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Seasonal changes in the ultrastructure of the thyrotroph in the woodchuck (*Marmota monax*) adenohypophysis

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INTRODUCTION

Annual cycles of endocrine function related to the seasonal activities of hibernators are well documented (Kayser, 1961; Mrosovsky, 1971). Many studies have been specifically concerned with the seasonal changes in thyroid activity of various hibernators (Zelasky, 1935; Wenberg & Holland, 1973; Hulbert & Hudson, 1976; Demeniex & Henderson, 1978*a*, *b*; Krupp, Young & Frink, 1977; Young *et al.* 1979). The thyroid gland of hibernators appears to be most active in early spring, gradually becomes less active during the late spring and summer, is almost completely inactive by the time the animals hibernate and commences activity again in late winter just before arousal from hibernation. The secretory activity of the woodchuck thyroid follicular cell, as estimated from electron microscopic observations, correlates closely with the amount of free active thyroid hormone in the blood during all seasons of the year (Krupp *et al.* 1977; Young *et al.* 1979).

Annual cycles of activity of the pituitary of several hibernators, including ground quirrels (Hoffman & Zarrow, 1958; Kadurina & Stefanox, 1974), the European marmot (Coninx-Giradet, 1927) and the American woodchuck (Rasmussen, 1921; Cushing & Goetsch, 1915), have been described on the light microscopic level and the trophic effect of thyroid-stimulating hormone (TSH) synthesized and secreted by the thyrotrophs of the anterior pituitary is well documented (Wissig, 1963; Dempsey & Peterson, 1955).

This study correlates the ultrastructure of the thyrotroph of the woodchuck pituitary during four seasons of the year with the previously reported data on thyroid follicular cell morphology and blood thyroid hormone levels in the same animals (Krupp *et al.* 1977; Young *et al.* 1979).

MATERIALS AND METHODS

Adult female and male woodchucks were captured in the spring (March), summer (July) and autumn (late September). Some of the autumn animals were allowed to hibernate in a dark, cold room at 7 °C until winter (December) when they were killed. The other animals were caged individually and fed Purina Rabbit Chow and water *ad libitum* for about 8 days before being killed by decapitation at midday. Pituitary glands were quickly excised, sliced in half horizontally and fixed for 4 hours in cold 4·2% glutaraldehyde in 0·1 M Millonig's buffer at pH 7·2. Small blocks of tissue

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from the peripheral portions of the anterior lobe were post-fixed in the above buffered 1% osmium tetroxide for 1 hour, processed and embedded in Epon 812. Thick sections (1 μm) were deplasticised and stained with aldehyde fuchsin to identify thyrotrophs (D'Angelo, 1955; Moriarty, 1973) and adjacent thin sections (80–100 nm) were stained in saturated aqueous uranyl acetate and lead citrate, examined and photographed with a Philips EM300. Cilia ratios were counted and calculated after the method of Dingemans (1969).

OBSERVATIONS

The thyrotrophs of the woodchuck pituitary are large, angular or polyhedral cells with an eccentric, pleomorphic nucleus, and a prominent nucleolus. They occur singly or in clumps and contain granules within their cytoplasm which measure from 40 to 160 nm in diameter, most measuring less than 100 nm. The granules are generally round and fairly uniformly electron-dense. Thyrotrophs tend to be located adjacent to blood vessels and frequently granule-filled cytoplasmic projections of these cells are observed between other cell types, often appearing to surround the cell completely.

In the *winter* thyrotroph, granules may be seen near the plasma membrane but are not frequently observed in contact with it. Most of the granules show a space between the dense core and its limiting membrane and some variation in electron density occurs between granules. Granular endoplasmic reticulum is sparse and scattered and Golgi zones are small. Crinophagic vessels are not prevalent but some granules appear to be fused together (Fig. 1). The cilia/nuclear ratio is 1:15·8.

The *spring* thyrotrophs appear larger and are more frequently encountered. They contain large Golgi zones, free ribosomes and granular endoplasmic reticulum frequently seen in parallel arrangement and sometimes dilated. Granules appear throughout the cytoplasm but are mainly at the periphery of the cell. They are uniformly dense and are frequently seen to be in contact with the plasma membrane. Mitochondria are numerous and there is an abundance of crinophagic vessels (Fig. 2). The cilia/nuclear ratio is 1:50.

