Virchows Arch. Abt. A Path. Anat. 356, 281—292 (1972)

Medullary Cancer of the Thyroid Gland and Its Possible Relations to Carcinoids

An Ultrastructural Study

E. Horvath, K. Kovacs, and R. C. Ross

Department of Pathology, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada

Received April 4, 1972

Summary. The ultrastructural study of a medullary cancer of the thyroid gland revealed some unusual features. The stroma of the tumor did not contain amyloid. An abundant amount of intracytoplasmic microfilaments was observed in the tumor cells, sometimes displacing the various organelles. The most prominent feature was the presence of very dense, pleomorphic ("biconcave") secretory granules, measuring an average of 250 mμ in their longest diameter. Mainly because of the type of secretory granules, this tumor showed a peculiar resemblance to ileal carcinoids. The possible significance of this finding is discussed.

Introduction

Medullary carcinoma of the thyroid gland was separated as an entity in 1959 by Hazard *et al.* The histogenesis, or possible hormonal secretion of this tumor were not entirely clear, and these questions have aroused considerable interest. In 1966, Williams and Williams *et al.* (1966) suggested that the tumor derived from the parafollicular cells which are believed to be responsible for calcitonin production. This hypothesis has been supported by the fact that some medullary thyroid cancers were capable of secreting calcitonin (Grimley *et al.*, 1969; Tubiana *et al.*, 1970). In other cases, instead of calcitonin, other hormones or hormone-like substances such as ACTH (Szijj *et al.*, 1969), serotonin (Moertel *et al.*, 1965) and prostaglandins (Sandler *et al.*, 1968) were produced.

The first electron microscope study of a medullary thyroid cancer was published in 1964 by Albores-Saavedra *et al.* These authors were dealing with the problem of amyloid production in these tumors and did not extend their examinations regarding possible hormone secretion. From 1968 onwards, however, several case reports appeared describing in detail the ultrastructural characteristics of this tumor including intracellular secretory granules indicative of hormone production (Braunstein, 1968; Gonzalez-Licea *et al.*, 1968; Meyer, 1968; Grimley *et al.*, 1969; McDermott and Hart, 1970; Lietz and Donath, 1970; Hachmeister and Zimmermann, 1970). The individual cases showed considerable differences rendering it impossible to generalize concerning electron microscopical characteristics of the tumor. Thus it appears that detailed analysis of additional cases might help to further elucidate the morphogenesis of this neoplasm.

In our case of medullary thyroid cancer an electron microscopic study was undertaken which revealed some unusual features: 1. lack of amyloid, 2. abun-

dance of intracytoplasmic microfilaments, 3. "biconcave" secretory granules, 4. close resemblance to ileal carcinoid tumors.

Case Report

This 22 year old female patient experienced a choking sensation and dysphagia. She noticed a lump in her neck. On scanning, the nodule did not show any evidence of I^{131}-uptake; the patient was euthyroid. Pre- and post-operative calcium levels were within the normal range (9.1, 9.9, 10.1 mg-%). There were no endocrine symptoms present. Calcitonin assay and 5-HIAA determination were not carried out.

During surgery the histological diagnosis of medullary carcinoma was established by frozen section and total thyroidectomy performed. The post-operative course was uneventful and she was discharged one week following surgery.

Methods

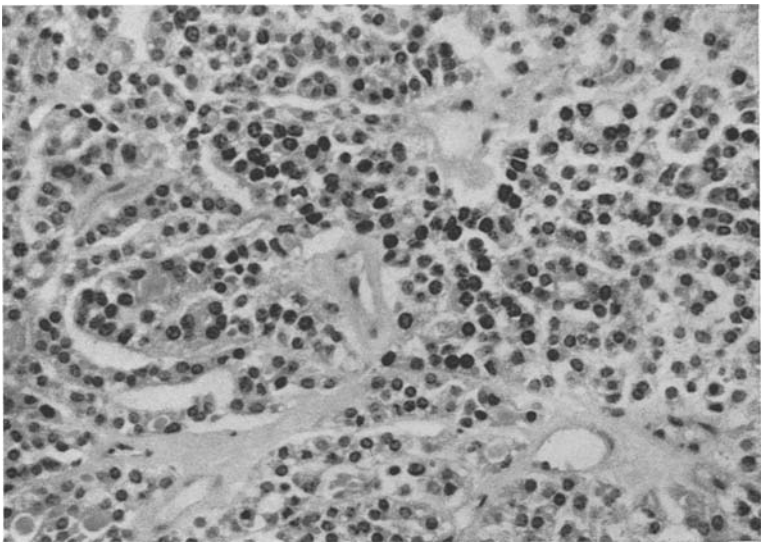
For light microscopic examination several pieces, including the tumor, adjacent thyroid and parathyroid tissue, were placed in formalin and post-fixed in Brasil routinely used in this laboratory. The blocks were dehydrated and embedded in paraffin. The following staining procedures were used: hematoxylin-phloxine-saffron, crystal violet, congo red, thioflavin-T, alcian blue, toluidine blue-polarized light method (Wolman, 1971), Masson's trichrome, PAS (with and without diastase digestion), mucicarmine, cresyl fast violet metachromasia (for the detection of C-cells) (Ljungberg, 1970a), argentaffin reaction (Fontana), Gomori's silver technique for argyrophilia, lead-hematoxylin (Solcia *et al.*, 1969) and masked metachromasia (Solcia *et al.*, 1968).

