ELECTRON MICROSCOPIC AUTORADIOGRAPHY

The Localization of I^{125} in Suppressed and Thyrotropin-Stimulated Mouse Thyroid Gland

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Iodide is concentrated from the blood by the thyroid gland; after oxidation it is incorporated in tyrosyl residues of the thyroglobulin molecule. Thus the halogen is stored as a component of thyroglobulin in the colloid of the thyroid follicle. Secretion of thyroid hormone is considered to require proteolysis of the thyroglobulin and release of iodinated thyronines from the base of the acinar cell; the substituted tyrosines are thought to be de-iodinated with retention of the released iodide. In experiments using radioisotopic iodine as a tracer, I^{125} has been found especially useful because its decay by K capture leads to the emission of a soft (27.4 kev) photon, particularly appropriate for autoradiography (1).

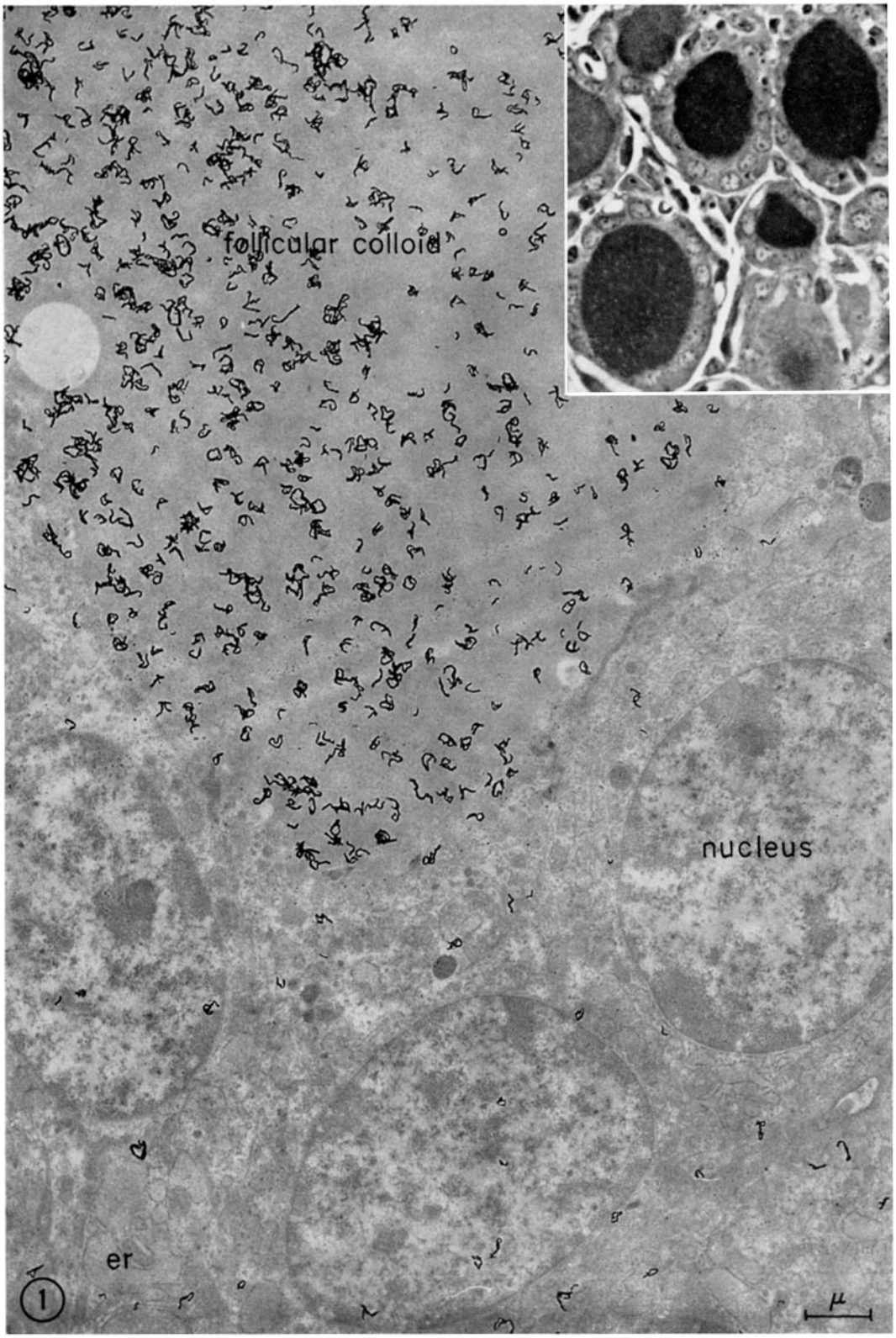
The application of autoradiographic methods to electron microscopy permits, under exceptional circumstances, a resolving power of 0.1 micron (2) and generally allows the localization of isotopically-labeled material to such cytoplasmic compartments as Golgi vacuoles, mitochondria, cisternae of the endoplasmic reticulum, or large granules or droplets. This communication concerns the use of I^{125} to identify the origin of the large granules which are seen in the follicular cells of the thyroid gland (3–5).

