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'SUBCELLULAR' LOCALIZATION OF RADIOIODINE IN THE THYROID FOLLICLE OF THE RAT FOLLOWING THE ADMINISTRATION OF LOW AND HIGH DOSES OF $^{125}$I

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ABSTRACT

The 'subcellular' localization of radioiodine was observed in the thyroid follicles of rats following the injection (i.p.) of 75 $\mu$Ci to 1000 $\mu$Ci of radioiodide ($^{125}$I). Twenty-four hours after the injection of the smaller doses (75, 150 and 250 $\mu$Ci), the radioiodine was distributed evenly throughout the follicular lumen. Twenty-four hours after the injection of the large dose (1 mc) of radioiodide, there were two histological patterns of follicular radioiodine localization. In the majority of follicles (about 85%) the radioiodine was distributed uniformly throughout the lumen. In the other follicles (15%) the activity was not uniformly distributed but was primarily localized along the cell-colloid border. At this interface, the radioactivity was entirely localized in the colloidal region. A possible explanation is that the follicles with an intra-colloidal ring of radioiodine are more sensitive to the early effects of radiation and could be reflecting some cellular biochemical alteration.

The iodination of amino acids is an essential reaction in the biogenesis of the thyroid hormones (De Groot 1965). Tyrosine molecules which are most likely bound to thyroglobulin by peptide linkage are iodinated to form monoiodotyrosine and diiodotyrosine which couple to form iodothyronines (tetraiodothyronine and triiodothyronine). These reactions occur in the basic histological unit of the thyroid gland, the thyroid follicle.

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Within the last few years it has become possible to apply electron microscopic radioautography to the study of the possible site(s) of iodination within the thyroid follicle. With this technique it has been demonstrated (Stein & Gross 1964) that 15 minutes after the injection of single doses of radioiodide, radioactivity was concentrated in the follicular lumen. After even a shorter interval (three minutes) following a single injection of radioiodide (Ibrahim & Budd 1965), or after long term administration of radioiodide (daily for 23 days) (Nunez & Becker, to be publ.), the radioactivity was localized primarily in the follicular lumen. These observations suggest that the follicular colloid rather than the epithelial cell may be the site of tyrosine iodination.

Employing light microscopic radioautography, it has been reported (Javano-vic et al. 1965 a, b) that following large doses of radioiodide (150 µc/100 g body weight) the radioactivity was primarily deposited in the follicular epithelium, but after the administration of smaller doses (10 µc/100 g body weight) radioiodine was distributed uniformly throughout the follicular colloid. These observations led the authors to conclude that the iodination process occurs in the cell and that large doses of radioiodide prevent the transfer of protein-bound iodine from the epithelial cell to the follicular lumen.

As part of a continuing study on the follicular localization of radioiodine in tumour and non-tumour bearing rats, high resolution radioautography was employed in rats that received low and high doses of radioiodide ¹²⁵I.

**Materials and Methods**

Four adult male Sprague-Dawley (Charles River) rats were studied. Two animals were bearing subcutaneously transplantable thyroid-tumours along their right flanks. Such tumour-bearing rats have thyroid glands that are normal both histologically and biochemically (Money et al. 1965). The animals were fed a Purina laboratory diet and weighed from 250–333 grams. Two rats (without tumours) were injected (i.p.) with 75 and 150 µc of ¹²³I respectively. The tumour-bearing animals were injected (i.p.) with 250 and 1000 µc of ¹²³I respectively. All animals were sacrificed 24 hours after the injection of radioiodide. The thyroid glands were removed and fixed in ice-cold one per cent osmic-sucrose buffered with phosphate (Millonig 1961) for two to four hours at 4°C. Following dehydration in graded alcohols and propylene oxide, the tissues were embedded in Epon 812 (Finch 1960). Thick (1–2 µ) and thin (gold colour) sections were cut with glass knives on a Porter-Blum ultramicrotome. The thin sections were transferred to carbon-coated parlodion prepared copper grids.

The copper grids were attached to microscopic slides which were dipped into a solution of Ilford L-4 emulsion that was diluted 1 to 6 with distilled water at 43°C (Caro & Van Turbegen 1962). Each slide was then held vertically in a rack and dried under a stream of warm air and subsequently placed in light-proof containers and stored at 4°C. After exposure from one week to several months, the grids were developed in Kodak Microdol, rinsed, fixed and washed with dilute solution of NaOH. The grids were stained with lead hydroxide from 20 to 30 minutes (Karnovsky 1961). The grids were examined in an RCA EMU 3B or 3D at 50 KV.
For light microscopy radioautography, the thick sections were placed on glass slides. In the dark the slides were coated with a solution of photographic emulsion (Ilford K-5) which had been diluted 1 to 1 with warm distilled water and the slides dried under a stream of warm air. The dried preparations were stored in light-proof containers and kept at 4°C. After exposure from 1 to 24 days, they were developed in Kodak D-19, rinsed, fixed and washed with distilled water. After drying on a hot plate, the sections were stained for 30 seconds with a dilute (1%) solution of toluidine blue.

