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Light-Electron Microscopic and Cytochemical Studies on the Morphogenesis of Familial Medullary Thyroid Carcinoma*

W. Schürch**, F. Babaï, Y. Boivin, and M. Verdy

Département de Pathologie, Université de Montréal, and Département de Pathologie, Hôpital Hôtel-Dieu, Montréal, Québec, Canada

Summary. Six cases of familial medullary thyroid carcinoma (MTC) were investigated by light and electron microscopy as well as by ultracytochemical methods. Light microscopic examination revealed multifocal C-cell proliferation in 5 subjects. These cells were mostly limited to thyroid follicles, but occasionally extended across the follicular capsule forming microscopic MTC. Electron microscopic examination showed that, in some follicles, the proliferating C-cells were still covered by a continuous layer of follicular cells, whereas in others the proliferation extended to the follicular center. C-cells were in direct contact with the colloid, and ultramicroinvasion of the follicular capsule was detected. These observations are consistent with the hypothesis that familial MTC seems to begin as multifocal C-cell proliferation, limited at first to thyroid follicles, between the capsule and the follicular epithelium. Later, the proliferation extends to the follicular center, and C-cells come in contact with the colloid, at which time an in situ carcinoma stage is reached. Some neoplastic cells invade the follicular capsule and, finally, multiple MTC appear and eventually conglomerate.

Generally, there were no constant morphologic criteria for a dysplasia or neoplasia among the proliferating C-cells limited to thyroid follicles, when compared with normal or even malignant C-cells. For these reasons, a hyperplastic or dysplastic process preceding MTC cannot be clearly distinguished from a neoplastic process. Our study, however, shows that a light microscopic, apparently hyperplastic process may be a malignant one.

Amyloid was present in the more voluminous MTC, associated with tumor cell necrosis, but it was not evident in small MTC and within the foci of C-cell proliferation.

For offprints contact: Dr. F. Babaï, Département de Pathologie, Université de Montréal, C.P. 6128, Succursale A, Montréal, Québec, Canada H3C 3J7

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** Present address: Department of Pathology, University of Maryland, 31 South Greene Street, Baltimore, Maryland 21201, U.S.A.

Ultracytochemical techniques revealed that the secretory granules of normal, proliferating and neoplastic C-cells contained polysaccharides and/or glycoproteins.

Key words: Thyroid gland – Familial medullary carcinoma – Morphogenesis – Electron microscopy – Cytochemistry of C-cells.

Introduction

Medullary thyroid carcinomas (MTC) were first clearly identified by Hazard et al. in 1959. A large number of studies in the last decade have pointed out the remarkable individuality of this particular variety of thyroid cancer, distinguishable by its amyloid stroma and relatively indolent clinical course (Williams et al., 1966; Ibanez et al., 1967; Steiner et al., 1968; Melvin et al., 1972). MTC can occur in hereditary or sporadic form. The former type is transmitted via an autosomal-dominant mode of inheritance with a reduced penetrance (Sarosi and Doe, 1968). Williams (1966) elucidated the histogenesis of MTC, i.e. its origin from C-cells, and Bussolati et al. (1969) using immunofluorescence techniques demonstrated the presence of calcitonin in the tumor cells with antibodies to the pure porcine hormone. The development of a highly sensitive radioimmunoassay (Tashjian et al., 1970) for the detection of serum calcitonin concentrations, after calcium and/or pentagastrin infusion (Hennessy et al., 1973), has proven to be of considerable value in the diagnosis of occult MTC among members of families with the disease (Jackson et al., 1973).

Although the histogenesis of MTC appears to have been clarified, its morphogenesis is less well-defined, and several questions still remain to be fully explained. It is well-known that hereditary MTC have a multifocal form and that the tumor is bilateral in a high percentage of cases. An increase of calcitonin in the thyroid tissue remote from the tumor was interpreted by Melvin et al. (1971) as an indication of a tumor associated with C-cell hyperplasia. Similarly, Ljungberg (1972) and Wells et al. (1975) presented morphologic evidence of an association between familial MTC and multifocal C-cell hyperplasia in some of their patients. Finally, Wolfe et al. (1973) reported morphologic, biochemical and immunohistochemical findings consistent with C-cell hyperplasia alone in 3 subjects faced with a possible risk of familial MTC. The latter team of investigators postulated that the multifocal C-cell hyperplasia might be a preinvasive or hyperplastic process.

We recently had an opportunity to study 6 patients with familial MTC. Of these subjects, 5 were clinically occult, and it therefore appeared relevant to follow the morphogenesis of the disease by light and electron microscopic investigation of the hyperplastic C-cell foci. Ultracytochemical techniques were also deemed necessary for an examination of C-cell content in familial MTC.

Materials and Methods

After discovery of the first case (a palpable tumor in the left lobe of the thyroid with regional lymph node metastases), the other 5 members of the family underwent stimulation of calcitonin

by calcium and pentagastrin infusion to detect occult MTC. The serum calcitonin concentration, measured by radioimmunoassay after stimulation, was abnormally high in these 5 subjects, none of whom showed a clinically detectable tumor in the thyroid. They were then thyroidectomized. The mean age of the patients was 29.5 years (range: 19 to 43 years). (Details of the clinical data and laboratory investigations will be published elsewhere by Verdy et al.)

Light Microscopy

In each case, the entire thyroid gland and regional lymph nodes were available for microscopic investigation. Immediately after removal, 3- to 4-mm thick sections were cut from the thyroids of patients 2 to 6 and carefully examined for macroscopic MTC. Following identification, the MTC nodules were fixed with a portion of adjacent thyroid tissue in neutral buffered formol for 8 to 12 h and postfixed in Bouin's solution for 4 to 6 h, then dehydrated and embedded in paraffin. Sections cut at 3 to 5 μ were stained by hematoxylin-phloxine-saffron (HPS), Masson's trichrome stain, Laidlaw's reticulin stain, Congo red stain and Periodic Acid-Schiff (PAS). In addition, chosen sections of thyroid tissue adjacent to the MTC, suggestive of C-cell hyperplasia, as well as sections of the MTC themselves, were impregnated by the silver nitrate method of Grimelius as modified by De Grandi (1970).

Electron Microscopy

A first set of fragments of each MTC, as well as fragments of adjacent thyroid tissue, were cut into 1-mm cubes, fixed for 2 h in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.3, and postfixed for 1 h in 1% osmium tetroxide in the same buffer. These cubes were embedded in Epon 812, after dehydration in graded alcohols. Sections were then cut on a LKB II ultramicrotome with a diamond knife. Semithin sections were stained with toluidine blue. The thin sections were picked up on Formvar-covered grids stabilized by a carbon coat and stained with uranyl acetate and lead citrate.

In two cases, a second set of fragments of MTC and adjacent thyroid tissue was fixed in glutaraldehyde alone and embedded in glycol methacrylate (GMA), according to the standard technique of Leduc and Bernhard (1967). For the visualization of polysaccharides, thin sections were stained for 30 to 60 min with a 1% solution of phosphotungstic acid (PTA) in 1 N HCL at pH 0.3 (Pease, 1966; Marinozzi, 1967). They were examined and photographed, using a Philips 300 electron microscope.