MATERIALS AND METHODS

Six female Swiss Webster albino mice weighing about 18 gm were fed Remington-type low iodine diet (Nutritional Biochemicals Corporation) for 9 days. They were then injected intraperitoneally with 15 μc carrier-free I^{125} and subcutaneously with 10 μg sodium l-thyroxine; concomitantly thyroid USP was added to the diet to a concentration of 0.066 per cent. Administration of thyroid hormone in these ways was previously shown to effect maximal thyroid gland

FIGURE 1. This survey electronmicrograph shows portions of three thyroid cells and a large area of follicular colloid from the gland of a mouse which received 15 μc of I^{125} 4 days before perfusion fixation of the tissue. The mouse also received exogenous thyroid hormone during the experimental period. The autoradiographic grains are concentrated over the follicular colloid. Very few grains can be seen over the thyroid cells. No colloid droplets are seen in the cytoplasm of these cells from the "suppressed" gland. Magnification, 10,000.

Inset: This phase-contrast photomicrograph of an autoradiograph from the same tissue prepared for light microscopy demonstrates intense blackening of the follicular colloid by the grains which are out of phase. Four days of exposure has given this result. ×400.



suppression[1] in about 4 days (6), at which time the mice would be optimally sensitive to injected thyrotropin. The mice so prepared in the present study were used on the 4th day of suppression when it was found that the thyroid glands contained on average 27.5 per cent of the dose of I^{125}, *i.e.* 4.13 μc per gland.

To establish that this dose of I^{125} did not cause early radiation damage, six mice were similarly treated and maintained for 2 weeks on the suppressive diet. Then the thyroid glands of three of the animals were examined by light microscopy which showed flat "resting" follicular epithelium, densely staining colloid, and no evidence of inflammation or necrosis. The diet of the remaining three mice was changed back to a diet without added thyroid hormone, and the excretion of I^{125} was studied. Within 4 days, the rate and pattern of excretion had returned to normal (7); this was accepted as evidence for the normal responsiveness of thyroid gland function and lack of significant radiation damage to those functions.

USP Reference Standard Thyrotropin (of bovine origin) was dissolved in 1 per cent albumin in 0.9 per cent sodium chloride solution. Thirty milliunits (mμ) in a volume of 0.2 ml was injected intravenously into the "stimulated" animals; control "suppressed" animals received 0.2 ml of the albumin solution only. The thyroids were fixed *in situ* by perfusion under ether anesthesia 40 minutes after the intravenous injections.

Perfusion fixation of the thyroid gland was effected by a modification of Palay's method (8). The fixative was a mixture of acrolein and buffered glutaraldehyde (9, 10) which caused an immediate rigidity of the entire animal. Fixation was continued *in situ* for 30 minutes by constant infusion. The tissue was then removed and fixed by immersion first in glutaraldehyde and then in osmium tetroxide to which salts had been added (10). This was followed by routine rinsing, dehydration in alcohols, and embedding in Epon (11). Sections were cut on a Porter-Blum microtome, mounted on carbon-coated formvar films, and then were dipped in Ilford L-4 emulsion, exposed, developed, fixed, and finally stained with alkaline lead solutions, all according to the method of Caro (2). Specimens for light microscope autoradiography were treated in a similar manner. Electron micrographs were taken with an RCA EMU 3E.

