STUDIES ON NICOTINIC ACID DEFICIENCY IN THE CHICK*

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In a previous report (1) it was demonstrated that chicks receiving purified rations low in nicotinic acid grew poorly and developed a typical chick blacktongue. The occurrence of these symptoms was prevented by the addition of nicotinic acid to the ration.

This paper presents further studies on this deficiency and gives data on the activity of several nicotinic acid esters, as well as studies on the possible synthesis of nicotinic acid within the chick.

The experimental procedure and the composition of the basal ration have been reported (1). Briefly, the nicotinic acid-deficient ration is composed of purified casein, gelatin, dextrin, salts, soy bean oil, cystine, crystalline vitamins (except nicotinic acid), vitamins A and D, and concentrates of biotin and the unknown vitamins. The nicotinic acid content of this basal ration varied between 0.2 and 0.3 mg. per 100 gm. Day-old white Leghorn chicks were used throughout.

Nicotinic acid assays were made according to the method of Snell and Wright (2). The tissues were prepared by autoclaving at 15 pounds for \( \frac{1}{2} \) hour with \( 1 \text{N} \) sodium hydroxide. The nicotinic acid content of the whole chick was determined by hydrolyzing the entire animal in boiling 10 per cent potassium hydroxide (alcoholic) for 45 minutes and analyzing a representative aliquot. Coenzyme I analyses were made by the method of Axelrod and Elvehjem (3).

Results

Growth Results (See Table I)—In confirmation of previous results, chicks receiving the basal ration (Group 1) showed a slow rate of growth (Column

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We are indebted to Merck and Company, Inc., Rahway, New Jersey, for the crystalline vitamins; to The Wilson Laboratories, Chicago, for solubilized liver (liver Fraction L); to Wilson and Company, Inc., Chicago, for gelatin; to Allied Mills, Inc., Peoria, Illinois, for soy bean oil; and to Dr. A. D. Welch, Sharp and Dohme, Inc., Glenolden, Pennsylvania, for the sulfasuxidine.

We are grateful to Miss Eleanor G. Anderson for the coenzyme I analysis and to Mr. Willard A. Krehl for the preparation of the nicotinic acid esters.

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### Table I

**Effect of Diet on Growth of Chicks and on Nicotinic Acid Content of Chick Tissues**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Ration</th>
<th>No. of chicks</th>
<th>No. dead at 4 wks.</th>
<th>Average weight at 4 wks.</th>
<th>No. with black tongue</th>
<th>Nicotinic acid in fresh breast muscle</th>
<th>Nicotinic acid in fresh liver</th>
<th>Carnitine in fresh breast muscle</th>
<th>Nicotinic acid in whole chicks at 4 wks.</th>
<th>Nicotinic acid in daily feed eaten at 4 wks.</th>
<th>Nicotinic acid balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basal (no nicotinic acid)</td>
<td>19</td>
<td>2</td>
<td>122</td>
<td>13</td>
<td>11.0</td>
<td>125</td>
<td>&lt;120</td>
<td>2.16</td>
<td>1.28</td>
<td>+0.88</td>
</tr>
<tr>
<td>2</td>
<td>Basal + 1.5 mg. nicotinic acid per 100 gm.</td>
<td>18</td>
<td>0</td>
<td>221</td>
<td>0</td>
<td>19.0</td>
<td>115</td>
<td>&lt;190</td>
<td>5.10</td>
<td>5.33</td>
<td>-0.23</td>
</tr>
<tr>
<td>3</td>
<td>Basal + 10 mg. nicotinic acid per 100 gm.</td>
<td>24</td>
<td>0</td>
<td>234</td>
<td>0</td>
<td>173.0</td>
<td>142</td>
<td>&lt;780</td>
<td>10.70</td>
<td>33.10</td>
<td>-22.40</td>
</tr>
<tr>
<td>4</td>
<td>Basal + 0.5 gm. sulfasuxidine per 100 gm.</td>
<td>4</td>
<td>0</td>
<td>124</td>
<td>4</td>
<td>8.4</td>
<td>127</td>
<td>&lt;80</td>
<td>1.80</td>
<td>1.34</td>
<td>+0.46</td>
</tr>
<tr>
<td>5</td>
<td>As for Group 4 + 1.5 mg. nicotinic acid per 100 gm.</td>
<td>6</td>
<td>0</td>
<td>191</td>
<td>0</td>
<td>16.0</td>
<td>129</td>
<td>&lt;140</td>
<td>5.18</td>
<td>5.33</td>
<td>-0.15</td>
</tr>
<tr>
<td>6</td>
<td>Practical chick starter</td>
<td>6</td>
<td>0</td>
<td>222</td>
<td>0</td>
<td>146.0</td>
<td>128</td>
<td>7.90</td>
<td>36.90</td>
<td>-29.00</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>As for Group 6 + 10 mg. nicotinic acid per 100 gm.</td>
<td>6</td>
<td>0</td>
<td>230</td>
<td>0</td>
<td>147.0</td>
<td>221</td>
<td>10.10</td>
<td>80.40</td>
<td>70.30</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>As for Group 6 + 100 mg. nicotinic acid per 100 gm.</td>
<td>5</td>
<td>0</td>
<td>222</td>
<td>0</td>
<td>134.0</td>
<td>334</td>
<td>11.90</td>
<td>482.90</td>
<td>-471.00</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Basal + 1.5 mg. ethyl nicotinate per 100 gm.</td>
<td>18</td>
<td>2</td>
<td>169</td>
<td>3</td>
<td>9.9</td>
<td>123</td>
<td>&lt;140</td>
<td>2.20</td>
<td>4.20</td>
<td>-1.91</td>
</tr>
<tr>
<td>10</td>
<td>Basal + 10 mg. ethyl nicotinate per 100 gm.</td>
<td>6</td>
<td>0</td>
<td>191</td>
<td>0</td>
<td>56.0</td>
<td>134</td>
<td>&lt;460</td>
<td>7.28</td>
<td>28.20</td>
<td>-20.92</td>
</tr>
<tr>
<td>11</td>
<td>Basal + 1.5 mg. propyl nicotinate per 100 gm.</td>
<td>6</td>
<td>0</td>
<td>174</td>
<td>0</td>
<td>9.9</td>
<td>123</td>
<td>&lt;140</td>
<td>2.20</td>
<td>4.20</td>
<td>-1.91</td>
</tr>
<tr>
<td>12</td>
<td>Basal + 1.5 mg. butyl nicotinate per 100 gm.</td>
<td>6</td>
<td>0</td>
<td>212</td>
<td>0</td>
<td>9.9</td>
<td>123</td>
<td>&lt;140</td>
<td>2.20</td>
<td>4.20</td>
<td>-1.91</td>
</tr>
</tbody>
</table>

