Morphological Aspects on the Site of Iodination of Thyroglobulin in the Thyroid Gland*

Hisao FUJITA**

Received October 26, 1971

Summary. The site of iodination of thyroglobulin in the thyroid gland revealed through morphological studies was critically reviewed in relationship to biochemical points of view. The phylogenetic and evolutional aspects of this problem were also reviewed and discussed. To conclude, the present author wishes to emphasize the following:

1. In adult vertebrates whose thyroid follicular structure has been well completed, the main site of the iodination of thyroglobulin is generally the follicular lumen and the apical plasma membrane region.

2. The iodination may also take place partly in the cytoplasm. Autoradiographic data from the chick embryo and from the dissociated thyroid cell of the rat and sheep, and the electron microscopic histochemical data for peroxidase reaction indicate the possibility that the iodination could occur also in the cytoplasm, such as in the rough endoplasmic reticulum, Golgi apparatus and subapical vesicles.

3. In usual adult animals, however, thyroglobulin in the follicular lumen is far larger in quantity than that in the cell cytoplasm and numerous molecules of thyroglobulin in the follicular lumen might have not yet completely iodinated and therefore injected iodine is considered to be combined with luminal colloid preferentially. This seems to be the reason why the iodination takes place almost entirely in the follicular lumen (and apical plasma membrane region) in adult animals having usual follicular structures.

4. Based on the data of larval lampreys and ascidians, iodine metabolism in the thyroid-homologous cells in cyclostomes and protochordates seems to show the similar pattern to that of higher vertebrates. The main site of iodination of protein in these animals is the apical plasma membrane region of certain types of endostylar cells, and the possibility of iodination taking place also in the endostylar lumen is not ruled out. Some of iodinated protein must be reabsorbed into the certain types of endostylar cells in these animals.

Contents

I. Orientation ..........................................................110
II. Site of iodination of thyroglobulin ..................................113
   1. Autoradiography ..................................................113
      a. Light microscopic level ........................................113
      b. Electron microscopic level ....................................116
   2. Relationship between morphological and biochemical data ....120

* A part of this study was supported by grants from the Dr. Henry and Bertha H. Buswell Research Fellowship and P. H. S. research grant Ca 3458 from the National Cancer Institute. This paper is dedicated to Distinguished Professor Oliver P. Jones upon his retirement as a Chairman of the Department August 31, 1971.

** The author would like to express his hearty thanks to Dr. Oliver P. Jones for giving him the opportunity to study in his department as a Visiting Research Professor (from Sept. 1, 1970 to Nov. 30, 1971) and for criticism of this manuscript.
I Orientation

In 1895 BAUMANN found by chance that the thyroid gland contains iodine. He isolated an iodine-binding amorphous compound and named it “Thyrojodin.” However, before him, the relationship between iodine and thyroid disease had been thought of by several people. It is said that exophthalmic goiter was described in Assyrian bas-reliefs and in medical script of ancient Egypt, China, India and Rome (Pre-Christian era), and a harmful quality of water was thought to be the cause of goiter in Rome (PLINIUS, A.D. 23–79; VITRUVIUS, Ist Century). ROGERIUS (1170), PROSSER (1769), COINDET (1820), PROUT (1834) and von BASEDOW (1840) tried to use an iodine preparation such as seaweed burnt sponges and others for the treatment of goiter or thyroid disorders. Since BAUMANN (1895), iodine has been considered to be an important component of the thyroid tissue. One of the hormones secreted from the thyroid gland was isolated as a crystal containing about 65% iodine in December, 1914 and named thyroxine by KENDALL (1915, 1919). However, a chemical structure he proposed (C₁₁H₁₉O₃N₃I₃; thyro-oxyindol) was wrong, and in 1926 HARRINGTON clarified it to be a derivative of tyrosine (C₁₅H₂₁O₄N₄I₂). Since then thyroxine, the molecule of which binds 4 iodine atoms has been regarded as the only substance to act as a hormone. Thyroxine is also named tetraiodothyronine (T4) owing to its chemical structure. In 1952, Gross and PITT-RIVERS, and ROCHE et al. found triiodothyronine (T₃) binding 3 iodine atoms as a substance having stronger hormonal action than thyroxine. Though diiodothyronine (T₂) also has very weak hormonal action, little of this material is secreted and now T₃ and T₄ are generally known as thyroid hormones. In addition another kind of thyroid hormone, thyrocalcitonin, has been discovered by COPP et al. (1962) and HIRSCH et al. (1964). Being the calcium-lowering factor quite different from the usual thyroid hormone in its chemical structure and function, this hormone is not discussed in the present paper.

\[
\begin{align*}
\text{HO} & \text{C}_2\text{H}_4\text{NH}_2\text{COOH} & & : \text{tyrosine} \\
\text{HO} & \text{O} & \text{C}_2\text{H}_4\text{NH}_2\text{COOH} & & : 3, 5, 3'\text{'-triiodothyronine (T₃)} \\
\text{HO} & \text{O} & \text{C}_2\text{H}_4\text{NH}_2\text{COOH} & & : 3, 5, 3', 5'\text{'-tetraiodothyronine (T₄)} = \text{thyroxine}
\end{align*}
\]
Both triiodothyronine and thyroxine being amino acid derivatives have a relatively simple chemical structure. Tyrosine and iodine are important materials for synthesizing the thyroid hormones. However, the production process of these hormones is very complicated and the hormones are not synthesized directly from tyrosine and iodine. First, a high molecular glycoprotein named thyroglobulin which incorporates a number of molecules of thyroid hormone is made and then it is hydrolyzed to liberate the hormone. There are many steps in the production of the hormones in this gland. These are: (1) synthesis of thyroglobulin in the follicular epithelial cell, (2) release of thyroglobulin into the follicular lumen, (3) iodination of thyroglobulin, (4) reabsorption of thyroglobulin into the follicular epithelial cell, (5) hydrolysis of thyroglobulin to liberate triiodothyronine (T3) and thyroxine (T4), (6) release of T3 and T4 from the basal part of the cell. Thyroglobulin, named by Oswald (1899) is a glycoprotein, about 660,000 in molecular weight, and its physicochemical structures have been clarified by numerous biochemists (Shulman et al. 1955; Robbins and Rall, 1960; Balls et al. 1960 and others). The iodoprotein in the thyroid is classified into several kinds of subunits: 3-6S, 12S, 19S, 27S and 33S. The 19S protein corresponds to thyroglobulin in a strict sense. A molecular model for 19S thyroglobulin has recently been proposed by Edelhoch (1965). It consists of two equal subunits (12S), each made up of two polypeptide chains which are held together by one or a few disulfide bonds (S-S). So it is now believed that thyroglobulin consists of 4 peptide linkages. The thyroid hormones, such as triiodothyronine and thyroxine, are incorporated into the peptide linkages of thyroglobulin, and the hydrolysis of thyroglobulin is needed to liberate the hormones. Thyroglobulin synthesized in the follicular epithelial cell is released into the follicular lumen to be stored there. The colloid in the lumen corresponds to thyroglobulin secreted from the follicular epithelial cell.