[1] Although it is recognized that the suppression of the thyroid gland following the administration of thyroid hormone is predominantly an indirect effect *via* the hypophysis, perhaps mediated by a hypothalamic influence, for convenience reference is made throughout the text solely to "thyroid gland suppression."

OBSERVATIONS

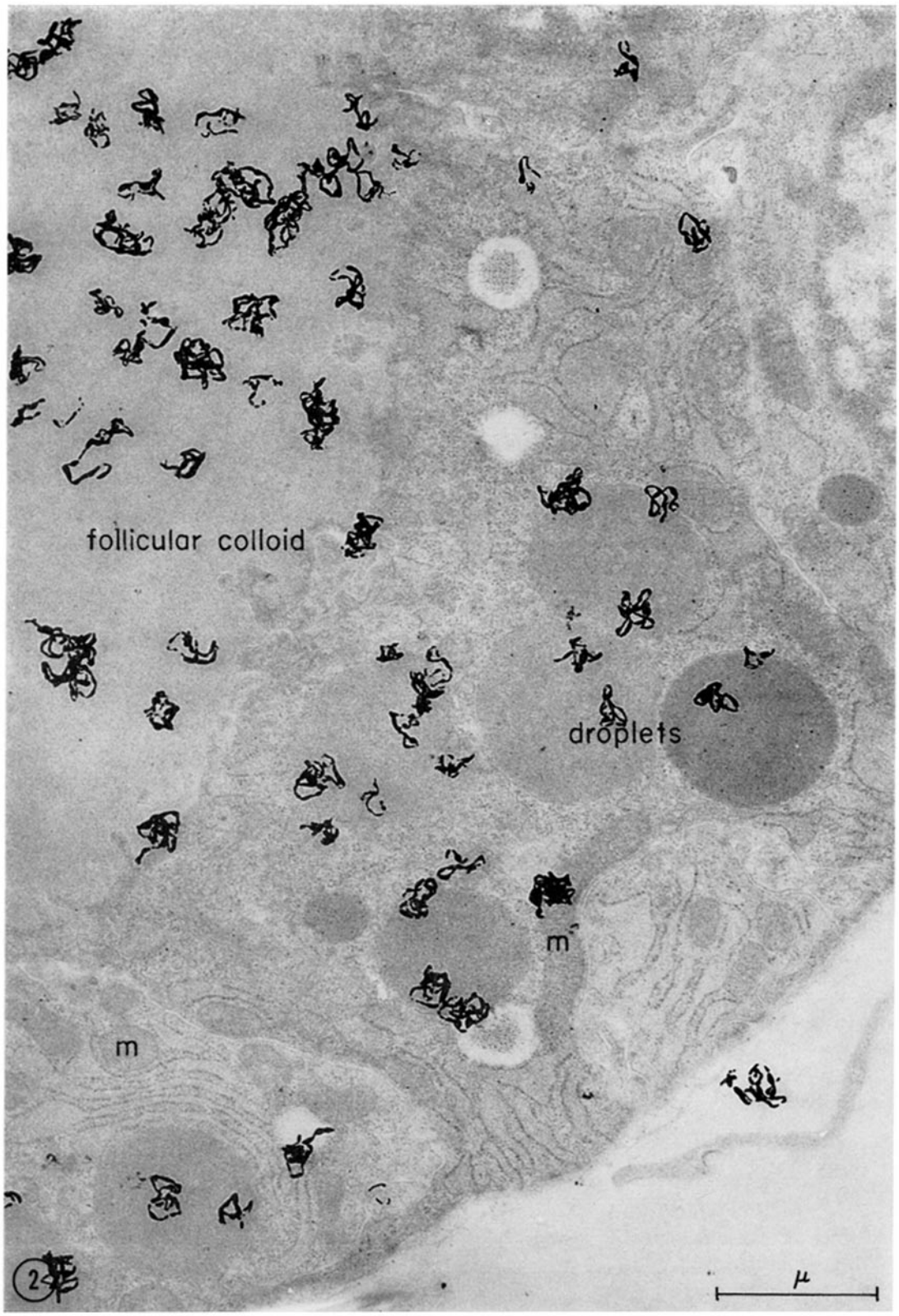
Light microscope autoradiography demonstrates an intense blackening of the colloid in the thyroid follicle after 4 days of exposure (Fig. 1). Background grains are negligible.

