Influence of Pyridoxine, Pyridoxal Phosphate, Deoxypyridoxine, and 2,4-Dinitrophenol on Methionine Absorption*

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It has been demonstrated by studies in vitro and in vivo that two absorptive processes are involved in the intestinal absorption of amino acids. It is also apparent that the vitamins of the Bg-group play a role in the "active" process of cellular uptake of amino acids (2) and that deoxypyridoxine (3) and 2,4-dinitrophenol (3, 4), have an inhibitory effect upon active absorptive processes.

We are presenting evidence that pyridoxal phosphate alleviates deoxypyridoxine- and 2,4-dinitrophenol-induced inhibition of methionine absorption, and that the inhibitory block is linked to the phosphorylation of the vitamin cofactor. These studies extend our investigations on methionine absorption (5) and lend further support to the hypothesis that the Bg-vitamins are involved actively in the absorptive process in the steady state.

EXPERIMENTAL PROCEDURE

Sodium chloride solutions of L-methionine were perfused through segments of the upper small intestine of the rat using a perfusion apparatus in situ previously described for the study of amino acid and sugar absorption (3, 6). With this apparatus the rate of perfusate flow and its temperature can be controlled, and samples can be removed for analysis without disruption of the system. Net load exchange and continuous absorption rates can be established for single animals in the steady state.

Sprague-Dawley adult male white rats, fasted 18 to 20 hours, were used in all experiments. These animals had been maintained on a stock regimen of Purina laboratory chow up to the time they were fasted just before the perfusion experiments. Animals were anesthetized before cannulation and perfusion of the amino acid. Perfusate sampling and the amount of absorption were determined by the usual method, starting with 20 mM L-methionine in 0.9% NaCl in the circulating perfusion system (5).

Methionine levels of the perfusate were determined chemically by Horn's modification (7) of Sullivan's procedure for the analysis of this amino acid. All determinations were made upon appropriately diluted perfusate samples; control standards were used for each analysis. Both the continuous rate and the net load absorbed over 1 hour periods were determined.

For these studies the animals were divided into a series of groups as follows: a group of control animals received no extra vitamins or antagonist; the second group received pyridoxine; the third, deoxypyridoxine; the fourth, deoxypyridoxine then pyridoxine; the fifth, DNP; the sixth, DNP then pyridoxine; the seventh, pyridoxine then DNP; the eighth, DNP then pyridoxal phosphate; the ninth, pyridoxal phosphate then DNP; and a group maintained on a pyridoxine-free diet.3

The vitamins and antagonists were injected intraperitoneally as aqueous solutions, deoxypyridoxine as its hydrochloride at a dose level of 0.5 mg and pyridoxine (as the hydrochloride) and pyridoxal phosphate in equimolar amounts. DNP was administered at a dose level of 10 mg per kg body weight.

RESULTS

Effect of Deoxypyridoxine, DNP, and Pyridoxine-free Diet upon Intestinal Absorption of L-Methionine

One group of this series was injected intraperitoneally with deoxypyridoxine, another group with DNP, each group having received the antagonist 1 hour before perfusion. The absorption patterns in these animals, a control group, and animals maintained on a pyridoxine-free regimen of 34 and 44 days duration are plotted in Fig. 1. There is a marked depression in the intestinal absorption of L-methionine induced by these antagonists and the vitamin-free dietary. This depression is exhibited both for the continuous rate of absorption and for the net load absorbed over the 1 hour experimental periods.

Alleviation of Deoxypyridoxine-induced Inhibition by Pyridoxine

One group of this series was injected with pyridoxine, two with deoxypyridoxine, one of which was followed ½ hour later by pyridoxine. In all instances the initial injections were administered 1 hour before perfusion with the amino acid. The resulting absorption rates are presented in Fig. 2 with the data obtained from the control group.

It was found that pyridoxine significantly stimulated the absorption to a level above that of control animals (p < 0.01) and that pyridoxine alleviated the effects of the antimetabolite, deoxypyridoxine.

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1 Manufacturer's analysis, pyridoxine 4.3 p.p.m.
2 The abbreviation used is: DNP, 2,4-dinitrophenol.
3 Pyridoxine-free Test Diet, manufactured by Nutritional Biochemicals Corporation.
oxypyridoxine, bringing the absorption pattern back to that of the controls.