Many papers have been published from morphological and biochemical aspects regarding the synthesis of thyroglobulin. Nadler et al. (1964) reported, using the electron microscopic autoradiography of 3H-leucine, that proteinous components of thyroglobulin are synthesized in the elements of rough endoplasmic reticulum and transported to the Golgi apparatus to become matured secretory granules (=subapical vesicles). Ekholm and Strandberg (1966, 1967a, b, 1968) using biochemical techniques and electron microscopy of the subcellular fractionations showed that in the guinea pig and rat thyroid, the thyroglobulin is synthesized within microsomal fractions (corresponding to rough endoplasmic reticulum) and then transported to the smooth surfaced vesicles. Recently the site for binding of the component of carbohydrate into thyroglobulin has been detected using the electron microscopic autoradiography of 3H-galactose, 3H-mannose (Whur et al., 1969) and of 3H-fucose (Haddad et al., 1971). They say that galactose and fucose are incorporated into thyroglobulin within the Golgi apparatus and mannose within the rough endoplasmic reticulum.

Then the thyroglobulin contained in the subapical vesicles is extruded into the follicular lumen and stored as a colloid there. As occasion demands, the thyroglobulin (colloid) in the follicular lumen is reabsorbed into the follicular cell. As to the mechanism of colloid reabsorption, phagocytosis and pinocytosis have been proposed, and intracellular large colloid droplets are now generally believed to be reabsorbed materials by many authors (Wollman and Spicer, 1961; Nadler et al., 1962; Bauer and
MEYER, 1964; SHELDON et al., 1964; WOLLMAN et al., 1964; WETZEL et al., 1965; EKHLÖM and SMEDS, 1966; SELJELID, 1967a, b, c, d; FUJITA, 1969; SELJELID et al., 1970). Lysosomes showing dense granular structures have been thought to fuse with the re-absorbed colloid droplet in order to hydrolyze its content and thereby liberate triiodothyronine and thyroxine (WOLLMAN et al., 1964; WOLLMAN, 1965; SELJELID, 1967d). These hormones are released from the basal part of the cell into the pericapillary

Fig. 1. A part of the normal chick thyroid follicular epithelial cell. Notice rough endoplasmic reticulum (R), Golgi apparatus (G), subapical vesicles (V) and reabsorbed colloid droplet (D). The protein is synthesized in the rough endoplasmic reticulum and transported to the Golgi apparatus where subapical vesicles containing thyroglobulin are formed. ×26,000
space. In addition, moniodotyrosine (MIT) and diiodotyrosine (DIT) are also liberated by hydrolysis of thyroglobulin. From these iodotyrosines, inorganic iodide and tyrosine are produced and used again for thyroglobulin synthesis.

There is another problem remaining as to the thyroglobulin synthesis. Tyrosine and thyronine incorporated into the thyroglobulin molecule should be iodinated. According to the degree of iodination, there are several kinds of iodotyrosine and iodothyronine; moniodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T3), tetraiodothyronine (T4) and so on. For making iodinate proteins, two steps are required: first, inorganic iodide is accumulated into the thyroid gland and then this iodide is oxidized and combined with protein. In the present review, the author will discuss chiefly the site for the second reaction, iodination of protein. For about 20 years many biochemical and morphological studies have been done concerning the site of iodination of tyrosine or thyronine. Nevertheless, there are some differences of opinion expressed in these studies. This problem has been one of the most important subjects in thyroid research and has not yet been clearly solved. Briefly speaking, some investigators have considered that the iodination of thyroglobulin (tyrosine or thyronine incorporated) takes place within the cell and the others, within the follicular lumen. In the present paper progress in the study as to the site of iodination of thyroglobulin will be traced critically and the present author’s opinion will be expressed.

II. Site of Iodination of Thyroglobulin

1. Autoradiography

   a. Light microscopic level

   One of the most powerful techniques for attacking this problem is autoradiography using radioactive iodine. Autoradiography is a method for tracing the route of certain material, using the radioisotope given into the living body, in the histological and cytological level. It was Bequerel (1896) who found first that the photographic plate is sensitized by the radioisotope, and Lacassagne (1924) succeeded in demonstrating the localization of injected radioactive polonium in animal tissue. They are pioneers in autoradiography. However, it was around 1940 that this method began to be used for biological research using many kinds of artificial radioisotopes.

   Autoradiography of the thyroid, using radioactive iodine, was first performed by Hamilton et al., (1940) and Gorbman and Evans (1941). They recognized the radioiodine incorporated into the thyroid colloid. The first works performed using autoradiography of radioactive iodine for detecting the site of iodination of thyroglobulin are those of Leblond and Gross (1948) and Doniach and Pelc (1949). Since then many investigators have applied this method to studying the iodine metabolism of the thyroid. Most of them injected inorganic iodide (131I or 125I) into the blood vessel or intraperitoneally, killed the animal certain minutes, certain hours or days after the treatment, and made an autoradiogram of the thyroid tissue. During the usual autoradiographic procedures, inorganic iodide must be washed away by fixative, alcohol and water, and only organic iodide bound with protein is detected by this method. So this technique seems suitable for the localization of organic iodide in the tissue. First 131I was used to greater advantage at the light microscopic level, and now 125I is
the favorite isotope in electron microscopy by many investigators, because $^{131}$I emits high energy $\beta$-particles with an energy conversion of a maximum energy of 608 KeV, while the energy radiation of $^{125}$I is low (3, 27 and 34 KeV) and more useful for detecting fine structural localization.

In the pioneer work done by Leblond and Gross (1948) using the light microscopic autoradiography, they showed that in rats receiving daily nonradioactive iodine or in hypophysectomized rats, the radioactivity is mostly present in the epithelium, especially in the apical portion of the cell, one hour after the injection of $^{131}$I. On the contrary in the iodine deficient animals, organic $^{131}$I was demonstrated mostly in the follicle luminal colloid as early as 2 minutes after the injection. Doniach and Pelc (1949) also found that organic $^{131}$I is present only in the follicular lumen of normal rat thyroids within 10 min after the injection. The resolving power of autoradiography was not so good in those days, though the results of Leblond and Gross (1948) seem to suggest the possibility that the iodination of thyroglobulin might take place in the follicular epithelium and those of Doniach and Pelc (1949) showed that the iodination might occur in the follicular lumen. Later, Nadler and Leblond (1954) and Van Heyningen and Sandborn (1963) reported that the iodination takes place in the follicular lumen. Many papers published since 1955 have concluded that the follicular lumen is the main site for iodination of thyroglobulin, though there are a few exceptions. These are briefly extracted as follows: Wolman and Wodinsky (1955), who found the autoradiographic image always localized in the colloid between 11 seconds and 1 hr after radiiodide injection, concluded that the iodination of thyroglobulin occurs in the luminal colloid. They noticed organic iodide especially localized in the narrow ring at the edge of the luminal colloid at 2 min and earlier, and then distributed throughout the colloid gradually. This "ring reaction" at the edge of the colloid has also been described by Nadler and Leblond (1954) and Nadler et al., (1954). However, in order to know the details of the site of this reaction, electron microscopic autoradiography was necessary. Lowenstein and Wolman (1967a, b; 1970) published several works dealing with the distribution and diffusion of the organic iodide in the lumen. They observed that there is a difference in the speed of iodination of thyroglobulin between the central follicles and peripheral follicles in the whole thyroid. The follicles in the central part of the gland react more promptly to the injected iodide.

