THE COMPARATIVE UTILIZATION OF PTEROYLGlutAMIC ACID AND PTEROYLTRIGLUTAMIC ACID BY CHICKS ON A PURIFIED DIET

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The isolation, identification, and synthesis of pteroylglutamic acid from liver have been described; simultaneously the isolation was reported of a compound, "fermentation Lactobacillus casei factor," which upon mild anaerobic alkaline hydrolysis yielded pteroylglutamic acid and 2 equivalents of glutamic acid (1). This compound has been termed pteroyltriglutamic acid (2).

Studies with chicks on a purified diet deficient in pteroylglutamic acid (3) indicated that pteroyltriglutamic acid, when fed at a level of 0.5 part per million of diet, was not markedly effective in promoting growth or preventing anemia. The addition of the lactone of either 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine (5-pyridoxic acid lactone) or 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine (4-pyridoxic acid lactone) together with pteroyltriglutamic acid produced a marked gain in weight over the controls and was completely effective in the prevention of anemia as measured by the hemoglobin level at 3 weeks. In another communication (4) it was reported that pteroylglutamic acid was fully effective for the prevention of anemia without the addition of the lactone. It was suggested (4) that the lactone of 4-pyridoxic acid functioned in an enzyme system required for the breakdown of "folic acid conjugates" and for the setting free of "folic acid" (pteroylglutamic acid).

However, other experiments have indicated that the chick is able to utilize pure pteroylheptaglutamic acid ("vitamin B₆ conjugate") as efficiently as pteroylglutamic acid ("vitamin B₆") (5) and that pteroyltriglutamic acid is utilized just as well as pteroylglutamic acid (6) for hemoglobin formation in chicks on purified diets without the addition of pyridoxic acid lactone. These experiments were made with basal diets differing from that employed by the Cornell group (3). The present investigation was undertaken in an attempt to repeat more closely the experimental conditions employed elsewhere (3).

EXPERIMENTAL

The basal diet was identical in composition with that described elsewhere (3) and consisted of starch, washed casein, gelatin, salt mixture, soy bean...
oil, cellophane, and fortified fish liver oil supplemented with pure vitamins and with succinylsulfathiazole, 1 per cent. The only known difference was that the soy bean oil used in the present investigation did not contain phosphoric acid as an antioxidant.

Day-old New Hampshire chicks were placed in electrically heated battery brooders and were fed the experimental diets immediately. Ten chicks were used in each group. The diets were mixed in small quantities at frequent intervals and were kept in a refrigerator. Acid-water-washed casein was supplied by Dr. M. L. Scott.

The following supplements, per kilo of basal diet, were used in the first experiment: Group 1, none; Group 2, 0.5 mg. of pteroyltriglutamic acid; Group 3, 1.0 mg. of pteroyltriglutamic acid; Group 4, 0.5 mg. of pteroyltriglutamic acid + 1.0 mg. of 4-pyridoxic acid lactone; Group 5, 1.0 mg. of pteroyltriglutamic acid + 1.0 mg. of 4-pyridoxic acid lactone; Group 6, 0.67 mg. of pteroylglutamic acid.

The purity of the pteroyltriglutamic acid was estimated by measurement of its absorption of ultraviolet light in aqueous solution at 365 mp. The preparation was approximately 95 per cent pure. It was obtained from a fermentation product (1). The pteroylglutamic acid preparation was 90 per cent pure, and 0.75 mg. was used to correspond to 0.67 mg. of the pure substance. This level was equivalent on a molar basis to 1.06 mg. of pteroyltriglutamic acid. Pteroyltriglutamic acid and pteroylglutamic acid were dissolved in water at a concentration of 0.1 mg. per ml., and the lactone at a concentration of 1.0 mg. per ml. The solutions were incorporated in the diets. Hemoglobin determinations, erythrocyte counts, and hematocrit measurements were made with blood from a wing vein. The results of the experiment are summarized in Table I.

Three cases of perosis were noted in seven surviving birds in Group 1 at 20 days. One case of perosis was simultaneously noted in each of Groups 2 and 4. Pteroyltriglutamic acid appeared to be fully effective in preventing anemia, even when fed at a level of 0.5 mg. per kilo of diet, which corresponded to only 0.32 mg. of pteroylglutamic acid.

The possibility remained that at a lower level pteroyltriglutamic acid, even if fully utilized, might be only partially effective in preventing anemia and that a condition more sensitive for measuring the supplementary effect of the 4-pyridoxic acid lactone might thus be established. Accordingly a second experiment was carried out 3 months later. The following supplements, per kilo of diet, were used: Group 7, none; Group 8, 0.3 mg. of pteroyltriglutamic acid; Group 9, 0.3 mg. of pteroyltriglutamic acid + 1.0 mg. of 4-pyridoxic acid lactone; Group 10, 0.2 mg. of pteroylglutamic acid; Group 11, 0.65 mg. of pteroylglutamic acid.

