Influence of Adrenal Cortex on Thyrocalcitonin Content of the Rat Thyroid

Thyrocalcitonin (TCT), a new factor which contributes to the regulation of calcemia, has recently been isolated and widely studied[1-4]. TCT storage in and secretion from the thyroid seem to be related mainly to the blood calcium level[5,6]. However, TCT content of the rat thyroid may be affected by other factors. Thus thyroid (thyroxine) lowers TCT content[7,8] while pituitary[9] and parathyroids[10] do not affect it.

The effect of the adrenal cortex is less known, although THOMPSON et al.[11] found that cortisone reduces the hypocalcemic effect of injected TCT. Since adrenocortical hormones play an important role in calcium homeostasis[12], it seemed of interest to study the effect of adrenalectomy and/or cortisone or ACTH treatment on TCT thyroid content of adrenalectomized and intact rats.

Materials and methods. Male albino rats, Wistar strain 60–70 g body weight were used. Each type of experiment (see Table) was performed on 1 to 3 groups of at least 10 animals each. All animals were individually caged and maintained at a constant temperature of 24 ± 1 °C. Each treatment (control animals received the vehicle only) lasted for 7 consecutive days. Treated as well as control animals were posted 24 h after the last treatment. Blood was obtained from the abdominal aorta under light ether anesthesia. The plasma samples of 2 animals were pooled together and blood calcium determined according to CLARK and COLLIP[13]. The thyroid glands of each group were pooled together, weighed, anidrified in acetone for 24 h at 4 °C and powdered. Preliminary experiments showed that TCT remained unchanged even when the thyroids in acetone or their powder had been stored for 20–30 days at 4 °C.

8 mg of thyroid powder were suspended in 4 ml of 0.1 *N* HCl by means of a Potter-Elvehjem homogenizer. The suspension was centrifuged for 15 min at 15,000 rpm in a refrigerated centrifuge[14]. TCT content of the supernatant was determined by assessing its hypocalcemic activity according to MUNSON and HIRSCH[15] in Sprague-Dawley rats, maintained on a low calcium diet[16]. Hog TCT, prepared according to TENENHOUSE et al.[17] and standardized in MRC (Medical Research Council) units was used as reference material.

Calcium content was determined[13] on blood obtained under light ether anesthesia 70 min after s.c. injection.

Results and discussion. The data are shown in the Table. In no instance was the blood calcium level affected by our experimental conditions. On the other hand, cortisone significantly increased TCT content of the thyroid in both adrenalectomized and intact rats, while ACTH exhibited a similar behaviour in intact animals only.

The postulate of GITTES et al.[6] must be taken into account to explain the variation of TCT content of the thyroid. Namely, a decrease in TCT content should be ascribed to its release not compensated by a concomitant synthesis, whereas TCT increase should probably be attributed to its increased synthesis. It is quite well known that corticosteroids affect both calcium and thyroid metabolism. As far as calcium is concerned, glucocorticoids inhibit its intestinal absorption in vitro[18,19], and presumably in vivo[20], increase its urinary excretion[20-22] by enhancing glomerular filtration rate[22] and inhibit its bone deposition[21]. Furthermore, they affect bone calcium metabolism by inhibiting bone collagen synthesis[23].