Results

Gross Appearance

The thyroids of patients 2 to 6 had a normal mean weight of 14.8 g, ranging from 12 to 16 g. [The average weight of thyroid glands in Quebec has been found to be 16.1 g for men and 13.6 g for women (Verdy et al., 1971).] In case 1, which presented clinically-palpable regional lymph node metastases and a palpable tumor in the left lobe of the thyroid, the weight of this organ was augmented to 46 g.

After sectioning of the thyroids of patients 2 to 6, MTC were detected deep in the lateral lobes at the limits of the middle and upper third. The MTC nodules were bilateral in 5 and multiple in 4 subjects (Table 1). Except for case 1, the MTC were circumscribed and round or oval in configuration. They were grayish-white with a semisolid consistency.

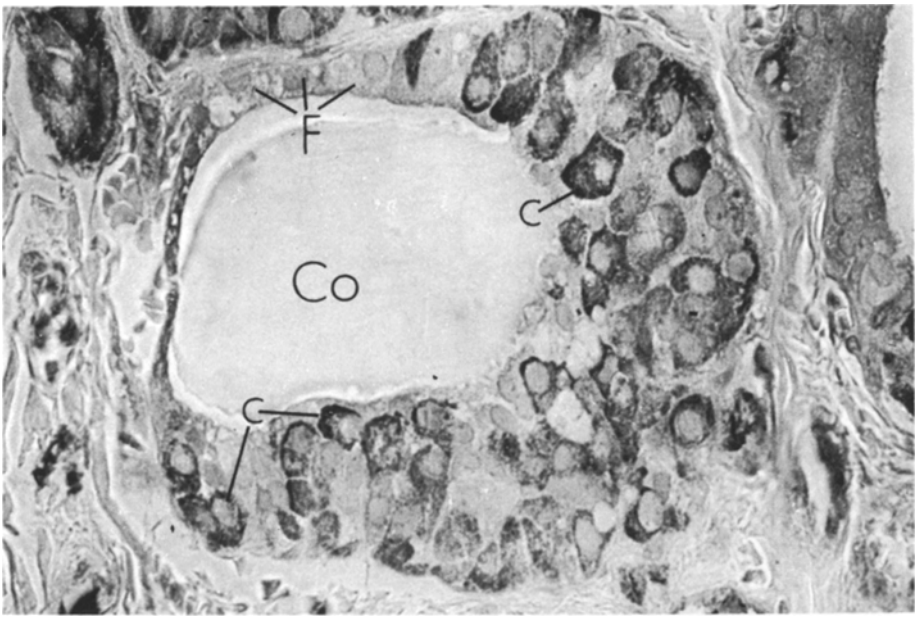
Table 1. Clinical, macroscopic and light microscopic findings in 6 patients with familial medullary thyroid carcinoma

Case	Age and sex	Weight of thyroid glands (g)	Size of tumors		Amyloid	Multifocal proliferation of C-cells		Lymph node metastases
			Right lobe (cm)	Left lobe (cm)		Right lobe	Left lobe	
1	25, M	46	1.0	4.5	+	?	?	+
2	43, M	15	1.0	0.7	+	+	+	–
			1.0	0.3				
			0.6	0.2				
3	29, F	16	0.3	1.2	+	+	+	–
4	31, M	16	0.4	–	–	+	+	–
			0.1					
5	19, M	12	0.3	0.2	+	+	+	–
			0.15	0.1				
6	32, M	15	0.6	0.6	+	+	+	–

Light Microscopy

The MTC nodules were usually well-circumscribed and sometimes partially encapsulated. Most of them displayed areas in which the tumor invaded the adjacent thyroid tissue. The nodules were composed of round, polyhedral or spindle-shaped cells, arranged in sheets, trabeculae or nests of variable size, usually separated by connective tissue septa. Frequently, in the well-demarcated but not encapsulated areas, as well as in distinct invasive regions, engulfed thyroid follicles were visible. Remnants of sequestered colloid could be observed, even in the center of the tumors. Occasionally, a pseudopapillary pattern was evident in the more voluminous MTC nodules, and this probably resulted from a degenerative process. The peripheral layers of the tumor cells of each nest or sheet remained in contact with the connective tissue stroma, while the cells in the central areas were disaggregated and, eventually, necrotic. Calcification was frequent in these areas, but psammoma bodies characteristic of papillary thyroid carcinoma were not seen.

The nuclei of the neoplastic cells were round or oval and exhibited a slight to moderate pleomorphism. The chromatin was finely granular, and the nucleoli were inconspicuous or slightly prominent. A few binucleate cells were noted in the more voluminous MTC. Mitoses were rare or absent. The cytoplasm varied from scant to moderately abundant and was finely granular and acidophilic. Argyrophilic granules were clearly visible in the cytoplasm, as shown by the silver nitrate impregnation technique of Grimelius. They differed greatly in number from one cell to another. Except for one patient, amyloid was present in all Congo red-stained sections examined under polarized light. It appeared as amorphous material between neoplastic cells and, more abundantly, as globoid masses in connective tissue septa, surrounded by collagen fibers. Calcified



Figs. 1–2. Light microscopy of paraffin-embedded section

Fig. 1. Case 5. Thyroid follicle showing marked proliferation of C-cells (*C*) which form several layers between the colloid (*Co*) and the follicular capsule. Argyrophilic granules are evident within the cytoplasm of C-cells. Follicular cells (*F*) do not contain silver-positive granules. Silver nitrate impregnation after Grimelius. × 500

deposits were also discernible in the amyloid substance. In the very small MTC with a diameter of less than 0.2 cm, amyloid was usually lacking.

Clusters of C-cells were detected in the deep portions of the lateral lobes in all but patient 1. (Thyroid tissue surrounding the carcinomas in this subject was not available for examination.) The clusters, as identified by the Grimelius stain, appeared in intrafollicular, parafollicular and interfollicular positions. C-cells were frequently present in several layers between the follicular capsule and the colloid (Fig. 1), but they sometimes also seemed to occupy entire follicles (Fig. 2). In areas with a high degree of C-cell proliferation, reticulin staining showed that the follicular pattern was usually preserved. Only in rare instances did the C-cell proliferation extend across the follicular wall; this was accompanied by a disappearance of the follicular pattern and the appearance of microscopic MTC nodules.

