THE ROLE OF THE COLLOID IN THE HORMONE-FORMING FUNCTION OF THE THYROID GLAND

M. F. Merkulov

From the Department of Pharmacology (Head — Active Member AMN SSSR the late V. I. Skvortsov) of the N. I. Pirogov Second Moscow Medical Institute

(Received February 18, 1959. Presented by Active Member AMN SSSR V. I. Skvortsov)

The role of the individual structural formations of the thyroid gland in the synthesis of its hormones is one of the more important problems in the histophysiology of this organ. After it had been proved that the colloid of the thyroid gland consists mainly of organic compounds of iodine, the function of a peculiar hormonal depot was ascribed to it. As a result of this, the cells of the follicular epithelium were endowed with the ability to secrete products both into the blood stream and into the cavity of the follicle. When there was an increased demand of hormone by the body, the cells of the follicular epithelium were called upon to mobilize reserves of hormone from the colloid.

This view of the role of the colloid was shaken by the first autoradiographic investigations of the hormone-forming function of the thyroid gland, using radioactive iodine [10, 13, 15, 17]. It became clear from these investigations that it is not only the surplus but practically the whole of the iodine entering the body that passes through the colloid. These findings made it necessary to study the role of the colloid in hormone formation in greater detail.

Bearing in mind that the bulk of the colloid consist of iodothyroglobulin, and using the method of microautoradiography, we attempted to trace the fate of its various component parts in the structures of the thyroid gland. As an indicator of the path of the protein part of the iodothyroglobulin we used S^{35}-methionine. Methionine, together with cystine, accounts for about 7% of the total amino acid content of iodithyroglobulin, and roughly the same or slightly less of the total is accounted for by tyrosine, diiodotyrosine and thyroxin taken together [1, 4, 9]. As a metabolite directly concerned in the formation of the hormonal products of the thyroid gland, we used radioactive iodine.

In this research we tried to show which structures of the thyroid gland first fix these compounds in the protein-bound form, for this would give some idea of the place of synthesis of thyroglobulin and of the site of its iodization.

EXPERIMENTAL METHOD

Experiments were conducted on male white rats weighing from 150 to 200 g. The radioactive substances were injected intravenously in the following doses: NaI^{131} without carrier — from 10 to 600 μC/kg, S^{35}-methionine — from 300 to 5000 μC/kg. The animals were sacrificed by decapitation after various intervals of time: 10 seconds, 5 and 20 minutes and, finally, 1, 4, 24 and 72 hours after injection of the labeled preparations.

The thyroid gland of the animal was extracted en bloc with the trachea as quickly as possible after death and placed in absolute ethanol, cooled to 0°. After fixation, the tissue was passed through a series of xylols and embedded in paraffin wax. Serial sections of the organ, 4-5 μ in thickness, were placed on glass slides,

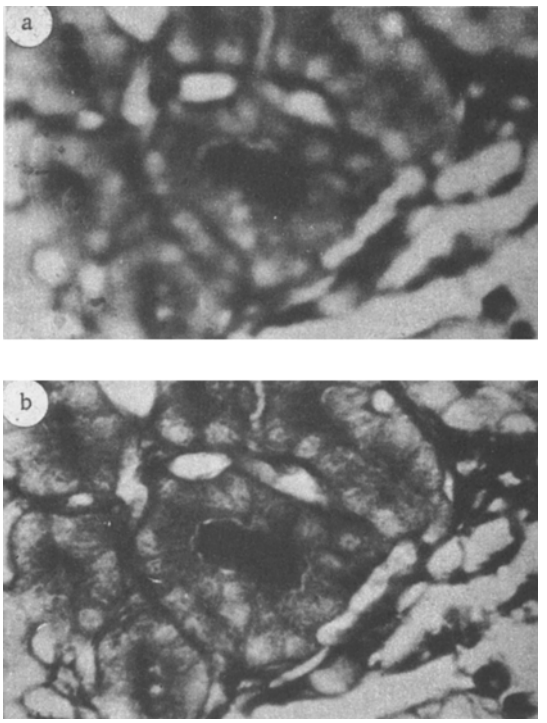

Fig. 1. Micro-autoradiograph of a follicle of the thyroid gland of a rat (the animal was sacrificed 10 seconds after the intravenous injection of 600 μC/kg NaI^{131}). a) focused on the photographic emulsion. Concentration of the silver grains is observed over the colloid only; b) focused on the tissue ; "Azane" stained; magnification 40 x 10.

and after removal of the paraffin wax, were stained with hematoxylin-eosin or by the "Azane" method. Micro-autoradiographs were obtained by the method which we have described previously [3].

