Ergosterol Synthesis in Yeast under the Action of Nystatin

That the general mechanism of ergosterol synthesis in yeast is similar to that of cholesterol in animals has been indicated by several lines of evidence[1]. While some of the manifold functions of cholesterol are well known, the significance of ergosterol in yeast is at best speculative, although it can be said that sterol production is connected with some fundamental function intimately associated with aerobiosis[2]. Moreover, it is one of the possible components subject to fluctuations under conditions of stress induced by drugs or other means[3]. In this connection, it was of interest to examine glycolytic inhibitors similar in structure to sterols, or their biosynthetic precursors like squalene, for their effect on sterol metabolism in yeast. KODICEK[4] has demonstrated a definite antagonism between unsaturated fatty acids and sterols in their inhibitory action on gram-positive bacteria. The polyene antibiotic, nystatin, is studied here, for its action on sterol synthesis in yeast.

GOTTLIEB et al.[5] have already advanced the hypothesis that the polyenes might prevent the biosynthesis of essential sterols by fungi, or compete with sterols serving as cofactors for vital metabolic reactions. LAMPEN et al.[6], on the other hand, suggest that the inhibition obtained with sterols in the action of polyenes on fungi would represent merely a polyene-sterol interaction. The present study with nystatin and a pure strain of *Saccharomyces cerevisiae* shows that there is indeed a metabolic disarrangement produced by nystatin and that this is evidenced in the enhanced sterol production under growing conditions.

The general plan of experiments was similar to that reported already by RAJAGOPALAN and SARMA[3]. Sterol was estimated with anthrone by the method of VAHOUNY et al.[7] after precipitation as the digitonide. The results are shown in the Table.

Ergosterol content of yeast grown under the influence of nystatin

Nystatin added	Dry weight of yeast	Ergosterol content free	Ergosterol content total
µg/ml	mg/100 ml medium	µg/100 mg dry cells	
0.0	292	81	140
6.0	253	130	170
10.0	210	128	210
14.0	172	124	208

If the antibiotic had inhibited sterol production, the metabolite-antimetabolite concept might be invoked to suggest an interference with squalene production or utilization. As it is, the results serve to underscore the paradoxical position of ergosterol, which can be considered as both essential and nonessential for life. However, in view of its continued presence and observed variations in various conditions—similar to the fluctuations in plasma cholesterol level in animals—it is tempting to ascribe a positive role to ergosterol in yeast. Since the antibiotic is toxic to yeast at low concentrations (1.5 to 2.0 µg/ml) and the production of more sterol is manifest even at a level causing 10% inhibition of growth, it is suggested that it may play a still undiscovered role either in the growth or in the perpetuation of the species, if not in its survival. It is interesting to compare in this context the suggested involvement of ribonucleic acid or a polyribonucleotide in sterol synthesis[8], and the effect of purine antagonists on the synthesis of sterols[9].

Zusammenfassung. Nystatin erhöht den Ergosterolgehalt in *Saccharomyces cerevisiae* auch bei einer Konzentration, die nur 10prozentige Wachstumshemmung bewirkt.

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The *in vitro* Inhibition of Pentobarbital Metabolism by Chlorpromazine

The importance of the hepatic microsomal enzymes for the metabolism of some drugs has recently been widely recognized. The enzymes have in common a TPNH and oxygen requirement[1]. The activities of numerous microsomal drug-metabolizing enzymes are inhibited by SKF 525 A, iproniazid, JB 516, isoniazid, and Lilly 18947, but the mechanism of inhibition is not yet known[2–5]. In the present work, evidence is given that the microsomal drug metabolizing enzyme responsible for the metabolism of pentobarbital is inhibited by chlorpromazine, a drug metabolized by a different microsomal enzyme.

Male rats of the Sprague-Dawley strain, weighing about 60 g were used. The enzyme activity was determined by measuring the metabolized pentobarbital in liver slices after an incubation for 1 h. Sliced livers (500 mg) were suspended in a Warburg flask which contained 6 ml of Krebs phosphate buffered Ringer (pH 7.4) and 0.2 ml of 308 µg sodium pentobarbital (final concentration was $2 \times 10^{-4} M$) and incubated in an atmosphere of oxygen at

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