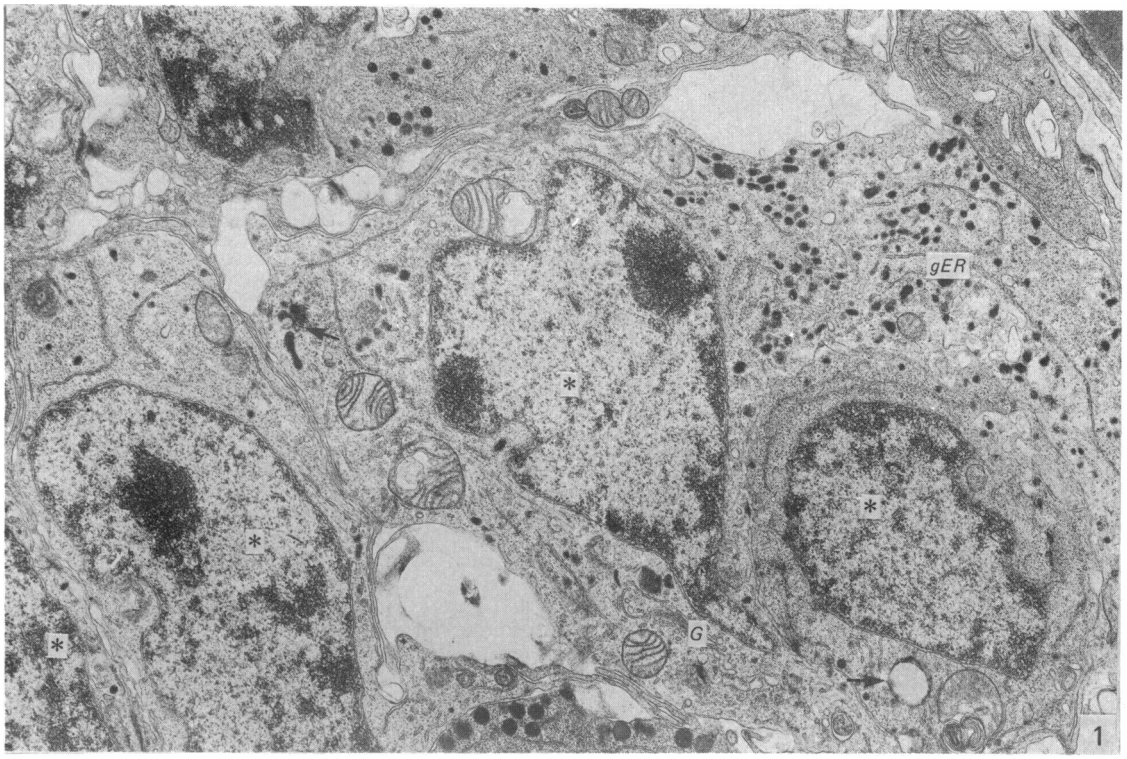
The *summer* thyrotroph is similar to the spring except that there are fewer granules and they are seen to be characteristically lined up around the periphery of the cell; there is usually a space between the central core and the limiting membrane of the granule and crinophagic vessels are sparse (Fig. 3). Cilia/nuclear ratio is 1:13·7.

Occasionally, cells similar to the summer thyrotroph are seen in the *autumn*, but most resemble those seen in the winter gland (Fig. 4). Cilia/nuclear ratio is 1:17·6.

DISCUSSION

The thyrotroph of the woodchuck pituitary is not unlike that described for the guinea-pig (Beauvallian, Mazzuca & Dubois, 1976), rat and human (Moriarty & Tobin, 1976), rabbit (Salazar, 1963) and the mouse (Barnes, 1962), the small granule size and its angular shape being rather consistent features of this cell type.

Even though this study was restricted to one of the basophils of the pituitary, the thyrotroph, our observations tend to confirm the findings of Rasmussen (1921). The ultrastructure of the pre-hibernatory gland did not differ significantly from that of the gland during hibernation, as Rasmussen observed at the light microscope level. He reported a tripling in the relative number of basophils in the spring and



Figs. 1–4. Thyrotrophs of woodchuck pituitary gland. Granular endoplasmic reticulum (*gER*), Golgi zone (*G*), Nuclei of thyrotroph (*) and crinophagic vessels (arrows).

Fig. 1. Winter. × 8600.

