Relationship of Vitamin B_6 to Adrenocortical Function in the Rat.* (24541)

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Certain experiments have indicated decreased steroid secretion by pyridoxine-depleted animals(1,2,3,4) while other observations led to the conclusion that adrenocortical hormone production is not impaired(5,6,7). In these earlier studies steroid secretion was determined by indirect methods and lack of precise measurement of adrenal hormone production may be responsible for the opposing views. The purpose of experiments reported here was to evaluate adrenocortical function of pyridoxine deficient rats by direct analysis of steroid secretion.

Methods. Young, male, Sprague-Dawley rats weighing 45-65 g were used. Animals were housed individually and weighed twice weekly. In each experiment there was a group fed a pyridoxine-deficient diet, a pair-fed control group, and an *ad lib.* fed control group. A synthetic pyridoxine-deficient diet complete with respect to other nutrients was prepared in this laboratory. Deficient and control groups were fed the same diet; however, control food was fortified with pyridoxine (22 mg/kg). At termination of each experiment, rats were killed by decapitation. The liver of each animal was excised and in Exp. I and III this organ was weighed. Hepatic tissue weighing approximately 400 mg was removed from left lateral lobe and homogenized in 10 volumes of phosphate buffer, pH 7.4 for 45 seconds. One ml of homogenate was diluted with 9 ml phosphate buffer and glutamic-pyruvic transaminase enzyme activity of this solution determined by the method of Karmen *et al.*(8). Protein determinations were also done on liver homogenate by the technic of Lowry(9). Adrenals were removed immediately after animals were sacrificed, dissected free of surrounding tissue, bisected and weighed. Both adrenals from each rat were placed in a beaker containing 2 ml of Krebs-Ringer-phosphate-glucose solution, pH 7.4, to which one unit of ACTH was added. Adrenals were incubated aerobically for 2 hours at 37°C. On completion of incubation, steroids secreted into the medium were recovered and quantitatively determined by method previously described(10). *Exp. I.* Animals were placed in 3 groups and given diets as described. The study was continued for 60 days. *Exp. II* was carried out according to plan except that 4-desoxypyridoxine, a pyridoxine antagonist, was added to food (30 mg/kg) given all animals. Desoxypyridoxine was used to reduce the time required to induce Vit. B_6 deficiency. Experiment was terminated after 19 days. *Exp. III.* Three groups of rats were fed diets according to plan for 62 days. At this time half the animals in each group were sacrificed. Desoxypyridoxine (30 mg/kg) was then added to food given all remaining animals and the study continued 4 additional days. Several times during the last 20 days of experiment, rats from each group were given 25 mg of dl-tryptophan orally and excretion of xanthurenic acid determined during the succeeding 24 hours(11).

Results. Exp. I. Results are summarized in Table I. Rats on Vit. B_6 deficient diet gained weight slowly for about 2 weeks. Subsequently, weight remained relatively constant. Other manifestations of deficiency appeared after 40-50 days had elapsed. At this time acrodynia was present, animals were less active than previously, and a few rats died. Pair-fed control rats gained weight more rapidly than did the deficient group and reached maximum weight after about 3 weeks. Thereafter, weight remained fairly constant, animals were active and showed no evidence of illness. *Ad lib.* fed controls gained weight steadily throughout the period and appeared

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TABLE I. Body, Adrenal and Liver Weights, Liver Glutamic Pyruvic Transaminase Activity, and Adrenocortical Hormone Secretion of Various Groups after 2 Months on Experimental Diets (Mean Values ± Standard Error of Mean).

Group	Body wt (g)	Adrenal wt (mg/100 g body wt)	Liver wt (g/100 g body wt)	Liver transaminase activity*	Adrenal steroid secretion (μg/100 mg adrenal wt)
B_6 deficient (9)†	81.1 ± 3.5	39.9 ±3.02	3.61 ± .14	.57 ± .03‡	76.1 ± 5.8
Pair fed control (9)	116.3 ± 1.3	26.5 ± .68	2.59 ± .07	.81 ± .05	77.8 ± 3.6
Ad lib fed control (9)	315.6 ± 9.5	13.6 ± .64	3.71 ± .07	.46 ± .03	62.6 ± 2.0

* μmoles pyruvate formed/hr/100 μg protein. † No. of animals in group. ‡ Mean of 7 determinations.

well. Adrenal weights, expressed in relation to body weight, demonstrate that marked adrenal hypertrophy occurred in the pyridoxine depleted group. Differences in adrenal weight between deficient and control rats, both pair-fed and *ad lib.* fed, were highly significant, P = <0.01. Liver weight to body weight ratio was almost the same in depleted rats as in *ad lib.* fed animals but was significantly greater (P = <0.01) than that of pair-fed controls. Liver glutamic pyruvic transaminase activity was significantly reduced in the deficient group when compared to pair-fed controls (P = <0.01) but was not significantly different from that of *ad lib.* fed rats. Adrenal steroid secretion by deficient and pair-fed rats was almost the same. Although adrenal hormone secretion of *ad lib.* fed controls was less than that of the other groups, differences were not statistically significant.

