THE RELATION OF THE DIETARY CA : P RATIO TO SERUM CA AND TO PARATHYROID VOLUME

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THREE FIGURES

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It has been shown by a number of investigations (Ham et al., '40; Carnes et al., '42) that various conditions associated with hypocalcemia or hyperphosphatemia, or both, lead to hyperplasia of the parathyroid glands. Evidence has also been given that parathyroid enlargement in such instances may be associated with increased secretory activity of the glands (Baumann and Sprinson, '39). The changes in the levels of blood Ca and P in these instances are usually reciprocal and the question has arisen whether the parathyroid enlargement is primarily related to the lowering of the blood Ca or to the increase in blood PO₄.

Ham and coworkers ('40), by adding phosphate to a stock diet, were able to obtain a small increase in the serum PO₄ of rats without changing the serum Ca. The parathyroids of these animals failed to enlarge. The glands did enlarge in rats on a Steenbock low Ca diet with low serum Ca values. It was concluded that hypocalcemia and not hyperphosphatemia is the stimulus to parathyroid enlargement.

In contrast to this, on adding graduated amounts of P to the Steenbock low P diet, Carnes and coworkers ('42) obtained stepwise increases in parathyroid volume in younger rats associated with rising serum PO₄ levels even in the absence of hypocalcemia. However at both extremes of the scale of dietary Ca/P ratio, the serum calcium changes were significant and the serum PO₄ values not always consistently altered. Further experience has cast doubt on the reliability of data obtained with the Steenbock diet which is inadequate in several respects and produces a very poor general condition of the experimental animals. Differences in the basal diet, form and quantity of added PO₄, and age of the experimental animals may have contributed to the discrepancies between the results of these two groups.
Recently Patt and Luckhardt ('42) concluded from a series of experiments on dogs that a low blood calcium is a direct stimulus for the parathyroid glands to produce more hormone. They lowered the blood calcium in their animals by injecting oxalate and also perfused decalcified blood through thyroid-parathyroid preparations, obtaining what they believed to be evidence for increased parathyroid activity. Possible alterations in blood PO₄, brought about by the procedures, were not excluded in these experiments.

The changes in Ca and PO₄ levels of the blood in response to variations in dietary Ca and P have been thoroughly studied by Shohl and Wolbach ('36) in an investigation of rickets in rats. They confirmed the findings of Kramer and Howland ('32) and of Bethke et al. ('32) that the blood levels of Ca and PO₄ reflect the dietary Ca/P ratio. Lowering this ratio decreased the serum Ca and increased the serum PO₄. Raising the ratio had the opposite effect on the serum values. They also found that, with a constant dietary Ca/P ratio, raising the absolute amounts of Ca and P in the diet produced a corresponding rise in both the serum Ca and serum PO₄.

If the blood level of either Ca or PO₄ is the primary stimulus of the parathyroid gland, it should be possible to determine which of these two factors is the essential one by experimentally altering the dietary intake of Ca and P independently over a wide range and by following the consequent changes in the blood levels and parathyroid volume. This can be done with a minimum of interference with the general nutrition in adult rats on a good basal ration.

**EXPERIMENTS**

Male albino rats, in most instances litter-mates, weaned at the age of 4 weeks and placed on our stock diet \(^1\) were selected from the stock colony at 10 weeks of age. They were fed for 4 weeks a diet \(^2\) devised by Zucker and Berg ('43) which is adequate in every essential except Ca and P and is thoroughly freed of vitamin D. This diet favors optimal growth (Zucker and Zucker, '42) if supplemented with proper amounts and Ca and P. The desired amounts of Ca and P were added in the form of CaCO₃ and KH₂PO₄. After 4 weeks the animals were anesthetized with ether and blood was drawn from the right auricle. Individual analyses were made for Ca (Clark and Collip, '25) and inorganic

\(^1\)Rockland rat diet (D-free).
\(^2\)Heated egg albumen (E) or alcohol extracted beef fibrin (F), 20%; modified Wesson salt mixture, 1.2%; Wesson oil, containing 5% carotene in oil, 2.0%; rice bran extract, 10%; cane sugar, to make 100%.
CALCIUM, PHOSPHORUS AND PARATHYROID

P (Kuttner and Cohen, '27) on fresh samples of serum. The parathyroid glands were fixed in Bouin's fluid, imbedded in paraffin, serially sectioned at 10 micra, and the volumes determined by tracings and planimeter measurements. Bones were sectioned and studied histologically and the bone ash was determined on the right femur in some of the groups. Serum protein estimations were made in some groups by the falling drop method.

