REVIEW ARTICLE

THE C CELLS (PARAFOLLICULAR CELLS) OF THE THYROID GLAND AND MEDULLARY THYROID CARCINOMA

The C Cells (Parafollicular Cells) of the Thyroid Gland and Medullary Thyroid Carcinoma

A Review

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NONFOLLICULAR CELLS of the thyroid gland have been recognized most commonly under the name *parafollicular cells*, the term applied by Nonidez [1] in 1932 in studies of the canine gland. In recent publications they have been identified as the *C cell*, as proposed by Pearse [2] in 1966; in conformity with their function in calcitonin secretion, this has now become a standard term. The term parafollicular cell, further, is not completely appropriate, as the cells also are found in an interfollicular location. Their importance in neoplasia was first recognized by Williams,[3] who proposed that they were the cells giving rise to medullary thyroid carcinoma.

The C Cells

The C cells were first identified by Baber in 1876 [4] in the thyroid of the dog in a parafollicular location and called *parenchymatous cells*. As further reported by Roediger (1975),[5] they have been recognized in the mammalian thyroid under a variety of names, among them parafollicular cell as indicated above,[1] interfollicular cell (Baillif, 1937),[6] neurohormonal cell (Sunder-Plassmann, 1939),[7] giant-light cell (Altman, 1940),[8] argyrophil cell (Sandritter and Klein, 1954),[9] light cell (Stux *et al.*, 1961),[10] and mitochondrion-rich cell (Foster *et al.*, 1964).[11] Foster *et al.*[11] related the cells to calcitonin production, demonstrating that they had "enzyme levels" sensitive to calcium concentration in the canine thyroid. Bussolati and Pearse [12] demonstrated the calcitonin content of the cells in the porcine thyroid by immunofluorescent studies. Thus the name C cell applied by Pearse [2] in 1966 is most appropriate, as it identifies them with the secretion of calcitonin.

Embryology

The C cells are now generally accepted as having their origin in the neural crest, as originally proposed by Pearse [2] in 1966, thus having a common origin with the adrenal medullary chromaffin cell, intestinal

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enterochromaffin cell, pituitary corticotrophs and melanotrophs, and islet cells. This entire series of cells was included under the descriptive term *APUD cells* by Pearse,[13] a term derived from their cytochemical characteristics (amine precursor uptake and decarboxylation). Origin in the neural crest in the mammals, including man, is considered most correct as presented by Smith.[14] The cells appear as clusters of neuroectodermal cells in the embryonic neural tube and do not take part in the infolding of the neural plate nor closure of the dorsal ectoderm. The thyroid, derived through infoldings and buddings of the primitive entoderm, becomes a site for inclusion of the C cells rather than their localization in distinct ultimobranchial bodies as found in some species.[14,15]

Histology

Most of the earlier information concerning C cells was derived principally from the study of animal thyroid glands. The C cells in the human thyroid, being of much smaller number than in that of other mammals, were not positively identified until recent years. This delay in their positive recognition was the result of their paucity in the human gland and also of their nonspecific appearance when stained with the usual histologic techniques.

Of the special stains, the Grimelius [16] silver nitrate argyrophil method is the most commonly reported and is specific in that it identifies the secretory granules of the cells. A variety of other methods have been employed to distinguish the cells from their follicular counterparts, however. The staining properties are those common to the APUD system [13] and are presented in depth and are correlated with their cytochemical characteristics by DeLellis and Balogh [17] in a consideration of the canine parafollicular cells and human medullary thyroid carcinoma.

With the hematoxylin and eosin stain, they are slightly paler than the follicular cells but the difference is not sufficient to identify them since they occur singly or in small groups, especially in the human gland. The Grimelius technique [16] is the most commonly used and provides identification of the cells through its dark granular staining of the cytoplasms associated with the localization in the secretory granules. The lead hematoxylin technique [18] was found useful by Pearse.[19] However, the argyrophilic property of the secretory granules of the cells supports the greater effectiveness of the silver staining technique. The specific immunoperoxidase technique [20,21] provides positive identification of the C cells based on their actual hormone content and was used in the detailed study of the thyroid glands of the human adult by Wolfe *et al.*[22] and Tashjian *et al.*[23] and of the neonate by Wolfe *et al.*[24] The correlations with the actual

calcitonin content of the thyroid tissue by bioassay and radioimmunoassay provided full proof of the accuracy of the cytologic method. Special histologic study of the thyroid was made by Englund *et al.*[25] utilizing fluorescent histochemistry and fluorometric determinations of dopamine following perfusion of the glands *ex vivo* with it.

The ultrastructure is of significant value in the differentiation of the C cells from their follicular counterparts and was employed by Braunstein and Stephens [26] for the recognition of the rare cells found in an extensive survey of the human thyroid in 1968. The results of this study, however, were doubted by Teitelbaum *et al.*,[27] who considered the differentiation reported as insufficient to distinguish the cells from the follicular cells.

Englund *et al.*,[25] using argyrophil and fluorescent microscopy, found an unequal distribution of the C cells in the adult human nontoxic and toxic gioters; the periphery of the lateral lobes was usually devoid of them, 5 mm beneath the thyroid capsule they were present in varying number, and the isthmus was free of the cells. In 1974, Wolfe *et al.*[22] and Tashjian *et al.*[23] made a detailed study of six entire human thyroid glands in adults without endocrine disorders, mapping the distribution of C cells in serial sections by immunoperoxidase localization and correlating the histologic findings by radioimmunoassay and bioassay of calcitonin in four of the glands in sections immediately adjacent to those used for microscopy. The histologic technique provides distinctive specific staining of the cytoplasm of the C cells without staining the follicular cells, colloid, or interstitial tissue. The cells comprised less than 0.1% of the epithelial mass and were found in both parafollicular and intrafollicular locations, most frequently in the former. Intrafollicular cells were applied to the basement membrane and were covered by a thin cytoplasmic layer of the adjoining follicular cells which separated them from the colloid. As a rule, they occurred singly and isolated, but they also were present in small clumps of not more than three cells in any section. The majority were predominantly located deep in the parenchyma of the middle third of each lobe. The calcitonin content correlated with the histologic distribution of the cells, with the extreme upper and lower portions containing less than 0.5 to 5 MRC mU/g (fewer than two C cells per low power field [10 ×]) and the middle third containing 10 to 160 MRC mU/g (four to ten C cells per low power field). The isthmus had low levels of calcitonin (one to four C cells per low power field). No immunoperoxidase-positive cells and no calcitonin were detected in the parathyroid glands, thymus, skeletal muscle, or kidney tissues used as controls.

The C cells were evident by the distinctly red-brown immunoperoxidase reaction filling the cell cytoplasms and were of polygonal or spindle

shape. They were not identifiable by hematoxylin and eosin staining without comparison with the specially stained sections, but with the latter, they had a clear to faintly granular cytoplasm with nuclei larger and more granular than in follicular cells.

Cytologic study of the C cells in toxic and nontoxic nodular goiters was made by Englund *et al.*[25] in smears obtained by fine needle aspiration biopsies of the lateral thyroid lobes. With fluorescence microscopy, the cells had a green fluorescence and occurred solitarily or in groups of two to four cells. The follicular cells, in contrast, showed an orange autofluorescence and were in large clusters. The May-Grünwald-Giemsa stain was not effective in distinguishing the cellular details.

The C cells of the neonate were studied by Roediger[28] using the Grimelius silver technique. They were present in clusters of 6 to 8 cells and were found in the lateral lobes but not in the isthmus. They comprised less than 1% of the thyroid mass, a much smaller portion than in the canine thyroid. Wolfe *et al.*[24] made a detailed study of six complete neonatal thyroids, obtained 3 to 12 hours postmortem from normocalcemic neonates without endocrine disorders. Serial sections were made of the lateral lobes and isthmus, and alternate sections were assayed for calcitonin. An immunoperoxidase localization technique was employed, and a correlation made by bioassay and radioimmunoassy of calcitonin. The C cells had a resemblance to those of the adult, with a finely granular red brown cytoplasm. They were restricted to the middle to upper thirds of the lateral lobes, and the cells were more numerous than in the adult, with up to 75 C cells per low power field (10 ×). The distribution of the cells was similar to that found in the adult: most frequent deep in the parenchyma of the lateral lobes and restricted to the middle to upper thirds along the hypothetical central axis. They were in both intrafollicular and parafollicular locations and, in contrast to those in the adult, were predominantly intrafollicular. The individual cells resembled those of the adult, often being of polygonal shape and up to 40 μ maximum diameter; some were of spindle shape with tapering cell processes. In the intrafollicular locations they were closely applied to the basement membrane. They occurred principally in clusters not exceeding six cells, and most of them intrafollicularly, with those in a parafollicular site comprising only 4 to 8% of cells of the total cell mass, whereas 75% of cells in the adult gland were in a parafollicular location. The calcitonin content in adjacent sections correlated strongly with the relative number of C cells.