Electron microscopic observations on thin sections of the thyroid cells from the animals which had received exogenous thyroid hormone (suppressed gland) show a centrally placed nucleus, a small Golgi complex, unremarkable mitochondria, and a moderate amount of the granular endoplasmic reticulum with cisternae which contain amorphous, moderately dense material. Very few large granules were seen in the cytoplasm of these cells.

Observations on thyroid cells from the animals which had been given thyrotropin 4 minutes before the tissue was fixed show that there are many large granules in the cytoplasm of these cells. These granules have the same density and homogeneous appearance as the colloid of the follicle. Sections show at least one and as many as seven large granules in almost every cell; they can be seen in the base as well as the apex of the thyroid cells.

Autoradiographs for electron microscopy show large numbers of grains over the colloid material of the follicle in both suppressed and stimulated glands (Fig. 2). Whenever the large granules are seen in the cells from the stimulated animals, there are grains over the granules. Occasionally there are granules in cells of the suppressed animal. Grains may be seen over these large granules too. As many as ten autoradiographic grains have been counted over some large granules. No localization over any other compartment of the cyto-

FIGURE 2. This electronmicrograph shows portions of three thyroid cells and an area of follicular thyroid from the gland of a mouse which received 15 μc of I^{125} 4 days prior to fixation and 30 milliunits of thyrotropin 40 minutes before the fixation. The cell in the lower left corner shows two large colloid droplets, the middle cell shows five large colloid droplets, and the portion of the cell in the upper right shows no droplets. Autoradiographic grains appear over each of the droplets in this micrograph and over the follicular colloid. Magnification, 25,000.



plasm has been seen in the present study. A few grains are found over the capillaries at the base of the acinar cells.

DISCUSSION

The dose of I^{125} used in this experiment appears appropriate for electron microscopic autoradiography. No radiation effect on the cells has been demonstrated in the acute study reported here.

The effect of thyrotropin on the suppressed thyroid gland cells can be recognized, as early as 40 minutes after administration of the hormone, by the different number of large granules in the cytoplasm when compared with the control (suppressed) group.

With mice prepared as described above, it was expected, and indeed was found, that 4 days after the injection of radioiodide all the I^{125} is in the follicular colloid in the suppressed animals. Forty minutes after the intravenous injection of thyrotropin, evidence of radioactivity is found in the follicular cells. The quantity of I^{125} in the blood of these animals is negligible, being about 0.05 per cent of that in the thyroid gland; clearly the radioactivity came from the colloid. In theory, this might be iodide which is known to be secreted by the thyroid gland under thyrotropic stimulation (12), or it might be free iodinated amino acids released by hydrolysis of thyroglobulin. However, the methods of fixation would be expected to wash out inorganic ions or free (alcohol soluble) amino acids. Consequently, it seems most likely that the isotope is in an organically bound form.

The release of thyroid hormone has been accepted as a process involving the proteolysis of thyroglobulin in the thyroid gland. De Robertis (13) described a protease in the luminal colloid and hypothesized that hydrolysis by this or a similar enzyme was necessary for the reabsorption of thyroxine by the follicular cell prior to secretion. Since the protein was such a large molecule (650,000 mol wt), this suggestion was appropriate, but recent findings have shown that equally large molecules may be identified in vacuoles of cells (14). The significance of the colloid droplets seen in the apex of the thyroid cell following stimulation by thyrotropin has been the subject of discussion (3, 5, 15). Whether they represent newly formed colloid about to be passed into the follicular lumen (3) or colloid derived from the lumen (4, 15) is in dispute.

Our data show that the large cytoplasmic granules which appear in cells of the thyroid gland after the injection of thyrotropin are the principal site for the intracellular I^{125}. Their rapid appearance and labeling with I^{125} leads us to conclude that they are derived from the colloid of the lumen. Whether or not this is the reflection of the normal process of secretion, or whether it is a phenomenon to be found only in the acutely stimulated gland, is a matter outside the present study.

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