Further Studies on the Localization of Peroxidase in Pig Thyroid Cells

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Synopsis

Previously the authors reported that thyroid peroxidase is bound mainly on the membranes of rough-surfaced endoplasmic reticulum. In the present paper, the question whether the enzyme is also contained in other subcellular organelles in the cells or not was investigated. Attempts to isolate plasma membranes and Golgi membranes were made, being monitored by following Na^+ , K^+ -ATPase and galactosyltransferase activities, respectively, through differential centrifugation and twice-repeated sucrose gradient centrifugation. The final preparations showed properties characteristic of these membranes and specific activities comparable to those of reported preparations of liver, but revealed practically no peroxidase activity. The buoyant densities of the Na^+ , K^+ -ATPase- and galactosyltransferase-associated particulates were around 1.136 and 1.154, respectively, while that of peroxidase-associated particulates (rough-surfaced endoplasmic reticulum) was about 1.192. Isolated nuclei did not show any peroxidase activity and the behaviour of both mitochondria and lysosomes was different from that of the peroxidase-associated particulates. Thus, all the results obtained in the present experiments are not favorable to the view that peroxidase is located in nuclei, mitochondria, lysosomes, Golgi apparatus and plasma membranes (microvilli) of thyroid cells.

It is well established that the thyroid gland involves a peroxidase (EC 1.11.1.7) which takes a central role in the iodination of thyroglobulin and synthesis of thyroxine. Most biochemical studies indicated that the enzyme is not in a soluble state in the cells but firmly bound to cytoplasmic particulates (Hosoya *et al.*, 1962; Klebanoff *et al.*, 1962; DeGroot and Davis, 1962; Benabdeljlil *et al.*, 1967; Ljunggren and Åkeson, 1968; Nagataki *et al.*, 1973). Attempts to solubilize the enzyme from the particulates and to purify it have been made in several laboratories, agreeing with each other on the point that the enzyme is of a hemoprotein nature (Hosoya and Morrison, 1967a, 1967b; Ljunggren and Åkeson, 1968; Taurog *et al.*, 1970; Nagasaka

et al., 1971; Danner and Morrison, 1971; Pommier *et al.*, 1972). However, a consensus has not been attained on the site of the enzyme in the cells. Peroxidase has been reported to be present in Golgi apparatus (Strum and Karnovsky, 1970), mitochondrial fraction (Taurog, 1970), nuclear envelopes (Nakai and Fujita, 1970; Strum and Karnovsky, 1970), colloid lumen (De Robertis and Grasso, 1946; Strum and Karnovsky, 1970) and microvilli (Benabdeljlil *et al.*, 1967; Taurog, 1970; Tice and Wollman, 1972). The location of peroxidase in microvilli was thought by these workers to be relevant to silver grains of radioiodine observed in a number of autoradiographic studies (Nadler and Leblond, 1955; Stein and Gross, 1964; Wollman, 1965; Fujita, 1969; Nadler and Chajut, 1972). On the other hand, the results the present authors obtained in biochemical experiments invari-

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ably indicated that the major site of the enzyme is presumably membranes of rER*¹) (Hosoya and Ui, 1961; Hosoya *et al.*, 1963; Hosoya and Morrison, 1967b; Hosoya *et al.*, 1971). These studies, however, did not rule out the possibility that the enzyme may be also present to even a small degree in other organelles in the cells. The present paper describes the results of a further survey for peroxidase activity in thyroid cells. A preliminary account of this work has already been presented (Hosoya *et al.*, 1973).

Materials and Methods

Porcine thyroid glands were obtained at the local slaughter house and transported on ice to the laboratory. The glands were freed of connective tissue and fat, sliced with a razor blade and washed well with cold saline. All manipulations in the following procedures were conducted at 0 ~ 4°.

