CYTOCHEMICAL LOCALIZATION OF
ENDOGENOUS PEROXIDASE
IN THYROID FOLLICULAR CELLS

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ABSTRACT
Endogenous peroxidase activity in rat thyroid follicular cells is demonstrated cytochemically. Following perfusion fixation of the thyroid gland, small blocks of tissue are incubated in a medium containing substrate for peroxidase, before being postfixed in osmium tetroxide, and processed for electron microscopy. Peroxidase activity is found in thyroid follicular cells in the following sites: (a) the perinuclear cisternae, (b) the cisternae of the endoplasmic reticulum, (c) the inner few lamellae of the Golgi complex, (d) within vesicles, particularly those found apically, and (e) associated with the external surfaces of the microvilli that project apically from the cell into the colloid. In keeping with the radioautographic evidence of others and the postulated role of thyroid peroxidase in iodination, it is suggested that the microvillous apical cell border is the major site where iodination occurs. However, that apical vesicles also play a role in iodination cannot be excluded. The in vitro effect of cyanide, amino-triazole, and thiourea is also discussed.

INTRODUCTION
Peroxidase activity was first demonstrated histochemically in granules within the cytoplasm of thyroid follicular cells by Dempsey (10). DeRobertis and Grasso confirmed these findings but also found peroxidase activity in the colloid (11). Additional investigations in a number of laboratories, particularly those by Alexander and co-workers (1, 3, 4), have resulted in the isolation and partial purification of thyroid peroxidase. The enzyme is thought to catalyze the iodination reactions that occur in the synthesis of the thyroid hormones, triiodothyronine and thyroxine. The major steps involved in thyroid hormone biosynthesis include the following (40). Iodide is actively transported into the thyroid follicular cell from the circulation. There it becomes oxidized, in the presence of H\textsubscript{2}O\textsubscript{2} and is presumably catalyzed by a peroxidase enzyme, to a different ionic species. The exact ionic state of the “oxidized iodide” is unknown, but it might be an iodinium ion (I\textsuperscript{+}) or a hypoiodite ion (I\textsuperscript{-}) (16). The “oxidized iodide ion” then iodinates the tyrosine residues of thyroglobulin (possibly catalyzed by the same peroxidase) to form first moniodotyrosine and then diiodotyrosine. Triiodothyronine is formed when one molecule each of moniodotyrosine and diiodotyrosine are coupled, whereas thyroxine results from the coupling of two molecules of diiodotyrosine. The reactions after iodide uptake are reported to take place after formation of the thyroglobulin polypeptide (43) and to occur within the thyroglobulin molecule (40). The synthesis of thyroglobulin follows a process similar to that observed for secretory proteins in general (35) and is dissociated from its iodination. Its synthesis is believed to involve subunit interaction, the thyroglobulin (a 19S protein) being formed from smaller subunits represented in 12S
and 3S–8S fractions (43, 47). Although most iodination is believed to occur after thyroglobulin synthesis has been completed (19S protein), it has been reported that iodination can also take place in vitro in the 12S protein (47).

The exact site of the thyroid peroxidase has been the subject of a number of investigations. Radioautographic studies have shown that, within 11 sec after injection of $^{131}$I, the radioactive label (representing protein-bound $^{131}$I) appears in the colloid (49). Sections of tissue examined in the light microscope reveal silver grains concentrated at the periphery of the follicular colloid. This has been called a “ring reaction” and has been taken as evidence that iodination occurs in the colloid (extracellularly) near the apical ends of the follicular cells (50).

High resolution electron microscopic radioautographs have repeatedly shown silver grains at the periphery of the colloid, near the cell-colloid interface (28, 44). These morphological observations have led to the theory that the site of iodination is the apical border of the follicular cells with their projecting microvilli.

Contrary to these observations are a number of biochemical findings that seem to indicate that iodination occurs at a different cellular site. Most biochemical investigators have localized peroxidase activity in a particulate fraction of thyroid tissue (associated with mitochondria and microsomes) where it appears to be firmly bound (4, 9, 19, 20, 25, 29). Moreover, saline extracts of thyroid do not exhibit peroxidase activity (28). These conflicting results make the in vivo site of this enzyme a subject of much controversy. Because peroxidase plays a role in the iodination reactions involved in the synthesis of thyroid hormones, (by possessing the ability to halogenate certain organic residues [40]), the location of this enzyme could contribute to our understanding of thyroid function. Therefore, the following study was undertaken in an attempt to identify cytochemically the sites of endogenous peroxidase activity within the rat thyroid gland.

