THE SITE OF THE SYNTHESIS OF PROTEIN-BOUND IODINE IN THE THYROID GLAND

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The process of synthesis of protein-bound iodine is a very important step in the formation of the hormone in the thyroid gland. This process embraces a whole series of successive enzymic reactions, in the course of which inorganic, ionic iodine, concentrated in the thyroid tissue, is oxidized to molecular iodine and takes part in substitution and oxidation reactions with the tyrosyl groups of the protein molecule, eventually forming hormonally active iodothyronines (throxin and tri-iodothyronine), entering into the composition of thyroglobulin [2, 7, 11]. The connection between this biochemical process and morphological structures, of great interest to the histophysiology of the thyroid gland, is still, however, uncertain.

METHOD

The present reserach comprises 3 series of experiments, carried out on male white mice weighing 150-200 g: 1) an autoradiographic investigation of sections of thyroid tissue containing colloid; 2) an autoradiographic investigation of sections not containing colloid; 3) a radiochromatographic investigation of protein hydrolyzates of the thyroid gland. In the first series of experiments (34 rats), the animals were injected intraperitoneally with 10 μC of NaI^{131} over a period of 2 minutes, 21 days before they were sacrificed. The thyroid gland was fixed in Carnoy's fluid and embedded in paraffin wax. Sections 5-7 μ in thickness were freed from wax and subjected to autoradiography by the method of covering with liquid emulsion (type R). The length of exposure was from 48 hours to 16 days at +4°. In the second series of experiments (17 rats) 10-20 μC of radioactive iodine was injected over a period of 2 minutes, 4 days before the animals were sacrificed. The thyroid gland was fixed in 10% formalin; sections were cut out to a thickness of 10-15 μ on a freezing microtome. Repeated treatment with 70° alcohol and water led to removal of the colloid from most of the follicles. After staining with hematoxylin-eosin, an autoradiographic investigation was undertaken (as in the first series) with prolonged exposure of the sections (up to 1 month). In the third series of experiments (8 rats) 20-25 μC of NaI^{131} was injected. Animals were sacrificed after 30 minutes to 1 hour (first group) and after 24 hours (second group).

The thyroid glands of each group (4 rats in each) were homogenized in 2-3 ml distilled water; the protein was precipitated by an equal volume of 10% trichloroacetic acid. The precipitate was centrifuged and washed, and suspended in 1 ml of borate buffer (pH = 8.4), (10-15 mg of crystalline trypsin, at a temperature of 38°, for 48 hours under a layer of toluene). The material was then extracted once only with 0.5-1.0 ml butanol at pH=1-2 acidfied with concentrated HCl). In volumes of 30-40 μl, the extract was treated by descending chromatography, in a system: butanol – CH_3COOH-H_2O (78:5:17, top layer) for 10-14 hours. The dried chromatograms were exposed for 10 days between two x-ray films and developed in the usual way. In order to identify the iodine-containing aminoacids, we employed the R_f ratios known from the literature [14] and we also used NaI^{131} and di-iodotyrosine as controls (the latter was developed with ninhydrin). The optic density of blackening along the length of the radiochromatograms was estimated by means of the MF-4 photometric apparatus.

RESULTS

In the first series of experiments an autoradiographic reaction was seen from 30 minutes to 1 hour after injection of radioactive iodine, in the form of rings around the follicles. After 24 hours continuous areas of blackening appeared, in the form of disks over the intrafollicular colloid. The reaction gradually became weaker and it became negative on the 21st day (Fig. 1).

The ring reaction is of great interest in the determination of the site of organic combination of iodine. Leblond and Gross [5] and also Pavlovic [9], Nandi et al. [8] interpreted it as the index of preferential distribution of protein-bound iodine in the epithelium of