Electron Microscopy

Fragments of MTC nodules and adjacent thyroid tissue were examined by conventional electron microscopic techniques (Epon embedding) in 5 of the 6 patients and by special procedures (GMA embedding and PTA staining) in

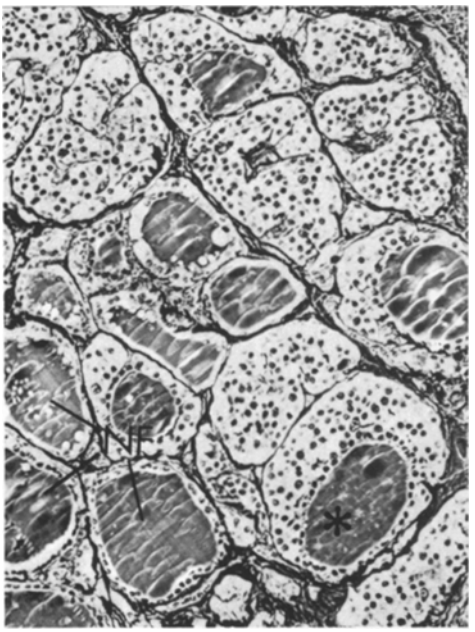
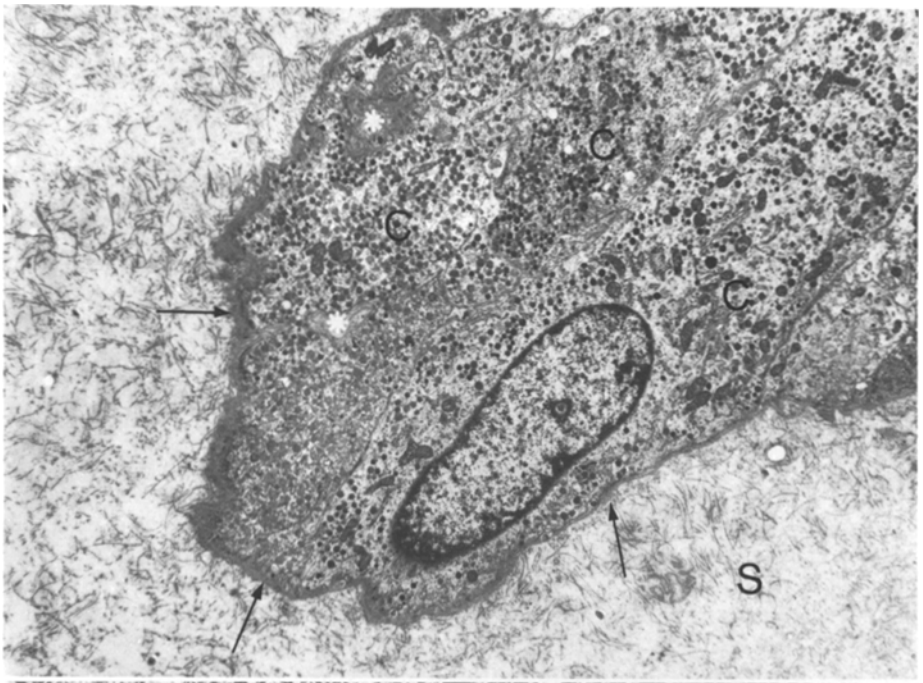


Fig. 2. Case 3. Multifocal C-cell proliferation apparently limited to the thyroid follicles. The follicular pattern seems to be preserved, as shown by the reticulin stain. A follicle similar to that seen in Figure 1 is present in the right inferior quadrant (*). A group of normal follicles (*NF*) is visible in the left inferior quadrant. Reticulin Stain. ×250

2 subjects. In order that our results may be more readily understood, we shall start by describing the ultrastructure of the capsule of thyroid follicles.

Thyroid follicles are surrounded by a capsule which consists of a basal lamina (basement membrane) and a connective tissue sheath, composed of fibroblasts and collagen fibers. The terminal parts of the capillaries frequently become incorporated in the connective tissue sheath of the capsule. The basal surface of the epithelial lining of follicles is often irregular with multiple finger-like projections of various length which are surrounded by continuous basal lamina.

Clusters of tumor cells were usually separated from the stroma by a continuous basal lamina (Figs. 3 and 4). Less frequently, the plasma membrane of the neoplastic cells was in direct contact with the stroma. The individual tumor cells were polyhedral, round or irregular in shape, with cytoplasmic processes. They were connected by junctional complexes of the desmosome type. The plasma membrane of adjoining tumor cells was generally straight, but interdigitations were also present and, in such instances, the desmosomes were less numerous. Canalicular spaces, observed between the neoplastic cells, contained extruded secretory granules and cellular debris. The neighboring tumor cells frequently showed cytoplasmic, microvillous-like processes in the canalicular spaces and formed junctional complexes in their vicinity (Figs. 5 and 6). Basement membranelike material was occasionally evident between the neoplastic cells, representing invaginations from the peripheral basal lamina which surrounded the tumor cell complexes (Fig. 6). The cytoplasm displayed typically-spherical secretory granules with a diameter of 100 to 350 nm, consisting of a membrane separated from an electron-dense core by a thin, clear zone. The



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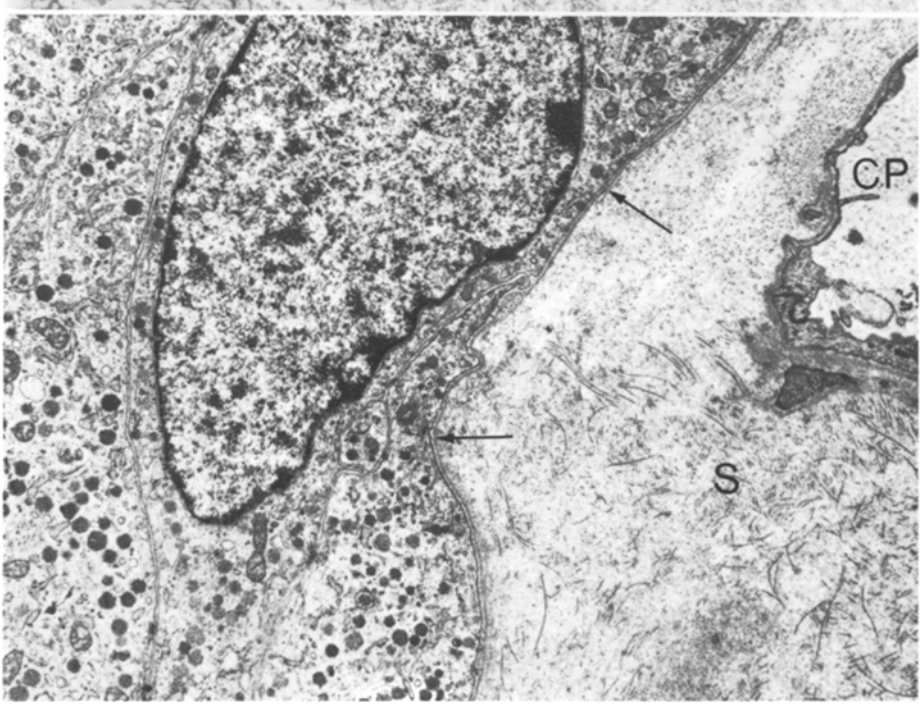

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Figs. 3–9. Electron microscopy of Epon-embedded sections stained with uranyl acetate and lead citrate

