DETECTION OF GLYCOPROTEINS AND AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]FUCOSE IN THE THYROIDECTOMY CELLS OF RAT ANTERIOR PITUITARY GLAND

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When the secretion of thyroid hormones is prevented by thyroidectomy or propylthiouracil (PTU) treatment, the anterior pituitary cells secreting the thyroid-stimulating hormone (TSH) become enlarged and their rough endoplasmic reticulum (RER) forms large dilated cisternae (1, 2). These cells are known as the thyroidectomy cells. The presence of granules in the dilated cisternae is a common finding 3 wk or more after suppression of thyroidal activity (1). These intracisternal granules are thought to be different from the secretory granules observed free in the cytoplasm (1). To our knowledge, little information is available on the composition of intracisternal granules. In this preliminary study, we report the histochemical detection of glycoproteins in the granules of thyroidectomy cells and the localization of [^{3}H]fucose, which is known to be a terminal sugar (3), in the carbohydrate side chains of TSH (4).

MATERIALS AND METHODS

Male Sprague-Dawley rats (275–325 g) were given tap water containing 0.05% PTU as well as Purina Chow. After 2 mo of treatment, two animals were killed and their pituitaries were quickly removed. Each anterior pituitary was cut into four parts and incubated for 2 min in 1 ml of Krebs-Ringer solution containing 1 mCi of [^{3}H]fucose (sp act 4.8 Ci/mmole) in a Dubnoff metabolic shaker at 37°C.

In one experiment, pituitary fragments were washed three times in a solution containing 0.20% nonradioactive L-fucose before fixation. Pituitary tissue was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. As control, pituitary fragments were incubated at 0°C in the presence of tritiated fucose and fixed 2, 30, and 120 min after the beginning of the incubation. After 1 h of fixation, the tissue was rinsed in the same buffer, postfixed in 1% OsO_4 in cacodylate, dehydrated in ethanol, and embedded in Epon. Semithin (1 μm) and ultrathin (silver) sections were autoradiographed, re-

spectively, with Kodak NTB-2 and Ilford L-4 emulsions. After an appropriate time of exposure, they were processed and poststained with toluidine blue (semithin section) or lead citrate (ultrathin section).

For histochemical detection of glycoproteins, pituitary tissue from two hypothyroid rats was fixed in glutaraldehyde or paraformaldehyde (4%) and embedded in glycol methacrylate (GMA) or Epon. The ultrathin sections or the tissue fixed in glutaraldehyde and embedded in glycol methacrylate were then reacted with phosphotungstic acid (PTA) at low pH according to the technique of Rambourg (5). The periodic acid-Schiff (PAS) staining procedure was also performed on 1 μm sections of the tissue fixed either in glutaraldehyde or paraformaldehyde after removal of the Epon for examination in the light microscope.

RESULTS

The pituitaries of hypothyroid rats were found to contain many thyroidectomy cells characterized by the presence of dilated cisternae of the RER (Fig. 1). It was not infrequent to observe cisternae which were very dilated (Figs. 1, 4, and 5). Many cisternae, including the megacisternae, contained granules. The secretory granules observed in the cytoplasm were relatively few in number. In unstained sections of the GMA-imbedded tissue, the density of the intracisternal granules was not very important (Fig. 2). Intracisternal granules as well as large lysosomes were regularly stained by the PTA technique (Fig. 3). The dense material contained in the dilated cisternae has little or no affinity for the stain. The secretory granules found in the cytoplasm gave a strong positive reaction. The PAS procedure also shows positive granules within the large cisternae of the thyroidectomy cells in both paraformaldehyde- and glutaraldehyde-fixed tissue (Figs. 4 and 5).