For electron microscopy, samples of tumor tissue were placed in 2.5% glutaraldehyde in 0.1 M Sorensen's buffer, minced into tiny pieces and fixed for 2 hours at 4°C. Following rinsing in buffer, the blocks were post-osmicated in 1% OsO^4 in Millonig's buffer. After washing in buffer, the tissue was processed through graded ethanol and propylene oxide, and then embedded in Epon 812. From the blocks, 0.5 μ thick sections were cut, stained with toluidine blue and examined under the light microscope. From selected areas, ultrathin sections were cut with a glass knife, stained with uranyl acetate and Reynolds' lead citrate. These sections were then studied with a Philips 300 electron microscope using 60 kV accelerating voltage.

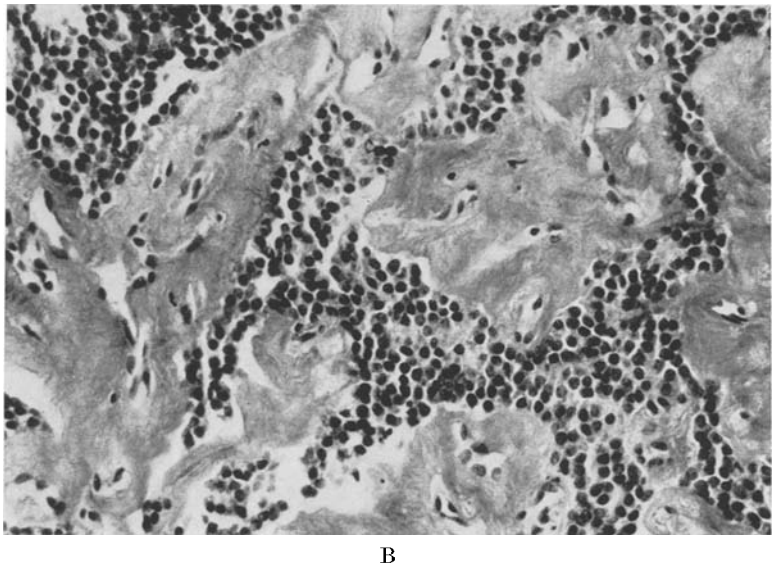
Light Microscopic Findings

The tumor was partly encapsulated and composed of sheets and solid nests of tumor cells separated either by thin fibrous septa (Fig. 1 A) or broad hyalin-collagenous stroma (Fig. 1 B). The tumor cells were rather uniform and exhibited a spherical shape. The cytoplasm was finely vacuolated. The nuclei were round, centrally located and moderately rich in chromatin. Mitoses were infrequent and pleomorphism was not marked. The amount of connective tissue stroma varied markedly in different parts of the tumor. In the more solid areas the neoplasm bore some resemblance to a carcinoid tumor.

The cytoplasm of the tumor cells contained a variable amount of diastase-digestible PAS-positive granular material. Intense argyrophilia was noted in a large percentage of the tumor cells. This change was also observed in several parafollicular cells of the adjacent normal thyroid. The results of argentaffin reaction, Ljungberg's method for C-cells, lead-hematoxylin and masked metachromasia were negative. However, this negativity is of dubious significance since the fixative containing picric acid, trichloracetic acid and ethanol makes the results rather uncertain.



A



B

Fig. 1 A and B. Histologic appearance of the medullary thyroid cancer. Solid part (A), tumor cell nests with an abundant connective tissue stroma (B). A Hematoxylin-Phloxine-Saffron; B Masson

The stroma stained blue with Masson's trichrome. The results of staining procedures applied for the detection of amyloid (crystal violet, congo red, thioflavin-T, toluidine blue-polarized light method) were negative.

Electron Microscopic Findings

Similar to the light microscopic picture, two different patterns of the tumor were recognized. In one region, small islets and a few rosettes of tumor cells were separated by broad bands of hyalin collagenous stroma. In other areas, only narrow septa of connective tissue were found between solid nests of tumor cells.

The part of tumor rich in hyalin collagenous stroma was composed of three somewhat different types of cells (types 1, 2, and 3 cells), the more solid part consisted of one ultrastructurally uniform cell type (type 4 cells).