Fig. 3. Case 2. Cluster of neoplastic C-cells (*C*) separated from the surrounding stroma (*S*) by a continuous basal lamina (*arrows*). Invaginations of the basal lamina are evident at several points between individual tumor cells (*). A large number of secretory granules is present within the cytoplasm of the neoplastic C-cells. ×4000

Fig. 4. Case 2. Three neoplastic C-cells with numerous secretory granules within the cytoplasm are separated from the stroma (*S*) by a thin, continuous basal lamina (*arrows*). The plasma membrane of adjoining tumor cells is mostly straight, but interdigitations are also present. A capillary (*Cp*) is visible in the right upper quadrant. ×8000

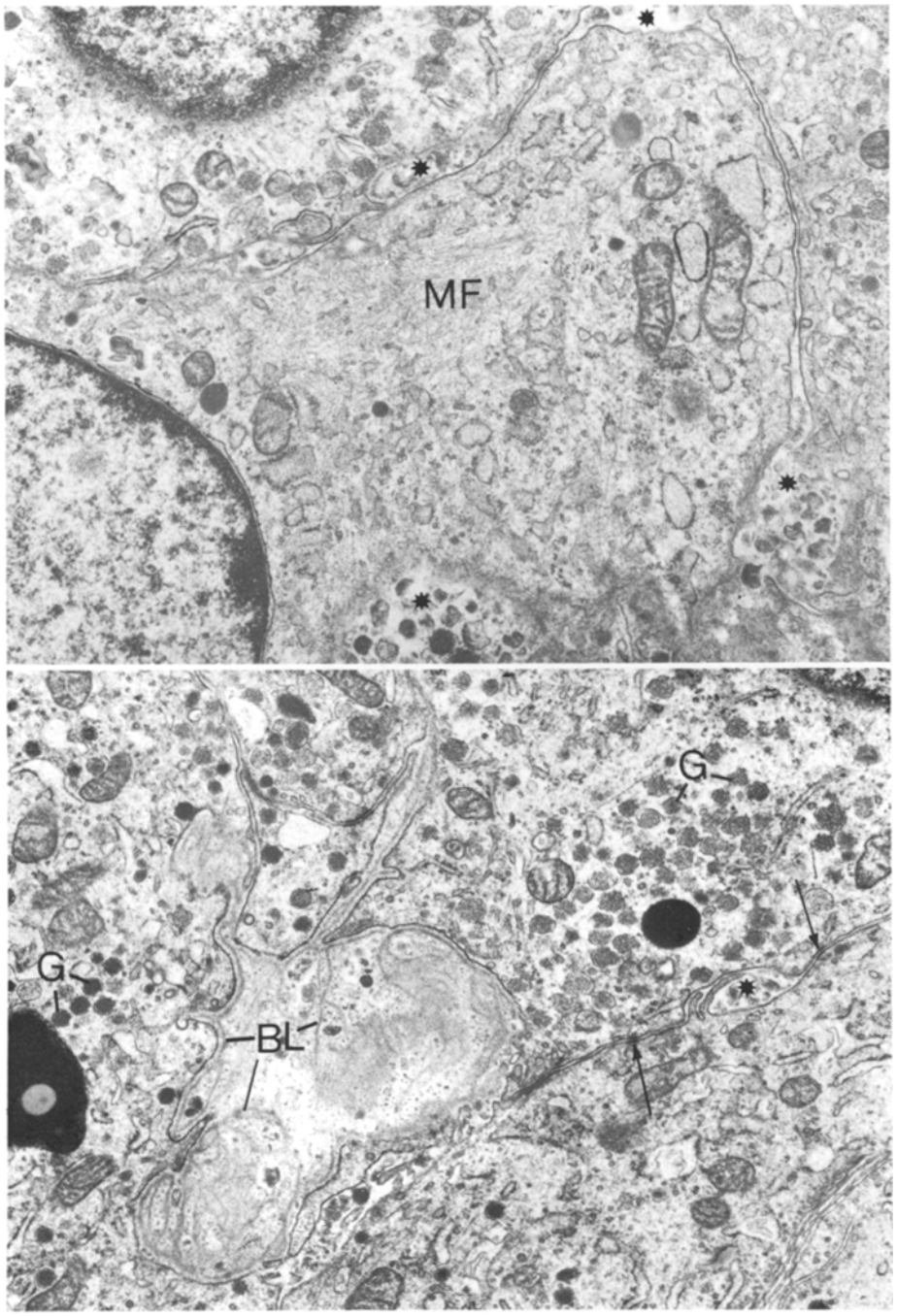


Fig. 5. Case 2. Neoplastic C-cell with aggregated microfilaments (*MF*) within the cytoplasm. The secretory granules are few in number. Several intercellular spaces (*) can be seen between adjoining tumor cells. The latter extend cytoplasmic microvillous-like processes into the intercellular spaces which contain, in addition, extruded secretory granules. × 14,000

Fig. 6. Case 2. Intercellular space between neoplastic C-cells showing coiled basal lamina (*BL*). The membrane bound secretory granules (*G*) of the adjoining tumor cells reveal variations in electron density and diameter. Two desmosomes (*arrows*) limit an intercellular space (*) which contains cytoplasmic microvillous-like processes of the adjoining neoplastic C-cells. × 12,000

electron density of the granules varied considerably. The smaller granules were usually more electron-dense than the larger ones (Fig. 6). Their number differed from cell to cell, being scanty in some cells and more or less abundant in others.

The cytoplasmic Golgi apparatus, mitochondria, microfilaments and microtubules mostly corresponded to those in normal human and other mammalian C-cells (Teitelbaum et al., 1971; Welsch, 1972). The differences between cytoplasmic organelles in normal and neoplastic C-cells seemed to be quantitative rather than qualitative. The ergastoplasm was usually less organized than in normal C-cells. The neoplastic counterpart seldom exhibited a well-developed, ribosome-studded ergastoplasm, stacked in a characteristic lamellar fashion that is typical of normal C-cells. The nuclei were moderately pleomorphic and showed membrane indentations. The nucleoli were irregularly-shaped and sometimes prominent, when compared to normal C-cells.

In some neoplastic cells, the cytoplasmic areas displayed numerous microfilaments (Fig. 5). These cells usually possessed a few secretory granules and frequently revealed irregular cytoplasmic processes extending between neighboring tumor cells. Some tumor cells exhibited irregular zones of cytoplasmic degeneration with focal discontinuities of the plasma membrane, partial disappearance of cytoplasmic organelles and the appearance of finely fibrillar material lacking the characteristic cross-hatched pattern of amyloid fibrils. Secretory granule alterations were observed in the vicinity of these areas. The granules seemed to be larger than normal, and had a weaker electron density, with discontinuities in their membranes. The fibrillar content of these granules frequently resembled the fibrillar material in the degenerative areas. Cells with such focal cytoplasmic degeneration, as well as entirely necrotic cells, were seen between preserved tumor cells and adjacent to the stroma. Completely dissolved tumor cells were infrequently noted within the stroma, as shown by the presence of randomly-distributed, partially-degenerated secretory granules intermingled with collagen fibers and amyloid fibrils. Elongated, spindle-shaped cells with morphologic characteristics of fibroblasts were observed within the stroma. They contained numerous longitudinal microfilaments near the plasma membrane and were surrounded by collagen fibers and amyloid fibrils arranged in a typical cross-hatched pattern.

