THE INFLUENCE OF VITAMIN B₆ ON THE FORMATION OF LIVER PYRIDINE NUCLEOTIDES*

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It has been shown that, when tryptophan is administered to rats, an increased excretion of niacin and some of its derivatives occurs (1–4). Isotope studies by Heidelberger et al. (5, 6) have given further evidence that niacin can be derived from tryptophan in the animal body. Vitamin B₆ has been implicated in this transformation, since in a pyridoxine deficiency the excretion of an abnormal metabolite, xanthurenic acid, has been observed by several workers (7–10). Both riboflavin and pyridoxine deficiencies have been shown to inhibit the excretion of niacin and quinolinic acid after the administration of either tryptophan or kynurenine (11), implicating these vitamins in the metabolism of intermediates past kynurenine (12). However, some workers (13–15) have been unable to demonstrate a striking connection between vitamin B₆ and tryptophan metabolism. Spector (15) reported that a simple pyridoxine deficiency in the rat apparently had little effect upon the conversion of tryptophan to niacin or N-methylnicotinamide as measured by the excretion of these metabolites.

Investigations in this laboratory (16, 17) have shown that in the rat tryptophan appeared to be more important than niacin in maintaining liver pyridine nucleotides (PN), even though the niacin structure is present in the PN molecules. Evidence from later studies (18) indicated that a simple pyridoxine deficiency in the rat did not disturb the normal conversion of tryptophan to liver PN to any demonstrable extent. The possibility existed, however, that the pyridoxine-“deficient” rat retained enough pyridoxine in its tissues to enable the synthesis of tissue PN to take place, especially with the high levels of dietary tryptophan employed in these studies. Under these conditions the apparently low concentrations of pyridoxine in the tissues would be efficiently utilized because of the high concentrations of dietary tryptophan. For this reason it appeared important to study the transformation of niacin and tryptophan to liver PN in animals in which the effects of the pyridoxine deficiency

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had been amplified by the use of the antivitamin, desoxypyridoxine (DB₆). That this compound may enhance a pyridoxine deficiency has been shown by Porter et al. (19).

EXPERIMENTAL

The experiment was designed to study the effects of pyridoxine upon the synthesis of liver PN from tryptophan, niacin, and a combination of the two metabolites. The niacin studies were included to observe whether pyridoxine is involved only in the conversion of tryptophan to the niacin moiety of PN and not in the actual synthesis of the niacin-ribose portion of the PN molecule. The experiment was divided into two parts because of the analytical limitations imposed by the large numbers of animals involved.

In the first part of the experiment, two groups of animals (Groups I and II) were employed in which both groups were fed a pyridoxine-deficient ration. In addition, Group II received a supplement of DB₆ to amplify the effects of the pyridoxine deficiency. In the second part of the experiment two groups of animals (Groups III and IV) were compared in which Group III received a complete vitamin supplement, while Group IV received a pyridoxine-deficient ration with a DB₆ supplement. Therefore, the effects of the pyridoxine inhibitor (DB₆) in addition to a pyridoxine deficiency can be compared with a simple pyridoxine deficiency (Groups I and II). Also a pyridoxine deficiency amplified by DB₆ can be compared with a complete vitamin supplement (Groups III and IV).

Weanling, male albino rats of the Sprague-Dawley strain weighing 40 to 50 gm. were employed as experimental animals. The rats were fed non-protein rations for 14 to 19 days to deplete them of tissue PN, as in previous work (17). Sulfasuxidine was incorporated into all the rations in an attempt to minimize bacterial synthesis of pyridoxine and niacin. The basal non-protein ration contained Salts 4 (20) 4 per cent, corn oil 5 per cent, sulfasuxidine 1.5 per cent, vitamin mix² 2 per cent, and sucrose to make 100 per cent.

Groups I and II received the basal ration, which lacked pyridoxine, for a period of 19 days. Group III received the basal ration supplemented with 0.25 mg. per cent of pyridoxine for 14 days, and Group IV the basal ration for 14 days.

At the end of the depletion period, the rats of each group were separated

¹ Whenever “complete” is used, it means a niacin-free otherwise complete vitamin mix.
² 100 gm. of vitamin mix contained the following vitamins in a sucrose base: thiamine hydrochloride 10 mg., riboflavin 15 mg., calcium pantothenate 100 mg., biotin 0.5 mg., folic acid 1 mg., choline chloride 5 gm., and i-inositol 0.5 gm.
into four subgroups (A, B, C, and D) and the various supplements incorporated into the rations at the expense of sucrose, as shown in Tables I and II. The supplementation period in each case lasted for 1 week. The groups in which the effects of desoxypyridoxine were studied (Groups II and IV) were supplemented with 2 mg. per cent of the antivitamin at the same time the rations were supplemented with niacin and tryptophan. The levels of tryptophan and niacin added to the rations are equivalent on a molar basis and have been shown to have equal effects upon the formation of liver PN in the rat when fed at this level (21).