Type 1 Cells

These tumor cells had relatively large, round or slightly oval, centrally located nuclei with prominent nucleoli. The moderately stained chromatin substance was evenly distributed or slightly clumped on the surface of the inner nuclear membrane. The rough surfaced endoplasmic reticulum (RER) was poorly or moderately developed within the scanty cytoplasm. However, a great number of ribosomes and polysomes were lying free without preference for any particular localization. The smooth surfaced endoplasmic reticulum (SER) was inconspicuous; only a few smooth walled vesicles were seen. The rod-shaped or oval mitochondria were moderate in number. They exhibited wavy or slightly ruffled limiting membranes, moderately dense matrix and closely spaced transverse cristae. Occasionally larger, more electron lucent mitochondria, showing signs of swelling, were also observed. The inconspicuous Golgi zones, when they were encountered, were composed of 3–4 flattened sacs and a few vesicles. Many cells of type 1 were completely devoid of secretory granules. In others, only a few pleomorphic dense granules were observed. (A detailed description of these structures will be given under the heading: Type 4 Cells.) Occasionally spherical secretory granules were observed. They had a moderately dense core and a strongly osmiophilic membrane with a halo between them and had an average diameter of 200 mμ. These granules were not seen in other cell types. Few lysosomal bodies were noted. The most characteristic feature of the type 1 cells was the presence of a large amount of fine microfilaments measuring 60 Å (50–75 Å) in width. They were usually arranged in parallel bundles and localized predominantly in perinuclear areas, although they were encountered also in other parts of the cytoplasm, sometimes displacing organelles. The microfilaments did not exhibit periodicity; however, using high power magnification, they showed a somewhat beaded appearance. All tumor cells had distinct cellular membranes which occasionally formed intercellular spaces, some of them showing microvillous projections between the neighboring cells. Complex interdigitations and desmosomes were regular features. Groups of tumor cells were surrounded by a well defined basement membrane which delimited the tumor cells from the connective tissue stroma (Fig. 2).

Type 2 Cells

In some regions, the tumor cells tended to form rosettes or follicle-like structures with a finely granular material in their "lumen". The nuclei of these cells were oval or slightly irregular. The relatively dark chromatin substance was clumped against the nuclear membrane. Nucleoli were prominent. The RER

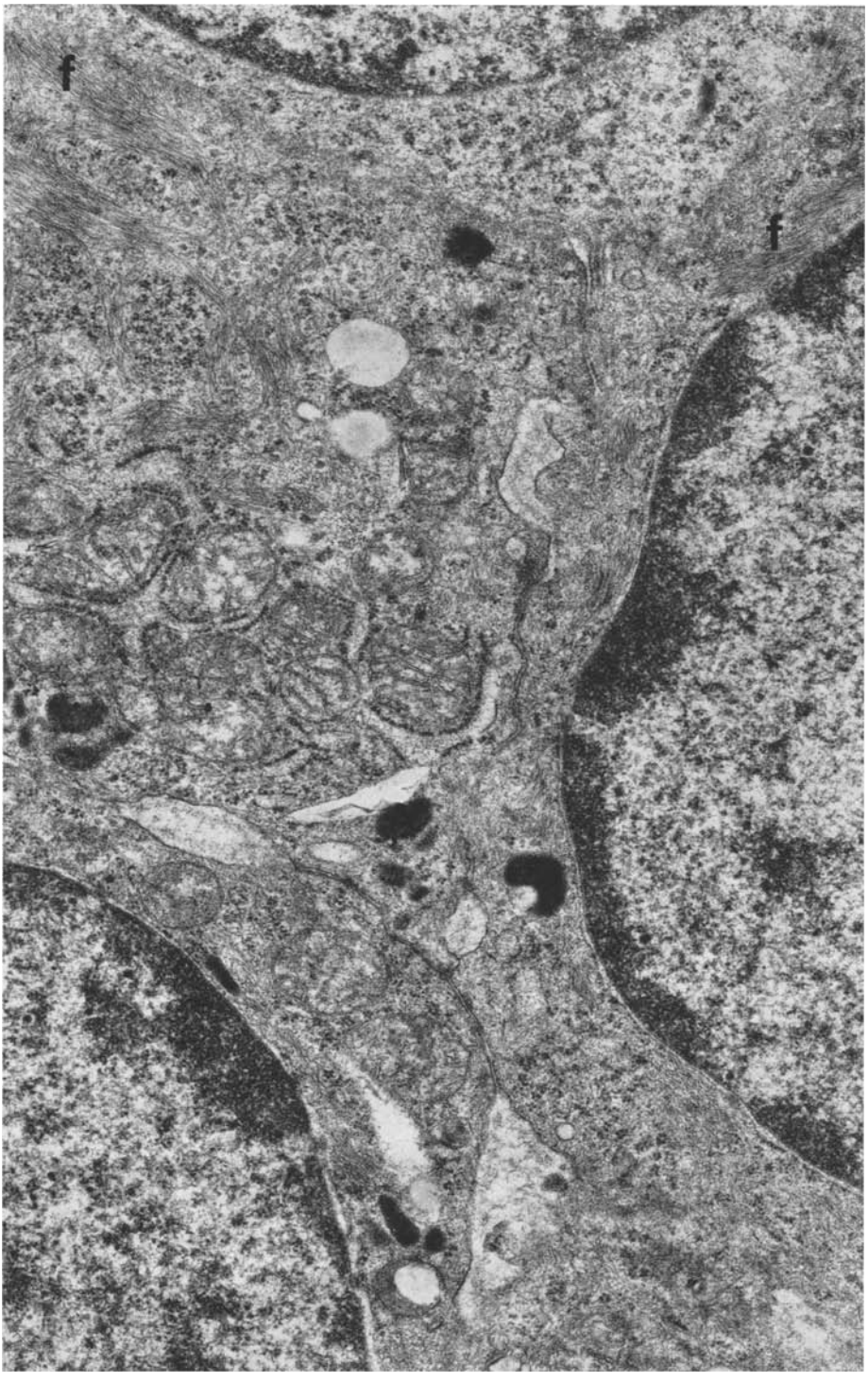


Fig. 2. Ultrastructural appearance of a type 1 tumor cell. Note several bundles of microfilaments (*f*) in the perinuclear region and among the cytoplasmic organelles. ×19600

exhibited cystic dilation in many of these cells but the amount of free ribosomes was as abundant as in type 1 cells. The SER was insignificant. The matrix of the oval or long-shaped mitochondria was relatively electron lucent. In a few mitochondria, rarefication, swelling and formation of membrane-bound empty vacuoles were observed. Golgi zones were well developed. A moderate number of strongly osmiophilic pleomorphic secretory granules, scattered throughout the cytoplasm, were also seen. The abundance of microfilaments was also a prominent feature in this cell type. The cell borders were similar to that described in the type 1 cells, except that here well developed terminal bars were also observed between the surface of cells facing the "lumen" of rosettes.

