152 (2112)

Can yeast grow in a chemically pure medium?

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Reports have lately appeared that yeast can grow on a medium composed of known ingredients, viz.: 50 grams cane sugar (Domino brand), 2 grams KH_2PO_4, 2.35 grams $(NH_4)_2SO_4$, 0.25 gram $CaCl_2$ and 0.25 gram $MgSO_4$, dissolved in a liter of distilled water. It was claimed that on the above medium, which we will call medium O, yeast could be grown in sufficient quantities to serve as a source of vitamine B in animal feeding experiments.

We have attempted to grow yeast on the above medium but have found it difficult to obtain sufficient yeast by this method. It grew very slowly in the incubator and hardly at all at room temperature. The yields were very small; for example, 1800 c.c. of nutritive solution distributed in twelve flasks gave, after growing for a month, 1.5 grams of dried yeast, whereas on addition of 1 c.c. autolyzed yeast to each flask, 900 c.c. in six flasks gave in four days 3 grams of product. Whereas the yeast in the first case developed a brown pigment and presented a spore-like shrunken appearance, in the second case the cells were colorless and in active budding.

In sub-culturing the yeast obtained on medium O, by introducing 5 c.c. into each of a series of flasks containing fresh media, the yield remained almost constant. This excluded the cause of the growth as being due to vitamine D introduced with the seeding, and suggested the possibility of an impurity in the medium. It therefore became necessary to investigate more carefully the purity of one or more of the three constituents of the medium, namely, salts, water and cane sugar.

Each one of the salts used as well as the cane sugar was dissolved separately in distilled water, shaken out with fullers earth and the filtrate evaporated to dryness. The distilled water of the laboratory was redistilled three times in an all-glass apparatus. The salts, sugar and the water were used to make up the neces-

sary solutions, 10 c.c. of each being inoculated with 0.5 c.c. of yeast suspension obtained from the 14th sub-culture. After suitable incubation, the growth was measured in millimeters by the centrifuge method. The results were as follows:

TABLE I.

	Growth in mm. after 3 days	4 days
1. Medium consisting of purified ingredients (Medium P)	1.5	1.5
2. Medium P with ordinary sugar replacing purified	5.0	5.0
3. Medium P with ordinary salts replacing purified	1.5	1.5
4. Medium P with ordinary water replacing purified	1.5	1.5
5. Medium O plus one drop autolyzed yeast. Viability test.	25.5	23.5
6. 10 c.c. sterile water plus 0.5 c.c. yeast suspension	1.4	1.5
7. Same as No. 6, but with yeast killed before incubation	1.0	1.0

These results prove that the cane sugar was the only vitamine D bearing factor in the medium and hence to this factor our whole attention was directed. The cane sugar was recrystallized three times from alcohol and each fraction tested in conjunction with the usual salts and distilled water. The same yeast suspension was used as in the previous experiment.

TABLE II.

	Growth in mm. after 3 days
1. Medium O	6.12
2. Medium O with once recryst. sugar replacing ordinary sugar	2.5
3. Medium O with twice recryst. sugar replacing ordinary sugar	2.37
4. Medium O with thrice recryst. sugar replacing ordinary sugar	2.12
5. Medium O plus one drop autolyzed yeast	23.0
6. Medium P plus ash from one gram of ordinary sugar	2.12
7. 10 c.c. sterile water plus 0.5 c.c. yeast suspension (killed)	2.0

We have also tested the activity of the residue obtained from the concentrated mother liquors, and found that this concentrate, when purified and added to medium P, influenced the growth of

yeast to a marked degree. This experiment has shown that the impurity in cane sugar can be practically eliminated after one recrystallization from alcohol and that the yeast growth promoting property of cane sugar is not likely to be in the ash. Our work has shown that cane sugar, purified either by fullers earth, by recrystallization from alcohol or by combination of both methods, is devoid of vitamine D.

We have also made some experiments on the influence of the medium on the subsequent growth of yeast. Yeast grown on a medium poor in vitamine D, as medium O, and then used for inoculation, gives a growth amounting to 0.4 mm.; while that grown on an agar-malt medium amounts to 2.25 mm. We can conclude therefore that the yeast which we, at least, have been using is unable to grow on a medium devoid of vitamine D. When growth does take place, it is invariably due to two factors: firstly, the amount of vitamine D introduced with the seeded cells, depending on the type of medium used; and secondly, the vitamine-like impurity found in cane sugar.

This impurity in cane sugar should be taken into account in any subsequent work on this subject. The strain of bakers' yeast that we have been using is unable to grow without vitamine D and hence is unable to synthesize vitamine B in the absence of vitamine D.

153 (2113)

Calcium in the blood.

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In our continued investigation of the calcium in the blood we have paid particular attention to furunculosis and to diabetes. The results here published are part of over 225 determinations made on human beings with the method described by Kramer and Tisdall.[1] They state the normal is 9.2—11.1 mg. in 100 c.c. of blood.

[1] Kramer, Benj., and Tisdall, F. F., *Jour. of Biol. Chem.*, 1921, xlvii, 475.