NET SYNTHESIS OF STEROLS IN RESTING CELLS OF SACCHAROMYCES CEREVISIAE*

by

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It has been known since the early classical experiments of LINDNER and his associates that fat formation in yeasts is greatly stimulated by aeration. The enhancing effect of oxygenation on the sterol content of yeasts has also been noted by many investigators (see Kleinzeller¹ for review).

Several years ago, it was noted that sterol formation could be induced in Saccharomyces cerevisiae by the vigorous aeration of cells that had been grown under strictly anaerobic conditions2. These observations have now been confirmed and extended. When cells of S. cerevisiae, strain LK2G12, are grown under nitrogen in a medium containing 2% peptone (Difco), 2% glucose and 1 % yeast extract, the sterol content. as determined by the LIEBERMANN-BURCHARD reaction, is 2-5 mg per gram of dried cells. The sterol content is about ten times higher when the cells are cultivated under aerobic conditions. Cells harvested from anaerobic cultures rapidly synthesize new sterol when aerated in phosphate buffer (pH 4.5 to 7.5) containing 1% glucose, whereas cells kept under anaerobic conditions show no net sterol synthesis (Fig. 1).

Using this system, it can be shown that the newly synthesized material is derived from the added glucose rather than from endogenous materials. After 4 to 8 hours aeration with radioactive glucose, the non-saponifiable fraction of the yeast cells is found to have a high specific activity, the carbon of this fraction having 75-90% of the specific activity of the carbon of the added glucose. Extensive labelling is found also in the fatty acids of these cells, while the proteins and polysaccharides contain considerably less radioactivity.

This procedure thus affords a relatively convenient method for the preparation of radioactive

yeast sterols of high specific activity. It appears to be simpler and more economical than the procedure of Hanahan and Wakil³ in which coenzyme A-reconstituted yeast cells⁴ are utilized for the synthesis of labelled sterol. Further details will be presented in a subsequent communication.

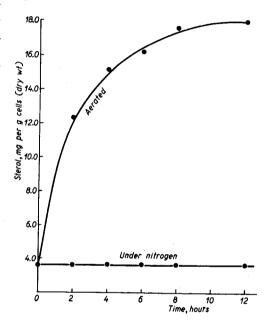


Fig. 1. Synthesis of sterols by Saccharomyces cerevisiae. Cells harvested after 48 hours growth under anaerobic conditions, washed twice in o. IM phosphate buffer (pH 6.5), resuspended in same buffer containing 1 % glucose. Half the cell suspension was aerated by bubbling air through the medium; the other half was kept under nitrogen. Temperature of incubation, 30 C.

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