PHYSIOLOGICAL HYPERTROPHY OF THE PARATHYROIDS, ITS CAUSE AND ITS RELATION TO RICKETS *

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Physiological hypertrophy of the parathyroid glands has been described in rickets by Erdheim,[1] Ritter,[2] and Pappenheimer and Minor,[3] and in renal disease by Bergstrand,[4] Hubbard and Wentworth,[5] Pappenheimer and Wilens,[6] and Shelling and Remsen.[7] Parathyroid hypertrophy has also been produced experimentally with low calcium diets by Marine,[8] and Luce,[9] and with phosphate injections by Drake, Albright and Castleman.[10] These last mentioned workers have established by experimental procedures that the opinion expressed by several former investigators was correct, namely, that hyperphosphatemia causes parathyroid hypertrophy. They state, however, that "it will require further studies to show whether hyperphosphatemia causes the hyperplasia directly or indirectly by producing a hypocalcemia." Pierre, de Boissezon and Lombard [11] have recently confined the findings of Drake, Albright and Castleman, and have found further that injections of calcium gluconate cause hypoplasia of the parathyroids of animals.

To settle whether hypocalcemia or hyperphosphatemia is the factor that directly controls parathyroid hypertrophy is by no means a simple matter. The difficulty lies in the reciprocal relationship which exists between the calcium and the phosphorus of the blood. This usually acts to effect a decrease in the concentration of one element if the concentration of the other is increased, and *vice versa*. By certain dietary procedures we have, however, attained a measure of success in obtaining each of these conditions without the other. Furthermore, we have performed experiments to clarify the relationship commonly assumed to exist between rickets and parathyroid hypertrophy. It is easy to visualize parathyroid hypertrophy in low calcium-high phosphorus rickets. But

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rickets can be produced with equal ease by a high calcium-low phosphorus diet, and in this type of rickets there is neither hyperphosphatemia nor hypocalcemia. We thought it would be interesting to study the parathyroids in both types of rickets to find whether they always enlarge in rickets, or only when rickets is produced by conditions leading to a hypocalcemia.

Material and Methods

Rats were used as experimental animals. They are admirably adapted to this type of work as each rat has only two parathyroid glands and these are embedded, one on each side, in the corresponding lobe of the thyroid gland. In only 1 of over 30 animals whose thyroid glands were serially sectioned was a parathyroid gland missing from its site in thyroid tissue. This animal was discarded. In none of the animals was there more than one parathyroid gland in each lobe of the thyroid gland.

The diets used are described with each separate experiment.

The method we employed to obtain figures relating to parathyroid volume requires explanation.

As the parathyroid glands of the rat are extremely small and embedded in thyroid tissue, they cannot be directly measured or weighed with any degree of accuracy. Hence, we employed an indirect method. Each thyroid gland was dissected out, fixed in Bouin's solution, embedded in paraffin and sectioned serially. The sections were stained with hematoxylin and eosin.

We were not concerned in this study with the attempt to determine the volumes of the various parathyroid glands in any standard units such as cubic millimeters. We merely desired figures that would compare with one another in the same ratios as would actual volumes. Our figures relating to parathyroid volume are therefore expressed in squares of graph paper, the number of squares being those located under a curve which was plotted as follows:

Sections of thyroids were examined consecutively until parathyroid tissue appeared. From this point on the sections were numbered consecutively. Each tenth section was examined and the greatest length and width of the oval parathyroid tissue present was measured with an eyepiece micrometer. Length multiplied by breadth gave a figure that was not the area of the parathyroid

tissue in the section but one proportional to the area. A curve was then plotted between the length-times-breadth figures and the serial numbers of the sections. The number of squares of graph paper under the curve so obtained is proportional to the volume of the gland, and hence satisfactory for our purpose. It should be pointed out that within any single experiment the graphs were all made on the same scale, but that the same scale was not used for all experiments. Hence figures of parathyroid size given for one experiment are not comparable with figures given in another, *i.e.* figures relating to parathyroid volume are only comparable within a single experiment.

EXPERIMENT 1

This experiment was performed to see if the parathyroids hypertrophy in low phosphorus rickets.

Ten young rats of approximately the same size and weight were selected and divided into two equal groups. The first group was fed a Steenbock diet with 3 per cent calcium carbonate plus 5 per cent yeast.* The second group was fed a normal diet. After 3 weeks the animals were anesthetized and the pooled blood from each group was taken for calcium and phosphorus estimations. The rats were then killed and their skeletons X-rayed. Knee joints were dissected out and fixed and decalcified in Bouin's solution, after which paraffin sections were prepared. The size of the parathyroid glands in these rats was determined by the method previously outlined.

