THE EFFECT OF GELATIN ON THE TRANSFORMATION OF TRYPTOPHAN TO Niacin IN Rats ON LOW CASEIN DIETS*

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It was demonstrated by Elvehjem and collaborators (1-4) that, if to a niacin-free diet containing 9 per cent casein and permitting moderate growth there was added an excess of tryptophan-deficient proteins (corn, gelatin, acid hydrolysates of proteins) or certain amino acids (glycine, threonine, phenylalanine), growth of young rats was impaired or stopped. Furthermore, this effect could be abolished equally well by the addition to the diet of approximately 1.5 mg. per cent of niacin or of 50 mg. per cent of tryptophan. This observation appears to imply that the superimposition of certain amino acids upon a diet containing barely adequate amounts of complete protein resulted in a niacin deficiency.

The question which still remained to be solved was whether the added amino acids raised the quantitative requirements for niacin, or, since tryptophan in the casein is the only known source of niacin in this diet, whether the transformation of tryptophan to niacin is impaired under these conditions. This question is partly answered by the data of Elvehjem et al. (4) who showed that the growth of rats receiving the basal 9 per cent casein diet amounted to 12 gm. per week, while the gain in rats receiving the same diet plus 1.5 mg. per cent of niacin was 16 gm. per week. Other data in the published studies of the Wisconsin group and our own unpublished data support the view that niacin added to a low protein diet enhances growth to a small extent, probably sparing a part of the food tryptophan as well as supplying the vitamin. This is further borne out by the growth data on the rats receiving 9 per cent casein plus added amino acids in which the addition of 1.5 mg. per cent of niacin restored, or even improved, the rate of growth, as compared with animals receiving the basal diet and the added niacin only ((4) Table I).

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The experiments described below furnish further evidence that the capacity of the organism to form niacin from tryptophan is impaired when a low protein (10 per cent casein-sucrose) diet is supplemented with a large amount of a tryptophan-free protein (gelatin).

**EXPERIMENTAL**

Weanling male albino rats of the Vanderbilt strain were kept singly in metal metabolism cages with tray bottoms. Fresh food and water were supplied daily and the rats were weighed at semiweekly intervals. Each rat was offered an adequate amount of food daily, and on the following day the residue was weighed and discarded. The basal diet used in these studies contained casein 10, sucrose 83, salts 4 (5), cottonseed oil 3, choline 0.5, L-cystine 0.4 gm., supplemented with 1 mg. each of thiamine, riboflavin, and pyridoxine, 6 mg. of Ca pantothenate, 15 mg. of inositol, 0.02 mg. of biotin, 0.02 mg. of folic acid, and 0.10 mg. of vitamin K per 100 gm. 2 drops of cod liver oil were given each week. The gelatin added to the diet was at the expense of the sucrose.

Urine was collected in 72 hour periods under 1 ml. of toluene and 0.5 ml. of glacial acetic acid. The urine and washings were made to a total volume of 50 ml., and after filtering, a suitable aliquot was taken from each of the six urines in the group, and the pooled sample stored in the refrigerator. The urinary excretion of N¹-methylnicotinamide was determined by the fluorometric acetone condensation method (6).

While the animals ingesting the basal diet (10 per cent casein), Group 1, and the casein + gelatin diet supplemented by niacin or tryptophan, in Groups 5 and 6, developed no outward symptoms of deficiency, the rats in Groups 2, 3, and 4 developed in the course of the first 3 weeks of the unsupplemented casein + gelatin diet rough coats, severe diarrhea, and incrustations of brownish red pigment about the mouth and paws. The addition of 5 mg. per cent of niacin to the diet produced remission of all of these symptoms; the addition of 50 mg. per cent of DL-tryptophan produced a temporary remission of the diarrhea only, while the other symptoms persisted and cleared gradually after the large 25 mg. dose of L-tryptophan.

