Occurrence of immunoreactive thyroglobulin in the parafollicular cells of dogs

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Summary. When the canine thyroid gland was stained by immunofluorescence and immunoperoxidase methods using undiluted thyroglobulin antiserum, a considerably stronger immunoreactivity was revealed in the parafollicular cells than in the colloid droplets and follicular cells. After induced hypercalcemia and antithyroid drug treatment, the immunoreactivity of the parafollicular cells coinciding with the movement of secretory granules containing calcitonin was conspicuously decreased. An application of diluted serum (above 1:10) produced a strong reaction in the colloid.

The mammalian thyroid gland contains, besides follicular cells, parafollicular or C cells which secrete calcitonin-serum calcium lowering polypeptide hormone. The parafollicular cells are derived from an ultimobranchial body which becomes incorporated into the thyroid during fetal life. In his view of APUD concept, Pearse[2] postulated that the parafollicular cells, as well as the cells of the ultimobranchial body which remains separated from the thyroid in lower vertebrates, might be offshoots from the neural crest. In any case, the parafollicular cells have been considered an independent cell line having a different function and origin from the follicular cells. In scanning electron microscopical appearance of vascular casts prepared with methyl methacrylate[3], the blood capillaries of parafollicular and follicular cells are closely anastomosed[4]. Why are the parafollicular cells, which have a function and origin different from the follicular cells, so intimately dispersed throughout the thyroid? We found by immunohistochemical methods, during investigation of the thyroid in dogs, that the parafollicular cells revealed thyroglobulin-like immunoreactivity in addition to calcitonin.

Materials and methods. Thyroglobulin was prepared from dog thyroid tissue according to a modified method of Ui and Tarutani[5], in which purification was performed on a column of Sephadex G-200 instead of DEAE cellulose as described by Berg[6]. The purified thyroglobulin antigen was injected s.c. in Freund's complete adjuvant (Difco) into the back of the neck of Japan white rabbits. After 4 injections at weekly intervals, the blood-sera samples were collected. Calcitonin was also extracted from dog thyroid according to the method of Potts et al.[7]. By means of Ouchterlony test (figure 1), 1-dimensional and 2-dimensional (Laurell method) immunoelectrophoresis, it was verified that antibody against purified thyroglobulin reacted only to thyroglobulin and not to calcitonin.

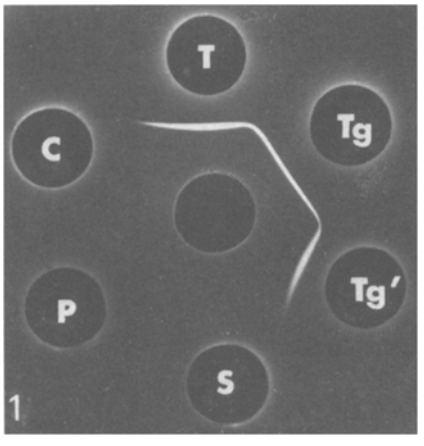

Fig. 1. Ouchterlony double diffusion in 1.5% agar and Tris-Na_2-EDTA-boric acid buffer, pH 8.4. Antiserum reacts against thyroglobulin but not calcitonin. Center well contains purified anti-thyroglobulin antiserum; T, thyroid homogenate in 0.9% NaCl; Tg, crude thyroglobulin; Tg', purified thyroglobulin; S, dog serum; P, pituitary homogenate in 0.9% NaCl; C, calcitonin.

For immunohistochemical methods, thyroid glands obtained from normal, chronically hypercalcemic (daily injection of 300,000 IU of vitamin D_3 and 0.5% $CaCl_2$ in drinking water for 20–30 days) and chronically antithyroid drug-treated (0.3–0.5% thiourea in drinking water for 1–4 months) dogs were fixed in Carnoy's, buffered (PBS) formalin, Bouin's and GPA (25% glutaraldehyde, 1 vol., saturated aqueous solution of picric acid, 3 vol., and acetic acid to give 1%) solutions at 4 °C. After dehydration, the tissues were embedded in paraffin and cut into 4–5 μm thick. Sections were first incubated at room temperature with anti-thyroglobulin antiserum for 30 min, then washed in PBS (0.15 M sodium chloride in 0.01 M phosphate buffer, pH 7.2). For immunofluorescence staining, the sections were reincubated for 30 min with fluorescein isothiocyanate labelled sheep anti-rabbit IgG (Medical and Biological Laboratories, Japan) which was purified on a column of Sephadex G-50 and absorbed with acetone-dried liver powder prior to use, rinsed in PBS and mounted in buffered polyvinyl alcohol. For immunoperoxidase staining, the sections were reincubated in goat antirabbit IgG for 15 min, rinsed in PBS and subsequently in peroxidase-antiperoxidase complex[8] for 30 min. After washing with PBS, the peroxidase reaction was developed with 50 mg of diaminobenzidine in 100 ml of 0.05 M Tris HCl buffer (pH 7.6) containing 1 ml of 1% H_2O_2. The specificity of the 2 immunohistochemical staining methods was tested by replacing the immune serum with pre-immune serum and absorbing the immune serum by crude thyroglobulin.