**Materials and Methods**

32 male and female Sprague-Dawley rats, weighing from 100 to 450 g and maintained on a diet of Purina Laboratory chow, were used. Most thyroid glands were fixed by perfusion (17) through the abdominal aorta (for 10 min) with one-fifth strength paraformaldehyde-glutaraldehyde fixative (23), with and without the addition of trinitro compounds (22), buffered by 0.1 M sodium cacodylate, pH 7.4. Immediately after perfusion, the thyroid was dissected free from the surrounding tissue and diced into tiny cubes that were placed for an additional 20 min in one-half strength paraformaldehyde-glutaraldehyde fixative. The tissue was then transferred to 0.2 M sodium cacodylate buffer, pH 7.4, where it remained overnight in the refrigerator. A few thyroid glands were perfused with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer and fixed for a total time of 30 min before being transferred to 0.2 M sodium cacodylate buffer, pH 7.4, in the refrigerator.

**Normal Morphology**

For fine structural study the perfused thyroid tissue was postfixed in Millonig’s osmium-containing fixative (32) for 2½ hr at 0–4°C, before being dehydrated in a graded series of ethanol and embedded in Araldite or Epon (27). Prior to dehydration and embedding, some tissue was stained en bloc with uranyl acetate according to the method of Farquhar and Palade (15) as slightly modified by Karnovsky (24).

**Cytochemistry**

** Peroxidase Activity:** For the cytochemical demonstration of endogenous peroxidase activity, small blocks of thyroid tissue were incubated at room temperature, for intervals ranging from ½ to 3 hr, in media containing 0.05% 3,3’-diaminobenzidine tetrahydrochloride (Sigma Chemical; Co., St. Louis, Mo.), in 0.05 M Tris-HCl buffer (ranging in pH from 7.6 to 8.5), and in concentrations of H$_2$O$_2$ ranging from 0.0003–0.0025 M. Blocks of tissue serving as controls for this technique were incubated in media without 3,3’-diaminobenzidine tetrahydrochloride (DAB) and without H$_2$O$_2$.

**Eliminating Endogenous H$_2$O$_2$:** In order to prevent a positive cytochemical reaction caused by the endogenous production of H$_2$O$_2$, tissue blocks were incubated in the medium just described which also contained catalase in concentrations of 0.05 and 0.1%.

To prevent a false positive reaction in the absence of exogenous H$_2$O$_2$, which might be caused by the nonspecific binding of DAB rather than by the endogenous production of H$_2$O$_2$, tissue was incubated for 1 hr in 0.05 M Tris-HCl buffer containing 0.1% catalase and 0.05% DAB. After three washes in buffer, the tissue was transferred for 1½ hr to 0.05 M Tris-HCl buffer containing 10$^{-2}$ M potassium ferricyanide.

**Inhibitors:** The inhibitory effect of various chemicals upon the cytochemical reaction for endogenous peroxidase was evaluated. Blocks of thyroid tissue were preincubated for 1 hr at room temperature in the presence of the particular inhibitor. They were then transferred for 2 hr at room temperature to
complete media that contained the same concentration of the appropriate inhibitor, either $10^{-2}\text{ M KCN}$, $10^{-2}\text{ M 3-amino-1,2,4-triazole}$, or thiourea in concentrations of $10^{-5}\text{ M}$ or $5 \times 10^{-3}\text{ M}$.

Following incubation the blocks of thyroid tissue were rinsed in Tris-HCl buffer before being postfixed, dehydrated, and embedded as described above. Thick (1-2 µ) sections of tissue were cut and examined in the light microscope in order to evaluate the cytochemical reaction product within thyroid follicular cells. Then thin sections of tissue were cut with glass knives on an LKB Ultrotome. These sections were placed on carbon-coated 200-mesh copper grids and either left unstained, or stained with lead citrate (45) before being examined in an RCA 3F or Philips 200 electron microscope.

**OBSERVATIONS**

**Normal Morphology of Thyroid Follicular Cell**

A major portion of the thyroid gland consists of rounded follicles. These are formed by single layers of follicular cells that envelop their extracellular secretory product, colloid.

