THE EFFECT OF SOME AMINO ACIDS ON THE GROWTH AND NICOTINIC ACID STORAGE OF RATS ON LOW CASEIN DIETS*

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(Received for publication, July 7, 1947)

Krehl et al. (1, 2) have observed that the inclusion of large amounts of corn grits in a low protein diet produced a growth retardation in rats, which was corrected by the addition of either nicotinic acid or L-tryptophan. The beneficial action of the amino acid is now attributed to its rôle as a biological precursor of nicotinic acid (3–5). It is not necessary to postulate the existence of a specific pellagragenic agent in corn for the rat, at least, because the deficiency syndrome with corn can be duplicated with non-corn rations by the addition of tryptophan-deficient proteins or acid-hydrolyzed proteins to a nicotinic acid-deficient diet containing suboptimal amounts of tryptophan (6). Inasmuch as nicotinic acid produced normal growth on the nicotinic acid-deficient diets only when these protein supplements were present, it appears probable that the unsupplemented diet was also inadequate with regard to some amino acids. In this respect, Hall and Sydenstricker (7) have observed normal growth in rats when lysine, valine, threonine, histidine, and tryptophan were added to low casein diets adequate in other respects.

We report here the production of a severe nicotinic acid deficiency in the rat on low protein diets supplemented with some amino acids and the effect of these substances on the storage of nicotinic acid in the liver.

EXPERIMENTAL

Wistar rats, 21 to 23 days old, were used in these experiments. The basal diet consisted of casein (Labco) 9, sucrose 82, salts (8) 4, L-cystine 0.2, cottonseed oil 3, and cod liver oil 2 parts. Vitamins were incorporated in 100 gm. of diet at the following levels: thiamine 1.0 mg., riboflavin 1.0 mg., pyridoxine 1.0 mg., calcium pantothenate 2.0 mg., choline chloride 200 mg., 2-methyl-1,4-naphthoquinone 0.5 mg., inositol 10 mg., biotin 0.02 mg., and folic acid 0.2 mg. α-Tocopherol was administered at a

* Acknowledgment is made of aid from the John and Mary R. Markle Foundation and the United States Public Health Service. Folic acid and biotin were generously contributed by Lederle Laboratories Division, American Cyanamid Company and Merck and Company, Inc., respectively.
level of 1.0 mg. per rat per week. Supplements of amino acids replaced an equal amount of sucrose in the diet.

The nicotinic acid content of liver was determined microbiologically (9) on tissue extracts prepared by autolysis aided by taka-diastase and papain (10).

From the results in Table I it is evident that the growth on the basal ration can be improved to a limited extent by the addition of either nicotinic acid or tryptophan. Even here, however, values remain below the rate of 21 gm. per week of our normal stock animals. The nicotinic acid content of the livers of these animals is similarly increased by supple-

Table I

Growth and Liver Nicotinic Acid of Rats on Various Diets

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>No. of rats</th>
<th>Diet</th>
<th>Gain per wk. for 4 wks.</th>
<th>Liver nicotinic acid γ per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Basal</td>
<td>9 (6-12)*</td>
<td>123 (90-155)*</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>+ 10 mg. % nicotinic acid</td>
<td>14 (11-18)</td>
<td>148 (126-173)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>+ 0.2 % L-tryptophan</td>
<td>15 (12-19)</td>
<td>177 (129-227)</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>+ amino acid mixture†</td>
<td>2 (-1 to +4)</td>
<td>148 (103-194)</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Diet 4 + 2.0 mg. % nicotinic acid</td>
<td>20 (17-25)</td>
<td>160 (142-180)</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>1 + 0.2 % L-tryptophan</td>
<td>24 (19-28)</td>
<td>218 (180-276)</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>4 minus L-lysine</td>
<td>3 (2-3)</td>
<td>126 (100-167)</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>7 + 2.0 mg. % nicotinic acid</td>
<td>20 (19-21)</td>
<td>172 (149-188)</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>7 + 0.2 % L-tryptophan</td>
<td>23 (21-25)</td>
<td>209 (182-263)</td>
</tr>
</tbody>
</table>

* The values in parentheses represent the range.
† Composition of amino acid mixture, L-histidine monohydrochloride 0.25 per cent, L-lysine monohydrochloride 0.52 per cent, DL-valine 0.30 per cent, and DL-threonine 0.40 per cent.

With tryptophan, however, normal storage is found, in spite of a suboptimal growth rate.

