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The ultrastructure of rat thyroid glands under experimental conditions in organ culture

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INTRODUCTION

Organ culture has been carried out with considerable success in studies of the functional anatomy of many glands (eg. ovary: Baker & Neal, 1969; pituitary: Tixier-Vidal, 1975; Baker & Young, 1979). An excellent review on the culture of thyroid glands was given by Cau, Michel-Béchet & Fayet in 1976.

In an earlier study (Baker & Young, 1973) we showed that the rat thyroid could be well maintained in a simple organ culture system. The present report is concerned with experiments in which the effects of thyroid-stimulating hormone (TSH) and an antithyroid drug (methylthiouracil) were assessed.

MATERIALS AND METHODS

A total of 28 hooded Wistar rats were used in this study. The animals were divided into five experimental groups.

Group 1. Normal untreated rats.

Group 2. Normal control rats injected with sodium hydroxide (NaOH) in saline daily for seven days before culture (see below).

Group 3. Rats injected daily with 0·02 g methylthiouracil (Sigma, London) for seven days before culture.‡

Group 4. Rats injected with 0·5 I.U TSH in Eagle's minimum essential medium (MEM) one day before the thyroid was removed for organ culture.

Group 5. 0·5 I.U TSH (Sigma, London) in Eagle's MEM was added to the explanted thyroids on the third day of culture.

The rats were killed with chloroform and the entire left lobe of the thyroid gland was cleanly and aseptically dissected into Eagle's MEM culture medium at 34 °C. The glands were then cut into explants measuring 1–2 mm^3 and were cultured for 6 days.

The techniques used for maintaining the explanted thyroid glands in organ culture have been described before (Baker & Neal 1969; Baker & Young, 1973, 1979).

At the end of the period of culture (6 days) the explants were fixed for electron microscopy with Millonig's buffered formalin, post-fixed with 1 % osmium tetroxide and embedded in Epon. Ultrathin sections, cut on a Reichert OMU 2 microtome, were stained with uranyl acetate and lead hydroxide and were examined with an AEI 801 electron microscope.

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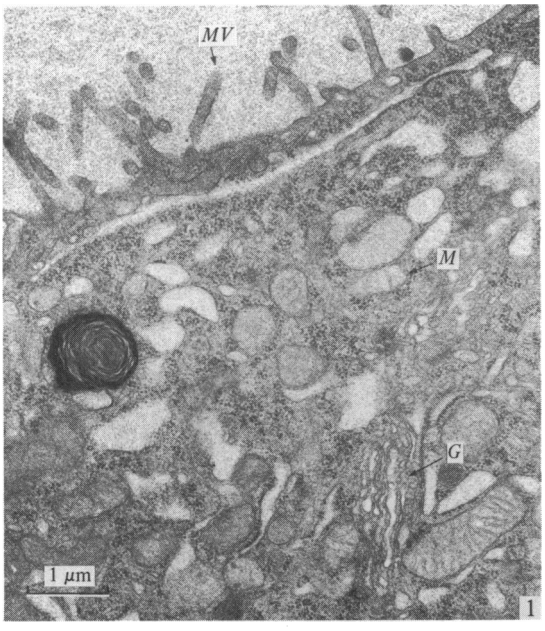
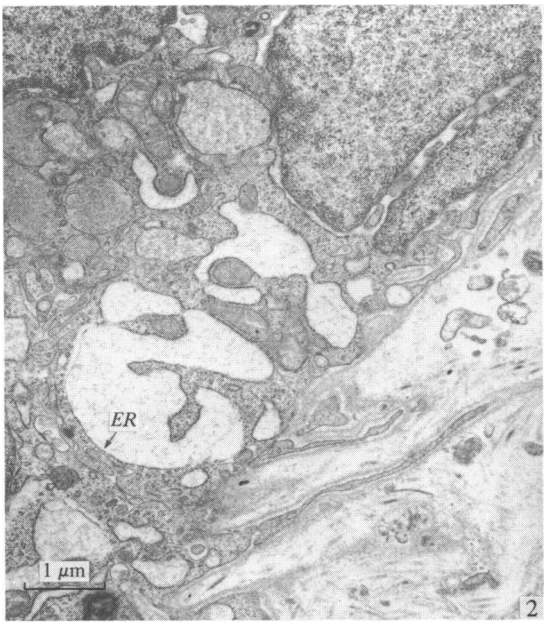
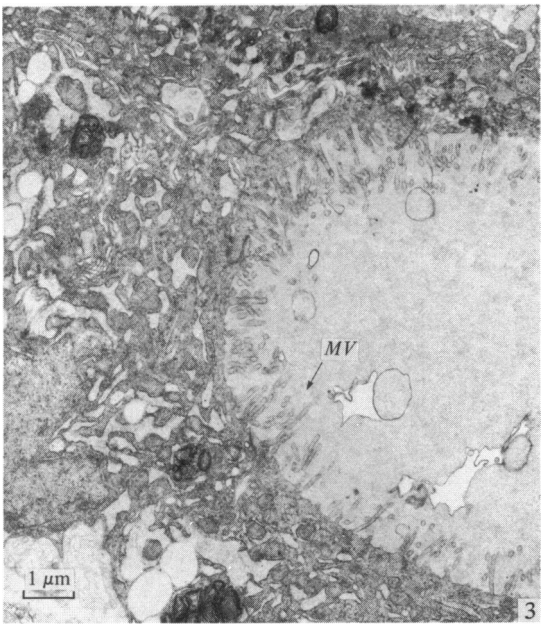
‡ The drug was prepared by shaking 1 g of the compound in sterile water and then *N* NaOH was added dropwise until the solution was just alkaline. Injections were given subcutaneously.