In patient 5, fragments of thyroid tissue adjacent to the MTC revealed marked C-cell proliferation within thyroid follicles – this observation was also made light microscopically and in semithin sections. The C-cells, usually well-differentiated with numerous secretory granules, a well-developed ergastoplasm and a prominent Golgi apparatus, formed several layers at the base of the follicles. In the central portion of the follicles, the proliferating C-cells were partially covered by flattened follicular cells with microvilli. Desmosomes were present not only between C-cells but also between C-cells and follicular cells. In some areas of the follicles, the C-cells had direct contact with the colloid (Fig. 7), which contained a few desquamated and necrotic cells, follicular cells and C-cells. In other areas, the C-cell proliferation extended centrally in the direction of the colloid, and a pseudofollicular pattern could be observed with colloid sequestered between the proliferating C-cells (Fig. 8). These follicles

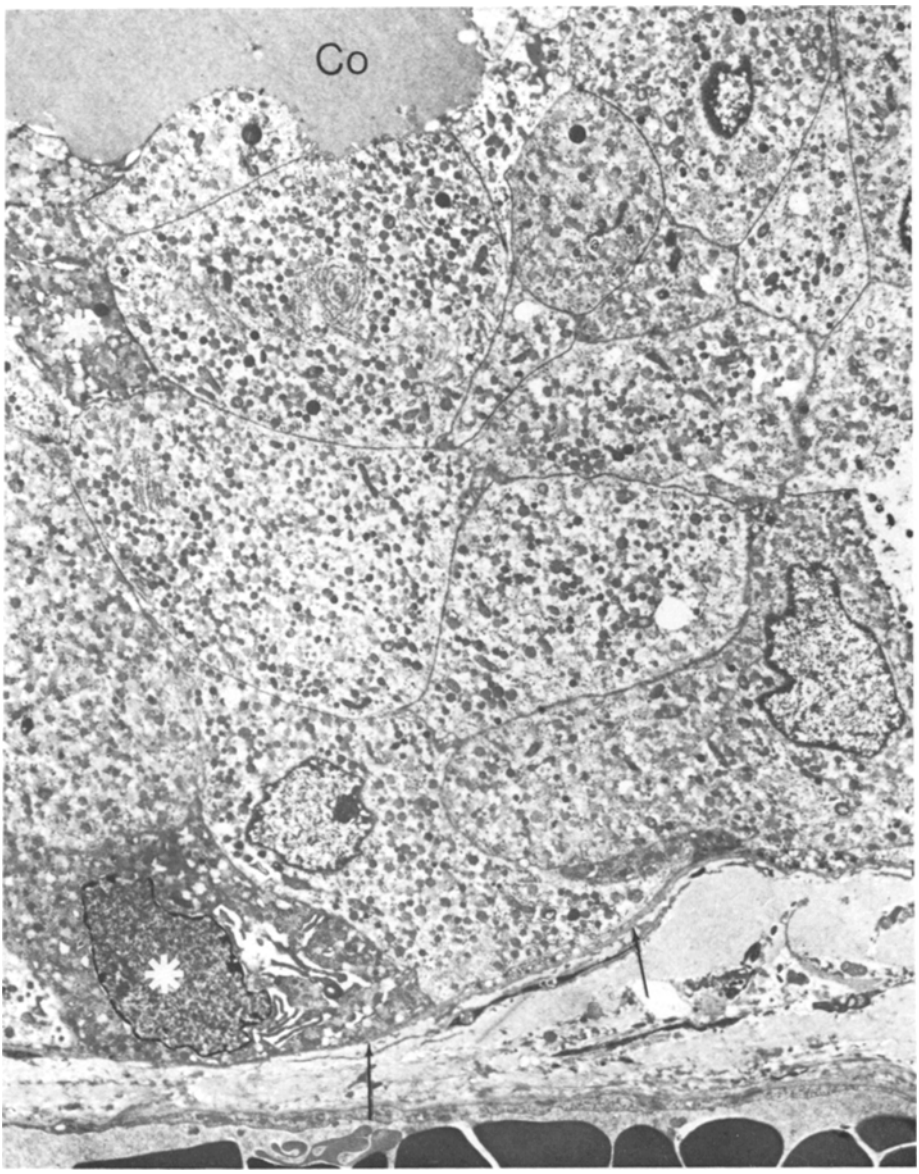


Fig. 7. Case 5. Part of a thyroid follicle with a high degree C-cell proliferation. Proliferating C-cells form several layers and are in direct contact with the colloid (*Co*). The cytoplasmic differentiation of the C-cells seems preserved, i.e. numerous secretory granules and a well developped rough surfaced endoplasmic reticulum are present within the cytoplasm. The nuclear membrane in some C-cells is irregular in outline. Two necrotic C-cells (*) are visible. There is no invasion of the follicular capsule (*arrows*). ×3700

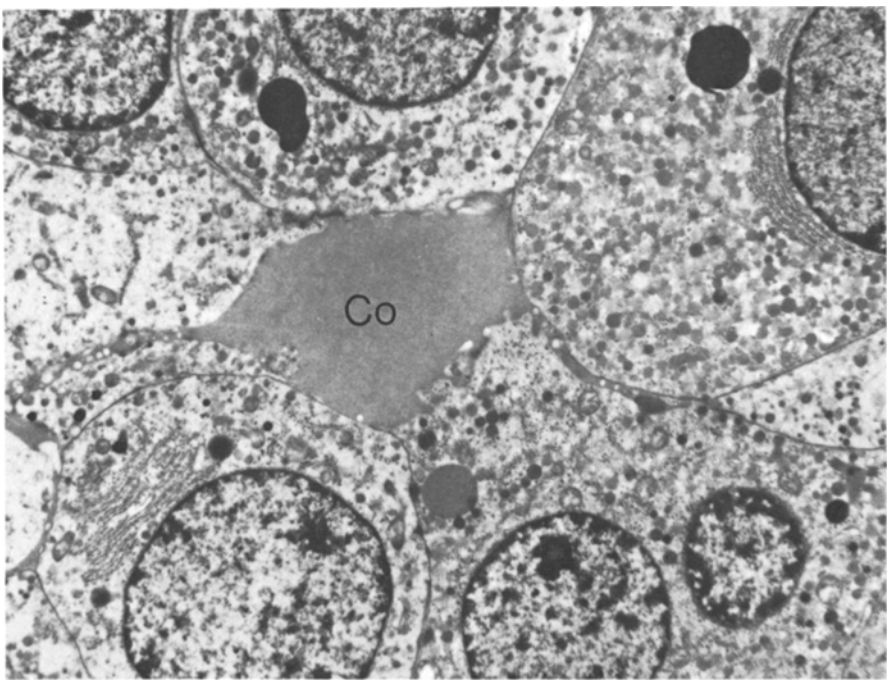


Fig. 8. Case 5. Several C-cells surround sequestered colloid (*Co*) forming a pseudofollicle in a thyroid follicle with high degree C-cell proliferation. ×4500