Effects of Pyridoxine upon DNP-induced Inhibition

Three groups of this series were injected with DNP 1 hour before perfusion. Of these groups one was injected with pyridoxine \( \frac{1}{2} \) hour before the administration of DNP and another, \( \frac{1}{2} \) hour after the DNP administration. The absorption patterns for these animals are shown in Fig. 3. There was no significant alleviation of the DNP-induced inhibition by pyridoxine, administered either before or after the DNP. All groups exhibited depressed absorptive ability.

Effects of Pyridoxal Phosphate upon DNP-induced Inhibition

Three groups of this series of rats were similarly injected with DNP. Of these, one was treated with pyridoxal phosphate \( \frac{1}{2} \) hour before the administration of DNP and another, \( \frac{3}{2} \) hour after DNP administration. The rate and net load of absorption are plotted in Fig. 4. The findings demonstrate that vitamin \( B_6 \) as pyridoxal phosphate both protects against and alleviates the DNP-inhibited absorptive process. This protection and alleviation is more pronounced in those instances where the pyridoxal phosphate is administered before the DNP rather than after; however, in either order of administration the resulting pattern of absorption was significantly different from the DNP-inhibited absorption (\( p < 0.001 \)) and not significantly different from the control group (\( p < 0.8 \) and \( < 0.3 \), respectively).

The data presented in the above series of experiments were grouped and analysed statistically for their significance. There was definite inhibition of intestinal absorption of L-methionine in those animals to which deoxypyridoxine, or DNP had been administered, or which had been maintained upon a pyridoxine-free regimen (8). Pyridoxine alleviated the inhibition induced by deoxypyridoxine but not that induced by DNP. Pyridoxal phosphate both protected against and alleviated DNP-induced inhibition. The presence of excess pyridoxine stimulated the absorption of methionine when administered to rats on an otherwise normal diet. These data are presented with their statistical analyses in Table I.

DISCUSSION

These experiments were designed to permit the simultaneous study both of the continuous absorption pattern and the net load exchange on the same animal in the steady state. They permit examination of the effects of the antimetabolites deoxypyridoxine and DNP upon the intestinal absorption of L-methionine and the alleviation of induced inhibition, by vitamin factors of the \( B_6 \) group.
Methionine Absorption

Inhibition of absorption induced by DNP was not significantly alleviated by treatment of the animal with pyridoxine but was by pyridoxal phosphate. Even though there was no significant alleviation when pyridoxine was administered before DNP, there was found to be a slight effect, as measured by the mean values for the group. This alleviation appeared to be greater both for the pyridoxine and pyridoxal phosphate groups before DNP than those following DNP administration.

The vitamin B₆ as a prosthetic group exists as pyridoxal 5'-phosphate. This coenzyme is apparently involved in the absorption of methionine induced by deoxypyridoxine, DNP, and extended periods of time on a pyridoxine-free diet (34 and 44 days). These findings in general might have been expected, but it is interesting to note the similarity between the groups of rats receiving either deoxypyridoxine or DNP. The inhibition exerted by dietary depletion is progressively greater with time, the longer this regimen is followed, the greater is the inhibition to a point of about 20% of the load perfused. This latter inhibition can be reversed by replacement with pyridoxine (8).

Inhibition of absorption induced by DNP was found to be quite rapid, since the rats had been injected with the antivitamins 1 hour before perfusion, and again a half hour before perfusion with pyridoxine. The degree of alleviation of the antagonist was statistically within the range of the control group, even though the mean values were slightly higher. The treatment with pyridoxine itself stimulated absorption significantly above that for the control group.

Since all animals but one group were maintained on a stock diet, the effects of treatment (antimetabolite or vitamin) were not due to dietary causes but the agents being used. We have shown that there is a significantly inhibited absorption of methionine induced by deoxypyridoxine, DNP, and extended periods of time on a pyridoxine-free diet (34 and 44 days). These findings in general might have been expected, but it is interesting to note the similarity between the groups of rats receiving either deoxypyridoxine or DNP. The inhibition exerted by dietary depletion is progressively greater with time, the longer this regimen is followed, the greater is the inhibition to a point of about 20% of the load perfused. This latter inhibition can be reversed by replacement with pyridoxine (8).

