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SCANNING ELECTRON MICROSCOPIC STUDY OF THE COLLAGEN SHEATH OF THE HUMAN THYROID GLAND AND ITS DISORDERS

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Abstract

A cell-maceration/scanning electron microscope (SEM) method was employed to demonstrate the collagen sheath around follicles (perifollicular sheath) of the human thyroid gland and its disorders. In the normal thyroid gland, the follicles were surrounded by spherical collagen sheaths composed of a framework of thick collagen bands 1-5 μm in width and fine solitary collagen fibrils 50-70 nm in diameter. In benign thyroid diseases (Graves' disease, Hashimoto's thyroiditis and adenomatous goiter), the perifollicular sheaths differed in size and in shape according to the disease, but they were always composed of thick collagen bands and fine fibrils as in the normal thyroid. On the other hand, the spaces surrounded by the perifollicular sheaths varied markedly in size in follicular adenoma, were small in oxyphilic adenoma, and irregularly shaped in embryonal adenoma. In all these adenomas, the perifollicular sheaths were mainly composed of fine fibrils 35-45 nm in diameter. In follicular carcinoma, the size and shape of the space surrounded by the perifollicular sheaths were irregular. In papillary adenocarcinoma, the collagen sheaths showed a papillary pattern. In medullary carcinoma, tumor nests were surrounded by well developed collagen sheaths. In all these carcinomas, the collagen sheaths were mainly composed of fine collagen fibrils 32-45 nm in diameter. In adenomas and follicular carcinoma, the perifollicular sheaths frequently had large holes through which the spaces surrounded by the collagen sheaths connected to each other. Such holes were, however, rare in the normal thyroid and benign non-neoplastic thyroid diseases.

Key Words: Human thyroid gland, scanning electron microscopy, collagen, thyroid disorders.

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Introduction

Scanning electron microscopic (SEM) studies on the structure of the normal thyroid gland and thyroid disorders have already been published (Nesland *et al.*, 1987; Weryha *et al.*, 1990). However, no reports have yet been made on the collagen sheath of the human thyroid gland and its disorders as observed by SEM. In 1987, Ohtani devised a cell-maceration/SEM method which employed a low temperature NaOH solution; using this method he successfully demonstrated the three-dimensional structure of the sub-basal laminar collagen tissue of the pancreas (Ohtani, 1987). Using this method, the three-dimensional structure of various organs has been reported (Ohtani, 1988; Ohtani *et al.*, 1988; Sugimoto and Ogata, 1989; Kawasaki and Ogata, 1992; Furuya and Ogata, 1993). In the present study, the same technique was applied to surgically resected specimens to investigate the structure of the collagen sheath of the normal human thyroid and of various thyroid disorders.

Materials and Methods

Thyroid specimens were obtained at surgery from 4 patients with Graves' disease, 3 with Hashimoto's thyroiditis, 7 with adenomatous goiter, 14 with follicular adenoma, 1 with oxyphilic cell adenoma, 1 with embryonal cell adenoma, 12 with papillary adenocarcinoma, 8 with follicular carcinoma, and 1 with medullary carcinoma. All Graves' disease patients were treated with thiamazole for about 6 weeks before operation. As control, normal thyroid specimens, obtained from the normal regions 20 patients with thyroid cancer, were used.

Light microscopic specimens were fixed in 20% formaldehyde solution for 1 week. Three μm sections were cut and stained by the silver impregnation method (Naoumenko and Feigin, 1974).

For NaOH cell-maceration, the resected thyroid tissues were immediately fixed in 2% glutaraldehyde solution in cacodylate buffer (pH 7.4) at 4°C for more than

4 hours, and then cut into small pieces (5 mm x 5 mm x 5 mm) and immersed in 10% NaOH solution at 20°C for 1 week (Ohtani *et al.*, 1988). After rinsing in running water for 2 days, the specimens were treated with a 1.5% aqueous solution of tannic acid for 12 hours and washed in running water for 4 hours. These tissue specimens were subsequently post-fixed in 1% OsO_4 solution at 4°C for 2 hours (Murakami, 1974), dehydrated through a series of graded ethanol and dried in a critical point drier (HCP-2, Hitachi, Tokyo). The dried specimens were mounted on metal stubs, coated with gold in an ion coater (IB-5, Eiko, Ibaraki) and observed under SEM (S-430, Hitachi, Tokyo) operated at an accelerating voltage of 15 kV.

Results

The normal thyroid gland

By light microscopy, the normal gland was observed to be composed of follicles lined with cuboidal epithelial cells and was filled with colloid. The interstitial surface of the epithelium was surrounded by thin argyrophilic fibers (Fig. 1a). In scanning electron micrographs of the NaOH cell-macerated specimens, the epithelium and basal lamina were removed and the collagen sheath was exposed. At low magnification, spherical collagen sheaths, 100-200 μm in diameter, surrounded the spaces which were occupied previously by the follicles. These structures are called perifollicular sheaths in this paper. The perifollicular sheath rarely had small holes (Fig. 1b). At higher magnification, the perifollicular sheaths were composed of a mixture of fine solitary collagen fibrils (50-70 nm in diameter, with a mean diameter of 55 nm) and thick collagen bands (1-5 μm width), which were composed of a parallel array of fine collagen fibrils (50-70 nm in diameter, with a mean diameter of 55 nm) (Fig. 1c). The thick collagen bands ran in various directions and formed the coarse framework of the perifollicular sheaths. Numerous solitary fine collagen fibrils ran in various directions around the thick collagen bands (Fig. 1d).

Graves' disease

Follicles varied in size and were surrounded by a thick layer of argyrophilic fibers (Fig. 2a). In scanning electron micrographs, the spaces surrounded by the perifollicular sheaths ranged from 50 to 600 μm in diameter. These perifollicular sheaths were composed of collagen bands and fine fibrils (50-70 nm in diameter, with a mean diameter of 55 nm) (Fig. 2b).