Exp. II. Results are summarized in Table II. Rats which consumed the B_6 deficient diet containing desoxypyridoxine failed to gain weight. These animals had diminished appetite and acrodynia after being on diet only 7-10 days. Although the control diet contained desoxypyridoxine in the same concentration as the pyridoxine-deficient food, pair-fed and *ad lib.* fed controls gained weight and appeared well. Adrenal hypertrophy was again marked in deficient rats and differences in adrenal weight between these rats and pair-fed and *ad lib.* fed controls were highly significant, P = <0.01. Hepatic transaminase activity was essentially the same in deficient and pair-fed controls; however, activity of this enzyme in *ad lib.* controls was significantly reduced (P = <0.01). Differences in adrenal steroid secretion by the 3 groups were small and not significant.

Exp. III. Results are summarized in Tables III and IV. B_6 deficient rats showed a small weight gain during the first 3 weeks but subsequently little change in weight occurred. Other manifestations of pyridoxine depletion appeared after 45 days but were not severe until desoxypyridoxine was added to the diet (day 63). No abnormalities were noted in control animals at any time.

Adrenals of deficient rats were larger than those of controls. The difference in adrenal weight between deficient and pair-fed control rats after 62 days was significant, P = <0.05. Adrenal weights of deficient and *ad lib.* fed controls at this time differed significantly,

TABLE II. Body and Adrenal Weights, Liver Glutamic Pyruvic Transaminase Activity, and Adrenal Steroid Hormone Secretion of Various Groups after 19 Days on Diets Containing Desoxypyridoxine (Mean Value ± Standard Error of Mean).

Group	Body wt (g)	Adrenal wt (mg/100 g body wt)	Liver transaminase activity*	Adrenal steroid hormone secretion (μg/100 mg adrenal wt)
B_6 deficient (11)†	57.2 ± .66	52.5 ± 1.73	.40 ± .02	71.0 ± 7.03
Pair fed control (10)	86.7 ± 1.33	30.0 ± .87	.43 ± .02	66.7 ± 3.87
Ad lib fed control (10)	172.8 ± 4.70	19.7 ± .78	.33 ± .01	61.5 ± 2.48

* μmoles pyruvate formed/hr/100 μg protein. † No. of animals in group.

TABLE III. Body, Adrenal and Liver Weights of Various Groups (Mean Values ± Standard Error of Mean; Each Mean Represents 5 Determinations Except Where Indicated by Number in Parentheses).

Group	Body wt (g) A*	Body wt (g) B*	Adrenal wt (mg/100 g body wt) A	Adrenal wt (mg/100 g body wt) B	Liver wt (g/100 g body wt) A	Liver wt (g/100 g body wt) B
B_6 deficient	89.1 ± 2.06	107.0 ± 8.46 (6)	35.8 ± 1.62	37.9 ± 2.32 (6)	4.29 ± .27	3.58 ± .13 (6)
Pair fed control	131.3 ± 3.01	131.2 ± 3.12	28.1 ± 2.27	25.0 ± .63	2.76 ± .18	2.28 ± .10
Ad lib fed control	313.1 ±11.3	333.9 ±23.0	15.3 ± .69	13.3 ± 1.03	3.78 ± .12	3.44 ± .13

* Values in columns labelled A are for rats fed experimental diets for 62 days; in columns labelled B for rats fed experimental diets for 62 days and were given experimental diets *plus* desoxypyridoxine for 4 additional days.

$P = <0.01$. Differences in adrenal weight of deficient and control animals on day 66 (after 4 days of consuming food containing desoxypyridoxine) were significant, $P = <0.01$. Liver weight-body weight ratios were almost the same in deficient rats and *ad lib.* controls on days 62 and 66; however, in each instance liver weight of pair-fed controls was significantly reduced ($P = <0.01$).

Liver glutamic pyruvic transaminase activity of pyridoxine-deficient rats was less than that of pair-fed controls on day 62, but this difference was not statistically significant. On day 66, after desoxypyridoxine feeding, liver transaminase activity of deficient rats was further reduced and the difference from the level in pair-fed controls was significant, $P = <0.01$. Hepatic transaminase enzyme activity of *ad lib.* controls was approximately the same as that of deficient rats at both times. Adrenocortical hormone secretion of deficient rats on days 62 and 66 was not significantly different from that of pair-fed or *ad lib.* fed controls.