On the other hand, there are several papers expressing the opinion that the iodination of thyroglobulin might take place in the follicular epithelial cell. Levenson (1960), who removed the follicle luminal colloid using the frozen section from the normal rat thyroid labelled with $^{131}$I in vivo for 1 hr, demonstrated that cellular organic iodine was present in the remaining epithelial cells. He also found the ring reaction at the peripheral zone of the luminal colloid of the rat thyroid 30 min-1 hr after the administration of $^{131}$I. From these data, he concluded that the formation of iodine-containing organic compounds (= iodinated thyroglobulin) in the thyroid begins in the epithelial cells and continues in the follicular lumen. Pitt-Rivers et al. (1964) showed, using autorography, that organic iodine is present in the thyroid epithelial cells of an untreated rat after 2 hrs labelling with $^{125}$I, and in the thyroid of rats pretreated with iodide and labelled with $^{131}$I for 10 or 15 min. They concluded that thyroglobulin is iodinated in the follicular epithelial cells.

An autoradiography was also used to learn the distribution of inorganic iodide in
the thyroid gland. For this purpose freeze dried sections are necessary because inorganic iodide is washed away by usual histological techniques. Pitt-Rivers and Trotter (1953) and Doniach and Logothetopoulos (1955) found silver grains for $^{131}\text{I}$ in the follicular cell as well as in the follicular lumen, using freeze dried sections, 15 min or 30–50 min after the injection of $^{131}\text{I}$ into thiouracil-treated or propylthiouracil-treated animals respectively. These drugs were used for inhibiting the organification of iodide. Andros and Wollman (1964) demonstrated that the accumulation of inorganic iodide was much higher in the follicular cells of some follicles than in the

![Fig. 2. An electron microscopic autoradiogram of a part of the mouse thyroid 1 hr after the injection of $^{125}\text{I}$ (200 $\mu\text{Ci}$). Numerous silver grains are localized only in the follicle lumen. $\times 15,000$]
Follicular lumen 5 min after the injection of $^{131}$I into propylthiouracil-treated mice, and they considered that the basal plasma membrane is responsible for iodine concentration and the apical plasma membrane exerts some control over the passage of radioiodide from the cell into the follicular lumen.

Fig. 3. A schematic diagram illustrating the iodine metabolism of the thyroid cell of an adult mouse or rat. a. A few minutes—a few hours after the injection of $^{125}$I. Most silver grains (red) are localized in the follicle lumen. The iodination of thyroglobulin takes place almost entirely in the follicle lumen. b. 24 hrs after $^{125}$I administration and 1 hr after TSH injection. Silver grains are found in the follicle lumen and in the reabsorbed colloid droplets.

b. Electron microscopic level

A technique for electron microscopic autoradiography was first tried by Liquiere-Milward (1956) using $^{60}$Co incorporated into nuclei of tumor cells. Since then many attempts have been made to improve the method by several investigators (O'Brien and George, 1959; Harford and Hamlin, 1961; Caro, 1961, 1962; Salpeter and Bachmann, 1964 and others). Electron microscopic autoradiography using radioactive iodide has been applied to thyroid research by many investigators (Kayes et al., 1962; Stein and Gross, 1963, 1964; Sheldon et al., 1964; Bauer and Meyer, 1964, 1965;
Site of Iodination of Thyroglobulin

IBRAHIM and BUDD, 1965; SIMON and DROZ, 1965; LUPULESCU and PETROVICI, 1965, 1968; NUNEZ, E. et al., 1966; EKHOLM, 1966; EKHOLM and SMEDS, 1966; TAKANO and HONJIN, 1968; FUJITA, 1969; SELJELID, 1970; NADLER, 1971; and others). This method was used for detecting the site of iodination of thyroglobulin or for determining whether the intracellular colloid droplet is the secretory substance or reabsorbed material. Among these authors STEIN and GROSS (1963, 1964), IBRAHIM and BUDD (1965), LUPULESCU and PETROVICI (1965), SIMON and DROZ (1965), EKHOLM (1966), TAKANO and HONJIN (1968), FUJITA (1969) and NADLER (1971) have applied this technique for detecting the site of iodination of thyroglobulin. By electron microscopic autoradiography, most of these authors found numerous silver grains only in the thyroid follicular lumen a few minutes to several hours after the injection of $^{125}$I or $^{131}$I into the mouse, rat or guinea pig and they considered that iodination of thyroglobulin takes place almost entirely in the follicular luminal colloid. TAKANO and HONJIN (1968) concluded that the iodination occurs only in the rough endoplasmic reticulum, though numerous silver grains were localized in the follicular lumen and very few in the cytoplasm in their photographs. They refused to consider the numerous grains in the follicular lumen. Though LUPULESCU and PETROVICI (1965) also found quite a few silver grains in the rough endoplasmic reticulum and FUJITA (1969) in the Golgi apparatus, they also attached importance to numerous grains in the follicular lumen. IBRAHIM and BUDD (1965) and FUJITA (1969) found silver grains for $^{125}$I in the follicular lumen and not in the cytoplasm 3–5 min after the injection into rats and mice. Grains in the follicular lumen and apical cell membrane region are increased in number with time, while none or few grains appear in the cytoplasm during a few hours after the injection (FUJITA, 1969). This fact tells us generally that the site of iodination of thyroglobulin is not in the cytoplasm but in the follicular luminal colloid and apical plasma membrane region in this case. In some of the follicles, silver grains are markedly numerous in the apical plasma membrane (microvillous) region and in the peripheral region of the follicular lumen. This phenomenon corresponds to the ring reaction mentioned above. So it is considered that active iodination of thyroglobulin takes place particularly in this region of some follicles. Here the question may arise whether the possibility exists or not that thyroglobulin is iodinated in the cytoplasm and then released into the follicular lumen rapidly. However, it has already been shown, using the autoradiography of $^3$H-leucine, that the synthesis of thyroglobulin in the cytoplasm does not proceed as rapidly (NADLER et al., 1962; FUJITA, unpublished data). According to the data of the present author, using electron microscopic autoradiography of $^3$H-leucine, it takes more than 30 min until a few silver grains appear in the follicular lumen and numerous grains remain in the cytoplasm during several hours after the injection of the isotope; on the contrary $^{125}$I appears within 3 min in the follicular lumen after the injection. This fact suggests that the protein synthesis and release do not proceed as quickly when compared with the iodination of the thyroglobulin. So the possibility mentioned above that thyroglobulin in synthesizing process might be iodinated in the cytoplasm and released into the lumen too rapidly to recognize the silver grains in the cytoplasm after the injection of radioactive iodine, can almost be ignored, though the possibility cannot be ruled out that thyroglobulin in some subapical vesicles might be iodinated and extruded into the follicular lumen during a shorter time. Here, the present author wishes to cite NADLER’s conclusion...
(1965) which is now believed by many morphologists, that protein moiety of thyroglobulin is secreted from the follicular epithelial cell into the periphery of the follicular lumen where it is iodinated.

