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Chemistry and Histology of the Thyroid Gland of Rats Fed High Levels of Calcium[1]

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Calcium as a cause of thyroid enlargement has remained a controversial subject. Thompson (1932, 1933) reported that high calcium intake in a diet low in iodine increased the size of the thyroid gland, and Taylor (1954) found the I^{131} uptake by the thyroid gland of rats fed low iodine diets was increased when 2% calcium carbonate was added. Remington and Levine (1936), however, reported that increasing the calcium in their goiter-producing diet fed to rats did not enlarge the thyroid glands or lower their iodine content below the iodine content of the basal diet. Sharpless *et al.* (1943) used several salts of calcium and found that only calcium chloride in the presence of vitamin D produced thyroid enlargement. From this they concluded that calcium in itself is not goitrogenic but that the acidity of the anion moiety of the calcium salt used could affect thyroid size and its iodine content.

In certain rural populations of Central America, the prevalence of goiter is very high (Muñoz, 1955), around 30%, and the effects of goitrogenic factors other than lack of iodine have not been studied. Due to the lime treatment of the corn used in tortillas, these rural people have a large calcium intake (Flores, 1961). Studies in man suggest a possible relationship between iodine deficiency and calcium intake, caused by drinking hard water and foods with a high content of available calcium (McClelland, 1935; Murray *et al.*, 1948). In view of this fact and because of the controversial results mentioned above regarding calcium as a goitrogenic factor, it was considered important to study in more detail the effect of calcium carbonate added to a low iodine diet on the weight, iodine content, and histology of the thyroid gland.

MATERIALS AND METHODS

Three experiments used female weanling rats of the Wistar strain distributed by weight among the experimental groups of 6 rats each, and an additional experiment used adult male and female rats. The animals, kept in individual cages, had access to feed and distilled water *ad libitum*, and all were weighed weekly. They were fed diets in which the levels of calcium ranged from 0.5 to 6%, and the levels of iodine varied from 0 to 3 mcg per rat per day. The basal diet was Steenbock's rickets-producing diet, which is also known to be goitrogenic (Remington and Levine, 1936), containing: yellow corn, 76 gm; wheat gluten, 18 gm; brewer's yeast, 2 gm; and sodium chloride, 1 gm. Vitamin D, in the form of a solution of "Vigantol" (E. Merck) in oil, was administered weekly. Changes in the level of calcium were made by

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substituting corn for calcium carbonate of reagent grade. Using a tuberculine syringe with a blunt needle, the potassium iodide solution, never exceeding 0.2 ml to insure complete intake, was given by mouth every day.

At the end of 35 days, the age at which rats on an iodine-deficient diet are reported to show the greatest thyroid enlargement (Remington and Levine, 1936), the animals were sacrificed and their thyroid glands were removed and weighed in an analytical balance. A segment of each lobe of the gland was then removed and fixed in 10% formalin for histological studies. The remainder of the gland was weighed and preserved in alkaline 95% ethyl alcohol and stored at 4°C pending iodine content analysis by the method of Walaszek-Piotrowsky and Koch (1952).

For the histological studies, paraffin sections at 4 μ were stained with hematoxylin-eosin and Mallory's P.T.A. stain. Variations in the size of the thyroid acini and the height of their epithelium lining were measured with a micrometer using 900 enlargements. Twenty to thirty measurements of the latter, chosen at random, were made in each thyroid, and the average in microns was calculated. The colloid content of the gland was estimated by the amount present in the acini and rated according to the arbitrary scale from 0 to 3: 0, no colloid at all; +, 1/3 of the acini, ++, 2/3 of the acini; and +++, all of the acini containing colloid.

RESULTS

Experiment I

In this experiment, the calcium content of the diet varied from 0.5 to 2%, and the iodine supplementation ranged from 0 to 3 mcg per rat per day. Table I shows the results on the weight and iodine content of the gland. It is evident that iodine supplementation decreased the weight of the thyroid and increased its iodine content; the differences were statistically significant ($P < 0.01$). The calcium level of the ration, however, had no effect on either variable.

