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.

REFERENCES

- Atkin, L., Williams, W. L., Schultz, A. S. & Frey, C. W. (1950). Unpublished work. Quoted by Snell, E. E. (1950). *Vitamin Methods*, vol. 1. New York: Academic Press Inc.
- Horowitz, N. H. & Beadle, G. W. (1943). *J. biol. Chem.* **150**, 325.
- Kaplan, N. O. & Lipmann, F. (1948). *J. biol. Chem.* **174**, 37.
- Klein, H. & Lipmann, F. (1953a). *J. biol. Chem.* **203**, 95.
- Klein, H. & Lipmann, F. (1953b). *J. biol. Chem.* **203**, 101.
- Ottke, R. C. (1949). *Arch. Biochem.* **23**, 49.
- Popp, E. M. & Totter, J. R. (1952). *J. biol. Chem.* **199**, 547.
- Reid, E. B., Pritchett, E. G. & Cushing, J. E. (1952). *J. biol. Chem.* **199**, 443.
- Sperry, W. M. & Webb, M. (1950). *J. biol. Chem.* **187**, 97.
- Stone, D., Hechter, O., Bonner, D. M. & Yanofsky, C. (1955). *Fed. Proc.* **14**, 147.
- Tatum, E. L. & Beadle, G. W. (1942). *Proc. Nat. Acad. Sci., Wash.*, **28**, 234.
- Tatum, E. L. & Giese, A. C. (1946). *Arch. Biochem.* **9**, 15.

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

By K. GUGGENHEIM AND E. J. DIAMANT

*Laboratory of Nutrition, Department of Biochemistry, Hebrew University,
Hadassah Medical School, Jerusalem, Israel*

(Received 16 September 1957)

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 & Behr (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 Behr, 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.

of diet): thiamine 0.2, riboflavin 0.3, pyridoxine 0.1, calcium pantothenate 1.6 and choline chloride 100. In some experiments 50 mg. of nicotinic acid was incorporated into each kilogram of the casein diet. The rats kept on the casein diet received additionally 100 i.u. of vitamin A twice weekly.

*N*¹-Methylnicotinamide in urine was determined by the method of Huff & Perlzweig (1947), and the pyridine nucleotides in the liver by the fluorometric procedure described by Robinson, Levitas, Rosen & Perlzweig (1947). Neither method is specific for derivatives of nicotinamide only, and the substances determined by them probably comprise the 3-acetylpyridine analogues as well.

RESULTS

Urinary excretion of N¹-methylnicotinamide-like substances. In the first experiment, three groups of rats were kept on the casein diet, supplemented with 50 mg. of nicotinic acid/kg. The first group was given daily oral doses of 5 mg. of nicotinamide, the second group received 5 mg. of 3-acetylpyridine daily and the third group remained untreated. At the end of the second, fourth and sixth week urine was collected for 3 days and the excretion of *N*¹-methylnicotinamide-like substances was determined (Table 1).

As may be seen from Table 1, administration of 3-acetylpyridine significantly depresses growth, in spite of the abundant dietary supply of nicotinic acid. Although rats treated with 3-acetylpyridine appear to be able to form *N*¹-methylnicotinamide-

like substances, the amounts excreted are much less than those of *N*¹-methylnicotinamide which are excreted by rats treated with the same dose of nicotinamide.

In a second experiment the efficiency of conversion of 3-acetylpyridine into *N*¹-methylnicotinamide-like substances was compared with that of nicotinamide. About 100 rats were kept on the casein diet, which was not supplemented with nicotinic acid, until they reached a weight of 150–250 g. At this stage they were divided into six groups, and each rat received a single oral dose of 0, 1, 2, 5, 10 or 20 mg. of either nicotinamide or 3-acetylpyridine/100 g. body wt. Urine was collected for 2 days, and the amount of *N*¹-methylnicotinamide-like substances excreted was determined. The results obtained are shown in Table 2.