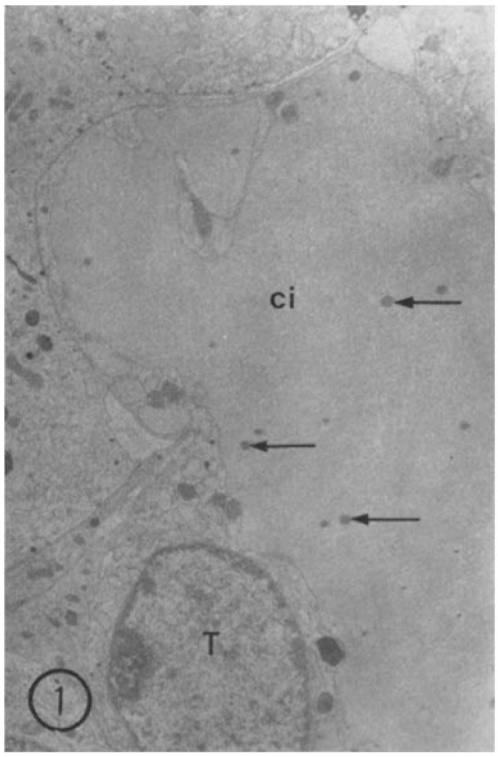
When the tissue has been incubated at 0°C with the [^{3}H]fucose to prevent the incorporation of the sugar, no autoradiographic reaction was observed at any of the time intervals studied, indicating no retention by glutaraldehyde of the free labeled sugar. The light microscope autoradiographs revealed that after 2 min of incubation in the presence of [^{3}H]fucose, radioactivity was found in the cytoplasm of every cell. No difference in the localization of silver grains was recorded in tissue washed in a solution containing cold fucose before the fixation. Many grains were regularly detected over the dilated cisternae of the thyroidectomy cells (Fig. 6). At the electron microscope level, the localization of 310 silver grains was studied in the thyroidectomy cells. About 30% of the grains were associated with the Golgi apparatus and 60% were associated with the RER (Figs. 7, 8, and 9). The radioactivity was localized over cisternae that both did and did not contain intracisternal granules (Figs. 8 and 9). Some grains were occasionally seen over mitochondria, lysosomes, nuclei, and plasma membranes.

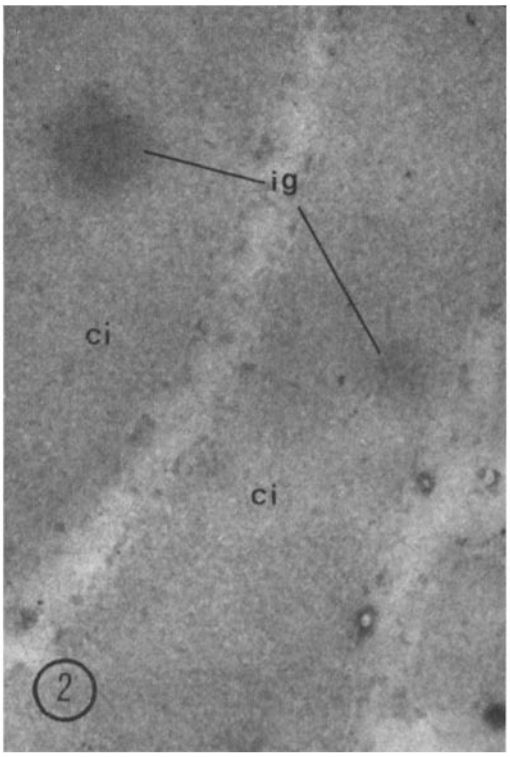
DISCUSSION

As evidenced by the PAS procedure, these results suggest that the intracisternal granules of the thyroidectomy cells contain glycoprotein. Since after glutaraldehyde fixation some aldehyde residues could give a false positive reaction, tissue fixed in paraformaldehyde was studied and also found to be PAS positive. These results were also confirmed at the ultrastructural level by the PTA reaction. Although there is still some controversy about the specificity of the PTA reaction (6), this technique is useful in order to detect glycoproteins. In fact, the present results provide another example of a correlation between the PTA and PAS techniques. The absence of positive reaction in the dense material contained in the cisternae could be due to a dilution of glycoproteins which can be detected only when packaged in the granules. The presence of PAS-positive intracisternal granules does not seem to have been observed yet (7, 8, 9). This failure may be explained by the general use of thick paraffin sections which do not allow a resolution such as that obtained here.

