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Thyroid and Blood Thyrocalcitonin Concentrations and C-Cell Abundance in Two Strains of Rats at Different Ages[1] (39525)

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Previous reports of changes in thyrocalcitonin (TCT) status in rats of different ages are conflicting. Some workers have reported that thyroid TCT levels increase with age (1) whereas others have observed (a) no differences in thyroid TCT in 60-, 120-, and 360-day-old rats (2) and (b) no difference in blood TCT between young and old rats (3). Since our own earlier studies employing the bioassay for TCT showed differences in the thyroid gland content of TCT between age-matched rats of different strains (4), and since the earlier conflicting studies (1-3) employed rats of different strains as well as different ages, we decided to examine the question of possible changes in TCT in young and old rats from two different strains commonly used in our laboratory.

Using a recently developed immunoperoxidase technique for light microscopic localization of rat thyroid C-cells (5) and an improved radioimmunoassay for rat TCT (6), we have evaluated and compared the relative area ratio between C-cells and follicular cells and the thyroid and blood TCT concentration in Fischer inbred rats (F344) and Holtzman rats.

Material and methods. Animals. Holtzman albino rats (descended from a Sprague-Dawley strain) were purchased from the Holtzman Co., Madison, Wis.; Fischer inbred rats (F344) were obtained from the Charles River Breeding Labs., Wilmington, Mass., (Charles River CDF rats). Following receipt, the rats were maintained on Purina laboratory chow and tap water.

Histology. Preparation of tissues and immunohistochemical staining have been described previously (5). The immunoperoxidase technique revealed brown-stained C-cells which were easily distinguished from unstained follicular cells. Comparison of area ratios of these two types of thyroid cells was performed according to the principle described by Chalkley (7). An ocular micrometer consisting of 5 horizontal lines and 20 vertical lines was attached to an eyepiece (10×), and the 100 points where the vertical lines intersected the horizontal lines were used as finders. The intersecting points constituted the point pattern. The numbers of C-cells and follicular cells in tissue sections that fell on these points were recorded as "number of hits." Cross sections obtained at the midline of the thyroid, in reference to the caudal and distal poles of the thyroid gland, were used, because this midline area has the highest concentration of C-cells (8). The total area of the cross sections was measured with the micrometer, and the results were expressed as the area ratio (7) between C-cells and follicular cells (C/F). Tissues for electron microscopic study were prepared and processed as described previously (9).

Blood collection and analysis. Generally, blood was obtained from each rat by cardiac puncture and allowed to clot. Serum was separated by centrifugation within 2 hr after blood collection. The serum levels of TCT and the thyroid gland content of TCT were measured by radioimmunoassay as described previously (6).

Statistical analysis. Experimental data were subjected to analysis of variance. Standard errors were calculated from the residual error term of the analysis of variance. The significance of differences between

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mean values was evaluated either by the F ratio or by a two-tailed t test. For the data shown in Fig. 1, standard linear regression analysis was employed with body weight designated the independent variable (X) and thyroid weight the dependent variable (Y). The value for the correlation coefficient (r) was calculated and analyzed for significance (P) by a 2-tailed test.

Results. Table I shows the TCT concentrations in thyroid glands and peripheral blood of 10-week-old Fischer rats and Holtzman rats. The mean thyroid TCT concentration in Fischer rats was slightly higher than that in Holtzman rats, but the difference was not statistically significant. The TCT level in the peripheral blood was below 0.24 ng/ml except for two out of eight Fischer rats and one of eight Holtzman rats.

Table II shows results of three experiments (A, B, and C) in which TCT concentrations in the thyroid glands and in the peripheral blood of 6- to 18-month-old rats of the two strains were measured. Results obtained from 6- and 18-month-old rats were combined for presentation since no differences between these two ages were found. In Experiment A, serum TCT was higher in Fischer rats than in Holtzman rats,

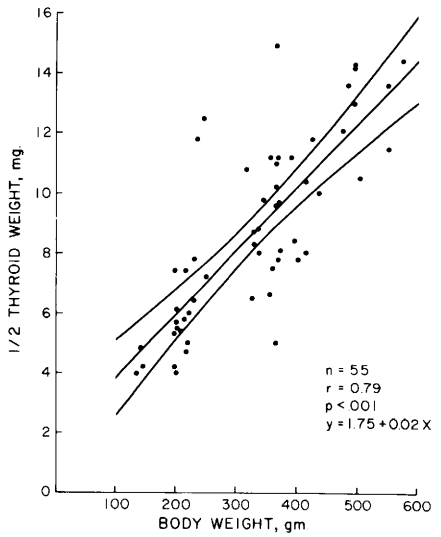

FIG. 1. Positive correlation between body weight and thyroid weight of both Fischer and Holtzman rats. Area between the two convex curves represents 95% confidence limits of the regression line.