It can be seen that excretion of *N*¹-methylnicotinamide-like substances was impaired only in those rats which received large doses of 3-acetylpyridine (10 and 20 mg.), whereas smaller doses of 3-acetylpyridine caused no significant decrease in the excretion of these substances. It would thus appear that the efficiency of formation of *N*¹-methylnicotinamide-like substances from 3-acetylpyridine decreases with increasing doses of the compound administered.

Liver pyridine nucleotides. Further evidence of the low efficiency of metabolic conversion of 3-acetylpyridine is found in Table 1, where it is

Table 1. *Effect of 3-acetylpyridine on growth, excretion of N¹-methylnicotinamide-like substances and liver pyridine nucleotides in rats kept on a casein diet containing nicotinic acid*

Results are given as the means, with the standard errors. Figures in parentheses indicate number of animals. NMNA, *N*¹-methylnicotinamide-like substances.

Treatment (5 mg./day)	Duration of experiment (weeks)	Weight increase		Excretion of NMNA		Liver		
		(g.)	P*	(μg./day)	P*	Weight (g.)	Pyridine nucleotides μg./g. of liver	P*
Nicotinamide	2	44 ± 2.0 (18)	—	1066 ± 100 (12)	—	4.3 ± 0.17 (6)	410 ± 46 (6)	—
3-Acetylpyridine	2	39 ± 1.1 (18)	0.05	481 ± 60 (18)	0.001	4.2 ± 0.17 (6)	211 ± 16 (6)	0.001
	2	40 ± 2.5 (6)	—	197 ± 146 (6)	0.001	4.3 ± 0.15 (6)	216 ± 16 (6)	0.001
Nicotinamide	4	84 ± 3.1 (12)	—	820 ± 185 (6)	—	5.3 ± 0.16 (6)	710 ± 39 (6)	—
3-Acetylpyridine	4	70 ± 4.1 (12)	0.01	398 ± 18 (12)	0.05	5.6 ± 0.12 (6)	430 ± 31 (6)	0.001
	4	82 ± 4.3 (6)	—	270 ± 82 (6)	0.02	6.1 ± 0.14 (6)	213 ± 20 (6)	0.001
Nicotinamide	6	128 ± 6.3 (6)	—	660 ± 165 (6)	—	6.5 ± 0.10 (6)	340 ± 28 (6)	—
3-Acetylpyridine	6	103 ± 4.2 (6)	0.01	158 ± 25 (6)	0.01	5.7 ± 0.12 (6)	240 ± 36 (6)	0.05

* Probability that the differences from animals treated with nicotinamide are due to chance.

shown that prolonged administration of it leads to a much lower level of liver pyridine nucleotides than does treatment with an equal amount of nicotinamide. Rats treated for 2 weeks with 3-acetylpyridine had no higher pyridine nucleotide levels in their livers than untreated control rats. Only after treatment for 4 weeks with 3-acetylpyridine did the pyridine nucleotide level in the livers rise above that found in untreated controls.

Effect on growth. In order to study the effect of 3-acetylpyridine upon growth, the corn-sucrose diet was employed. The results are summarized in Table 3.

The table shows that the increase in weight of rats maintained on this corn-sucrose diet is depressed unless nicotinic acid is also incorporated (expts. 1 and 2). Small doses of 3-acetylpyridine (0.5 mg./day) significantly increased growth rates of animals kept on the corn-sucrose diet free from nicotinic acid (expt. 3), whereas large doses (5 mg. of 3-acetylpyridine/day) proved to be toxic and led to a much smaller increase in weight (expt. 4), which was only insignificantly improved by further supplementation of the diet with

nicotinic acid (expt. 5). The administration of correspondingly large doses of nicotinamide, on the other hand, exerted no toxic effects (expt. 6).