For the isolation of Na⁺, K⁺-ATPase-rich plasma membrane fraction, the slices were processed as follows. The slices were homogenized in 3 volumes of medium A with 5 sec burst of Ultra Turrax at 73 volts twice and rehomogenized by one stroke of a glass-Teflon homogenizer. The sediments obtained by centrifugation at 50 × g*²) for 5 min were resuspended in medium A, homogenized with the glass-Teflon homogenizer and centrifuged again. The supernatants were combined and spun at 600 × g for 10 min. The sediments were washed twice with medium A by repeating homogenization and centrifugation. The supernatants were combined and the volume was adjusted by adding enough medium to give a cytoplasmic extract (E), which was 10 ml per g starting slice. The cytoplasmic extract was centrifuged at 25,000 × g for 10 min and the sediments obtained

were suspended in medium A with one-tenth volume of the cytoplasmic extract used. The suspension was referred to as Fraction M.*³) The suspension was diluted with 0.25 M sucrose to give a protein concentration of 5 mg/ml and 20 ml of the suspension was overlaid on 20 ml of 0.32 M (10.6%)*⁴) sucrose placed on 50 ml of 1.2 M (35.6%) sucrose in a 94 ml-centrifuge tube. After centrifugation at 95,000 × g for 50 min in a Type 42 rotor, a milky band at the interface was collected and diluted with 2 volumes of cold distilled water. The suspension was spun at 75,000 × g for 30 min and the pellets obtained was resuspended in a small volume of 0.25 M sucrose and designated as M 3-1. Three ml of M 3-1 suspension was mixed with 2 ml of 0.1 M Tris-HCl buffer, pH 8.5, and then 50.0% sucrose was added to give a 35.0% sucrose suspension. Thirteen ml of the suspension was placed in the bottom of 38.5 ml-centrifuge tube and overlaid with the following step-gradient: 15 ml of 32.0% sucrose and 8 ml of 8.4%. After centrifugation at 81,500 × g for 150 min, the interface layer between 8.4% and 32.0% was collected, diluted with 3 volumes of distilled water and centrifuged at 75,000 × g for 30 min. The pellets obtained were suspended in a small volume of 0.25 M sucrose and designated as M 3-1-1.

For the isolation of Golgi membrane-rich fraction, the thyroid slices (usually 30~40 g) were homogenized in one volume of medium B with a 5 sec burst of Ultra Turrax at 73 volts and rehomogenized by one stroke of a loosely-fitted glass-Teflon homogenizer. The homogenates were passed through four layers of nylon cloth and the volume adjusted to become 4 ml per g of wet tissue by the addition of medium B, and the suspension was centrifuged at 600 × g for 10 min. The supernatant (cytoplasmic extract) was separated into three particulates fractions (F 1, F 2 and F 3) and a soluble fraction by successive centrifugation at 2,000 × g for 10 min, 10,000 × g for 10 min and 105,000 × g for 60 min, respectively. The second fraction, F 2, was richest in galactosyl-transferase activity and subjected to discontinuous sucrose gradient centrifugation. Fifty ml of F 2 suspension in 16.0% sucrose (5 mg protein per ml) was layered on 40 ml of 37.0% sucrose in a 90ml-centrifuge tube. After centrifugation at 95,500 × g for 90 min, a milky band at the interface of the discontinuous sucrose gradient, F 2-1, and pellets at the bottom, F 2-2, were recovered. The former was

*1) Abbreviations used are: ER, endoplasmic reticulum; rER, rough-surfaced endoplasmic reticulum; sER, smooth-surfaced endoplasmic reticulum; Na⁺, K⁺-ATPase, Na⁺, K⁺-stimulated adenosine triphosphatase (EC 3.6.1.3); medium A, 0.25 M sucrose containing 0.1 mM MgCl₂ and 20 mM Tris-HCl buffer, pH 7.4; medium B, 0.5 M sucrose containing 37.5 mM Tris-maleate buffer, pH 6.5, and 5 mM MgCl₂; medium C, 0.25 M sucrose containing 3 mM CaCl₂; medium D, 2.2 M sucrose containing 3 mM CaCl₂.

*2) Indicating always average values for gravity force as used in our previous papers.

*3) Previously, the corresponding fraction was referred to as Fraction M + L (Hosoya *et al.*, 1971), but here this fraction is referred to as Fraction M for the sake of convenience.