Results. In both immunofluorescence and immunoperoxidase histochemistry using undiluted anti-thyroglobulin antiserum, the strongest reaction throughout the thyroid was found in cells which were readily identified as parafollicular cells on the basis of their location, shape and size as well as the feature of their nucleus (figure 2). The immunoreactivity of the follicular cells and colloid droplets was much lower than that of the parafollicular cells. Furthermore, the reaction was confirmed in the para-

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follicular cell complexes[9], probable remnants of the ultimobranchial body, consisting of the parafollicular cell masses (figure 3). The parafollicular cells in the complexes were in various stages of differentiation and the immunoreactivity varied similarly from cell to cell. The undifferentiated cells[10] in the complex did not react. Reactions were observed in the cytoplasm of the parafollicular cells and not in the nucleus.

After chronically induced hypercalcemia or chronic injection of the antithyroid drug, the parafollicular cells displayed a more or less severe depletion of the secretory granules[11,12], which could be revealed by silver impregnation, HCl-basic dye and lead-hematoxylin stainings. The reactions of the immunofluorescence and immunoperoxidase histochemistry in these experimental conditions entirely coincided with the movement of the secretory granules revealed by the staining methods. In chronic hypercalcemia the immunoreactivity almost completely disappeared from the parafollicular cells (figure 4), and in hypothyroidea it was faint (figure 5). A diluted serum (above 1:10) provoked a strong reaction in the colloid droplets, especially at the margin of the luminal colloid, which was the main locus of the thyroglobulin iodination. In both immunofluorescence and immunoperoxidase stainings, no reaction was observed in the

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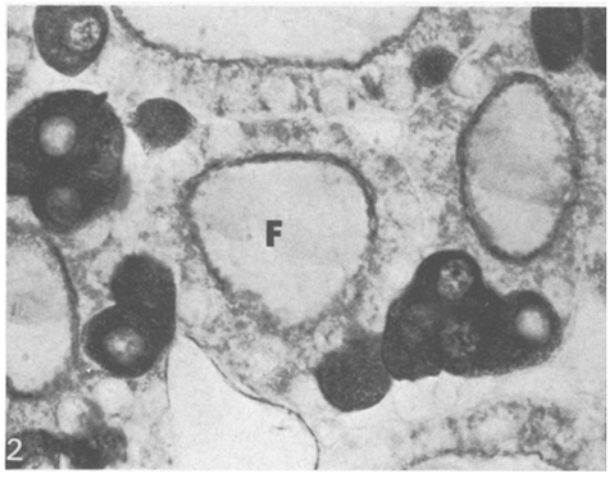