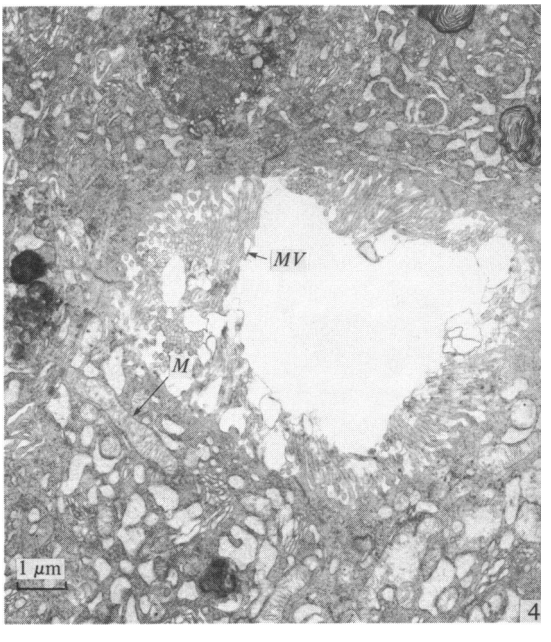


Fig. 1. Electron micrograph of a follicular cell from a control culture. *MV*, microvilli; *G*, Golgi zone; *M*, mitochondria.

Fig. 2. Electron micrograph of a follicular cell from a control culture. *ER*, endoplasmic reticulum.

Fig. 3. Electron micrograph of follicular cells when methylthiouracil was given before culture. Note the microvilli (*MV*).

Fig. 4. Electron micrograph of follicular cells when methylthiouracil was given before culture. Note the numerous microvilli (*MV*) and the elongated mitochondria (*M*).