Type 3 Cells

In this cell type, seen only infrequently, the main feature was the presence of monoparticulate glycogen in abundant amount, occupying a considerable portion of the cytoplasm. The RER was inconspicuous and consisted only of a few dilated cisternae located usually around the small, round mitochondria, the number of which seemed to be less than that in the other cell types. Golgi zones were distinct and well developed. The amount of intracytoplasmic microfilaments, detected in the perinuclear areas and amongst the clumps of glycogen, was considerably less than in the first two cell types. Dense pleomorphic secretory granules were occasionally seen. Cell borders showed the same characteristics as in the type 1 cells (Fig. 3).

Type 4 Cells

This type of cell constituted the basic cell composing the solid area of the tumor. These cells were polygonal in shape and closely apposed. The nuclei were relatively large, round or oval, and showed irregular rarefications with clumping of their chromatin. Nucleoli were large and dense. The RER was represented by a few moderately dilated rough surfaced cisternae and widely dilated tubules. A great number of free ribosomes and polysomes were also observed. The SER was poorly developed. There were many round and oval mitochondria present, showing considerable variation in size. Their matrix was moderately dense with irregularly arranged and shaped cristae. The majority of the mitochondria were small. However, a few large relatively lucent mitochondria were also found having long parallel cristae. Swelling and granular inclusions in a few mitochondria were also seen. The distinct Golgi zones appeared to be normal. A great number of pleomorphic ("biconcave", Ferreira, 1971) secretory granules, having an average longest diameter of 250 mμ (118–503 mμ), were observed in the cytoplasm of these cells. They were usually arranged in large groups located at one pole of the tumor cells. Their matrix was homogenous and uniformly dense. Closely fitted limiting membranes were ill-defined, and sometimes could not be seen. Occasionally less dense granules were present with well recognizable membranes. These cells also contained a moderate amount of glycogen, mainly of the monoparticulate form. Bundles of microfilaments were also encountered, although their presence was not as prominent as in the types 1 and 2 cells. The cell borders were similar to those found in the other cell types (Fig. 4).