TABLE I. TCT CONCENTRATIONS IN THYROID GLANDS AND PERIPHERAL BLOOD OF 10-WEEK-OLD FISCHER AND HOLTZMAN RATS.

	Fischer	Holtzman
Thyroid (ng/mg)	86 ± 13.4[a]	54 ± 13.4
Serum (ng/ml)	6 of 8 = N.D.[b] (2 of 8 = 0.31, 0.49)	7 of 8 = N.D. (1 of 8 = 0.29)

[a] Values of TCT concentration in thyroid glands are shown as mean ± SE (N = 8).
[b] N.D. = not detectable or <0.24 ng/ml.

and this difference was highly significant ($P < 0.001$). In Experiment B, TCT concentrations in both peripheral blood and thyroid glands were measured in Fischer rats. A high serum TCT in Fischer rats, similar to that found in Experiment A, was associated with a high thyroid TCT concentration. In Experiment C, TCT concentrations in peripheral blood and thyroid glands were measured in Holtzman rats. A low serum TCT level, similar to that obtained in Experiment A, and a low thyroid TCT concentration were found.

Figure 1 shows the positive correlation observed between the body weight of the rats used and the wet weight of one of their two thyroid lobes (1/2-thyroid) which was analyzed for TCT. Figure 2 shows results of thyroid weight and TCT content measurements in 10-week-old rats. The 1/2-thyroid weight of Holtzman rats and Fischer rats was different, but no significant difference in 1/2-thyroid TCT was found between Holtzman and Fischer rats. Figure 3 shows the thyroid weight and TCT content in 6- to 18-month-old rats of the two strains. Again the 1/2-thyroid weights of Holtzman rats and Fischer rats were different ($P < 0.001$). The 1/2-thyroid TCT content also was different ($P < 0.05$).

We also examined the distribution of C-cells in Fischer rats. In 10-week-old rats, C-cells were localized chiefly in the central position of the thyroid lobes, a distribution similar to that which we described previously for 4- to 5-week-old Holtzman rats (5). In 6- to 18-month-old Fischer rats, there was a significantly higher area ratio of C-cells to follicular cells (0.15 ± 0.026;

TABLE II. TCT Concentrations in Thyroid Glands and Peripheral Blood of 6- to 18-Month-Old Fischer and Holtzman Rats.

Experiment	Fischer		Holtzman	
	Serum (ng/ml)[a]	Thyroid (ng/mg)[a]	Serum (ng/ml)[a]	Thyroid (ng/mg)[a]
A	1.52 ± 0.06	–	0.68 ± 0.06	–
B	1.37 ± 0.06	461 ± 38	–	–
C	–	–	0.48 ± 0.06	182 ± 38

[a] Values of TCT concentration in serum and thyroid glands are shown as mean ± SE.

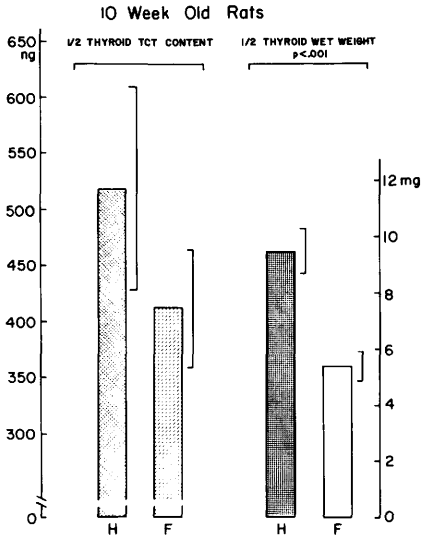

FIG. 2. TCT in thyroid glands of 10-week-old Holtzman rats (H) and Fischer rats (F). The two bars on the right represent the mean weights of one thyroid lobe (1/2-thyroid gland) and the two bars on the left show the total TCT content obtained in those thyroid lobes (1/2-thyroid TCT content). Brackets denote ± SE.