1 P. L. MUNSON and P. F. HIRSCH, Clin. Orthopaedics *49*, 209 (1966).
2 D. H. COPP, Am. J. Med. *43*, 648 (1967).
3 G. V. FOSTER, I. MACINTYRE and A. G. E. PEARSE, Nature *203*, 1029 (1964).
4 G. BUSSOLATI and A. G. E. PEARSE, J. Endocrin. *37*, 205 (1967).
5 T. MATSUKAWA and K. KUROSUMI, Nature *213*, 927 (1967).
6 R. F. GITTES, S. U. TOVERUD and C. W. COOPER, Endocrinology *82*, 83 (1968).
7 S. YASUMURA, M. BURK, A. CHAUSMER, R. LITTLEMAN and S. WALLACH, Endocrinology *81*, 256 (1968).
8 L. DE GIUSEPPE and G. RINDI, unpublished results.
9 G. MILHAUD and H. S. MOUKHTAR, C. r. Acad. Sci. *260*, 3179 (1965).
10 R. F. GITTES, P. L. MUNSON and S. U. TOVERUD, Fedn Proc. *25*, 496 (1966).
11 J. S. THOMPSON, G. M. A. PALMIERI and L. P. ELIEL, Clin. Res. *15*, 415 (1967); Endocrinology *83*, 870 (1968).
12 H. RASMUSSEN and H. F. DE LUCA, Ergebn. Physiol. *53*, 108 (1963).
13 M. REINER, *Standard Methods of Clinical Chemistry* (Academic Press, New York 1953), vol. 1.
14 P. HIRSCH, E. VOELKEL and A. P. MUNSON, Science *146*, 412 (1964).
15 P. MUNSON and P. HIRSCH, Am. J. Med. *43*, 367 (1967).
16 P. HIRSCH, Fedn Proc. *26*, 237 (1967).
17 A. TENENHOUSE, C. ARNAUD and H. RASMUSSEN, Proc. natn. Acad. Sci. *53*, 818 (1965).
18 D. SCHACHTER, in *The Transfer of Calcium and Strontium Across Biological Membranes* (Ed. R. H. WASSERMAN; Academic Press, New York 1963), p. 167.
19 H. E. HARRISON and H. C. HARRISON, Am. J. Physiol. *199*, 265 (1960).
20 J. CLARK and M. R. SMITH, Endocrinology *74*, 421 (1964).
21 J. CLARK, R. F. GEOFFROY and W. BOWERS, Endocrinology *64*, 849 (1959).
22 H. LAAKE, Acta Endocrin. *34*, 60 (1960).
23 R. P. CANNON, L. E. FRAZIER and R. H. HUGHES, Arch. Path. *61*, 271 (1956).

Effect of adrenalectomy and cortisone or ACTH treatment on plasma calcium and TCT content of thyroid glands of intact or adrenalectomized rats

Treatment	No. of groups[c]	Body weight (g) Initial	Final	Plasma Ca^{++} (mE/l)	Thyroid Weight (mg/100 g body wt.)	TCT content (MRC U/g)
None	1	70.5 ± 1.09[d]	106.5 ± 2.99	6.08 ± 0.10	8.4 ± 0.45	12.30 ± 2.33
Adrenalectomy	3	71.6 ± 1.56	104.0 ± 2.19	6.39 ± 0.06	9.3 ± 0.33	12.08 ± 3.08
Adrenalectomy + cortisone[a]	3	69.6 ± 1.36	65.6 ± 1.41	6.25 ± 0.05	11.1 ± 0.46	59.55 ± 6.66
Adrenalectomy + ACTH[b]	2	70.0 ± 1.12	97.0 ± 2.37	6.24 ± 0.08	9.8 ± 0.26	6.87 ± 2.33
ACTH[b]	3	71.0 ± 1.33	97.3 ± 2.23	6.15 ± 0.06	9.0 ± 0.42	30.80 ± 6.00

[a] 5 mg/day s.c. for 7 days. [b] 2.5 U/day s.c. for 7 days. [c] At least 10 rats per group. [d] Average $\pm$ S.E.

In so far as thyroid metabolism is concerned, cortisone reduces the weight of the gland and inhibits its protein synthesis[24]; on the other hand, it is well known that ACTH, as well as cortisone, has a goitregenic action[25] with lower iodine uptake by thyroid gland.

Thus, the increase in TCT thyroid content may be considered a result of (a) calcium negative balance due to hypercorticism, and/or (b) the inhibiting action of cortisone or ACTH on thyroid metabolism. In fact, a decrease in thyroid function is probably generally followed by an increased TCT content[7]. On the other hand, thyroxine is directly involved in calcium metabolism[26-29], increases bone catabolism in intact rats without modifying calcemia[30] and enhances the hypocalcemic effect of exogenous TCT[31-34]. It is likely that in our experimental conditions thyroid metabolism had been inhibited by hormonal treatment resulting in a decreased thyroxine production and increased TCT content of the gland. If this assumption is correct, a reduction in bone catabolism and subsequent reduction in TCT demand should be expected.

It should be emphasized that experimentally induced hypercorticism is likely to affect, at one time, calcium, thyroid and bone metabolism thus producing the very sharp and significant increase in TCT content of the thyroid which we were able to demonstrate. In our opinion, the accumulation of TCT is probably ascribable to a sharp decrease in its output, since its synthesis was presumably inhibited[24] by corticoids. From a practical viewpoint, our results suggest that, during cortisone therapy, the administration of TCT might delay the onset of osteoporosis.