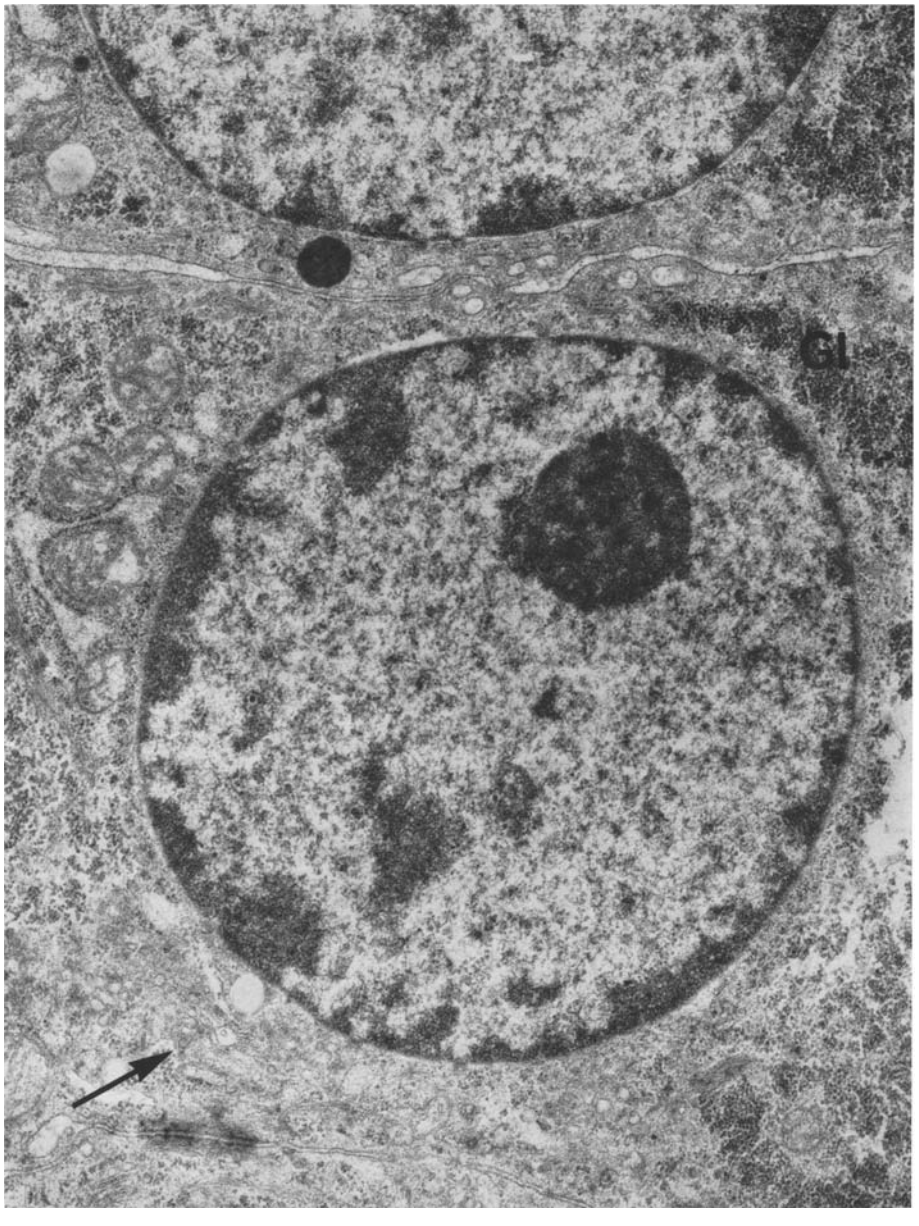


Fig. 3. Type 3 cell exhibiting large clumps of glycogen (*Gl*) in the cytoplasm. Only a few microfilaments are seen, the Golgi apparatus (arrow) is conspicuous. ×18600

Stroma

The bulk of the stroma was composed of a very fine fibrillar-granular material with varying amount of collagen fibers. Fibrils showing the size and morphologic characteristics of amyloid were not found.

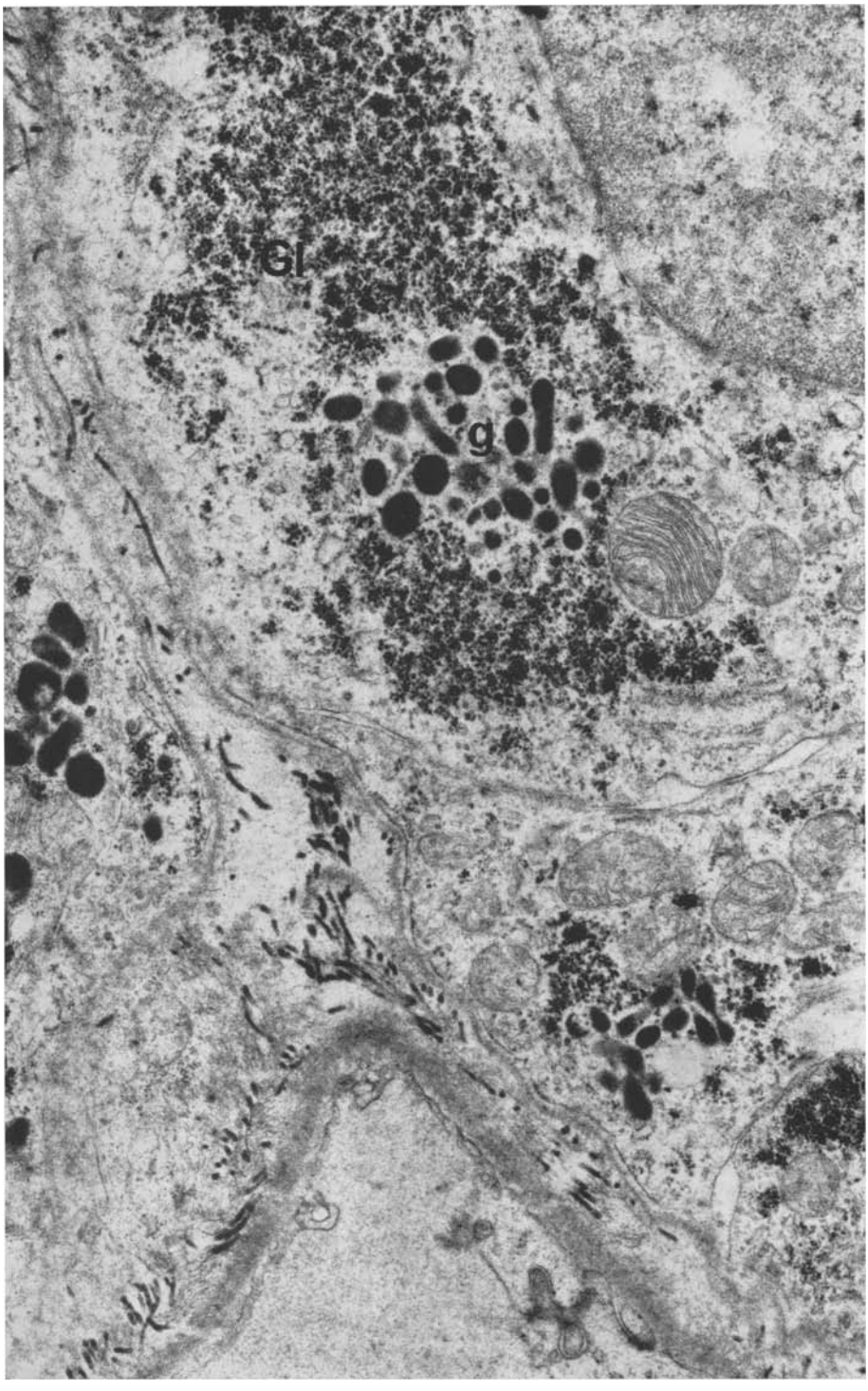


Fig. 4. Vascular poles of type 4 cells showing clusters of "biconcave" granules (*g*). There are also clumps of glycogen (*Gl*) present. ×19200