Xanthurenic acid excretion of depleted rats following oral administration of 25 mg of dl-tryptophan averaged 3.2 mg per day. When control animals were given equivalent doses of tryptophan, only traces of xanthurenic acid were excreted.

Discussion. It was decided to use liver glutamic pyruvic transaminase enzyme activity as evidence of Vit. B_6 deprivation since it has been shown that activity of this enzyme is directly related to B_6 intake(12). Although transaminase activity was less in pyridoxine-deprived animals than in pair-fed controls in all experiments, differences were significant only in Exp. I and the second part of Exp. III. Furthermore, in most instances transaminase activity of deficient rats was greater than that of *ad lib.* controls. Since a marked reduction of transaminase activity did not occur in rats fed the deficient diet, the question arises as to whether B_6 deficiency was present in these animals. That deficiency did occur is evident for the following reasons. First, rats were fed a synthetic diet containing no pyridoxine and clinical manifestations of the vitamin deficiency were present. Finally, B_6

TABLE IV. Liver Glutamic Pyruvic Transaminase Activity and Adrenocortical Hormone Secretion of Various Groups (Mean Values ± Standard Error of Mean; Each Mean Represents 5 Determinations Except Where Indicated by Number in Parentheses).

Group	Liver transaminase activity* A†	Liver transaminase activity* B†	Adrenal steroid secretion (μg/100 mg adrenal wt) A	Adrenal steroid secretion (μg/100 mg adrenal wt) B
B_6 deficient	.40 ± .07	.30 ± .02(6)	70.3 ± 12.48	67.9 ± 5.39(6)
Pair fed control	.51 ± .05	.53 ± .02	80.2 ± 5.44	82.1 ± 4.29
Ad lib fed control	.35 ± .07	.34 ± .05	68.1 ± 9.75	62.4 ± 7.89

* μmoles pyruvic acid formed/hr/100 μg protein.

† Values in columns labelled A are for rats fed experimental diets for 62 days; in columns labelled B for rats fed experimental diets and were given experimental diets *plus* desoxypyridoxine for 4 additional days.

depletion was clearly demonstrated in deficient animals of Exp. III by measuring urinary excretion of xanthurenic acid following oral administration of dl-tryptophan. Animals in the depleted group excreted large quantities of xanthurenic acid following oral tryptophan whereas both pair fed and *ad lib.* fed controls excreted only minute amounts of this substance. Therefore, despite the small changes in liver glutamic pyruvic transaminase activity which occurred in depleted rats, it is apparent that Vit. B_6 deficiency was present in these animals.

Liver weight to body weight ratio of pair fed rats was significantly less than that of either the pyridoxine-deficient group or *ad lib.* fed controls. Earlier studies have shown that partial starvation results in decreased liver size hence the occurrence of smaller livers in pair-fed rats than in *ad lib.* fed animals is not surprising(14). It was not anticipated, however, that liver weight of deficient rats would be greater than that of pair-fed controls and results of this investigation do not provide an explanation for this observation. Histologic study of various organs in B_6 deficient rats conducted by other investigators has demonstrated abnormal accumulation of fat in the liver and it may be for this reason that hepatic weight was increased in depleted animals(5).

Marked adrenal enlargement was observed in experiments described herein. Such hypertrophy must occur as a result of B_6 deficiency and is not due simply to partial starvation since adrenal enlargement was greater in depleted rats than in pair-fed controls. In contrast to previous investigations, adrenocortical function was assessed in these studies by direct determination of steroid secretion. The results obtained in all experiments show that hormone production by adrenals of B_6 deficient rats was equal to that of pair-fed and *ad lib.* fed control animals. It is therefore concluded that function of the adrenal cortex is not altered in Vit. B_6 deficiency even though hypertrophy of this gland occurs.

Summary. Vit. B_6 deficiency was induced in growing rats by feeding a pyridoxine deficient diet. In certain experiments desoxypyridoxine, a metabolic antagonist of Vit. B_6, was added to shorten the period required to induce this vitamin deficiency. In each experiment pair-fed and *ad lib.* fed controls were given the same diet as consumed by deficient rats except that pyridoxine was added. After deficiency became manifest adrenocortical function of experimental animals was measured by determining steroid secretion of isolated adrenals in response to ACTH. The results show that while marked adrenal hypertrophy occurs in B_6 deficient rats adrenocortical hormone secretion is not impaired.

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