The results are summarized in Table II. The level of pteroylglutamic
acid fed to Group 10 was stoichiometrically approximately equivalent to the level of pteroylglutamic acid fed to Groups 8 and 9. Group 11 as a "positive control" group received a level of pteroylglutamic acid in excess of the requirement for growth and hemoglobin formation; this level is in

**Table I**

Response of Chicks to Pteroyltriglutamic Acid, with and without 4-Pyridoxic Acid Lactone, and to Pteroylglutamic Acid

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Average weight at 1 day gm.</th>
<th>21 days gm.</th>
<th>28 days gm.</th>
<th>Hemoglobin at 21 days gm. per cent</th>
<th>28 days gm. per cent</th>
<th>Erythrocyte count 21 days millions per c.m.</th>
<th>28 days millions per c.m.</th>
<th>Mean corpuscular volume $\times 10^3$ cu. microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>82*</td>
<td>74†</td>
<td>5.0</td>
<td>1.3</td>
<td>0.50</td>
<td>$\dagger$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>222</td>
<td>299</td>
<td>8.7</td>
<td>8.5</td>
<td>2.50</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>252</td>
<td>344</td>
<td>8.7</td>
<td>8.5</td>
<td>2.65</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>235</td>
<td>331</td>
<td>8.9</td>
<td>8.5</td>
<td>2.43</td>
<td>1.05</td>
<td></td>
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<tr>
<td>5</td>
<td>40</td>
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<td>338</td>
<td>8.8</td>
<td>8.5</td>
<td>2.59</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>244</td>
<td>335</td>
<td>8.6</td>
<td>8.6</td>
<td>2.73</td>
<td>0.92</td>
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</tbody>
</table>

* Five survivors.
† One survivor.
$\dagger$ Not determined.

**Table II**

Results of Second Experiment in which Insufficient Levels of Pteroyltriglutamic Acid and Pteroylglutamic Acid Were Fed in Groups 8, 9, and 10

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Average weight at 1 day gm.</th>
<th>21 days gm.</th>
<th>28 days gm.</th>
<th>Hemoglobin at 21 days gm. per cent</th>
<th>28 days gm. per cent</th>
<th>Erythrocyte count 21 days millions per c.m.</th>
<th>28 days millions per c.m.</th>
<th>Mean corpuscular volume $\times 10^3$ cu. microns</th>
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<tr>
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<td>45</td>
<td>102</td>
<td>122</td>
<td>5.7</td>
<td>5.0</td>
<td>1.12</td>
<td>1.23</td>
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<tr>
<td>8</td>
<td>44</td>
<td>158</td>
<td>211</td>
<td>8.2</td>
<td>9.0</td>
<td>2.62</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>135</td>
<td>191</td>
<td>8.2</td>
<td>8.2</td>
<td>2.16</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>144</td>
<td>190</td>
<td>7.1</td>
<td>8.1</td>
<td>2.14</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>45</td>
<td>174</td>
<td>248</td>
<td>8.9</td>
<td>9.5</td>
<td>2.87</td>
<td>0.93</td>
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</table>

The growth of the chicks in the second experiment was markedly slower than in the first experiment. It may be speculated that this was due to a diminished carry over of some unidentified factor from the maternal diet in the second experiment. Again, the utilization of pteroyltriglutamic acid appeared to be complete as com-
pared with the corresponding level of pteroylglutamic acid, and the addition of 4-pyridoxic acid lactone appeared to have no effect. Three cases of perosis at 28 days were observed in Group 7, three in Group 8, one in Group 9, two in Group 10, and none in Group 11.

**DISCUSSION**

In a single experiment involving a comparison between two groups of six chicks each, it was noted that 4-pyridoxic acid had a supplementary effect on growth and hemoglobin formation when added to a purified diet containing pteroyltriglutamic acid. No supplementary effect on yeast concentrate was obtained by adding 4-pyridoxic acid (7).

In another investigation (8) it was noted that 4-pyridoxic acid lactone had no supplemental effect on the action of a suboptimal amount of “vitamin B₆” (pteroylglutamic acid) in promoting growth, feathering, and hemopoiesis in the chick. This result is in agreement with the report published elsewhere (4) and has no bearing on the claim that the lactone is concerned with the breakdown of pteroylglutamic acid conjugates.

The present series of experiments fails to confirm the results published elsewhere (3), although an attempt was made to duplicate the diet. It may be noted, however, that New Hampshire chicks were used in the present investigation, while white Leghorn chicks were used by the Cornell group. Another possibility is that pyridoxic acid lactone was present in some constituent of the diet or was carried over from the eggs into the newly hatched chicks.

**SUMMARY**

1. Chicks were found to utilize pteroyltriglutamic acid and pteroylglutamic acid equally well on a molar basis for growth and the prevention of anemia.

2. The addition of 4-pyridoxic acid lactone had no measurable effect on the utilization of pteroyltriglutamic acid under the conditions of the experiments.

Our thanks are due to Mr. Sidney Upham for synthesizing 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine. Hematological observations were made by Miss Margaret Belt. Dr. M. L. Scott kindly supplied the purified casein.

**BIBLIOGRAPHY**


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