*4) Percentage of sucrose solution is always expressed by weight/weight.

diluted with 3 volumes of cold distilled water and the suspension was centrifuged at $105,000 \times g$ for 60 min to obtain precipitates, which was then suspended in a small volume of 37.0% sucrose. Fifteen ml of the suspension (1.4 mg protein per ml), 14 ml of 33% sucrose and 5.5 ml of 27.0% sucrose were successively placed in a 38.5 ml-centrifuge tube and centrifuged at $81,500 \times g$ for 120 min in a SW 27 rotor. Milky bands at each interface layer were withdrawn and diluted separately 3 volumes of distilled water, centrifuged at $105,000 \times g$ for 60 min and the pellets were suspended in 1.6 ml of medium B and designated as F 2-1-1 (from the upper layer) and F 2-1-2 (from the lower layer), respectively. Occasionally, F 2-2 was also separated into two subfractions, F 2-2-1 and F 2-2-2, as follows. The pellets, F 2-2, were suspended in medium B to become 2.5 mg protein per ml and 50 ml of the suspension was layered over 20 ml of 37.0% sucrose which was placed on 20 ml of 42.0% sucrose at the bottom of a 90 ml-centrifuge tube. After centrifugation at $95,500 \times g$ for 90 min, two layers at the interfaces of discontinuous sucrose were recovered and treated as described in the case of F 2-1.

The isolation of thyroid nuclei was performed as follows. Thyroid glands were well perfused with cold 0.9% NaCl solution, sliced and washed in the NaCl solution. About 10 g of the slices were homogenized in 10 volumes of medium C with two 10 sec burst of a Waring blender, rehomogenized by 2 strokes of a glass-Teflon homogenizer and the homogenate was passed through two layers of gauze and centrifuged at $600 \times g$ for 10 min. The pellets obtained were suspended in 20 ml of the same medium and spun at $600 \times g$ for 10 min. This washing was repeated twice. An aliquot of the pellets (crude nuclei) was suspended in 0.25 M sucrose and used for the assay of peroxidase. Most of the pellets remaining were suspended in 12 ml of medium D. Each 4 ml of the suspension was layered over 1 ml of the same medium in a 5 ml-centrifuge tube to obtain pellets (purified nuclei) by centrifugation at $73,500 \times g$ for 30 min in a Hitachi swinging rotor (RPS 40A). In a few cases, the pellets were suspended in the medium D to repeat the same process for obtaining further purified nuclei.

The activities of peroxidase, cytochrome oxidase (EC 1.9.3.1) and Na^+ , K^+ -ATPase were measured and expressed as described previously (Hosoya *et al.*, 1971). The assay procedure and expression of enzyme unit of acid phosphatase (EC 3.1.3.2) was the same as De Duve *et al.* (1955) employing β -glycerophosphate (British House Ltd) as the substrate. Galactosyltransferase was assayed by the method of Morré *et al.* (1970) and the enzyme amount which catalysed the transfer of one nmole of galactose per hr under the conditions was defined as one unit. In this reaction,

the formation of N-acetyllactosamine was checked using an authentic compound (a gift from Prof. Yoshizawa) by paper chromatography (Solvent system, *n*-propanol: ethylacetate: water = 7: 1: 2 v/v; R_f = 0.313, 0.388 and 0.524 for N-acetyllactosamine, galactose and N-acetyl-D-glucosamine, respectively). UDP [^{14}C] galactose, uniformly labeled (234 mCi/mmole), was obtained from the Radiochemical Center, Amsterdam, UDP-galactose from Sigma Chemical Corp. and N-acetyl-D-glucosamine from Nakarai Chemical Ltd. Protein and RNA were determined as described previously (Hosoya *et al.*, 1971). DNA was determined by Burton's method (1956) using calf thymus DNA (Sigma Chemical Corp.) as standard.

Results

Na^+ , K^+ -ATPase-associated plasma membranes

The isolation of plasma membranes is usually conducted by assaying 5'-nucleotidase, Na^+ , K^+ -ATPase, alkaline phosphatase, leucine aminopeptidase or adenyl cyclase (Depierre and Karnovsky, 1973). Among these enzymes, we employed Na^+ , K^+ -ATPase because the enzyme was biochemically confirmed to be located in the plasma membrane fraction of thyroid (Wolff and Jones, 1971) and stained histochemically in microvillous cell border of apical plasma membranes (Shimazaki *et al.*, 1967). The chemical and enzymatic composition, together with the electron microscopic observation, indicated that our preparation consists mainly of plasma membranes, as described elsewhere (Nagai and Hosoya, 1974). The data shown in Table 1 indicate that peroxidase was excluded by the second sucrose gradient centrifugation and not present in the final preparation, M 3-1-1.

