which gave satisfactory results on the basis of agreement between estimates based on absorption at 260 m μ and organic phosphorus content.

2. The chemical composition of a preparation of disrupted sarcosomes from pig heart was determined.

3. Microsomes from rat liver and from mammary gland of the lactating cow were found to contain relatively high proportions of phospholipid and of nucleic acids, whereas microsomes from cow's milk contained much less lipid and considerably less nucleic acid.

4. Incubation of mammary-gland microsomes in milk serum caused some loss of lipid and considerable loss of nucleic acid, so that the product had a composition closely resembling that of microsomes from milk.

5. The significance of these results in relation to the origin of milk microsomes is discussed.

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Effect of Sulphanilamide on Formation of Sterols in *Saccharomyces cerevisiae* and *Neurospora crassa*

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Dietary administration of sulphonamides has been shown to produce deficiencies of various vitamins in experimental animals. In particular, sulphaguanidine and succinylsulphathiazole have been widely used for inducing a deficiency of folic acid in rats. In the course of an investigation on the relationship between folic acid and coenzyme A, Popp & Totter (1952) observed that the addition of succinylsulphathiazole to an otherwise normal diet resulted in a significant increase in the liver content

of coenzyme A in rats. The increase was taken to represent the response of the animal to an enhanced demand for coenzyme A required for the acetylation of the free sulphonamide released by the breakdown of absorbed succinylsulphathiazole.

Klein & Lipmann (1953a, b) have demonstrated the dependence on coenzyme A of sterol synthesis in both yeasts and rats and its sensitivity to changes in the tissue levels of the coenzyme. In the present studies it has been found that there is a significant

elevation in the coenzyme A content of yeast cells grown in a synthetic medium supplemented with sulphanilamide. The ergosterol content of the cells also goes up steeply in the presence of the drug. The enhanced formation of sterol has also been observed in the mould *Neurospora crassa* under similar conditions. These results clearly point to the production of adaptive responses in these micro-organisms when subjected to sulphanilamide toxicity.

EXPERIMENTAL

Organisms

The strain of *Saccharomyces cerevisiae* used in these investigations was maintained on malt-agar medium by fortnightly subculture and preserved in the ice-chest between transfers. The wild strain of *Neurospora crassa*, variant 8a, was maintained by fortnightly subculture on agar slants prepared from the basal medium of Horowitz & Beadle (1943), supplemented with 0.2% of yeast extract and 0.2% of malt extract.

Methods

Experiments with Saccharomyces cerevisiae. The synthetic medium of Atkin, Williams, Schultz & Frey (1950) was employed and portions (100 ml.) of the medium, prepared in single concentration and adjusted to pH 5.2, were distributed into 1 l. conical flasks. Sulphanilamide was added to the appropriate flasks in specified amounts. After sterilization, the flasks were inoculated with a suspension of *S. cerevisiae* cells from a 24 hr. growth. After incubation for 18–20 hr. at 30°, the cells from each flask were collected by centrifuging and washed repeatedly with water. The cells were dried by spreading as a thin layer in an evacuated desiccator, for coenzyme A determinations, or by placing in an oven at 100° for 90 min., for ergosterol determinations.

The coenzyme A content of the yeast cells was determined by the method of Kaplan & Lipmann (1948). For determination of ergosterol, the dried cells were repeatedly ground up with separate portions of ethanol-acetone (1:1) mixture, and the extracts pooled in each case and made up to volume. Portions of these extracts were used for determination of free and total ergosterol by the digitonide-precipitation method (Sperry & Webb, 1950); the colour developed with the Liebermann-Burchard reagent was read on the Klett-Summerson photoelectric colorimeter, with filter 64, between 60 and 90 sec. after addition of the reagent.

Experiments with Neurospora crassa. Stone, Hechter, Bonner & Yanofsky (1955) have recently reported that the capacity of mycelia of *N. crassa* to synthesize ergosterol increases with the age of the growing culture. It was therefore considered essential that, in studies on the relative abilities of mycelia of the mould grown under different conditions to synthesize ergosterol, cultures of the same age should be taken for comparison. Sulphanilamide added to the culture medium before inoculation has been shown to suppress the germination of spores of *Neurospora* (Tatum & Beadle, 1942), resulting in failure of growth during an initial period. In order to obtain mycelial growth of the same age with and without sulphanilamide in the medium, the technique employed by Tatum & Giese (1946) in their studies on the effect of sulphanilamide on respiration in *N. crassa* was adopted. This consisted in growing the organism in the basal medium for different periods before addition of sulphanilamide.