The general ultrastructural characteristics of a thyroid follicular cell are seen in Fig. 1. Typically, the cell is cuboidal in shape and rests upon an extracellular basal lamina. Its apical border is lined by microvilli, which project into the follicular colloid. A very prominent organelle is the endoplasmic reticulum that often displays dilated cisternae filled with a finely granular or filamentous material. Ribosomes are attached to the membranes along some portions of the endoplasmic reticulum while other areas lack ribosomes. Ribosomes are also scattered throughout the cytoplasm where microtubules are occasionally seen. The nucleus, limited by the two membranes that form its envelope, lies basally and in its vicinity Golgi regions are often observed. Near the Golgi areas many vesicles and membrane-bounded inclusions of varying density exist. Other inclusions, which contain a substance similar in appearance to the follicular colloid, are also present. Just at the base of the microvillous border the cell often displays a number of small vesicles. Occasionally, in this apical area, other dense inclusions are also seen. For a more detailed description of the fine structural features of thyroid follicular cells, see Ekholm and Sjöstrand (12), Wissig (48), and Heimann (10).

**Cytochemistry**

**Endogenous Peroxidase Activity**

Thyroid follicles are known to be heterogeneous, differing in their functional states of activity (49). However, it appears that in tissue demonstrating a positive cytochemical reaction for peroxidase activity, the follicular cells uniformly demonstrate the reaction product if the substrate has had an opportunity to penetrate the block to a depth sufficient to reach them. Parafollicular cells are negative for the reaction product, but mast cells and red blood cells are consistently positive. Thyroid follicular cells that have been incubated in the presence of DAB demonstrate a dark reaction product in the following intracellular sites.

**Perinuclear cisternae:** A positive reaction for peroxidase activity is characteristically seen localized within the perinuclear cisternae of thyroid follicular cells (Figs. 2, 3). Within the same cell, some portions of the perinuclear cisterna give an intensely positive reaction, while other areas appear to be free of reaction product. All cells displaying a positive cytochemical reaction seem to demonstrate peroxidase activity in this site.

**Endoplasmic reticulum:** Since the cisternae of the rough endoplasmic reticulum are continuous with the perinuclear cisternae, it is not surprising that follicular cells exhibiting a positive reaction consistently have the electron-opaque reaction product in their endoplasmic reticulum (Figs. 2-5). Sections of thyroid tissue not stained with lead clearly reveal the reaction product in the cisternal elements of this organelle. The endoplasmic reticulum in thyroid follicular cells is not highly ordered, and its cisternae are often dilated (Figs. 1-3). Although ribosomes are attached to the membranes along some portions of the endoplasmic reticulum, other areas of this organelle are free of ribosomes. In sections not stained with uranyl acetate or alkaline lead, the ribosomes comprising the rough endoplasmic reticulum do not appear to demonstrate a positive peroxidase reaction (Figs. 2, 5). A few follicular cells contain profiles of endoplasmic reticulum that do not display the dark reaction product.

**Golgi apparatus:** Electron micrographs of thyroid follicular cells displaying a positive reaction for endogenous peroxidase activity do not always reveal the electron-opaque product within
The morphology of a thyroid follicular cell is illustrated in this electron micrograph. Profiles of endoplasmic reticulum (ER) displaying dilated cisternae are evident throughout the cytoplasm. A Golgi complex (Go) lies to the left of the nucleus (N). Ribosomes, mitochondria, vesicles, and various inclusions (some thought to be colloid droplets [CD]) are also present. Microvilli (MV) project apically from the cell into the colloid (C) that is contained within the lumen of the follicle. × 20,000.

the Golgi apparatus. However, this observation appears to be related to the plane of the section through the cells. Many follicular cells do demonstrate the dark reaction product within their Golgi regions (Fig. 3). The location of the product within this organelle is quite specific. The inner two or three lamellae forming a portion of the concave face of the Golgi complex react in a positive manner. Both the lamellar membranes and their cisternae appear to have peroxidase activity (Fig. 3). Vesicles closely associated with the concave inner surface of the Golgi complex also contain the dense reaction product. The outer convex Golgi lamellae were not observed to
Figure 2. A section through a thyroid follicular cell that has been incubated 2 hr for the presence of endogenous peroxidase activity. Sites exhibiting the dark reaction product are the perinuclear cisterna (PC), endoplasmic reticulum (ER), apical vesicles (arrow), lateral vesicles (LV), and the microvillous apical cell border. Section not stained with uranyl acetate or alkaline lead. $\times 23,000$. 
FIGURE 3 The Golgi complex exhibits the dark reaction product only within its inner few concave lamellae (arrows) and associated vesicles. The perinuclear cisternae (PC) clearly demonstrate cytochemical evidence of peroxidase activity. Other inclusions that are thought to represent colloid droplets (CD) do not stain, but nearby peripheral lateral vesicles (LV) stain intensely. × 28,000.