The histological studies corroborated these findings. There was no change in the histology of the gland due to calcium levels. Iodine, on the other hand, had a marked effect. In the groups that were not fed iodine, the thyroid showed marked hyperplasia with small acini containing none or very tiny amounts of dilute colloid. Figures 1A and 1B show the thyroid glands from an animal fed 2% calcium and no iodine; there is marked hyperplasia as described above. Figures 1C and 1D show the thyroid of a rat fed the same 2% calcium but with 3 mcg per day of iodine added. The acini are enlarged, have different sizes and shapes, are rich in colloid, and are lined with low cuboidal epithelium.

Experiment II

Since the highest level of calcium used in Experiment I was 2% of the diet, it was important to determine whether higher levels would affect the weight, iodine content, and histology of the thyroid gland. In Experiment II, the levels of calcium were 2, 4, and 6% of the diet with the same intake of iodine as was used in Experiment. Table II again shows that iodine had a significant effect on both the weight and iodine content of the thyroid, but the calcium levels of the ration had no effect at the different iodine supplementation levels.

TABLE I

EFFECT OF IODINE AND INTERMEDIATE LEVELS OF CALCIUM INTAKE ON THE THYROID GLAND OF YOUNG ALBINO RATS

Experiment I

Treatment						
Calcium (gm %)	Iodine (mcg/day)	Thyroid weight (mg)	Thyroid weight (mg/100 gm body wt)	Iodine in thyroid (mcg/mg)	Colloid	Epithelium height (μ)
0.5	0	70.9 ± 9.7[a]	44.3 ± 6.8	0.015 ± 0.004	0	15.73 ± 1.02
1.0	0	47.2 ± 6.1	29.3 ± 3.4	0.026 ± 0.016	0	15.23 ± 1.02
1.5	0	59.6 ± 10.0	44.6 ± 7.8	0.009 ± 0.001	0	14.54 ± 0.49
2.0	0	49.8 ± 12.4	32.4 ± 5.7	0.044 ± 0.016	0	15.34 ± 0.84
0.5	1	17.6 ± 1.3	10.9 ± 0.5	0.306 ± 0.07	++	6.90 ± 0.62
1.0	1	19.6 ± 2.4	11.1 ± 1.2	0.287 ± 0.03	++	7.82 ± 0.23
1.5	1	19.1 ± 1.4	12.1 ± 0.6	0.359 ± 0.03	++	7.10 ± 0.82
2.0	1	17.9 ± 1.6	11.8 ± 0.8	0.332 ± 0.03	++	7.22 ± 0.48
0.5	2	20.0 ± 2.0	11.2 ± 0.7	0.440 ± 0.06	++	7.48 ± 0.74
1.0	2	17.6 ± 1.7	10.6 ± 0.7	0.546 ± 0.08	++	6.33 ± 0.67
1.5	2	16.6 ± 1.8	10.7 ± 0.7	0.525 ± 0.04	++	6.28 ± 0.18
2.0	2	15.5 ± 0.9	10.5 ± 0.6	0.523 ± 0.04	++	7.35 ± 0.64
0.5	3	16.9 ± 0.9	9.6 ± 0.6	0.574 ± 0.08	++	7.04 ± 0.28
1.0	3	17.2 ± 2.0	10.8 ± 1.1	0.528 ± 0.08	+++	6.05 ± 0.96
1.5	3	16.1 ± 1.5	9.9 ± 0.6	0.519 ± 0.07	+++	5.65 ± 0.48
2.0	3	16.8 ± 1.4	10.8 ± 0.9	0.536 ± 0.10	+++	6.15 ± 0.60

[a] Standard error of the mean.

As in the first experiment, there was no change in the histology of the gland as a result of the calcium intake. The lack of iodine in the first group again shows evidence of thyroid hyperplasia and the same histological characteristics as shown in Fig. 1A.

