INTESTINAL CHEMISTRY.

VII. THE ABSORPTION OF CALCIUM AND PHOSPHORUS IN THE SMALL AND LARGE INTESTINES.

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The problems involved in calcium and phosphorus absorption possess an unusual interest because of the fact that the di- and tricalcium phosphates are very insoluble in neutral solutions and because a condition of experimental rickets can be produced either by low calcium, high phosphorus or low phosphorus, high calcium diets, this condition involving a loss of calcium or phosphorus or both to the body by way of the intestinal tract. Especially noteworthy is the fact that minute amounts of anti-rachitic substance such as may be found in a few mg. of cod liver oil may swing the balance of these elements from strongly negative to strongly positive. At the present time controversy continues as to whether the defect in rickets lies in the inability of the bone cells to utilize calcium and phosphorus brought to them or in a failure of absorption of these elements from the intestines.¹

The study of the intestinal phase is complicated by the fact that calcium and phosphorus are not only absorbed from but are also excreted into the intestines so that fecal analyses represent only a summation of these processes. The procedure described by the author in which unabsorbable iron oxide is mixed with the diet and determined along with calcium and phosphorus in different parts of the intestinal tract² makes it possible readily to study absorption of these elements in different parts of the tract even in small animals such as the albino rat. The present paper

² Bergeim, O., J. Biol. Chem., 1926, lxx, 47.
presents some results on the influence of antirachitic substance on the absorption of calcium and phosphorus from different parts of the intestinal tract.

Six albino rats each weighing about 120 gm. were placed on a calcium-high phosphorus-low diet (wheat gluten 15, gelatin 15, whole yellow corn 33, whole wheat (soft) 33, NaCl 1, CaCO₃ 3, Fe₂O₃ 0.25, all ground to pass a 20 mesh or finer sieve and thoroughly mixed, the inorganic salts being first ground together in a mortar. Especial care in grinding and mixing was necessary only for the food given during the last few days of the experiment.) The animals were kept on this diet for 25 days, a rachitic condition being induced. They were then divided into two groups of three each. The rats of one group were given 10 drops each per day of cod liver oil for 5 days and the other group 10 drops each of olive oil beside the usual diet. During the last 24 hours of the experiment the animals were fed individually pellets of the moistened diet at 10 p.m., 8 a.m., 12 noon, and 1 p.m. Sufficient food was not given at any time so that the animals would not consume the next meal. Each animal received in all 10 gm. of food. The animals were killed with chloroform at 5 p.m. The gastrointestinal tracts were removed and separated into stomach, middle small intestine (second and third quarters), lower small intestine (fourth quarter), cecum, upper large intestine, and lower large intestine. The contents of each section were transferred to silica crucibles and ignited at a low temperature. The ash was dissolved in HCl and calcium, phosphorus, and iron determined by methods previously described.³

³ Bergeim, O., J. Biol. Chem., 1926, lxx, 35.
Three other non-rachitic animals were fed in the same way a diet consisting of powdered milk 50, corn-starch 50, and ferric oxide 1. This diet is of course high in both calcium and phosphorus.

From the analyses the Ca:Fe and P:Fe ratios were calculated for each section of the intestines and also for the food and the percentage absorption of each element determined. Thus a
ratio of 8:1 in the food and 4:1 in the intestines indicated 50 per cent absorption. The results obtained are presented in Figs. 1 to 3. Results are expressed as percentages of the amounts of calcium and phosphorus in the food. As the Ca:P ratio in the food was 5.7:1 the phosphorus percentages would have to be divided by 5.7 to make them more absolutely comparable with those of calcium. The atomic ratios in the diet were Ca:P:4.4:1.
The atomic Ca:P ratios for the intestinal contents are given in Table I.

On the milk diet an acid condition generally prevailed throughout the intestines due presumably to lactic acid fermentation, and both calcium and phosphorus were absorbed to a considerable extent in the small intestines. Calcium and phosphorus were excreted into the cecum and large intestines, the final balance being positive or negative depending apparently upon the ability of the tissues of the animals to retain these elements.

From the data it appears:
1. Both normal animals (those fed cod liver oil) and rachitic
animals show a considerable degree of calcium absorption in the small intestines. On this diet therefore (a calcium-high phosphorus-low diet) the rachitic condition could not be due to a failure of calcium absorption.