*The figures given in Columns 5 and 6 represent the average values obtained from individual analyses of tissues of three chicks and those given in Columns 7 and 8 represent averages obtained from two chicks.*
3) and chick blacktongue (Column 4). When 1.5 mg. of nicotinic acid per 100 gm. were added to the ration, nearly maximum growth was obtained, while a higher level, 10 mg. per 100 gm., gave only slightly better growth. Fig. 1 shows representative chicks with and without nicotinic acid.

Sulfasuxidine (succinyl sulfathiazole), known to diminish the growth of certain intestinal organisms (4), was fed to chicks in Groups 4 and 5 to determine whether synthesis of nicotinic acid occurred in the intestine of chicks receiving a purified ration. The growth results with this drug, compared with those without the drug, showed that if intestinal synthesis of nicotinic acid does occur only small amounts are produced.

To determine whether or not larger amounts of nicotinic acid had any effect on the nicotinic acid content of tissues, a good practical chick starter mash (Wisconsin No. 45) was fed with high amounts of this vitamin. (This mash contained 7.7 mg. of nicotinic acid per 100 gm. as measured by the bacterial assay.) As far as growth was concerned, additions of nicotinic acid up to 100 mg. per 100 gm. (Groups 6 to 8) caused neither improvement nor detrimental effect.

The growth obtained by feeding ethyl, propyl, and butyl nicotinates (Groups 9 to 12) showed that the nicotinic acid activity of these compounds increased as the length of the carbon side chain increased. The reason for the difference in the activity of the esters cannot be explained from our data, but it is possible that the shorter chain esters resisted enzymatic action in the intestine to a greater extent than the longer chain esters.

Influence of Diet on Nicotinic Acid Content of Tissues—The amount of nicotinic acid per gm. of fresh breast muscle and liver taken from the chicks that had received the various diets for 4 weeks is shown in Columns 5 and 6, Table I. When 1.5 mg. of nicotinic acid per 100 gm. of ration were added, the amount in the breast muscle increased only slightly over that obtained with the basal ration. When an excess of this vitamin was fed, 10 mg. per 100 gm. of ration, the amount in the breast muscle was in-

![Fig. 1. Nicotinic acid deficiency in the chick. (Both chicks are the same age; the chick on the right did not receive nicotinic acid.)](image-url)
creased 15-fold. The amount of nicotinic acid in the breast muscle of the chicks receiving sulfasuxidine (Groups 4 and 5) was slightly lower than that of the corresponding group without the drug (Groups 1 and 2). The amount of nicotinic acid in the liver did not increase appreciably unless high amounts of nicotinic acid were fed with the practical ration. It appears that the nicotinic acid content of the breast muscle increases to a saturation point, at which point further storing of nicotinic acid occurs in the liver. Dann and Handler (5) have reported nicotinic acid values for chicken breast muscle and liver which agree closely with the values we have obtained for these tissues in the normal control groups. These normal values may, of course, be raised or lowered, depending on the nicotinic acid content of the ration.

The coenzyme I analysis (Column 7) of the breast muscle showed that the concentration of this enzyme, like the nicotinic acid content, was markedly influenced by the amount of nicotinic acid available to the tissues.