The functional implication of the increase in the number of C cells in the neonate as compared with the adult is not known. Wolfe *et al.*[24]

suggests that it might be relative to the size of the gland, since in the neonate it is smaller in size and volume than in the adult, about one tenth that of the latter. Samaan *et al.*[29] reported calcitonin levels higher at term in cord blood than maternal blood and speculated that calcitonin might be an important hormone for the human fetus, possibly in regulation of fetal ossification.[30]

Ultrastructure

The ultrastructure of the parafollicular cells of the thyroid of several mammals had been reported, but apparently the first study of the C cells of the human thyroid was made by Braunstein and Stephens [26] in 1968. In a detailed study of four human thyroids, two from surgery and two autopsy specimens, they found two cells with ultrastructure they regarded as characteristic in a search of several hundred cells in one section of a normal surgically removed thyroid. Light microscopy was of no value in locating them. They described the cells as having pale cytoplasm, separated from the colloid by a thin rim of follicular cell cytoplasm, and with no contact with colloid in step sections. Numerous large mitochondria were present, secretion vesicles and dilated cisternae were prominent features, but no Golgi apparatuses were observed. There were relatively few secretory granules, which they described as having a dark central core separated from the external membrane by a narrow space and occurring in clusters. The granules averaged 100 to 200 mμ in diameter. Occasional desmosomes were seen in the cell membrane separating the cell from the follicular cell. Occasional prominent fine filamentous structures were present in the cytoplasm near the cell membrane.

Teitelbaum *et al.*[27] made a detailed study of thyroid tissue and found parafollicular cells only after examining 200 or more random sections of normal thyroid tissue fixed in glutaraldehyde. Localization of the cells was made by toluidine blue staining. The cells were 1.5 to 2 times the size of follicular cells and were clustered along the follicular basement membrane with no contact with colloid. Ultrastructurally, there were many electron-dense secretory granules 200 to 300 Å in diameter and an abundant ergastoplasm with a characteristic lamellar stacking. The importance of glutaraldehyde fixation was emphasized. They questioned the report by Braunstein and Stephens [26] because of the lack of demonstration of the diffuse granulation and lamellar endoplasmic reticulum. The identification of the cell as parafollicular on the basis of few membrane-bounded granules was not considered sufficient for positive identification of the cell type since the granules may be found in the cytoplasm of

follicular cells and they considered the illustrated, dilated endoplasmic reticulum to be characteristic of follicular cells.

Function

The primary secretory function of the C cells is the production of calcitonin. The important features of this hormone were reviewed in depth by Haymovits and Rosen.[31] Copp *et al.*[32] in 1962 demonstrated the polypeptide in canine thyroid-parathyroid preparations and interpreted the source to be the parathyroid glands. Foster *et al.*[11] found the parafollicular cells of the dog thyroid responsive to hypercalcemia and the source of calcitonin; they proposed the name *thyrocalcitonin* for possible distinction from the hormone believed by Copp to be of parathyroid origin. Bussolati and Pearse [12] studied the production of calcitonin by an immunofluorescent method in the pig and dog, demonstrating it in cells morphologically indistinguishable from C cells. Bauer and Teitelbaum [33] noted secretory association with the cytoplastic granules in particulate fractions of hog thyroid homogenates. Matsuzawa and Kurosunu [34] found degranulation of the parafollicular cells of the rat as a specific effect of acute hypercalcemia. Assays of calcitonin in the human thyroid were made in 1966 by Alaipoulios *et al.*[35] demonstrating evidence of calcitonin in the normal human thyroid glands, finding none in two thyroid adenomas and one hyperplastic gland; and low values in colloid goiter. The presence of calcitonin in pooled human glands was reported by Haymovits and Rosen.[36] Detailed studies and partial purification of porcine and human calcitonin were made by Barrett and Bell.[37] In a thorough correlation with C cells of the thyroid, Wolfe *et al.*[22] found a variation in the content of the hormone in conformity with the distribution of the cells, with the extremes of the upper and lower poles of the lateral lobes (less than two cells per low power field) having less than 0.50 MRC mU/g to 5.0 and 10 to 160 MRC mU/g in the midlateral lobes (four to ten cells per low power field).

Calcitonin is a polypeptide with 32 amino acids and contains a seven-member disulfide ring at the carboxy terminal. Human calcitonin has a largely different amino acid sequence than the porcine type; leucine and isoleucine are present; there is a seven-member ring, with only one amino acid substitution, in the amino-terminal; and leucine and glycine are in identical positions in the chain.

The basic function of calcitonin is the regulation of plasma calcium by a feedback mechanism. The effect in this regard is obtained through the inhibition of bone resorption, thus reducing the amount of circulating

calcium. An elevation of the latter stimulates secretion of the hormone and a decrease in the content and granulation of the C cells although the full physiologic importance is not known. With the reduction of circulating calcium, the parathyroid glands are stimulated to function and with continued calcitonin secretion may develop hyperplastic or perhaps neoplastic changes.

Therapeutic use for calcitonin has been found in the treatment of certain manifestations of Paget's disease of the bone. The extended use of porcine calcitonin, however, is limited by the production of antibodies to this variety of the polypeptide.

Hyperplasia

Hyperplasia of the C cells occurs with hypercalcemia, chiefly in association with hyperparathyroidism and toxic goiter and in members of families with a high incidence of medullary thyroid carcinoma.

Experimental hypercalcemia of long duration has been reported to result in C cell hyperplasia in several mammalian species, including the rat, dog, and cow as noted by Ljungberg and Dymling.[38] Capen and Young[39] produced hyperplasia of the parafollicular cells of the bovine thyroid by daily administration of vitamin D_2; cellular hypertrophy was found after 5 days and hyperplasia after 30 days of hypercalcemic stimulation.

Kracht *et al.*[40] (1969) described C-cell hyperplasia in an extensive study of the thyroids of patients with hyperparathyroidism. They found the increase in the cells to be restricted to the areas in which they are most frequently found in the normal gland, especially evident in the upper third of the lateral lobes. The cells occurred in "knots and clusters" and individually were hypertrophied. Some follicles were surrounded by them and follicular cells replaced. The hyperplasia of the C cells was properly considered to be associated with thyrocalcitonin hypersecretion and decreased content of the hormone in the gland related to its release in response to hypercalcemia.

Ljungberg and Dymling[38] described in detail C-cell hyperplasia in a nontoxic adenomatous goiter in association with hypercalcemia due to hyperparathyroidism which was known to have been present for over 2 years. Studies of the thyroid were made using several histochemical techniques—Grimelius silver nitrate, acid metachromatic staining using azure A and pseudoisocyanin, and cresyl violet. The C cells occurred in clusters of variable size, mostly located between the follicles, but some were intrafollicular. In some areas, they were of sufficient number to

completely surround an atrophic follicle. The histologic character of the cells varied with the size of the foci. In those of smaller type the cells had an abundant pale-staining vacuolated cytoplasm, rather large pale-staining nuclei, some in an eccentric location, with delicate chromatin and one or several small nucleoli. The larger clusters were formed of C cells with smaller dense nuclei and a cytoplasm that was more granular and eosinophilic with frayed margins. No amyloid was demonstrable in the clusters. Ljungberg and Dymling [38] stated that the reduction in the intensity of staining of some cells is in accord with the findings reported in hypercalcemic animals and also in C cells exposed to a high concentration of calcium in organ culture.[41]

The ultrastructural changes in the parafollicular cells associated with experimental hypercalcemia induced by oral administration of vitamin D_2 to cows were reported by Capen and Young.[39] A reduction in the number of secretory granules and aggregation of ribosomes was found after 3 days; after 5 days of feeding followed by a recovery period of 48 hours, there was an accumulation of secretory granules, and thyrocalcitonin levels in the thyroid glands were slightly higher than those of controls.