The present author, however, does not deny the possibility that the cell cytoplasm has also an ability to iodinate thyroglobulin. Some studies using tissue culture or biochemical methods have shown that the iodination of thyroglobulin takes place intracellularly. Pulvertaft et al. (1959) have reported that isolated pathological human thyroid cells grown in tissue culture in the presence of $^{131}$I form very small amounts of iodinated tyrosine, thyroxine or triiodothyronine, and Pastan (1961) noticed the formation of monoiodotyrosine (MIT) in peptide linkage in the single cell dispersed from the calf thyroid when incubated with $^{131}$I. Tong et al. (1962b) reported that iodine is incorporated in the sheep thyroid cells which had been dispersed and isolated by trypsinization, and they interpreted that the presence of colloid and follicular structure is not essential for iodine-concentrating function and hormone synthesis by the thyroid gland. Raghupathy et al. (1965) also showed biochemically in isolated cultures of sheep thyroid cells that they are able to incorporate $^{131}$I into a thyroglobulin-like protein.

Tixier-Vidal et al., (1969), learning that sheep thyroid cells isolated by trypsinization and incubated in the presence of $^{125}$I exhibit a few silver grains in the rough endoplasmic reticulum by electron microscopic autoradiography, have the opinion that the site of thyroglobulin iodination is intracellular, at the level of the rough endoplasmic reticulum. The present author (Fujita, unpublished data), who also

---

**Fig. 4.** A part of the thyroid of a 10-day-old chick embryo 30 min after the injection of 50 μCi of $^{125}$I. Grains are seen over the cytoplasm as well as over the primitive follicular lumen. ×14,000
obtained a similar result in the dissociated cultured rat thyroid cell, considers that the cell cytoplasm has also a capability to iodinate thyroglobulin. He has a view that the iodination of thyroglobulin could take place not only in a certain strict part but also in some other regions of the gland, according to various experimental or natural conditions. Even in the normal adult rat, mouse or guinea pig, Lupulescu and Petrovici (1965), Ekholm (1966), Takano and Honjin (1968) found a few silver grains over the rough endoplasmic reticulum in the follicular epithelial cell.

Similar results were obtained in vivo in the chick embryo thyroid whose follicular structure is not well organized (Fujita, 1969). As Hilfer (1965), and Fujita and Tanizawa (1966) reported, the most primitive follicular lumen appears in an 8-day-old chick embryo. By light microscopic autoradiography, organic iodide is first recognized in an 8-day-old embryo (Fujita and Nagata, 1962). According to Fujita and Tanizawa (1966), the typical follicular structure is completed in a 14-day-old embryo. Fujita (1969) reported, using electron microscopic autoradiography, that the thyroid of a 10-day-old chick embryo disclosed several silver grains over the

---

**Fig. 5.** A part of the thyroid of a 13-day-old chick embryo 2 hrs after the injection of $^{131}I$ (200 μCi). Grains are seen over the cytoplasm as well as the small follicular lumen. × 20,000
thyroid cell cytoplasm, especially over the Golgi apparatus, apical small vesicles, rough endoplasmic reticulum as well as over the follicular lumen 15, 30, 45 and 60 min after the injection of $^{125}$I. These data show that iodination of thyroglobulin could take place not only in the follicular lumen but also in the cell cytoplasm.

2. Relationship between morphological and biochemical data

The question may arise why iodination occurs almost entirely in the follicular

Fig. 6. A part of the thyroid of a 10-day-old chick embryo 24 hrs after injection of $^{125}$I (50 $\mu$Ci). Grains are localized in the follicle lumen. $\times$15,000
lumen of the adult mouse and rat. It is well known that thyroglobulin is synthesized in the elements of the rough endoplasmic reticulum, transported to the Golgi apparatus and extruded into the follicular lumen. Seed and Goldberg (1965) showed biochemically in vitro that puromycin which blocks the synthesis of thyroglobulin does not block its iodination, and propylthiouracil which blocks the iodination of thyroglobulin does not block the incorporation of amino acid into thyroglobulin. Many biochemical studies have demonstrated that these two reaction processes (synthesis and iodination) are independent of each other, and the iodination takes place after the synthesis of its peptide backbone has been done (Lissitzky et al., 1964, 1965; Nunez et al., 1965; Seed and Goldberg, 1965; Sellin and Goldberg, 1965; Cavalieri and Searle, 1967). Mauchamp et al. (1965) reported that the site of thyroglobulin synthesis is different from that of its iodination. Now it is believed that the tyrosine which has already been incorporated in the peptide linkage is only iodinated and the iodination of tyrosine does not likely occur at the amino acid level. The heterogeneity of thyroglobulin in iodination has been shown by numerous investigators (Uit et al., 1961; Robbins, 1963 and others). In various vertebrates it has been demonstrated that iodination could occur not only in 19S but also in 12S and eventually in other proteins (Lachiver et al., 1965). They considered that these different proteins in iodination could be dependent upon the species and the physiological state. Nunez et al. (1965), who found the iodination of prethyroglobulin could occur and the polymerization of the precursors (3–8 S, 12S) of thyroglobulin does not depend on their iodination, suggested the existence of two iodination sites; the first is intracellular particles and the second is the luminal colloid. Perelmutter et al. (1965), Pierce et al. (1965), De Crombrugghe et al. (1967) Tarutani and Uit (1968, 1969), and Olien et al. (1970) obtained poorly iodinated or non-iodinated thyroglobulin from the thyroid of goitrous patients, goitrogen-treated animals and CR 60 mm human embryo. Mauchamp et al. (1965) reported that thyroid slices from sheep synthesize thyroglobulin which is still not iodinated.