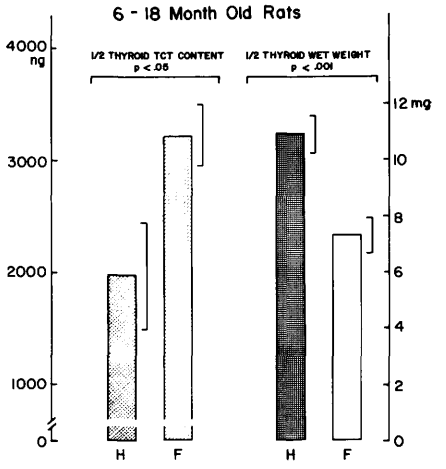

FIG. 3. TCT in thyroid glands of 6- to 18-month-old Holtzman rats (H) and Fischer rats (F) are shown. For details see legend to Fig. 2.

mean ± SE) than in 10-week-old Fischer rats (0.08 ± 0.013). Electron microscopic study of thyroid tissue from an 18-month-old Fischer rat showed abundant clusters of C-cells which were easily found (Fig. 4). Like C-cells in young rats, they were located intrafollicularly close to the basal membrane.

Discussion. The results show that in Fischer and Holtzman rats, most of the 10-week-old animals had undetectable levels of blood TCT (0.24 ng/ml, Table I). In contrast, blood TCT of all older rats from both strains was much higher and easily measured (0.5–1.5 ng/ml, Table II). Our findings are not in agreement with a recent report showing no differences in blood TCT between young (120–140 g) and old (2 years) Wistar rats (3). These discrepancies may be due to strain differences, since in our study a significantly ($P < 0.001$) higher blood TCT was observed in Fischer rats compared with Holtzman rats of the same age (Table II, Experiment A). Not only the concentration of TCT in blood but also the concentration of TCT in thyroid tissue increased with age (Tables I and II). Since the size (weight) of the thyroid glands also increased with age (body weight) (Fig. 1), we also examined the relationship between thyroid weight and thyroid TCT content (Figs. 2 and 3). In 6- to 18-month-old rats, the results clearly showed that Fischer rats had smaller thyroid glands but a higher TCT content than Holtzman rats (Fig. 3). It has been reported previously that in Wistar rats thyroid TCT content increases with age (1). However, it is not clear whether these earlier results are in agreement with our present findings or whether the apparent in-