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**TABLE I**

**Intestinal absorption of 20 mM L-methionine**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Water exchange</th>
<th>Continuous absorption rate</th>
<th>Net load absorbed in 1 hour</th>
<th>Probability values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>mL 15 min</td>
<td>% 30 min</td>
<td>% 45 min</td>
<td>% 60 min</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>9</td>
<td>18.10 ± 6.80</td>
<td>39.24 ± 6.84</td>
<td>53.60 ± 9.81</td>
<td>65.20 ± 9.96</td>
</tr>
<tr>
<td>Deoxypyridoxine</td>
<td>0</td>
<td>9.78 ± 4.59</td>
<td>18.50 ± 6.77</td>
<td>35.67 ± 4.30</td>
<td>54.43 ± 4.18</td>
</tr>
<tr>
<td>Deoxypyridoxine then pyridoxine</td>
<td>8</td>
<td>13.45 ± 7.14</td>
<td>28.81 ± 9.49</td>
<td>46.38 ± 8.13</td>
<td>55.98 ± 7.34</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>12</td>
<td>-0.10</td>
<td>9.35 ± 3.65</td>
<td>17.39 ± 5.17</td>
<td>24.27 ± 9.73</td>
</tr>
<tr>
<td>DNP then pyridoxine</td>
<td>4</td>
<td>-0.20</td>
<td>8.09 ± 2.22</td>
<td>15.22 ± 4.65</td>
<td>24.83 ± 3.13</td>
</tr>
<tr>
<td>Pyridoxine then DNP</td>
<td>5</td>
<td>±0.00</td>
<td>13.61 ± 2.79</td>
<td>21.54 ± 3.47</td>
<td>27.60 ± 3.49</td>
</tr>
<tr>
<td>DNP then pyridoxal phosphate</td>
<td>4</td>
<td>-0.13</td>
<td>17.43 ± 2.88</td>
<td>36.42 ± 4.17</td>
<td>47.24 ± 10.13</td>
</tr>
<tr>
<td>Pyridoxal phosphate then DNP</td>
<td>5</td>
<td>±0.07</td>
<td>18.38 ± 3.89</td>
<td>33.31 ± 13.23</td>
<td>54.24 ± 16.08</td>
</tr>
<tr>
<td>Deficient diet:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 days</td>
<td>1</td>
<td>-0.45</td>
<td>6.81</td>
<td>12.28</td>
<td>15.00</td>
</tr>
<tr>
<td>44 days</td>
<td>1</td>
<td>-0.45</td>
<td>2.96</td>
<td>11.76</td>
<td>16.20</td>
</tr>
</tbody>
</table>

* Absorption is expressed in respect to intestinal segments 10 cm long, located approximately 3 to 13 cm distal to the pylorus.

* Negative values indicate the mean loss in the circulating perfusate volume as measured at the termination of perfusion experiments.

* These mean values are expressed as the percentage of the initial concentration of methionine perfused (zero time) in respect to the times indicated.

* The values for the net load absorbed are based upon measured volume and concentration, taking into account samples removed for the continuous absorption studies.

* These values are calculated standard deviations of the mean values given in each instance.

* Probability derived from the test of significance applied in respect to the net load exchange.
tive process studied here. The B₆ vitamins by way of a reaction with ATP are converted to their respective phosphates (9). DNP, a substance capable of blocking this system of phosphorylation, should then interfere with the conversion of pyridoxine to pyridoxal phosphate. If this metabolic block is responsible for pyridoxal phosphate formation or reformation, then the DNP should block absorption, assuming that this cofactor is necessary for active absorption. More pyridoxine would not be expected to pass through the blocked sequences after DNP poisoning, but might be converted to pyridoxal phosphate to a limited extent if administered before the DNP. However, pyridoxal phosphate itself, independent of the synthetic phosphorylation system, should then alleviate or remove the DNP block to absorption, regardless of whether it were administered shortly before or after the DNP. This was found to occur in our preparations in situ in rats in the steady state. We cannot explain, at this time, the stimulatory effect of pyridoxine by itself. Pyridoxal phosphate by itself does not seem to stimulate absorption above that which is normal.⁴

**SUMMARY**

Intestinal absorption of L-methionine has been studied by a perfusion technique in situ which allows for study of the continuous rates and net load absorbed from perfused upper small intestinal segments. Absorption was markedly inhibited by deoxypyridoxine and 2,4-dinitrophenol (DNP) when these antimetabolites were administered to rats before perfusion with the amino acid. Rats maintained on a pyridoxine-free diet likewise exhibited a depressed ability to absorb.

Pyridoxine was shown to alleviate the inhibited intestinal absorption induced by deoxypyridoxine, but failed to alleviate DNP-induced inhibition. DNP-induced inhibition was alleviated by the injection of pyridoxal phosphate either before or after DNP was administered.

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