Effects of Desoxy- and Methoxypyridoxine in the Mouse.* (17681)

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A powerful anti-pyridoxine activity of desoxypyridoxine and methoxypyridoxine was observed by Ott(1,2) in the chick. The effects of these compounds in the rat were studied by Porter and co-workers(3). These workers concluded that desoxypyridoxine interferes with some phase of tryptophan metabolism. They also found that, though methoxypyridoxine may act similarly, there was some cleavage of this substance *in vivo* to pyridoxine. The finding reported from this laboratory(4) that the vitamin B_6 deficient mouse, in contrast to the rat, does not develop a typical set of symptoms has been confirmed by others(5,6). The observations of Ott suggested to us the possibility of producing a more severe deficiency in the mouse by means of desoxy- and methoxypyridoxine.

Experimental. (a) *The effect of desoxypyridoxine and methoxypyridoxine in vitamin B_6 deficient mice.* In this study, 3 strains† of albino mice were used, namely, Swiss-Webster, Rockland, and our own strain. The animals were placed at weaning on a vitamin B_6-free diet (diet A) of the following composition: Casein (Labco), 25; sucrose, 53; salts(7), 5; roughage material (Ruffex) 2; hydrogenated vegetable fat (Crisco or Primex), 10; lard, 5. This diet was supplemented with the following vitamins added per kg diet: Thiamine, 10 mg; riboflavin, 10 mg; calcium pantothenate, 100 mg; choline, 1.5

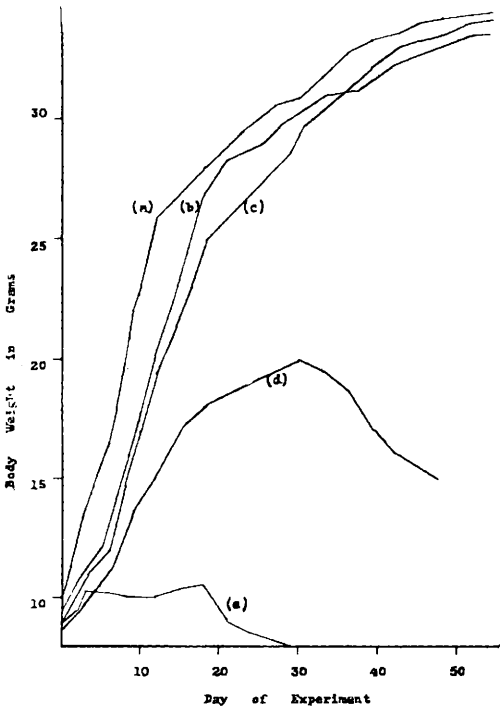

FIG. 1.

Growth curves showing the effects of desoxypyridoxine (DP) and methoxypyridoxine (MP) in vit. B_6 deficient mice.

(a) Growth curve of mice receiving the deficient diet (diet A) supplemented with 20 mg MP per kg diet; (b) growth curve of mice receiving diet A and a subcutaneous injection of 50 γ MP daily; (c) growth curve of mice receiving diet A supplemented with 20 mg DP and 20 mg MP per kg diet; (d) growth curve of mice receiving diet A, and, (e) growth curve of mice receiving diet A supplemented with 20 mg DP per kg diet.

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† The data for the three strains are grouped together, since no differences were observed.

‡ A mixture of 90% β- and 10% α-carotene was used.

TABLE I.
Effects of Desoxypyridoxine (DP) and Methoxypyridoxine (MP) on Vit. B_6 Deficient Mice.

Diet	No. of mice	Time of appearance of the acrodynia (avg in days)	Survival time (avg in days)
Control	12	—	40
Control + DP	24	23	32
Control + MP	11	—	*

* All the mice in this group were normal. Their avg body wt on 60th day of experiment was 34.6 g.

g; β carotene‡ 20 mg; α tocopherol, 40 mg; vitamin D (Drisdol) 2500 I.U. This group served as controls. Two other groups received the basal diet supplemented with 20 mg desoxypyridoxine§ and 20 mg methoxypyridoxine,‖ respectively, per kg diet. The results of these experiments are shown in Fig. 1 and Table I.

