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Alterations in Thyroidal Calcitonin Content of Rats Fed Diets of Varying Ca:P Ratios[1,2] (39582)

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It is well established that secretion of calcitonin (CT) from the mammalian thyroid gland is srongly influenced by the calcium concentration of the serum and is stimulated by hypercalcemia (1). In most mammalian species, thyroidal stores of CT are relatively large with respect to rates of secretion (1, 2). However, large changes in thyroid gland content of CT can be produced in the rat by inducing a sustained hyper- or hypocalcemia. Chronic hypercalcemia produces depletion of thyroidal CT content whereas chronic hypocalcemia following parathyroidectomy is associated with a progressive increase in CT content (3).

Since it is well known that changes in the dietary Ca:P ratio can influence the concentration of blood calcium (4-8), we initiated the present study to examine possible effects of alterations in the dietary Ca:P ratios on the content of CT in the rat thyroid gland. Parathyroidectomized (parex) and adrenalectomized (adrex) rats, as well as intact rats, were studied. The results show that alterations in the dietary Ca:P ratio produce large changes in thyroidal CT and that these changes cannot be explained simply on the basis of chronic changes produced in the serum levels of calcium.

Materials and methods. Male Holtzman rats (Madison, Wisc.) were grouped so that their mean body weight at the beginning of the study was the same. Experimental diets were prepared as previously described (7, 8) and rats were pair-fed for 1 to 2 months. Dietary ratios of Ca:P were varied between 1:8 and 8:1 either by changing the calcium content and keeping the phosphorus content constant at 0.4% (7) or by changing the phosphorus content and keeping the calcium content constant at 0.4% (8). Six rats from each group were used for serum analyses, and for the assay of thryoidal CT, glands from two to three rats in each group were pooled for analysis.

Surgery was performed under ether anesthesia. Some rats were bilaterally adrenalectomized 7 days before the feeding experiments, and these were given 0.15 *M* NaCl in place of distilled drinking water during the experimental period. Parathyroidectomy was performed by surgical excision and only rats having serum calcium levels below 8 mg/100 ml and serum phosphorus levels above 11 mg/100 ml 1 week after surgery were used. In this study involving different surgical procedures and high phosphate diets (Table I), initially there were 10 rats per group. At the end of 1, 2, and 4 weeks, two animals from each group were killed by cardiac exsanguination under ether anaesthesia, and determinations of serum calcium, phosphate, and CT were performed. The thyroid glands of both rats in each group were pooled and either analyzed immediately for CT or frozen rapidly on dry ice and kept at −20° until assayed. At the end of 8 weeks, the remainder of the animals were killed and similar analyses were performed. The number of animals remaining in each group at this time ranged from one to four. Since the results were similar at 1, 2, and 4 weeks, only the data obtained at the end of 4 and 8 weeks are given.

Serum calcium was determined by atomic

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absorption spectrophotometry, and serum phosphorus, by a modification of the Fiske–Subbarow method (9). For CT assays, pooled thyroid glands were analyzed for CT by bioassay and radioimmunoassay. Bioassays with porcine CT as a reference standard were performed as previously described (10). Radioimmunoassays were conducted as described earlier (11) using highly purified rat CT for both radioiodination and as unlabeled reference standard. Statistical evaluation of differences in calcium and phosphate levels in sera was done by Student's t test.

Results. Analyses of CT content of thyroid glands. Figure 1 shows the CT content in thyroid extracts analyzed by both bioassay and radioimmunoassay. Values obtained by the two methods showed excellent correlation ($r = 0.86, n = 18$). The ratios obtained by dividing the bioassay value (milliunits per gland) by that for the radioimmunoassay (micrograms per gland) gave a mean of 271 $\pm$ 16 mU/μg, a value which corresponds closely to the specific biologic activity of pure rat CT (11).

Effects of varying dietary Ca:P on thyroidal CT of intact rats. Figure 2 summarizes the findings obtained in intact rats fed experimental diets varying in Ca:P for 4 weeks. In this experiment, Ca:P was varied by altering dietary Ca. No significant differences in body weight were found. However, as dietary Ca:P ratio fell from 8:1 to 1:8, serum Ca decreased slightly while serum P rose. Thyroidal CT remained quite constant at all ratios between 8:1 and 1:4, but with a Ca:P ratio of 1:8 there was a marked elevation in thyroidal CT. Figure 3 shows results of a similar study in which the Ca:P ratio of the diet was altered by changing dietary P. Rats fed diets with Ca:P ratios of 1:8, 4:1, and 8:1 had very little weight gain. Although the patterns of change in serum Ca, serum P, and thyroidal CT were similar to those observed in the study shown in Fig. 2, changing the dietary Ca:P ratio by altering the phosphorus content of the diet appeared to have a greater effect on these variables. In this study, thyroidal CT rose progressively as the dietary Ca:P ratio fell below 1:1, and at a dietary Ca:P of 1:8, the levels of serum phosphorus were significantly higher ($P < 0.01$) and those of serum cal-