DISCUSSION

From this study it seems that the rat is able to convert 3-acetylpyridine into *N*¹-methylnicotinamide, thus confirming the results obtained in earlier studies on dogs (Beher *et al.* 1952; McDaniel *et al.* 1955). It may, however, be questioned whether the fluorescent product obtained in our experiments by the method of Huff & Perlzweig (1947) is *N*¹-methylnicotinamide or its 3-acetylpyridine analogue. Our results do not provide any definite answer. Beher *et al.* (1952), however, using ¹³C-labelled 3-acetylpyridine, conclusively proved its conversion into *N*¹-methylnicotinamide in the dog. The efficiency of the conversion of 3-acetylpyridine into either *N*¹-methylnicotinamide or its 3-acetylpyridine analogue is much less than that of nicotinamide into *N*¹-methylnicotinamide. Repeated small doses or one single large dose of 3-acetylpyridine induce a much lower excretion than

Table 2. *Urinary excretion of N¹-methylnicotinamide-like substances after an oral test dose of nicotinamide or 3-acetylpyridine*

Means and standard errors are given. NMNA, *N*¹-methylnicotinamide-like substances.

No. of rats	Weight (g.)	Nicotinamide given (mg./100 g. body wt.)	Excretion of NMNA (μg./100 g. body wt./day)	No. of rats	Weight (g.)	3-Acetylpyridine given (mg./100 g. body wt.)	Excretion of NMNA (μg./100 g. body wt./day)	P*
6	243	—	23 ± 6	12	226	—	23 ± 3	—
10	212	1.0	65 ± 12	6	190	1.0	40 ± 10	0.1
10	219	2.0	182 ± 37	12	194	2.0	105 ± 27	0.1
10	221	5.0	225 ± 64	12	205	5.0	120 ± 28	0.2
6	160	10.0	700 ± 119	6	185	10.0	218 ± 71	0.01
6	173	20.0	1950 ± 171	6	196	20.0	376 ± 60	0.001

* Probability that the differences between the animals treated with nicotinamide and those treated with 3-acetylpyridine are due to chance.

Table 3. *Weight increase of rats treated for 6 weeks with nicotinamide or 3-acetylpyridine and maintained on a corn-sucrose diet*

Means and standard errors are given.

No. of expts.	No. of rats	Supplement (mg./kg. of ration)	Treatment (mg./day)	Weight increase in 6 weeks (g.)
1	12	—	—	33 ± 3.6
2	12	Nicotinic acid, 50	—	66 ± 3.9
3	12	—	3-Acetylpyridine, 0.5	66 ± 4.6
4	12	—	3-Acetylpyridine, 5.0	48 ± 5.1
5	12	Nicotinic acid, 50	3-Acetylpyridine, 5.0	56 ± 2.4
6	12	—	Nicotinamide, 5.0	64 ± 3.3
P*	1 vs. 2: 0.001		1 vs. 3: 0.001	1 vs. 4: 0.02
	3 vs. 4: 0.01		4 vs. 5: 0.2	2 vs. 5: 0.05
	1 vs. 6: 0.001			

* Probability that the differences between the groups compared are due to chance.

equal doses of nicotinamide. Likewise, rats treated with 3-acetylpyridine have less pyridine nucleotides in their livers than rats receiving equal doses of nicotinamide. It is true that the analytical method employed by us does not distinguish between pyridine nucleotides proper and their 3-acetylpyridine analogues. However, Kaplan, Goldin, Humphreys, Ciotti & Venditti (1954) have reported that the injection of 3-acetylpyridine analogues into mice, although increasing the pyridine nucleotide content of the liver, did not result in the formation of the 3-acetylpyridine analogues of the pyridine nucleotides. It may therefore be assumed that the pyridine nucleotides found in the liver after administration of 3-acetylpyridine result from the conversion of the analogue into the vitamin. This conversion, though inefficient, may partially explain the vitamin-like effect of 3-acetylpyridine.

In our growth experiments with rats kept on a corn-sucrose diet small doses of 3-acetylpyridine had a vitamin-like effect, whereas large doses impaired the normal growth. This growth-inhibiting effect of 3-acetylpyridine was observed on rats maintained on either the casein or the corn-sucrose diet, and even supplementation of these diets with nicotinic acid did not lower the toxicity of 3-acetylpyridine.