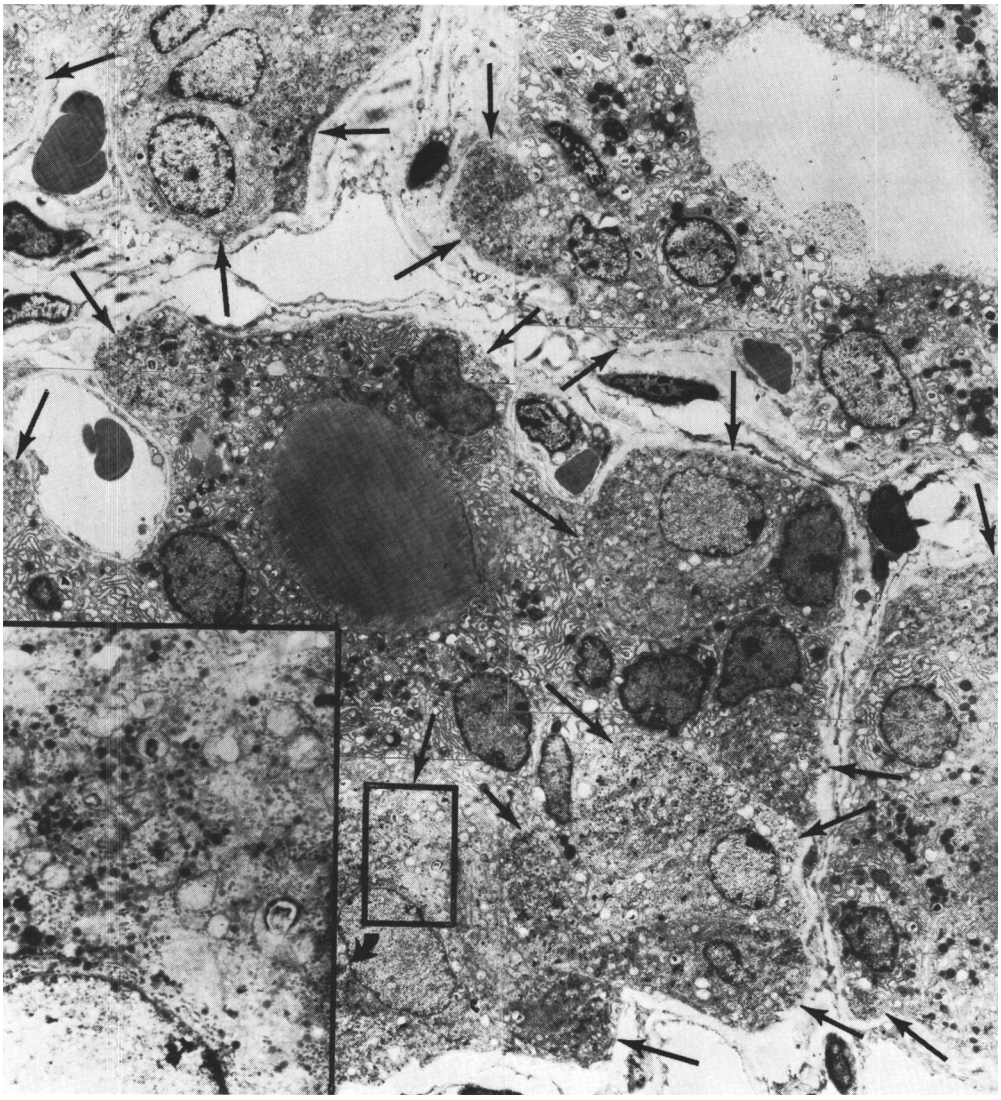

FIG. 4. Electron micrograph (montage) of thyroid tissue from an 18-month-old Fischer rat shows abundant C-cells readily identified by the presence of secretory granules. C-cells (arrows) are found intrafollicularly and localized close to the basal membrane (uranyl acetate and lead citrate; montage ×2430; inset ×9000).

crease in thyroid TCT content reported was due merely to an increase in the size of the thyroid glands with increasing age of the rats. In contrast, other investigators have *not* found differences in thyroid TCT content, as measured by bioassay in 60-, 120-, or 360-day-old Sprague–Dawley rats (2). We have used male and female rats of both strains in our present study. The data were combined since no sex differences with respect to blood and thyroid TCT were observed.

Because of the high level of TCT in blood and in the thyroid gland, Fischer rats may represent an especially useful strain of rat for the study of TCT secretion. The significance of the differences in TCT between Fischer rats and Holtzman rats and the factors responsible for the increases in TCT in blood and thyroid gland with age remain to be elucidated.

Summary. We have shown an increase in TCT in the blood and thyroid gland with increased age in two different strains of rats.

Fischer (F344) rats, 6 to 18-months old, have a higher level of circulating TCT (~1.5 ng/ml) than young 10-week-old Fischer rats (<0.2 ng/ml). Thyroid TCT concentrations in old Fischer rats also were significantly higher than in young Fischer rats, and this was associated with a significantly higher area ratio of C-cells to follicular cells in the older rats. In Holtzman rats a similar increase in blood and thyroid TCT with age was also observed. Comparing the two strains, both thyroid and serum TCT in old Fischer rats were significantly higher than in old Holtzman rats.

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1. Milhaud, G., Perault-Staub, A. M., and Moukhtar, M. S., C. R. Acad. Sci. Ser. D **264,** 110 (1967).
2. Frankel, S., and Yasumura, S., Endocrinology **87,** 602 (1970).
3. Triggs, S. M., Hesch, R. D., Woodhead, J. S., and Williams, E. D., J. Endocrinol. **66,** 37 (1975).
4. Cooper, C. W., Endocrinology **82,** 1015 (1968).
5. Peng, T.-C., Cooper, C. W., Petrusz, P., and Volpert, E. M., Endocrinology **97,** 1537 (1975).
6. Cooper, C. W., Obie, J. F., and Hsu, W., Proc. Soc. Exp. Biol. Med. **151,** 183 (1976).
7. Chalkley, H. W., J. Nat. Cancer Inst. **4,** 47 (1943–1944).
8. Peng, T.-C., Fed. Proc. **34,** 761 (1975).
9. Peng, T.-C., Mol. Cell. Endocrinol. **2,** 1 (1974).

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