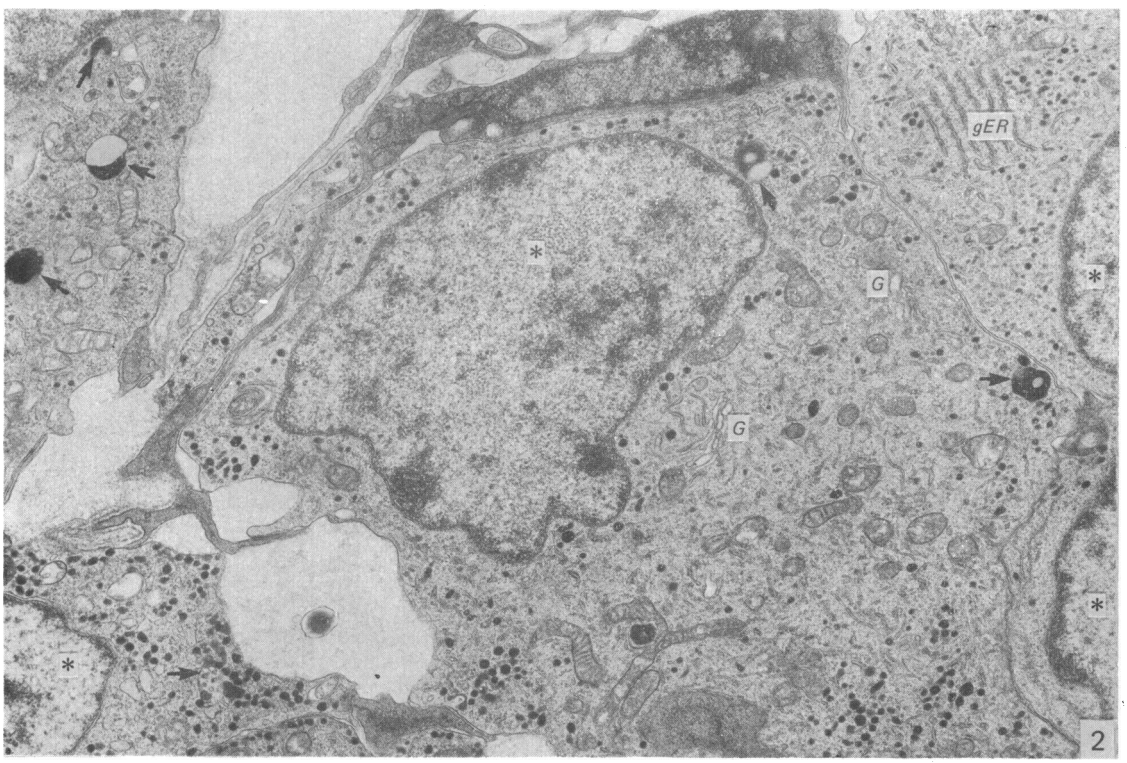


Fig. 2. Spring. × 8600.