The data in Table I and Fig. 1 show the growth-depressing effect of 6 per cent gelatin added to the 10 per cent casein diet. They also demonstrate that the addition of either 5 mg. per cent niacin or 50 mg. per cent of tryptophan at the beginning of the experiment is equally effective in abolishing this inhibitory action of gelatin. It is worth noting that the addition of the niacin or of the tryptophan to the gelatin-containing diet (Groups 5 and 6) results in better growth than on the basal diet alone without gelatin (Group I). Thus, the growth-depressing effect of gelatin appears to be due almost entirely to a lack of niacin, and not to any other-
Fig. 1. Growth responses of rats in Groups 2, 3, and 4 maintained for the first 3 weeks on a 10 per cent casein + 6 per cent gelatin diet. Beginning in the 4th week, to the diet of Group 2 were added 50 mg. per cent of \( \text{DL-tryptophan} \) and to the diet of Group 3 were added 5 mg. per cent of niacin; the diet of Group 4 remained unchanged. At the beginning of the 5th week all animals received a single 25 mg. dose of \( \text{L-tryptophan} \) intraperitoneally. At the beginning of the 7th week, three animals in Group 4 were given another 25 mg. dose of \( \text{L-tryptophan} \) orally (\( \text{O} \)), and three animals received the same amount intraperitoneally (\( \text{IP} \)).

Table I

**Modifying Effects of Niacin and Tryptophan upon Growth Inhibition Produced by Gelatin in Rats**

The average values for six rats in each group, the gain being measured in gm. per rat per week.

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<thead>
<tr>
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<td>per wk.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3rd</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>4th</td>
<td>9</td>
<td>-6∗</td>
<td>17†</td>
<td>-3</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>5th-6th</td>
<td>13‡</td>
<td>11‡</td>
<td>16‡</td>
<td>4‡</td>
<td>12‡</td>
<td>11‡</td>
</tr>
</tbody>
</table>

* 50 mg. per cent of \( \text{DL-tryptophan} \) added to diet.

† 5 mg. per cent of niacin added to diet.

‡ On the first day of the 5th week all the animals received a single dose of 25 mg. of \( \text{L-tryptophan} \) intraperitoneally.

wise toxic effect of the large amount of gelatin in the diet. Table I further shows the striking differences in response to supplements of niacin and
tryptophan added to the casein-gelatin diet in the beginning of the experiment (Groups 5 and 6), as compared with the results obtained when these supplements were added after 3 weeks of progressing growth depression produced by the gelatin (Groups 2 and 3). Whereas the animals in Groups 5 and 6 continued to grow well (15 to 18 gm. per week), the rats in Group 2, when given a supplement of 50 mg. per cent of tryptophan in the diet, actually lost weight to about the same extent as the control Group 4, which received no supplement to the gelatin diet. On the other hand, the animals in Group 3, when supplemented with 5 mg. per cent of niacin, showed an immediate and large gain in weight (Fig. 1).

At the beginning of the 5th week all animals received intraperitoneally a single dose of 25 mg. of L-tryptophan. The rats in Groups 2 and 4 showed an immediate positive gain in weight. The animals in Group 2 which were receiving 50 mg. per cent of tryptophan showed a larger gain and maintained it during the next 2 weeks (Fig. 1), whereas the control animals in Group 4 (casein-gelatin diet alone) responded with an increase in weight during the first 6 days after the injection and then declined again. As shown in Fig. 1, the animals in Group 4 were given at the beginning of the 7th week another single dose of 25 mg. of L-tryptophan, three rats receiving the dose orally and three intraperitoneally. All animals showed about equally rapid growth responses during the first 5 days, but lost weight just as rapidly in the next 4 days. These results confirmed those observed previously in the same animals in the 5th week, and also demonstrated that the effect of tryptophan is the same, regardless of the mode of administration.

The excretion of N1-methylnicotinamide was measured in all groups for the 3 day period following the single intraperitoneal dose of 25 mg. of L-tryptophan at the beginning of the 5th week. The control urines were collected during the preceding 3 day period. The results summarized in Table II indicate that in Group 4 the effect of the gelatin in the diet is to depress the excretion of extra N1-methylnicotinamide after the test dose of tryptophan. The addition of niacin to the diet of the animals in Group 3 restores the excretion of extra N1-methylnicotinamide to the level found in Group 1. However, the animals in Group 4 supplemented with tryptophan did not respond to the test dose. In Groups 5 and 6, where supplements of niacin and tryptophan, respectively, were given from the beginning of the experiment, significantly large increases in the excretion of N1-methylnicotinamide were observed. The reason for the different responses in Groups 5 and 6 is not apparent.