From these facts, it is easy to consider that thyroglobulin could be iodinated in various sites as mentioned above. The present author has speculated that the thyroglobulin molecule extruded into the follicle lumen has not yet been iodinated satisfactorily and has numerous spaces in itself for binding iodine (Fujita, 1969). In thyroids of many species of animals (13 mammals and 2 lower vertebrates), Salvatore et al. (1965) reported that 19S is the major component, varying form 82–100% of thyroglobulin-like iodoproteins. The majority of 19S is the follicular luminal colloid and so follicular luminal thyroglobulin is by far the larger in quantity than cytoplasmic thyroglobulin. Hence, when iodine is injected into the adult animal, a large amount of the iodide ion is considered to enter the follicular lumen to combine with thyroglobulin of the luminal colloid. This speculation coincides well with the biochemical data that thyroglobulin obtained from the follicular colloid shows heterogeneity in degree of iodination (Uit et al., 1961; Assem et al. 1965).

3. Enzyme of iodination

Some enzymes have been known to be necessary for the iodination of thyroglobulin. First, the iodide ion must be taken into the thyroid cell from the blood capillary. The iodide ion in the blood capillary must pass through the endothelium,
endothelial basal lamina, pericapillary space, follicle basal lamina, and plasma membrane of the follicular epithelial cell. Now it is not clear why the iodide ion in the blood capillary is easily taken up into the follicular epithelial cell passing through these many kinds of structures. The uptake of iodide ion is called iodide trapping. Though the mechanism of iodide trapping is not clear, active transport (ion pump) has been proposed to explain this mechanism (Doniach and Logothetopoulos, 1955; Woodbury and Woodbury, 1963), and ouabain sensitive ATPase seems to be implicated in the take-up of the iodide ion into the cell (Wolff and Halmi, 1963). However, no one has yet succeeded in demonstrating ATPase in the basal plasma membrane of the thyroid follicular epithelial cell histochemically. This is a problem to be solved in the future.

Then the iodide ion should be organized. It has been believed that iodide ion taken up into the follicle epithelial cell is oxidized to become I₂ before being bound with thyroglobulin. Since the works of Serif and Kirkwood (1958) and Alexander (1959), the oxidation of iodide ion is accepted to occur using H₂O₂ with the enzymatic action of peroxidase. The oxidized iodine is the active form of iodide. Reactions are summarized as follows:

\[
\begin{align*}
\text{NADH (or NADPH)} + \text{H}^+ + \text{O}_2 & \xrightleftharpoons{\text{peroxidase}} \text{NAD}^+ \text{ (or NADP}^+) + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + \text{I}^- & \xrightarrow{\text{peroxidase}} \text{oxidized iodide} + \text{H}_2\text{O} \\
\text{Oxidized iodide} + \text{tyrosine} & \xrightarrow{\text{tyrosine iodonase (?)}} \text{idotyrosine}
\end{align*}
\]

Histochemically endogenous peroxidase reaction has been recognized in the follicular epithelial cell of the rat thyroid (Dempsey, 1944; DeRobertis and Grasso, 1946) and in the follicle colloid (DeRobertis and Grasso, 1946). The fine structural localization of a peroxidase in the thyroid gland has been demonstrated electron microscopic histochemically by Strum and Karnovsky (1970, 1971), Nakai and Fujita (1970) and Shin et al. (1970). Using 3, 3'-diaminobenzidine tetrahydrochloride (DAB), they all found the reaction products for endogeneous peroxidase in the cisternae of rough endoplasmic reticulum, in dense bodies and around the external surface of the microvilli in the apical lumen of the rat thyroid. In addition, Strum and Kharnovsky (1970) and Nakai and Fujita (1970) described the DAB positive reaction in the Golgi vesicles and subapical vesicles. These facts suggest that the iodination of thyroglobulin could take place in many parts of the cell such as the cisternae of rough endoplasmic reticulum, Golgi vesicles, subapical vesicles and follicular lumen, while Strum and Karnovsky (1970) considered that the peroxidase is also synthesized in the rough endoplasmic reticulum and transported to the Golgi apparatus to be condensed, and a matured peroxidase exists only in the apical vesicles and external surface of the microvilli. From this opinion, they explain that iodination takes place in the apical vesicles or in the peripheral luminal colloid. Biochemically the peroxidase activity has been demonstrated in the thyroid homogenate by Alexander (1959, 1961, 1965) and Hosoya (1961) and this enzyme is isolated and purified by Hosoya and Morrison (1967). It has been shown that the enzyme for iodine-binding is present in
the subcellular particulate fractions (or microsome-mitochondrial fractions) by Kondo (1961), Suzuki et al. (1961), Alexander and Corcoran (1962) DeGroot and Davis (1962), Hosoya et al. (1962), Klebanoff et al. (1962), Maloof et al. (1964) and Alexander (1965). According to Hosoya et al. (1962) the subcellular fraction sedimented between the limits of 5,000 g for 10 min and 19,000 g for 30 min was richest in the enzyme in the sheep thyroid. This microsomal fraction must correspond to the rough endoplasmic reticulum. The Golgi vesicles and subapical vesicles may also be included in the small particles of the microsomal fraction. Furthermore, Ben Abdeljlil et al. (1967), who succeeded in purifying the apical particles (that might contain microvilli also) by using the sheep thyroid, demonstrated the existence of iodide-peroxidase activity in these particles. Olin et al. (1970) who studied the embryogenesis of the human thyroid using morphological and biochemical methods, concluded that the iodination of thyroglobulin parallels the formation of follicles and the structural maturation of the apical cell zone of the follicular cells. In summary the data obtained from electron microscopic histochemistry almost coincides with those from the histochemistry mentioned above.

As for the iodination of thyroglobulin, besides peroxidase, another enzyme, tyrosine iodinase has been proposed (Fawcett and Kirkwood, 1954). However, the reality of the existence of this enzyme is now obscure. This enzyme has been considered necessary for iodinating the tyrosine which is incorporated in thyroglobulin. The localization of tyrosine iodinase has not yet been demonstrated histochemically, though Fawcett and Kirkwood (1954), Serif and Kirkwood (1958) and Yip (1964, 1965) described the occurrence and properties of the enzyme, tyrosine iodinase, in the subcellular fraction biochemically. It is not clear whether the enzymic action of tyrosine iodinase is only one of the functions of peroxidase or not. In other words, it remains to be decided whether tyrosine iodinase and peroxidase are merely the same thing or two different things. As Taurog and Howell (1966) and Nunez et al. (1966a, b) observed, iodination of thyroglobulin takes place with the enzymatic action of crystalline chloroperoxidase and horseradish peroxidase respectively. Furthermore, Hosoya (1968) clarified that purified thyroid peroxidase (Hosoya and Morrison, 1967a, b) has the activity for iodination of thyroglobulin. These results suggest that a single enzyme can act for the oxidation of iodide as well as the iodination of thyroglobulin. In regard to the localization of enzymes necessary for iodine metabolism, further studies should be done in the future.

