Effect of Dietary Egg Albumin on the Phytase Activity of Rats Fed on Rice Diet

By

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The amount of phosphorus in rice, though large in comparison with that of calcium, is not enough for the normal growth of rats and calcification of their bone. Moreover, the greater part of phosphorus in rice is phytin form of low digestibility. The authors have investigated the effect of addition of egg albumin and vitamin D to the rice diet on the availability of phosphorus in rice by rats.

The experiments provide evidence that the addition of egg albumin to the rice diet greatly enhanced the phosphorus utilization as resulted from that of vitamin D. The phytase of small intestine has been activated by the supplement of egg albumin as well as vitamin D to the diet. The effect of egg albumin and that of vitamin D are cumulative. In contrast to the effect of egg albumin the addition of phosphate decreased the phytase activity.

Phytic acid (inositol hexaphosphoric acid), which occurs in cereal grains, forms insoluble salts (phytin) with Ca, Mg and Fe, with consequent impairment of absorption of these elements including phosphorus. In areas of the world where unrefined cereals form large part of the diet and few animal protein is consumed, it may result in serious calcium and phosphorus deficiency inclusive of so-called “cereal rickets”. Calcium and phosphorus of these substances is unavailable nutritionally for the most part. Vitamin D apparently counteracts the effect of phytic acid. Krieger et al.1,2 demonstrated that in the absence of vitamin D, phytic acid phosphorus was not available to the rat, but when vitamin D was fed, more of the phosphorus was utilized. Steenbock et al.3 found that vitamin D increased the amount of intestinal phytase.

In the present work, the effect of addition of egg albumin to rice diet on the intestinal phytase in rats was studied.

EXPERIMENTAL

Measurement of intestinal phytase: At the end of the feeding period (4 weeks) the rats were anesthetized with ether, and the whole of the small intestine was
removed and the proximal 30 cm cut off from the remainder. After washing in ice cold water it was then homogenized by the technique of Potter and Elvehjem. A portion of homogenate was taken for the determination of phytase by the method of Roberts and Yudkin. The activities of phytase were expressed as mg of inorganic P liberated by 1 g wet tissue in 16 hours at 37°C.

Measurement of intestinal phosphatase: Another portion of the homogenate was used for determination of alkaline phosphatase activity by the method of Motzok. The activities of tissue phosphatase were expressed as mg of inorganic P liberated in 15 min at 30°C from Na-glycerophosphate by the enzyme in extracts corresponding to 1 g (wet weight) of intestine.

Determination of phosphorus and calcium: Each carcass except the intestine taken for the measurement of phytase was solubilized in a beaker containing concentrated hydrochloric acid warming the beaker on boiling bath for 5 hours. The exact amount of HCl depended upon the weight of the animal before it was killed, 1 ml of acid being added for each gram of body weight. After cooling the hydrolysate was placed in a 2 l separating funnel and extracted with 3 times with 50 ml of light petroleum. The combined extracts were then washed with distilled water. After the washings were added to the acid hydrolysate placed in 500 ml volumetric flask it was made to volume with distilled water. Aliquot was taken and digested with perchloric acid solution, and phosphoric acid was determined by the method of Allen. Since the content of phosphorus in the petroleum extract was negligibly small, the analysis of the extract was omitted.

In the first series of experiments, thirty-three male albino rats, Wistar strain, weighing about 64 g were housed individually in screen bottomed cages. They were divided in 6 groups (4 to 6 animals each) and fed as follows:

- Group 1, basal diet;
- Group 2, basal-plus-egg albumin;
- Group 3, basal-plus-egg albumin and phosphate;
- Groups 4, 5 and 6 correspond to Groups 1, 2 and 3 respectively except they receive vitamin D.

The basal diet consisted of finely ground rice (polished and washed) 94.3; soybean oil, 2; salts, 3.7 (g); thiamine. HCl, 0.3; riboflavin, 0.3; vitamin B6, 0.3; Ca-pantothenate, 1.5 (mg); vitamin A, 100 i.u. Vitamin A was administered as palmitate in drops of soybean oil per week. Vitamin D (100 i.u.), when required, administered orally as crystalline calciferol in less than 0.2 ml of soybean oil per 100 g of diet. Salts were provided as Hegsted salts from which the phosphorus-containing salts had been eliminated by substitution with KCl and CaCO3. The addition of egg albumin (10%) was made at the expense of rice. Egg albumin (Wako Pure Chemical Industries, Ltd, Osaka) contained 116 mg of phosphorus per 100 g. Phosphorus content of rice powder was 112 mg per 100 g and of which 43 mg was phytin-form. Since the phosphorus content of egg albumin and rice powder was nearly equal, no adjustment for making the same phosphorus content by adding phosphate was made.
The diet was made into a paste by heating with 140 ml of water per 100 g. The diet and drinking water were given ad libitum. The amount of diet given was restricted to the amount of diet that consumed by rats in the basal group.

In the second series of experiments, exactly the same experiments as in the first series of experiments, were repeated with rats weighing about 57 g, but alkaline phosphatase in intestine also was determined.