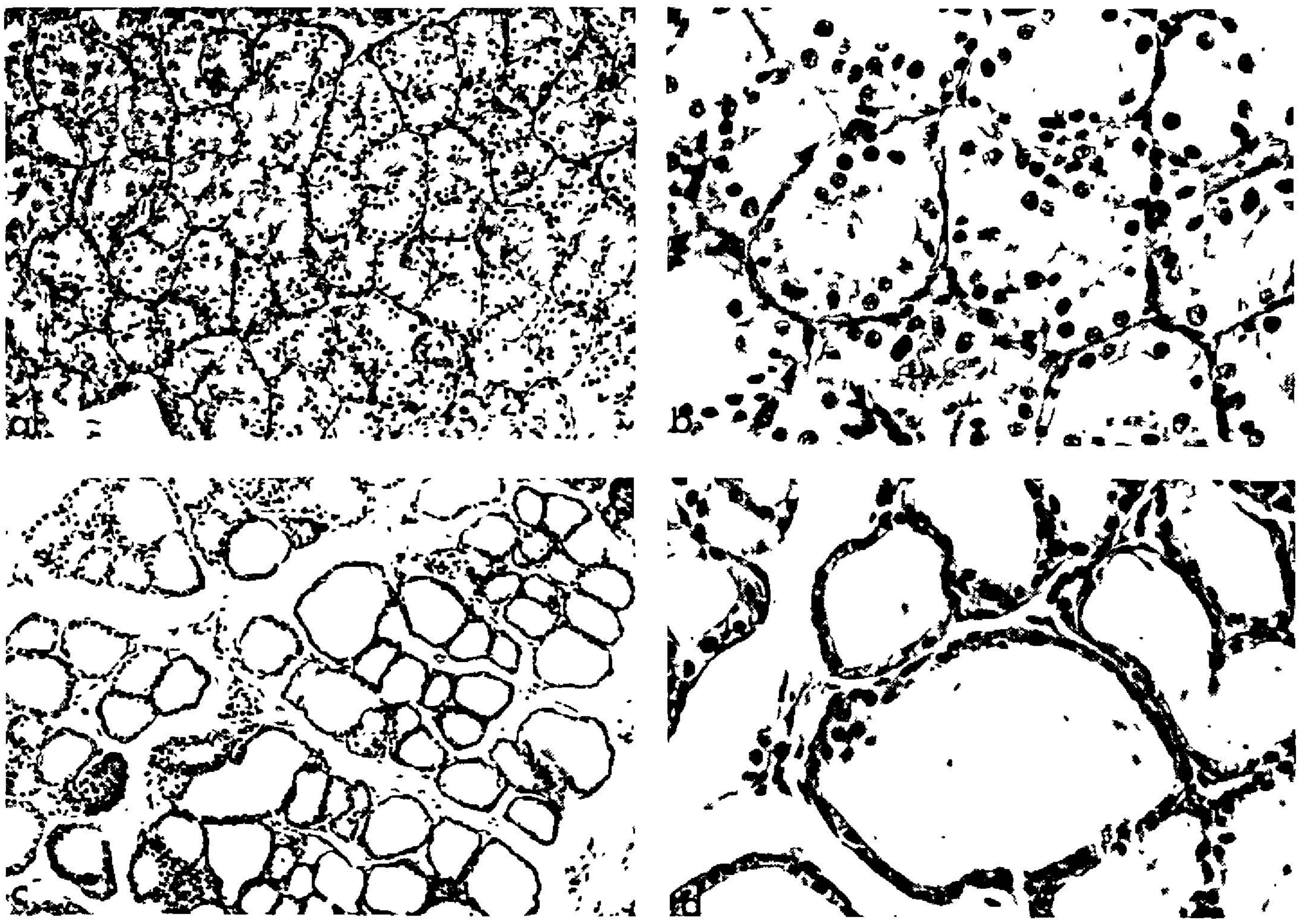

FIG. 1. (A) Thyroid gland from an animal fed 2% Ca and no iodine. There is marked hyperplasia with small acini and no colloid (× 100). (B) Same as (A). The acini lined by columnal cells with active vesicular nuclei and abundant cytoplasm. In the center, a cell undergoing mitosis (× 250). (C) Thyroid gland from an animal fed 2% Ca and 3 mcg per day of iodine. The thyroid acini are of different size and rich in colloid (× 100). (D) Same as (C). The cells lining the acini are flat or low cuboidal and the nuclei pycnotic (× 250).