An increase in the number of C cells in toxic goiters has been reported by several investigators. The association may basically be related to the tendency to hypercalcemia and osteoporosis related to the increased thyroxin as noted by Adams *et al.*[42] Reduced levels of calcitonin in thyrotoxic goiters has been reported by Alaiapoulios and Rose [43] and Laljee *et al.*[44] Englund *et al.*[25] made a detailed study of diffuse goiters of patients with hyperthyroidism, all of whom had received treatment with carbimazole and with L-thyroxine added before the operation. After surgical removal, the glands were oxygenized and perfused with L-Dopa, and studies were made by silver staining, fluorescence histochemistry, and fluorometric determinations of dopamine, and examination of fine needle aspiration biopsies. The cells were found in small clusters of two to three cells or were isolated, sometimes they were present between the follicle cells. They were of increased number in comparison with nontoxic nodular goiters. The cells were evident 5 mm below the thyroid capsule, in conformity with their normal anatomic location. There was a variation from case to case, but they occurred with a mean of 5.3 ± 0.5 cells per field as compared with 1.8 ± 0.5 in nodular atoxic goiters. The hyperthyroid patients had a tendency to hypercalcemia, with plasma calcium levels of 4.9 to 5.6 (mean, 5.3) mEq/liter as compared with a normal range of 4.3 to 5.2 mEq/liter. Thus, the study of Englund *et al.*[25] fully demonstrated a significant increase in the number of C cells in the toxic

goiter. They noted that the relationship to carbimazole therapy in this study had not been fully decided, but a direct relationship is supported by the association of hypercalcemia with thyroxine.[42]

Hyperplasia may occur in those individuals genetically susceptible to medullary thyroid carcinoma and is strongly indicated by elevation of serum calcitonin levels either directly or after stimulation.[45,46] Wolfe *et al.*[47] made a detailed report of hyperplasia of the C cells in the study of 3 young adult patients with a familial background indicating a high risk for medullary thyroid carcinoma. Whole glands were available for study, and complete evaluation was made by serial sections of lateral lobes and isthmus. No tumor masses were found grossly. An increase in the number of C cells was evident, with the hematoxylin and eosin stain only in areas where they were present in greatest number, i.e., the upper two-thirds of the lateral lobes. The specific evaluation was made through the use of immunofluorescent and immunoenzymatic techniques for localization of calcitonin and the Grimelius silver stain. The morphologic findings were substantiated by quantitative calcitonin assay of immediately adjoining sections. The C cells were predominantly located in a parafollicular location, but occasionally they were focally present intrafollicularly. Rarely, they formed follicle-like structures. The C-cell clusters were predominant in the upper and middle thirds of the lateral lobes, the remainder of the gland showing only zero to two C cells per high power field. The Grimelius technique demonstrated argyrophilic intracytoplasmic granules with the same distribution as the immunohistochemical methods. Special stains for amyloid demonstrated the presence of extracellular amyloid focally within the C-cell clusters in all 3 cases but was more prominent in one. In sections of a normal gland, both the numbers of C cells and the calcitonin content were considerably less than in the glands of the 3 patients, with a greatest density in the midportions of the lateral lobes and lowest in the inferior lateral lobes and isthmus. In the normal gland, the calcitonin content, as measured by radioimmunoassay, for the left lobe was 24 to 27 MRC mU/g in the midportion and less than 0.6 in the upper and lower poles; for the midthird of the right lobe there was a maximum value of 50, with other portions of the gland showing values less than 100 MRC mU/g. In contrast, the values in the second of the 3 cases were 1600 for the midthird of the left lobe, 3200 for the upper third, 17 for the lower third, and 1100 for the upper midthird of the right lobe, 670 for the upper third, and less than 50 for the lower midthird and lower third. The clusters of C cells correlated strongly with the increased calcitonin content. Examination of the parathyroid glands in 2 cases showed an absence of fat but no gross enlargement. In 2 patients the basal serum calcitonin values were

within normal range but rising with time, especially in 1 case. The provocative calcium infusion test showed a progressive increase in response. In Case 3, there were abnormally elevated levels of serum calcitonin, both basal and after calcium infusion.

Wolfe *et al.*[47] note that from the localization of the C cells, neoplasia would predominantly occur in the upper and middle thirds of the lateral lobes; the multifocal nature of the hyperplasia is in conformity with the multicentric origin of familial medullary thyroid carcinoma.[45] In hyperplasia, as evidenced by the detailed study of Wolfe *et al.*,[47] the increase in C-cell mass conforms with the distribution of C cells in the normal thyroid, with the major increase evident in the mid and upper middle thirds of the lateral lobes.

Adenoma

Beskid *et al.*[48] have reported the occurrence of C-cell adenomas in the human thyroid, based on the study of 12 surgically resected nontoxic adenomas. However, as Roediger[5] notes, the cells occurred only as nests between the thyroid follicles, without encapsulation or compression of adjoining thyroid tissue. In the illustrations, the follicle-like structures resemble those of true follicle cells with a content resembling colloid. This, apparently, is the only report regarding C-cell adenoma of the thyroid gland.

Medullary Thyroid Carcinoma

Medullary carcinoma was first recorded as a specific pathologic diagnosis for a thyroid tumor in 1947[49] and was stated to be of an intermediate or lower grade of malignancy. The solid arrangement of cells originally had caused it to be included in the group of undifferentiated carcinomas and thus of a high malignancy. Review of sections revealed an unusual uniformity of cell type, a moderate number of mitoses as compared with the undifferentiated or anaplastic carcinoma, a uniform arrangement of cell sheets separated by stroma, and especially, the presence of amyloid in the tumor—features that identified it as a distinctive histologic type. The clinical evidence of a relatively long survival of patients, despite the undifferentiated appearance of the neoplasm, established it as a clinicopathologic entity.

On the basis of the histologic structure, *solid carcinoma* was considered an appropriate diagnostic term but one that did not distinguish it from the highly malignant anaplastic carcinoma, which also had such an appearance. The sheet-like arrangement of the neoplastic elements and the discrete gross character of the tumor were suggestive of the histology of the mammary neoplasm and the distinctive term *medullary carcinoma*

was applied. There was a delay in the complete acceptance of the term and, thus, of a publication referable to the tumor, because of the lack of "softness" of the neoplasm. However, the histologic and clincial features demanded a specific identity and the designation *medullary (solid) carcinoma* was used in the title of the publication concerning the neoplasm by Hazard *et al.*[50] in 1959.

Ljungberg [51] found reports on malignant goiter with amyloid published in the German literature early in the twentieth century by Burk,[52] Jaquet (1906),[53] and Stoffel (1910).[54] A series of 7 cases of distinctive type of carcinoma of the thyroid was published in 1951, by Horn,[55] who recognized the general gross and histologic structure of the neoplasm and the moderate grade of malignancy but gave no indication of the presence of the amyloidic substance. In a case report by Brandenberg [56] in 1954, apparently the tumor was described as *metastasizing amyloid struma.* Williams *et al.*[57] noted that the term *carcinoma thyroideum hyalinicum* had been proposed by Laskowski [58] in 1957.

Origin of the neoplasm from the parafollicular cell was suggested in 1966, by Williams,[3] who advised that the neoplasm was a source of the hypocalcemic agent. This was fully supported 2 years later by ultrastructural studies of the tumor made by Meyer [59] and was further confirmed by Meyer and Abdel-Bari [60] on the bases of the resemblance of the cytoplasmic granules of the tumor cells to those of parafollicular cells, biochemical considerations, and the thyrocalcitonin-like activity demonstrated in the neoplasm (both directly in the tumor and on tissue culture studies). Substantiation was also made by Cunliffe *et al.*[61] and Melvin and Tashjian.[62] The occurrence of medullary carcinoma as the most frequent type with a familial association was established by Schimke and Hartmann in 1965,[63] and Williams [64] reported the occurrence of the neoplasm with pheochromocytoma that year. The association of medullary carcinoma, parathyroid adenopathy, and pheochromocytoma, and the familial relationship was reported as a distinct entity by Steiner *et al.* in 1968,[65] designating it *multiple endocrine neoplasia, Type 2.* A second type of familial multiple endocrine adenopathy associated with medullary carcinoma was also recognized at this time by Schimke *et al.*[66] and Gorlin *et al.*[67] in which there was pheochromocytoma and multiple mucosal neuromas.