VESICLES: Many cytoplasmic inclusions exist in thyroid follicular cells (Figs. 1, 2). Aside from the bodies that seem to represent colloid droplets (Figs. 1, 3), darkly stained round or ovoid profiles are also observed. Some of these dense inclusions probably represent lysosomes (41), including autophagic and/or heterophagic vacuoles (42). A few cannot be identified with certainty, and their nature remains unclear. Because dense inclusions are part of the normal morphology of many thyroid follicular cells, it is sometimes difficult to be certain whether darkly stained bodies in tissue that has been incubated represent sites of the reaction product. However, in sections not stained with lead it is evident that within the cells, just beneath the apical microvilli, many vesicular inclusions display a positive reaction product (Figs. 2, 4, 5). These inclusions are not so dark
FIGURE 4 An electron-opaque reaction product is evident along the apical border of this thyroid follicular cell. The dark material appears to be associated with the outer surface of the microvillous membranes and the colloid adjacent to these structures. The endoplasmic reticulum (ER) and apical vesicles (arrow) also stain for peroxidase activity. × 29,000.

FIGURE 5 The apical portions of three thyroid follicular cells are sectioned tangentially in this electron micrograph. Peroxidase activity is demonstrated cytochemically in apical vesicles (arrow) and clearly outlining the microvilli that project from the cells. Section not stained with uranyl acetate or alkaline lead. × 30,000.
as other dense bodies seen in control cells and therefore can be distinguished from them. A few very intensely stained vesicles, often appearing as elongate, narrow profiles, are consistently observed just within the lateral cell borders (Figs. 2, 3). These lateral cytoplasmic inclusions are striking because they exhibit such an intense reaction product. The presumed colloid droplets do not stain for peroxidase (Fig. 3).

**APICAL CELL BORDER:** Electron micrographs of certain thyroid follicles reveal a most interesting finding. Outlining the surface of the apical microvilli that project from the follicular cells into the colloid is a dark electron-opaque substance (Figs. 2, 4, 5). At low magnification (Fig. 2), this material exhibits itself as a distinct ring of reaction along the periphery of the colloid contained within the thyroid follicle. The dark material is closely associated with the external surface membranes of the microvilli. At higher magnification (Fig. 4), the dark substance appears as a fine flocculent material that often has a distribution reminiscent of the presumed mucopolysaccharide coating (glycocalyx, "fuzz") associated with cell surface membranes (7).

The reaction product is seen along the apical border of follicular cells after both short and long incubation periods. However, the intensity of the reaction product at this site appears to increase with the longer incubation interval. Cells displaying the most intense apical border reaction are those lining follicles near the edge of the tissue block (within approximately 50 µ of the surface). Such cells have been in contact with the substrate for the longest period of time.

**THYROID TISSUE INCUBATED IN MEDIUM WITHOUT H2O2**

Omitting H2O2 from the incubation medium still resulted in a positive cytochemical reaction. The distribution of this dark reaction product within follicular cells was identical to that observed in tissue incubated in the presence of exogenous H2O2. This result was interpreted to indicate that endogenous H2O2, the source of which is not known, was present. Catalase was therefore added to incubation medium lacking exogenous H2O2 in order to inhibit a positive reaction that could be caused by a source of endogenous H2O2. The presence of catalase did completely inhibit the cytochemical reaction. Moreover, when thyroid tissue that had been inhibited by catalase was placed into the usual incubation medium containing exogenous H2O2, the dark cytochemical reaction product was again found in the follicular cells, indicating that the reaction was reversible.

A negative cytochemical reaction followed the incubation of tissue in the presence of DAB and catalase, with subsequent incubation in the presence of potassium ferricyanide (a strong oxidizing agent). This ruled out the possibility that the positive reaction might be the result of DAB being nonspecifically bound and subsequently oxidized by OsO4.

**INHIBITORS**

Thyroid tissue incubated in medium containing 10^-4 M KCN did not visibly appear to darken. However, 1 µ thick sections of such tissue examined in the light microscope did display some questionable evidence of peroxidase activity. Thin sections of these same areas examined in the electron microscope revealed that the thyroid follicular cells were totally negative, but a slightly positive reaction did occur in red blood cells. Thus KCN was considered to largely inhibit the endogenous peroxidase.