Hashimoto's thyroiditis

The histological appearance of Hashimoto's thyroiditis varied according to the stage. In most cases, the follicles were small. In some areas, follicles had almost disappeared and were replaced with abundant argyrophilic fibers (Fig. 3a). In scanning electron micrographs, the spaces surrounded by perifollicular sheaths were small, ranging from 20 to 100 μm in diameter. In some areas, the collagen tissue predominated and the follicles were scarce (Fig. 3b). At higher magnification, the perifollicular sheaths were mainly composed of thick collagen bands (3-5 μm in width) (Fig. 3c).

Figure 1 (on the facing page). Normal thyroid gland. **a.** Light microscopic view of a silver-impregnated specimen. Most follicles are polyhedral and filled with colloid. The follicles are surrounded by argyrophilic fibers darkly stained by silver deposits (arrows). Bar = 50 μm. **b.** Low magnification scanning electron micrograph of normal, NaOH-macerated, thyroid tissue. The collagen tissue forms spherical perifollicular sheaths around the spaces previously occupied by the follicles. Inter-lobular collagen septa (S). Bar = 100 μm. **c.** Medium magnification view of the perifollicular sheath. Thick collagen bands of varying widths run circularly and form the framework of the perifollicular sheaths. Fine collagen fibrils are seen around the thick collagen bands and filling the spaces between the bands. Bar = 10 μm. **d.** High magnification of the perifollicular sheaths. A thick collagen band (B) formed by bundles of fine collagen fibrils. Solitary fine collagen fibrils (arrows) run in various directions encompassing the thick collagen bands. Bar = 0.1 μm.

Adenomatous goiter

Marked variations in the size of the follicles were observed. The amount of argyrophilic fibers around follicles was variable (Fig. 4a). In scanning electron micrographs, the spaces surrounded by the perifollicular sheaths varied in size markedly, ranging from 30 to 500 μm. Most perifollicular sheaths were composed of abundant thick collagen bands (3-10 μm in width) and fine collagen fibrils (Fig. 4b). Each lesion was partly encapsulated by a thin collagen capsule (Fig. 5a).

Follicular adenoma

The follicles were of various sizes, filled with colloid, and surrounded by a thin layer of argyrophilic fibers (Fig. 6a). In scanning electron micrographs, the margin of the adenoma was well demarcated by a thick collagen capsule (Fig. 5b). The spaces surrounded by perifollicular sheaths varied in size from 40 to 200 μm (Fig. 6b). At higher magnification, the perifollicular sheaths were mainly composed of fine collagen fibrils (35-45 nm in diameter, with a mean diameter of 42 nm) (Figs. 6c and 6d).