Since there is no retention of [^{3}H]fucose by glutaraldehyde fixation, only the sugar incorporated into glycoproteins is likely to be detected. In the hepatocytes, duodenal, and thyroid cells (10, 11, 12), at short time intervals after [^{3}H]fucose injection, the labeled sugar seemed to be localized exclusively over the Golgi apparatus. The same localization was observed in the follicular cells of thyroid lobes incubated for 15 min in the presence of [^{3}H]fucose (12). In the present experiments, it was clearly demonstrated that incorporation of fucose occurred in both RER and Golgi apparatus of thyroidectomy cells.[1] To our knowledge, this is the first demonstration of the incorporation of a terminal sugar in the RER. Presumably a fuco-

[1] Unpublished studies have recently shown that, in thyrotrophs of normal rats, [^{3}H]fucose was localized in the Golgi apparatus 2 min after sugar was added to the incubation medium.





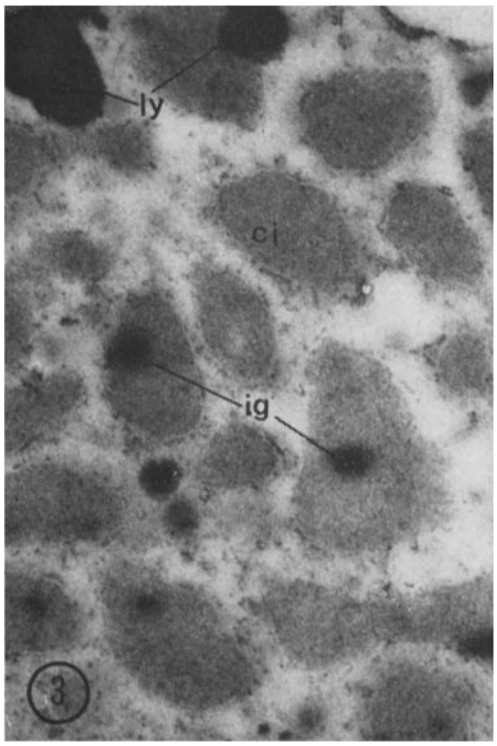
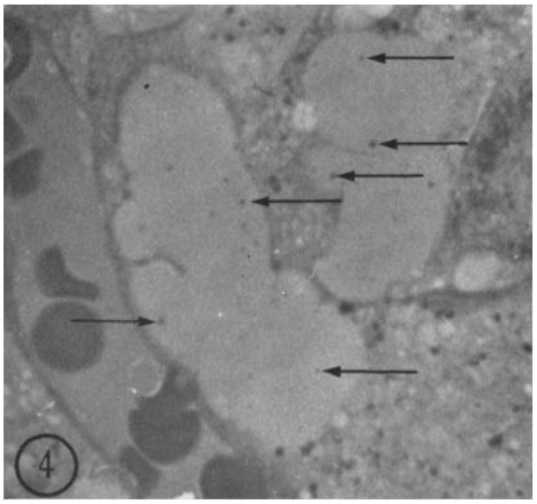
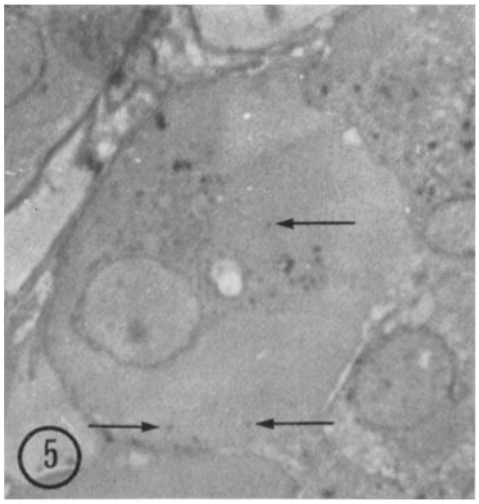