Throughout the entire experiment the animals were fed and given water ad libitum and received 2 drops of fortified haliver oil per rat per week. At the end of the supplementation period, the animals were sacrificed and the liver PN concentration determined by the method of Feigelson, Williams, and Elvehjem (22).

RESULTS AND DISCUSSION

The results of the liver PN analyses for Groups I and II are presented in Table I. It can be seen that Group IA, which received no pyridoxine and no niacin or tryptophan, was depleted during the initial depletion period from a normal level for normal weanling rats of about 900 µg per gm. of liver to 515 µg per gm. of liver. Upon supplementation with either niacin (Group IB) or tryptophan (Group IC), the liver PN levels were increased significantly and to the same extent over that in the unsupplemented group. When both niacin and tryptophan were fed simultaneously, the total increase in PN was almost equal to the sum of the increase with the individual supplements, as shown in Group ID. Thus, when niacin was fed, the increase in PN over the unsupplemented group was 416 µg, with tryptophan the increase was 421 µg, and with the two supplements together the increase was 747 µg. Therefore, it can be seen that in Groups I, in which the pyridoxine deficiency was brought about only by feeding a ration lacking in pyridoxine, no difference in the degree of conversion was noted between niacin and tryptophan. Essentially the same results had been observed earlier by Williams et al. (18), who studied the effects of a simple pyridoxine deficiency on the conversion of tryptophan to liver PN.

That the vitamin deficiency was probably not severe enough to have any apparent effect on the conversion of tryptophan to PN is indicated in Groups II, which received the same supplements as Groups I, except that DB₆ was also included in the supplement rations. Group IIA, unsup-
plemented except with DB₆, gave liver PN values slightly lower but not greatly different from Group IA, indicating that the effect of DB₆ alone upon the endogenous formation of liver PN was probably negligible. If one compares Groups IB and IIB, both of which received the same niacin supplement, it can be observed that the liver PN level was increased by essentially the same amount, whether DB₆ was included in the supplemental ration or not. However, from a comparison of the results of Groups

### Table I

**Effects of Simple Pyridoxine Deficiency and Deficiency Enhanced by Desoxypyridoxine upon Conversion of Tryptophan and Niacin to Liver Pyridine Nucleotides**

The basal ration was given to all the groups minus vitamin B₆ and niacin.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Supplement per 100 gm. basal ration after PN depletion</th>
<th>No. of animals</th>
<th>PN concentration γ per gm. fresh liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>0</td>
<td>8</td>
<td>515 ±34*</td>
</tr>
<tr>
<td>IB</td>
<td>163† mg. niacin</td>
<td>7</td>
<td>981 ±31</td>
</tr>
<tr>
<td>IC</td>
<td>270† mg. tryptophan</td>
<td>7</td>
<td>936 ±100</td>
</tr>
<tr>
<td>ID</td>
<td>163 mg. niacin + 270 mg. tryptophan</td>
<td>8</td>
<td>1262 ±49</td>
</tr>
<tr>
<td>IIA</td>
<td>2 mg. desoxypyridoxine</td>
<td>12</td>
<td>454 ±33</td>
</tr>
<tr>
<td>IIB</td>
<td>2 mg. desoxypyridoxine + 163 mg. niacin</td>
<td>10</td>
<td>822 ±67</td>
</tr>
<tr>
<td>IIC</td>
<td>2 mg. desoxypyridoxine + 270 mg. tryptophan</td>
<td>10</td>
<td>700 ±44</td>
</tr>
<tr>
<td>IID</td>
<td>2 mg. desoxypyridoxine + 163 mg. niacin + 270 mg. tryptophan</td>
<td>7</td>
<td>1067 ±57</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† These levels of niacin and tryptophan are equimolar.

IC and IIC, it is apparent that the presence of DB₆ inhibited the conversion of tryptophan to liver PN markedly. In Group IC the increase in liver PN over the unsupplemented control was 421 γ, whereas in Group IIC the increase over the control (Group IIA) was only 246 γ. This smaller increase was also noted in Group IID, which received both niacin and tryptophan, so that, although the observed PN level was higher than that with either of the supplements alone, it did not reach the level attained by Group ID in which no DB₆ was fed. The increase of 613 γ in Group IID over the control was approximately the sum of the individual increases in Group IIB of 368 γ and in Group IIC of 246 γ for niacin and tryptophan, respectively.
Groups III and IV comprise a separate experiment begun a few weeks after Groups I and II. Past experience (20) has shown that in order to compare the absolute PN values of one group of animals with another in this type of experiment the groups must be run simultaneously. Therefore, the absolute PN values of Groups I and II are comparable as well as those of Groups III and IV but not those of the first experiment with the second. The results for Groups III and IV are presented in Table II.

