

# CXLVII. THE SYNTHESIS OF ANTINEURITIC VITAMIN BY YEAST.

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RECENTLY Hawking [1927] has shown that yeast can synthesise the factor which cures the symptoms of head retraction in pigeons fed upon polished rice. These experiments were not quite conclusive on two accounts, viz.: (1) the amount of "bios extract" used in growing the yeast contained so much of the antineuritic factor that the amount of the latter which was synthesised appeared to be small; and (2) there was some contamination of the yeast by bacteria. The following experiment was performed under conditions which were more satisfactory in these respects.

## EXPERIMENTAL.

The cane sugar, salts and "bios extract" were precisely the same as Hawking employed, and the experiment may be regarded as a continuation of his work. A basal medium was used, of which the composition was:

$\text{KH}_2\text{PO}_4$	...	...	5.0 g.	$\text{CaCl}_2$	...	...	0.25 g.
$\text{NH}_4\text{Cl}$	...	...	2.5 g.	Cane sugar	...	...	50.0 g.
$\text{MgSO}_4$	...	...	0.35 g.	Glass-distilled water	to 1000 cc.		

The yeast was the same strain of *Saccharomyces cerevisiae*, and the stock cultures were grown at 25° in 5 cc. portions of basal medium containing 0.05 cc. of "bios extract." A small loopful of stock culture (21 days old) was transferred to a fresh tube of this medium and grown at 25° for one day. The supernatant fluid was pipetted off and replaced by 5 cc. of basal medium, the resulting suspension being used for inoculation of the flask in the following experiment.

*Experiment.* 0.5 cc. of "bios extract" was added to 1500 cc. of basal medium in a 2-litre flask, the neck of which had been drawn out to a diameter of 15 mm. in order to minimise the risks of accidental infection. After sterilisation (by steam-heat on three successive days) the flask was inoculated with approximately 3000 yeast cells and maintained at 25° for 10 days with occasional shaking. Samples were then removed to agar slopes, and the culture was at the same time examined in wet and dry films. The latter showed that there was no bacterial contamination, and the growth on the agar slopes consisted in all cases of pure yeast. The greater part of the fluid was syphoned

off, and the remainder separated on the centrifuge. The resulting yeast weighed 5.7 g. (wet). This yeast was immediately extracted by boiling for one minute with 7 cc. of 1 % acetic acid, and the extract separated from the cell-residue by centrifuging. The fluid was decanted, made up to a volume of 10 cc. with water, and divided into two equal parts.

Two pigeons were used, each receiving 5 cc. of the extract. In both the symptoms of head retraction disappeared, to reappear in one case after 2.75, and the other after 3.25 days. The yeast therefore contained at least 6 day doses.

Hawking's experiments showed that the "bios extract" did not contain more than 0.7 day doses in 0.5 cc. The cells used for inoculation represented a thousandth part of the suspension from which they were obtained, and therefore did not contain more than 0.00007 day doses. Thus, before the development of the yeast, the flask did not contain more than 0.70007 day doses. Of the 6 day doses found in the yeast, 5.3 day doses were presumably synthesised.

#### SUMMARY.

An experiment is recorded in which 5.7 g. of yeast were produced in a medium which did not contain more than 0.7 day doses of antineuritic vitamin. Bacterial contamination was avoided, and the yeast contained at least 6 day doses. Considerable synthesis can therefore occur.

I wish to thank Prof. Peters for his interest and advice, and for his kindness in testing the extract on the pigeons.

#### REFERENCE.

Hawking (1927). *Biochem. J.* 21, 728.