In addition to the secretion of calcitonin, the neoplasm had been recognized as a source of prostaglandins by Williams *et al.*,[68] and a secretion of adrenocorticotropic hormone has been the subject of several reports as reviewed by Melvin *et al.*[69] Elevated levels of histaminase activity in the tumor was reported by Baylin *et al.* in 1970.[70]

The neoplasm has been reported in the rat and the dog. Lindsay and Nichols [71] found it as a natural occurrence, particularly in the Long-Evans strain of rats and in five other strains; the neoplasm at times contained amyloid. Leav *et al.*[72] found it to be a rare occurrence in the dog; in the study of 94 primary carcinomas of the canine thyroid, only two were of the medullary type.

Medullary carcinoma, generally, comprises about 7 to 10% of thyroid carcinomas; a range of 3.5 to 11.9% was recorded in the extensive review by Ljungberg.[51] It occurs in a wide range of ages, most within the limits of 10 and 80 years, with the majority of cases in the fifth or sixth decade. In the familial group, however, the neoplasm has been detected in a younger age group because of the suspicion of its possible presence and utilization of special diagnostic tests, especially calcitonin assay, for earlier detection. Ljungberg [51] found the average sex distribution (female/male) to be 1.5 : 1, but with a reported range of 0.9 to 3.7 : 1. Fletcher,[73] in a survey of 247 reported cases, found a frequency of 6.8% of thyroid carcinomas, occurring slightly more frequently in women, with the ratio of 1.3 : 1. The ages of the patients varied from 10 to 82 years, with a mean of 36 years; most patients were in the fourth to sixth decades. Hill *et al.*,[74] in a report of 73 cases of a total of 777 thyroid carcinomas found an age distribution of 15 to 82 years, with an average of 47.2 years; the sex distribution (female/male) was 1.4 : 1. Chong *et al.*[75] found a wide age range for the tumor, 2 to 73 years, with a median of 51 years; 29 of 139 cases were of familial type. For the latter, the age range was 2 to 60 years, with a median of 21 years; there was a slight predominance of females. The precedent history of radiation of the thyroid in childhood is a quite rare occurrence; Ibanez *et al.*[76] found it recorded in only 1 of 53 patients.

There may be no clinical evidence of the first manifestation of the neoplasm, especially in the younger patient in the familial group, or there may be thyroid enlargement or evidence of cervical lymph node metastases. The patients are mostly euthyroid, Ibanez *et al.*[76] reporting 46 of 53 patients without thyroid functional abnormality, 6 that were hypothyroid, and 1 with hyperthyroidism. The thyroid evidence may be merely enlargement of a lobe or the gland, or it may be the presence of a nodule. Ibanez *et al.*[76] reported thyroid enlargement in 25 of 53 patients; Ljungberg,[51] however, found a nodular enlargment of the thyroid gland in 34 of 39 surgical cases. The thyroid tumor had been detected or suspected in 38 patients and was an incidental finding (a 5-mm-diameter tumor) in 1 patient undergoing an operation for toxic goiter. Diarrhea was a presenting symptom in some patients, reported as severe in 12 of 67 cases by Williams;[77] in 4 of the patients there was facial flushing. In one type of the

familial variant, mucosal neuromas are present, and the patients may have a marfanoid habitus.

The neoplasms generally belong to the group of thyroid carcinomas with an intermediate degree of malignancy, and mostly, there is no variant that exceeds this except for the spindle cell histologic type recognized by Williams *et al.*[57] as being of higher malignancy.

Cervical lymph node metastases may be a presenting symptom and were reported in 35 of 139 patients (25%) by Chong *et al.*;[75] 63 of 128 patients with operation for cure had cervical metastases at the initial surgery. Distant metastases were reported by Ibanez[78] in a study of pathologic material from 52 living patients and 20 autopsy subjects; metastases were found in the mediastinum in 22, the lung in 17, and the liver in 12, with other occurrence in a variety of sites.

The survival of patients with the neoplasm varies from a few months, as a rare instance, to many years. The survival of 60 patients is recorded by Ibanez;[78] 32 survived at 3 or more years, with 8 living for up to 15 to 20 years; 32 were dead as a result of the neoplasm. Histologic features were not regarded as a reliable indication of prognosis, but an abundance of amyloid and polyhedral cell structures, and the presence of calcification usually indicated a more favorable prognosis; numerous mitoses, as well as the presence of necrosis of the neoplasm, were indications of a high degree of malignancy.

Pathologic Findings

Gross Appearance

Grossly the neoplasms range from a few millimeters in diameter to 8 cm as reported by Ljungberg[51] and rarely of larger size. Chong *et al.*[75] in a study of 139 cases, reported a range of less than 0.01 cu cm to greater than 92 cu cm. Hazard *et al.*,[50] in the original report of 21 neoplasms, found them to be 1.5 to 8 cm.

The tumor is of quite firm to hard consistency. It varies somewhat in color but is usually white or gray-white, at times with a purplish mottling, occasionally pale tan or brown, and lacks the central scar seen with some papillary carcinomas.[50]

Most neoplasms occur as a single mass, usually quite circumscribed but not encapsulated. Hill *et al.*[74] noted some infiltration without a distinct border and Ljungberg[51] found them occasionally to be of irregular shape with gross infiltration of the adjoining thyroid tissue. Granular calcification was present in 22 of 47 cases reported by Ljungberg;[51] bone formation was present in 3. In a rare instance, the neoplasms may completely replace a lobe and be adherent to surrounding structures, but without invading

them grossly. The cut surface is flat or slightly bulging; occasionally there may be some hemorrhage and fibrosis. The thyroid capsule is usually intact, with perhaps some adherence to adjoining structures without penetration of them by the neoplasm. Hazard *et al.*[50] reported gross capsular vein invasion in 2 of 21 neoplasms and invasion of the innominate vein from an adjoining metastatic focus. The capsule of involved lymph nodes is usually intact, but adherence to the adjoining structures is greater than that seen with papillary carcinoma; in 2 of 21 cases,[50] infiltrating tumor masses were present in areas of lymph node involvement.

The primary neoplasm mostly is limited to one of the lateral lobes, although metastases to the other lobe may occur. However, in the familial variant, primary neoplasms are frequently found in both lobes. Origin in the isthmus is of great rarity; Ljungberg [51] reported such an origin in 1 of 42 tumors. Chong *et al.*[75] found bilateral tumors in 34 of 139 cases, 28 being of familial type. In the latter group, all but 1 patient had bilateral thyroid neoplasia; the remaining patient had a congenital absence of one lobe of the thyroid. Hill *et al.*[74] found apparently early neoplasms in the upper portions of the lateral lobes. Ljungberg [51] found no predilection for any special part of these lobes. Chong *et al.*[75] reported the neoplasm removed from the right lobe in 69 cases and from the left in 42.

Microscopic Characteristics

The neoplasm is composed of a basically sheet-like arrangement of tumor cells with irregular stromal trabeculae varying in number and density.[50] Thus the neoplastic masses are divided into groups of variable size and configuration. In the study by Hazard *et al.*,[50] amyloid was present in the stroma in all 21 of the cases, evidenced as a bright red staining substance in the hematoxylin and eosin–stained preparations, and with a granular or homogeneous appearance. In a few of the neoplasms the crystal violet stain was necessary for the recognition of the amyloidic substance. An occasional foreign body giant cell reaction to the amyloid was found. The cells were of moderate size and varied from 9 to 16 μ in greatest diameter; they were polyhedral, angular, round, or spindle shaped. In one tumor the latter cells were markedly elongated, resembling a spindle cell sarcoma. The nuclear/cytoplasmic ratio was generally low. Nuclei were oval or round and of uniform size and staining properties. Rarely, a giant nuclear form was seen in one neoplasm. Mitoses were usually sparse. There were no tumor giant cells or atypical mitoses. An artifact in the older formalin–fixed material resulted in a pseudopapillary configuration caused by the separation of cells from the stroma and from

each other; however, there were no psammoma bodies in these areas. In several tumors, the cells were predominantly polyhedral, resembling a carcinoid configuration. In one neoplasm, there were anastamosing cords of cells about stromal vessels simulating the appearance of an islet cell tumor. Sparse follicular elements were found in 3 of 21 tumors, some resembling included thyroid follicles. In all neoplasms, the adjoining thyroid tissue was infiltrated by tumor cells *en mass* rather than individual tumor cell components.