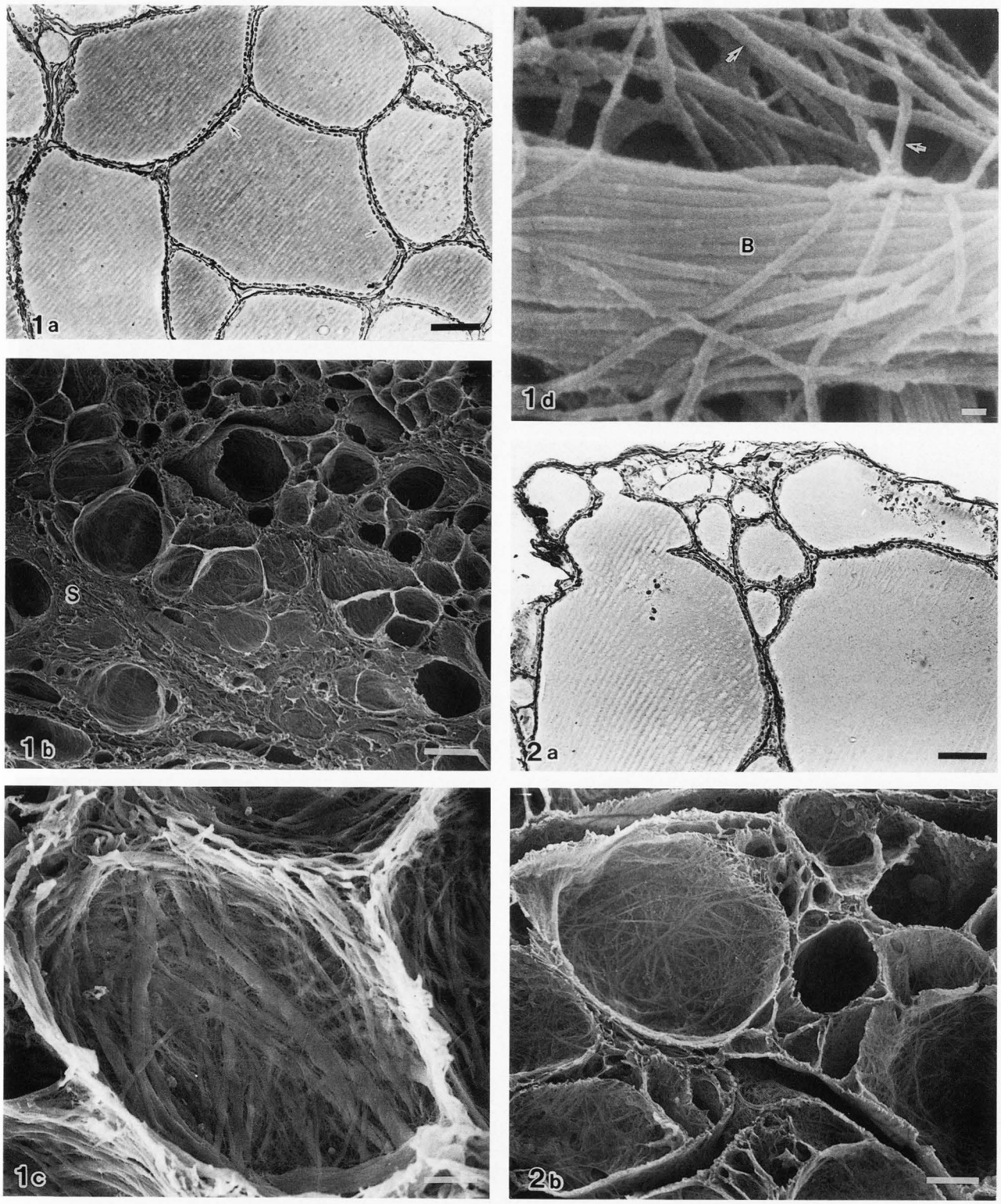


Figure 2. Graves' disease. **a.** Silver-impregnated specimen. The follicles are enlarged. Bar = 50 μm. **b.** Cell-macerated specimen. The spaces surrounded by the perifollicular sheaths are variable in size but most of them are larger than those of the normal thyroid. Bar = 100 μm.

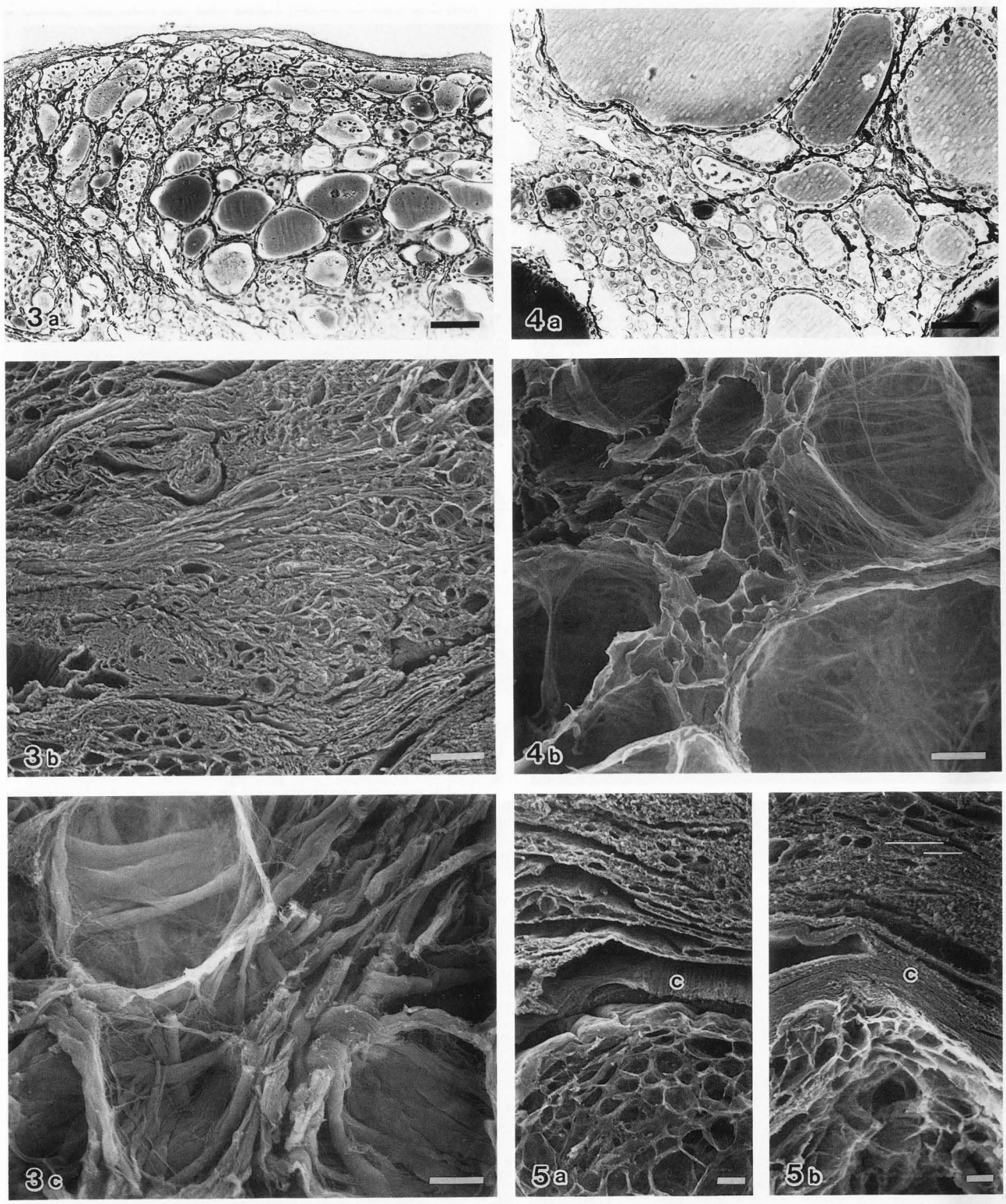
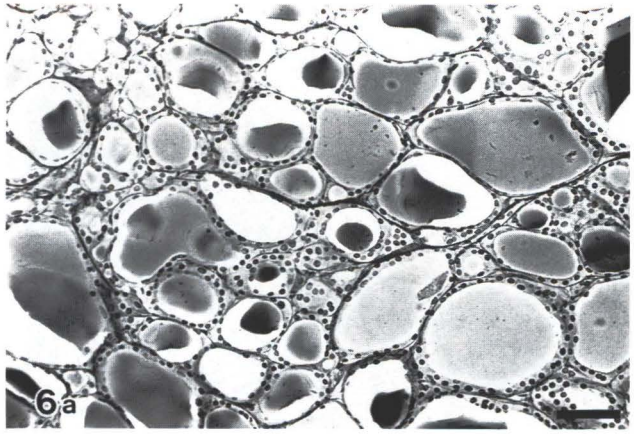