These data indicate clearly that the presence of an excess of gelatin in the 10 per cent casein diet results in an impairment of the metabolic pathway from tryptophan to niacin. This defect, if permitted to develop for several
weeks, cannot be remedied by the addition of a small amount of tryptophan (50 mg. per cent) but can be abolished either by adding a small amount of niacin or by relatively very large amounts of tryptophan (25 to 100 mg. daily).

**Table II**

*Excretion of N1-Methylnicotinamide in Rats Fed Low Protein Diet containing 6 Per Cent Gelatin and Supplements of Niacin and Tryptophan*

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet</th>
<th>Average excretion of N1-methylnicotinamide per 100 gm. rat</th>
<th>Control increase over control value after 25 mg. L-tryptophan intraperitoneally</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basal (10% casein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&quot; + 6% gelatin ( + 50 mg. % DL-tryptophan, beginning 4th wk.)</td>
<td>41</td>
<td>465</td>
</tr>
<tr>
<td>3</td>
<td>Basal + 6% gelatin ( + 5 mg. % nicotinic acid, beginning 4th wk.)</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>Basal + 6% gelatin</td>
<td>344</td>
<td>465</td>
</tr>
<tr>
<td>5</td>
<td>&quot; + 6% gelatin + 5 mg. % nicotinic acid</td>
<td>60</td>
<td>72</td>
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<tr>
<td>6</td>
<td>&quot; + 6% gelatin + 50 mg. % DL-tryptophan</td>
<td>316</td>
<td>764</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Presumably the growth-depressing effect of whole corn, corn proteins, acid-hydrolyzed proteins, gelatin, or amino acids such as glycine, DL-threonine, or DL-phenylalanine (4), when added to a low casein diet, is primarily due to the ensuing biochemical defect in the metabolic relationship of tryptophan to niacin in rats. In all cases described in the literature and in our observation, this defect is restored by small amounts of niacin alone. While added tryptophan may produce somewhat enhanced growth as compared with niacin, it is apparent that the major effect of tryptophan is due to its yield of niacin, and the further increment in growth may be due to the availability of extra tryptophan for tissue synthesis in animals kept on a tryptophan-poor diet. From this viewpoint, the pellagragenic effect of an excess of corn in the diet is explicable on two accounts: the deficiency of tryptophan as a source of niacin, and the imbalance of amino acids leading to an impairment in the transformation, tryptophan → niacin, in a diet which is already poor in niacin.

Schweigert and Pearson (7) recently reported that the addition of 12 per cent gelatin to a 12 per cent casein-sucrose diet depressed the growth of
rats and that the addition of 1 mg. per cent of niacin or the feeding of 100 mg. doses of DL-tryptophan daily restored the growth. It is to be noted that this restoration was obtained with relatively huge doses of tryptophan. In the present study, while this effect of large doses of free tryptophan is confirmed, the inadequacy of smaller amounts of tryptophan equivalent to concentrations to be found in diets containing 15 to 20 per cent casein in overcoming the syndrome in niacin-depleted rats is clearly demonstrated.

SUMMARY

The inhibition of growth and of the transformation of tryptophan to niacin, as determined by the urinary excretion of N′-methylnicotinamide after a test dose of tryptophan, in young rats on a diet containing 10 per cent casein and 6 per cent gelatin can be adequately prevented by the initial inclusion of either 5 mg. per cent of niacin or 50 mg. per cent of tryptophan. If the same amount of tryptophan is added after the animals have been on the above diet for 3 weeks and have developed deficiency symptoms, the growth and the excretion of N′-methylnicotinamide remain depressed. The addition of 5 mg. per cent of niacin to the diet after 3 weeks does restore both growth and the excretion of N′-methylnicotinamide to normal levels. A single large parenteral or oral dose (25 mg.) of tryptophan restores for a few days only the normal growth curve of the animals. These observations in conjunction with the data of other investigators are interpreted to demonstrate that the major deleterious effect of gelatin, of other tryptophan-deficient proteins, and of amino acids when added to a low casein diet is due to the impairment of the metabolic process involved in the tryptophan-niacin interrelationship.

We wish to thank Miss Betty Jean Peck for technical assistance.

BIBLIOGRAPHY

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