti-beriberi vitamine to see if it could not act as coferment in alcoholic fermentation. The experiments failed at that time, but now the subject through the work of Williams, Bachmann and Eddy has attained a new interest. Their results seem to prove that vitamine activity can be measured by growth of yeast cells and would permit of dispensing with animal experimentation in the initial stages of vitamine fractionation.

Because of the uncertainty of the Bachmann fermentation test, and the complicated procedure of Eddy's test, it was thought desirable to develop a simple macro method. Our procedure is as follows: A yeast suspension is prepared according to the method of Eddy by shaking a loopful of 48 hours yeast culture in a definite amount of Naegeli's solution for 4 hours on a shaking machine. Duplicate tubes are then prepared containing: (1) yeast suspen-

sion + Naegeli, (2) vitamine sol. + Naegeli, and (3) vitamine sol. + yeast suspension + Naegeli. The tubes are incubated for 20 hours at 30° and the fermentation then interrupted by heating to 70° for a few minutes. The contents of the test tubes are then transferred to special centrifuge tubes, the bottom part of which ends in a capillary 2½ cm. in length and divided in mm. The tubes are centrifuged at 2600 r.p.m. for 15 min. and the reading made without much delay as there is a tendency for the yeast cells to swell up slightly after a time. Sterility during the entire process, excepting centrifugation, is of paramount importance. The results obtained with this method were as follows: The procedure was so well standardized that the controls with yeast suspension alone were practically constant. Duplicate experiments checked very well so that the method was applicable for a quantitative determination. In developing the test, autolyzed yeast was used as a source of vitamine. It was noticed, however, that the amount of cells obtained, as shown by the reading on the centrifuge tube, does not increase double if the vitamine amount is doubled. By trying varying amounts of vitamine a curve was established, using autolyzed yeast as standard, which permits of comparison between an unknown vitamine content and our standard. The sensitivity of the method is .0001 c.c. of autolyzed yeast, and the amount to be tested is best made so that it corresponds to our curve between 0.01 and 0.1 c.c. of autolyzed yeast. In this interval the curve climbs up abruptly and small differences can be read with facility. It was found also that contrary to the method of Eddy, where the number of cells incubated varies the controls to an undesirable extent, this is not the case in our method. The variation of cells in our yeast suspension has very little effect on the final result.

First of all, we were interested to know in how specific this test is for B-vitamine. Among the substances found negative in their action were allantoin, hydantoin, nicotinic acid, several purine and pyrimidine bases and several aminoacids. Pilocarpine found by Dutcher to be curative in pigeon beriberi was found inactive, while thyroid gland was active, as it contained the cellular constituents. An extract of pituitary gland was also found active for the same reason. In a second series the substances isolated

from the vitamine fraction from yeast in 1912 and 1913 were tested. Those of 1913 were analyzed at that time and their formulas established. These substances showed an attenuated but definite activity. Polished rice was entirely negative as opposed to the statement of H. H. Green. Saliva was found inactive and urine as already reported by Muckenfuss was active. The latter test may prove of clinical value later on, but possibly blood could be used to greater advantage for diagnostic purposes. A few discrepancies were found however; for instance, yeast treated with Lloyd's reagent still retained a large portion of its activity and also it was found that corn and wheat, separated from the germ by the method of Voegtlin and Myers, still exhibited a large activity, although several times less than the germ containing portion. Further experimentation is under way to clear up these matters.

By comparing the method of extraction of Osborne and Wake-man from yeast with an extract prepared from autolyzed yeast and then heated over 50° to coagulate proteins (we found some heat coagulable protein in autolyzed yeast) we found that Osborne's preparation contains only one-fifth of the nitrogenous substances but exhibits only one-fifth of the activity of the autolyzed yeast extract. It remains to be seen therefore whether it is advisable to use very purified extracts for vitamine fractionations, since it appears that the concentration of vitamins in such crude extracts as above varies with the amount of impurities present.

94 (1554)

**The site of the cardiac lesion in two instances of intraventricular heart block.**

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The hearts of two cases were examined in order to determine the site of the lesion associated with electrocardiograms suggesting partial block of either the right or the left branch of the auriculo-