EXPERIMENT III

Since in the previous experiments the variation in iodine intake from 0 to 1 mcg per rat per day was abrupt, and since 1 mcg is almost the rat's normal requirement (Levine *et al.*, 1933), it was possible that, with lower levels of iodine and relatively higher levels of calcium, the effect of the latter could be more easily demonstrated. In Experiment III, therefore, the levels of calcium were set at 0.5, 1.5, and 2% of the diet, while iodine supplementation was set at 0.25, 0.50, 0.75, and 1.0 mcg per rat per day. Table III shows that only iodine supplementation decreased the thyroid weight and increased the iodine content of the gland significantly.

The results of the histological studies show that neither calcium nor iodine had an effect; apparently, 0.25 mcg of iodine per rat per day is sufficient to maintain normal low cuboidal epithelium and normal amounts of colloid.

TABLE II

EFFECT OF IODINE AND HIGH LEVELS OF CALCIUM INTAKE ON THE THYROID GLAND OF YOUNG ALBINO RATS

Experiment II

Treatment						
Calcium (gm %)	Iodine (mcg/day)	Thyroid weight (mg)	Thyroid weight (mg/100 gm body wt)	Iodine in thyroid (mcg/mg)	Colloid	Epithelium height (μ)
2.0	0	38.4 ± 5.3[a]	31.2 ± 3.3	0.020 ± 0.004	0	13.52 ± 0.50
4.0	0	35.8 ± 3.3	28.9 ± 2.8	0.025 ± 0.001	0	13.72 ± 0.87
6.0	0	31.5 ± 6.4	25.8 ± 3.8	0.033 ± 0.004	0	11.98 ± 1.31
2.0	1	20.6 ± 2.1	13.2 ± 0.9	0.286 ± 0.05	++	7.67 ± 0.88
4.0	1	16.7 ± 2.0	12.3 ± 1.3	0.455 ± 0.10	++	7.18 ± 0.64
6.0	1	14.7 ± 1.8	13.2 ± 0.9	0.481 ± 0.13	++	6.24 ± 0.73
2.0	2	15.3 ± 1.6	11.3 ± 1.0	0.553 ± 0.06	++	7.35 ± 1.62
4.0	2	11.8 ± 0.9	10.2 ± 0.5	0.680 ± 0.08	++	5.64 ± 0.41
6.0	2	12.5 ± 1.0	11.3 ± 0.7	0.600 ± 0.09	++	5.50 ± 0.28
2.0	3	14.2 ± 0.9	10.6 ± 0.7	0.637 ± 0.04	++	6.13 ± 0.59
4.0	3	13.3 ± 1.1	11.4 ± 0.5	0.719 ± 0.06	++	5.73 ± 0.30
6.0	3	12.4 ± 1.6	9.7 ± 0.8	0.786 ± 0.08	++	5.58 ± 0.31

[a] Standard error of the mean.

TABLE III
EFFECT OF IODINE AND INTERMEDIATE CALCIUM INTAKES ON THE THYROID GLAND OF YOUNG ALBINO RATS
Experiment III

Treatment						
Calcium (gm %)	Iodine (mcg/day)	Thyroid weight (mg)	Thyroid weight (mg/100 gm body wt)	Iodine in thyroid (mcg/mg)	Colloid	Epithelium height (μ)
0.5	0.25	2.14 ± 1.3[a]	11.8 ± 0.6	0.156 ± 0.02	+	8.38 ± 0.49
1.5	0.25	22.9 ± 2.4	14.2 ± 1.4	0.170 ± 0.02	+	9.51 ± 1.17
2.0	0.25	18.7 ± 1.1	12.9 ± 1.0	0.198 ± 0.03	++	8.47 ± 1.15
0.5	0.50	23.8 ± 3.4	12.8 ± 1.1	0.207 ± 0.03	+	8.03 ± 0.36
1.5	0.50	15.4 ± 1.0	10.1 ± 0.7	0.341 ± 0.04	++	7.13 ± 0.50
2.0	0.50	18.8 ± 2.0	12.8 ±1.0	0.282 ± 0.05	++	8.21 ± 0.61
0.5	0.75	12.3 ± 2.1	10.2 ± 0.7	0.288 ± 0.02	+	7.43 ± 0.23
1.5	0.75	19.8 ± 1.8	12.0 ± 1.2	0.295 ± 0.03	++	8.02 ± 0.33
2.0	0.75	17.7 ± 1.6	12.0 ± 1.4	0.348 ± 0.03	+	7.45 ± 0.51
0.5	1.0	18.0 ± 1.2	10.5 ± 0.5	0.350 ± 0.03	++	8.41 ± 0.88
1.5	1.0	15.0 ± 1.0	9.6 ± 0.6	0.455 ± 0.04	++	6.79 ± 0.24
2.0	1.0	15.2 ± 1.2	10.5 ± 0.8	0.411 ± 0.03	++	7.20 ± 0.47