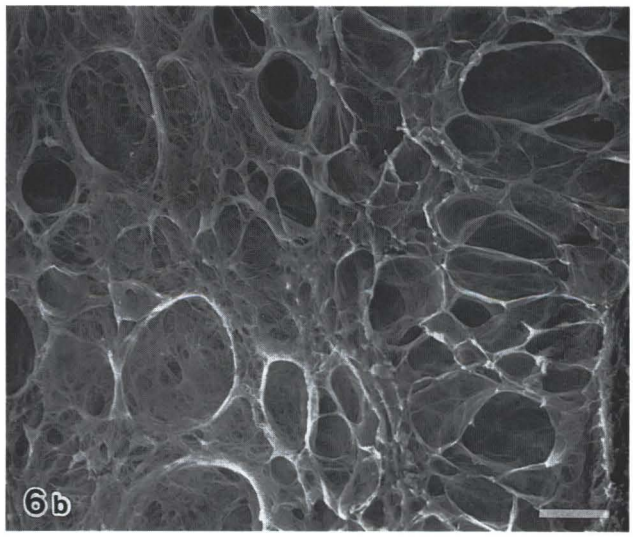
Figure 5. Comparison of the capsules in adenomatous goiter and follicular adenoma. **a.** The adenomatous goiter is partly encapsulated by a thin collagen capsule (C). Bar = 100 μm. **b.** The follicular adenoma is encapsulated by a thick collagen capsule (C). Bar = 100 μm.

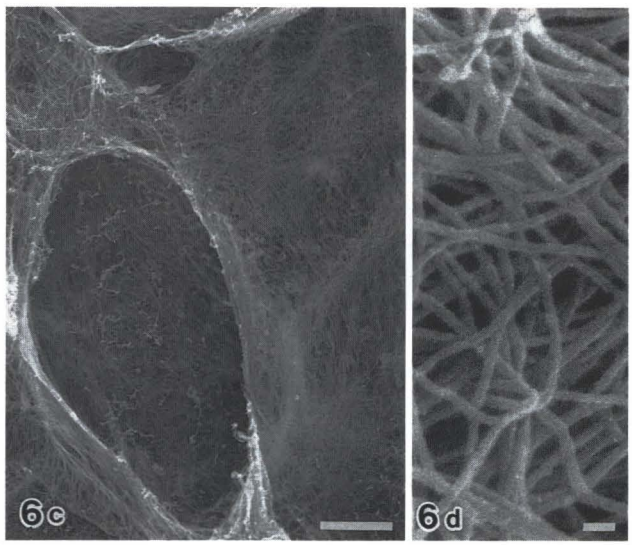
Figure 3 (on the facing page, left). Hashimoto's thyroiditis. **a.** Silver-impregnated specimen. The follicles are small. The argyrophilic fibers are well developed. Bar = 50 μm. **b.** Low magnification scanning electron micrograph. In some areas, collagen tissue predominates over the glandular tissue. The spaces surrounded by the perifollicular sheaths are small. Bar = 100 μm. **c.** High magnification view of the same specimen. Perifollicular sheaths are composed of abundant thick collagen bands. Bar = 10 μm.

Figure 4 (on the facing page, top right). Adenomatous goiter. **a.** Silver-impregnated specimen. The size of the follicles is markedly variable. The amount of argyrophilic fibers around the follicles varies. Bar = 50 μm. **b.** Cell-macerated specimen. There is a marked difference in the size of the spaces surrounded by the perifollicular sheaths. Bar = 100 μm.

Figure 6 (at right). Follicular adenoma. **a.** Silver-impregnated specimen. The follicles are surrounded by argyrophilic fibers and are variable in size. Bar = 50 μm. **b.** Cell-macerated specimen. The spaces surrounded by the perifollicular sheaths are variable in size. Bar = 100 μm. **c.** The collagen sheaths are composed of fine fibrils. Bar = 10 μm. **d.** High magnification. The fine fibrils run in various directions. Bar = 0.1 μm.







Oxyphilic cell adenoma (Hürthle cell adenoma)

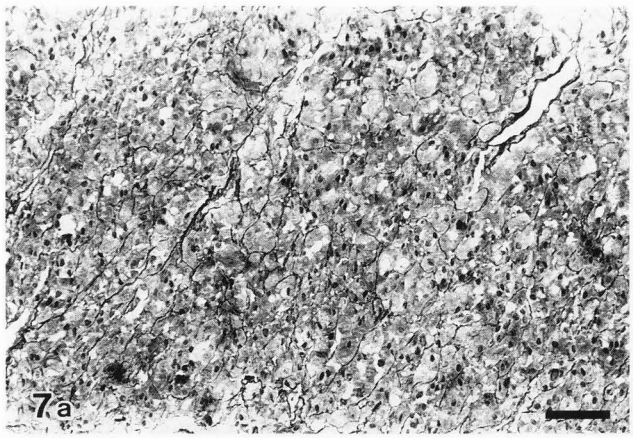
This adenoma was composed of very small follicles (Fig. 7a). In scanning electron micrographs, the spaces surrounded by the perifollicular sheaths were small, 15-60 μm in diameter (Figs. 7b and 7c), and were mainly composed of fine collagen fibrils (40-45 nm in diameter, with a mean diameter of 43 nm).

