Time Sequence of Ultrastructural Changes in the Stimulated Dog Thyroid*

P. Nève and J. E. Dumont++

Laboratory of Pathological Anatomy and Electron Microscopy, Laboratory of Experimental Medicine and Laboratory of Nuclear Medicine, University of Brussels, and Biology Department***, Euratom, Brussels, Belgium

Received September 15, 1969

Summary. The kinetics of the effects of acute administration and of multiple injections of TSH on the ultrastructure of the thyroid of the dog were studied. The results are correlated with data obtained by light microscopy and by biochemical study of the tissue. The response to TSH is more delayed in the dog than in the rat; it is characterized by interfollicular and intrafollicular heterogeneity: only after 3 hours do all thyroid cells demonstrate signs of stimulation. The stimulation of each thyroid cell results in three overlapping stages: colloid phagocytosis, colloid digestion and morphological changes suggesting increased biosynthetic activity. The former effects are concomitant with the effects of TSH on the secretory process in vivo and the metabolic activation of thyroid slices in vitro; the latter effect parallels an increased RNA content of the tissue. Thyroids stimulated for 3 and 6 days by TSH exhibit marked hyperplasia and cellular hypertrophy. The colloid is scant and no colloid droplets are evident. After three days of TSH administration, numerous intracellular spaces, containing colloid are observed.

Key-Words: Thyroid stimulation dog — Ultrastructure.

During the last few years, the electron microscopy of the thyroid of rat, mouse, dog, and guinea pig, after in vivo stimulation by thyrotropin, has been described in several articles (Wissig, 1963; Tashiro and Sugiyama, 1964; Bauer and Meyer, 1965; Wetzel et al., 1965; Ekholm and Smeds, 1966; Seljelid, 1967a—f; Kosanovic et al., 1968; Lupulescu and Petrovici, 1968). Careful histochemical and autoradiographic techniques applied to electron microscopy have demonstrated that most intracellular droplets observed in stimulated glands result from the phagocytosis of luminal colloid by the follicular cells; these droplets fuse with lysosomes in the cells and are progressively digested thereafter (Nadler et al., 1962; Wollman et al., 1964; Wetzel et al., 1965; Ekholm and Smeds, 1966; Seljelid, 1967a, b). These convincing observations have been restricted to acutely stimulated thyroid and were not correlated with physiological or biochemical data.

For several years, the effects in vivo and in vitro of thyrotropin on the histology, physiology, and biochemistry of the dog thyroid have been studied in this laboratory (Dumont and Rocmans, 1964a, b). The purpose of the present work

* Work carried out in part under contract Euratom — University of Brussels — University of Pisa n° 026-63-4 BIAE, and supported by grant 1011 of the Fonds de la Recherche Scientifique Médicale and a grant of the Fonds National de la Recherche Scientifique (Crédit aux Chercheurs: P. Nève).

++ Our thanks are due to Professor P. Dustin for his help, to Doctor P. Rocmans for his surgical assistance, to Miss E. Bricourt and Mr. G. Vienne for their technical assistance, and to Miss Ch. Borrey for the typing of the manuscript.

*** Contribution n° 496 of the Biology Department, Euratom.
was to define by light and electron microscopy the time sequence of the acute and chronic effects of thyrotropin on the morphology of the dog thyroid in vivo and to compare histological and ultrastructural observations. These results will be correlated, with available data on the physiological and biochemical effects of thyrotropin.

**Material and Methods**

Sixteen dogs weighing about 20 Kg were used. Each dog received 300 mg of thyroid extract per day during the three days before the experiment. On the day of the experiment, the dogs were anesthetized with pentobarbital: one lobe of the thyroid was resected and used as a control, after which thyrotropin 15IU (Ambinon, Organon, Osa, Nederland) was injected intravenously; the other lobe was removed at the end of the stimulation period. The stimulation periods studied were 10, 30, 60, 120, 180 and 240 minutes and 24 hours. For the study of chronic stimulation, the surgical wound was closed after the removal of the control lobe and 15IU of thyrotropin were administered daily by intramuscular injection; the experimental lobes were resected at the end of the stimulation period (3 and 6 days respectively).

Each thyroid sample was studied by light and electron microscopy. For light microscopy, the pieces were fixed in Bouin’s solution, embedded in paraffin, sectioned and stained with PAS. The fragments of tissue for electron microscopy were transferred within one minute after excision to a drop of fixative (glutaraldehyde 4.2% in 0.1 M Millonig buffer solution, pH = 7.4), and divided with a razor blade into small pieces of about 1 mm³, which were placed in fresh chilled fixative for 4 hours. The fragments were rinsed overnight with Millonig’s (1962) buffer solution, containing glucose 30 mM and postfixed for 30 minutes with 2% osmium tetroxyde in the same Millonig’s buffer. Dehydration took place in rising concentrations of ethanol. The pieces were embedded in epon according to Luft (1961). Ultrathin sections were made with a diamond knife on a LKB microtome. The staining of the sections was carried out with both uranyl acetate and lead citrate (Reynolds, 1963), or by the method of Karnovsky (1961). A Siemens ElmiskopI electron microscope was used. Semi-thin sections stained with toluidin blue at pH = 12 were studied in all cases.

Physiological and biochemical investigations were carried out on similar dogs; the methods used and many of the results have been reported previously (Dumont and Rocmans, 1964a). RNA was measured by the method of Fleck and Begg (1965) and DNA by the method of Burton (1956). In these experiments, the stimulation period has been evaluated as the sum of the delay between TSH injection and removal of the stimulated lobe and of the incubation time.

**Results**

1. Resting Thyroid

As treatment with thyroid extract for 3 days can be assumed to suppress the stimulation of the thyroid by the pituitary, the thyroid control lobes can be considered as “resting thyroids” (Freinkel, 1964). Light microscopy showed rounded follicles of middle size with a low epithelium (cell height: 5—8 μ) and a wider interfollicular space than in the normal human thyroid (Nève, 1965; Heiman, 1966). Survey electron micrographs confirmed the relative importance of the interfollicular space which contained mainly blood capillaries and connective tissue. Numerous parafollicular cells were observed. The follicular cells were flattened or cuboidal (Fig. 1). A basement membrane, about 800 Å thick, enclosed the whole follicle. The lateral plasma membrane formed infoldings between adjacent cells. Occasional desmosomes were seen peripherally to the typical junctional complexes (Wissig, 1960; Heiman, 1966; Seljelid, 1967c) which closed at their apex the intercellular spaces. The classical apical microvilli whose maximal length was 0.8 μ showed an interior longitudinal striation. No central