2. Both normal and rachitic animals show a considerable excretion of phosphate into the small intestines. As calcium absorption took place most rapidly where this excretion of phosphate was most marked and hence the Ca:P ratio was lowest it would appear that the excretion of phosphate into the intestine was an important factor in promoting calcium absorption.

3. In the normal animals there was an approximate balance between excretion and absorption of calcium in the lower bowel so that a high positive calcium balance was maintained. Phosphorus was also reabsorbed so as to bring about a positive balance of this element.

In the rachitic animals on the other hand the excretion of calcium into the lower bowel was much more marked than absorption and led to very low retentions or losses of this element. Coincident with this marked excretion of calcium into the lower bowel there was a failure of phosphate to be adequately reabsorbed and hence considerable losses to the body resulted.

The results obtained support the following interpretation.

1. The secretion of phosphate into the gastrointestinal tract favors the absorption of calcium. This was suggested by the author some years ago on indirect evidence.4

2. In rickets calcium may be absorbed from the upper intestine but reexcreted into the lower intestine so that a loss of calcium to the body results. This reexcretion of absorbed calcium must be due to a failure of the bones to take up this element for purposes of calcification. As rachitic bones are able to take up calcium and phosphorus in vitro1 the failure of calcification must lie at least in part outside the bone cells and be due to the low phosphate content of the blood which is so uniformly noted in rickets. This low blood phosphate is the result of the inability of the tissues of the body to maintain the proper concentration through breakdown of organic phosphates which they contain. That the body tissues are low in phosphorus in rickets has been shown.5

Antirachitic vitamin would therefore appear to act by

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facilitating breakdown of organic phosphates of the tissues, thus elevating the blood phosphate and enabling absorbed calcium to be deposited in the bones. This calcium being deposited in the bones is not reexcreted in the lower bowel to hinder phosphate absorption which is therefore favored so that the phosphate balance also becomes positive and healing of rickets begins. In rickets of the phosphorus-low type therefore tissues other than the bones or intestinal mucosa would appear to be predominantly concerned, although we should expect these tissues also to be affected, and in other types of rickets they may be primarily involved.

This view would correlate the hypothesis presented by us\(^4\) as to the importance of phosphoric esterase in calcium absorption, calcification, and decalcification with the evidence presented in this paper, with the direct demonstration of hexose phosphatase in bone cells by Robison \textit{et al.}\(^1\) and with the finding of Shipley, Kramer, and Howland\(^1\) that in experimental rickets the defect is not primarily in the bones. It is of interest also that interpretation of the parathyroid effect as involving an increased breakdown of organic phosphates of the tissues which we presented some years ago\(^4\) is supported by the finding of Greenwald of a phosphate loss in long continued parathyroid treatment. The favorable effect of fasting on calcification in rickets may in part be due to the fact that blood phosphate will be less constantly drained away by intestinal content high in calcium and some more phosphate will be liberated from the breakdown of body tissues. Considerable more work is necessary for a solution of the questions raised.

CONCLUSIONS.

1. The absorption of calcium and phosphorus from different parts of the gastrointestinal tract of albino rats was studied. Animals rendered rachitic by phosphorus-low diets as well as such animals given cod liver oil showed a considerable degree of calcium absorption from the small intestine. The rachitic condition could not therefore be due to a failure of calcium absorption.

2. Both groups of animals showed a considerable secretion of phosphate into the upper tract. This secretion appears to be
an important factor in promoting calcium absorption as the latter was most rapid where the P:Ca ratio was highest.

3. The animals given cod liver oil showed a positive calcium balance throughout the intestines. Phosphorus secreted into the upper tract was absorbed in the lower intestines to produce an ultimate positive balance of this element also.

4. In the rachitic animals the calcium absorbed in the upper intestine was excreted into the lower intestine, leading to a negative or subnormal balance. Coincident with this marked excretion of calcium into the lower bowel there was a failure of phosphate to be adequately reabsorbed and hence a loss of the latter to the body.

5. The failure of absorbed calcium to be used in calcification is believed to be due to the low phosphate concentration of the blood. Antirachitic substance may act by elevating blood phosphate by promoting the breakdown of organic tissue phosphates thus leading to increased deposition of calcium with lessened excretion into the gut and consequent better absorption of phosphate therefrom.
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