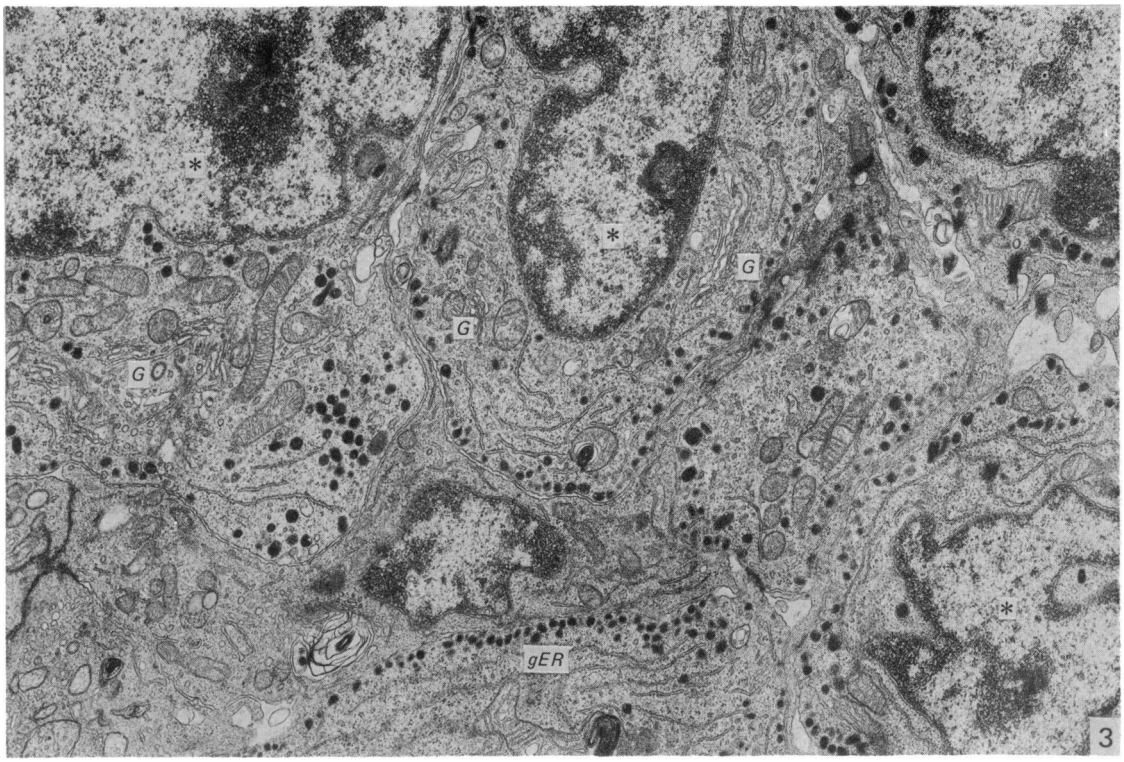


Fig. 3. Summer. × 8600.

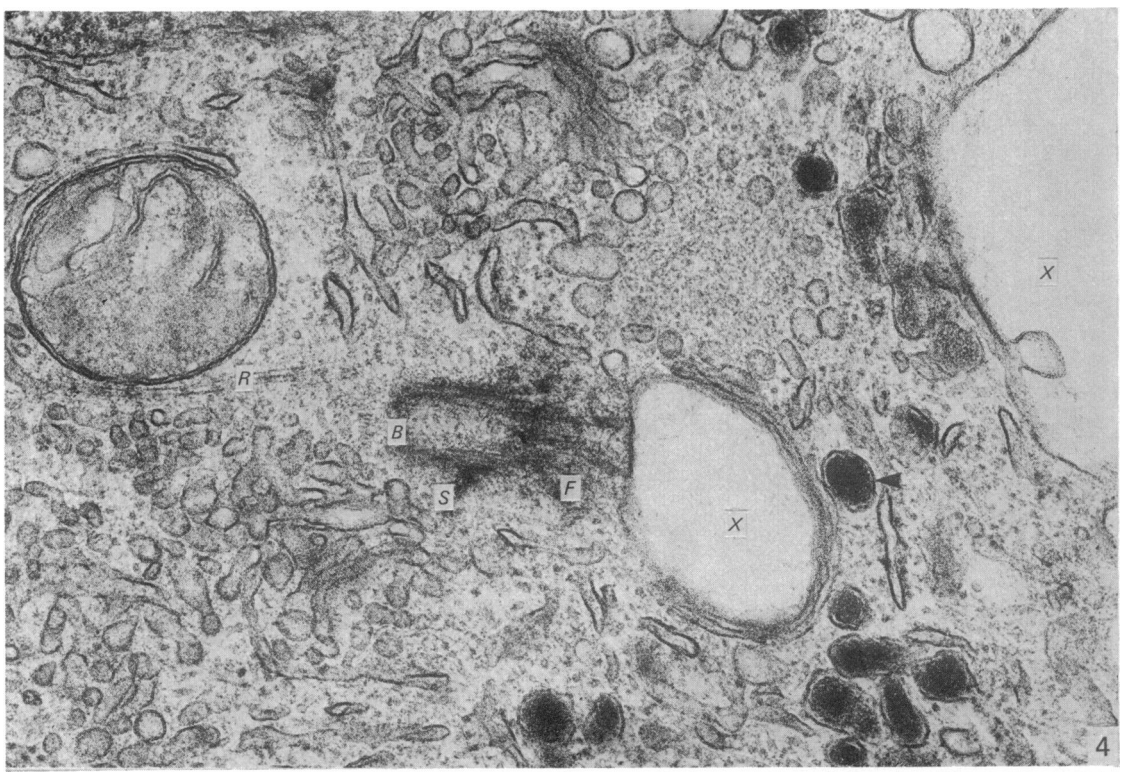


Fig. 4. Autumn, showing basal body (*B*), satellites (*S*), transitional fibres (*F*), rootlet (*R*) of cilia, extracellular space (*X*) and granules (arrowhead). × 55000.

an increase in the intensity of their staining reaction. Our results show confirming ultrastructural evidence in the form of cilia/nuclear ratio and cytoplasmic granule content in the thyrotroph.

