PRODUCTION OF NICOTINIC ACID DEFICIENCY WITH 3-ACETYLPYRIDINE, THE KETONE ANALOGUE OF NICOTINIC ACID

By D. W. WOOLLEY*

(From the Laboratories of The Rockefeller Institute for Medical Research, New York)

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Since it has been shown during the past two years that diseases with signs characteristic of specific vitamin deficiencies may be produced by the feeding of certain structural analogues of various vitamins, and that these diseases may be cured by adequate doses of the vitamin concerned (1–4), it has seemed desirable to study the types of structural change which, when applied to a vitamin or other metabolite, will result in the formation of antagonistic agents. Although the substitution of sulfonic acid or sulfonamide groups for the carboxyl groups of acidic vitamins has given rise to compounds (*e.g.* sulfonilamide (5), thiopanic acid (6, 7), and pyridine-3-sulfonic acid (8)) that cause specific vitamin deficiencies of certain microorganisms, this type of change has not been very successful for the formation of agents capable of producing vitamin deficiency diseases in animals (9). Therefore, attempts have been made to learn what alterations of the carboxyl group will result in the realization of antagonistic analogues which are effective in animals.

Two previous observations led to the finding that 3-acetylpyridine will cause nicotinic acid deficiency in animals. In 1938 Woolley *et al.* (10) found that dogs suffering from nicotinic acid deficiency were promptly killed by a single dose of 3-acetylpyridine, while normal dogs were unharmed by similar doses of the compound. In 1942 Auhagen (11) reported that *p*-aminoacetophenone was bacteriostatic, and that this action was reversed by *p*-aminobenzoic acid. These facts indicated that the exchange of —$COCH_3$ for —COOH might be a general method for the formation of inhibitory analogues. The observations in this paper lend support to such a suggestion, but more cases will be required to establish this type of structural change as one of general applicability.

3-Acetylpyridine was observed to cause a disease in mice characterized by many of the signs seen in nicotinic acid-deficient dogs and humans. This disease was very rapid in onset following administration of the drug. When sufficient quantities of nicotinic acid or nicotinamide were given, the signs of the disease did not appear. This fact indicated that the signs resulted from a deficiency of nicotinic acid or nicotinamide in the

* With the technical assistance of M. L. Collyer.

animal. It was of interest to note that despite these reactions in the animal, 3-acetylpyridine did not inhibit the growth of microorganisms in a manner subject to specific reversal by nicotinic acid. Auhagen (11) observed results similar to these with bacteria.

EXPERIMENTAL

3-Acetylpyridine was synthesized according to the directions of Strong and McElvain (12).

Production of Disease of Mice with 3-Acetylpyridine—Weanling mice (10 to 12 gm.) were caged on screen floors and fed a ration composed of sucrose 75 gm., vitamin-free casein (Labco) 18 gm., salts (13) 5 gm., fortified corn oil (14) 1 gm., thiamine 0.2 mg., riboflavin 0.5 mg., pyridoxine 0.2 mg., calcium pantothenate 2 mg., choline chloride 100 mg., and inositol 100 mg. Since mice grew as rapidly on this ration as on the ration plus nicotinic acid (15), it was concluded that mice, as do rats, synthesized their own supply of nicotinic acid. 3 days after the start of the tests, 3-acetylpyridine was given orally in single daily doses.

The signs of the disease which resulted were in some degree dependent on the size of the daily dose. When more than 10 mg. per mouse per day were given, the animals exhibited rapid respiratory rate, soon followed by loss of control of the hind legs, and death within the 1st day. The best sequence of events was seen with 2 to 4 mg. doses, for this amount allowed the signs to develop for a number of days before death ensued. Very soon after the first dose the animals began to breathe quite rapidly. In a few hours difficulties in control of the hind legs were seen. Occasionally an animal would stand upright in the manner of a begging dog. Within 2 days almost complete paralysis of the hind legs resulted. At about the same time the mice appeared extremely wet and unkempt. Emaciation was usually prominent. The skin, first on the chest wall, and then on the sides and legs, became very red and inflamed. Fiery red tongues did not develop until the 4th to 7th day, and then they appeared in only about half of the animals. It was of interest that these red tongues were never seen in animals given sufficient drug to cause fatal disease in 1 or 2 days, but that a relatively long period of onset was necessary for their appearance.