### TABLE 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DIET</th>
<th>% DIETARY CA</th>
<th>% DIETARY P</th>
<th>DIETARY CA/P RATIO</th>
<th>BODY WEIGHT (GM.)</th>
<th>PARATHYROID VOLUME (MM.³)</th>
<th>SERUM PO₄ (MO. %)</th>
<th>SERUM BONE ASH RATIO</th>
<th>NO. RATS</th>
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1 Rickets present.

RESULTS AND DISCUSSION

The complete data are given in table 1. By adding either Ca or P, or both simultaneously to the basic diet, nine variations of the dietary Ca/P ratio were obtained ranging from 15 to 0.032. The absolute level of dietary Ca varied from approximately 2% to 0.015% and the P from approximately 1.2% to 0.07%. Because of the age of the animals and the relatively short duration of the experiment, growth was only moderately retarded on low Ca and low P variations of the diet. Both types of rickets were mild as judged histologically and by the bone ash values.

On the basis of parathyroid size the animals fall into three distinct divisions. The glands were very small when the dietary Ca/P ratio was far above optimal (groups 1 and 2). The glands were very large when this ratio was far below optimal (groups 9-11). When the dietary

1 We are indebted to Miss Margaret Young for the bone ash determination.
Ca/P ratio approached the optimal range (9), even though the absolute quantities of Ca and P were varied over an extremely wide range, the glands were intermediate in size and the groups did not differ significantly between each other (groups 3–8). Figure 1 illustrates the interdependence of parathyroid volume and the per cent of dietary Ca and P. Figure 2a shows, incidentally, that the relationship between parathyroid volume and the logarithm of the dietary Ca/P ratio closely approximates a straight line.

The effect of the diets on serum Ca level was inverse to their effect on the parathyroid volume. The table shows that on the basis of serum values the animals fall into the same three divisions that were made on the basis of parathyroid size. On a diet of high Ca/P ratio there was a mild hypercalcemia (groups 1 and 2). On a diet of very low Ca/P ratio there was a moderate hypocalcemia (groups 9–11). On a diet with a Ca/P ratio approaching the optimal, the serum Ca fell within the accepted range of normal, even though the absolute quantity of dietary Ca was varied within very wide limits (groups 3–8). Figure 2b shows that the relationship between the serum Ca level and the logarithm of the dietary Ca/P ratio also approximates a straight line. Under the conditions of our experiment the serum Ca level was not proportional to the per cent of dietary Ca at a constant Ca/P ratio. The serum Ca was not significantly different on diets of 2, 1, and 0.6% Ca, respectively (groups 3–7) and it was not greatly depressed with as little as 0.053% dietary Ca (group 8) when the dietary Ca/P ratio remained in the neighborhood of 1. These findings are confirmed by unpublished data on a sufficient number of animals which at weaning (age 4 weeks) were placed on the experimental diets for 4 weeks and then killed.

The serum phosphate level has been found, in agreement with Shohl and Wolbach ('36), to depend on both the dietary Ca/P ratio and the absolute quantity of dietary P up to a certain maximal level of serum PO₄. This maximum is apparently dependent upon factors other than dietary Ca and P and is also a function of the age of the animal. It was approximately 7–8 mg. % in this experiment but in immature rats it is about 10–12 mg. %. The level of serum PO₄ thus does not bear a simple inverse relationship to the serum Ca level.

Figure 3 shows that there is a fairly close inverse proportionality between the parathyroid size and the level of serum Ca over the entire range investigated. This relationship deviates somewhat from a straight line, perhaps due to an inherent limitation of maximal para-
Fig. 1 Relationship between the per cent dietary calcium, the per cent dietary phosphorus, and the parathyroid volume.

Fig. 2a Relationship between the parathyroid volume and the logarithm of the Ca/P ratio of the diet.