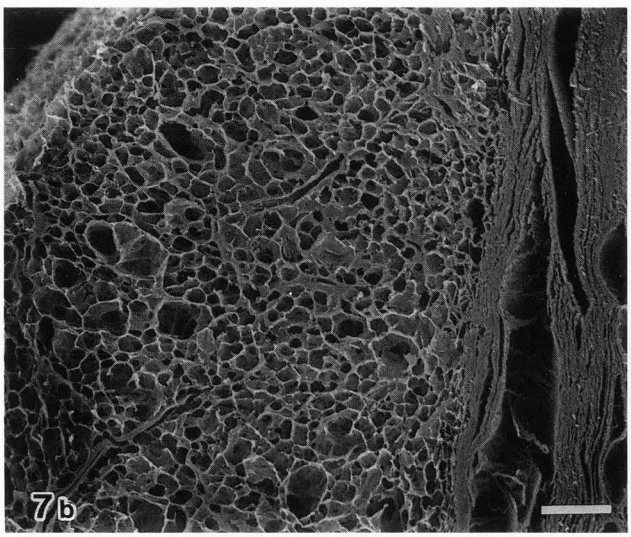
Embryonal adenoma

Tumor cells were arranged forming small follicles which were partially surrounded by well developed argyrophilic fibers. Only a few of these follicles contained colloid (Fig. 8a). In scanning electron micrographs, thin collagen sheaths extended from the trabecula and formed the perifollicular sheaths (Fig. 8b); these perifollicular sheaths had large holes, 20-30 μm in diameter and they were mainly composed of fine collagen fibrils (40-45 nm in diameter, with a mean diameter of 43 nm) (Figs. 8c and 8d).

Follicular carcinoma

The follicles were small and partially surrounded by thin argyrophilic fibers (Fig. 9a). A few of them were filled with colloid. In scanning electron micrographs, the size of the spaces surrounded by the perifollicular





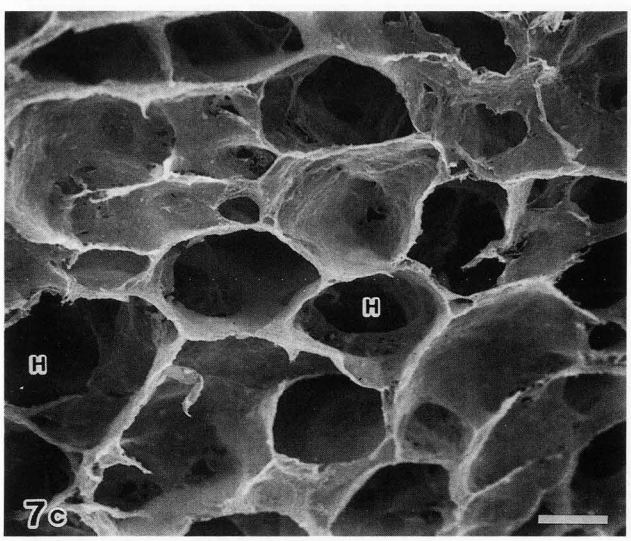


Figure 7 (at left). Oxyphilic adenoma. **a.** Silver-impregnated specimen. The follicles are very small and are surrounded by thin argyrophilic fibers. Bar = 50 μm. **b.** Cell-macerated specimen. The spaces surrounded by the perifollicular sheaths are small and variable in size. Bar = 100 μm. **c.** High magnification. Perifollicular sheaths have large holes (H) and are mainly composed of a delicate meshwork of solitary fine collagen fibrils. Bar = 10 μm.

Figure 8 (on the facing page, at left). Embryonal adenoma. **a.** Silver-impregnated specimen. Tumor nests are partially surrounded by argyrophilic fibers. Bar = 50 μm. **b.** Cell-macerated specimen. The spaces surrounded by the perifollicular sheaths are small and irregular. Bar = 100 μm. **c.** Perifollicular sheaths have large holes (H). Bar = 10 μm. **d.** High magnification. Fine fibrils run in various directions. Bar = 0.1 μm.

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sheaths ranged from 20 to 300 μm in diameter (Fig. 9b). Most perifollicular sheaths had large holes, about 10-50 μm in diameter, through which the lumina of adjacent perifollicular sheaths were continuous with each other (Fig. 9c). These perifollicular sheaths were mainly composed of fine collagen fibrils (40-45 nm in diameter, with a mean diameter of 42 nm) (Fig. 9d).

Papillary adenocarcinoma

Papillary and follicular growth were mixed in some areas. In the typical papillary growth area, well developed thin argyrophilic fibers were seen (Fig. 10a). In low magnification scanning electron micrographs, the collagen sheaths showed a papillary structure (Figs. 10b and 10c). At higher magnification, the collagen sheaths were mostly composed of fine collagen fibrils (32-37 nm in diameter, with a mean diameter of 35 nm) (Fig. 10d).

Medullary carcinoma

The tumor showed lobular or trabecular growth (Fig. 11a). The stroma contained variable amounts of argyrophilic fibers. Well developed septa were seen. In scanning electron micrographs, the collagen sheaths extended from the well developed collagen septa and partially surrounded the tumor nests (Figs. 11b and 11c). The collagen sheaths were composed of fine collagen fibrils (35-40 nm in diameter, with a mean diameter of 38 nm) (Fig. 11d).

Discussion

The present study has first succeeded in showing the shape of both the follicles and tumor nest three-dimensionally by SEM. Our findings clearly show that the collagen fibril arrangement of the sheath is characteristic of each disorder.