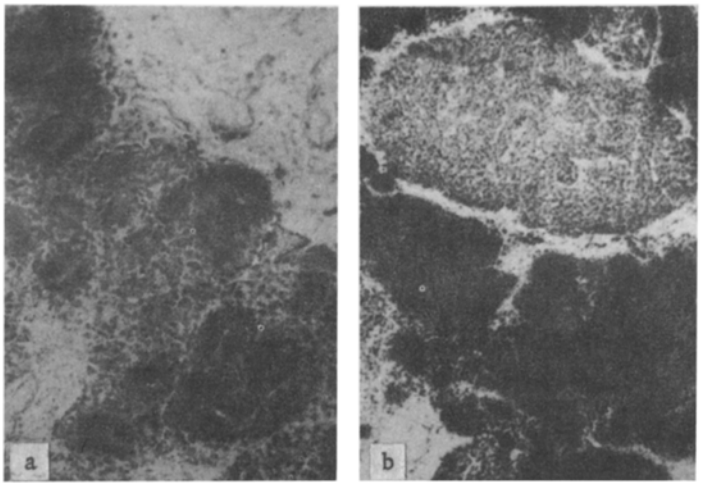

Fig. 1. Autoradiograms of paraffin wax sections of the thyroid glands of rats. Magnification: ocular 7× objection 10×. a) One hour after injection of I^{131}; b) after 24 hours.

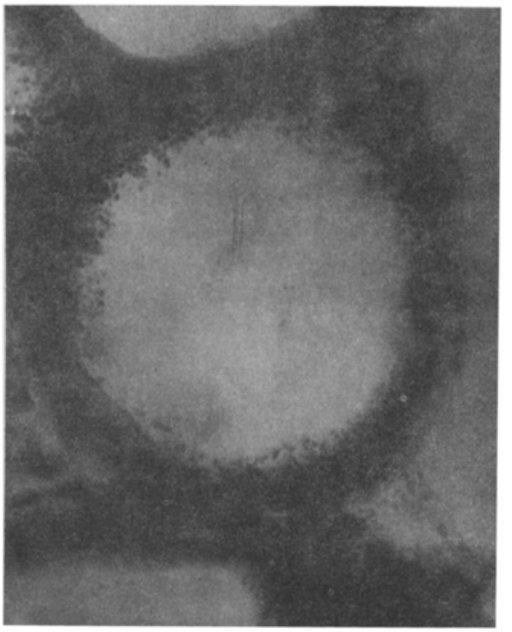

Fig. 2. Autoradiogram of a frozen section of the thyroid gland of a rat (colloid removed) one hour after injection of I^{131}. Magnification: ocular 10 ×, objective 40 ×. An obvious but weak autoradiographic reaction is observed over the cells of the epithelium.

the follicles. However, Wollman and Wodinsky [15] and also M. F. Merkurov [1] have pointed out that in those follicles in which the colloid has become separated from the epithelium of the wall by the action of the fixing agent, the ring of the autoradiographic reaction does not correspond to the epithelial cells, but to the peripheral zone of the intrafollicular colloid. These authors consider that the iodization of the protein molecule is brought about in the colloid and not in the cytoplasm of the thyroid epithelium.

In our material the picture of displacement of the ring together with the peripheral zone of the colloid was repeatedly encountered. This gives grounds for considering that the iodized protein, producing this type of reaction, was localized in the colloid. It is, however, unjustifiable to conclude that the intrafollicular colloid plays an indispensable role in the process of organic combination of iodine, because of certain considerations which arise from the method itself. The autoradiographic resolution of such closely situated microobjects as the follicular epithelium and the follicular colloid is achieved by the use of fairly thin layers of emulsion and short periods of exposure. In these conditions, only areas of high activity or of higher concentration of radioactive isotope are found. If the concentration of organic I^{131} in the peripheral zone of the colloid is much higher than in the cells, then no autoradiographic reaction takes place over the cells. The autoradiographic investigation of paraffin wax sections thus gives no grounds for denying the presence of organic iodine in the epithelial cells of the thyroid gland.

In the second series of experiments, in which we made autoradiographic investigations of sections obtained by the freezing microtome, the colloid was removed from the sections by treatment with alcohol and distilled water. Completeness of removal of the colloid was controlled by histological examination. An obvious positive reaction over the cells was observed after prolonged exposure of the sections, considerably less in intensity than the corresponding autoradiograms of paraffin wax sections that were investigated (Fig. 2). These results show that the cells of the thyroid epithelium contain iodized protein, although in a considerably smaller concent3ation than the colloid.

There are three possible explanations of these facts: 1) the iodized protein enters the cells from the colloid, in which the process of iodization takes place; 2) the organic iodine is entirely synthesized in the cells and is reapily excreted into the colloid; 3) the process of iodization takes place in both cells and colloid.