To determine the extent of synthesis of nicotinic acid occurring within the chick, the amount of the vitamin in the entire 4 week-old chick was determined (Column 8). This value was compared to the total nicotinic acid taken in over the 4 week period plus the amount present in the day-old chick (Column 9) and thereby the nicotinic acid balance was determined (Column 10). The amount of nicotinic acid in the day-old chick was determined by analyses of four representative chicks. They were found to contain, on the average, 27.4 $\gamma$ (27.2 to 27.7 $\gamma$) per gm. of fresh tissue, or a total of 0.9 mg. (0.80 to 0.91 mg.) of nicotinic acid per chick. This total value plus the nicotinic acid taken in over the 4 week period by chicks on the basal ration was somewhat smaller than the nicotinic acid content of the 4 week-old chick, showing that about 0.9 mg. of nicotinic acid was synthesized during the experimental period. This amount of nicotinic acid is about one-sixth of the total amount of nicotinic acid needed in the diet for normal growth over a 4 week period. These data, in addition to the weight results, give direct evidence that the young chick on purified rations can synthesize only a part of its total nicotinic acid requirement. The nicotinic acid balance of chicks on the 1.5 mg. level of nicotinic acid (Group 2) was slightly negative, showing again that this amount of nicotinic acid is just borderline. Chicks in Group 3, receiving 10 mg. of nicotinic acid per 100 gm., had a balance of $-22.4$ mg., indicating that at this level a large excess of the vitamin was taken in.

The results obtained with Groups 4 and 5, receiving sulfasuxidine, were similar to the results with Groups 1 and 2. It is evident, however, that sulfasuxidine retarded synthesis of nicotinic acid by about 50 per cent. (Compare Group 1 with Group 4, Column 10.) The results from the chicks on the practical rations showed that regardless of how much nico-
tinic acid was in the diet the content of the whole chick appeared to reach a saturation point above which excess nicotinic acid was largely excreted.

Other Results—Since our last report, we have noticed in our nicotinic acid-deficient groups a dermatitis occurring in spite of ample pantothenic acid and biotin in the ration. The dermatitis first appeared on the upper part of the feet and on the legs after the chicks had been on the diet for 2 to 3 weeks. About 40 per cent of the birds on the basal ration has been so affected. Some (eight out of thirty) of the chicks receiving the esters of nicotinic acid at low levels (Groups 9, 11, and 12) had a severe dermatitis not only of the feet and legs but over the entire body, especially under the wings, where large hard scales were formed (see Fig. 2). Since the chicks in Group 10 which received a high amount of ethyl nicotinate did not have the dermatitis, it was concluded that this condition could not be due to a toxicity of the esters.

The occurrence of some perosis in the nicotinic acid-deficient chicks (six out of nineteen) suggested that nicotinic acid may be another factor necessary for the prevention of this condition, since the control group, receiving the nicotinic acid, had no perosis. There was no correlation between the occurrence of the perosis and the dermatitis mentioned in the preceding paragraph. Other symptoms seen in chicks receiving the basal ration were slow feather development and a decrease in food consumption.

**DISCUSSION**

Since the discovery that nicotinic acid was necessary for the cure and prevention of canine blacktongue and human pellagra, there has been a great deal of work on the measurement of this vitamin in all types of food-
stuffs. There is need for a good animal assay to corroborate the shorter and more efficient microbiological and chemical assays. For this reason we suggest the use of the chick as a possible assay animal and we have found (unpublished data) that this animal may be used for this purpose to advantage.

The reason the dermatitis which occurred in chicks receiving low levels of the nicotinic acid esters was more severe than the dermatitis in chicks on the basal ration is not yet clear. It may be a matter of effective nicotinic acid levels or it is possible that since nicotinic acid was low within the tissues the unhydrolyzed esters were used in making a coenzyme I-like substance which caused the blocking of certain true coenzyme I reactions.

The question arises whether or not the finding that chicks on purified rations require a dietary source of nicotinic acid is of any practical value as far as the commercial feeding of poultry is concerned. As yet we cannot fully answer this question, but it would seem that since the minimum requirement is small compared to the amounts found in most foodstuffs a deficiency of nicotinic acid would be rare in chicks receiving an average ration. It is entirely possible, however, that such a deficiency could exist along with a deficiency of other vitamins of the B complex in chicks receiving a poorly balanced ration, provided the synthesis of nicotinic acid within the body of the chick is as low as it is on purified rations.

SUMMARY

1. The young chick, when fed purified rations, requires a dietary source of nicotinic acid for the prevention of chick blacktongue and for optimal growth. Other deficiency symptoms are a decreased food consumption, a marked lowering of the nicotinic acid and coenzyme I content of breast muscle, poor feather development, and occasionally perosis or scaly dermatitis.

2. Chicks receiving the basal ration synthesized about one-sixth of their total nicotinic acid requirement.

3. Several esters of nicotinic acid were found to have partial nicotinic acid activity which varied with the length of the carbon side chain.

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