Riassunto. Il trattamento con ATCH o cortisone causa un aumento del contenuto in tirocalcitonina delle tiroidi di ratto. Vengono discusse le possibili implicazioni dei risultati ottenuti.

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[24] N. B. YATVIN, R. W. VANNEMACHER and N. V. BROWN, Endocrinology *79*, 1079 (1966).
[25] M. SONENBERG, Vitam. Horm. *16*, 205 (1958).
[26] H. L. MONEY, Brokhaven Symposium Biology *7*, 137 (1955).
[27] P. ADAMS and J. JOWSEY, Endocrinology *81*, 735 (1967).
[28] R. F. GITTES and G. L. IRVING, Endocrinology *79*, 1033 (1966).
[29] A. D. CARE, Fedn Proc. *27*, 153 (1968).
[30] G. MILHAUD, L. T. MING and M. S. MOUKHTAR, C. r. Acad. Sci. *264*, 846 (1967).
[31] H. MILHAUD, A. M. PERAULT and M. S. MOUKHTAR, C. r. Acad. Sci. *261*, 813 (1965).
[32] C. C. JOHNSTON and W. P. DEISS, Endocrinology *78*, 1139 (1966).
[33] A. W. WASE, A. PETERSON, E. RICKES and J. SOLEWSKI, Endocrinology *79*, 687 (1966).
[34] G. MILHAUD and M. S. MOUKHTAR, Proc. Soc. exp. Biol. Med. *123*, 207 (1966).

Oxygen Consumption of Genetically Obese Rats[1]

Oxygen consumption is significantly reduced in 2 strains of gentically obese mice when compared to their lean littermates (obese[1] and A^{y}[2]). This finding in genetically obese mice led us to investigate the uptake of oxygen in genetically obese rats[3]. Obesity in these rats is inherited as a Mendelian recessive trait and the affected members of each litter can be detected within the first few weeks of life. We found that the oxygen consumption of these genetically obese rats was reduced when compared to littermates which became fat following hypothalamic lesions.

Materials and methods. Animals: Obese female rats of the Zucker Strain and their lean female littermates[2] were housed in a room with controlled light and temperature and given Purina Laboratory Chow and tap water. In the first experiment oxygen consumption was determined on 12 rats from 6 litters, there being one lean and one obese rat from each litter. At the end of this experiment bilateral lesions were placed in the ventromedial nuclei of each of the 6 lean littermates[4]. These lesions were made using the coordinates of DEGROOT[5] and applying a 2 mAmp current through the tip of an insulated platinum needle for 15–30 sec. All 6 rats became hyperphagic but only 3 became as obese as their genetically obese littermates. The second experiment utilized these 3 rats and their genetic obese littermates for the measurement of oxygen consumption. Experimental procedures were conducted on rats at 2 and 8 months of age.

Oxygen consumption of obese and thin rats

Experiment No.	Animals	No. of rats	Age (month)	Body weight (g)		Oxygen consumption ml O_2/g per h		ml O_2/$g^{0.7}$ per h	
1	Obese-genetic	6	2	314 ± 5[a]	< 0.01	1.11 ± 0.025[a]	< 0.05	6.22 ± 0.45[a]	n.s.
	Lean	6	2	207 ± 6		1.48 ± 0.029		7.35 ± 0.47	
2	Obese-genetic	3	8	550 ± 22	n.s.	1.03 ± 0.042	< 0.05	7.15 ± 0.29	< 0.05
	Obese-lesioned	3	8	517 ± 27		1.22 ± 0.038		7.95 ± 0.25	

[a] Mean ± S.E.M.

[1] J. MAYER, R. E. RUSSELL, M. W. BATES and M. M. DICKIE, Endocrinology *56*, 318 (1952).
[2] A. BARTKE and A. GORECKI, Am. J. Physiol. *214*, 1250 (1968).
[3] L. M. ZUCKER and T. F. ZUCKER, J. Heredity *52*, 275 (1961).
[4] G. A. BRAY, J. Lipid Res. *9*, 681 (1968).
[5] J. DEGROOT, *The Rat Brain in Stereotoxic Coordinates* (N.V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam 1963).