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Evidence for Participation of Vit. B_6 in Glutamine-α-Keto Acid Transamination-Deamidation Reaction. (22166)

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In a previous study(1) it was found that, although livers of vit. B_6 deficient rats exhibited markedly reduced glutamate-pyruvate transaminase and cysteine desulfhydrase activities, the glutamine-α-keto acid transamination-deamidation reaction proceeded at about equal rates with livers of control and experimental rats.† This finding, and our inability to resolve the glutamine enzyme by fractionation, were consistent with the concept that vit. B_6 was not involved in the glutamine-α-keto acid reaction, or alternatively, that affinity of the glutamine system for coenzyme was greater than that of the other enzymes studied. The present study supports the latter interpretation.

Patients given isonicotinic acid hydrazide (INAH) for treatment of tuberculosis developed a peripheral neuritis, ameliorated by administration of pyridoxine; such patients were also found to excrete in their urine greater than normal quantities of vit. B_6, apparently in a conjugated form involving INAH(2). These findings suggested to us that administration of INAH might represent a useful experimental procedure for depleting an animal of vit. B_6. We have therefore administered large doses of INAH to several groups of rats, and after periods ranging from 8 to 30 days, examined the livers for glutamine transaminase-deamidase activity in the presence and absence of coenzymes.

Methods. Weanling rats of Sprague-Dawley strain weighing 30 to 45 g, were used in Exp. 1 to 4, and adult Buffalo rats weighing 150 to 250 g were employed in Exp. 5 and 6. Animals were housed in individual cages and given water and diet *ad libitum*. *Exp. 1*: 26 rats were given stock Purina Chow diet; 13 of these were given 600 mg of INAH/k/day by gastric tube, and 13 received approximately same amount in drinking water. After

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† We have subsequently confirmed this observation using weanling rats of the Sprague-Dawley strain; adult Buffalo rats were used in the earlier work(1).

30 days, 5 animals of each group remained alive. *Exp. 2*: 30 animals received stock diet and 300 mg of INAH/k (by gastric tube) daily for 20 days; all survived. *Exp. 3*: 30 rats given vit. B_6 deficient diet and deoxypyridoxine as previously described(1), as well as 300 mg of INAH/k by tube per day for 14 days; 20 rats survived. *Exp. 4*: 30 rats were given same regimen as in Exp. 3 for 10 days; 25 rats survived. *Exp. 5*: 39 rats given stock diet and 480 mg of INAH/k by tube per day for 8 days; 26 survived. *Exp. 6*: 60 rats given stock diet and 480 mg of INAH/k by tube for 10 days; 37 rats survived.

Results. Large doses of INAH were employed because preliminary experiments indicated that smaller doses, at least over periods of 10-20 days, did not result in lowered transaminase activity. In general, rats given high doses of INAH were sluggish, developed moist snouts, and failed to gain weight or lost weight (0 to 0.7% body weight/day); death usually occurred shortly after INAH administration and was preceded by convulsions. Typical physical signs of vit. B_6 deficiency did not appear, even in Exp. 3 and 4, in which vit. B_6 deficient diet was administered; however, the duration of these studies was relatively short.

Enzyme assays were carried out essentially as described previously(1). The homogenate system consisted of 0.5 ml each of homogenate, L-glutamine (20 μM), sodium pyruvate (40 μM), and 0.1 M veronal buffer (pH 7.8), and incubated at 37° for 2 hours. The purified enzymes were isolated and lyophilized(3,4), and assayed in a system consisting of 20 μM of sodium pyruvate, 10 μM of L-glutamine, 10 mg of enzyme, in 0.4 ml of 0.025 M veronal buffer; the incubation period was 2 hours. Pyridoxal phosphate and pyridoxamine phosphate were used at a concentration of 100 γ/0.4 ml.

In Exp. 1, the livers of the treated rats exhibited greatly reduced glutamine-pyruvate transaminase-deamidase activity and increased glutaminase activity (Table I). Addition of pyridoxal phosphate or pyridoxamine phosphate increased glutamine-pyruvate activity in some instances, but not significantly in others. Because of the variable response to addition of the coenzymes, and because of the possibility that increased glutaminase activity might interfere with the determination of the glutamine-pyruvate reaction, subsequent studies were carried out using purified enzyme preparations obtained from pooled livers of a group of experimental rats and of their untreated controls.