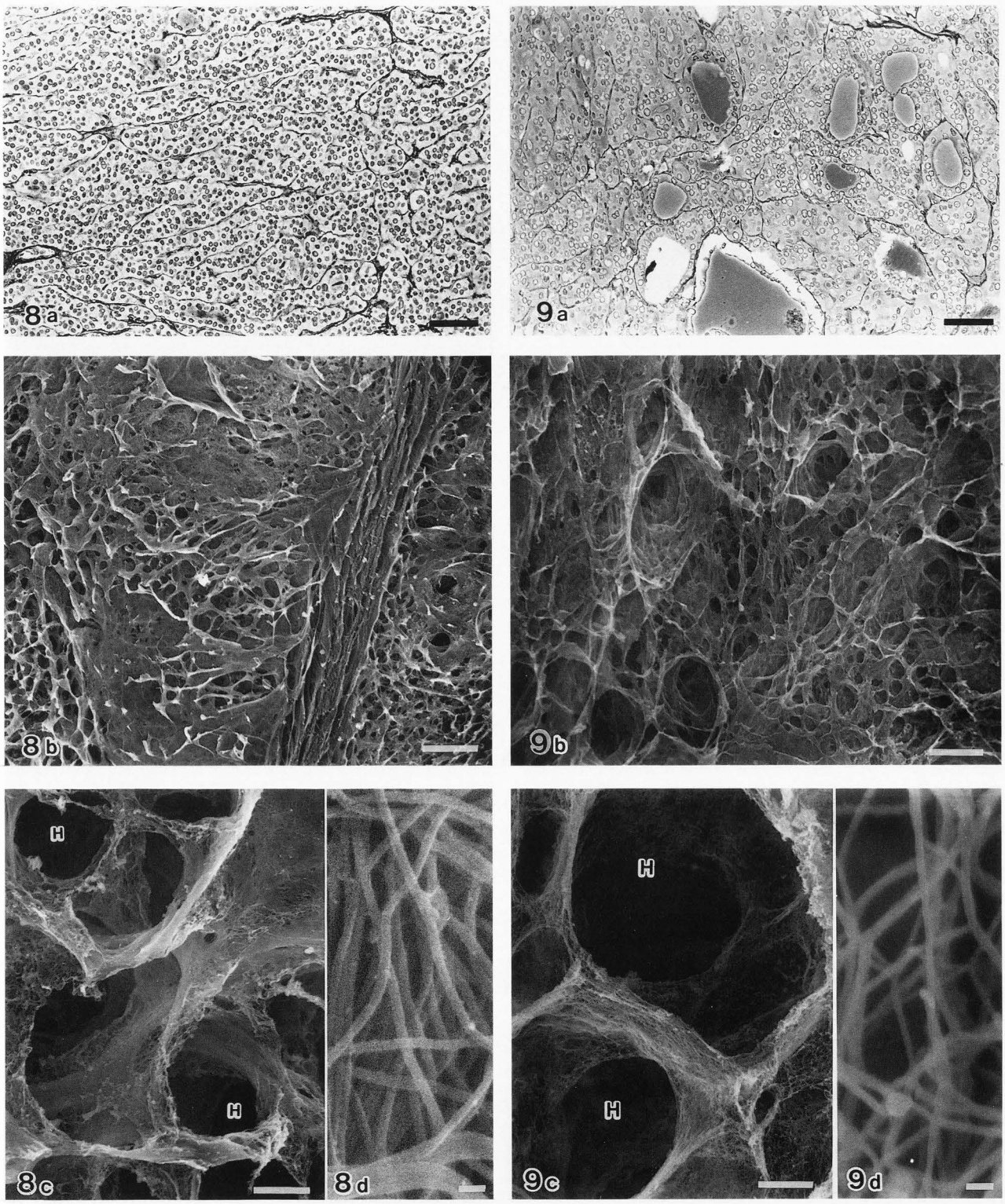


Figure 9. Follicular carcinoma. **a.** Silver-impregnated specimen. Follicles are irregular in shape; some contain colloid. Bar = 50 μm. **b.** Cell-macerated specimen. The spaces surrounded by the perifollicular sheaths are irregular in shape and various in size. Bar = 100 μm. **c.** Perifollicular sheaths have large holes (H). Bar = 10 μm. **d.** High magnification. The collagen sheaths are mostly composed of loosely arranged fine collagen fibrils. Bar = 0.1 μm.

