XCVII. THE SYNTHESIS OF THE ANTINEURITIC FACTOR (TORULIN) BY YEAST.

By FRANK HAWKING.

From the Rockefeller Department of Biochemistry, Oxford.

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THERE has been some uncertainty as to whether yeast could synthesise vitamin B when grown on a medium which did not contain it. Macdonald [1922], Heller [1923], and Nelson, Fullmer and Cessna [1921] tested for the factor promoting growth in rats and found it present. Harden and Zilva [1921] and Heller [1923] tested for the antineuritic factor, and found this to be synthesised. But Eijkman, Hoogenhuijze and Derks [1922] found that the antineuritic factor did not occur in yeast unless the medium contained either it or its decomposition products.

In all these experiments there seems to have been no test at the end as to the success of the precautions against bacterial contamination, although it is known that some species of bacteria can synthesise vitamin B [Damon, 1923; Heller, Elroy and Garbeck, 1925]. In some of the experiments it is almost certain that unsuspected impurities containing bios were present [Hoet, le Clef and Delrue, 1924; Funk and Freedman, 1923; Willaman and Olsen, 1923]. Hence further experiments were undertaken to investigate whether yeast could synthesise the antineuritic factor, if the possibility of active impurities in the medium and of bacterial contamination was excluded or controlled. The antineuritic factor is considered here to be the factor curative for symptoms of head retraction in pigeons fed upon polished rice.

Method.

The medium used was:

KH_2PO_4	5 g.	Cane sugar ...	50 g.
NH_4Cl	2·5 g.	Water	1 litre
$MgSO_4$	0·35 g.	Bios extract ...	3 cc.
$CaCl_2$	0·25 g.		

The cane sugar was recrystallised three times from 80 % alcohol. The salts were either purified by double recrystallisation, or Kahlbaum's preparations were used. All glassware was cleaned with chromic acid. The water used was glass-distilled from alkaline permanganate.

The bios extract deserves further explanation. It was prepared by Mr G. L. Peskett from baker's yeast, according to the method of Eddy, Kerr, and Williams [1924], up to the "fuller's earth" stage, and its activity may be

judged from the following experiment (Table I). Flasks containing 100 cc. of the salt solution and various concentrations of the extract were inoculated, and after growing for 6 days at 33° the yeast was centrifuged off, dried at 140° overnight, and weighed.

Table I.

Conc. of bios extract %	Dry wt. of culture g.	Wt. of yeast / Conc. of bios
1·0	0·10, 0·15 = 0·125	0·125
0·5	0·10, 0·14 = 0·12	0·240
0·2	0·07, 0·10 = 0·085	0·425
0·1	0·04, 0·04 = 0·04	0·400
0·025	hardly any growth	—

The extract also contained a certain amount of vitamin. The attempt was made to control this by using low concentrations of extract in the medium. The content of vitamin may be obtained from the following results (Table II), obtained by the curative and protection test [Kinnersley and Peters, 1925].

Table II.

Pigeon	Dose of extract cc.	Duration of cure
1	1	No cure
2 (re-treated bird)	5	2 days
3	5	7 „
4	4·5	6 „
5	4·5	1 day

Prof. Peters makes the following comment upon the figures.

"The bios extracts are distasteful to the birds and the results obtained exceptionally variable. This is apt to be so with inactive concentrates, and it is usually found that re-treated birds react less readily to curative extracts than birds fresh from the dealers. For the purpose of this paper it would appear wisest to take the least favourable results, namely, experiments 3 and 4."

Hence, for the purpose of this paper, it may be concluded that the extract contained not more than 1·4 day doses per cc., *i.e.* 2·1 day doses per 500 cc. medium as a maximum.

The strain of yeast used was *Saccharomyces cerevisiae* Nat. Type Culture No. 381/21, from the Lister Institute. One drop of a 48 hours' growth in the above medium was transferred to 10 cc. of the salt solution, and one drop of this was used to inoculate each of the 500 cc. culture flasks. The inoculation contained about 20,000 cells. The flasks were incubated for 5 days at 30–33° and then the yeast was centrifuged off and extracted twice with 70 % alcohol as described by Funk, Harrow and Paton [1923].