TABLE I. Glutaminase and Glutamine-Pyruvate Transaminase-Deamidase Activity of Rat Liver Homogenates.*

Procedure	Substrates†	No. of rats	Range	Mean
Controls	Gl	9	.60–1.52	1.01
	Gl + P	9	2.95–4.54	3.56
INAH by tube	Gl	4	2.16–3.91	2.80
	Gl + P	4	.89–2.90	1.81
INAH in water	Gl	6	1.33–2.49	2.03
	Gl + P	6	.67–2.86	1.91

* Values are expressed in terms of μM of ammonia formed (corrected for blank ammonia) per ml of homogenate per hr; experimental details are given in the text.
† Gl = Glutamine; P = Pyruvate.

Experiments on several such purified enzyme preparations are described in Table II. These preparations did not possess glutaminase activity, and the effects observed with added coenzymes were readily reproducible. Preparations of the enzyme from livers of INAH treated rats exhibited a considerably reduced activity, and showed a significant increase in activity when pyridoxal phosphate or pyridoxamine phosphate was added. In

TABLE II. Glutamine-Pyruvate Transaminase-Deamidase Activity of Purified Liver Preparations.*

Exp. No.	Procedure†	Initial activity	Activity with coenzymes: Pyridoxal PO4	Activity with coenzymes: Pyridoxamine PO4
2	Con.	7.50	8.10	—
	Exp.	3.07	4.50	—
3	Con.	7.44	8.06	8.04
	Exp.	1.49	3.16	3.36
4	Con.	6.09	5.75	5.50
	Exp.	1.38	3.38	3.37
5	Con.	6.46	6.25	—
	Exp.	2.06	5.66	—
6	Con.	5.26	5.73	—
	Exp.	2.44	5.77	—

* See footnote, Table I.
† Con. = Control; Exp. = Experimental.

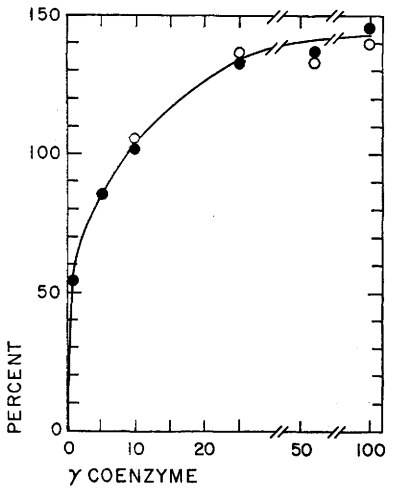

FIG. 1. Effect of pyridoxal and pyridoxamine phosphates on activity. Abscissa: γ coenzyme; ordinate: % increase in activity. Closed circles = pyridoxal phosphate; open circles = pyridoxamine phosphate.

confirmation of earlier studies(1,3,4) values of transaminase activity were in close agreement with those for ammonia formation. Maximal activation was observed with 25 γ of coenzyme (Fig. 1).

Discussion. The present results are consistent with the concept that the glutamine transaminase, like other transaminases, requires a vit. B_6 coenzyme. Failure of earlier attempts to demonstrate this may probably be ascribed to strong binding of the coenzyme by the enzyme. It is apparent that a relatively severe deficiency was required to remove vit. B_6 from this enzyme. It is of interest that concentration of coenzyme required for maximal activation of the partially resolved enzyme is of approximately the same order of magnitude as that required by other transaminases. It thus appears that deamidation of glutamine in this system may be made to depend on the presence of vit. B_6. It is probably the first step of the transamination-deamidation reaction (transamination of glutamine to α-ketoglutaramic acid) which requires vit. B_6. The second step, in which ammonia is formed, is hydrolysis of α-ketoglutaramate to α-ketoglutarate(5).