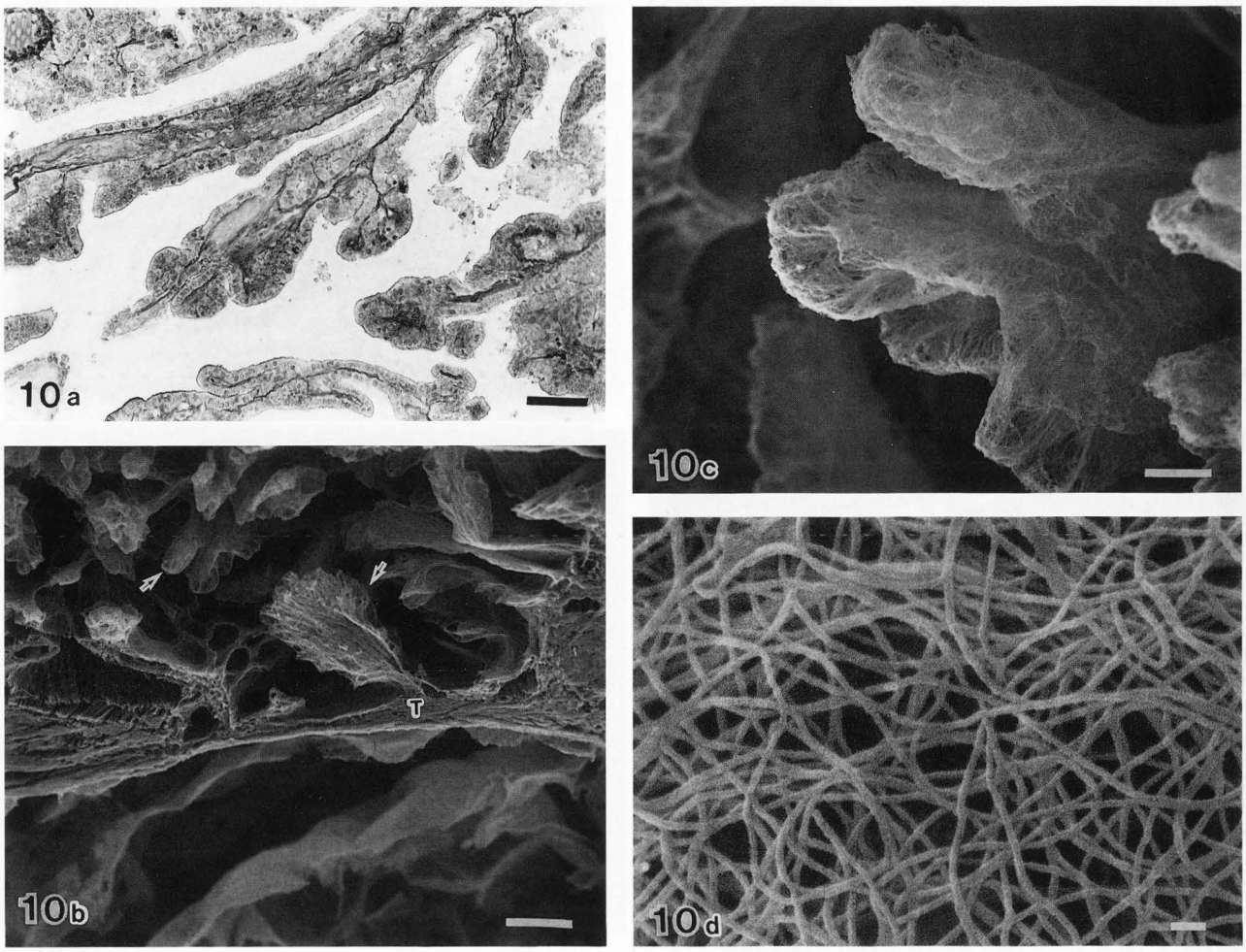
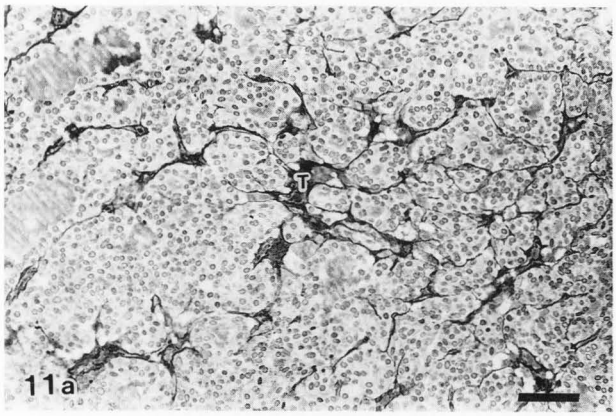


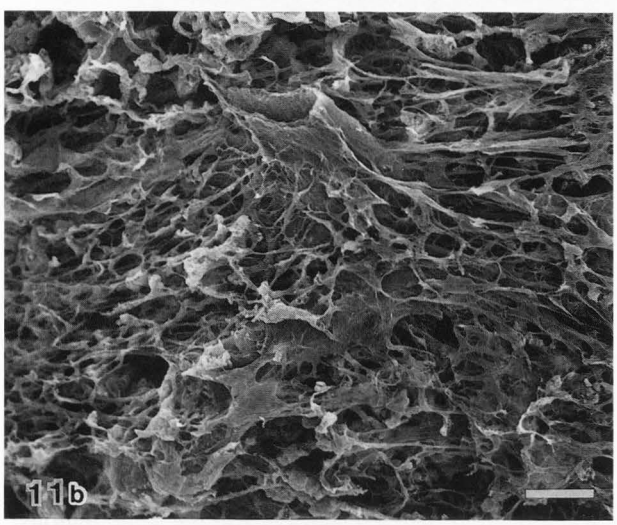
Figure 10. Papillary adenocarcinoma. **a**. Silver-impregnated specimen. Typical papillary growth area. Bar = 50 μm. **b**. Cell-macerated specimen. Papillary shaped collagen sheaths (arrows) protrude from the trabecula (T). Bar = 50 μm. **c**. Medium magnification. The collagen sheath appears as a cactus-like structure. Bar = 10 μm. **d**. High magnification. The protruding collagen sheaths consist primarily of fine fibrils. Bar = 0.1 μm.

The spaces surrounded by the perifollicular sheaths of the normal thyroid gland are spherical in shape, and measure 100-200 μm in diameter. According to Meissner and Warren (1969), in untreated Graves' disease, the follicles vary in size, but there is a tendency toward smaller follicles. However, in the present study, large follicles predominated and consequently, the spaces surrounded by the perifollicular sheaths were also large. This may be attributable to the fact that all specimens were obtained at surgery after using anti-thyroid drugs, which causes the enlargement of the thyroid follicles. In Hashimoto's thyroiditis, collagen tissues are generally abundant and the perifollicular sheaths are formed by thick collagen bands. In adenomatous goiter, the histological findings vary considerably depending upon the pathophysiological stages of the disease (Meissner and Warren, 1969). In our series, the spaces surrounded by the perifollicular sheaths varied significantly in size. SEM observations revealed that, in contrast to adenoma, adenomatous goiter has a very thin collagen capsule, which is in agreement with previous reports (Meissner and Warren, 1969).

The size of the follicles in benign adenomas is generally smaller than in the normal thyroid (Meissner and Warren, 1969). In follicular adenoma and oxyphilic cell adenoma, the perifollicular sheaths retain a similar structure to those of the normal thyroid, although the sizes of the spaces surrounded by them are small, especially in oxyphilic adenoma. Embryonal adenoma is the most poorly differentiated of the follicular group (Meissner and Warren, 1969). The spaces surrounded by the perifollicular sheaths are very small and show a rather irregular structure.