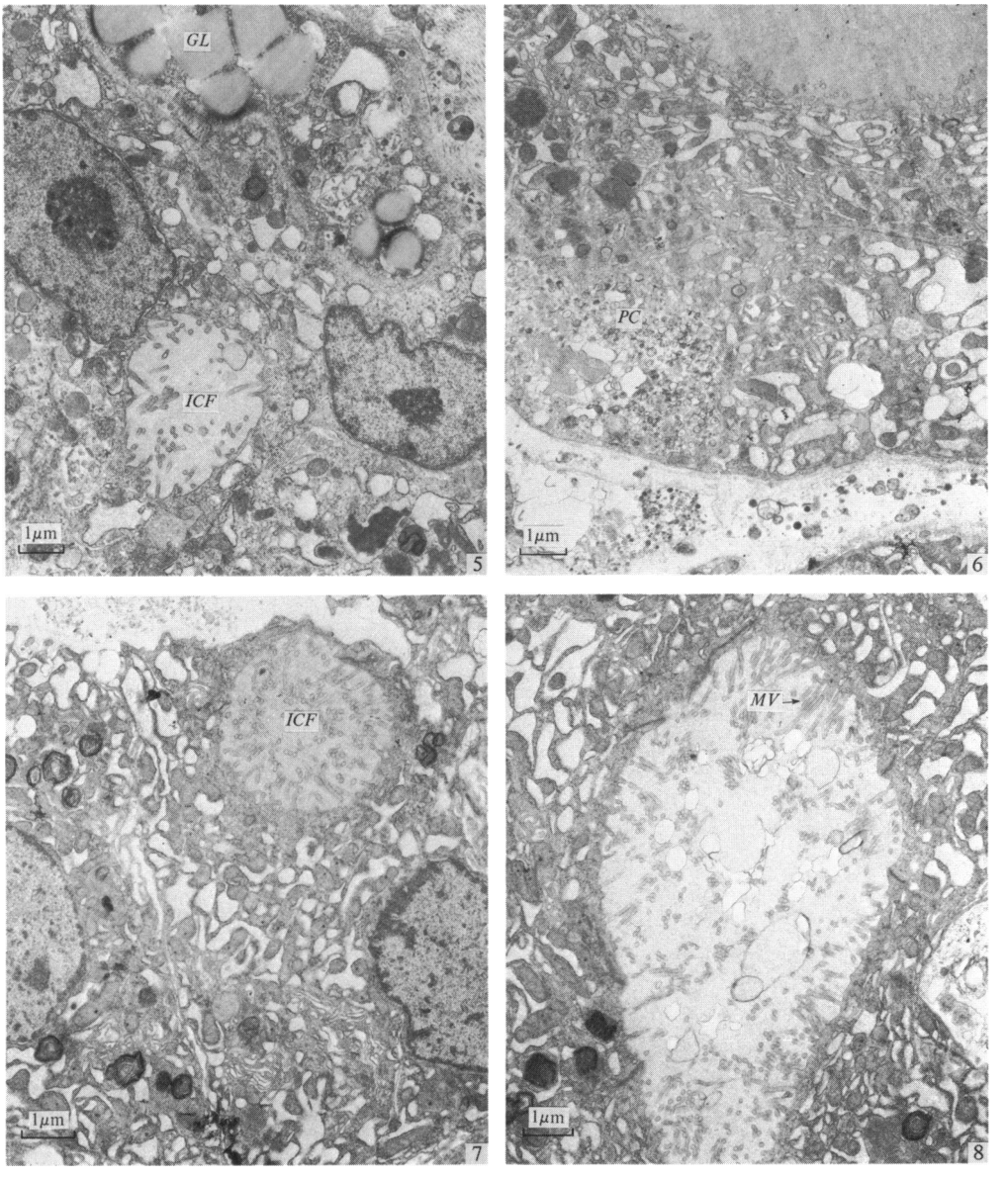


Fig. 5. Electron micrograph of follicular cells when TSH was given one day before culture. Note the 'intracytoplasmic' follicle (*ICF*) and the large globules (*GL*).

Fig. 6. Electron micrograph when TSH was given one day before culture. Observe the parafollicular cell (*PC*).

Fig. 7. Electron micrograph of a follicular cell with TSH injected into culture. Note the 'intracytoplasmic' follicle (*ICF*).

Fig. 8. Electron micrograph of a follicular cell after TSH had been injected into culture. Note the great length of the microvilli (*MV*).

RESULTS

(1) *Control cultures*

Because the antithyroid drug methylthiouracil was dissolved in saline containing NaOH, two control groups of rats were required to assess the potential cytotoxic actions of the vehicle. No ultrastructural differences were detected between rat thyroids in Groups 1 and 2 and thus NaOH was seemingly without effect. The following description of the ultrastructure of the thyroid gland applies to both control groups.

The rat thyroid glands consisted of typical follicles which contained colloid. The epithelium of the follicle consisted of two types of cell within a basement membrane: the main follicular cell and the so-called parafollicular or 'light' cells. In the rat, light cells form 1–5 % of the total population of epithelial cells (Young & Leblond, 1963).

In our material, the follicular cells were cuboidal and their apices were characterised by numerous irregularly arranged microvilli (up to 1·4 μm in length: see Fig. 1). Typical tight junctions and intracellular channels were found between adjacent cells (Young, 1966).

The endoplasmic reticulum of the follicular cell was distributed throughout the cytoplasm and towards the base of the cell its cisternae were dilated (Fig. 2). Free ribosomes were detected and a prominent Golgi zone was apparent. Numerous typical elongated mitochondria and lysosomes were observed. The cells contained apical vesicles but intracellular colloid droplets were not detected.

(2) *Methylthiouracil administered before culture*

Thyroid glands removed from animals in Group 3 were larger than those in controls (Groups 1 and 2).

The follicular cells possessed even more numerous and larger (up to 2·0 μm) microvilli than controls (Fig. 3). No obvious changes were detected in the Golgi region or in the incidence of lysosomes but the endoplasmic reticulum consisted of more dilated cisternae. The mitochondria were elongated (up to 2·3 μm: see Fig. 4) and the nuclei were more oval or indented.