### Table II

**Comparison of Ability of Rats Fed Complete Vitamin Supplement with Ability of Rats Lacking Pyridoxine and Receiving Desoxypyridoxine to Convert Niacin and Tryptophan to Liver Pyridine Nucleotides**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Basal ration</th>
<th>Supplement per 100 gm. basal ration after PN depletion</th>
<th>No. of animals</th>
<th>PN concentration (mg. fresh liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIA</td>
<td>+ vitamin B₆ and niacin</td>
<td>0.25 mg. pyridoxine</td>
<td>10</td>
<td>643 ± 22*</td>
</tr>
<tr>
<td>IIIB</td>
<td>+ &quot; &quot; &quot; &quot; &quot;</td>
<td>0.25 &quot; &quot; &quot; &quot; + 163 mg. niacin</td>
<td>8</td>
<td>1130 ± 66</td>
</tr>
<tr>
<td>IIIC</td>
<td>+ &quot; &quot; &quot; &quot; &quot;</td>
<td>0.25 mg. pyridoxine + 270 mg. tryptophan</td>
<td>9</td>
<td>1089 ± 74</td>
</tr>
<tr>
<td>IIID</td>
<td>+ &quot; &quot; &quot; &quot; &quot;</td>
<td>0.25 mg. pyridoxine + 163 mg. niacin + 270 mg. tryptophan</td>
<td>9</td>
<td>1387 ± 160</td>
</tr>
<tr>
<td>IVA</td>
<td>- &quot; &quot; &quot; &quot; &quot;</td>
<td>2 mg. desoxypyridoxine</td>
<td>8</td>
<td>683 ± 15</td>
</tr>
<tr>
<td>IVB</td>
<td>- &quot; &quot; &quot; &quot; &quot;</td>
<td>2 &quot; &quot; &quot; &quot; + 163 mg. niacin</td>
<td>8</td>
<td>1074 ± 75</td>
</tr>
<tr>
<td>IVB</td>
<td>- &quot; &quot; &quot; &quot; &quot;</td>
<td>2 mg. desoxypyridoxine + 270 mg. tryptophan</td>
<td>6</td>
<td>882 ± 35</td>
</tr>
<tr>
<td>IVB</td>
<td>- &quot; &quot; &quot; &quot; &quot;</td>
<td>2 mg. desoxypyridoxine + 163 mg. niacin + 270 mg. tryptophan</td>
<td>8</td>
<td>1217 ± 71</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

Although the absolute values are somewhat higher than those obtained in the first experiment for Groups I and II, the actual differences between the control and the supplemented group are almost the same. Thus, in Groups III, which received a complete vitamin supplement, little difference was observed in the increase in liver PN between the group receiving the niacin supplement (Group IIIB) and that fed the tryptophan supplement (Group IIIC). In Group IIID, which received both niacin and tryptophan, the synthesis of liver PN was again approximately equal to the sum of the individual stimulation by niacin and tryptophan, respectively.

From Groups IV, which received DB₆ in addition to the niacin and tryptophan supplements, it can be observed that a significantly smaller increase
in liver PN over the control (Group IVA) occurred with the tryptophan supplement (Group IVC) than with the niacin supplement (Group IVB). With niacin the increase in PN was 391 γ of PN per gm. of liver, while with tryptophan the increase was only 149 γ. The increase in PN over the control group when both niacin and tryptophan were fed together was 530 γ, which is approximately equal to the sum of the individual stimulation by niacin and tryptophan.

As already observed by Williams et al. (18), a simple dietary deficiency of pyridoxine did not appear to affect the conversion of tryptophan to PN. In the experiments presented here, again no demonstrable effect was apparent unless the antivitamin, desoxypyridoxine, was used to enhance the effects of the pyridoxine deficiency. The results reported in this paper are generally in accord with the results of Ling et al. (23), who observed a decrease in blood pyridine nucleotides after the administration of tryptophan to pyridoxine-deficient rats. It is evident from the present results, however, that even the inclusion of DB₆ in the ration does not completely block the conversion of tryptophan to liver PN. If the antivitamin had been fed throughout the whole experiment rather than only during the supplementation period, possibly the suppression would have been greater. However, the weanling rats would probably have been unable to survive the combined effects of the non-protein diet and the antivitamin for the entire feeding period, since some mortality was observed even among the animals receiving the complete vitamin supplement.

**SUMMARY**

It has been shown that a simple dietary deficiency of pyridoxine in the rat has little demonstrable effect upon the conversion of tryptophan to tissue pyridine nucleotides. However, if the pyridoxine deficiency is enhanced by feeding desoxypyridoxine, the synthesis of tissue pyridine nucleotides from tryptophan can definitely be shown to be inhibited. The synthesis of these coenzymes from niacin is unaffected either by a simple pyridoxine deficiency or by feeding desoxypyridoxine to pyridoxine-deficient rats, indicating that the reactions involved in the synthesis of the ribose moiety or the reactions connecting the ribose to the niacin moiety are probably not influenced by pyridoxine.

**BIBLIOGRAPHY**


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