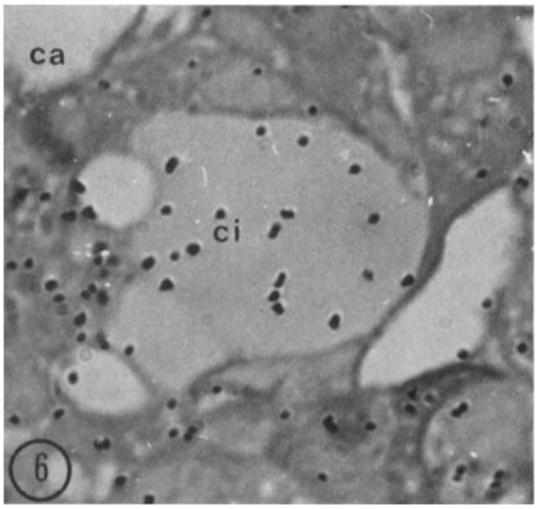
FIGURE 1 A thyroidectomy cell (*T*) showing dilated cisternae of the RER. One cisternae (*ci*) is very enlarged and contains intracisternal granules (arrows). × 4000.

FIGURE 2 Section of the GMA-embedded tissue which has not been stained with PTA. The cisternae (*ci*) of the thyroidectomy cell show no reaction, nor do the intracisternal granules (*ig*) which can be detected by their own density. × 60,000.

FIGURE 3 Portion of a thyroidectomy cell stained with PTA at low pH. The intracisternal granules (*ig*) and the lysosomes (*ly*) are stained. The dense material contained in the cisternae (*ci*) is not PTA positive. × 24,500.







FIGURES 4 and 5 Light micrographs of the thyroidectomy cells after PAS staining. In Fig. 4, the tissue has been fixed with formaldehyde, and in Fig. 5 it has been fixed with glutaraldehyde. Positive granules (arrows) are seen in the dilated cisternae. × 1500.

FIGURE 6 Light microscope autoradiograph of pituitary tissue incubated for 2 min in a medium containing [^{3}H]fucose. Silver grains are found over cells. Many grains are localized over cisternae (*ci*) of thyroidectomy cells. *ca*, capillary. × 2000.

syltransferase is present in both Golgi apparatus and RER. It could tentatively be suggested that the TSH chains are completed in the Golgi apparatus under basal conditions of TSH secretion when the granules package TSH in the normal way, and also in the RER under conditions of increased TSH secretion when the hormone could be packaged in granules within the dilated cisternae.

SUMMARY

In the thyroidectomy cells of rat anterior pituitary, granules are present in the dilated cisternae of the RER. These granules display a positive reaction for glycoprotein at both the light (PAS procedure) and electron microscope (PTA technique) level. After the addition of [^{3}H]fucose to the incubation medium, radioactivity is localized in both RER and Golgi apparatus. These results suggest that some glycoproteins have their synthesis completed in the cisternae of the RER.