Aminotriazole (10^-2 M) was effective in completely inhibiting the cytochemical reaction in follicular cells observed in the electron microscope, but red blood cells remained positive, due to the peroxidatic effect of hemoglobin. The addition of 10^-5 M thiourea to the incubation medium was not effective in inhibiting the cytochemical reaction. At a concentration of 5 X 10^-5 M (11) the cellular reaction appeared to be inhibited in 1 µ thick sections, although red blood cells were clearly positive. In the electron microscope, most follicular cells in this tissue displayed a negative cytochemical reaction, but a few follicular cells exhibited a slightly positive reaction in their perinuclear cisternae, endoplasmic reticulum, a few vesicles, and outlining their microvilli. Therefore, the higher concentration of thiourea markedly inhibited the reaction in vitro.

Endogenous (14, 37) and exogenous catalase (46) may be demonstrated cytochemically by a reaction utilizing DAB. However, under the conditions used in this experiment the exogenous catalase did not exhibit peroxidatic activity, and a nonspecific reaction was not observed.
DISCUSSION

Particular sites within thyroid follicular cells demonstrate cytochemical evidence of endogenous peroxidase activity. These sites include the perinuclear cisternae and endoplasmic reticulum, the inner few lamellae of the Golgi complex, the vesicles (particularly within the apical cytoplasm), and the outer surfaces of the rough endoplasmic reticulum.

The organelar distribution of peroxidase activity is consistent with what one would expect for a cell engaged in the synthesis of a protein (38). The ribosomes attached to the membranes of the rough endoplasmic reticulum synthesize proteins that are transferred into the cisternae of this organelle. It is known from many morphological studies that the perinuclear cisternae and the cisternae of the rough endoplasmic reticulum are continuous. In differentiating plasma cells, Leduc and coworkers found that antibody was first localized in the perinuclear space, but that with maturation it disappeared from this site and appeared in the endoplasmic reticulum (26). The present study would seem to indicate that peroxidase is being synthesized continuously by thyroid follicular cells, since it is invariably found within the perinuclear cisternae. The few portions of the perinuclear cisternae and endoplasmic reticulum cisternae within the same cell that do not contain reaction product could indicate functional heterogeneity among these elements, or might relate to the technical procedures being employed.

From the endoplasmic reticulum, proteins frequently are transported to the Golgi regions of cells (38) where they undergo condensation and become “packaged” into granules prior to secretion. In the present study, only the inner few lamellae of the Golgi complex in thyroid follicular cells stain for peroxidase activity. This observation implies that the Golgi apparatus has some role in the segregation and/or synthesis of thyroid peroxidase. The presence of peroxidase activity only in the inner few lamellae of the Golgi complex suggests that the lamellae are functionally heterogeneous. A similar example of functional polarity existing within the Golgi complex has been demonstrated in studies of polymorphonuclear leukocytes, where two morphologically distinct granule populations have been shown to originate from opposite faces of the Golgi apparatus (5).

The present cytochemical study indicates that enzymatic specialization exists within the Golgi apparatus of thyroid follicular cells.

In addition to its role in the condensation and packaging of proteins into secretory granules, the Golgi apparatus has also been implicated in the synthesis and incorporation of carbohydrates in many different cells (21, 36). Lactoperoxidase, an enzyme closely related to thyroid peroxidase, is known to contain a high content of carbohydrate (33). Plant peroxidases also have significant amounts of carbohydrate, and it has been suggested that this might be a characteristic of peroxidases in general (33). Therefore, it is possible that thyroid peroxidase also contains carbohydrate moieties and that these are incorporated into the enzyme in the Golgi apparatus of follicular cells.

Vesicles that exhibit a positive cytochemical reaction are found throughout follicular cells and can be distinguished from the blacker, very dense inclusion bodies, some of which presumably are lysosomes (42). These vesicular inclusions displaying the reaction product might be portions of the endoplasmic reticulum and/or be vesicles derived from the Golgi apparatus (as opposed to packaged secretory granules). The packaging of peroxidase protein into typical secretory granules, as occurs in the Golgi apparatus of eosinophils (6, 31), does not take place in thyroid follicular cells.

Several workers have obtained experimental evidence indicating that apical vesicles are involved in the discharge of thyroglobulin from thyroid follicular cells into the colloid (13, 35). When leucine-3H was used as a radioautographic label to follow the synthesis of thyroglobulin, it was shown that the label appeared in the apical vesicles just before it appeared in the colloid (35). These workers concluded that uniodinated thyroglobulin is transferred from apical vesicles to the colloid where it is then iodinated. Other studies of microsomal subfractions from thyroid labeled in vivo with leucine-3H led these authors also to suggest that apical vesicles are involved in the transfer of thyroglobulin from its site of synthesis into the colloid (13). Presumably the membranes of the apical vesicles fuse with the cell surface membrane at the base of the microvilli. Then, by a process of reverse pinocytosis (exocytosis), the content of the vesicles is transferred externally.