Golgi membranes

For the isolation of Golgi membranes, galactosyltransferase was employed as a marker enzyme because this enzyme was found to be involved in the thyroidal Golgi apparatus (Herscovics, 1969; Bouchilloux *et al.*, 1970). The cytoplasmic extract in 0.5 M sucrose was

Table 1. Activities of Na⁺, K⁺-ATPase and peroxidase, and amount of protein in each step for isolation of Na⁺, K⁺-ATPase-rich particulates. The isolation procedure is described in the text.

Fraction	Relative values of		
	Protein	Na ⁺ , K ⁺ -ATPase	Peroxidase
Cytoplasmic extract (E)*	(100)	(100)	(100)
M	12.4	110.2	38.2
M 3-1	0.93	55.9	8.8
M 3-1-1	0.19	15.0	~0

* Absolute values were 108 mg, 0.65 units and 0.075 GU per g tissue for protein, Na⁺, K⁺-ATPase and peroxidase, respectively.

Fig. 1. Distribution of galactosyltransferase and peroxidase in subcellular fractions obtained by differential centrifugation, (A), and by sucrose gradient centrifugation, (B). The isolation procedure of these fractions is described in Materials and Methods. In the left hand figure, (A), the sum of F 1, F 2 and F 3 was taken as 100 (absolute values of galactosyltransferase and peroxidase being 107 units/g tissue and 0.100 GU/g tissue, respectively). In the right hand figure, (B), the specific activity of F 2 was taken as 1.0 (absolute values of galactosyltransferase and peroxidase being 7.82 units/mg protein and 0.0056 GU/mg protein, respectively). Mean values for the two experiments were presented.

separated by the successive centrifugation into three particulate fractions (F 1, F 2 and F 3), and then the second fraction, F 2, was further subjected twice to discontinuous sucrose gradient centrifugation. The result depicted in Figure 1 indicates that galactosyltransferase-associated particulates showed a tendency to sediment at a lower centrifugal force and to be

of lower density than peroxidase-associated particulates. Thirteen per cent of the total activity of galactosyltransferase in the original particulates fraction was recovered in the final fraction, F 2-1-1, and its specific activity was comparable to that of the preparations derived from the liver tissue, 230~350 nmoles per mg protein, (Fleischer and Fleischer, 1970; Kato

Fig. 2. Isopycnic centrifugation of five subcellular fractions.

Each 2 ml of 0.5 M sucrose suspension of four fractions derived from F 2 (F 2-1-1, F 2-1-2, F 2-2-1 and F 2-2-2) and of M 3-1-1 (0.72, 3.0, 6.0 and 1.16 mg protein/ml, respectively) was separately layered on the top of 30 ml of 25.0 to 45.0% linear sucrose gradient which was placed on 5 ml of 55.0% sucrose in the 38.5 ml-centrifuge tubes. After centrifugation at $81,500 \times g$ for 15 hr in a Beckman SW 27 rotor, fourteen drop fractions were collected from the bottom and an aliquot was used for the determination of protein and of sucrose density by a refractometer. The data for the five centrifuge tubes are superposed in this figure.

et al., 1971), while peroxidase activity in F 2-1-1 was extremely small. Confirming these results, it was found by electron microscopy that the fraction scarcely contains any rER but contains sac- and tubular-like vesicles and small fragments similar to those found in the case of liver by Fleischer and Fleischer (1970).

In order to compare the buoyant density of galactosyltransferase-associated particulates with that of peroxidase-bound ones, F 2-1-1 and F 2-1-2 were subjected to isopycnic centrifugation, together with F 2-2-1 and F 2-2-2 which were derived from the peroxidase-rich fraction, F 2-2. For comparison, a Na^+ , K^+ -ATPase-rich fraction, M3-1-1, prepared as described above, was also run parallel with them. After 15-hr centrifugation, protein in each fraction was distributed as depicted in Figure 2, showing each of the main bands located at different levels. The peaks of the protein concentration in these fractions were at densities of 1.136, 1.154, 1.163, 1.192 and 1.218 g/cm^3 for M 3-1-1,

F 2-1-1, F 2-1-2, F 2-2-1 and F 2-2-2, respectively. Specific activities of four enzymes at the peak are shown in Figure 3. This figure indicates that peroxidase was not encountered in galactosyltransferase-rich fraction while the latter enzyme was somewhat distributed in F 2-1-2 (presumably sER) and F 2-2-1 (rER). No peroxidase was found in the peak fraction of M 3-1-1 (plasma membranes) or of F 2-2-2 (mitochondria).

