Although some of the compounds studied are quite active as indirect inhibitors of NSF and cholesterol biosynthesis there is little possibility that an inhibitor of oxidative phosphorylation would have utility in practical control of cholesterol biosynthesis. Oxidative phosphorylation is of such fundamental significance in the metabolism of most cells in an aerobic environment that no basis of selective action against cholesterol biosynthesis in particular would appear to exist.

Summary. Classical inhibitors of oxidative phosphorylation particularly 2,4-dinitrophenol inhibit the biosynthesis of non-saponifiable material and cholesterol from mevalonic acid by rat liver homogenates. Presumably in the presence of inhibitors a level of ATP is not maintained for the phosphorylations and the concerted decarboxylation and dehydration essential in utilization of mevalonic acid.

1. Wright, L. D., Cleland, M., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 219.
2. Wright, L. D., Cleland, M., Dutta, B. N., *ibid.*, 1958, v98, 425.
3. Wright, L. D., Cleland, M., Norton, J. S., *J. Am. Chem. Soc.*, 1958, v80, 3485.
4. Wright, L. D., Cleland, M., Norton, J. S., Loeb, M., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 299.
5. Wright, L. D., Loeb, M., Norton, J. S., *ibid.*, 1959, v101, 866.
6. Wright, L. D., Loeb, M., *ibid.*, 1959, v102, 540.
7. Chaykin, S., Law, J., Phillips, A. H., Tchen, T. T., Bloch, K., *Proc. Natl. Acad. Sci. U. S.*, 1958, v44, 998.

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Utilization of Pyridoxine N-Oxide by Rats.* (25453)

TAKETAMI SAKURAGI AND FRED A. KUMMEROW
Dept. of Food Technology, University of Illinois, Urbana

When pyridoxine was treated with a mixture of glacial acetic acid and hydrogen peroxide, addition of one atom of oxygen resulted (1). Based on analogous reactions observed with a variety of pyridine derivatives(2), the product was believed to be pyridoxine N-oxide(1). The currently available data indicate that some biological systems are capable of reducing a heterocyclic compound containing N-oxide functions(3,4). In addition, the biological formation of N-oxides as a normal constituent has been reported(4,5). By means of bioautography and microbiological assay, pyridoxine N-oxide retained some Vit. B_6 activity for growth of *Saccharomyces carlsbergensis*, suggesting that yeast could reduce a portion of the N-oxide(1). In the present study, the metabolism of pyridoxine N-oxide by rats has been investigated.

Methods. Male rats 3 weeks of age were placed on basal diet† for 10 days and then evenly divided into 5 groups of 6 rats each with respect to body weights. During assay period, the basal ration and water were fed *ad lib.* In addition, rats were supplemented once each week for 4 weeks with distilled water, or distilled water which contained 20 μg of pyridoxine hydrochloride, 100 μg of pyridoxine hydrochloride, or equivalent amounts of pyridoxine N-oxide hydrochloride. *Paper chromatography and bio-autography.* Paper chromatography was conducted on Whatman No. 1 filter paper at room temperature in a descending system. The solvent was the upper layer of a mixture of isoamyl alcohol, pyridine and water (2:1:2, *v/v*). The spots of Vit. B_6 components as well as pyridoxine N-oxide were detected by spraying the papergram with

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† Each 10 lbs. of basal ration free from both fats and vit. B_6 contained 7.8 lb glucose, 1.8 lb vitamin-free casein, 0.4 lb Wesson salts(6), 9 g choline chloride, 4.5 g inositol, 10 mg thiamine chloride hydrochloride, 15 mg riboflavin, 100 mg nicotinic acid, 90 mg D-calcium pantothenate, 45 mg menadione, 0.5 mg biotin, 15,000 I. U. vit. A, 0.15 mg calciferol and 30 mg alpha-tocopheryl acetate.

TABLE I. Average Body Weight Gains of Male Rats Supplemented with Pyridoxine or Pyridoxine N-oxide over a Period of 4 Weeks.