It is generally agreed that the iodination of thyroglobulin involves endogenous thyroid per-
oxidase (47). In an effort to learn more about the iodination reactions that occur in the synthesis of the thyroid hormones, most experimental studies have utilized radioactive iodide. Radioautographic studies employing such tracers (28, 34, 44) have repeatedly shown the radioactive label in the colloid, rather than within the follicular cells. However, radioautographs of thyroids from animals experimentally treated with thyroxine (in an effort to slow the metabolism of iodine and the turnover of thyroglobulin) revealed silver grains at the colloid-cell boundary, overlying the microvilli of the cells (44). This finding correlates with the present study where endogenous peroxidase activity was observed along the microvillous apical border of thyroid follicular cells. The cytochemical peroxidase reaction product was observed very near the surface membranes of the microvilli, perhaps associated with their presumed mucopolysaccharide coating. From this cytochemical evidence and the investigations discussed above, it appears that the major site of thyroglobulin iodination is the apical microvillous border of thyroid follicular cells. However, cytochemically positive apical vesicles were also frequently observed in these cells. From the present study it is not possible to ascertain whether or not any one vesicle contains both thyroglobulin and peroxidase, or whether different populations of apical vesicles exist. Iodination is a very rapid process, occurring within a matter of seconds following the administration of radiiodide (50), and exocytosis might also occur so rapidly that ultrastructural evidence for it would be extremely difficult to obtain. Therefore, one cannot overlook the possibility that some iodination of thyroglobulin might occur within apical vesicles and that the product might be rapidly discharged into the colloid.

The intimate association of peroxidase activity with the membranes of the microvilli suggests that some of the enzyme could be firmly bound, while the remainder of it appears to be carefully compartmentalized within the cell. Biochemical fractionation studies of thyroid tissue have led to the conclusion that the thyroid peroxidase is localized in a particulate mitochondrial-microsomal cell fraction. These findings should be interpreted with caution, since the techniques employed are often harsh. For example, during such a procedure the endoplasmic reticulum could be ruptured and the peroxidase content released, thus allowing the peroxidase to become nonspecifically adsorbed on other organelles.

A positive cytochemical reaction in the absence of exogenous H₂O₂ was shown to be due to the presence of endogenous H₂O₂ within the blocks of thyroid tissue. The source of this H₂O₂ is not known. Likewise, the physiological source of endogenous H₂O₂ within the intact thyroid gland remains unknown, but flavine-linked enzymes might well be involved (2). Flavines have been shown to stimulate iodination in vitro and it has been proposed that the autoxidation of their reduced forms might generate H₂O₂ (2). Such a mechanism might be operating in the experimental system used in this study, but it is also possible that aldehyde fixation could be giving rise to peroxides (39).

Cyanide, aminotriazole, and thiourea were tested in vitro for their inhibitory effects upon the cytochemical reaction, KCN was found to be less effective as an inhibitor of thyroid peroxidase than aminotriazole or the higher concentration of thiourea. Alexander also reported that KCN was less effective as an inhibitor of H²¹I incorporation into tyrosine by thyroid homogenates than was aminotriazole or thiouracil (1). Thiouracil is an antithyroid compound chemically related to thiourea (40).

Aminotriazole has been shown to inhibit peroxidase activity in vitro in homogenates of thyroid and salivary gland (1). This substance was also observed to inhibit the cytochemical reaction in thyroid follicular cells. However, aminotriazole is also an inhibitor of catalase (30), and since thyroid tissue contains both peroxidase and catalase, the use of this substance alone does not enable one to distinguish between the two enzymes. Moreover, catalase has been shown to possess peroxidatic activity (6) that can be demonstrated by a modified DAB reaction (14, 37). Although the reaction product visualized in thyroid follicular cells is evidence of peroxidatic activity, one cannot be absolutely certain that it does not also represent catalase. However, studies showing that thiourea inhibits catalase activity are lacking. It has been reported that thiourea (in vitro at a concentration of 5 × 10⁻⁶M) inhibits thyroid peroxidase in tissue observed in the light microscope (10, 11), and this was confirmed in the present study. In vivo studies with thiourea are now being under-
taken. Additional studies involving attempts to stimulate and inhibit thyroid peroxidase activity are also presently in progress.