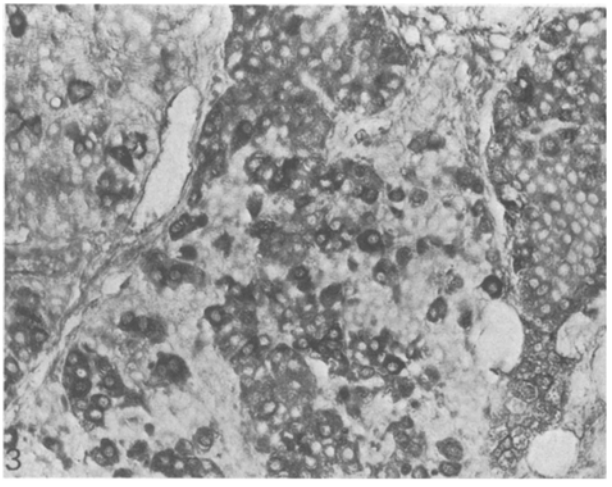

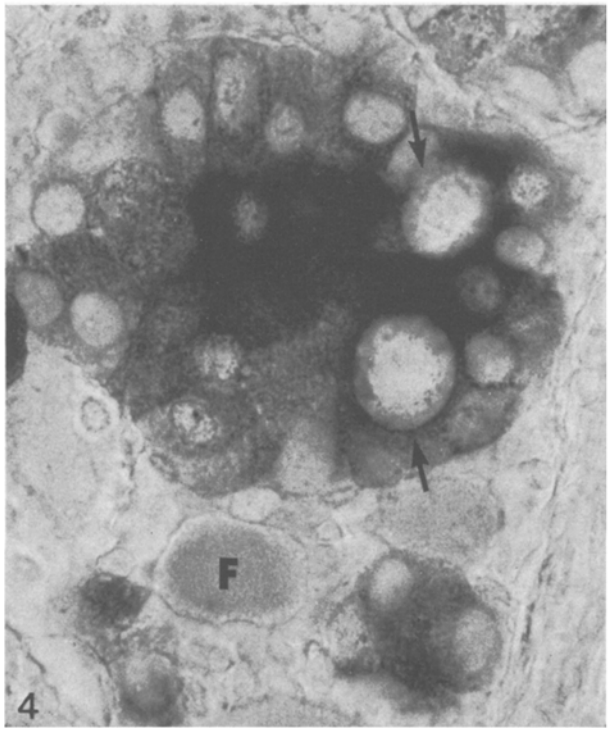

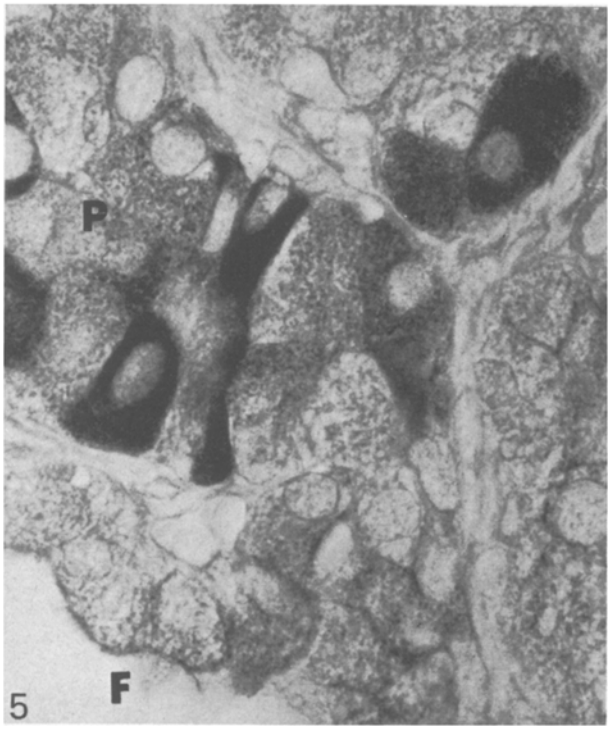

Fig. 2–5. The dog thyroid gland stained with immunoperoxidase method using undiluted anti-thyroglobulin antiserum. Fixed in Bouin. *2* Normal dog thyroid. Fine immunoreactive granules fill the cytoplasm of the parafollicular cells. F, follicle. × 800. *3* The parafollicular cell complex. Variably intense immunoreactivity corresponds to variable developmental stages of the parafollicular cells. × 300. *4* Dog after combined administrations of calcium and vitamin D_3 for 20 days. The enlarged parafollicular cells display conspicuous decrease of immunoreactive granules, especially at capillary side. Note 2 mitotic parafollicular cells (arrows). F, follicle. × 1000. *5* Dog treated with thiourea for 2 months. Some of the parafollicular cells (P) show roughly dispersed immunoreactive granules and enlarged cytoplasm. F, follicle. × 1000.

thyroid when the anti-thyroglobulin antiserum was absorbed with thyroglobulin or replaced by normal rabbit serum. Neither was a reaction evident in other tissue such as parathyroid, anterior and posterior lobes of pituitary and pars tuberalis after incubation with the antiserum.