The first hypothesis is contrary to the established mechanism of mobilization of the colloid, which is brought about by the proteolytic decomposition of the protein molecule and resorption of the decomposition products of low molecular weight [4, 10, 14, etc.]. Meanwhile, these products cannot form the substrate of the autoradiographic reaction, for they are washed from the section by the action of the histological reagents. The protein-bound iodine, thus detected autoradiographically in the cells, is an indication of the intracellular formation of iodothyroglobulin.

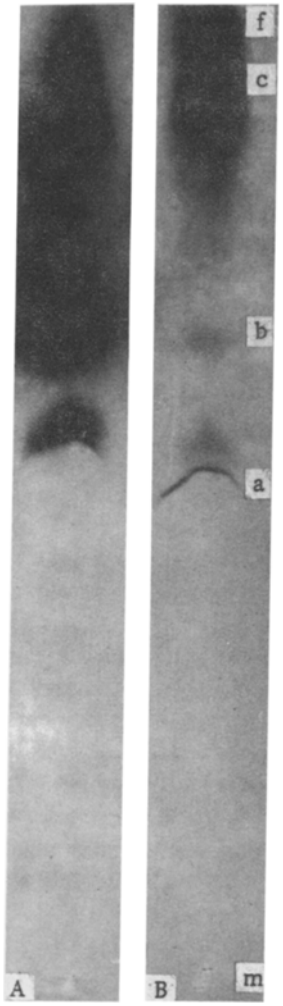

Fig. 3. Radiochromatograms of tryptic hydrolyzates of the thyroid glands of rats. In the period from 1 to 24 hours, the activity of the iodothyronines increases at the expense of a considerable fall in the activity of mono- and di-iodotyrosine. A) One hour after injection of I^{131}; B) after 24 hours; a) Stain corresponding to MIT; b) stain corresponding to DIT (identified by control tests); c) stain corresponding to iodothyronine; M) line of application of hydrolyzate; F) front of solvent (34 cm).

Is the epithelial cell the only site for formation of the thyroid hormone, or does this process also take place in the intrafollicular colloid? The results of the third series of experiments, shown in Fig. 3, tend to support the latter. As was shown in the first series of experiments, at all times of the chromatographic investigation (from 1 to 24 hours) the organic iodine was present almost entirely in the colloid, although one hour after its administration it was mainly situated in the peripheral zone, and later on, almost uniformly throughout the whole colloid. Meanwhile, in the intervening period (from 1 to 24 hours) the proportion between the activity of the iodine-containing aminoacids of the thyroglobulin underwent an essential change: The activity of the iodothyronines (thyroxin and tri-iodithyronine - Tx and TIT) rose at the expense of a fall in the activity of mono- and di-iodityrosine (MIT and DIT). These findings agreed with the results of the extensive investigations of Roche and his co-workers, who found that the level of labeled MIT reaches its maximum 8-10 hours after injection of radioactive iodine, that of DIT a few hours later, that of TIT after 12-14 hours and that of Tx after 24 hours [12]. Thus, in the period between 8 and 24 hours when nearly all the labeled thyroglobulin is present in the colloid, condensation of the iodityrosines takes place, with the formation of iodothyronines and the conversion of MIT and TIT into DIT and Tx, i.e., the further iodization of the monoiodo-substituted tyrosine groups of the protein molecule. It is highly probable that combination of iodine with those tyrosine radicals of the protein molecule that were not, generally speaking, subjected to halogenization before secretion into the colloid, may take place also in the colloid.

SUMMARY

The author conducted an autoradiographic investigation of the thyroid gland at various intervals after the administration of radioiodine to rats. The method of liquid emulsion coating was used. Paraffin sections prepared at intervals of 30 minutes to 1 hour produce an autoradiographic ring reaction which, in the main, corresponds to the peripheral zone of the intrafollicular colloid. By using frozen sections devoid of the colloid the author established the presence of small quantities of protein-bound iodine in the thyroid epithelium. Radiochromatographic investigation of thyroid tissue hydrolyzates at different intervals after radioiodine administration points to the fact that, during the interval of 1-24 hours, when the bulk of the protein-bound iodine is in the colloid, the amount of radioiodothyronines increased at the expense of radioiodotyrosine. A conclusion is drawn that the formation of iodine-containing organic compounds in the thyroid gland begins in the epithelial cells and continues in the follicular lumen.

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