4. Conclusion

To conclude, the author wishes to repeat that iodination of thyroglobulin takes place almost entirely in the follicular luminal colloid, especially in its peripheral region (apical plasma membrane region) in normal adult animals, though several regions of the follicular epithelial cell have also the ability for iodination of proteins.

III. Phylogenetic Aspects on the Site of Iodination

1. Orientation in phylogeny

Several excellent reviews have been published about the comparative aspects of thyroid function (Bargmann, 1939; Gorbman, 1959; Barrington, 1959, 1964, 1968;
GORBMAN and BERN, 1962; DODD and MATTY, 1964 and others). However, we could not find any review dealing with the site of iodination of thyroglobulin-like protein in phylogenetic aspects.

Throughout all the vertebrates (fish, amphibia, reptilia, aves and mammalia), the thyroid is known as a morphologically simple organ consisting of numerous follicular structures. The cyclostome, the lowest vertebrate, is the most interesting in the evolutionary meaning of the thyroid. One species of the cyclostome is the hagfish and other is the lamprey. The thyroids of both animals show scattered follicles. In the lamprey the thyroid structures are quite different between larval and adult animals. The thyroid of an adult lamprey consists of extremely large and small follicles scattered in the hypobranchial region. On the other hand, in the larval lamprey called ammocoetes, no follicular structures are formed in this organ and the organ is not called a thyroid but an endostyle. So, morphologically the most primitive thyroid is thus that of the adult lamprey. The endostyle of the larval lamprey was already described as an organ homologous to the thyroid by MÜLLER (1871), DOHRN (1886), and Bargmann (1939). Iodotyrosine (MIT, MIT) and iodothyronine (T3, T4) have been known to be present in extracts of the endostyle of a larval lamprey, ammocoetes of Lampetra planeri or of Petromyzon marinus (LELOUP and BERG, 1954; Leloup, 1955; Roche et al. 1961). The endostyle of this animal consists of two hollow organs extending from the first to fifth gill arches and separated by a medial septum. Each hollow communicates with the pharynx through a narrow duct, the ductus hypobranchialis, which is closed at metamorphosis.

The epithelial cells of this organ in the larval lamprey have been classified into five or six types; type 1 to type 5 or 6 (MARINE, 1913; LEACH, 1939). Among them

**Fig. 7.** Transverse section through one of the anterior chambers of the endostyle of a larval lamprey, ammocoete of Lampetra japonica. 1–5 Type 1–5 cells. Type 2c and type 3 cells are considered to be homologous to the thyroid cell.

**Fig. 8.** Transverse section of the endostyle of an ascidian, Ciona intestinalis. 1–8 Zone 1–8 cells. Zone 7 and 8 cells are considered to be homologous to the thyroid cell.
only type 2c and type 3 cells have been considered to be homologous to the thyroid follicular epithelial cell. LELoup (1952, 1955), STERBA (1953), OLIVEREAU (1955), BARRINGTON and FRANCHI (1956) and CLEMENTS-MERLINI (1960a, b) established, by light microscopic autoradiography, that the type 2c and type 3 cells, especially type 3 cells, are the main iodine concentrating cells. At metamorphosis type 3 cells are considered to become chiefly follicular epithelial cell (STERBA, 1953; CLEMENTS-MERLINI, 1960; HONMA, 1960). Electron microscopically type 2c and type 3 cells are fairly similar to the follicular epithelial cell of the adult lamprey (FUJITA and HONMA, 1968).

Endostyles of further lower animals, such as ascidians and amphioxus, have also been regarded as homologous to the thyroid. These animals are included in the category of phylum chordata like the vertebrate and are known as a species of a protochordata. The ascidians belong to the category of subphylum tunicata and the amphioxus to subphylum cephalochordata. The light microscopic structures of their endostyles have been described by many investigators (BARRINGTON, 1957, 1959, 1964; BARRINGTON and FRANCHI, 1956; BARRINGTON and THORPE, 1965a; OLSSON, 1963 and others). The endostyle of these animals is a groove in the floor of the gill chamber and its general structure is quite different from that of the larval lamprey. The main function of the endostyle of these animals has been considered to be gathering foods, mixing them with mucoproteinous substances secreted from some kinds of cells, and sending them into the digestive canal using numerous cilia. By light microscopy 8 zones are distinguished in the transverse section of the endostyle of the ascidian and 6 zones in amphioxus. Zone 1 is a median region forming the bottom of the floor of an endostyle in the ascidian, Ciona intestinalis. Each zone from zone 2 to zone 8 consists of two symmetrical rows respectively arranged in order forming the lateral walls of the endostyle. Cells in zones 2, 4 and 6 are glandular elements secreting mucoproteinous or proteinous substances and those in zones 1, 3 and 5 to be supporting elements in Ciona. Zone 7 cells, or zone 7 and 8 cells are considered to be homologous to the thyroid cell of vertebrates (BARRINGTON and THORPE, 1965a; FUJITA and NAMBA, 1971). In amphioxus, zone 2, 4 and 5 cells are glandular cells which might be equivalent to zone 2, 4 and 6 cells of Ciona intestinalis, and THOMAS (1956) reported that iodine is accumulated in the mucous cell of the dorsal gland tract corresponding to zone 5 and BARRINGTON (1958) also emphasized that the zone 5 cells bind iodine. Recent chemical studies have shown that endostyles of these animals produce thyroid hormones which are quite the same as those in higher vertebrates.

KENNEDY (1966) found monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T3) and thyroxine (T4) in ascidian blood. In addition, BARRINGTON and THORPE (1956b, and BARRINGTON, 1968) demonstrated thyroxine in extracts of the endostyle of an ascidian, Ciona intestinalis using radiochromatography. COVELLI et al. (1960) and TONG et al. (1962a) reported the existence of MIT, DIT, T3 and T4 in the endostyle of an amphioxus.