In this connexion it is of interest that the diphosphopyridine nucleotide analogue occurs in intact animals fed with 3-acetylpyridine. Kaplan & Ciotti (1954) have shown that 3-acetylpyridine can be exchanged for nicotinamide in pyridine nucleotides by pig-brain diphosphopyridine nucleotidase. In contrast with results with liver, administration of 3-acetylpyridine induced some analogue of diphosphopyridine nucleotide in brain and spleen as well as in neoplastic tissue (Kaplan *et al.* 1954). Furthermore, Kaplan, Ciotti & Stolzenbach (1956) demonstrated that 3-acetylpyridine-pyridine nucleotide reacts much more slowly with yeast alcohol dehydrogenase but more rapidly with liver glutamic dehydrogenase than the pyridine nucleotide. Similar observations have recently been reported for an analogue of pyridoxine by Sandmann & Snell (1955). After administration of ω -methylpyridoxine to rats, ω -methyl-4-pyridoxic acid appears in the urine in amounts similar to those found for 4-pyridoxic acid after pyridoxine administration. The ω -methyl-analogue of pyridoxine phosphate was found to be much less able to activate glutamic-aspartic transaminase. Also, the organs of rats treated with the pyridoxine analogue showed less transaminase and thionase activity than was found in control animals. On the other hand, the analogue sup-

ported the growth of rats kept on a diet deficient in pyridoxine.

The two-sided effect of both 3-acetylpyridine and ω -methylpyridoxine may be interpreted in terms of the dual character of the coenzymes containing the analogue: they may activate some of the vitamin-dependent enzymes but not others; moreover, they may function as metabolic antagonists in those enzymic reactions which they do not activate.

SUMMARY

1. Rats treated with 3-acetylpyridine excrete more N^1 -methylnicotinamide-like substances in their urine than do untreated controls. Repeated small doses or one single dose of 3-acetylpyridine lead to a lower excretion of these substances than that observed after administration of corresponding doses of nicotinamide.

2. The pyridine nucleotide level in the livers of rats treated with 3-acetylpyridine are lower than in animals given equal amounts of nicotinamide.

3. Small doses of 3-acetylpyridine promote the growth of rats maintained on a corn-sucrose diet, whereas large doses depress it. Supplementation of this diet with liberal amounts of nicotinic acid has no significant ameliorating effect on this inhibition of growth.

4. The two-sided role of 3-acetylpyridine is explained in terms of its partial conversion into the vitamin and the dual character of the coenzymes containing the analogue.

The skilled technical assistance of Mr Yeheskel Fattal is gratefully acknowledged.

REFERENCES

- Behr, W. T., Holiday, W. M. & Gaebler, O. H. (1952). *J. biol. Chem.* **198**, 573.
Braun, K. J. (1949). *J. Pharmacol.* **95**, 58.
Gaebler, O. H. & Behr, W. T. (1951). *J. biol. Chem.* **188**, 343.
Huff, J. W. & Perlzweig, W. A. (1947). *J. biol. Chem.* **167**, 157.
Kaplan, N. O. & Ciotti, M. M. (1954). *J. Amer. chem. Soc.* **76**, 1713.
Kaplan, N. O., Ciotti, M. M. & Stolzenbach, F. E. (1956). *J. biol. Chem.* **221**, 833.
Kaplan, N. O., Goldin, A., Humphreys, S. R., Ciotti, M. M. & Venditti, J. M. (1954). *Science*, **120**, 437.
McDaniel, E. G., Hundley, J. M. & Sebrell, W. H. (1955). *J. Nutr.* **55**, 623.
Robinson, J., Levitas, N., Rosen, F. & Perlzweig, W. A. (1947). *J. biol. Chem.* **170**, 653.
Sandmann, R. & Snell, E. E. (1955). *Proc. Soc. exp. Biol., N.Y.*, **90**, 63.
Woolley, D. W. (1945). *J. biol. Chem.* **157**, 455.
Woolley, D. W. (1946). *J. biol. Chem.* **162**, 179.