(3) *Animals injected with TSH one day before the onset of organ culture*

These thyroids were characterised by what have been called 'intracytoplasmic follicles' (Cau *et al.* 1976: see Fig. 5). These consisted of large cavities or 'vacuoles' within the cytoplasm of the follicular cell and possessed numerous microvilli protruding into a faintly granular 'secretion' which was paler than colloid (Fig. 6). The nuclei of the follicle cells were often elongated and indented while a few droplets and several large globules of colloid were seen. Between these cells, collagen fibres, connective tissue cells and even those resembling mast cells (Hentzer & Kobayasi, 1975) were observed. Partially degranulated parafollicular ('light') cells were found on two occasions (Fig. 6). They were characterised by the presence of electron-dense granules of 0·16 μm diameter and were associated with the basement membrane (Young, 1980). Endoplasmic reticulum was not detected in these 'light' cells.

(4) *Addition of TSH to rat thyroid glands in organ culture*

The most conspicuous feature of these glands was the presence of the 'intracytoplasmic follicles', referred to above (see Fig. 7). The 'true' follicles possessed

numerous prominent microvilli of up to 1·8 μm in length (Fig. 8). Only a few colloid droplets were seen, although the cisternae of the endoplasmic reticulum were dilated (Fig. 7), which might be indicative of secretion. Lysosomes were frequently observed.

DISCUSSION

In the control cultures the microvilli were numerous and elongated (up to 1·4 μm), a feature which was noted by Baker & Young in 1973. Fujita (1975) gives the normal length of microvilli in the rat as 0·44 μm. The mitochondria were of the elongated form and appeared to be increased in number: a prominent Golgi zone was seen. These changes would be consistent with increased stimulation of the thyroid by TSH. Endogenous TSH fixed to the cell membrane at the time of isolation would be activated more slowly by thyroid cells *in vitro* than *in vivo* (Cau *et al.* 1976). The same features were observed in the explanted thyroids which had received TSH in organ culture.

In our experiments, TSH was used to stimulate activity in the glands (Yoshimura, Yomestsu & Nakamura, 1962), while methylthiouracil was used to inhibit thyroid activity (Millar, Roblin & Astwood, 1945). The latter authors had also assessed the action of antithyroid drugs *in vitro*.

When TSH was injected into rats 24 hours before the onset of organ culture, few ultrastructural changes could be detected compared to the control group. The most conspicuous feature was an increase in colloid droplets, indicating that secretory processes can be demonstrated by using organ culture. However, Wissig (1960) has shown that the maximum incidence of these droplets is found about 2 hours after injection of TSH, indicating that our injections were carried out too late to demonstrate actual secretion.

The most noteworthy feature of the explanted thyroids that had received TSH in organ culture was the presence of what have been called 'intracytoplasmic follicles' (Cau *et al.* 1976). In micrographs, these structures resemble small vacuoles – seemingly filled with the colloid material – within the follicle cells. However, when these structures were carefully followed through serial sections they were found to be pouches which were continuous with the main follicle (Cau *et al.* 1976). We have retained the name given to the pouches by Cau *et al.*, to avoid confusion, and have confirmed that the structures contain numerous elongated microvilli. Their significance in the Cervidae has been discussed by Young (1976).

Of particular interest was the finding of parafollicular ('light') cells in the organ cultured thyroid glands. There has been some confusion in the literature as to whether these cells are influenced by TSH. Thompson, Isler & Sarkar (1962) found no evidence for an effect of TSH on the ultrastructure of 'light' cells, but Yoshimura, Yachi, Ishikawa & Kiguchi (1972) found hyperplastic changes at the light microscope level following addition of TSH to cultured thyroids. In the present study, two partially degranulated 'light' cells were detected when TSH was administered to the rat 24 hours before the onset of culture. The significance of the observation is limited by the paucity of these cells in the thyroid (especially at the high magnification used in electron microscopy) but TSH might be implicated in at least the release of secretory granules from the parafollicular cells. Similar observations have been made for the thyroid of the Plains viscacha (*Lagostomus maximus*) *in vivo*, where treatment with TSH results in the formation of spherical empty vesicles in 'light' cells (Young, 1980).

Numerous changes at the light and electron microscope levels have been reported when the effect of goitre-inducing agents is studied *in vivo* (Wissig, 1960). The agent that we used (methylthiouracil) cannot be added to organ cultures because of its effect on the pH of the medium and its limited solubility. Nevertheless, changes similar to those reported by Wissig (1960), including numerous elongated microvilli and elongated mitochondria, can be detected *in vivo* following treatment with methylthiouracil *in vivo*. It would seem therefore that organ culture provides a simple model or assessing thyroid physiology and biochemistry.

SUMMARY

A technique has been described which permits the maintenance for up to 6 days in organ culture of explanted thyroid glands. This provided a suitable model for an electron microscope study to be carried out on glands under experimental conditions. The appearance of the control gland was consistent with stimulation by TSH. The formation of 'intracytoplasmic' follicles was caused by injection of TSH into the culture. Parafollicular (light) cells were described for the first time in organ cultured thyroid glands.

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