Iodotyrosine and rarely iodothyronine have been demonstrated chiefly in the body surfaces of some invertebrates. The synthesis of iodothyronine is interesting and important for thyroid function, but the present author does not deal with iodine metabolism in the invertebrate in this review.
2. Site of iodination in phylogenetic aspects

From mammalia to elasmobranchs

There are many papers dealing with the fine structure of thyroid glands in higher as well as lower vertebrates. The follicular epithelial cells of mammals (Ekhholm and Sjöstrand, 1957; Wissig, 1960, 1963; Heimann, 1966; Seljelid, 1967a; Klinck et al., 1970); aves (Fujita, 1963); reptilia (Watari, 1960; Muramoto, 1964); amphibia (Herman 1960; Coleman et al., 1968; Larsen, 1968; Hearing and Epper, 1969; Nakai et al., 1970); teleost (Fujita and Machino, 1964; Fujita et al., 1966; Suemasu et al., 1966) and chimaeroid (Nakai and Gorbman, 1970) show a similar pattern to one another in their fine structures. The rough endoplasmic reticulum where thyroglobulin is synthesized is well developed in the basal and lateral parts of the cell and the Golgi apparatus where thyroglobulin is transported from the rough endoplasmic reticulum is located in the supranuclear region. In addition to these, subapical vesicles (secretory granules), large reabsorbed colloid droplets, and dense granules (lysosomes) are seen in the apical region of the cytoplasm. Electron microscopic autoradiography of 125I of lower vertebrates such as amphibia, Rana nigromaculata nigromaculata, and chimaeroid fish, Hydrolagus colliei, shows a similar pattern to that of mammalia (Nakai et al., 1970; Nakai and Gorbman, 1969). They considered that in these animals the main site of the iodination of thyroglobulin is the follicular luminal colloid as in higher vertebrates.

Cyclostomes and protochordates

As for the thyroid of cyclostomes, the fine structure of the follicular epithelial cell has been described in the adult lamprey (Fujita, 1966; Fujita and Honma, 1966; Hoheisel, 1970) and in the adult hagfish, Myxine glutinosa (Fujita and Honma, unpublished data). In the adult lamprey, Lampetra japonica, three kinds of follicular epithelial cells were classified; ciliated taller, non-ciliated taller, and non-ciliated lower cells. However, these cells are considered to be essentially the same in their function. Though the rough endoplasmic reticulum is somewhat well developed in these cells, the cisternae are not so dilated as those of the higher vertebrate. For this reason, Fujita and Honma (1966) have the idea that the thyroid function of this animal is not so active. The cell has large or small dense bodies in the cytoplasm which might be lysosomes. By light microscopic autoradiography of 125I, the main site of the iodination of thyroglobulin is thought to be the follicular luminal colloid (Fujita, unpublished data).

Concerning the endostyle of a larval lamprey, ammocoetes of Lampetra japonica, which is homologous to the thyroid, Egeberg (1965), Fujita and Honma (1968), and Hoheisel (1969) have described the fine structure. Type 2c and type 3 cells of the endostyle of this animal, which have been considered to become thyroid cells at metamorphosis show a good deal of similarity to the thyroid cell of the adult lamprey in the electron microscopic figures. These cells show numerous cilia in the apical part and flattened elements of rough endoplasmic reticulum in the infranuclear region. Though the large colloid droplets are not found, some dense bodies which might be lysosomes are distributed chiefly in the supranuclear parts. This dense body seems to correspond to the PAS-positive granules described by light microscopy.

In the ascidians, Ciona intestinalis, zone 7 cells or zone 7 and 8 cells are considered
to be homologous to the thyroid cell (Barrington and Thorpe, 1965a; Fujita and Nanba, 1971). Both cells, though cuboidal and lower in height, are somewhat similar to the type 2c and type 3 cells of the endostyle of a larval lamprey in their fine structure. They contain poorly developed rough endoplasmic reticulum, a small Golgi apparatus, a few lysosomes and multivesicular bodies, and numerous small vesicles. In addition, only zone 8 cells bear cilia on their apical surface. Concerning the fine structure of the endostylar cells of amphioxus and oikopleura, further studies are needed, though some descriptions have been made electron microscopically (Olsson, 1965; Welsch and Storch, 1969).

The iodine metabolism of the endostyles of cyclostomes and protochordates has been studied using light microscopic autoradiography of $^{131}$I or $^{125}$I in the larval lamprey (Gorbman and Creaser, 1942; Oliverneau, 1955; Barrington and Franchi, 1956; Clements-Merlini, 1960) and in the ascidian, Ciona intestinalis (Barrington and Thorpe, 1965a). Clements-Merlini (1960) and Barrington and Thorpe (1965a) considered that the protein iodination takes place in the cytoplasm of the type 2c and 3 cells of the endostyle in a larval lamprey, and of the zone 7 cell in Ciona intestinalis.
Fig. 10. Parts of type 3 (right) and 4 (left) cells of a larval lamprey 2 hrs after injection of $^{125}$I (200 $\mu$Ci). Silver grains are localized over the apical cell membrane region of only a type 3 cell. $\times 16,000$
respectively. Barrington and Franchi (1956) described the PAS-positive granules in the type 3 cell in the larval lamprey as being radioactive, in animals having been immersed 48 hrs in water containing 200 μCi/1 of 131I. It is necessary to consider whether these PAS-positive granules are secretory materials or reabsorbed ones. This problem will be discussed later. Clements-Merlini (1960) reported that the type 2c and type 3 cells in the larval lamprey show intracellular radioactivity several hours after the injection of 131I. As to the endostyle of Ciona intestinalis, Barrington and Thorpe (1965a) believed that the zone 7 cell is a specialized region with exceptional capacity for binding iodine and the main site of iodine-binding is intracellular. To detect the exact localization of iodide in the cell electron microscopic autoradiography of 125I is necessary.

Fujita and Honma (1969), who found by electron microscopic autoradiography numerous silver grains for organic iodide over the apical cell membrane region of type 2c and type 3 cells 30 min, 1 and 2 hrs after the intraperitoneal injection of 200 μCi of 125I in the larval lamprey, considered that iodination of thyroglobulin takes place almost entirely in the apical cell membrane region. In addition, from the fact

![Fig. 11. A schematic diagram of type 2c or 3 cells (3) and type 5 cells (5) of a larval lamprey. Silver grains (red dots) are located over the apical plasma membrane region of type 2c and 3 cells 1–2 hrs after the injection of 125I, and over the dense bodies (lysosomes), multivesicular bodies 6–24 hrs after the injection. In some of the type 5 cells, grains are found in large vacuoles, multivesicular bodies, and lysosomes 6–24 hrs after the injection.](image)
that a few apical small vesicles were labeled, they speculated that the apical small vesicles which might be secretory granules containing thyroglobulin-like protein is also slightly iodinated. After 6-24 hrs, they noticed silver grains over the small and large dense bodies which might be lysosomes in the cytoplasm and suggested the possibility of the occurrence of the reabsorption and hydrolysis of iodinated thyroglobulin in the type 2c, type 3 and type 5 cells. The present author wishes to add the following possibility; the endostylar lumen is not closed but opened to the digestive canal and so the materials in this lumen are washed away during the fixation and dehydration in autoradiographic procedures of the tissue. Therefore, it is not ruled out that the iodination of protein takes place also within the endostylar lumen. The present author considers that iodination of thyroglobulin-like protein in the endostyle of the larval lamprey takes place almost entirely in the apical plasma membrane region (and in the endostylar lumen), and the iodinated materials are reabsorbed into these cells to be hydrolyzed by lysosomes for producing the thyroid hormone. Though Clements and Gorbman (1955), Gorbman (1959), and Thomas (1962) suggested that the thyroglobulin-like protein is mostly transported to and hydrolyzed in the alimentary canal and reabsorbed through the digestive epithelium, the present author wishes to emphasize that the endostyle cells also reabsorb the iodinated protein and hydrolyze it to liberate the thyroid hormone. The PAS-positive granules, which Barrington and Franchi (1956) described in the type 3 cell, might correspond to the lysosomal dense bodies containing reabsorbed materials. As Clements-Merlini (1960a) reported, some of type 5 cells also contain accumulated iodine. However, this type of cells were labeled only in large vacuoles and dense bodies 6 and 24 hrs after the