Lysosomes

Our previous results suggested that, although the distribution of acid phosphatase activity considerably overlapped with that of peroxidase activity, peroxidase may not be related to lysosomes in view of a difference in the pattern of specific activity of the two enzymes in subcellular fractions (Hosoya, 1963; Hosoya *et al.*, 1971). This has been further confirmed from the results depicted in Figure 4. In this experiment, an acid phosphatase-rich fraction, Fraction L_a , (see Hosoya,

Fig. 3. Specific activities of four enzymes and densities at the position of the protein peak shown in Figure 2.

Fractions assayed were No. 7 of F2-2-2, No. 11 of F2-2-1, No. 18 of F2-1-2, No. 20 of F2-1-1 and No. 24 of M3-1-1. Densities were calculated from the values for sucrose concentration of these fractions as shown in Figure 2.

Fig. 4. Sucrose gradient centrifugation of an acid phosphatase-rich particulate fraction.

The particulate fraction used was sediments between 100,000 g·min and 250,000 g·min from cytoplasmic extract, corresponding to Fraction L_a of the previous paper (Hosoya *et al.*, 1963). 0.5 ml of the suspension (10 mg protein/ml) was layered on the top of multi-layer sucrose gradient (0.5 ml each of 1.8, 1.6, 1.4, 1.3, 1.2, 1.0, 0.8 and 0.6 M from bottom to top) in a 5 ml-centrifuge tube. After centrifugation at 101,000 × g for 2 hr in a SW 39 rotor, five drop fractions were collected from the bottom and used for the assay of peroxidase and acid phosphatase and for the determination of protein.

Table 2. Contents of protein and nucleic acids, and activity of peroxidase in nuclear preparations. The procedure for the preparation is described in the text.

	Fraction	Peroxidase activity		Protein: DNA: RNA
		Total ($\frac{\text{GU} \times 10^3}{\text{g thyroid}}$)	Specific ($\frac{\text{GU} \times 10^3}{\text{mg protein}}$)	
Exp. 1	Cytoplasmic extract (E)	422.0	6.76	
	Crude nuclear fraction	2.2	0.77	5.7: 1: 0.091
	Purified nuclear fraction		0.037	4.7: 1: 0.091
	Further purified nuclear fraction		~0	4.9: 1: 0.071
Exp. 2	Cytoplasmic extract (E)		5.49	
	Purified nuclear fraction		~0	2.9: 1: 0.077

1963), was centrifuged at $101,000 \times g$ for 2 hr in a sucrose gradient. The centrifugation was not enough to attain a state of equilibration and to estimate the buoyant density of acid phosphatase-associated particulates, but sufficient to indicate that those were heavier than peroxidase-bound particulates. That the former contains so-called lysosomes of thyroid (Wollman *et al.*, 1964; Seljelid, 1965; Ekholm *et al.*, 1966) was confirmed from the following observations: (1) Electron microscopic examination showed that No. 5 fraction, which was highest in acid phosphatase activity, consisted mainly of spherical or ovoid dense bodies similar to those found in liver tissue. (2) When Gomori's histochemical method (Gomori, 1952) was applied to the No. 5 fraction, black reaction products of acid phosphatase was deposited within the dense bodies. The reaction product was entirely eliminated by heat treatment or in the presence of KF. (3) Treatment with pH 5 medium at 37° before incubation caused leakage of the dark reaction product outside of the dense bodies. On the other hand, the main components in the No. 9 fraction of Figure 4 were rough microsomes as found in Fraction IV of the previous paper (Hosoya *et al.*, 1971).

Nuclei

The nuclei of thyroid cells were isolated and purified essentially by the method of Amano (1967). When examined with both light and electron microscopes, the crude preparation

was still seen to be contaminated with cytoplasmic elements, while the purified preparation contained practically no contaminants. In agreements with these observations, peroxidase activity was not found in the purified nuclei, as shown in Table 2.