The results of this experiment are recorded in Table I.

* The Steenbock diet used in these experiments consisted of

Ground yellow corn	76%
Gluten flour	20%
Calcium carbonate	3%
Sodium chloride	1%

Dried brewer's yeast (5 per cent) was added to this diet to aid growth. To obtain definite rickets the animal must grow during its regimen on the deficient diet.

It should be noted that a diet deficient only in vitamin D does not produce rickets in the rat. To produce rickets the diet must be unbalanced in other respects. The above diet, for instance, contains sufficient calcium carbonate to interfere with phosphorus absorption. Without the calcium carbonate, or with greatly reduced calcium carbonate, the diet is deficient in calcium. Thus, by varying the amounts of calcium carbonate either low phosphorus or low calcium rickets can be produced.

The normal serum phosphorus of the rat is higher than it is in man. The normal figure is higher in young rats than in older ones.

TABLE I

Effect on the Parathyroid Glands, Blood and Bones of Feeding Young Rats for 3 Weeks (1) a Diet Designed to Produce Low Phosphorus Rickets, and (2) a Normal Diet

	Volume * of two parathyroid glands			Average weight of rats in group	Serum calcium	Serum phosphorus	Histological examination of bone
	Smallest volume in any rat	Largest volume in any rat	Average volume in group				
				gm.	*mg./100 cc.*	*mg./100 cc.*	
Group 1. Rats fed a diet to produce low phosphorus rickets	83	182	121	101	9.4	2.3	Severe rickets
Group 2. Rats fed a normal diet	146	209	168	135	10.2	8.5	Normal

* Volume expressed in squares of graph paper (see Material and Methods).

Conclusions from Experiment 1

The parathyroid glands of the rachitic animals were smaller than those of the controls, but the relationship between size of the parathyroid and body weight was approximately the same in both groups. There was no evidence in this experiment to show that low phosphorus rickets is associated with parathyroid hypertrophy.

Experiment 2

This experiment was performed to find out if parathyroid hypertrophy developed in rats with low calcium rickets. It was controlled by a group of animals with low phosphorus rickets.

Ten young rats of approximately the same age and size were selected from the colony and were divided into two groups of 5 each. Both groups were fed a Steenbock rickets-producing diet plus 5 per cent yeast. Three per cent calcium carbonate was included in the diet of the first group, but only 0.5 per cent calcium carbonate was included in that of the second group (see footnote). At the end of 3 weeks the rats in this experiment were treated similar to those of Experiment 1.

The results of this experiment are recorded in Table II.

Conclusions from Experiment 2

Rats with low calcium rickets demonstrated marked hypertrophy of the parathyroid glands, in comparison with rats with low phosphorus rickets. As we have shown in Experiment 1 that low phosphorus rickets does not affect the size of the parathyroid glands, and as the blood phosphorus level in the animals with hypocalcemia was within normal limits, we can conclude that hypocalcemia is the cause of the parathyroid enlargement.

Experiment 3

This experiment was designed to confirm that hypocalcemia is the cause of parathyroid enlargement, and that it is effective in animals somewhat older than those used in rickets experiments.

Twelve rats, 4 weeks old, were divided into two groups of 6 each. One group was fed an adequate diet, the other group a mineral deficient diet (Robertson [12]). The animals were fed these diets for about 6 weeks, when they were killed. The blood from

TABLE II

Effect on the Parathyroid Glands, Blood and Bones of Feeding Young Rats for 3 Weeks Diets Designed to Produce (1) Low Phosphorus Rickets, and (2) Low Calcium Rickets

	Volume * of two parathyroid glands			Average weight of rats in group	Serum calcium	Serum phosphorus	Histological examination of bone	X-ray observations on bone
	Smallest volume in any rat	Largest volume in any rat	Average volume in group					
				gm.	*mg./100 cc.*	*mg./100 cc.*		
Group 1. Rats fed a diet to produce low phosphorus rickets	78	98	89	85	10.1	2.0	Severe rickets	Severe rickets
Group 2. Rats fed a diet to produce low calcium rickets	191	297	258	70	3.9	8.0	Severe rickets	Severe rickets

* Volume expressed in squares of graph paper (see Material and Methods).