In follicular carcinoma, the follicular structure is





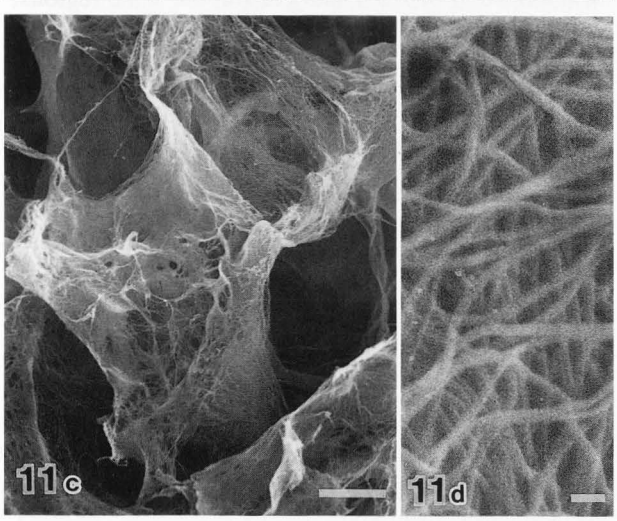


Figure 11 (at left). Medullary carcinoma. **a.** Silver-impregnated specimen. Tumor nests are partially surrounded by argyrophilic fibers extending from the trabecula (T). Bar = 50 μm. **b.** Cell-macerated specimen. Irregular shaped collagen sheaths extend from the trabecula. Bar = 100 μm. **c.** Collagen sheaths are irregular in shape. Bar = 10 μm. **d.** The collagen sheath is composed of fine fibrils. Bar = O.1 μm.

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still retained, but the shape and size of the spaces surrounded by the perifollicular sheaths are markedly irregular. In papillary carcinoma, the collagen sheaths exhibit a papillary pattern with various irregular protrusions. In medullary carcinoma, each tumor nest is surrounded by a well developed collagen sheath. Anaplastic carcinoma was not included in the study because this highly malignant cancer is not an indication for surgical therapy.

In the normal thyroid, the perifollicular sheaths rarely have small holes which interconnect the empty spaces surrounded by the perifollicular sheaths. These holes are preferentially seen in the smaller follicles. In Graves' disease, Hashimoto's thyroiditis and adenomatous goiter, these holes are also frequently observed in the smaller follicles and are rare in the larger follicles. Such holes are also seen in benign adenomas, being abundant in embryonal adenoma. In follicular carcinoma, most perifollicular sheaths have large holes. Their function, however, is still unknown.

The thick collagen bands are predominantly seen in the perifollicular sheaths of the normal thyroid and non-neoplastic thyroid diseases. However, they are less frequent in adenomas and very scarce in carcinomas. In addition, the diameter of the fine collagen fibrils in adenomas and carcinomas is 32-45 nm, whereas in the normal thyroid and non-neoplastic thyroid diseases, it is 50-70 nm. Flint *et al.* (1984) reported that the fibril diameters in rat tail skin, immediately after birth, are small (~ 34 nm), but increase rapidly during the first month (diameter ~ 100 nm), and within five months of birth, the average diameter of the collagen fibrils in rat tail skin is ~ 155 nm. The smaller diameter of the collagen fibrils in neoplastic thyroid diseases may thus indicate that their collagen tissue is less mature than that in the normal thyroid.

Acknowledgments

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Discussion with Reviewers

O. Ohtani: The collagen fibrils possess bands which are determined by an alternative succession of elevated and depressed segments along the fibrils. The bands can be demonstrated under a scanning electron microscope. Why do you think that your electron micrographs do not show any band along the collagen fibril?
Authors: Bands along the collagen fibril are unclear by SEM, although they are clearly seen in the same tissue by TEM. The reason is unknown.

O. Ohtani: Tannic acid is indeed very good for hardening the collagenous materials prepared by the cell-maceration technique, as well as for increasing the conductivity of the specimen by the aid of osmium tetroxide. But tannic acid deposited on the collagen fibrils will grow into large granules. The longer the treatment period with tannic acid, the larger the granules formed by tannic acid. Why did you treat your specimens with tannic acid for such a long period as 12 hours?
Authors: We treated our specimens with tannic acid for 12 hours in order to increase the conductivity of the specimens. Although, large granules were occasionally seen on the specimens, the areas we selected for observation were without granules.

R. Wroblewski: Do you think that these holes represent a new connection (effect of disease) between the follicles which earlier were separated or are just narrower portions of previously existing follicles now elongated and deformed?
T. Ushiki: You note the presence of the "hole" in the perifollicular collagen sheath. Does this mean the pore (fenestra) of the sheath that open to the stromal space?
Authors: We think that holes are just narrower portion of previously existing follicles now elongated and deformed. We do not think that the fenestra of the sheath open to the stromal space.

R. Wroblewski: Do you think that changes in the collagen sheath are accompanied by the loss of the basal membrane and the follicular cells in the same regions?
Authors: Yes, we think so.

R. Wroblewski: Are there any immunohistochemical studies of the changes in collagen type described by your pathological conditions which could be related to the fine and gross morphological changes in the collagen sheath structure?
Authors: To our knowledge, there are no immunohistochemical studies of collagen in thyroid diseases.

T. Ushiki: According to the diameter of the collagen fibrils, you consider that the collagen fibrils of the perifollicular sheath in neoplastic diseases are less mature than those in the normal thyroid. However, collagen fibrils beneath the basal lamina are generally thin (about 30-50 nm in diameter). Did you also find abnormal collagen fibrils in your materials?
Authors: Please refer to the mean diameter and distribution of fibril diameter in each disorder provided in **Results**. According to Flint *et al.*, the larger the diameter of the collagen fibrils, the more matured it is. Therefore, the collagen fibrils of the normal thyroid are more matured than that of neoplastic diseases. We did not observe the collagen fibrils that terminated in neoplastic diseases.