Prevention of Disease with Nicotinic Acid or Nicotinamide—When sufficient nicotinic acid or nicotinamide was added to the ration, the signs of the disease were prevented, and the mice grew at a rate comparable to that seen in animals getting no 3-acetylpyridine. It was found advisable to feed the rations containing nicotinic acid or nicotinamide for 3 or 4 days before the drug was administered. If this was not done the animals frequently had not begun to eat well, and hence were not sufficiently fortified to withstand the effects of the 3-acetylpyridine. Only partial success was

had in attempts to save animals ill from the drug by oral administration of relatively large doses of ethyl nicotinate, although this ester was effective in prevention of the disease if its use was begun 3 days before the 3-acetylpyridine was given. Some representative data on the responses of mice to 3-acetylpyridine and to this compound plus nicotinic acid or nicotinamide are shown in Table I. Whenever nicotinic acid was used, enough sodium bicarbonate was added to the ration to neutralize the acid.

Effect of 3-Acetylpyridine on Microbial Growth—3-Acetylpyridine inhibited the growth of *Lactobacillus casei* cultured in the nicotinic acid-free

TABLE I

Effect of 3-Acetylpyridine and 3-Acetylpyridine Plus Nicotinic Acid on Growth and Survival of Mice

3-Acetylpyridine	Nicotinic acid	Nicotinamide	Animals	Deaths	Average change in weight	Survival time
mg. per day	*per cent of ration*	*per cent of ration*			*gm. per wk.*	*days*
0	0	0	10	0	+3.5	>14
10	0	0	19	19		1
4	0	0	24	21		3
2	0	0	4	1		4*
1	0	0	10	0	+1.0	>9
10	2.0	0	3	0	+3.8	>7
10	0.2	0	6	4		1–3*
4	2.0	0	10	0	+3.0	>14
4	0.2	0	10	0†	+2.2	>14
4	0	0.5	9	0	+1.9	>7

* Survival time of the animals that died.

† One of the mice developed redness of the skin on the ventral surface, and unkempt hair.

medium of Landy and Dicken (16). Half maximal inhibition of growth was produced by 2 mg. of the compound per cc. Small amounts of nicotinic acid did not diminish the inhibition of growth caused by 2 mg. of 3-acetylpyridine per cc., but as the concentration of nicotinic acid was raised above 200 γ per cc. the inhibition of growth was reversed. When the concentration of 3-acetylpyridine was doubled, the quantity of nicotinic acid necessary for reversal likewise was doubled. However, this antagonistic action of nicotinic acid was a non-specific one because it was likewise produced by acetic acid and because the effect was not produced by nicotinamide or by sodium nicotinate.

3-Acetylpyridine was even less active against *Saccharomyces cerevisiae*, *Escherichia coli*, *Staphylococcus aureus*, and *Lactobacillus arabinosus* than against *Lactobacillus casei*. No detectable effect was observed when the

last two organisms were cultivated in the presence of 4 mg. of the ketone per cc., and the slight inhibition of growth of the first two species which resulted from large doses of the compound was not reversed by nicotinic acid.

Effect of Growing Cultures of Lactobacillus arabinosus on 3-Acetylpyridine—To determine whether the resistance of microorganisms to the action of 3-acetylpyridine was due to the ability of such species to inactivate the compound, the following experiment was performed. *Lactobacillus arabinosus* was grown at 30° for 48 hours in the medium of Landy and Dicken (16) plus 4 mg. of 3-acetylpyridine per cc. The luxuriant crop of organisms was filtered off and the filtrate was made alkaline and extracted four times with chloroform. The chloroform was removed from the extract and the resulting residue was assayed on mice. When it was fed to each of six mice at a level equivalent to 10 mg. of the 3-acetylpyridine originally added to the culture medium, five of the six exhibited characteristic signs and died in 20 to 40 hours. Six mice which were fed the basal ration plus 0.5 per cent of sodium nicotinate remained well when they were given the same amount of the culture extract as was used for the former animals. It was therefore concluded that the majority of the 3-acetylpyridine was unchanged by the growing bacteria.