Lysine, valine, histidine, and threonine, when added to the basal ration to improve the amino acid composition, produce a marked growth depression, which is not reflected in a further decrease in the liver nicotinic acid. In fact, these values are somewhat above those obtained on the basal diet alone. Within this former group the growth rates of -1 to +4 correspond to values of 194 to 103 γ for the vitamin content of the liver,

1 The liver nicotinic acid of our stock animals is 160 γ per gm. of tissue.
2 Most of these animals exhibit a reddish staining of the nose and whiskers and a reddening of the paws, apart from any porphyrin-like accumulation. Corneal vascularization is absent.
indicating that within this group an inverse relationship exists between the growth rate and nicotinic acid storage. The addition of nicotinic acid (Diet 5) or tryptophan (Diet 6) not only corrects the growth depression, but permits a normal growth rate, which is not possible in the absence of these amino acids (Diets 2 and 3). The synergistic effect of nicotinic acid and these amino acids is also reflected in normal nicotinic acid storage in the liver. With supplementary tryptophan, storage values are considerably above normal, indicating perhaps either excessive synthesis or retention of biologically active intermediates in the synthetic process. It is of interest to note that in animals on Diet 3 tryptophan can maintain a normal level of nicotinic acid in the liver, in spite of a subnormal growth rate, whereas this is not possible if nicotinic acid is preformed in the diet (Diet 2).

Inasmuch as nicotinic acid will promote normal growth when the lysine-deficient protein zein is added to a low protein diet (6), this amino acid was omitted from the amino acid supplement (Diet 7). The effect of this modified amino acid supplement on the growth of rats on the basal diet resembles that observed with the amino acid mixture containing lysine. However, there is no change in the nicotinic acid storage in the liver. With supplementary nicotinic acid or tryptophan the growth depression is corrected and normal growth ensues. It is also evident that the amount of lysine in a 9 per cent casein diet is adequate for normal growth when optimal amounts of other amino acids are present.

In unpublished experiments we have found values of liver nicotinic acid as high as 353 γ per gm. in rats on diets containing from 0.5 to 1.0 per cent L-tryptophan.

### Table 11

**Effect of Acid Hydrolysis on Liver Nicotinic Acid Values**

<table>
<thead>
<tr>
<th>Diet No.*</th>
<th>Liver nicotinic acid</th>
<th>By autolysis</th>
<th>By autolysis + acid hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>γ per gm.</td>
<td>γ per gm.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>144</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>227</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>161</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>152</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>239</td>
<td>232</td>
<td></td>
</tr>
</tbody>
</table>

The values represent data obtained from one litter of six rats. The “autolyzed” data are included in Table I.

* As given in Table I.

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In a previous communication from this laboratory (5) it was shown that after the administration of tryptophan to rats there was an increased urinary excretion of an unidentified substance, which was converted to nicotinic acid after acid hydrolysis. Apparently this derivative is not present in significant amounts in the livers of rats on various diets in the present work (Table II).

**DISCUSSION**

Sarett and Perlzweig (11) and Wright and Skeggs (12) have shown that the nicotinic acid storage in the liver, regardless of vitamin intake, is directly related to the protein level of the diet. Our results indicate that on a 9 per cent casein diet the addition of nicotinic acid will increase the storage to a limited extent. This restriction of action is apparently related to the existence of deficiencies of some amino acids on the low protein ration, for when these deficiencies are satisfied, dietary nicotinic acid not only permits normal growth, but also normal storage. It is of interest to note in this respect that Salmon (13) observed that nicotinic acid has a more marked growth-promoting action in rats on 12 per cent than on 9 per cent casein rations. It is presumed that at the higher level of casein, with which deficiencies of some amino acids are less evident, nicotinic acid can exert more fully its growth-promoting action.

The production of a severe nicotinic acid deficiency in the rat by the addition of some amino acids to a 9 per cent casein ration may explain the similar deleterious effects of zein, gelatin, or acid hydrolysate of fibrin when added to a low protein ration. Krehl et al. (14) have observed that of a number of amino acids tested glycine was particularly effective in depressing growth of rats under similar conditions. In this respect we have found that sodium benzoate does not prevent the growth-inhibitory effect of gelatin in rats on low casein diets. Groschke et al. (15) indicate that most amino acids depress growth in chicks on diets low in nicotinic acid. In rat experiments involving the addition of amino acids to a low protein diet, it is well to consider not only the growth-depressing effects of these substances, but also their ability to promote normal growth upon the addition of nicotinic acid.

**SUMMARY**

The growth of rats on a 9 per cent casein diet is improved to a limited extent by supplementary nicotinic acid or L-tryptophan. This is associated with an increased level of liver nicotinic acid. The addition of histidine, valine, threonine, and lysine to the low protein diet produces a

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4 Unpublished data.
marked growth depression, which is not accompanied by a decrease in liver nicotinic acid. The addition of either nicotinic acid or tryptophan not only corrects the growth retardation, but also permits normal growth. When lysine is omitted from the amino acid mixture, essentially similar results are obtained.

No evidence is obtained for the presence in liver of an acid-hydrolyzable derivative of nicotinic acid previously reported present in the urine of rats receiving tryptophan.

BIBLIOGRAPHY

THE EFFECT OF SOME AMINO ACIDS ON THE GROWTH AND NICOTINIC ACID STORAGE OF RATS ON LOW CASEIN DIETS
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