RENAL GLUTAMINASE ACTIVITIES IN VITAMIN B₆-DEFICIENT RATS*

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Glutamine has been implicated in the functioning of the central nervous system (1), in muscle metabolism (2), in transamination (3), in detoxification (4), and as a precursor of urinary ammonia (5). An excellent review of these and other possible roles of glutamine in metabolism has been presented by Archibald (6). It would seem that glutamine acts as a mobile reserve of ammonia and glutamic acid in the body and would therefore be expected to effect a number of physiological functions.

In this laboratory, a lowered plasma glutamine level has been found consistently in rats deprived of vitamin B₆ and given deoxypyridoxine for a period of approximately 21 days (7-10), but this lowering has not been observed in pair-fed controls. Simple deprivation of the vitamin for a period of 20 days did not elicit this lowering in the absence of deoxypyridoxine (11). A less marked but relatively consistent observation has been an elevated level of plasma glutamic acid in vitamin B₆-deficient rats (7-10). These findings suggested the possibility of an alteration in renal glutaminase activity consequent to vitamin B₆ deficiency in the rat. Decreased levels of plasma glutamine in vitamin B₆-deficient rats given deoxypyridoxine, but not in those simply deprived of the vitamin, might be attributable to differences in the degree of deficiency, since deoxypyridoxine has been shown to hasten the onset of external deficiency signs (12), or to an effect of deoxypyridoxine separate from antivitamin action. To investigate these possibilities, renal glutaminase activities have been measured in vitamin B₆-deprived and pair-fed control rats with and without provision of dietary deoxypyridoxine. Renal glutaminase activities were also measured in thiamine-deficient rats in order to investigate one other vitamin deficiency.

Methods

Albino rats of the Wistar strain and of both sexes were employed. All animals were housed in individual, screen-bottomed cages, and were provided with drinking water ad libitum. The diet employed was the 20 per

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cent casein, 20 per cent corn oil, vitamin B₆-free basal diet previously described (7). When vitamin B₆ was supplied, this was added to the food as pyridoxine hydrochloride at a level of 50 γ per rat per day; in one experiment deoxypyridoxine hydrochloride was added to the food of two groups at a level of 100 γ per rat per day.

After the indicated period of experimental feeding, the animals were fasted for 20 hours and killed by stunning and decapitation. In each case, the left kidney was removed, and phosphate-activated glutaminase activities of homogenates were determined by the method of Archibald (13) as modified by White and Rolf (14). Since in most cases analyses were carried out over a 2 or 3 day period, care was taken to select animals from each group and of each sex each day to prevent biasing of results. The possibility of differences in the amounts of preformed ammonia in tissues from the experimental groups was investigated with blanks containing tissue, phosphate buffer, and phosphate-cyanide buffer. No consistent or significant differences were noted among groups. Indeed the amounts of preformed ammonia were very small. A blank containing phosphate buffer, phosphate-cyanide buffer, and glutamine was employed to eliminate errors which might arise from nesslerization in the presence of glutamine. It is realized that even in the absence of added phosphate slight apparent glutaminase activity of renal tissue can be detected by the method employed. However, under these experimental conditions this apparent activity was very low and far below that noted in the presence of added phosphate. Thus it is safe to state that the data reported are representative of phosphate-activated glutaminase activities.

EXPERIMENTAL

Renal Glutaminase Activities in Vitamin B₆ Deficiency—54 rats were divided into six groups, equal with respect to sex distribution, number, and an initial average body weight of 111 gm. Two groups were provided with deoxypyridoxine and one of these groups was further provided with pyridoxine. Two groups received only pyridoxine supplements, and the two remaining groups received no supplements. All groups were pair-fed, and the deprived group received deoxypyridoxine in order to eliminate differences consequent to variations in the amount of food consumed. Following 20 to 22 days of experimental feeding, four groups of animals (one control, one deprived, and one control and one deprived both given deoxypyridoxine) were fasted and killed and the renal glutaminase activities determined. It should be noted that at this time only those animals deprived of vitamin B₆ and receiving deoxypyridoxine had acrodynia, the external manifestation of vitamin B₆ deficiency. The two remaining groups were maintained for a total of 35 days with continuous pair-feeding.
At the end of this time these animals were fasted and killed and the renal glutaminase activities determined. A moderate degree of acrodynia was evident in the second group of deprived animals after 35 days of vitamin restriction. The average daily food consumption of all groups was 11.6 gm. per rat. The results of this study are presented in Table I.