In conclusion, this study demonstrates endogenous thyroid peroxidase activity within apical vesicles and associated with the outer surfaces of follicular cell microvilli. Correlating this cytochemical evidence with a number of other investigations thus provides additional support for the theory that iodination occurs at the microvillous border of thyroid follicular cells.

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REFERENCES

1. ALEXANDER, N. M. 1959. Iodide peroxidase in rat thyroid and salivary glands and its inhibition by antithyroid compounds. J. Biol. Chem. 234:1530.
2. ALEXANDER, N. M. 1961. The mechanism of iodination reactions in thyroid glands. Endocrinology. 68:571.
3. ALEXANDER, N. M. 1965. Studies on the purification and characterization of thyroid peroxidase. In Current Topics in Thyroid Research. C. Cassino and M. Andreoli, editors. Academic Press Inc., New York. 43.
4. ALEXANDER, N. M., and B. J. CORCORAN. 1962. The reversible dissociation of thyroid iodide peroxidase into apoenzyme and prosthetic group. J. Biol. Chem. 237:243.
5. BAINTON, D. F., and M. G. FARQUHAR. 1966. Origin of granules in polymorphonuclear leukocytes. J. Cell Biol. 28:277.
6. BAINTON, D. F., and M. G. FARQUHAR. 1967. Segregation and packaging of granule enzymes in eosinophils. J. Cell Biol. 35:6A.
7. BENNETT, H. S. 1963. Morphological aspects of extracellular polysaccharides. J. Histochem. Cytochem. 11:2.
8. DE DUVE, C., and P. BAUDHUIN. 1966. Peroxisomes (microbodies and related particles). Physiol. Rev. 46:523.
9. DEGROOT, L. J., and A. M. DAVIS. 1962. Studies on the biosynthesis of iodotyrosines: a soluble thyroidal iodide-peroxidase tyrosine-iodinase system. Endocrinology. 70:492.
10. DEMPSEY, E. W. 1944. Fluorescent and histochemical reactions in the rat thyroid gland at different states of physiological activity. Endocrinology. 34:27.
11. DE ROBERTIS, E., and R. GRASSO. 1946. Peroxidase activity of the thyroid gland under normal and experimental conditions. Endocrinology. 34:137.
12. EKHOHL, R., and F. SJOSTRAND. 1957. The ultrastructural organization of the mouse thyroid gland. J. Ultrastruct. Res. 1:178.
13. EKHOHL, R., and U. STRANDBERG. 1968. Studies on the protein synthesis in the thyroid. III. In vivo incorporation of leucine-3H into thyroglobulin of microsomal fractions of the rat thyroid. J. Ultrastruct. Res. 22:252.
14. FAMHI, H. D. 1968. Cytochemical localization of peroxidase activity in rat hepatic microbodies (peroxisomes). J. Histochem. Cytochem. 16:547.
15. FARQUHAR, M. G., and G. E. PALADE. 1965. Cell junctions in amphibian skin. J. Cell Biol. 26:253.
16. FAWCETT, D. M., and S. KIRKWOOD. 1952. Mechanism of the antithyroid action of iodide ion and of the aromatic thyroid inhibitors. J. Biol. Chem. 204:787.
17. GRIFFITH, L. D., R. E. BULGER, and B. F. TRUMP. 1967. The ultrastructure of the functioning kidney. Lab. Invest. 16:220.
18. HEIMANN, P. 1966. Ultrastructure of human thyroid. A study of normal thyroid, untreated and treated diffuse toxic goiter. Acta Endocrinol. 33:(Suppl. 110).
19. HOSOYA, T., and M. MORRISON. 1967. A study of the hemoproteins of thyroid microsomes with emphasis on the thyroid peroxidase. Biochemistry. 6:1021.
20. HOSOYA, T., Y. KONDO, and N. Ui. 1962. Peroxidase activity in thyroid gland and partial purification of the enzyme. J. Biochem. (Tokyo). 52:180.
21. Ito, S. 1959. Structure and function of the glycoalyx. Fed. Proc. 28:12.
22. Ito, S., and M. J. KARNovsky. 1965. A formaldehyde-glutaraldehyde fixative containing trinitro compounds. J. Cell Biol. 39:168a.
23. KARNovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27:137A.
24. KARNovsky, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. 35:213.
25. KLEBANOFF, S. J., C. YIP, and D. KESSLER. 1962.