Fig. 2b Relationship between the serum calcium concentration and the logarithm of the Ca/P ratio of the diet.
thyroid growth in the hypocalcemic groups within the period of 4 weeks. The same deviation in the line could be due to a limitation, by factors other than the diet, in the maximal serum Ca attainable. The poorest alignment of the points occurs in the midportion of the curve within the normal range of serum Ca. This scattering of the points is not eliminated by expressing the parathyroid volume per unit of body weight.

![Graph showing relationship between serum calcium concentration and parathyroid volume.](image)

**Fig. 3** Relationship between the serum calcium concentration and the volume of the parathyroid glands.

Table 1 shows that the relationship between parathyroid size and serum PO₄ level is not a close one over the entire range, due to the low maximal level of serum PO₄ attainable. The apparent dependence of parathyroid volume on serum PO₄ concentration in younger animals was possibly due to the much higher maximum attainable at that age (Carnes et al., '42). Under the present conditions no such dependence is apparent.

It has been suggested, on the basis of considerable circumstantial evidence that the stimulus for the parathyroid glands to produce more hormone is a serum Ca ion level below normal (Albright, '42). The chief factor influencing the ionization of serum Ca is the serum protein concentration (McLean and Hastings, '35). The serum protein was not determined in all the groups of this experiment but in groups 6, 7, and 10 (diets 918, 907, and 909) the average value was 6.6, 6.6, and 6.8%, respectively, and in unpublished experiments on rats of different ages on diets 908 and 913, no significant deviations in serum protein
concentration have been encountered in well-nourished animals. It is unlikely that the serum \( \text{PO}_4 \) concentration influences the ionization of serum \( \text{Ca} \) under the present experimental conditions. McLean and Hastings ('35) found no evidence of a non-ionized \( \text{Ca-PO}_4 \) complex in the plasma under a variety of experimental and spontaneous pathological conditions. Such a complex is producible in the blood under certain conditions, however (McLean and Hinrichs, '38) and the development of manifest tetany in the parathyroidectomized dog has been said to be closely correlated with a critically low ratio of serum \( \text{Ca} \)/serum \( \text{PO}_4 \) (Reed et al., '28). It has also been suggested that the parathyroid hyperplasia in renal disease is a result of a disturbance in the \( \text{Ca}/\text{P} \) ratio of the plasma (Jaffee and Bodansky, '43). The table contains the serum \( \text{Ca}/\text{PO}_4 \) ratios of most of the groups in the present experiment. When the various suboptimal diets (groups 1, 2, 8, 9) are considered, a rather close inverse proportionality is observed between this ratio and the parathyroid volume. But this is no closer relationship than that between the serum \( \text{Ca} \) and parathyroid size in the same groups and the alignment of these groups with those on the more nearly optimal diets (groups 3, 4, 5) is poor. Moreover, the difference in serum \( \text{Ca}/\text{PO}_4 \) ratio between some of the animals with greatly enlarged parathyroids and those with normal-sized glands is not great enough to be significant.

Whereas the present experiment does not yield a decisive answer to the fundamental question of what constitutes the physiological stimulus to parathyroid activity, the data do indicate that over a wide range, both above and below the normal, there is a close correlation between the total serum \( \text{Ca} \) concentration and parathyroid volume under closely controlled conditions. The possibility remains that the \( \text{Ca} \) ion concentration is the important moiety. These results are essentially in accord with those of Ham and associates ('40) who concluded that hypocalemia is the stimulus to physiological hypertrophy of the parathyroid glands. It may be anticipated that other factors, especially the specific growth essentials, will influence the translation of this stimulus into anatomical enlargement. The failure to obtain high enough values of serum \( \text{PO}_4 \) in this experiment prevents any further conclusions on the possible influence of serum \( \text{PO}_4 \) level on the size of the glands under other circumstances.

CONCLUSIONS

A close direct proportionality has been found between the logarithm of the dietary \( \text{Ca}/\text{P} \) ratio and the serum \( \text{Ca} \) concentration in mature rats.
on an adequate basal ration over a very wide range of dietary Ca and P. A similarly close inverse proportionality has been found between the logarithm of the dietary Ca/P ratio and the volume of the parathyroid glands. The parathyroid volume and serum Ca concentration accordingly varied inversely and with a nearly linear relationship between serum Ca levels of 7.3 and 11.9 mg. %. The serum inorganic PO₄ level was less uniformly related to the parathyroid size under the present experimental conditions.

LITERATURE CITED