Discussion

The main ultrastructural characteristics of medullary cancer of the thyroid are an amyloid stroma and the presence of spherical secretory granules or vesicles with an average diameter of 200 mμ in the cytoplasm of the tumor cells (Albores-Saavedra *et al.*, 1964; Black, 1968; Dayan and Woodhouse, 1968; Gonzalez-Licea *et al.*, 1968; Meyer, 1968; Grimley *et al.*, 1969; McDermott and Hart, 1970). Intracellular fibrillar material, believed to represent amyloid by some authors (Gonzalez-Licea *et al.*, 1968; McDermott and Hart, 1970), was detected only in smaller amount in a few cases (Meyer, 1968). Considering the cell borders, the findings varied. Meyer (1968) could not find desmosomes in his two published cases. McDermott and Hart (1970), however, observed a considerable number of desmosomes between neighboring tumor cells.

The medullary carcinoma studied by us exhibited ultrastructural features different from any of the cases hitherto published. The first striking difference was the lack of amyloid in the tumor stroma. The presence of amyloid in the stroma was considered a prerequisite for the diagnosis of medullary cancer by several authors (Hazard *et al.*, 1959; Williams, 1966; Williams *et al.*, 1966). On the other hand, Tubiana *et al.* (1970) have found that some cases of medullary cancer do not contain amyloid. They even felt it justifiable to consider this group as a separate entity, since, according to their data, these tumors differed from the amyloid producing variant not only morphologically but also in their clinical and biological behavior.

The other characteristic finding in our case was the presence of bundles of microfilaments in the cytoplasm of practically every tumor cell. This is a common phenomenon in several neoplasms (Luse and Lacy, 1960; Greider and Elliott, 1964; Johnston and Waisman, 1971), and microfilaments are also regular structural elements of some normal cells, e.g. in the endocrine cells of the gastrointestinal tract (Forssmann *et al.*, 1969; Pearse *et al.*, 1970; Ferreira, 1971). Despite this fact, no data are available concerning their origin and possible biological significance.

The groups of very dense, pleomorphic ("biconcave", Ferreira, 1971) secretory granules, localized usually at one pole of the tumor cells, represented the most peculiar feature of the neoplasm. It is known that medullary carcinomas have some histologic similarities to carcinoids (Williams, 1966; Williams *et al.*, 1966; Ibanez *et al.*, 1967). This resemblance is even more prominent ultrastructurally and, according to Gonzalez-Licea *et al.* (1968), sometimes it is hardly possible to distinguish between the electron microscopic appearance of medullary carcinomas of the thyroid and some of the carcinoid tumors. Morphologic similarities are also known between Langerhans islet tumors and medullary carcinomas. The fact that the pancreas and thyroid are derived from gut primordium might explain this phenomenon (Moertel *et al.*, 1965; Gonzalez-Licea *et al.*, 1968). Beyond their common origin and morphologic similarities, islet cells of the pancreas, endocrine cells of the gastrointestinal tract and parafollicular cells of the thyroid belong to the APUD (Amine, Precursor, Uptake and Decarboxylation) cell system, sharing important biological and biochemical properties (Pearse *et al.*, 1970). Relatively little is known about the exact function and regulation of the endocrine cells of the digestive system, although their histological, histochemical and ultrastructural

features have been extensively studied by several authors (Carvalheira *et al.*, 1968; Black, 1968; Forssmann *et al.*, 1969; Pearse *et al.*, 1970; Ferreira, 1971; Vassallo *et al.*, 1971) in the last few years. It has been demonstrated that at least five types of intestinal endocrine cells exist showing specific localization and well-distinguishable ultrastructural characterisitcs (Forssmann *et al.*, 1969). The neoplastic cells of carcinoids usually exhibit the same electron microscopical appearance as endocrine cells occurring normally in the same parts of the gastrointestinal tract (Black, 1968). It is important to note that the medullary carcinomas investigated so far had spherical (200 mμ diameter) secretory granules and vesicles of moderate or low density in the cytoplasm resembling gastric carcinoids. On the other hand, the shape, size, electron density and intracellular distribution of the granules observed in our case correspond to those found in ileal carcinoids (Black, 1968; O'Neal *et al.*, 1968) and also in the normal endocrine cells, most common in the small intestine, called EC (Vassallo *et al.*, 1971), EC (diazonium) (Pearse *et al.*, 1970), I serotonin (Forssmann *et al.*, 1969), EC 1 (Ferreira, 1971) or type 2 cells (Black, 1968). These cells are believed to be responsible for the production of enteroserotonin and give positive results with argentaffin reactions. The tumor cells in our case did not show argentaffinity; they only gave a well defined argyrophilia. This does not exclude, however, the possibility that the cells might be derived from the same cell group mentioned before. It is very probable that the matrix or membrane of granules is responsible for the argyrophilia (Pearse *et al.*, 1970), while argentaffinity of the cells might depend upon the quantitiy of secretory product (serotonin) stored in the granules (Rubin *et al.*, 1971). In our case, unfortunately, no biochemical or biological determinations were done. Clinically, however, there were no symptoms indicating excessive release of any biologically active substance.