Discussion. From the late 1950's to early 1960's, numerous immunofluorescence studies using anti-thyroglobulin antiserum were performed on the thyroid. However, since the existence of parafollicular cells in the thyroid was not noticed by previous investigators, there was no report on the immunoreactivity of these cells to antithyroglobulin. The present study is the first to demonstrate that the parafollicular cells contain thyroglobulin-like or immunologically similar protein, which seems to imply a close metabolic relationship between the parafollicular and follicular cells.

It is well-known that the thyroid gland shows conspicuous hyperplastic changes following the application of antithyroid drugs. One of the authors[12] previously found that the injection of thiourea for 1–4 months causes cytological changes not only in the follicular but also in the parafollicular cells of the canine thyroid: decrease in secretory granules, enlarged cell bodies and dilation of cisterns of rough endoplasmic reticulum. The significance of the parafollicular cell reaction to antithyroid drug could not be explained at that time. However, if the synthesis of the thyroglobulin-like substance takes place in rough endoplasmic reticulum-Golgi complex system of the parafollicular cells and if it is affected by the antithyroid drug, this parafollicular cell reaction, similar to that of the follicular cells, might well be acounted for.

It has been reported recently that various polypeptide hormones may be synthesized by way of prohormones, as in the case of proinsulin and insulin. Neurophysins associated with oxytocin and vasopressin in neurosecretory granules have been known as carrier proteins. Thus, the existence of inactive proteins in addition to active polypeptide hormone has been attracting more and more attention in different polypeptide hormone-secreting cells. It will be necessary to elucidate the cell-biological significance of the thyroglobulin-like immunoreactivity in the parafollicular cells as well as its relation to calcitonin.

Androgen dependency of hepatic hydroxysteroid dehydrogenases in the rat: Prepubertal responsiveness and unresponsiveness towards exogenous testosterone[1]

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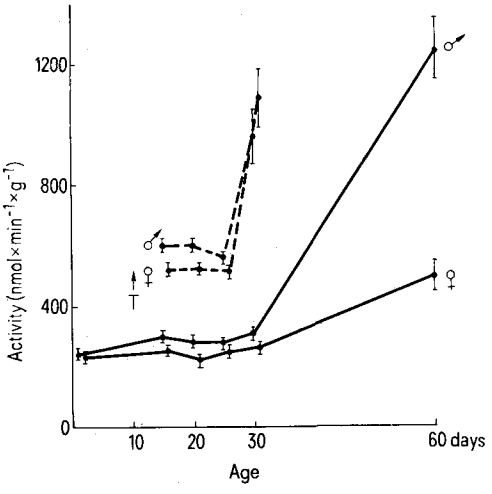
Summary. The prepubertal responsiveness of 3 typical androgen-dependent enzyme activities, namely 20-ketoreductase, 3α- and Δ^4-3β-hydroxysteroid dehydrogenase, towards a large dose of testosterone was investigated. The androgen induced the activity of the 3α-enzyme prepubertally in both sexes.

A phase of sexual indifference, lasting from day 1 to 30 of life, is observed in the ontogenesis of the sexually differentiated enzyme activities of hepatic steroid metabolism[2–4]. Androgen or oestrogen-dependency becomes apparent only after this phase has been completed (for criteria of androgen or oestrogen dependency see Lax et al. and Ghraf et al.[5,6]). In order to test whether this prepubertal indifference is due to either insufficient levels of circulating androgen or androgen unresponsiveness, 3 typical androgen-dependent enzyme activities were investigated after administration of a large dose of testosterone between day 10 and 13 of life.



Prepubertal responsiveness of NAD-dependent microsomal 3α-hydroxysteroid dehydrogenase activity of rat liver towards exogenous testosterone (T); (—), untreated animals; (---), treated animals. Bars represent means ± SD.

Material and methods. Male and female rats of the strain Chbb: THOM with intact gonads were used. Between day 10 and 13 of life, treated animals were administered 2 doses of Testoviron-Depot® s.c. (corresponding to a total amount of 100 mg testosterone) and the activities of the following microsomal liver enzymes were tested on day 15, 20, 25 and 30: NAD-dependent 3α-hydroxysteroid dehydrogenase[7], NADP-dependent Δ^4-3β-hydroxysteroid dehydrogenase[8] and NADP-dependent 20-ketoreductase[2]. Microsomal protein was estimated by the method of Lowry et al.[9]. The degree of significance between 2 mean

1 This investigation was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 87, Endokrinologie).

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