RESULTS

A. Light Microscopic Radioautography

Light microscopic radioautograms of the thyroids from the rats injected with 75, 150, and 250 µc of radioiodide revealed that most of the radioiodine was distributed homogeneously throughout the follicular lumen. Occasional

![Light radioautograms of thyroid gland of rat sacrificed 24 hours after the injection of 1 mc of radioiodide (131I). The reduced photographic grains are primarily distributed evenly in the lumen of the follicles in the field except for the large follicle at the left. In this follicle there is a light luminal area surrounded by a dark ring of radioiodine at the periphery.](image-url)
randomly scattered reduced radioautographic grains were visible in the follicular epithelium.

Radioautograms from the thyroid of the animal injected with one mc of radioiodide demonstrated that approximately 15 per cent of the follicles had a non-homogeneous distribution of radioiodine throughout the follicle with a dark ring of radioiodine concentration near the epithelium (Fig. 1).

B. *Electron Microscopic Radioautography*

Electron radioautograms from the three animals injected with the lower
doses of radioiodide consistently revealed that the radioautographic grains were distributed evenly throughout all the follicular lumen (Fig. 2). Epithelial radioactivity was not associated with any particular organelle. In the animal injected with one mc, electron radioautograms showed that about 85 per cent of the follicles also had homogeneous distribution of radioiodine within the follicular lumen (Fig. 3). In the other 15 per cent, as was seen with light microscopy, however, there was a definite accumulation of radioactivity along the cell-colloid border (Fig. 4). Higher magnification electron micrographs (Fig. 5) revealed that the radioactivity was clearly localized in the colloid

Fig. 3.
Electron radioautogram showing the pattern of radioiodine distribution noted in about 85 per cent of the follicles of the thyroid gland from a rat injected with a 1 mc dose of $^{131}I$. The radioautographic grains were evenly distributed (in high concentration) in the colloid. (18 000 ×).
at the cell-lumen interface and not in the epithelial cells. The density of the follicular intra-colloidal ring of radioactivity was the same throughout any given follicle, although it differed between follicles.

**DISCUSSION**

In the present study, electron radioautograms revealed that small doses of radioiodide were associated with evenly distributed radioactivity throughout

![Electron radioautogram shows the pattern of radioiodine distribution](image)

*Fig. 4.*

Electron radioautogram shows the pattern of radioiodine distribution seen in approximately 15 per cent of the follicles from the rat injected with 1 mc of $^{131}$I. The lumen is practically free of reduced silver grains except for an intra-luminal ring of radioactivity at the periphery of the follicular lumen. (12 000 X).
the colloid. There was a distinct difference in the thyroid gland of an animal treated with 1 mc where in approximately 15 per cent of the follicles the radioactivity was accumulated in an intra-colloidal ring. The zone of reduced radioautographic grains was clearly limited to the periphery of the lumen. These electron radioautograms support the observation (Javanovic et al. 1965 b) of a demonstrable difference in the follicular localization of radioactivity after the administration of dose of radioiodide greater than 150 µc/100 g body weight. This difference consisted of a ring of radioactivity in the lumen

![Fig. 5.](image-url)

At high magnification, the intra-luminal ring of radioactivity was limited to the colloid. The cytoplasm of the epithelial cell is almost completely devoid of radioiodine. (28 500 X).
adjacent to the epithelium. This variation may be related to differences in radiosensitivity. Since radiation damages the internal structure of the thyroid cell (Sobel 1964; McQuade & Evans 1959) these follicles may have undergone a biochemical alteration resulting in a decreased ability to trap circulating iodine. This could account for the reduced radioiodine deposition in the follicles as demonstrated in the radioautograms.

Furthermore, since it has been reported (Jovanovic et al. 1965 a, b) that relatively high doses of radioiodide inhibit thyroxine synthesis to a much greater extent than the iodination of tyrosine, one might speculate that the accumulation of radioactivity at the periphery of the lumen could represent that area of the follicle where iodination of tyrosine is occurring without subsequent coupling.

REFERENCES

Stein O. & Gross J.: Endocrinology 75 (1964) 787.

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