Argyrophilia is not a unique finding among medullary carcinomas of the thyroid. Williams *et al.*, (1966) found it in about 50% of their cases. Ljungberg (1970b, c) showed a great number of argentaffin cells in the two medullary carcinomas he observed and claimed that the tumors were composed of C-cells and another type of parafollicular cells assumed to contain monoamines. He also showed argentaffin cells to be present in the non-neoplastic portion of the same thyroid. Similarly, in our case, a considerable number of argyrophil cells with parafollicular location were demonstrated in the non-neoplastic thyroid gland, adjacent to the tumor. There are also other data indicating the heterogenity of medullary carcinomas. Tubiana *et al.* (1970) succeeded in detecting calcitonin in all cases of medullary carcinoma with amyloid stroma when it was looked for. On the other hand, the results were entirely negative in tumors without amyloid stroma (Tubiana *et al.*, 1970). In another published case of medullary carcinoma without amyloid stroma (Moertel *et al.*, 1965), "malignant carcinoid syndrome" developed in the advanced phase of the disease indicating excessive production of serotonin by the metastatic tumor. It is also known that, besides calcitonin and serotonin, medullary carcinomas are capable of producing other hormones or hormone-like substances, such as ACTH and prostaglandins (Williams *et al.*, 1968; Grimley *et al.*, 1969; Szijj *et al.*, 1969)—another characteristic shared by these tumors and carcinoids (O'Neal *et al.*, 1968; Sandler *et al.*, 1968; Johnston and Waisman, 1971). Based on these data, it seems obvious that, as Gonzalez-Licea

et al. (1968) pointed out, there are many similarities between medullary carcinomas of the thyroid and carcinoid tumors, since these neoplasms appear to arise from different cell types within the same system. Compared with the medullary carcinomas studied ultrastructurally so far, our case showed the unique feature of exhibiting a striking resemblance to carcinoids of the small intestine arising from the serotonin producing cells.

Another problem awaiting further study is whether the parafollicular cells in the normal thyroid gland belong to a homogeneous cell population or more than one type of parafollicular cell exist. Extensive histochemical and ultrastructural studies could help clarify this problem, hindered by the fact that parafollicular cells are difficult to find in normal adult thyroid tissue (Braunstein and Stephens, 1968). Apart from this question, it seems established that the tumors arising from the parafollicular cells represent more than one type of neoplasms which can be distinguished from each other only by means of electron microscopy. This problem, beyond its theoretical interest, has important practical implications. An adequate ultrastructural classification of medullary thyroid cancers might also help in determining the biological behavior of the tumor, which is unpredictable in many cases.

The authors are grateful to Mr. P. Wong and Miss Eva Sekulova for their technical assistance and to Miss Diane Karch for the valuable secretarial help.

References

Albores-Saavedra, J., Rose, G. G., Ibanez, M. L., Russell, W. O., Grey, C. E., Dmochowski, L.: The amyloid in solid carcinoma of the thyroid gland. Staining characteristics, tissue culture, and electron microscopic observations. Lab. Invest. **13**, 77–93 (1964).

Black, W. C. (III): Enterochromaffin cell types and corresponding carcinoid tumors. Lab. Invest. **19**, 473–486 (1968).

Braunstein, H., Stephens, C. L.: Parafollicular cells of human thyroid. Arch. Path. **86**, 659–666 (1968).

Braunstein, H., Stephens, C. L., Gibson, R. L.: Secretory granules in medullary carcinoma of the thyroid. Electron microscopic demonstration. Arch. Path. **85**, 306–313 (1968).

Carvalheira, A. F., Welsch, U., Pearse, A. G. E.: Cytochemical and ultrastructural observations on the argentaffin and argyrophil cells of the gastrointestinal tract in mammals, and their place in the APUD series of polypeptide-secreting cells. Histochemie **14**, 33–46 (1968).

Dayan, A. D., Woodhouse, M. A.: Amyloid and medullary carcinoma of the thyroid. Electron microscope observations in one case. Path. et Microbiol. (Basel) **31**, 93–96 (1968).

Ferreira, M. N.: Argentaffin and other "endocrine" cells of the small intestine in the adult mouse. I. Ultrastructure and classification. Amer. J. Anat. **131**, 315–330 (1971).

Forssmann, W. G., Orci, L., Pictet, R., Renold, A. E., Rouiller, C.: The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. An electron microscope study. J. Cell Biol. **40**, 692–715 (1969).

Gonzalez-Licea, A., Hartmann, W. H., Yardley, J. H.: Medullary carcinoma of the thyroid. Ultrastructural evidence of its origin from parafollicular cells and its possible relation to carcinoid tumors. Amer. J. clin. Path. **49**, 512–520 (1968).

Greider, M. H., Elliott, D. W.: Electron microscopy of human pancreatic tumors of islet cell origin. Amer. J. Path. **44**, 663–678 (1964).