became tortuous with infoldings of the capsule, which gave them an irregular form. The nuclei of proliferating C-cells showed some pleomorphism, as compared to normal C-cells, but the cytoplasmic differentiation seemed to be preserved (Figs. 7 and 8). Serial sections of a follicle with marked C-cell proliferation revealed, at two different points, morphologic evidence of ultramicroinvasion of the basal lamina (Babaï, 1976). At these points, the C-cells were less differentiated, had fewer secretory granules and contained numerous microfilaments. The nuclei were irregularly-shaped. Cytoplasmic extensions of C-cells across the basal lamina, which became discontinuous, entered into direct contact with collagen fibers of the capsule and the stroma (Fig. 9).

Ultracytochemical Study

Further information was obtained from GMA-embedded, PTA-stained sections of MTC and thyroid tissue, taken from patients 5 and 6. C-cells were easily identified in sections of thyroid tissue stained with PTA at pH 0.3 (Fig. 10). The cell coat was clearly visible, and the secretory granules displayed a strikingly-reactive dense core. The intensity of the PTA stain varied within the granule population of a single cell (Fig. 10). The vesicles had a greater diameter, an

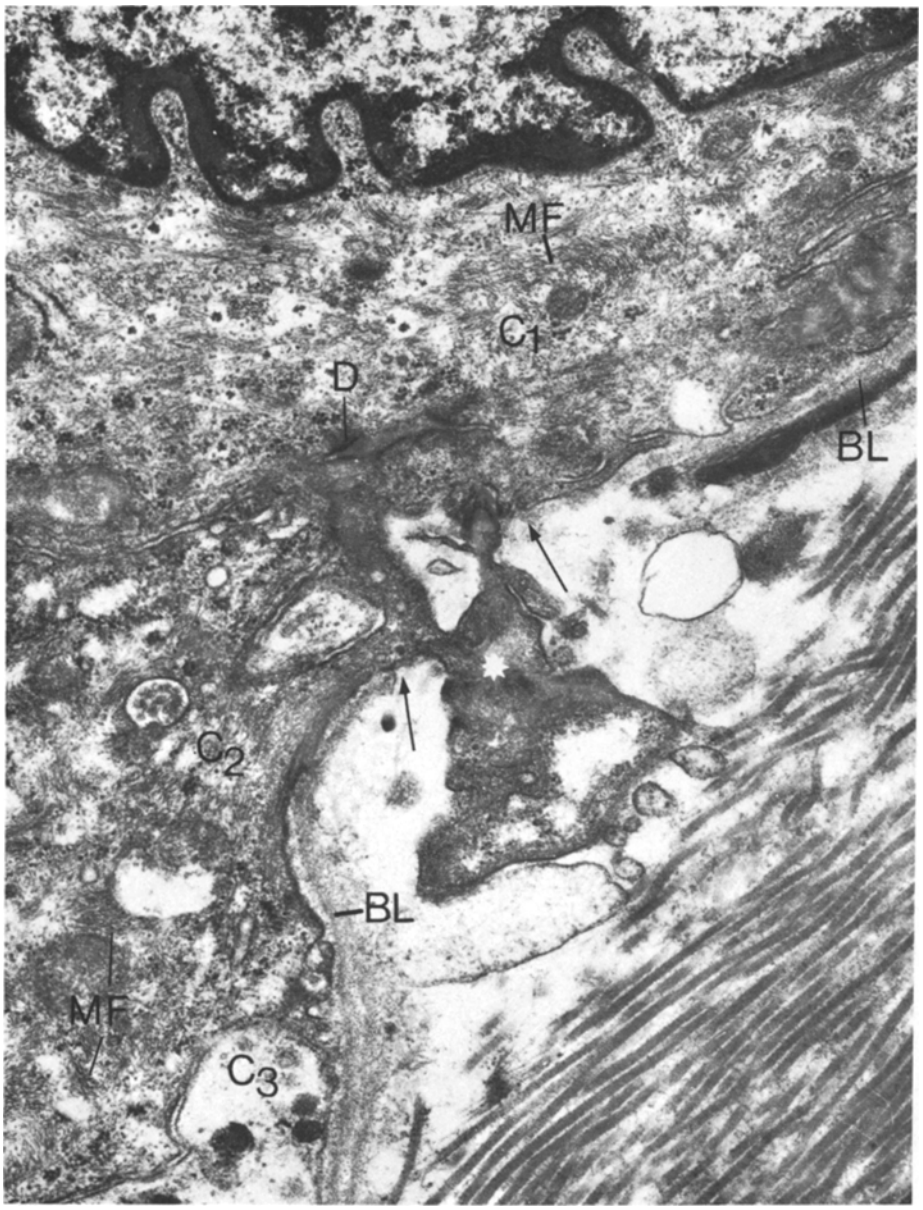
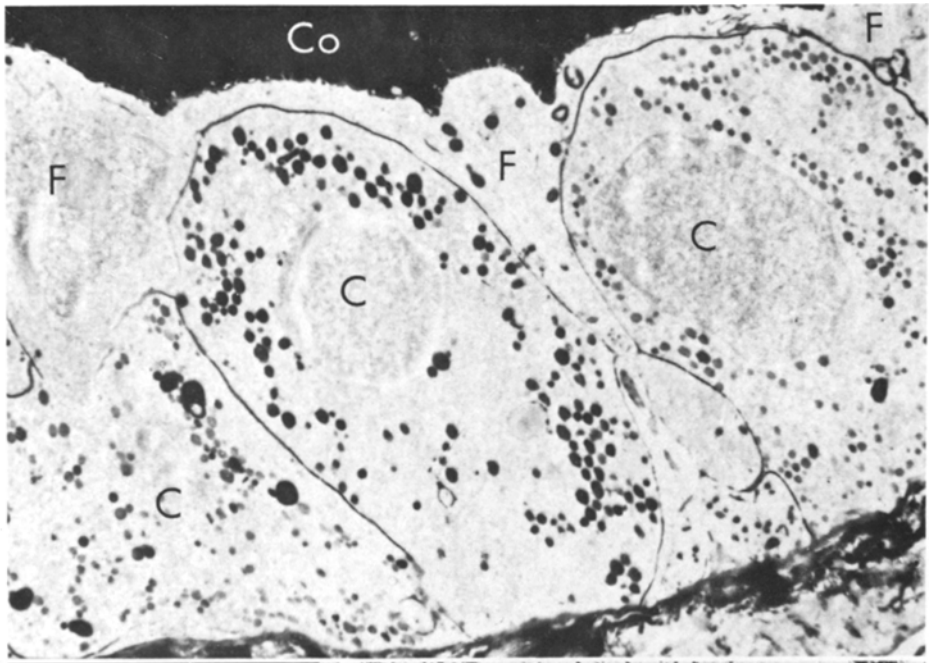