[a] Standard error of the mean.

TABLE IV

EFFECT OF CALCIUM AND IODINE INTAKE ON THE THYROID GLAND OF ADULT ALBINO RATS

Experiment IV

Treatment						
Calcium (%)	Iodine (mcg/day)	Thyroid weight (mg)	Thyroid weight (mg/100 gm body wt)	Iodine in thyroid (mcg/mg)	Colloid	Epithelium height (μ)
0.5	0	26.2 ± 1.3[a]	9.5 ± 0.6	0.168 ± 0.05	++	9.28 ± 1.36
1.5	0	24.4 ± 2.6	9.6 ± 0.6	0.272 ± 0.08	+	11.33 ± 1.00
2.0	0	21.9 ± 3.0	8.7 ± 0.6	0.338 ± 0.06	++	8.42 ± 0.53
0.5	1	24.1 ± 4.0	8.3 ± 0.8	0.643 ± 0.24	++	8.71 ± 0.97
1.5	1	18.5 ± 1.5	6.8 ± 0.4	0.730 ± 0.37	++	7.01 ± 0.73
2.0	1	19.6 ± 1.8	7.7 ± 0.8	0.413 ± 0.03	++	8.05 ± 0.32
0.5	2	17.4 ± 2.1	6.4 ± 0.1	0.536 ± 0.14	++	7.24 ± 1.11
1.5	2	16.5 ± 1.8	8.7 ± 0.8	0.560 ± 0.12	++	8.01 ± 1.11
2.0	2	16.2 ± 0.9	6.9 ± 0.4	0.437 ± 0.08	++	8.21 ± 0.45

[a] Standard error of the mean.

EXPERIMENT IV

The possibility that age might be an important factor related to the effect of calcium on the thyroid gland was studied in this experiment, in which male and female adult rats were given three levels of iodine supplementation. The results shown in Table IV agree with those obtained with young rats; only iodine had an effect on the weight and iodine content of the thyroid.

The histological studies show that in all groups there was no effect due to calcium and a slight effect due to iodine.

DISCUSSION

It is evident from the results presented here that calcium had no effect on the weight of the thyroid gland or on its iodine content. On the other hand, increasing the iodine intake resulted in a decrease in size of the thyroid and in an increase in its iodine content. The height of the epithelium and the colloid content of the gland were affected by the iodine intake, but calcium had no effect on the histology of the gland. These results, therefore, agree with those published previously by Remington and Levine (1936).

Goitrogenic factors as those present in legumes and species of the genus *Brassica* are of importance in this area since such species are consumed as part of the daily diet of the rural population. Likewise, deficiency of vitamin A has been reported to cause thyroid enlargement in human populations (Hovart and Maver, 1958) whose vitamin A consumption is inadequate. These factors are now under study in our laboratories.

SUMMARY

Three experiments with weanling female rats and one with adult rats in which the diets contained from 0.5 to 6% of calcium and the iodine intake was from 0 to 3 mcg per rat per day were conducted. In none of the experiments did calcium cause a change in the size of the thyroid, its iodine content, the histology of the gland as measured by the height of the epithelium lining of the acini, or the amount of colloid present in the gland. Iodine intake on the other hand, decreased thyroid size, increased the iodine content of the gland, decreased the epithelium height, and increased the colloid content of the gland.

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