In Fig. 1 are shown the growth curves of mice of the 3 groups which may be considered typical of the majority of mice studied. Also included is the growth curve of 5 mice which received both analogues at the same level in the deficient diet. Finally, the growth curve of 6 mice given methoxypyridoxine subcutaneously (50 γ/day) is also included. The anti-vitamin effect of desoxypyridoxine manifests itself both by its effect on growth and its ability to produce the typical vitamin B_6 deficiency syndrome (Table I). On the other hand, the methoxy analogue displays pyridoxine activity as indicated by its ability to stimulate growth and counteract the anti-vitamin action of desoxypyridoxine.

(b) *The effect of the protein level in the diet, and the effect of supplementing the diet with methionine, on the deficiency symptoms induced by desoxypyridoxine.* The same experimental procedure was used here as in (a) except that the casein level was varied from 15 to 60% at the expense of sucrose. One group of mice was given the 25% casein diet supplemented with DL-methionine to bring the total methionine content up to the amount present in a 60% casein diet (1.05% DL-methionine). In each case, a control group was run without desoxypyridoxine. The results of these experiments are summarized in Table II. They show that the deficiency symptoms do not develop unless the anti-vitamin is included in the diet. They also show that an increase in the protein intake shortens the survival period of vitamin B deficient mice. This is in agreement with the findings of Miller and Baumann(6). It is worthy of note that a supplement of methionine reproduced the effects of the 60% casein diet.

(c) *The inhibition index of desoxypyridoxine in the mouse.* By 'inhibition index' we mean the molar ratio of anti-vitamin to vitamin, where the quantity of anti-vitamin is just capable of counteracting an amount of the vitamin sufficient to cover minimal daily needs. The procedure used in determining the inhibition index was as follows: Weanling mice were placed on the 25% casein vitamin B_6-deficient diet, and kept on this ration for a depletion period of 30 days. At the end of this period, the animals were ready for the experiment. Beginning on the 30th day, and daily thereafter, they were injected subcutaneously with solutions containing desoxypyridoxine and pyridoxine in ratios (anti-vitamin/vitamin) of 50, 25, 15, 10, and 5. Preliminary experiments had shown that an injection of 1 γ pyridoxine‖ per day was insufficient for maintenance in most cases, whereas a dose of 2 γ would cause some growth, and in some cases a fairly substantial increase in weight. Accordingly, the experiments were performed with both these levels of pyridoxine in the ratios of anti-vitamin/vitamin mentioned above. Table III shows the growth response of the mice to the various ratios of desoxypyridoxine/pyridoxine. It was observed that injections with the higher ratios caused an immediate and sharp decrease in the weights of the animals. When

§ We are indebted to Dr. Karl Folkers of Merck & Co. for these substances.

‖ Expressed as pyridoxine HCl.

TABLE II.
Data Showing the Effects of the Casein Level in the Diet, and of a Supplement of Methionine, on the Acrodynia Induced by Desoxypyridoxine (DP).

No. of mice	Level of casein	Appearance of the acrodynia (Avg, days)	Survival time (Avg, days)
15	15% (Control)	—	49
16	15% + DP	24	31
12	25% (Control)	—	40
24	25% + DP	23	32
11	45% (Control)	—	35
21	45% + DP	24	31
12	60% (Control)	—	30
18	60% + DP	18	25
10	25% + DP + DL-methionine	18	23

TABLE III.
Data Showing the Response of Vitamin B_6-Deficient Mice to Pyridoxine and to Various Ratios Desoxypyridoxine/Pyridoxine.

No. of mice	Ratio D.P./P.	γ Pyridoxine per day	Growth response* No. of mice	
			+	—
19	—	1	5	14
14	—	2	12	2
7	50	1	0	7
7	25	1	0	7
6	15	1	0	6
5	10	1	0	5
4	25	2	0	4
5	15	2	0	5
11	10	2	2	9
7	5	2	5	2

* The (+) sign denotes a gain, the (—) sign a loss in weight.

desoxypyridoxine was administered at a ratio of anti-vitamin : vitamin of 10 to mice receiving 2 γ pyridoxine per day, they still lost weight, though at a slower rate, and then died. On the other hand, when the ratio was 5, under the same conditions, there was a slight increase in the weights of the animals, and they survived for relatively long periods.