Supplement*	Levels of supplement,† μg/rat/wk		
	0	20	100
	g		
Pyridoxine	6.2 ± 4.9‡(5)§	37.3 ± 4.1 (6)	83.7 ± 6.7 (6)
" N-oxide		32.3 ± 4.4 (6)	79.7 ± 6.2 (6)

* Supplementation was conducted once a week at beginning of every week by oral administration of aqueous solution of vitamin preparations.

† Vitamin level is indicated as pyridoxine hydrochloride.

‡ Stand. dev. calculated according to the formula: $\sqrt{\Sigma d^2/(n-1)}$.

§ Figures in parentheses indicate No. of rats used in each group.

N,2,6-trichloro-*p*-quinoneimine dissolved in benzene followed by exposure to ammonia vapor, or ferric chloride dissolved in ethanol. For bioautography, the papergram which had been dried was cut into 21 sections so that each section represented 0.05 of an R*f* unit. These sections were numbered from 0 to 20 as the R*f* values increased, and individually placed in a tube containing 9 ml of assay medium(7). After 1 hr at room temperature, the paper sections were removed and the tubes steamed in autoclave at 100°C for 10 min. After cooling, each tube was inoculated with 1 ml of a suspension of a 24 hr culture of *S. carlsbergensis* (A.T.C.C. 4228). Incubation was conducted at 30°C for 18 hr and growth of yeast in each tube was measured, using Coleman No. 9 Nepho-Colorimeter, degree of growth expressed in terms of optical density against distilled water at 655 mμ. *Excretion of Vit. B_6 from rats after administration of pyridoxine and pyridoxine N-oxide.* Six normal male rats weighing between 395 and 410 g were divided into 3 groups of 2 rats each. Two ml of distilled water, 2 ml of distilled water containing 5 mg of pyridoxine hydrochloride, or 2 ml of distilled water containing equivalent amount of pyridoxine N-oxide hydrochloride was administered *via* stomach tube. Urine was collected for 15 hr; urine samples were pooled within each group and diluted to 100 ml. To each paperstrip, 0.01 ml of urine thus prepared was applied and developed for bioautography. One-tenth ml of urine specimen after supplementation with pyridoxine N-oxide was also mixed with 0.1 ml of concentrated hydrochloric acid. Five mg of sodium nitrite was added to the acidic solution and the mixture heated on steam bath for 10 min., 0.02 ml of final solution was used for bio-autograph.

Results of growth assay with rats using pyridoxine or pyridoxine N-oxide as sole source of Vit. B_6 indicated that pyridoxine N-oxide retained Vit. B_6 activity (Table I). Differences between body weight gains which resulted upon supplementation with pyridoxine and pyridoxine N-oxide were statistically insignificant at the 5% level.

Efficient utilization of pyridoxine N-oxide as source of Vit. B_6 by intact rats was further supported by data of urine samples collected after administration of pyridoxine hydrochloride or pyridoxine N-oxide hydrochloride. Supplementation with pyridoxine and its oxide apparently gave similar bioautographic patterns (Fig. 1: *B, C*). When the urine specimen, obtained after oral administration of pyridoxine N-oxide, was treated with nitrous acid before bioautography, the low R*f* peak disappeared, while the high R*f* peak remained (Fig. 1; *D*). This confirms that the former peak represented pyridoxamine. Under the experimental conditions, peaks of pyridoxal and pyridoxine tended to fuse, and thus the high *Rf* peak as observed represented pyridoxal and/or pyridoxine. Although the N-oxide was far less active than pyridoxine in supporting growth of assay organism,‡ the appearance of a large, high R*f* peak after administration of the N-oxide suggested that excretion of pyridoxine N-oxide as such in urine, if any, was negligible (Fig. 1; *C*). The patterns as presented were obtained using 0.01 ml

‡ Apparent activity of pyridoxine N-oxide in supporting growth of assay organism, *S. carlsbergensis,* varied from time to time, usually ranging 5-17% of that of pyridoxine on a molar basis.

