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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37°C for 1 h, with shaking. At the end of the incubation period, the reaction mixture was homogenized with a Potter-Elvehjem type homogenizer and 2 ml of the homogenate were used. Chlorpromazine was added in volume of 0.1 ml after being disolved in distilled water. The determination of pentobarbital was carried out according to BRODIE et al.[6].

The pentobarbital metabolism had a 44% inhibition by the same concentration of chlorpromazine (2×10^{-4}) and 81% with high concentration (8×10^{-4}) as shown in the Table.

It is well known that the major metabolic pathway of pentobarbital is the side chain oxidation and that of chlorpromazine is the sulfoxide formation, but both reactions take place in hepatic microsome and require TPNH and oxygen[7,8]. The inhibition of pentobarbital metabolism by chlorpromazine may not be due to a competition in requirement of TPNH. The addition of TPN, glucose-6-phosphate and nicotinamide did not reverse the inhibition.

A similar inhibition was also obtained in the experiments with the microsomal fraction[9]. Our preliminary results indicate that the type of inhibition seems not to be a competitive one[9].

It was further demonstrated that chlorpromazine also inhibits the metabolism of meprobamate, carisoprodol and hexobarbital, while, on the other hand, meprobamate metabolism was inhibited by pentobarbital, phenobarbital, strychnine, amphetamine, and aminopyrine[9]. The *in vitro* inhibition of microsomal drug-metabolizing enzymes by the drugs which are also metabolized by microsomal drug-metabolizing enzymes must therefore be quite a general phenomenon in the drug metabolism.

On the other hand, 15 mg/kg of chlorpromazine, unlike SKF 525 A, does not significantly inhibit the pentobarbital metabolism *in vivo*.

It is also of interest that the inhibition of pentobarbital metabolism by chlorpromazine is more remarkable in phenobarbital pretreated rats, which have high enzyme activity, than in normal rats[10–15].

Further studies on the mechanism of the observed evidence may present some information on the nature of the microsomal drug-metabolizing enzymes and especially on the action of SKF 525 A or on the mechanism of enzyme induction by the phenobarbital pretreatment.

The work in detail will be published elsewhere.

Effect of chlorpromazine on the *in vitro* metabolism of pentobarbital

Chlorpromazine concentration (Mol)	Pentobarbital metabolism (μg/g/h)	Inhibition (%)
0	202 ± 3.5 (8)	
5×10^{-5}	182 ± 4.9 (6)	10
1×10^{-4}	152 ± 4.8 (6)	25
2×10^{-4}	123 ± 3.3 (9)	44
4×10^{-4}	83 ± 4.0 (6)	59
8×10^{-4}	38 ± 3.1 (6)	81

The numerals in brackets show number of determination.

Riassunto. Si è osservato che il pentobarbital alla concentrazione di $2 \times 10^{-4} M$ può inibire per il 44% il metabolismo *in vitro* della cloropromazina alla medesima concentrazione.

Si è discussa quindi la possibilità di inibire il metabolismo *in vitro* di alcuni farmaci propria di altre sostanze che vengono metabolizzate in maniera del tutto simile da sistemi enzimatici a livello microsomale epatico.

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Über einen phytotoxisch wirkenden Stoff aus dem Extrakt von *Brachycaudus napelli* Schrk. (Insecta, Aphidoidea)

Versuche mit wässrigen Extrakten verschiedener Blattlausarten ergaben, dass dieselben das Welken der abgeschnittenen und in die Extrakte gestellten Wirtspflanzensprosse hervorrufen können (KAZDA[1]). Eine nähere Prüfung dieses Effekts – mit dem Extrakt aus *Brachycaudus napelli* Schrk. – ergab auch an abgeschnittenen Tomatensprossen das Auftreten von Welkeerscheinungen, Blattnekrosen und Störungen im Wasserhaushalt. Im Anschluss an Untersuchungen über Thermostabilität, Löslichkeit und Diffusionsfähigkeit des welkeaktiven Agens, die an anderer Stelle veröffentlicht werden, wurde eine Methode zur teilweisen Entfernung der Ballaststoffe aus dem Extrakt entwickelt.

Methode. Die in der Natur gesammelten Blattläuse wurden mit destilliertem Wasser zerrieben, im Vakuum bei 60°C sofort eingedampft, 2 h bei 110°C getrocknet und dreimal mit Äther extrahiert. Der ätherunlösliche Rückstand wurde zunächst dreimal je 3 h mit Wasser und nachfolgend dreimal mit Azeton extrahiert. Die Wasser- und Azetonextrakte wurden im Vakuum zum Eintrocknen eingeengt, die Rückstände in 5 ml destilliertem Wasser/g frischem Aphidenmaterial gelöst, in den Dialysierschlauch übertragen und 48 h in destilliertem Wasser dialysiert. Es erfolgte eine sechsmalige Wassererneuerung. Das Wasser mit den durch den Dialysierschlauch durchgegangenen Stoffen wurde sofort nach dem Absetzen von der Dialyse durch einen bakteriologischen Filter filtriert und im Vakuum bis zum Eintrocknen abgedampft. Der Rückstand, der nach den vorherigen Untersuchungen einen welkeaktiven Stoff (oder Stoffe) enthalten sollte, wurde unter-

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