**Effect of Adding Pyridoxal Phosphate to Homogenates** Since pyridoxal phosphate has been stated to act as the coenzyme of several enzymes (15–18), it seemed logical to investigate the possibility of an effect of this substance on renal glutaminase activity when added to the homogenates. The diammonium salt of pyridoxal phosphate (62 per cent pyridoxal phosphate) was obtained from the California Foundation for Biochemical Research. This compound was added to an aliquot of each homogenate at a level of 33 γ per 100 mg. of tissue. Calculated as pure pyridoxal phosphate, this level (20 γ per 100 mg.) is approximately 2 times that found effective for certain transaminase systems (15). Addition of pyridoxal phosphate to the aliquots of homogenates was made 1 hour prior to the assay of glutaminase activity. During this 1 hour period the homogenates with and without added pyridoxal phosphate were stored in a cold room at 4°. It was found necessary to make a blank correction in the glutaminase assay for the addition of pyridoxal phosphate, since this compound contributed *per se* to color formation, presumably through the presence of ammonia in the salt.

Seventeen rats were divided into two groups comparable with respect to sex distribution, number, and an initial average body weight of 105 gm.

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**TABLE I**

<table>
<thead>
<tr>
<th>Group</th>
<th>Days on diet</th>
<th>No. of rats</th>
<th>Average gain in body weight</th>
<th>Renal glutaminase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>gm.</td>
<td>Range</td>
</tr>
<tr>
<td>− B6</td>
<td>20–22</td>
<td>9</td>
<td>63</td>
<td>29–46</td>
</tr>
<tr>
<td>+ B6</td>
<td>20–22</td>
<td>9</td>
<td>85</td>
<td>30–46</td>
</tr>
<tr>
<td>− B6 + DB₆†</td>
<td>20–22</td>
<td>9</td>
<td>54</td>
<td>16–36</td>
</tr>
<tr>
<td>+ B6 + DB₆</td>
<td>20–22</td>
<td>9</td>
<td>78</td>
<td>27–47</td>
</tr>
<tr>
<td>− B₆</td>
<td>35</td>
<td>9</td>
<td>54</td>
<td>30–38</td>
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<tr>
<td>+ B₆</td>
<td>35</td>
<td>9</td>
<td>101</td>
<td>32–47</td>
</tr>
<tr>
<td>No thiamine</td>
<td>21</td>
<td>8</td>
<td>36</td>
<td>30–62</td>
</tr>
<tr>
<td>+ thiamine</td>
<td>21</td>
<td>8</td>
<td>58</td>
<td>32–52</td>
</tr>
</tbody>
</table>

* Expressed as per cent of glutamine split in 20 minutes by 20 mg. of kidney tissue.
† DB₆ signifies dietary provision of deoxypyridoxine.
One group was deprived of vitamin B6; the other group was given pyridoxine in the food and was pair-fed with the deprived group. After 35 to 36 days of experimental feeding, during which time the average daily food consumption of both groups was 10.5 gm. per rat, the animals were fasted and killed, and renal glutaminase activities were determined with and without addition of pyridoxal phosphate to the homogenates. At this time, the deprived rats exhibited moderate acrodynia. The results of this study are set down in Table II.

**Renal Glutaminase Activities in Thiamine Deficiency**—Sixteen rats were divided into two groups, equal with respect to sex distribution, number, and an initial average body weight of 102 gm. The basal diet employed was that described previously (7), with the exception that 100 mg. of pyridoxine hydrochloride replaced the 100 mg. of thiamine hydrochloride in the vitamin powder. One group was fed this basal diet ad libitum; the remaining group received 50 γ of thiamine hydrochloride per rat per day in the food and was pair-fed with the deprived group. After 21 days of experimental feeding, the animals were fasted and killed, and renal glutaminase determinations were carried out. The average daily food intake of both groups was 11.0 gm. per rat. The results of this study are presented in Table I.