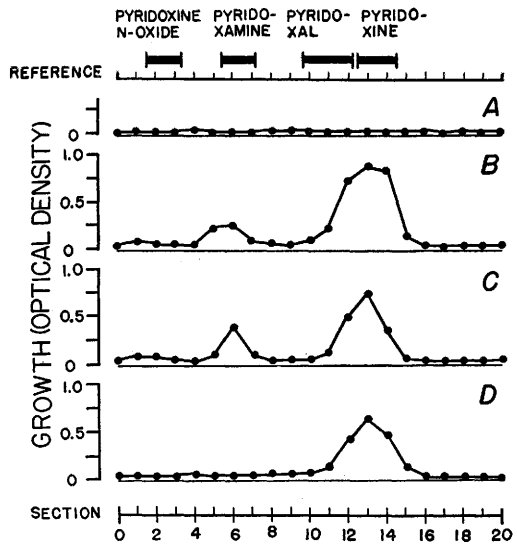

FIG. 1. Bioautographic pattern of vit. B_6 in rat urine. Urine samples were collected over a period of 15 hr after oral administration of *(A)* none, *(B)* pyridoxine · HCl, or *(C)* pyridoxine N-oxide · HCl. The pattern *D* represented urine collected after administration of pyridoxine N-oxide and subsequently treated with nitrous acid. The low R*f* peak represents pyridoxamine and the high R*f* peak represents pyridoxal and/or pyridoxine.

each of urine specimens. Even 0.05 ml of urine specimen from rats which had been supplemented with pyridoxine N-oxide failed to reveal a peak corresponding to pyridoxine N-oxide.

Since it became evident that reduction of pyridoxine N-oxide to Vit. B_6 readily occurred in intact animals, its possible transformation *in vitro* was investigated. Fifty μg of pyridoxine N-oxide hydrochloride was incubated with 0.5 ml of a 0.1 M phosphate buffer (pH 7.4) for 4 hr at 37°C in an open tube in the presence of 50-55 mg of fresh rat liver slice (approximately 1.5 mm thick) and of other additives. The liver slices were prepared from 45-55 day old normal male rats weighing 180-230 g. After incubation, degree of reduction was calculated from the increase in apparent Vit. B_6 activity in the digest, as measured directly with *S. carlsbergensis*(8). Reference tubes were concurrently prepared with pyridoxine hydrochloride as well as pyridoxine N-oxide hydrochloride. The calculation was based upon the assumption that Vit. B_6, presumably pyridoxine, which had been formed from pyridoxine N-oxide did not undergo further changes. Under conditions employed, 50 μg of pyridoxine hydrochloride, which had been incubated in place of pyridoxine N-oxide hydrochloride, retained its full activity when assayed with *S. carlsbergensis*.

Liver slices alone were unable to reduce pyridoxine N-oxide. In the presence of liver slice plus 800 μg of DPNH§ or 5 mg of a preparation of alcohol dehydrogenase, reduction of pyridoxine N-oxide was noted. In both cases, however, only approximately 5% of N-oxide originally added was reduced. When the alcohol dehydrogenase was used as an additive, further addition of DPN and/or ethyl alcohol, or addition of Mg^{++} and/or Mn^{++} failed to enhance the reduction. It was also noted that reduction of pyridoxine N-oxide by rat liver slices did not occur in the presence of one or more of the following additives: ATP, DPN, TPN, TPNH, glucose 6-phosphate and glucose 6-phosphate dehydrogenase. Under the variety of conditions tested, whenever no increase in Vit. B_6 activity after incubation was observed, Vit. B_6 activity attributable to the original pyridoxine N-oxide was fully recovered, suggesting that the N-oxide remained unchanged.

A variety of Vit. B_6 derivatives retaining vitamin activity for growth of rats are known (7,9-11). Except for the ω-methyl analogs of Vit. B_6, they are believed to regenerate free Vit. B_6 *in vivo*(7,11). Our results seemed to indicate that *in vitro* pyridoxine N-oxide *per se* could not replace the biochemical functions of Vit. B_6, which, however, is readily formed *in vivo* from the N-oxide. It has been reported that the N-oxide of a metabolite sometimes serves as an antimetabolite(12). Pyridoxine N-oxide, however, does not seem to act as an antivitamin B_6 in rats as well as in yeast.