In a study of 67 cases, Williams confirmed the above observations but noted several additional features. The cells had ill-defined cytoplasmic margins and cytoplasm that was granular and eosinophilic. The latter staining property, of course, may have been a variation in the staining technique compared with the report of Hazard *et al.*[50] Binucleate or trinucleate forms were not infrequent, and nuclear size had a moderate to occasionally a marked variation. Areas of necrosis were quite unusual and were found extensively in only one neoplasm. Focal calcification was common, present in over half the cases. Many of the calcium deposits were small and resembled psammoma bodies, but without lamination. The spindle cell variant was dominant in 14 of the 67 neoplasms. A carcinoid or islet cell pattern was evident in some areas of a few tumors. True tubular structures were infrequent and present in only 5 cases. In three tumors, cells were present that resembled goblet cells with intracellular mucin. An occasional rosette-like arrangement of cells was found about small areas of amyloid. Staining with the periodic acid–Schiff technique revealed small amounts of positive material in the cell cytoplasms and many granules in clear macrophage-like cells; this appearance was partly due to the presence of glycogen identified by the Best's carmine method. With the alcian blue stain, the tumor cells were weakly positive. Over half the 34 neoplasms studied by the argyrophil technique had positive cells, but only a few were strongly positive.

The histochemical characteristics of human medullary carcinoma were studied in detail in two tumors by DeLellis and Balogh.[17] The stains employed were those for previous studies of the polypeptide hormone–producing cells of the APUD system,[13] the cells having common histochemical characteristics including masked metachromasia and argyrophilia. Studies of both human and rat medullary carcinoma revealed similar histochemical properties, with only slight variations in the intensity of reaction. With coriophosphine O staining, after acid hydrolysis, the carcinoma cells had an intense orange-red metachromasia with greater intensity after fixation in glutaraldehyde than in formaldehyde; the reac-

tion was classed as + to ++. Toluidine blue staining after acid hydrolysis showed a red-purple metachromasia in the cytoplasms of the cells, with a grading of ++. The cells were negative to the cresyl fast violet stain. With lead hematoxylin they had a ++ intensity. Argyrophilia was demonstrated by both the Grimelius and Azzopardi techniques but the staining intensity was much stronger with the former with the intensity of +++, compared to ± to +; the rat medullary carcinoma had slightly less staining intensity with the Grimelius technique than did the human carcinoma, and about equal intensity with the Azzopardi method.

Specific methods of demonstration of the cells of the tumor or hyperplasia of the C cells is by the use of immunofluorescent and immunoenzymatic techniques identifying the calcitonin in the cells,[22,47] as noted by Wolfe *et al.*,[22] utilizing methods reported by Sternberger *et al.*[20] and Mason *et al.*[21]

A second type of cell was recognized by Ljungberg [79,80] in an histochemical study of medullary carcinoma and was basically identified by an agentaffin rather than argyrophilic granulation; they were regarded as entirely distinct from the C cells. In the hematoxylin and eosin–stained preparations, the cells were identified in consecutive sections to those stained by the argentaffin method and were evident as having small hyperchromatic nuclei, often eccentric and with a cytoplasm filled with rather coarse, red-brown granules of sufficient number to completely hide the nuclei. In unstained sections, they had a weak intrinsic yellow color. The cells were found in 20 of 34 medullary carcinomas including 10 of familial type. The cells were identified in nontumorous thyroid tissue in 13 of 25 glands containing medullary carcinoma, 7 with familial, and 6 with sporadic type. They were diffusely distributed, usually singly, with typical polyhedric, often spider-like shapes. The cell bodies were large, as much as ten times the size of an erythrocyte, but most only evidenced by the slender cytoplamsic processes. They were located in an interfollicular location adjoining or at a distance from the walls, and in no instance were they intermingled with the follicular cells, although they did have a close relationship to the parafollicular capillaries. This cell type was not present in any of forty other examples of thyroid carcinoma and was not present in the portions of the glands not involved by tumor. However, in 6 of 50 normal glands or those with benign disease, a few of the argentaffin cells were demonstrable in a parafollicular location. The cells were identified in 5 of 8 of the parathyroid glands from patients with medullary carcinoma, as being present in the stroma or intermingled with the parathyroid cells but not in the soft tissue about the gland.

Ultrastructure

Several studies of the ultrastructure of medullary thyroid carcinoma have been reported.[59,81,82] The special characteristic feature differentiating medullary thyroid carcinoma from other thyroid neoplasms is the presence of secretory granules and amyloid fibrils.[78]

Details of the electron microscopic structure based on the study of two tumors were reported by Meyer.[59] Basement membranes were present about the cells except in the presence of amyloid. The nuclei were usually of wavy outline and with clumping of the chromatin, especially against the nuclear membrane. The Golgi apparatus had smooth membranes in lamellar arrangement with formation of flattened sacs, vacuoles, and small vesicles. Granular endoplasmic reticulum was moderate or less in amount, lamellar, or in groups of rounded or flattened sacs. Mitochondria were numerous, and many had open ends. The secretory granules were often surrounded by distinct membranes and were largely electron dense, with a mean diameter of 100 mμ in one tumor and 150 mμ in the other. Many of the limiting membranes had two lamellae separated by a lucent zone. The membranes closely adjoined the cores or were separated from them by lucent zones of 20 mμ in width. Smaller granules had a frequent association with the Golgi apparatus and were considered to possibly have had origin there. Many granules adjoined the plasma membrane and occasionally their membranes fused with it. The amyloid fibrils had a mean diameter of about 100 Å and were arranged in bundles. They were in an intercellular location in the tumor and occurred in large clumps in the stroma; in one tumor, the amyloid fibrils were mingled with the collagen fibrils. Intracellular fibrils were evident once in each tumor.

Amyloid

Amyloid was reported to be present in all of the neoplasms in the series of Hazard *et al.*;[50] Williams *et al.*[57] found a considerable amount in the majority of primary tumors and the metastases. Ljungberg [51] found it to be present in at least one primary neoplasm in the thyroid gland but not in all if there were several separate tumors. In some cases, the amyloid may be of such uneven distribution as to require an extensive search of multiple blocks and use of special stains for its detection.

Williams *et al.*[57] detailed the histologic features of 67 cases and demonstrated amyloid, in both the primary neoplasms and the metastases. The smaller deposits were found between tumor cells, adjoining cell membranes or within the cell cytoplasms. The larger amounts were present as interlacing trabeculae between cells or occurring in irregular masses in the sheets of tumor cells or stroma. In some areas there was a pseudofollicular

appearance caused by the grouping of the tumor cells about the small amyloid focus. Scattered deposits of amyloid were present in the stroma and in the adventitia of small vessels in and adjoining the tumor. In a few instances, there was a foreign body–like reaction to the amyloid; it could not be definitely determined whether the multinucleate cells were neoplastic or of macrophage origin. An unusual finding was the presence of amyloid deposits in the renal glomeruli in 2 patients with long standing and widespread secondary tumor.

The amyloid stains in the same manner as usual amyloid, but it has been suggested that it is of a different type, resembling that seen in islet cell neoplasms and designated APUD amyloid by Pearse *et al.*[13] and associated with the polypeptide cells of neural crest origin.[83] Pearse *et al.*[84] have also noted that the amyloidic substance formed by these cells (designated *apudamyloid*) is histochemically different from the secondary amyloid (immunamyloid).

Amyloid is not evident, generally, in other thyroid carcinomas, although Polliack and Freund [85] report the presence of amyloid in a papillary carcinoma.

Ibanez,[78] in an ultrastructural study, found that tumor cells in the areas devoid of amyloid had well-preserved organelles and secretory granules but those in apposition to it had an altered morphology showing distention of endoplasmic reticulum and mitochondrial changes indicative of cellular degeneration; no secretory granules were present and intracellular fibrils were found resembling those of extracellular amyloid. Some of the cells in the amyloid had preserved organelles, with an increased density of cytoplasm due to the presence of more numerous fibrils, suggesting an early stage of amyloid production; the secretory granules appeared unrelated to the fibrils, although the amyloid was apparently a product of the tumor cells.