Cilia have been reported in human and animal pituitary glands (Rasmussen, 1929; Wheatley, 1967; Salazar, 1963; Kagayama, 1965; Dahl, 1967). It has been reported that most cells in the pituitary contain a cilium but that cells with a high mitotic activity contain one less frequently, since centrioles cannot be involved in both mitosis and in the basal structure of a cilium at the same time (Dingemans, 1969). Our cilia/nuclear ratios lay between 1:13·7 and 1:17·6 in all seasons except spring where the ratio was significantly lower at 1:50.

The cessation of hibernation in the spring coincides with the onset of the breeding season. The woodchuck continues to lose weight as food is not yet easily available to it. The serum free (active) T3 and T4 concentrations are the highest of any season (Young *et al.* 1979), and both the thyrotroph of the pituitary and the majority of the thyroid follicular cells ultrastructural morphology indicate great synthetic and secretory activity. The number and frequency of crinophagic vessels seen in the spring thyrotroph may indicate that a regulation of the secretory process is being effected by lysosomal disposal (Farquhar, Skutelsky & Hopkins, 1975) or the disposal of secretory granules that had 'wintered through', i.e. synthesized prior to hibernation.

The serum thyroid hormone levels are lower in the summer than at any other time of year (Young *et al.* 1979), and we observe the expected synthetic and secretory activity of the thyrotroph, which is similar to the spring cell but is sparse in crinophagic vessels. Now that the food supply is plentiful and the physiological demands of the breeding season have passed, the thyroid follicular cells are synthesizing and storing colloid and the animal fattens (Krupp *et al.* 1977).

The morphological evidence of synthetic and/or secretory activity and granule storage noted in other endocrine organs of the woodchuck (Frink, Krupp & Young, 1978*a*, *b*) is not seen in the fall thyrotroph; rather, there are indications of a gradual lessening of activity prior to the hibernatory period. This parallels the changes seen in the thyroid follicular cell during this time of year (Krupp *et al.* 1977).

As cold weather approaches and food becomes scarce in the autumn, the woodchuck has nearly doubled in weight since spring. An expected response to the onset of cold weather would be an increase in TSH production and a concomitant increase in follicular cell activity (Melander & Rerup, 1968; Straw & Gregly, 1967). That this is not seen may be due to the high plasma concentrations of bound thyroid hormones in the autumn animal (Young, 1975; Young *et al.* 1979; Wenberg & Holland, 1973), which inhibit TSH synthesis. The metabolic rate of the woodchuck is also low at this time (Bailey, 1965) as food supplies lessen and the animal prepares to hibernate. Hulbert & Hudson (1976) report that in the ground squirrel, the hypothalamic–pituitary–thyroid axis may not be the same in hibernators as in other mammals since the thyroid does not respond to cooling at any time of the year.

During the hibernatory period, the demands for thyroid hormones occasioned by periodic arousals may be taken care of by the conversion of existing bound plasma T3 and T4 to the free mode (Young *et al.* 1979), and the ultrastructural quiescence observed in both the thyroid follicular cell and the thyrotroph of the anterior pituitary may be a manifestation of the resting stage of the cell's secretory cycle. However, Demeniex & Henderson (1978*a*, *b*), studying the annual cycle of Richardson's ground squirrel, report that their results indicate evidence of continued thyroid function during hibernation in this animal.

The concomitant cyclic activity of the woodchuck thyroid, pituitary and serum T3 throughout the year is summarized in the following chart:

		Thyroid activity		
	Free T3	Secretory	Synthetic	Pituitary activity
Spring	+++	++	—	++
Summer	++	+	+++	+++
Autumn	+	+	+	+
Winter	—	—	—	—

These data appear to support the literature on hibernators previously cited in that (1) correlation is observed between pituitary and thyroid activity, and (2) the thyroid in hibernation does not appear to be responsive to low temperatures. In addition the ultrastructure of the thyrotroph of the winter animals supports the conclusions of Hulbert & Hudson (1976) and Krupp *et al.* (1977) that the winter thyroid is inactive.

SUMMARY

Thyrotrophs of the woodchuck anterior pituitary are described through the four seasons of the year. Evidence of an increase in number and activity in the spring is presented. Activity of the thyrotroph is seen to continue through the summer and decline in the autumn to quiescence during the hibernatory period of winter. The significance of these findings is discussed in relation to thyroid follicular cell morphology, serum T3 and T4 levels and the physiological activities of the animal. High levels of thyroid hormones in the serum may be consistent with the appearance of great activity seen in the thyrotroph and follicular cell when taken in context with breeding season, ambient temperature and lack of food.

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