Fig. 12. Parts of zone 8 cells of an endostyle of the ascidian, Ciona intestinalis, 6 hrs after immersion in sea water containing $^{125}$I ($1 \text{ mCi/l}$). Grains are seen over the apical plasma membrane region. $\times 12,500$
injection of $^{125}$I (FUJITA and HONMA, 1969). It is difficult to consider the vacuoles and dense bodies to be secretory materials. The vacuoles are probably implicated in reabsorbed materials and the dense bodies might be lysosomes, being necessary for hydrolysis of reabsorbed materials. This type of cell is very poor in indications of protein-synthesis (FUJITA and HONMA, 1968). Like OLSSON (1963) and EGEBERG (1965) suggested, the present author has the opinion that the type 5 cell also absorbs the iodinated thyroglobulin from the endostylyar lumen, though he does not deny the reabsorption of the thyroglobulin in the intestine. Concerning the iodine metabolism of the endostyle of Ciona intestinalis, a similar pattern to that of the larval lamprey was recognized by electron microscopic autoradiography. FUJITA and NANBA (1971) found numerous silver grains in the apical cell membrane region of zone 7 and zone 8 cells, especially of zone 8 cells, 1, 4, 6, 16 and 24 hrs after immersion in sea water containing 1 mCi/1 of $^{125}$I, and in the multivesicular bodies and lysosomes in these cells, especially 16 and 24 hrs after immersion. From these facts they thought that the main site of iodination of protein is the apical plasma membrane region, and they suggested the possibility that iodination takes place in the endostylyar lumen because the materials in the lumen are washed away during the autoradiographic procedures and the detection of this material for radioactivity is very difficult. They believed that the labeled multivesicular bodies and labeled large lysosomes might contain reabsorbed materials from the endostylyar lumen.

Based on the data of larval lampreys and ascidians, the present author wishes to conclude that iodine metabolism in the thyroid and its homologous organs throughout the vertebrates and protochordates shows a fundamentally similar pattern in phylogenetic aspects (FUJITA, 1971). Furthermore, the electron microscopic autoradiography of radioactive iodine in amphioxus and myxine is needed to clarify the phylogeny of the iodine metabolism of the thyroid gland. It is also necessary to examine whether the materials in the endostylyar lumen have the ability to combine with iodine or not.

---

**甲状腺におけるチログロブリンのヨード化の場について**

藤田 尚男

甲状腺において チログロブリンがヨード化される場（チログロブリンに組み込まれたチロシンにヨードが結合する場）についての形態学的研究を 基本的な研究との関連のうえにおいて批判的に概観し、著者の見解を明らかにした。ついで この問題を統制的に発生的立場から検討した。すなわち 原始動物や円口類にみられる甲状腺と相営の器官である内柱の ヨード代謝についての研究を概観し、著者の見解を示した。著者の見解を要約すると 次の通りである。

1. 脊椎動物の正常の状態では、ヨードの有機化（チログロブリンのヨード化）の場は 主に濾胞腔 および 濾胞腔面する細胞膜の領域である。
2. しかし 細胞内でもヨードの有機化起こる可能性を否定はしない。濾胞の充分に完成しないニットリ胚子や、濾胞上皮細胞をバラバラにして濾胞構造をなくした場合に
は、細胞内（粗面小胞体、ゴルジ装置、分泌小胞など）で少量ながらヨードの有機化が起こる。ヨードの有機化に必要な酵素であるペロキシダーゼは粗面小胞体、ゴルジ装置、分泌小胞(subapical vesicles)、および濾胞腔に存在する。

3. それにかかわらず、正常の成熟動物に125Iや131Iを投与したときに、細胞内を素通りしてすぐに濾胞腔に入り、蛋白と結合すると考えられる成績が得られるのは、濾胞腔内には細胞内とくらべてはるかに大量のヨード（チロロブリン）があり、その分子には充分にヨードが結合しきっておらず、ヨードの入りこむ余地が多数に存在するために、投与した同位素がまずここに入りこむためと考えられる。生化学的にもチロロブリンにはヨードとの結合の度合によって数多くの段階があり、さらに無ヨード化チロロブリンの存在さえも報告されていることはこのことを裏づける。私はヨードの有機化の場を一箇所と断定して議論することは無意味であると考えている。

4. 円口類チャツメウナギの幼生や原索動物の一一種ホヤの、甲状腺濾胞上皮細胞と相同である、内柱細胞におけるヨードの有機化の場も、高等動物の甲状腺のそれとほぼ同じパターンをとるものと考えられる。すなわちある種の内柱細胞（一部の内柱細胞はヨード代謝と関係がない）では、おもに細胞の表面でヨードと蛋白の結合が起こり、なお少量ではあるが細胞内でも有機化が起こる可能性がある。また内柱の腔は消化管に関いている標本作製中に内容物が流出するので、オートグラフの標本では125Iを認められないが、内柱腔内でヨードの有機化が起こる可能性を否定はできない。内柱腔内に出されたヨード蛋白の一部は、内柱細胞内に再吸収される。

References


———: Electron microscopic observations on the most primitive thyroid gland in phylogenetic aspect. (On the thyroid of a lamprey, Lampetra japonica). In: Sixth International Congress for Electron Microscopy II. Tokyo, Maruzen, 1966 (p. 529-530).


———: Some observations on the fine structure of thyroids of hibernating and aroused bats. Z.
Site of Iodination of Thyroglobulin


Hirsch, P. F., G. F. Gauthier and P. L. Munson: Thyroid hypocalcemic principle and recurrent


Leloup, J.: Fixation du radioiode par la glande thyreoide de la lamproie marine (Petromyzon marinus)


---


---


---


---


---


---

Nakai, Y., H. Nanba and H. Fujita: Fine structure and functional properties of the thyroid of the...


---


Site of Iodination of Thyroglobulin 139


Strum, J. M. and M. J. Karnovsky: Aminothiazole goiter. Fine structure and localization of


Present address:
Dr. Hisao Fujita
Department of Anatomy
Hiroshima University School of Medicine
Kasumi, Hiroshima
734 Japan