Tissue cultures of medullary carcinomas were made by Albores-Saavedra *et al.*[86] demonstrating the presence of amyloid at the end of 4 to 5 weeks and becoming more prominent later; no secretory granules were observed. The amyloid appeared to lie peripherally in the cytoplasm, attached to the cell, and later dispersed into the medium.

Histogenesis

The neoplasm now is fully accepted as being of C-cell (parafollicular cell) origin. This was first proposed by Williams in 1966 [3] and definitely stated to be so with respect to the medullary carcinoma of the rat thyroid. The ultrastructure of the tumor cells conforms with that of the normal C cell [59,60] and has similar histochemical properties, including argyrophilia

and masked metachromasia. Immunohistochemically, the cells also are identical with the C cells.[87] Functionally both the C cells and medullary carcinoma are sensitive to calcium stimulus.

Function and Diagnostic Relationships

The cells of medullary thyroid carcinoma primarily produce calcitonin. Histaminase [70,88] is produced by some tumors, and prostaglandin secretion was first demonstrated by the studies of Williams *et al.*[68] Ectopic hormone secretion has occurred as a rare event.

Calcitonin

This hormone occurs in the tumor in greatly excess amounts, many times that of the normal thyroid; it is demonstrable in venous drainage from the tumor and in the peripheral circulation. In the neoplasm, it is not autonomous and may be increased by calcium infusion and other stimulating agents, especially gastrin. Despite the abundance of calcitonin secretion, hypocalcemia is of rare occurrence,[89,90] through parathyroid hormone production or, possibly, development of tolerance to the hormone.

The secretion of calcitonin is an important factor in the diagnosis of the tumor as evidenced by its increase over the normal level in the blood either before or after stimulation; radioimmunoassay has largely replaced bioassay in its measurement. Deftos [91] reported the application of radioimmunoassay for calcitonin in 33 patients, assaying peripheral plasma and that from veins draining the tumor. Normal plasma basal concentrations were near the detection limit of the assay (100 $\mu\mu$g/ml); in 3 patients, plasma values were in this range but were increased to diagnostic levels by calcium infusion, in 1 patient requiring only a 10-minute infusion. The basal levels of plasm calcitonin were abnormally elevated in 26 of 33 patients. The ratio of calcitonin in blood draining the tumor to that peripherally ranged from 4.3 to 57.0.

The assay of calcitonin in the peripheral blood, with or without secretory stimulation, is a commonly used method for the diagnosis of the presence of the neoplasm and is especially effective in the diagnosis of tumors too small for clinical detection. It is especially valuable in the study of subjects at high risk through familial relationship. Wells and Ontjes [92] have noted there have been reports of elevation of the hormone in other disease states: subacute thyroiditis,[93] Zollinger-Ellison syndrome,[94] and oatcell carcinoma,[95] and other nonthyroid tumors.[96]

The report of Miller *et al.*[97] illustrates the value of the radioimmunoassay of calcitonin in the serum in the diagnosis of early familial

medullary carcinoma of the thyroid. Patients with medullary carcinoma, with but few exceptions, have basal levels of the hormone exceeding 0.38 ng/ml. In those with questionably low levels the provocative test with calcium infusion over a 4-hour period has been commonly used, resulting in a marked increase in calcitonin secretion in patients with neoplasm.[99] Miller *et al.*[97] record, in the study of 63 normal subjects, the stimulated level of the calcitonin in the serum is less than 0.55 ng/ml; in patients with medullary carcinoma it was always greater than 2 ng/ml. In the study of 83 members of a family without previous demonstrable presence of the neoplasm, 12 were found to have elevated serum calcitonin levels consistent with the presence of the tumor, 9 with elevated basal levels, and 3 with abnormally high levels after calcium infusion. Despite the absence of clinical evidence of thyroid enlargement or other indications of the presence of the tumor in 11 of 12 patients, surgical exploration confirmed the diagnosis of medullary thyroid carcinoma. ^{131}I iodide or ^{99}Tcm pertechnitate scan and ^{75}Se methionine scan were negative except in 1 patient (with the largest tumor) in whom bilateral cold nodules were evident with the ^{131}I iodide scan.

In some patients, the abnormality of calcitonin production by the neoplasm may not be revealed without a provocative test. Calcium chloride infusion is most often used. An effective infusion used by Deftos[91] was 5 mg/kg body weight/hr for 2 to 4 hours; as noted previously, 1 patient in the series required only a 10-minute infusion.

In addition to the calcium infusion test, other methods of calcitonin stimulation have been reported as being effective in diagnosing the neoplasm.

Pentagastrin. Pentagastrin is a potent stimulator of calcitonin secretion, as first demonstrated by Cooper *et al.*[100] in the pig. Hennessey *et al.*[101] found it to be more effective than calcium infusion in application to secretion of the hormone in patients with medullary thyroid carcinoma, with a peak response occurring 1 to 5 minutes after injection, compared with the longer period after calcium infusion. Hennessey *et al.*[102] and Wells *et al.*[103] reported it to be of especial value in the detection of a neoplasm of small size with no clinical indication of its presence. In a study of a family with familial medullary thyroid carcinoma (multiple endocrine neoplasia, Type II), Wells *et al.*[103] found it of great diagnostic value in the recognition of the thyroid neoplasm in 4 children, in whom the neoplasm occurred in very small foci, bilaterally. It was also found to have even greater diagnostic accuracy when used conjointly with selective catheterization of the inferior thyroid vein.

Glucagon Infusion. Deftos[91] found a variable response of calcitonin to

glucagon infusion, with no consistent effect on the calcium concentration in the plasma, and he concluded that it was not a reliable provocative test. Further, it could be dangerous in the presence of pheochromocytoma because of its potential for excessive catecholamine release.[104]

Ethanol. Ethanol has been reported to be an effective agent in the stimulation of calcitonin secretion. Cohen *et al.*[105] report the occurrence of facial flushing after ingestion of alcohol by a patient with medullary thyroid carcinoma; a six- to sevenfold fold increase in the hormone in the blood was found. There was no elevation of prostaglandin E_2. Wells *et al.*[106] also report the stimulation of calcitonin by ethanol in patients with the neoplasm and that it was not an effect secondary to increased secretion of gastrin, which also is elevated by alcohol ingestion. They regarded the stimulatory effect not as great as that obtained by gastrin. Dymling *et al.*,[107] in a study of a large family with familial chromaffinosis (multiple endocrine neoplasia, Type 2), found that ingestion of whiskey, in a 50-ml amount, was a potent stimulator of calcitonin secretion with a magnitude similar to that during the 4-hour calcium infusion test.

Histaminase

In 1970, Baylin *et al.*[70] reported elevated levels of histaminase activity in the serum of 4 patients with metastatic medullary thyroid carcinoma and also in ten tissue specimens of the neoplasm. In 2 patients with metatastic medullary carcinoma, the activity was totally inhibited by oral doses of the histaminase inhibitor aminoguanidine, with a long duration of this effect. Measurement of the enzyme was proposed as a possibility of a new diagnostic approach to the presence of the neoplasm and for the postoperative follow up of patients.

Normally, the mean serum histaminase activity was 1.4 pmoles/ml/hr; in the 4 patients with metastatic medullary carcinoma there were elevated levels, with values ranging from 3.4 pmoles/ml/hr to 60 pmoles in a patient with widespread metastases. Normal activity was present in a patient whose disease was limited to the thyroid and normal values were found postoperatively in 2 patients without residual disease. With other neoplasms, only 1 patient had an elevated level of the enzyme, a woman with metastatic breast adenocarcinoma. In the tumors studied, the tissue values were exceptionally high: in 6 of 7 patients, 1385 to 1960 pmoles/g/hr, with all but one value 1620 or above, the adjacent normal thyroid had a low level with a range of 15 to 170.

The elevation of the enzyme in the tissue from the neoplasm as indicated by Baylin *et al.*[70] could be of diagnostic value in the identification of the specific type of tumor when histologic diagnosis might be difficult as in the absence or sparsity of amyloid. Baylin *et al.*,[88] in a later study, again

emphasized the importance of the demonstration of histaminase activity, which thus served as a specific marker for the presence of medullary thyroid carcinoma.

An abnormal intradermal reaction to histamine injection was reported by Baum [108] in a patient with medullary thyroid carcinoma, evidenced by the absence of the flare that normally occurs. This was considered possibly related to the increase in histaminase levels in patients with the neoplasm.