It has been reported that INAH inhibits *E. coli.* arginine decarboxylase(6), tryptophanase(7), and glutamate-oxalacetate transaminase of BCG(8); inhibition was reversed by pyridoxine, which is presumably converted to pyridoxal phosphate in the cell. It was also observed that patients being treated with INAH excreted unusually large amounts of xanthurenic acid in the urine after a test dose of tryptophan(2). The present results appear to provide general confirmation of the concept that this compound has an anti-vit. B_6 effect.‡ It is clear, however, that INAH has other effects (*cf*(9)) some of which may be related to the present observation of increased liver glutaminase and the fact that transaminase activity of preparations obtained from INAH-treated animals was often lower, even with added coenzyme, than that of controls. In 2 experiments in which INAH treatment was of relatively short duration, pyridoxal phosphate restored the activity to virtually control levels. (Exp. 5 and 6, Table II).

Beaton and Ozawa(10) reported that liver extracts of vit. B_6-deficient rats exhibited decreased ability to catalyze the glutamine-pyruvate reaction. Addition of pyridoxal phosphate to the extracts produced only very small and erratic activation.§ Our present results, which show a clear-cut reproducible *in vitro* effect of pyridoxal phosphate and pyridoxamine phosphate, lead to a conclusion which is not essentially different than that of Beaton and Ozawa, namely that vit. B_6 is involved in the mechanism of glutamine-α-keto acid transamination reaction. The present findings also suggest that INAH treatment may be a useful tool in the study of vit. B_6-catalyzed enzymatic reactions.

‡ We have also found that livers of INAH-treated rats exhibited reduced glutamate-pyruvate and aspartate-ketoglutarate transaminase activities; the control level of activity was restored by addition of pyridoxal phosphate.

§ It was observed several years ago that livers of *very markedly* vit. B_6 deficient rats and mice exhibited reduced glutamine transaminase-deamidase activity. It may not be concluded from this evidence that the enzyme specifically required vit. B_6 because addition of pyridoxal phosphate did not increase the activity of enzyme preparations obtained from these very sick animals. (Meister, A., Morris, H. P., and Tice, S. V., unpublished data).

Summary. 1. Liver homogenates prepared from rats given large doses of isonicotinic acid hydrazide (INAH) exhibited reduced glutaminepyruvate transaminase-deamidase activity and increased glutaminase activity. 2. Purified enzyme preparations (free of glutaminase) prepared from livers of INAH-treated rats catalyzed the glutamine-pyruvate reaction at markedly reduced rates compared to controls. Addition of pyridoxal (or pyridoxamine) phosphate produced a considerable increase in activity of the preparations obtained from INAH-treated rats, but not of that of the untreated controls. 3. The evidence supports the concept that vit. B_6 participates in the glutamine transaminase system.

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Effect of Dihydrocholesterol and Soybean Sterols on Cholesterol Metabolism in Rabbit and Rat.* (22167)

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Because considerable evidence exists showing that such sterols as dihydrocholesterol and soybean sterols are absorbed from the gut, it was considered worthwhile to restudy the effects of these sterols on cholesterol metabolism in rabbit and rat. The data in this paper provide an explanation for the conflicting reports heretofore published and demonstrate the fallacy inherent in attempts to produce a negative cholesterol balance by inhibition of cholesterol absorption from the gut.

Experimental. Preparation of sodium acetate-1-C^{14}. Methylmagnesium bromide was carbonated with $C^{14}O_2$. Control diets consisted of Purina Rabbit Chow for rabbits and Purina Laboratory Chow for rats. Sterol diets were prepared by dissolving the requisite amount of dihydrocholesterol‡ or mixed soybean sterols§ in ether, adding the solution to control diets and evaporating the ether. Sera and tissues were prepared for sterol analysis as described previously(1). *Sterol determination.* Digitonin precipitable, Liebermann-Burchard (L.B.) positive sterols were determined by the method of Sperry and Webb(2) and total digitonin precipitable sterols by a modification of the Pollak-Wadler turbidimetric method(3). The latter method was modified as follows: Sterols were precipitated with digitonin in graduated glass-stoppered tube at 10°C. After precipitation the volume was adjusted in the tube, and one drop

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‡ Dihydrocholesterol (Lot no. 105-220 I MRS), generously supplied by Schering Corp., contained 98% Liebermann-Burchard negative sterols.

§ The mixed soybean sterols (Control no. S-7577 and S-9707), generously supplied by Distillation Products Industries, contained 79% and 94% Liebermann-Burchard positive sterols respectively.