RESULTS

The results obtained from the first series of experiments are given in Table I. The addition of egg albumin in the rice diet increased the phytase activity, whereas the supplementation of phosphate decreased it. With the administration of vitamin D the phytase activity increased in intestine as Steenbock et al. reported (Group 4). The level of increase in phytase with inclusion of egg albumin in the diet was comparable to that gained with vitamin D. When albumin was given together with vitamin D the phytase activity was increased still further. In any event, egg albumin exerted the same relative effect with both rations, i.e., with or without

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement</th>
<th>Weight gain g</th>
<th>Phosphorus in body mg</th>
<th>Calcium in body mg</th>
<th>Phytase mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>12.7 (5)</td>
<td>401±38**</td>
<td>593±23</td>
<td>38.9±19.9</td>
</tr>
<tr>
<td>2</td>
<td>Egg white</td>
<td>44.6 (6)</td>
<td>433±30</td>
<td>629±37</td>
<td>51.9±0.6</td>
</tr>
<tr>
<td>3</td>
<td>Egg white, phosphorus</td>
<td>45.5 (6)</td>
<td>783±57</td>
<td>1188±60</td>
<td>35.5±5.2</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin D</td>
<td>17.0 (4)</td>
<td>408±54</td>
<td>623±41</td>
<td>50.0±2.1</td>
</tr>
<tr>
<td>5</td>
<td>Vitamin D, egg white</td>
<td>45.4 (6)</td>
<td>474±31</td>
<td>673±60</td>
<td>59.9±5.1</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin D, egg white, phosphorus</td>
<td>49.6 (6)</td>
<td>797±75</td>
<td>1214±173</td>
<td>40.6±12.1</td>
</tr>
</tbody>
</table>

* Figures in the parentheses indicate number of rats.
** Mean value ± standard deviation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement</th>
<th>Weight gain g</th>
<th>Phytase mg</th>
<th>Phosphatase mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>15.6 (6)</td>
<td>22.4±13.8**</td>
<td>19.8±10.5</td>
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<tr>
<td>2</td>
<td>Egg white</td>
<td>46.9 (6)</td>
<td>24.9±8.6</td>
<td>20.3±10.5</td>
</tr>
<tr>
<td>3</td>
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<td>48.4 (6)</td>
<td>34.4±10.7</td>
<td>22.9±8.4</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin D</td>
<td>19.4 (6)</td>
<td>29.1±7.7</td>
<td>18.9±8.7</td>
</tr>
<tr>
<td>5</td>
<td>Vitamin D, egg white, phosphorus</td>
<td>47.8 (6)</td>
<td>39.4±12.8</td>
<td>35.6±11.5</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin D, egg white, phosphorus</td>
<td>52.9 (6)</td>
<td>35.2±4.6</td>
<td>25.3±7.4</td>
</tr>
</tbody>
</table>

* Figures in the parentheses indicate number of rats.
** Mean value ± standard deviation.
vitamin D. The supplementation of phosphate, on the contrary, decreased the intestinal phytase activity.

In the second series of experiments alkaline phosphatase also was determined together with phytase. The results are shown in Table II. By an accident the temperature of the deep freezer in which the intestine was preserved had not been kept low enough for some time. The lower activities of phytase in these experiments than those of phytase in the first series of experiments might be due to the imperfect preservation. In the presence of vitamin D, however, the addition of egg albumin in the rice diet increased significantly the intestinal phytase.

The results of determination of alkaline phosphatase shows that its activity paralleled closely with that of phytase.

**DISCUSSION**

Experiments with the rice diets suggest that availability of phosphorus of rice increases significantly by the addition of egg white to the rice diet of rats, and that the increase appears to correlate to the intestinal phytase activity.

The increase of phytase activity by supplementation of diet with vitamin D was reported by Steenbock et al. The evidence is now presented that the phytase activity increases as well by increase of protein in the diet as by that of vitamin D and that the effect seems to be cumulative, i.e., it increases independently in the presence of vitamin D.

McCance et al. found that the low protein diets depressed Ca absorption in healthy adults. Platt and Stewart reported that the osteoporosis in pigs, brought about by the protein deficient diets, could not be corrected by the addition of calcium, whereas the replacement of carbohydrate by protein, without extra Ca, resulted a considerable improvement, suggesting that in the prevention or treatment of osteoporosis an increase in the protein value of the diet may be as important as, or more important than, supplementation with Ca.

In the northern part of Japan (Tohoku), winter is long and severe, and an infant born in October is kept indoors during the winter months. In the northwestern part of Japan (Hokuriku), cloudy weathers last long and are followed by heavy snow fall in winter. People who have small income in these areas live usually on a monotonic diet consisting rice, miso (fermented soybean) and salted vegetable with very little animal protein. The deficiency of animal protein besides sunshine perhaps accounts for their especial tendency to produce rickets in children. Animal protein may furnish them an available phosphorus and activate their intestinal phytase.

Recently Koyanagi et al. reported that the increase of creatine and creatinine concentration in urine and the improvement of adaptation to darkness in children living in Tohoku district were the results of administration of phosphate, suggesting deficiency of phosphorus in their diet.
References

7) Allen, R.J.L. *ibid.*, 1940, 34, 858.