N. crassa 8a was first grown at 30° in 250 ml. conical flasks containing 30 ml. of the basal medium (Horowitz & Beadle, 1943) at a concentration corresponding to 40 ml. of the single-strength medium. After a specified period of growth, either 24 hr. or 48 hr., the medium in one group of flasks was supplemented with 10 ml. lots of sterile water, and that in the remaining flasks with 10 ml. lots of a sterile solution of sulphanilamide (15 mg./ml.). The contents of each flask were gently mixed and the incubation was continued. Duplicate sets of flasks from the control and the sulphanilamide-treated cultures were removed periodically, the mycelia washed with water and dried at 100° for 90 min. The total ergosterol content of the dried material was then determined.

RESULTS AND DISCUSSION

The results of the experiment on the effect of sulphanilamide on the coenzyme A content of yeast cells are presented in Table 1. The inclusion of sulphanilamide in the growth medium produced a significant effect on the coenzyme A levels, which showed a progressive rise with increased levels of the drug. The enhancement of the coenzyme A content of the yeast cells obviously represented a metabolic response by the organism to the administration of the drug. These results, in conjunction with the observation by Popp & Totter (1952) of a similar increase in levels of liver coenzyme A in rats fed with succinylsulphathiazole, point to a general adaptive response in living organisms to a stress on the acetylating system.

Table 1. *Effect of sulphanilamide on coenzyme A and ergosterol contents of Saccharomyces cerevisiae cells*

Figures for coenzyme A content are the averages of two values each and those for the ergosterol content are averages of four values each.

Sulphanilamide added (mg./100 ml. of medium)	Growth (mg. dry cells)	Coenzyme A content (units/g. dry wt.)	Ergosterol content (µg./100 mg. dry wt.)	
			Free	Total
0	412	127	110	245
5	322	154	120	306
10	206	249	138	384
20	108	328	159	786

The effect of sulphanilamide on the ergosterol content of yeast cells was also very marked (Table 1), increased levels of the drug producing a steep rise in the capacity of the organism to synthesize ergosterol. A major portion of the enhanced ergosterol content in the presence of sulphanilamide was accounted for by the esterified form of sterol. In view of the intimate relationship between sterol synthesis and coenzyme A, it is reasonable to conclude that the increase in the ergosterol content of *S. cerevisiae* cells in the presence of sulphanilamide is a consequence of the effects of the drug on formation of coenzyme A by the organism.

The addition of sulphanilamide to the medium of growing cultures of *N. crassa* resulted in a significant increase in the capacity of the mycelia to synthesize ergosterol (Tables 2 and 3), indicating the similarity of the action of the drug on *S. cerevisiae* and *N. crassa*. As with yeast, the increased ergosterol content of *N. crassa* in the presence of sulphanilamide was probably the result of an identical adaptive response to the toxicity of the drug.

The capacity of the mould to synthesize ergosterol increased with the period of growth (Tables 2 and 3), as was observed by Stone *et al.* (1955). Ottke (1949), who identified ergosterol as the main sterol of *Neurospora*, found that the sterol represented 0.13 % of the dry weight of the mould grown for 5 days under vigorous aeration. In the present

experiments it was found that the ergosterol content of *N. crassa* in stationary surface growth was nearly 1 % after 5 days of growth.

In experiments with the larvae of the rice moth, *Corcyra cephalonica* St., the inclusion of sulphanilamide at a level of 0.25 % in a whole-wheat-flour diet resulted in an increase of 22 % in the cholesterol content of the larval tissue after a growth period of 3 weeks. If the effect of the drug on the sterol content represented an alteration in the capacity of the tissue to synthesize sterol, these results would again seem to indicate that the production of adaptive responses to the administration of the drug is a phenomenon of wide occurrence.

Reid, Pritchett & Cushing (1952) showed that when *N. crassa* was grown in presence of toxic concentrations of *p*-aminobenzoic acid, the culture medium contained some compounds associated with abnormal metabolism induced by excess of the amine. One of the compounds isolated from the medium was *N*-acetyl-*p*-aminobenzoic acid. In view of this evidence for the presence of an amine-acetylating system in *N. crassa*, it is conceivable that if a stress is imposed on the system, as in the presence of large amounts of sulphanilamide, there will be changes in the factors associated with acetylation, especially in the levels of coenzyme A. Under these circumstances, when there is an increased formation of coenzyme A, there may be a resultant alteration in the systems which involve the coenzyme. The changes in

Table 2. *Effect of sulphanilamide on synthesis of ergosterol by growing cultures of Neurospora crassa*

The organism was grown initially for 24 hr. before addition of sulphanilamide (150 mg./40 ml. of final medium). Incubation was then continued and the total ergosterol content of normal and sulphanilamide-treated mycelia determined periodically. Growth at the time of additions was less than 3 mg. dry wt./flask. Figures for ergosterol content are the averages of two values each.