Discussion

Although the presence of thyroid peroxidase on nuclear envelopes was reported in some histochemical studies at the electron microscopic level (Strum and Karnovsky, 1970; Nakai and Fujita, 1970; Tice and Wollman, 1972), the results of most biochemical studies were not favorable to the view (Hosoya *et al.*, 1962; DeGroot and Davis, 1962; Hosoya *et al.*, 1971; Nagataki *et al.*, 1973). The present experiments on purified nuclei also failed to demonstrate the enzyme in the nuclear preparation (Table 2) and caused us to focus our attention on cytoplasm and plasma membranes. Since the major site of the enzyme has been found in ER (Hosoya *et al.*, 1971, 1972), the present study has been devoted to examining whether the enzyme is located in other organelles like mitochondria, lysosomes, Golgi apparatus or plasma membranes.

The present experimental results obtained by sucrose gradient centrifugation (Fig. 2 and Fig. 3) have almost eliminated the possibility that mitochondria and lysosomes carry peroxidase. The peroxidase activity often found in

the mitochondrial fraction obtained by differential centrifugation may be well explained by contaminating microsomes (Hosoya *et al.*, 1971) or fragments of rER which were incompletely detached from surrounding mitochondria (Hosoya *et al.*, 1972). The absence of peroxidase in lysosomes seems to diminish the possibility that thyroglobulin incorporated into colloid droplets may be iodinated when they fuse with lysosomes (Wetzel *et al.*, 1965; Seljelid, 1965).

It is well known that the cytoplasm of liver, kidney and other tissues contain a kind of subcellular vesicles called peroxisomes (morphologically microbodies), which possess catalase, L- α -hydroxy acid oxidase and uricase and can produce hydrogen peroxide (De Duve and Baudhuin, 1966). However, to our knowledge, the presence of such particles in thyroid cells has not been reported and we ourselves have been able to detect neither catalase nor L- α -hydroxy acid oxidase activity in cytoplasmic particulates, as mentioned previously (Hosoya *et al.*, 1971).

It is reported that the ends of Golgi apparatus and tubular sER are connected to each other and share some kinds of enzymes (Fleischer and Fleischer, 1970). This awakened an expectation that thyroidal Golgi apparatus may involve peroxidase, but the results presented in Figure 2 and Figure 3 tend to deny this possibility. Nevertheless, galactosyltransferase may be distributed to some degree in sER since its specific activity in F 2-1-2 attained to about one-sixth of that of F 2-1-1.

In connection with the distribution of these enzymes, it is interesting to note the densities of subcellular particulates in thyroid cells and to compare them with those of liver. The density of Golgi membranes of pig thyroid seems to correspond to that of liver, 1.12~1.14, (Fleischer and Fleischer, 1970; Morré *et al.*, 1970), because the galactosyltransferase activity had a peak in a fraction with density of 1.154 (Fig. 3). The densities of rough and smooth microsomes of rat liver were reported to be around 1.206 and 1.167, respectively,

(Lee *et al.*, 1969; Breillat, 1973), and these coincide with densities at a peak peroxidase activity of F 2-2-1, 1.192, and of F 2-1-2, 1.163. The density of mitochondria of pig thyroid, 1.218, also agrees with the reported values for rat liver, 1.217 (Beaufay *et al.*, 1959).

Whether peroxidase exists on microvillous cell border is an important problem, in view of its relationship to the iodination of thyroglobulin. Although some workers reported the presence of the enzyme on microvilli by histochemical studies (Strum and Karnovsky, 1970; Tice and Wollman, 1972) and by biochemical studies (Benabdeljlil *et al.*, 1967; Taurog, 1970), the present experimental results on isolated plasma membranes did not offer evidence favorable to the idea. In the manipulation for isolation, we employed, as a marker enzyme, Na⁺, K⁺-ATPase which was reported to be located on apical membranes as well as basal membranes (Shimazaki *et al.*, 1967) and took advantage of the difference in density for its separation from other organelles. Therefore, it is expected that the fragments of microvilli, as a portion of apical membranes, should come into the preparation. It seems to be quite improbable that the density of microvilli is as great as that of rough microsomes.

In conclusion, the reasonable deduction from this study is that it is unlikely that peroxidase is contained in nuclei, mitochondria, lysosomes and plasma membranes, although it is usually difficult to draw conclusion about the complete absence of anything. Thus, the site of peroxidase must be almost confined to rER and sER as mentioned previously (Hosoya *et al.*, 1971, 1972). It remains to explain the relationship of the site of peroxidase to the iodination of thyroglobulin. We wish to deal with this problem in the next paper which is to describe the experimental results concerning the site of iodination of thyroglobulin.

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