Fig. 9. Case 5. Follicle with high degree C-cell proliferation. Three C-cell (C1, C2, C3) are connected together by desmosomes (*D*) at the base of the follicle. Numerous microfilaments (*MF*) but only a few secretory granules are present within the cytoplasm. C2 extends a cytoplasmic process (*) across the basal lamina (*BL*) which is discontinuous between the two arrows (ultramicroinvasion). The cytoplasmic process of C2 is in direct contact with collagen fibers of the follicular capsule. ×24,000



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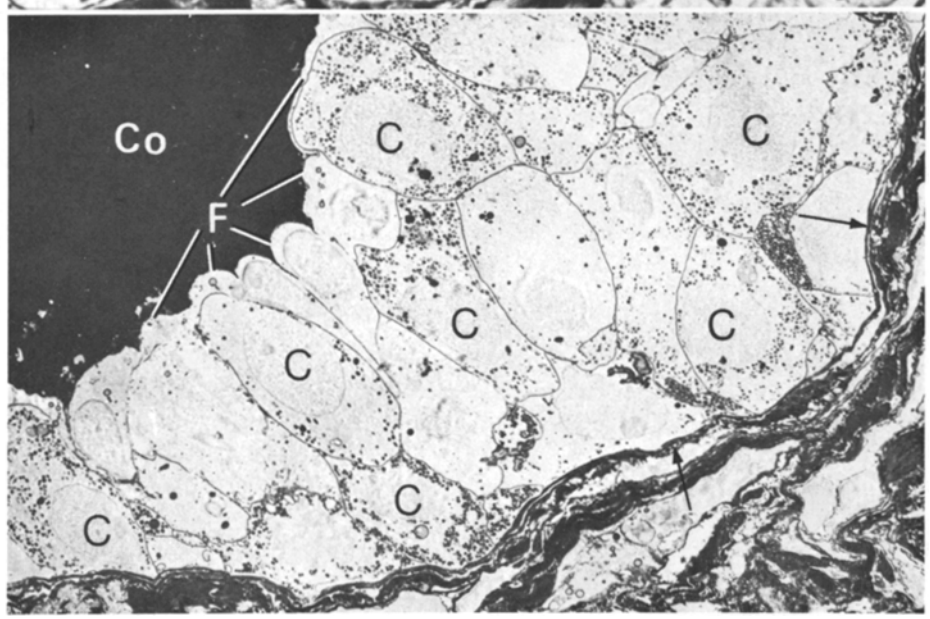

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Figs. 10–12. Ultracytochemical study of GMA-embedded sections stained with PTA (pH 0.3)

Fig. 10. Case 6. Three C-cells (*C*) in a parafollicular position within a thyroid follicle. They are covered by follicular cells (*F*) and are not in contact with the colloid (*Co*). Numerous PTA-positive secretory granules are present within the cytoplasm of the C-cells. × 5500

Fig. 11. Case 6. Thyroid follicle with several layers of proliferating C-cells (*C*) which are covered by a continuous layer of follicular cells (*F*). The C-cells are not in contact with the colloid (*Co*). They contain PTA-positive secretory granules within the cytoplasm. Their number varies from one C-cell to the other. The capsule (arrows) of this follicle and the cell coat of C-cells and follicular cells are also stained with PTA. × 1300

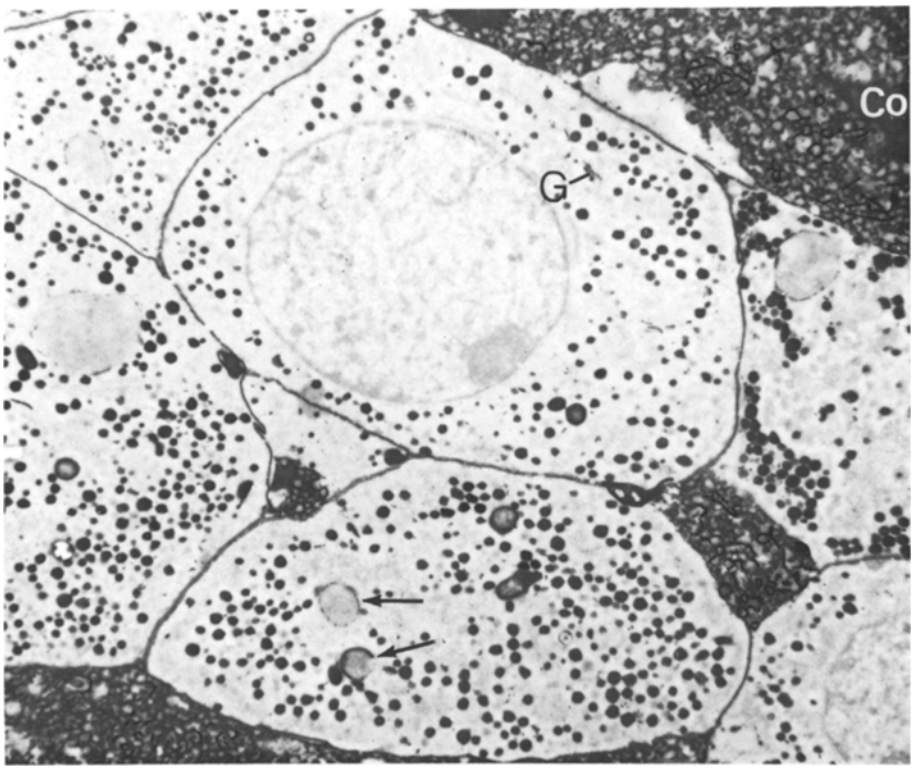

Fig. 12. Case 6. Several neoplastic C-cells from a small tumor nodule. The intensity of the PTA stain varies greatly within the granule population. The Golgi apparatus (*G*) is also PTA-positive. In addition, vesicles with a greater diameter are visible showing a PTA-positive periphery (*arrows*). These vesicles most likely represent lysosomes. Between the neoplastic C-cells, PTA-positive homogeneous colloid-like material (*Co*) and nonhomogeneous granular material are visible. × 5000

unstained core and a strongly-stained, ring shaped periphery. They probably corresponded to lysosomes. The Golgi apparatus became apparent after PTA staining of appropriate sections. The other cytoplasmic organelles remained unreactive to PTA at pH 0.3.

The C-cells were localized between follicular cells (intrafollicular position) as well as between follicular cells and the basal lamina of the capsule.