All animals receiving the higher ratios of desoxypyridoxine to pyridoxine developed the typical vitamin B_6 deficiency syndrome (except those which succumbed rapidly). Curiously enough, even some of the mice receiving the anti-vitamin at the ratio of 5, mentioned in the preceding paragraph, showed the symptoms, but these later disappeared. Such a ratio of 5, in the case of animals receiving only 1 γ pyridoxine per day, was still effective. However, this is not significant, since, as mentioned above, 1 γ pyridoxine is not adequate for maintenance.

Discussion. Contrary to what has been found in the case of rats, simple deprivation of vitamin B_6 apparently does not impose a sufficiently severe strain on the mouse to produce the gross symptoms of the deficiency. The experiments described above show that this syndrome may be produced in mice by the administration of desoxypyridoxine. The animals displayed the typical acrodynia of the paws and snout, ring tail, and the spastic gait. In confirmation of the findings of Miller and Baumann(6), our experiments also show that an increase of the casein level in the diet will shorten the survival period of vitamin B_6 deficient mice. It is interesting, however, that this increase had no effect on the symptoms induced by the anti-vitamin except when the casein level was increased to 60%, when some slight effect was observed. Apparently at the level of desoxypyridoxine administered, the inclusion of large amounts of casein had only a slight effect on the acrodynia. This is in contrast to the pronounced effect which the

casein level exerts on the skin lesions produced in the vitamin B_6 deficient rat not receiving desoxypyridoxine(8). The results obtained with the methionine supplement are not surprising in view of our observations on rats(9) which clearly showed the deleterious effect of sulfur amino acids on the vitamin B_6 deficient animal.

In contrast to the anti-vitamin action shown by desoxypyridoxine, methoxypyridoxine exhibits pyridoxine activity in the mouse. Thus, the mouse differs from the chick in its reaction to this analogue. The results of Porter and co-workers(3) are of interest in this connection in that they give evidence for both a vitamin and anti-vitamin action of this compound in rats. Results of experiments now in progress in our laboratory indicate that methoxypyridoxine is not quantitatively cleaved to pyridoxine in the mouse, since injection of an amount equivalent to 2 γ pyridoxine (which gives a fairly good growth response in the vitamin B_6 deficient mouse (Table III)) is without effect.

Summary. (1) Desoxypyridoxine has been shown to exert a powerful antipyridoxine effect in the mouse. With the aid of this substance, typical symptoms of vitamin B_6 deficiency (acrodynia) have been produced in this species. (2) A high casein level in the diet was found to have a detrimental effect on the acrodynia thus produced, but not to the same extent as that observed in the case of the acrodynia of vit. B_6 deficient rats, not receiving desoxypyridoxine. Methionine, in amounts equivalent to those present in the casein, had a corresponding detrimental effect. (3) The pyridoxine requirement of the mouse was found to be, under the conditions of our experiments, between 1 and 2 γ per day. (4) For mice receiving 2 γ pyridoxine per day, the inhibition index of desoxypyridoxine was found to be 10. (5) Methoxypyridoxine acts as a pyridoxine precursor in the mouse.

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Hypoalbuminemia and Renal Lesions in Experimental Hyperglobulinemia.* (17682)

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Recent observations have stimulated interest in the role of proteins in the pathogenesis of renal disease(1-5). This report is concerned with changes in blood serum and kidneys which occur after the intraperitoneal injection of normal rabbit globulin. When the rabbit antipneumococcic serum† employed was subjected to chemical and electrophoretic analysis, it was found to be a 10% globulin solution with a salt content of 0.9% at pH 7.4 consisting chiefly of gamma and traces of beta globulin preserved in 0.4% phenol and 1:50,000 phenyl mercuric borate.

Method. Rabbits weighing 2 to 3 kg were fed pellets, lettuce and water. Fifty to 60 cc of the test material were injected intraperi-

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