By this method of histological treatment of the material all the radioactive iodine compounds not combined with protein were completely washed from the tissues. We satisfied ourselves on this point by preliminary experiments in which we injected animals with mercazole, which blocks the iodization of thyroglobulin but does not affect the power of the thyroid gland to concentrate iodides from the plasma. Such treatment also washes from the tissues practically all the radioactive conversion products of S^{35}-methionine or the free labeled amino acid [6, 16]. In this way we recorded on micro-autoradiographs the distribution in the tissues of the protein-bound compounds of I^{131} and S^{35} alone.

EXPERIMENTAL RESULTS

Only 10 seconds after the intravenous injection of radioactive iodine, the thyroid gland contained well-defined amounts of protein-bound radioactive iodine compounds (Fig. 1), and the iodine was shown to be concentrated entirely in the cavity of the follicles — in the colloid. In sections of the follicles which were large in size, the radioactivity was sometimes distributed in the form of a closed circle along the border of the apical parts of the cells and the colloid. In sections in which, in the course of preparation, the colloid had become separated from the follicular epithelium, it was seen particularly clearly that the radioactivity was wholly bound up with the colloid. Sometimes the accumulation of protein-bound compounds of radioactive iodine in the colloid had the appearance in the sections of an open circle or semicircle. It is interesting to observe that at such an early stage as 10 seconds after the injection of radioactive iodine, it was not present in all the follicles in protein-bound form. The number of follicles containing radioactive thyroglobulin was 5-10 % of the remainder.

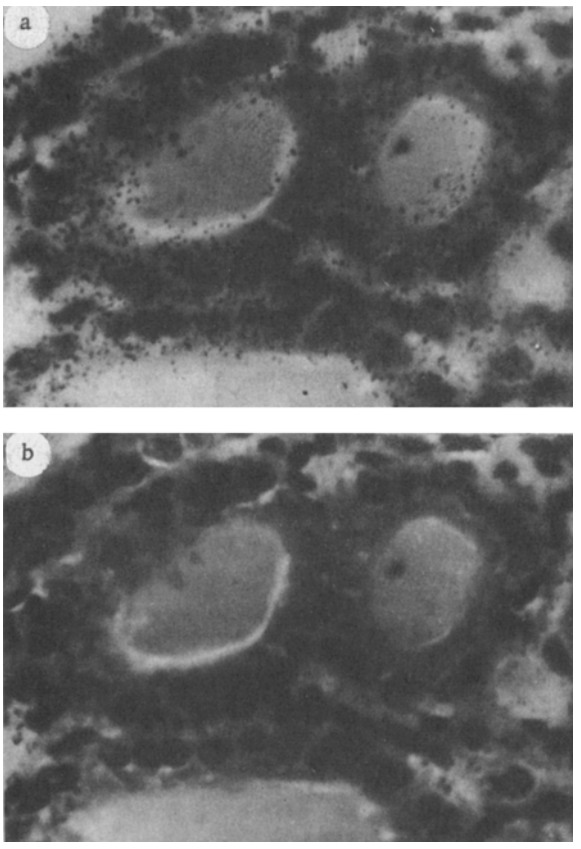

Fig. 2. Micro-autoradiograph of the follicles of the thyroid gland of a rat (the animal was killed 5 minutes after the intravenous injection of 5 mC/kg of S^{35}-methionine). a) focused on the photographic emulsion. Concentration of the silver grains is observed over the follicular epithelium only; b) focused on the tissue; stained with hematoxylin-eosin; magnification 40 x 10.

At later periods an increase was observed in the number of follicles containing protein-bound radioactive iodine compounds. For instance, in rats sacrificed after 1 hour, practically all the follicles were radioactive. At this stage protein-bound radioactive iodine compounds also appeared in the cells of the follicular epithelium. The main accumulation of the iodized protein in the colloid was maintained, however, at whatever time the animals were sacrificed.

It was impossible to obtain micro-autoradiographs of the protein-bound S^{35}-methionine compounds 10 seconds after the intravenous injection of the labeled amino acid, even when an activity of 5-10 μC/g was used. Five minutes after injection of S^{35}-methionine, however, an obvious accumulation of sulfur-labeled protein was seen in the cells of the follicular epithelium. At this time hardly any radioactivity was present in the colloid (Fig. 2).

With lengthening of the interval between the injection of S^{35}-methionine and death of the animals, the concentration of labeled protein in the colloid rose, and after 24 hours it was considerably greater than its accumulation in the cells of the follicles. The greater accumulation of labeled protein in the colloid was also maintained at later stages after the injection of S^{35}-methionine.