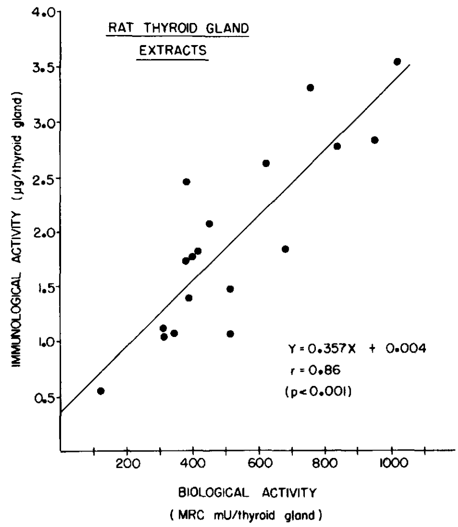

FIG. 1. Positive correlation between values for CT obtained by radioimmune assay and by bioassay. Various dosages of each thyroid gland extract (see Methods) were analyzed by both assay methods.

cium were significantly lower ($P < 0.01$) than when dietary calcium was varied. In both Figs. 2 and 3, the results indicate that thyroidal CT rose as (i) the dietary Ca:P ratio fell, (ii) the serum Ca fell, and (iii) the serum P rose. Detectable serum levels of CT were found only in rats fed the diet with Ca:P = 1:8.

Influences of parathyroidectomy and adrenalectomy on thyroidal CT. Table I shows alterations in serum Ca and thyroidal CT in parex and adrex rats fed diets having a Ca:P ratio of 2:1 (0.8% Ca and 0.4% P) or 1:8 (0.4% Ca and 3.2% P) for 4 or 8 weeks. Within each treatment group (intact, parex, or adrex), mean thyroidal CT was higher and mean serum Ca lower in rats fed a ratio of 1:8 compared to those fed diet with a ratio of 2:1. Overall, however, CT levels showed no significant correlation with serum Ca. For example, similarly high CT levels were observed in rats fed a ratio of 1:8 for 8 weeks regardless of their surgical treatment, despite the fact that their serum Ca levels were quite different. In general, thyroidal CT levels in each of the three groups appeared to be related to the length of time that the diet with the Ca:P ratio of 1:8 was fed, those on diet 8 weeks having a higher level than those fed the diet for only 4 weeks.

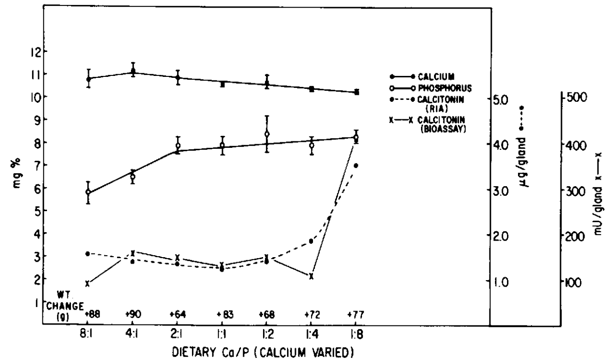

FIG. 2. Thyroidal CT as measured by radioimmunoassay (●- - - -●) and bioassay (×——×), and the serum levels of calcium (●——●) and phosphorus (○——○) are plotted against the dietary Ca:P ratios shown on the abscissa. The vertical lines represent the standard deviation of the mean. Mean changes in body weight (grams are shown at the bottom of the graph.

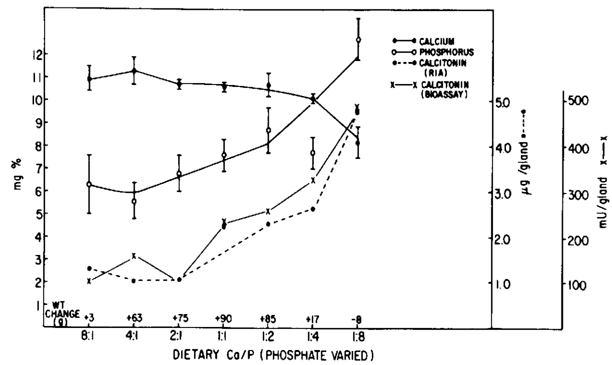

FIG. 3. Same legend as for Fig. 2.