DISCUSSION

From the foregoing experiments it appears that 3-acetylpyridine is an effective agent for causing signs of nicotinic acid deficiency, even in species such as the mouse for which nicotinic acid is not a dietary essential. In this respect it resembles glucoascorbic acid, an analogue of vitamin C which allows the production of a scurvy-like disease in animals which do not require ascorbic acid in the diet (2). If nicotinic acid and ascorbic acid are regarded as hormones in species such as the mouse (and they are certainly not vitamins for this species), then 3-acetylpyridine and glucoascorbic acid may be viewed as compounds with structures analogous to, but with actions antagonistic to, hormones. With 3-acetylpyridine it may be possible to study some of the manifestations of nicotinic acid deficiency in species where this would otherwise be impossible.

In view of the action of 3-acetylpyridine in causing signs of nicotinic acid deficiency in animals, its ineffectiveness against bacteria is puzzling. Even against microbial species for which nicotinic acid is an essential growth factor it is relatively inert. Furthermore, this is not due to any ability of the bacteria to inactivate the substance. Either the microorganisms have effective means of preventing the entry of this harmful compound, or they lack those metabolic reactions involving nicotinic acid with which 3-acetylpyridine interferes in the animal organism. If either of these possibilities is correct, an elucidation of the process involved would be illuminating.

The contrast in the action of 3-acetylpyridine and pyridine-3-sulfonic acid is noteworthy. The former is effective against mice and ineffective against microorganisms, while for the latter the reverse is true (9).

SUMMARY

Typical signs of nicotinic acid deficiency as seen in susceptible species were caused by 3-acetylpyridine when 2 or more mg. per day were fed to mice. This species was not susceptible to nicotinic acid deficiency produced by the usual dietary means. The signs of the disease were prevented by sufficient amounts of nicotinic acid or of nicotinamide in the ration. 3-Acetylpyridine was regarded as the structural analogue of nicotinic acid in which the —COOH group of the vitamin had been exchanged for —$COCH_3$. In contrast to the results with animals, 3-acetylpyridine was relatively ineffective for the inhibition of growth of microbial species, and in those instances in which inhibition of growth was produced it was not reversed specifically by nicotinic acid. This ineffectiveness against bacteria was not due to the ability of these organisms to inactivate the compound.

BIBLIOGRAPHY

1. Woolley, D. W., and White, A. G. C., *J. Biol. Chem.*, **149,** 285 (1943).
2. Woolley, D. W., and Krampitz, L. O., *J. Exp. Med.*, **78,** 333 (1943).
3. Woolley, D. W., *J. Biol. Chem.*, **154,** 31 (1944).
4. Emerson, G. A., and Tishler, M., *Proc. Soc. Exp. Biol. and Med.*, **55,** 184 (1944).
5. Woods, D. D., *Brit. J. Exp. Path.*, **21,** 74 (1940).
6. Snell, E. E., *J. Biol. Chem.*, **139,** 975 (1941).
7. McIlwain, H., *Biochem. J.*, **36,** 417 (1942).
8. McIlwain, H., *Brit. J. Exp. Path.*, **21,** 136 (1940).
9. Woolley, D. W., and White, A. G. C., *Proc. Soc. Exp. Biol. and Med.*, **52,** 106 (1943).
10. Woolley, D. W., Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Biol. Chem.*, **124,** 715 (1938).
11. Auhagen, E., *Z. physiol. Chem.*, **274,** 48 (1942).
12. Strong, F. M., and McElvain, S. M., *J. Am. Chem. Soc.*, **55,** 816 (1933).
13. Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, **109,** 657 (1935).
14. Woolley, D. W., *J. Biol. Chem.*, **143,** 679 (1942).
15. Woolley, D. W., *Proc. Soc. Exp. Biol. and Med.*, **46,** 565 (1941).
16. Landy, M., and Dicken, D. M., *J. Lab. and Clin. Med.*, **27,** 1086 (1942).