TABLE III

Effect on the Parathyroid Glands and Blood of Feeding Rats 4 Weeks Old Mineral Deficient and Normal Diets for 6 Weeks

	Volume * of two parathyroid glands			Average serum calcium of large group of similar rats	Average serum phosphorus of large group of similar rats
	Smallest volume in any rat	Largest volume in any rat	Average volume in group		
				mg./100 cc.	*mg./100 cc.*
Group 1. Rats fed a mineral deficient diet	253	468	318	7.3	8.5
Group 2. Rats fed an adequate diet	205	306	243	10.2	7.7

* Volume expressed in squares of graph paper (see Material and Methods).

these animals unfortunately met with an accident so that accurate calcium and phosphorus readings could not be made. But as this adequate diet and this particular mineral deficient diet was being used in a great number of similar animals in the course of an extensive experiment, we were able to obtain calcium and phosphorus estimations from a large number of animals from both groups.

The results obtained in this experiment are recorded in Table III.

Conclusions from Experiment 3

We did not obtain the weights of the rats whose parathyroids were sectioned in this experiment. But in a large group of rats being fed similar diets it was apparent that the animals that received an adequate diet were more than half as large again as those that received the mineral deficient diet for the length of time noted in our experiment. Thus, the enlargement of the parathyroid glands in relation to body weight was even greater in the mineral deficient animals than appears by directly comparing the volume of their parathyroid glands with those of the control animals. This experiment, we think, shows that parathyroid enlargement is caused by hypocalcemia because the blood phosphorus in the mineral deficient animals was within normal limits. But even so, it was slightly higher than the blood phosphorus level of the control animals; so, to exclude the possibility that this slight elevation of phosphorus caused the parathyroid enlargement, we planned another experiment.

Experiment 4

This experiment was designed to determine whether a hyperphosphatemia in the absence of a hypocalcemia would cause parathyroid hypertrophy.

Ten young rats of approximately the same weight and size were selected and divided into two groups of 5 each. The first group was fed a normal diet, and the second group was fed a normal diet plus 3.5 parts of tricalcium phosphate and 2.5 per cent phosphoric acid. At the end of 3 weeks the rats of both groups were killed, the pooled blood from each group was tested for calcium and phosphorus, and the size of the parathyroid glands of 3 animals of the

TABLE IV

Effect on the Parathyroid Glands and Blood of Feeding Young Rats for 3 Weeks (1) a Diet Designed to Raise the Blood Phosphorus without Lowering the Calcium, and (2) a Normal Diet

	Volume * of two parathyroid glands			Average weight of rats in group	Serum calcium	Serum phosphorus
	Smallest volume in any rat	Largest volume in any rat	Average volume in group			
				gm.	*mg./100 cc.*	*mg./100 cc.*
Group 1. Rats fed a normal diet	214	307	269	150	11.6	9.7
Group 2. Rats fed extra calcium phosphate and phosphoric acid	209	294	265	147	11.3	11.7

* Volume expressed in squares of graph paper (see Material and Methods).

first group and of 4 of the second group was estimated by the method previously described.

The results of this experiment are recorded in Table IV.

Conclusions from Experiment 4

Hyperphosphatemia in the absence of hypocalcemia does not cause physiological hypertrophy of the parathyroid gland.

Comment

Relationship of Parathyroid Enlargement to Rickets: Experiment 1 showed that parathyroid hypertrophy does not occur in low phosphorus rickets. Experiment 2 showed that extreme parathyroid hypertrophy develops in low calcium rickets. Hence, we conclude that parathyroid hypertrophy is not necessarily related to rickets but occurs in that condition only when there is a deficiency of calcium in the blood.

Hypocalcemia as the Cause of Parathyroid Hypertrophy: It was pointed out in our introduction that there is no clear evidence as to whether parathyroid hypertrophy is instituted by a low blood calcium or by a high blood phosphorus. In the absence of proof to the contrary, it would be hazardous to assume both conditions were not present in all the studies that have been reported. In two of our experiments (2 and 3) we showed that a low blood calcium caused parathyroid hypertrophy in the absence of hyperphosphatemia, and in our last experiment (4) we showed that hyperphosphatemia in the absence of a hypocalcemia did not cause parathyroid hypertrophy. Therefore, we conclude that hypocalcemia and not hyperphosphatemia is the primary cause of physiological hypertrophy of the parathyroid gland.

Summary and Conclusions

1. Parathyroid hypertrophy is not necessarily associated with rickets; it occurs only in low calcium rickets, and not in low phosphorus rickets.

2. Parathyroid hypertrophy develops when the blood calcium level is low and the phosphorus at normal levels.

3. Parathyroid hypertrophy does not occur when the blood phosphorus is raised and the blood calcium is kept at normal levels.

4. Hypocalcemia, instead of hyperphosphatemia, is the primary cause of physiological hypertrophy of the parathyroid glands.

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