TABLE I. CALCITONIN CONTENT OF THYROID GLANDS AND LEVELS OF SERUM CALCIUM IN RATS FED DIETS WITH VARYING Ca:P RATIOS.[a]

Treatment	Dietary Ca:P (ratio)	Weeks on test	Body wt (g)	Serum Ca (mg/100 ml)	Serum P (mg/100 ml)	Radioimmune assay (μg/gland)	Bioassay (mU/gland)
Group 1							
Parex [3]	1:8	8	299	5.0	13.7	3.56	1010
Parex [2]	1:8	4	353	7.2	14.3	2.61	662
Parex [2]	2:1	8	417	7.9	8.6	1.07	315
Parex [2]	2:1	4	348	8.2	11.1	1.10	506
Group 2							
Intact [4]	1:8	8	336	8.8	13.4	2.79	832
Intact [2]	1:8	4	322	8.4	12.1	1.49	509
Intact [2]	2:1	8	418	9.6	5.8	1.77	398
Intact [3]	2:1	4	416	10.2	8.1	1.10	303
Group 3							
Adrex [3]	1.8	8	377	9.6	6.5	2.82	947
Adrex [2]	1:8	4	325	9.5	10.3	2.09	457
Adrex [4]	2:1	8	475	10.0	7.2	1.40	393
Adrex [2]	2:1	4	415	10.0	8.7	1.10	331

[a] The diet with Ca:P = 1:8 contained 0.4% Ca and 3.2% P and that with Ca:P = 2:1 contained 0.8% Ca and 0.4% P. The numbers in brackets refer to the number of animals in each group.

Discussion. These findings clearly show that thyroidal stores of CT were altered by feeding rats diets which varied in their Ca:P ratios (Figs. 2 and 3; Table I). Furthermore, the results indicate that thyroidal CT content increased as the dietary Ca:P ratio was lowered irrespective of the blood calcium concentration which the animals were able to maintain.

It has been shown that CT levels in rat thyroid gland varied inversely with the existing levels of blood calcium in rats chronically maintained under conditions of hyper- or hypocalcemia and it was suggested that the increased CT content during hypocalcemia perhaps resulted from the absence of a stimulus for CT release in the presence of continued biosynthesis of CT (3). Conversely, decreased CT content with chronic hypercalcemia was presumed to be caused by continued high stimulus for release together with an inability of biosynthetic mechanisms to maintain normal thyroid levels of CT (3). In the present study, measurable amounts of CT in sera were found only in rats fed diets with Ca:P ratio of 1:8. This suggests that the capacity of the thyroid gland to store CT may have been exceeded resulting in its spillage into the serum. Our findings agree with those of Gittes *et al.* (3) in showing that CT levels in the thyroid gland increase as blood Ca falls. However, the present findings also indicate that the changes in thyroidal CT levels observed are not explained simply by changes in blood Ca. Rather, changes in CT levels in the thyroid in the present study appeared to be correlated more closely with the dietary Ca:P ratio and the length of time the diet was fed. This conclusion is supported by the finding that although parex rats fed the high phosphate diet had severe hypocalcemia (5.0 mg/100 ml) and the highest content of thyroidal CT, the adrex rats fed the same diet had almost the same thyroidal content of CT but were essentially normocalcemic (9.5 mg/100 ml). Although adrenalectomy had no effect on serum calcium or phosphate of rats fed diet with Ca:P = 2, of interest is the finding, for which we have no adequate explanation, that when adrenalectomized rats were fed diet with Ca:P = 1:8, the fall in serum calcium and rise in serum phosphate that was observed in intact rats fed the same diet was prevented to a large extent. At the same time, no apparent differences were observed in thyroidal CT between intact and adrenalectomized rats fed this diet. Further studies will be required to clarify the mechanisms responsible for changes in thyroidal CT content and the factors responsible for the partial protection of adrex rats fed a Ca:P ratio of 1:8 against hypocalcemia and hyperphosphatemia but not against the increase in thyroidal CT.

Summary. The thyroidal content of CT was measured by bioassay and radioimmunoassay in intact, parathyroidectomized, and adrenalectomized rats fed diets with varying Ca:P ratios for 4 to 8 weeks. There was a good correlation between values obtained with the two assay methods. In intact, parex, and adrex rats, thyroidal CT content was higher 4 and 8 weeks after feeding a diet with a Ca:P ratio of 1:8. However, there was a little difference in overall CT content between intact, parex, and adrex rats despite marked differences in the levels of serum calcium. CT content appeared to be related to the length of time the rats were fed the high phosphate diet. In intact rats fed diets with Ca:P ratios ranging from 8:1 to 1:8 and prepared by varying the calcium content (P constant at 0.4%), the CT content of thyroid gland and serum was elevated only in those animals fed the 1:8 diet. In other intact rats fed with diets having the same range of ratios but prepared by varying the phosphorus content (Ca constant at 0.4%), thyroidal CT increased progressively with the diets having a Ca:P ratio below 1:1. The results indicate that changes in thyroidal CT stores produced by feeding diets with varying Ca:P ratios cannot be explained solely on the basis of changes in the levels of serum calcium.

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