### RESULTS AND DISCUSSION
As shown in Table I, following 20 to 22 days of experimental feeding, no alteration in renal glutaminase activity was apparent in those animals simply deprived of vitamin B6. However, vitamin B6 deprivation coincident with the feeding of deoxypyridoxine for a similar period resulted in a significant decrease in mean activity ($t = 2.92$; significant at the 1 per cent

### Table II

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Average gain in body weight</th>
<th>Pyridoxal phosphate*</th>
<th>Renal glutaminase activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deprived</td>
<td>9</td>
<td>56</td>
<td>−</td>
<td>21–36</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td>+</td>
<td>30–51</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>92</td>
<td>−</td>
<td>30–40</td>
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<tr>
<td></td>
<td>8</td>
<td></td>
<td>+</td>
<td>37–43</td>
</tr>
</tbody>
</table>

* 20 γ per 100 mg. of kidney tissue.
† Expressed as per cent of glutamine split in 20 minutes by 20 mg. of kidney tissue.
level). No significant difference in renal glutaminase activity was noted between control groups with and without deoxypyridoxine. Simple deprivation of vitamin B₆ for a total of 35 days resulted in a significant decrease in mean renal glutaminase activity ($t = 4.80$; significant at the 1 per cent level). Thus it would seem that deoxypyridoxine hastened not only the appearance of acrodynia but also the lowering of glutaminase activity. Other studies in this laboratory have indicated that deoxypyridoxine does not act in the manner expected of an antivitamin for all enzyme systems affected by vitamin B₆ deficiency (19, 20), although a longer period of simple deprivation might have altered the interpretation. The present study suggests that the effect of deoxypyridoxine on renal glutaminase activity in the absence of vitamin B₆ is that of an antivitamin. It should be noted that, since pair-feeding was followed throughout, differences in enzyme activities between groups in this and the subsequent experiments cannot be attributed to differences in food consumption.

Owing to the large amount of blood required for determination of plasma glutamine and glutamic acid, it was not possible to measure these metabolites in the animals sacrificed individually in these studies. The findings on renal glutaminase activities are entirely in accord with observations reported previously on these metabolites after 21 days of experimental feeding (7–11). Under similar experimental conditions, a low mean plasma glutamine level, an elevated mean glutamic acid level, and a low renal glutaminase activity are compatible. Although findings in vitro do not of necessity represent the situation in vivo, it would appear that the phosphate-activated glutaminase of renal tissue is affected by vitamin B₆ deficiency either directly or secondarily.

The results of the first experiment do not indicate the manner in which vitamin B₆ deficiency brings about the alteration in renal glutaminase activity. This effect could be of a direct or coenzyme-like nature or could simply represent an adaptation of activity to a low level of available substrate, namely plasma glutamine. The second study, in which pyridoxal phosphate was added to the homogenates, investigated the possibility that the lowering in renal glutaminase activity might be a direct effect of vitamin B₆ deficiency. The data set down in Table II confirm the finding that deprivation of vitamin B₆ for 35 days results in a depression in renal glutaminase activity ($t = 2.98$; significant at the 1 per cent level). Addition of pyridoxal phosphate to the homogenates caused an apparent stimulation of activity in the homogenates of deprived rats ($t = 3.30$; significant at the 1 per cent level) and a smaller stimulation in homogenates of control rats ($t = 2.48$; significant at the 5 per cent level). Addition of pyridoxal phosphate restored the mean enzyme activity of tissue from deprived rats to the level of tissue from pyridoxine-fed control rats.
As shown in Table I, thiamine deficiency had no apparent effect on renal glutaminase activity in rats, at least in the time interval used in this study. Application of a t test to the difference between means yielded a t value of 0.688, which is not significant. Based on body weight, the metabolic effects of thiamine deficiency were more severe than those of vitamin B₆ deficiency; yet no change in renal glutaminase activity was noted in thiamine-deficient rats.

SUMMARY

Renal phosphate-activated glutaminase activities were significantly lower in vitamin B₆-deprived rats than in pair-fed controls after 35 days of experimental feeding. Addition of deoxypyridoxine to the diet made apparent this abnormality in 20 to 22 days of deprivation. Addition of pyridoxal phosphate to homogenates significantly increased and restored to normal the mean renal glutaminase activity of kidney tissue from vitamin B₆-deprived rats, while it caused a small increase in activity of the enzyme in tissue from control rats. Thiamine deficiency did not alter renal glutaminase activity.

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