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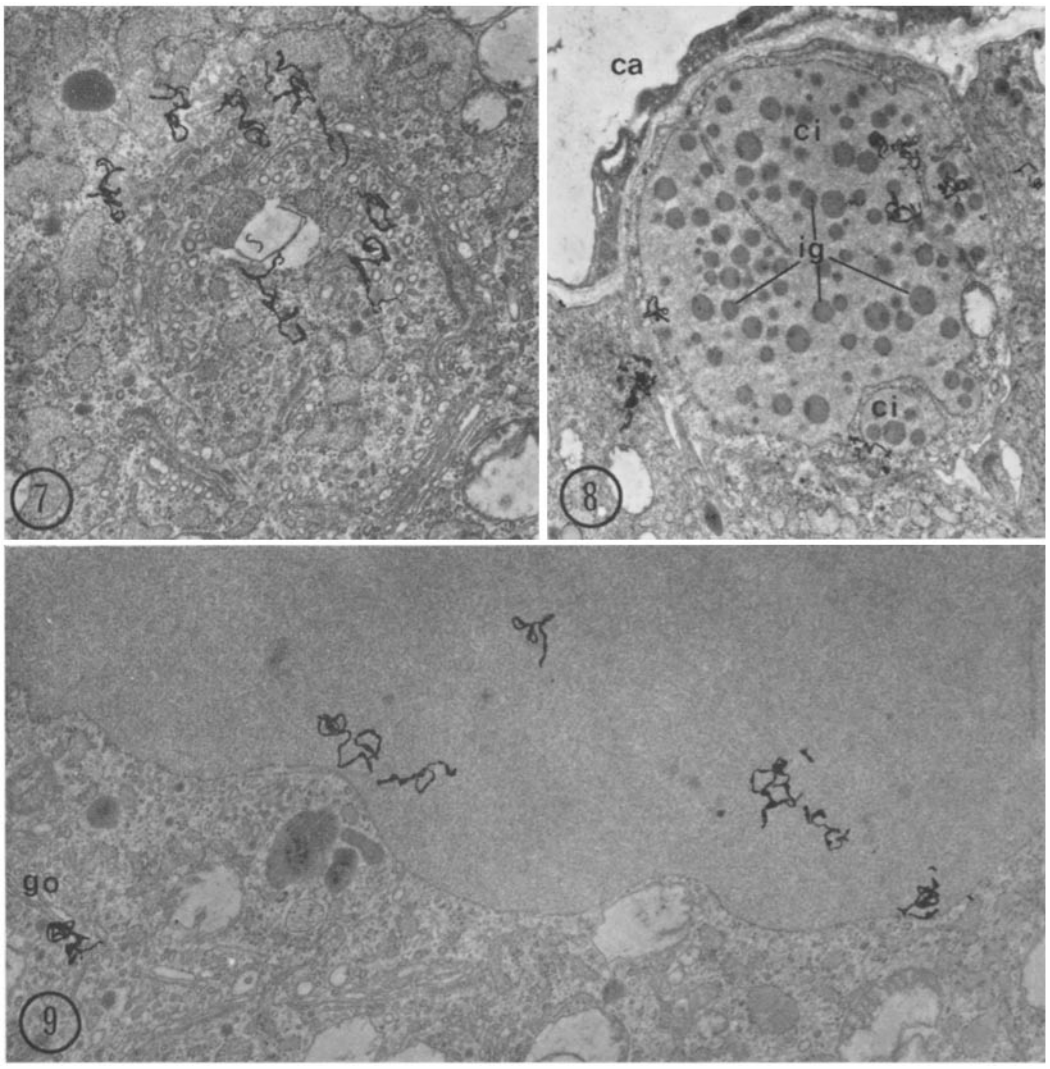

FIGURES 7–9 Electron microscope autoradiographs of pituitary tissue incubated as in Fig. 6.

FIGURE 7 Portion of a thyroidectomy cell. The Golgi apparatus is well developed, but contains no immature granules. The silver grains are localized over the Golgi area. × 17,000.

FIGURE 8 Dilated cisternae (*ci*) of a thyroidectomy cell. The intracisternal granules (*ig*) are abundant. Silver grains are seen almost exclusively over the cisternae. *ca*, capillary. × 10,200.

FIGURE 9 In this portion of a thyroidectomy cell, the dilated cisternae (*ci*) contain no granules. The grains are localized over the dense material of the cisternae. One grain is seen over the Golgi apparatus (*go*.) × 19,000.

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