Before centrifuging, smears were made of the yeast grown and subcultures of the supernatant fluid were also made on to agar slopes. These were incubated for 2 days at 33° and one at 37°, and then a composite smear was made from

each tube. The two sets of slides thus obtained were examined for bacterial contamination, staining with carbol-thionine blue. Careful inspection showed it slight, but an attempt was made to give some objective indication of the degree to which it was present. Three microscope fields on each slide were taken at random, and everything that might possibly be a micro-organism was counted, the result being expressed as a percentage of the number of yeast cells present in the same field (300–500). When expressed thus, the contamination of the actual yeast extracted was about 1–2 % (see below). This is an outside figure and is probably excessive, especially as the agar slopes —presenting conditions favourable to bacterial growth—gave a figure only slightly higher (2–3 %). In any case, the proportion by volume was very much less, since a yeast cell is at least 250 times as large as an average micro-organism.

Results.

A series of earlier experiments was spoilt by the presence of too much bios extract, and so, of too much vitamin in the culture medium. The latest results obtained with the most improved technique were as follows:

Table III.

Pigeon	Yeast wt. g.	Cured or protected for days	Contamination	
			Smear %	Agar slope %
5	3·7	3	1·3	—
6	3·9	5	1·3	3·0
7	3·85	3+	1·5	2·2
8	2·95	7	2·1	—

Pigeon 8 came from an irregular batch of which several other birds gave abnormally high results, and must therefore be discarded. For the purpose of the experiment, pigeons 5 and 7 gave the lowest result, namely, 3 days. If this lowest figure is therefore taken for the curative effect of the yeast, and compared with the highest figure obtainable for the amount of possible vitamin in the bios present in the medium, the results obtained will be the most unfavourable to synthesis found under the conditions of the experiments. The facts are that not more than 2·1 day doses/500 cc. were originally present, whereas not less than 3 day doses were found in the yeast after growth. A balance of synthesis of 1 day dose out of three is therefore indicated.

Discussion.

The increase of activity observed is small, and it might be suggested that it could be attributed to unrecognised impurities present in the medium. Though the salts and sugar had been purified as described, an attempt was made to extract the medium with successive fractionations with 70 % alcohol. This had to be abandoned owing to the difficulty of freeing from sugar sufficiently to give to a bird. Against the possibility of slight contamination in the medium, however, may be set the probability that Funk's method does

not extract all the torulin present. Further, no allowance can be made for a possible utilisation of the factor by the yeast in the course of its metabolism. The figures given for the bacterial contamination which are maximal suggest that this possible source may be neglected.

A general consideration of the problem of synthesis by the yeast cell suggests that such synthesis is likely to be small unless a much purer bios preparation can be obtained. It is inherently probable that an optimal concentration of the vitamin in the yeast cell can be obtained either by intracellular synthesis or adsorption from the environment. Fluid in which yeast has grown is notably free from vitamin, a fact which was confirmed in some of the earlier experiments. Two lots of 300 cc. of medium, after growth of the yeast, were extracted with 66 % alcohol and taken up in aqueous solution but failed to cure birds, although initially 4 day doses were present in each. Adsorption of vitamin by the yeast has been stressed by Eijkman *et al.* [1922], Randoin and Lecoq [1926] and Southgate [1924]. It may be concluded that yeast preferentially adsorbs the factor, merely synthesising when necessary to meet the optimal requirements. Therefore until a bios preparation can be obtained containing a growth-promoting factor of the bios type in much larger concentration than it does torulin or its decomposition products, the problem would seem impossible of final settlement. There were two other contentions, contained in Eijkman's paper. It was suggested that yeast could not synthesise the antineuritic factor, but merely reactivated its decomposition products. In these experiments, treatment with alkali was avoided, and the method of preparation of the bios should not be destructive of torulin. It is, however, still possible that the apparent synthesis is merely a reactivation of some decomposition product. The further contention that, as the antineuritic factor is not synthesised by yeast, it is to be distinguished from the growth-promoting factor, is puzzling to interpret in the light of recent work upon the duality of vitamin B. If both factors are needed for the growth of the rat, some synthesis of the antineuritic vitamin must have occurred.

SUMMARY.

Evidence is produced to show that yeast (*S. cerevisiae*) can synthesise the curative factor for pigeons (torulin) in small amounts.

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