The results obtained show that only 10 seconds after the intravenous injection of radioactive iodide, iodized protein was found in the colloid of the thyroid gland. We chose a time of 10 seconds because, after this time, the iodide would have passed from the place of injection through the remainder of the greater circulation and the lesser circulation and would have entered the tissue of the gland. It might have been expected that decapitation, leading to an instantaneous arresting of the circulation, would not have such a rapid paralyzing effect on the course of the metabolic processes in the thyroid gland. Extraction of the thyroid gland and trachea en bloc, and placing it in the cold fixing agent under our experimental conditions took about 10 seconds. A certain length of time was necessary also for diffusion of the cold fixative into the tissue. From the foregoing it follows that under these circumstances the usual course of the metabolic processes in the thyroid gland could continue for a further 15-20 seconds after the entry of the radioactive iodine into the gland. The obtaining of micro-autoradiographs of the thyroid gland at later times will evidently be fraught with great technical difficulties.

The experiment thus showed that for at least 15-20 seconds after the entry of the radioactive iodine into the thyroid gland, its protein-bound compounds were to be found exclusively in the colloid. The method of micro-autoradiography, like any other method, has certain limitations. For this reason it may be suggested that its sensitivity in this particular case was insufficient to show a low concentration of labeled protein in the cells of the follicles. By considering the density of the darkening of the photographic emulsion over the colloid, the background of the photographic emulsion, and length of the exposure, comparatively simple calculations will show what gradations of concentration of iodized protein between the colloid and the cells of the follicular epithelium would be necessary for it to be impossible to detect the presence of labeled protein in the cells. The calculations in our experiments showed that it would be possible to detect the presence of labeled protein in the cells in a concentration of 100-500 times less than in the colloid. It follows that 15-20 seconds after the entry of the iodine into the gland, not less than 99% of the newly formed iodized protein was present in the colloid.

On the basis of these findings, two hypotheses can be put forward regarding the site of iodization of the protein in the thyroid gland: iodization either takes place in the apical parts of the cells of the follicles of the thyroid gland at the moment that the protein molecule passes into the colloid, or iodization of the protein takes place in the cavity of the follicle in the colloid itself. There are reasons to believe that the uptake of labeled amino acids in the body mainly reflects the process of protein synthesis [6, 16]. The appearance of labeled protein in the follicle cells first may indicate that this is the place where thryroglobulin is synthesized, after which it passes into the cavity of the follicle.

The results obtained suggest that the processes of synthesis of the thyroglobulin molecule and its iodization in the thyroid gland occur in different places. Synthesis of the protein part takes place in the follicle cells, and iodization occurs either in the cavity of the follicle or at the border of the apical parts of the cells and the colloid at the moment when the thyroglobulin molecule passes into the cavity of the follicle.

For this reason the reports [7, 8] that the tissue of the thyroid gland has the power to accumulate radioactive iodine in a protein-bound form, the presence of which is coincidental with the time of appearance of follicular structures in the symplast are of interest. Hence it follows that colloid filling the cavity of the follicles may be regarded not as a depot for hormone but as a structural unit of the gland having a definite function, in which takes place one of the more important stages in hormone formation – the iodization of the protein.

The droplets of matter found in the follicle cells which resemble colloid in their staining properties are regarded by several workers as being fully identical with colloid and as products secreted into the cavity of the follicle. There are reports [6] that organic iodine compounds are present in these droplets of matter. We consider that the organically combined iodine found in the follicle cells must be regarded as a product of hydrolysis of colloid destined for secretion into the blood stream. This is suggested by the later appearance of protein compounds of iodine in the follicle cells than in the colloid.

In our investigations we never found labeled protein-bound iodine compounds in the cells of the symplast which were not organized into follicular structures. This fact may be evidence that, in normal conditions, they do not form part of the organ and do not participate in the formation of the hormone.

SUMMARY

Experiments were performed on rats. Using the micro – autoradiographic method the author determined the localization of the protein-combined I^{131} and S^{35}-methionine compounds in the thyroid gland 10 seconds, 5-20

minutes and 1, 4, 24 and 72 hours after the intravenous injection of the labeled preparations. The iodine-thyroglobulin with radioiodine inclusion appears first in the colloid. Inclusion of S^{35}-methionine into the thyroglobulin molecule occurs in the follicular epithelium cells. The data obtained demonstrate that thryoglobulin synthesis and its iodization in the thyroid gland occur at different sites. Thyroglobulin synthesis takes place in the follicular cells, while protein iodization — either in the cavity of the follicles or at the border of the apical parts of the cells and colloid. The above led to the convulsion that the thyroid gland colloid should be regarded not as a place of hormone storage but as a structural unit of the organ which participates in hormone formation.

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