Period of incubation after addition (hr.)	Growth in flasks (mg. dry wt. of growth)		Ergosterol content of mycelia (μg./100 mg. dry wt.)	
	Control	Sulphanilamide-treated	Control	Sulphanilamide-treated
24	78.2	17.4	612	822
48	112.3	23.2	762	994
96	137.2	37.4	974	1240

Table 3. *Effect of sulphanilamide on synthesis of ergosterol by growing cultures of Neurospora crassa*

Experimental conditions were as described in Table 2 except that addition of sulphanilamide was made after an initial growth period of 48 hr. Growth at the time of addition: 81.8 mg. dry wt./flask. Ergosterol content of mycelia at the time of addition: 513 μg./100 mg. dry wt. Figures for ergosterol content are the averages of two values each.

Period of growth after addition (hr.)	Growth in flasks (mg. dry wt. of growth)		Ergosterol content of mycelia (μg./100 mg. dry wt.)		Increase in ergosterol content over initial level (μg./100 mg. dry wt.)	
	Control	Sulphanilamide-treated	Control	Sulphanilamide-treated	Control	Sulphanilamide-treated
48	103	90.6	681	773	168	260
96	121	101.0	787	945	274	431
120	124	102.0	935	1010	422	498

ergosterol content of *N. crassa*, as also of *S. cerevisiae*, in the presence of sulphanilamide may be taken to represent secondary effects of the drug caused by changes in the levels of coenzyme A. It is possible that the various other biochemical systems involving the function of coenzyme A will also be affected under similar conditions. The results of the present studies seem to indicate that an investigation on these lines is warranted.

SUMMARY

1. The coenzyme A content of *Saccharomyces cerevisiae* cells grown in the presence of sulphanilamide was significantly greater than that of normal cells.
2. The total ergosterol content of the yeast cells showed a progressive rise with increasing degree of sulphanilamide toxicity.
3. A similar enhanced capacity for sterol formation in the presence of sulphanilamide was observed in growing cultures of *Neurospora crassa*.
4. It is suggested that these changes are the

result of an adaptive response on the part of the organisms to overcome the toxicity of the drug.

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Effects of 3-Acetylpyridine on Metabolism of Nicotinic Acid in Rats

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Woolley (1945) reported the appearance of symptoms resembling nicotinic acid deficiency in mice treated with 3-acetylpyridine, which could be prevented by administration of nicotinic acid, nicotinamide or tryptophan (Woolley, 1946). Similarly, 3-acetylpyridine, when incorporated in the perfusion fluid of the isolated heart, produced electrocardiographic changes, which were reversible upon addition of nicotinamide (Braun, 1949). Gaebler & Beher (1951) found a marked increase in urinary excretion of *N*¹-methylnicotinamide in dogs after oral doses of 3-acetylpyridine. Normal dogs suffered no deleterious effects after administration of 3-acetylpyridine, whereas manifestations of blacktongue could be precipitated in nicotinic acid-deficient dogs. In subsequent studies Beher, Holiday & Gaebler (1952) demonstrated that ¹³C-labelled 3-acetylpyridine serves as a source of nicotinic acid and forms urinary *N*¹-methylnicotinamide containing ¹³C. Recently, McDaniel, Hundley & Sebrell (1955) have shown that 3-acetylpyridine may act as either substitute or antagonist of nicotinic acid in the dog, the

ultimate effect depending upon the dog's state of nutrition with regard to this vitamin. 3-Acetylpyridine was most effective as an antagonist in deficient animals, thus suggesting an impaired capacity to transform it into nicotinic acid in this condition. 3-Acetylpyridine seems therefore unique in being an antimetabolite that can be detoxified by means of its conversion into a vitamin.

In view of the dual role of 3-acetylpyridine in the metabolism of nicotinic acid the present study was carried out in order to determine more clearly the activity of the compound in rats.

METHODS

All the animals used were albino males with an initial weight of about 40 g. Two kinds of diet were employed: a casein diet and a corn-sucrose diet. The casein diet consisted of (g./100 g. of diet): casein 18, cornstarch 38, glucose 35, vegetable oil 5 and salt mixture (U.S.P. XIII) 4. The corn-sucrose diet was composed as follows (g./100 g. of diet): casein 5.4, sucrose 49.2, ground yellow corn 40.0, vegetable oil 3.0 and salt mixture (U.S.P. XIII) 2.4. These diets were fortified with the following vitamins (mg./100 g.