Grimley, P. M., Deftos, L. J., Weeks, J. R., Rabson, A. S.: Growth *in vitro* and ultrastructure of cells from a medullary carcinoma of the human thyroid gland: transformation by Simian virus 40 and evidence of thyrocalcitonin and prostaglandins. J. nat. Cancer Inst. **42**, 663–680 (1969).

Hachmeister, U., Zimmermann, H. D.: Elektronenmikroskopische Untersuchungen an einem C-Zellencarcinom. Verh. dtsch. Ges. Path. **54**, 371–375 (1970).

Hazard, J. B., Hawk, W. A., Crile, G., Jr.: Medullary (solid) carcinoma of the thyroid: clinicopathological entity. J. clin. Endocr. **19**, 152–161 (1959).
Ibanez, M. L., Cole, V. W., Russell, W. O., Clark, R. L.: Solid carcinoma of the thyroid gland. Analysis of 53 cases. Cancer (Philad.) **20**, 706–723 (1967).
Johnston, W. H., Waisman, J.: Carcinoid tumor of the vermiform appendix with Cushing's syndrome. Ultrastructural study of a case. Cancer (Philad.) **27**, 681–686 (1971).
Lietz, H., Donath, K.: Zur Ultrastruktur und Entstehung des Amyloids im medullären Schilddrüsencarcinom. Virchows Arch. Abt. A. **350**, 261–274 (1970).
Ljungberg, O.: Cresyl fast violet—a selective stain for human cells. Acta path. microbiol. scand., section A, **78**, 618–620 (1970a).
Ljungberg, O.: Two histochemically different human parafollicular cells. Acta path. microbiol. scand., section A, **78**, 621–624 (1970b).
Ljungberg, O.: Two cell types in familial medullary thyroid carcinoma. A histochemical study. Virchows Arch. Abt. A **349**, 312–322 (1970c).
Luse, S. A., Lacy, P. E.: Electron microscopy of a malignant argentaffin tumor. Cancer (Philad.) **13**, 334–346 (1960).
McDermott, F. T., Hart, J. A. L.: Medullary carcinoma of the thyroid with hypercalcaemia. Clinical and ultrastructural observations. Brit. J. Surg. **57**, 657–661 (1970).
Meyer, J. S.: Fine structure of two amyloid-forming medullary carcinomas of thyroid. Cancer (Philad.) **21**, 406–425 (1968).
Moertel, C. G., Beahrs, O. H., Woolner, L. B., Tyce, G. M.: "Malignant carcinoid syndrome" associated with noncarcinoid tumors. New Engl. J. Med. **273**, 244–248 (1965).
O'Neal, L. W., Kipnis, D. M., Luse, S. A., Lacy, P. E., Jarett, L.: Secretion of various endocrine substances by ACTH-secreting tumors—gastrin, melanotropin, norepinephrine, serotonin, parathormone, vasopressin, glucagon. Cancer (Philad.) **21**, 1219–1232 (1968).
Pearse, A. G. E., Coulling, B., Weavers, B., Friesen, S.: The endocrine polypeptide cells of the human stomach, duodenum, and jejunum. Gut **11**, 649–658 (1970).
Rubin, W., Gershon, M. D., Ross, L. L.: Electron microscope radioautographic identification of serotonin-synthesizing cells in the mouse gastric mucosa. J. Cell Biol. **50**, 399–415 (1971).
Sandler, M., Karim, S. M. M., Williams, E. D.: Prostaglandins in amine-peptide-secreting tumors. Lancet **1968II**, 1053–1054.
Solcia, E., Capella, C., Vassallo, G.: Lead-hematoxylin as a stain for endocrine cells. Significance of staining and comparison with other selective methods. Histochemie **20**, 116–126 (1969).
Solcia, E., Vassallo, G., Capella, C.: Selective staining of endocrine cells by basic dyes after acid hydrolysis. Stain Technol. **43**, 257–263 (1968).
Szijj, I., Csapo, Z., Laszlo, F. A., Kovacs, K.: Medullary cancer of the thyroid gland associated with hypercorticism. Cancer (Philad.) **24**, 167–173 (1969).
Tubiana, M., Parmentier, C., Gerard-Marchant, R., Lacour, J., Milhaud, G., Bok, B., Weiler, J.: Etude comparative des épitheliomes médullaires du corps thyroide avec et sans stroma amyloide. Europ. J. Cancer **6**, 39–47 (1970).
Vasallo, G., Capella, C., Solcia, E.: Endocrine cells of the human gastric mucosa. Z. Zellforsch. **118**, 49–67 (1971).
Williams, E. D.: Histogenesis of medullary carcinoma of the thyroid. J. clin. Path. **19**, 114–118 (1966).
Williams, E. D., Brown, C. L., Doniach, I.: Pathological and clinical findings in a series of 67 cases of medullary carcinoma of the thyroid. J. clin. Path. **19**, 103–113 (1966).
Williams, E. D., Karim, S. M. M., Sandler, M.: Prostaglandin secretion by medullary carcinoma of the thyroid. A possible cause of the associated diarrhoea. Lancet **1968I**, 22–23.
Wolman, M.: Amyloid, its nature and molecular structure. Comparison of a new toluidine blue polarized light method with traditional procedures. Lab. Invest. **25**, 104–110 (1971).

Dr. E. Horvath
Department of Pathology
St. Michael's Hospital
30 Bond Street
Toronto 2, Ontario, Canada