Summary. The evidence shows that intact rats are capable of reducing pyridoxine N-oxide to Vit. B_6 and utilizing it as a source of vitamin. Pyridoxine N-oxide supported growth of rats almost as efficiently as did an equivalent amount of pyridoxine.

§ Preparations of DPNH (Lot No. 79-741), yeast alcohol dehydrogenase (Lot No. 79-700), DPN, TPN, and TPNH were purchased from Sigma Chemical Co., St. Louis, Mo.

1. Sakuragi, T., Kummerow, F. A., *J. Org. Chem.*, 1959, v24, 1032.

2. Ochiai, E., *ibid.*, 1953, v18, 534.

3. May, A., *Enzymologia,* 1957, v18, 142.

4. Chaykin, S., Bloch, K., *Biochim. et Biophys. Acta,* 1959, v31, 213.

5. Clemo, G. R., Dalglish, A. F., *J. Chem. Soc.*, 1950, 1481.

6. Wesson, L. G., *Science,* 1932, v75, 339.

7. Sakuragi, T., Kummerow, F. A., *Arch. Biochem. Biophys.,* 1958, v73, 43.

8. Atkin, L., Schultz, A. S., Williams, W. L., Frey, C. N., *Ind. Eng. Chem., Anal. Ed.,* 1943, v15, 141.

9. Sandman, R., Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 63.

10. Heyl, D., Luz, E., Harris, S. A., Folkers, K., *J. Am. Chem. Soc.,* 1952, v74, 414.

11. Sakuragi, T., Kummerow, F. A., *Arch. Biochem. Biophys.,* 1956, v63, 32.

12. Brown, G. B., Clarke, D. A., Biesele, J. J., Kaplan, L., Stevens, M. A., *J. Biol. Chem.,* 1958, v233, 1509.

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Serologic Comparison of Mammary Tumor Viruses from 3 Strains of Mice.* (25454)

PHYLLIS B. BLAIR (Introduced by Howard A. Bern)

Dept. of Zoology and Its Cancer Research Genetics Lab., University of California, Berkeley

The antigenic nature of the mouse mammary tumor virus is recognized. Sera of rabbits, rats, and guinea pigs(1-3) previously injected with extracts of virus-containing tissues are capable of neutralizing the virus in tumor extracts, both *in vivo* and *in vitro*. Normal sera of these animals, on the other hand, have little or no effect on the virus. The present experiment was designed to investigate possible antigenic differences between mammary tumor viruses possessed by different strains of mice. To eliminate any possible effect arising from tissue antigen differences, one strain (BALB/cCrgl) served as donor for all viruses. A comparison was made between the antigenicity of the viruses carried by 2 different strains of mice (C3H/Crgl and A/Crgl) and also between the viruses carried in a strain (A/Crgl) and a subline of it (A/Crgl/3, formerly designated A/vi), which are known to differ in effect upon tumor development(4). In addition, possible tissue differences were investigated by a comparison of one virus in 2 genetically different hosts (A/Crgl virus in A/Crgl and in BALB/cCrgl mice). The effectiveness of rabbit antisera in neutralizing the activity of the viruses was measured by tumor incidence in susceptible virus-free test mice injected with virus and antiserum.

Materials and methods. Antigen preparation: Mammary tissue was taken from the following groups of mice (Table I): BALB/c females (virus-free C); BALB/c females fostered on C3H (C3H virus in C); BALB/c females fostered on A/3 (A/3 virus in C); BALB/c females fostered on A (A virus in C); and A females (A virus in A). The donor females were permitted to breed, and were sacrificed during the third week of their second lactation. Mammary gland pairs #2, 3, and 4 were dissected out. The mammary glands from 2 females in a group were homogenized in a Waring blendor with 24 ml sterile saline for a total of 2 min. The mixture was spun in an International Clinical Centrifuge for 30 min at 1540 g. The pellets were discarded and the supernatants were then spun for 90 min in the multispeed attachment of an International Model U Centrifuge (maximum 30,000 g). The supernatants were discarded, and the pellets were resuspended in saline (.5 ml/g of original tissue weight for injection into rabbits, 2 ml/g of original tissue weight for injection into mice). The solu-

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