Prostaglandins

The association of diarrhea with medullary carcinoma was recognized by Williams,[77] who found that there was a correlation with the extent of the tumor and the relief that occurred after resection. In 1968, Williams and his associates [68] reported appreciable amounts of prostaglandins E_2 and $F_{2\alpha}$ in tumor tissue from 4 of 7 cases and demonstrated raised blood levels in 2 patients with higher levels in blood draining the neoplasms than in the general circulation; both had diarrhea which was possibly due to production of prostaglandins by the tumor.

Diarrhea occurs in about 30% of patients [74] with the neoplasm; in the severe form, it may result in large losses in fluid electrolytes and the weight of the patient, and steatorrhea may occur. The cause of the diarrhea has not been fully established and may lie in a combination of hormonal factors,[109] but there are considerable indications that prostaglandins are involved in the etiology, as originally reported by Williams *et al.*[68] Calcitonin does not appear to influence gut motility.[110] Bennett and Fleshler [111] have stated that prostaglandins do have such a function and have recognized evidence for their role, especially E_2 and $F_{2\alpha}$, and further in relation to diarrhea, injection of $F_{2\alpha}$ has been reported to cause diarrhea.[112] Barrowman *et al.*[109] found further support for such an etiology in the successful treatment of diarrhea in a patient with medullary thyroid carcinoma of spindle cell type extensively involving the thyroid and with pulmonary metastases, using oral administration of ground nutmeg,[113] a potent prostaglandin inhibitor. Large amounts of prostaglandin E_2 had been demonstrated in the peripheral blood and some extracted from the tumor; the diarrhea had persisted after thyroidectomy, though the blood levels had returned to normal. The beneficial effect of nutmeg may be a direct action on prostaglandins or a nonspecific effect on gut motility.[113] Bernier *et al.* [110] found a patchy partial villous atrophy of small intestinal mucosa on peroral biopsy in 2 cases and a normal mucosa and submucosa in a third. The immediate cessation of diarrhea after removal of the tumor in certain cases provided evidence for a definite relation with the neoplasm. Prostaglandin activity in the blood of 1 patient was minimal and in another, negative.

Thus, there are occasional reports of elevation of prostaglandins, especially F_2 and $F_{2\alpha}$, in association with medullary thyroid carcinoma and evidence for a relation to diarrhea. However, information regarding the frequency of their presence and true relation to the syndrome associated with this neoplasm is not complete.

Ectopic Hormone Secretion

Medullary thyroid carcinoma has been reported occasionally to secrete several hormonally active substances in addition to calcitonin. In this relatively rare event, adrenocorticotrophin is the most frequent; serotonin and, possibly, melanin-secreting hormone have been reported with an association.

Adrenocorticotropin. Medullary thyroid carcinoma is one of several nonpituitary neoplasms that may cause hyperadrenocorticism and Cushing's syndrome through the production of an adrenocorticotropic hormone. Szijj *et al.*[114] state that only about 200 cases of ectopic secretion of the hormone had been reported to 1969, and in the survey of available literature, only 15 were associated with medullary thyroid carcinoma; they added an additional case.

In 1968, Williams *et al.*[115] reported 2 cases associated with carcinoma of the thyroid of medullary type. He found 9 other published cases of the syndrome in association with thyroid carcinoma, 1 described as medullary, 2 as papillary, and the other 6 variably as anaplastic, undifferentiated, atypical, or of solid type. A review of 5 of these cases proved them to be medullary thyroid carcinoma. In the 2 cases with papillary carcinoma, he noted that the syndrome and tumor may have been unrelated; in 8 of 9 the medullary carcinoma presented concommitantly with Cushing's syndrome. Two of the 9 cases had bilateral adrenal pheochromocytoma.

In a review and a case report in 1970, Melvin *et al.*[69] found previous reports of 16 cases, with 14 having neoplasm definitely medullary thyroid carcinoma; 1, described as anaplastic but with long survival; and 1 papillary but with a doubtful relation to Cushing's syndrome. Identification of an adrenocorticotropin by assay had been made in 2 cases reported by Donahower *et al.*,[116] high levels were found in both the thyroid tumor and plasma.

As noted by Szijj *et al.*[114] the evidence of Cushing's syndrome with ectopic ACTH production may show variations from the usual type. The clinical signs and symptoms are generally more severe, muscle weakness is frequent, obesity usually not present, and abdominal striase absent; skin pigmentation is common.

The pituitary in the presence of such ectopic ACTH production has been rarely described. Williams *et al.*[115] found hyalinization of basophils in their 2 cases, with a microscopic chromophobe adenoma in 1. Szijj *et al.*[114] recorded a pituitary weight of 0.67 g; several large Crooke's cells with vacuolated cytoplasm were present.

Serotonin. Flushing of the face suggestive of carcinoid syndrome was reported by Ibanez *et al.*[76] in 3 of 15 patients with medullary thyroid carcinoma and diarrhea and by Williams [77] as a symptom associated with the neoplasm. However, results of measurement of serotonin have rarely been reported. Bernier *et al.*[110] found blood serotonin increased, but not significantly, in 1 patient with severe flushing and diarrhea. However, there is a report by Moertel *et al.*[117] of a case in which the tumor was associated with the production of the malignant carcinoid "syndrome." The patient had attacks of intense flushing and diarrhea and high levels of serotonin were found in the skin and liver metastases from the tumor and in the blood. The tumor was diagnosed as "metastatic solid carcinoma" and assumed to have had origin in the thyroid, from which there had been a primary carcinoma, 2 cm in diameter, of the left lobe removed 2 years previously by subtotal thyroidectomy. The histologic appearance of the neoplasm and the presence of parathyroid adenoma and hyperplasia are in full support of a diagnosis of medullary thyroid carcinoma.

Melanotropin. There is no direct biochemical proof of the production of melanin-stimulating hormone by the neoplasm, but pigmentation of the skin may occur with the tumor. Cunliffe *et al.*[118] reported a case in which the patient had the general features found with multiple endocrine neoplasia, Type 2b, and with pigmentation, unlike freckles, about the mouth and on the hands and feet. The pigmentation disappeared after thyroidectomy and was regarded as possibly a result of a humoral agent produced by the medullary carcinoma.

Familial Medullary Thyroid Carcinoma and Multiple Endocrine Adenopathy

The association of thyroid carcinoma with pheochromocytoma was first reported by Sipple [119] in 1961, and later recognized as the Sipple syndrome, but the specific type of thyroid carcinoma was not indicated. In 1965, Williams [64] reported the association with medullary thyroid carcinoma as the specific type and recognized the possible familial relationship. As noted by Ljungberg,[51] the familial occurrence of thyroid carcinoma in association with pheochromocytoma had been reported by Cushman [120] and by Friedell *et al.*[121] in 1962. A tumor in the former report was stated to conform with medullary (solid) carcinoma and two of the tumors in the latter to be medullary or solid carcinoma. Schimke and

Hartman [63] reported the occurrence of "familial amyloid-producing" medullary thyroid carcinoma with pheochromocytoma and recognized it as a distinct genetic entity with an autosomal dominant mode of inheritance. Recognition of a possible familial relationship is of great importance because it is a significant indication that the neoplasm may be present in the thyroid despite the absence of clinical signs. Calcitonin assays are of a special importance in such early recognition of the presence of the tumor and have lead to its identification in an early age group and at an early stage in the development of the neoplasm, thus promoting higher rates of patient survival.