The iodination of tyrosine by beef thyroid preparations. Biochim. Biophys. Acta. 58:563.
26. LEDUC, E. H., S. AVRAMEAS, and M. BOUTEILLE. 1968. Ultrastructural localization of antibody in differentiating plasma cells. J. Exp. Med. 127:109.
27. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:209.
28. Lupulescu, A., D. Andreani, and M. Andreoli. 1967. Thyroglobulin: synthesis, iodination and hydrolysis. Folia Endocrinol. (Roma). 20:385.
29. Maloof, F., and M. Soodak. 1964. The oxidation of thiocyanate by a cytoplasmic particulate fraction of thyroid tissue. J. Biol. Chem. 239:1995.
30. Margoliash, E., A. Novogrodsky, and A. Schejter. 1960. Irreversible reaction of 3-amino-1:2:4-triazole and related inhibitors with the protein of catalase. Biochem. J. 74:339.
31. MILLER, P., and V. HERZOG. 1969. Die Lokalisation von Peroxydase und saurer Phosphatase in eosinophilen Leukocyten während der Reifung. Z. Zellforsch. Mikroskop. Anat. 97:94.
32. MILLONG, G. 1962. Further observations on a phosphate buffer for osmium solutions in fixation. In Proceedings of the Fifth International Congress of Electron Microscopy. S. S. Breese, Jr., editor. Academic Press Inc., New York. 2:2-8.
33. Morrison, M., and W. F. Steele. 1968. Lactoperoxidase, the peroxidase in the salivary gland. In Biology of the Mouth. P. Person, editor. American Assn. for the Advancement of Science. Washington D.C. 89.
34. NADLER, N. J. 1965. Iodination of thyroglobulin in the thyroid follicle. In Current Topics in Thyroid Research. C. Cassano and M. Andreoli, editors. Academic Press Inc., New York. 73.
35. NADLER, N. J., B. A. YOUNG, C. P. LEBLOND, and B. MITMAKER. 1964. Elaboration of thyroglobulin in the thyroid follicle. Endocrinology. 74:333.
36. NEUTRA, M., and C. P. LEBLOND. 1969. The Golgi apparatus. Sci. Amer. 220:100.
37. Novikoff, A. B., and S. Goldfischer. 1968. Visualization of microbodies for light and electron microscopy. J. Histochem. Cytochem. 16:507.
38. PALADE, G. E. 1966. Structure and function at the cellular level. J. Amer. Med. Ass. 198:815.
39. PEARSE, A. G. E. 1960. Histochemistry. J. A. Churchill Ltd., London. 2nd edition. 320.
40. Pitt-Rivers, R., and R. R. Cavaleri. 1964. Thyroid hormone biosynthesis. In The Thyroid Gland. R. Pitt-Rivers and W. R. Trotter, editors. Butterworth & Co., Ltd., London. 1:37.
41. Seljelid, R. 1965. Electron microscopic localization of acid phosphatase in rat thyroid follicle cells after stimulation with thyrotropic hormone. J. Histochem. Cytochem. 13:687.
42. Seljelid, R. 1967. Endocytosis in thyroid follicle cells. I. Structure and significance of different types of single membrane-limited vacuoles and bodies. J. Ultrastruct. Res. 17:195.
43. Sellin, H. G., and I. H. Goldberg. 1965. Biosynthesis of thyroglobulin. III. Intracellular localization and properties of labelled thyroid proteins. J. Biol. Chem. 240:774.
44. Stein, O., and J. Gross. 1964. Metabolism of 125I in the thyroid gland studied with electron microscopic autoradiography. Endocrinology. 75:207.
45. Venkatasubbu, M. A., and H. D. Fahim. 1969. The use of beef liver catalase as a protein tracer for electron microscopy. J. Cell Biol. 25:407.
46. Venkataram, M. A., and H. D. Fahim. 1969. The use of beef liver catalase as a protein tracer for electron microscopy. J. Cell Biol. 42:480.
47. WERNER, S. C., and J. A. NAUMAN. 1968. The thyroid. Ann. Rev. Physiol. 30:213.
48. Wissig, S. L. 1964. Morphology and cytology. In The Thyroid Gland. R. Pitt-Rivers and W. R. Trotter, editors. Butterworth & Co., Ltd., London. 1:32.
49. Wollman, S. H. 1965. Heterogeneity of the thyroid gland. In Current Topics in Thyroid Research. C. Cassano and M. Andreoli, editors. Academic Press Inc., New York. 1.
50. Wollman, S. H., and I. Wodinsky. 1955. Localization of protein-bound 1131 in the thyroid gland of the mouse. Endocrinology. 56:19.