In PTA-stained sections, the follicular cells showed an irregular, apical cell coat with numerous microvilli. The innermost portion of the cytoplasm contained intensely-stained vesicles, usually much smaller than the secretory granules of C-cells, as well as larger vesicles with an unstained core and a strongly-reactive periphery. The larger vesicles, probably representing lysosomes, were surrounded by the smaller ones, which most likely corresponded to endocytosed colloid. The follicular basal lamina and the colloid itself were significantly PTA-positive.

Sections from patient 6 revealed marked C-cell proliferation within the follicles. Several layers of C-cells at the base of the follicle were entirely covered

by flattened follicular cells (Fig. 11). The proliferating C-cells never came in contact with the colloid. Occasionally, granular, PTA-positive material was apparent between the C-cells, and this probably represented necrotic cellular debris or extruded secretory granules.

The number of secretory granules varied greatly from one cell to another within the MTC nodules (Fig. 12). Granular material, stained intensely by PTA, was visible between the neoplastic cells (Fig. 12). It was frequently associated with partially-degenerated tumor cells. In addition, the basement membrane-like material between neoplastic cells was similar to that observed by conventional techniques. Occasionally, the PTA stain revealed a homogeneous material which in all probability represented sequestered colloid.

Discussion

The present study showed convincingly an association between multifocal C-cell hyperplasia and familial MTC in 5 out of 6 patients at the light microscopic level. To comprehend fully the morphogenesis of this disease, it is necessary to know the exact topographic relationship of C-cells and thyroid follicles. Light microscopically, clustered and individual C-cells are found in intrafollicular, parafollicular and interfollicular positions (Roediger, 1973). Electron microscopically, however, single and clustered proliferating C-cells are limited to thyroid follicles, are enclosed by follicular basal lamina, and are seen in intrafollicular and parafollicular positions.

Thyroid follicles are frequently irregular in shape with extensions of various length. In accordance with the findings of Klinck et al. (1970), we noted that the basal surface of the epithelial lining of follicles was often irregular, with finger-like projections, surrounded by a continuous basal lamina. The apparent interfollicular position of C-cells could be explained by the incidence of the sections. Thus, parts of a follicle may appear to be separated from the main portion. Through electron microscopic studies of human fetal thyroids at various gestational periods, Chan and Conen (1971) found that C-cells migrate from the ultimobranchial body to the follicles and that they occupy definite parafollicular and intrafollicular positions. It is well-known that, under normal conditions, C-cells never come in contact with the colloid of thyroid follicles. The cytoplasmic portion of follicular cells covering the C-cells may be thin enough to make it impossible to ascertain their intrafollicular or parafollicular position by light microscopy.

Our light and electron microscopic study was focused on the morphogenesis of familial MTC. Therefore, we were principally concerned with the peripheral portions of the carcinomas and the adjacent thyroid tissue in the deep regions of the lateral lobes, where C-cells are normally concentrated (Wolfe et al., 1975). Based on our observations, we believe that the following morphogenic sequence occurs in the development of familial MTC. The disease seems to begin as *multifocal C-cell proliferation,* limited at first to thyroid follicles, forming multiple layers between the follicular capsule and the follicular epithelium. Proliferation appears to be more aggressive in some follicles, and the proliferating C-cells

enter in direct contact with the colloid and extend to the follicular center, achieving an in situ carcinoma stage. The probability of this phase is suggested by the observed ultramicroinvasion of the follicular capsule and is documented by the invasive potential of proliferating C-cells (Babaï and Tremblay, 1972; Babaï, 1976). Finally, multiple microscopic MTC appear and conglomerate.

It would be tempting to compare this morphogenic sequence with that of carcinomas in the uterine cervix. However, the biological behavior of endocrine tumors is virtually impossible to predict by morphologic criteria alone. We did not generally observe constant morphologic criteria for dysplasia or neoplasia among the proliferating C-cells limited to thyroid follicles, when compared with normal or even neoplastic C-cells. For these reasons, a hyperplastic or dysplastic (premalignant) process preceding MTC, as proposed by Wolfe et al. (1973), cannot be clearly distinguished from a neoplastic process. Our study suggests that a light microscopic, apparently hyperplastic process may be a malignant one. In the absence of cytologic criteria indicating a benign or malignant neoplastic process, it is theoretically impossible to distinguish between benign and malignant neoplastic cells, except when ultramicroinvasion or cellular invasion is observed.

Although the ultrastructural features of MTC are well-known (Alborez-Saavedra et al., 1964; Gonzales-Lica et al., 1968; Meyer, 1968; Ibanez, 1974; Normann et al., 1976), we need to emphasize the different patterns of intercellular spaces lined by tumor cells. It becomes clear from our observations that a pseudofollicular pattern frequently emerges in early developing MTC. The neoplastic C-cells in the nascent MTC extend to the center of the follicle, surrounding sequestered colloid. Even in the more advanced stages, that is, in small tumor nodules, pseudofillicles are occasionally observed. Freeman and Lindsay (1965) stressed the intrafollicular growth tendency of MTC as it invades adjacent thyroid follicles at the light microscopic level. Our ultrastructural findings, however, support a multifocal origin of MTC within thyroid follicles and subsequent confluence of the nascent tumor nodules. Other intercellular spaces are found between tumor cells and contain extruded secretory granules, cellular debris as well as colloid-like material. The adjoining tumor cells exhibit cytoplasmic microvillus-like processes and form junctional complexes in the vicinity of these canalicular spaces. Finally, a basal lamina-like material is frequently seen between tumor cells and it represents an invagination of the basal lamina surrounding tumor cell clusters.

Amyloid seems to be a product of tumor cells according to most investigators (Alborez-Saavedra et al., 1964; Meyer, 1968; Meyer et al., 1973; Ibanez, 1974), but Lietz and Donath (1970) suggest that it may originate from a stromal cell resembling a smooth muscle cell. Our results indicate that amyloid is associated with tumor cell degeneration, as was observed by Ibanez (1974). Contrary to the findings of Wolfe et al. (1973), we were unable to detect amyloid within the multiple foci of C-cell proliferation limited to thyroid follicles and in the samll tumor nodules.

Kalina and Pearse (1971) showed that the antibody to calcitonin attaches to the secretory granules, indicating that at least the antigenic sites of this polypeptide hormone are present in the granules. Our ultracytochemical study with PTA at a low pH revealed that the dense cores of the granules contained an intensely-stained material. It is thought by many investigators that polysaccharides and glycoproteins are stained in aldehyde-fixed tissues by PTA at a low pH (Pease, 1966; Marinozzi, 1967; Rambourg, 1967; Babaï and Bernhard, 1971). We can only conclude that the secretory granules of normal, proliferating

and neoplastic C-cells contain polysaccharides and/or glycoproteins. McDowell et al. (1976) noted PTA-staining of dense core granules present in small granule cells, carcinoid cells and oat cell carcinomas of the bronchus. The granules of adrenal medullary A-cells were also found to ebe stained with PTA at a low pH (Cantin and Benchimol, 1975), whereas the granules of N-cells were shown to remain unreactive. Our results indicate that another member of the APUD series – the C-cells – also contain polysaccharides.

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