In 1968, Steiner *et al.*[65] reported in detail the concomitant occurrence of medullary carcinoma, parathyroid hyperplasia or adenoma, pheochromocytoma, and Cushing's disease, as a distinct entity and designated it *multiple endocrine neoplasia, Type 2,* in contrast to the type with anterior pituitary, parathyroid, and pancreatic islet cell adenopathy which had previously been recognized by Wermer [122] and Ballard *et al.*[123] and is now identified as Type 1 (MEN-I). The occurrence of Cushing's disease with the triad of MEN, Type 2, is extremely rare and not included in that type of adenopathy. In the original report, it was present as a single case, and the authors stated it was associated with excess ACTH produced by the pituitary; none of the hormone was found in the thyroid neoplasm. The association of medullary thyroid carcinoma with pheochromocytoma conforms with the common embryonic origin of the C cells and neurochromaffin cells of the adrenal medulla from the neural crest.[45]

The association of mucosal neuromas with medullary thyroid carcinoma and pheochromocytoma had been reported by Williams and Pollock in 1966.[124] Ljungberg *et al.* in 1967 [125] reported the association of medullary thyroid carcinoma and pheochromocytoma as a familial chromaffinosis and with multiple fibroma-like tumors in some cases. In 1968, the association with mucosal neuromas was reported by Schimke *et al.*[66] and Gorlin *et al.*,[67] who recognized it as a distinctive syndrome, at times with a marfanoid habitus. Later this type of multiple endocrine adenopathy, without parathyroid involvement, was identified by Sizemore *et al.*[126] as multiple endocrine neoplasia, Type 2b. Khairi *et al.*[127] reported it as Type 3, but the two types of adenopathy are commonly recognized as variants of Type 2 and designated Type 2a (MEN-IIA) and Type 2b (MEN-IIB).

Medullary thyroid carcinoma has been found in some families apparently without pheochromocytoma, although the data with regard to an adrenal medullary component are not always complete. Ljungberg [51] found reports of six families, including 21 cases, compared with 22 families where there was coexistent pheochromocytoma. Block *et al.*[128] de-

scribed two families in which medullary carcinoma occurred apparently without pheochromocytoma.

Medullary carcinoma as a part of a familial multiple endocrine adenopathy has had an incidence of usually about 10% of reported cases. Chong *et al.*,[75] however, in a study of 139 surgically treated patients found a definite family history in 20 (14%); multiple endocrine adenopathy was found in 29, about equally divided between the two types. In previous reports, however, Type 2a has had a much higher incidence. There may be no clinical indication of the adenopathy or evidence through the presence of a thyroid tumor, symptomatic metastases, or occasionally, a functioning pheochromocytoma.[92]

The neoplasm in the thyroid is usually bilateral and multicentric, arising in areas of C-cell hyperplasia. Palpable nodules may be present but not with the frequency of the nonfamilial type. Twenty-nine patients with the multiple endocrine adenopathy were reported by Chong *et al.*;[75] 16 patients (55%) had palpable thyroid nodules and in 27 the neoplasm was bilateral and multicentric. In 1 patient, a child, only bilateral C-cell hyperplasia was present. There was a developmental absence of a thyroid lobe in 1 case. Lymph node metastases were found in 16 at surgery; patients without metastases included the three youngest (2 to 11 years).

The asymptomatic type is often suspected by the history of familial association of the medullary carcinoma and diagnosed by the demonstration of hypercalcitoninemia, either on direct determination or after stimulation of calcitonin secretion. Söderstrom *et al.*[129] have reported fine needle aspiration of the thyroid as an effective method of diagnosis in patients with verified hereditary or sporadic medullary carcinoma but suggested that it might be difficult to identify the tumor cells in small nonpalpable tumor foci, though such an application would be worth a trial. Possible seeding of the needle tract by tumor cells in cases with a positive cytology was not mentioned, but might be a consideration and require excision of the tract as well as the neoplasm at surgery.

Adrenal Medullary Disease

Changes in the adrenal medulla are generally bilateral and mostly reported as pheochromocytoma. However, detailed studies of the medulla have revealed alterations varying from hyperplasia of the diffuse and nodular type to true neoplasia.

Carney *et al.*[130] studied the adrenal glands of 19 patients with the diagnosis of multiple endocrine neoplasia, Type 2. Bilateral pheochromocytoma was present in 10 and was metastatic in 4; unilateral pheochromocytoma with contralateral diffuse and nodular hyperplasia was present

in 2; unilateral pheochromocytoma with contralateral diffuse hyperplasia in 2; bilateral diffuse hyperplasia in 1; and bilateral nodular hyperplasia in 1. Homolateral pheochromocytoma was identified in 1 patient but the changes in the other adrenal were not known. An apparently normal adrenal medulla was found in 2 patients. The significance of the presence of the pheochromocytoma was emphasized by the deaths of 5 patients as a result of the tumor: 2 from pulmonary metastases, 1 from cerebral hemorrhage, and 2 from hypotensive crisis.

DeLellis *et al.*[131] reported the adrenal medullary changes in 10 patients with familial medullary thyroid carcinoma, using technics assuring early detection of an abnormality. Estimates of adrenal medullary and cortical volume were obtained by serial blocking of the adrenals and histologic analysis of representative sections; urinary catecholamine studies were also made. Adrenal medullary hyperplasia of diffuse and multinodular type was found mostly in the head and body regions of the gland. The adrenal medullary volumes in the three cases of the morphometric study ranged from 25 to 31% of the total adrenal volume as compared with the normal value of 8.1 ± 0.4. Thus the study fully supported the derivation of the pheochromocytoma from medullary diffuse or nodular hyperplasia. The catecholamine studies of the urine provided evidence that the earliest manifestations of adrenal medullary hyperfunction was the increased urinary epinephrine/norepinephrine ratio.

Parathyroid Adenopathy

Parathyroid adenoma or hyperplasia is generally much more frequent in multiple endocrine neoplasia, Type 2, and is present in 50% or more of the cases of this type. Wells and Ontjes[92] reported a 60% incidence; Catalona *et al.*,[132] in 6 of 12 documented cases; and Melvin *et al.*,[99] in 9 of 11 cases, 6 with elevated levels of parathormone. Chief cell hyperplasia is the most common change with the familial disease. The hyperplastic changes may be only slightly evident, detected by a relative increase of chief cell elements in proportion to fat cells, as this may be evaluated in the adult.

The parathyroid changes may be a primary alteration as a part of the adenopathy or a result of stimulation of the parathyroid glands through the calcium alterations induced by the excess calcitonin. The rarity of hypocalcemia with medullary carcinoma and the occurence of hyperplasia and adenoma with the neoplasm provide support for the latter, although there is a varied opinion in this regard. Keiser *et al.*[138] consider the parathyroid changes as a result of the genetic origin of the disorder; Melvin *et al.*,[45] finding an unexpected high incidence of such a change in a kindred

with medullary thyroid carcinoma, also favored this. Schimke *et al.*[66] believed it might be related to the increased level of calcitonin and not be of genetic etiology.

Multiple Endocrine Neoplasia, Type 2b (MEN-IIB)

This variant, as stated previously, is basically found in patients of the familial group with medullary thyroid carcinoma, pheochromocytoma, and mucosal neuromas. Parathyroid alterations are absent or rare as compared with Type 2a (MEN-IIA).

The patients have a characteristic appearance, with marfanoid habitus and a distinctive facies, with thick lips, prognathism, multiple neuromas of the tongue, and enlarged corneal nerves.[134] Intestinal ganglioneuromatosis may occur, possibly as a result of the mucosal neuromatous changes; Whittle and Goodwin [135] found reports of 8 proven and 2 probable cases, and added an additional case. As reported by Bartlett *et al.*,[136] the mucosal neuromas appear first and are often evident at birth. Because of the early origin of evident changes, particularly the characteristic facies and neuromatous changes, MEN-IIB may be diagnosed more often, in childhood, than Type 2a.[92] The intestinal involvement may be indicated by gastrointestinal complaints in a patient with the external features of MEN-IIb; and, as noted by Whittle and Goodwin,[135] radiographic abnormalities were common; there had been two reports in which the diagnosis was made by rectal biopsy.[136,137]

Treatment of Multiple Endocrine Adenopathy

Treatment of the thyroid abnormality is surgical and is most successful in the preclinical phase. Wells and Ontjes [92] have stated that a neck exploration should not be done before the possibility of adrenal pheochromocytomas has been investigated and the abdomen explored if tests have indicated its presence.

Since the thyroid neoplasm is usually bilateral, total thyroidectomy is the operation of choice.[92] The potential for C-cell hyperplasia giving rise to neoplasia is a further indication for removal of all thyroid tissue, even though the hyperplastic changes are more dominant in the mid- and upper thirds of the lateral lobes. The presence of positive lymph node metastases requires a neck dissection on the involved side, though prophylactic radical dissection is not advised.[92]

The parathyroid glands are explored and evaluated and usually only those grossly enlarged removed; if all are enlarged, total parathyroidectomy is advised by Wells and Ontjes [92] with heterotopic parathyroid transplantation.[138]

When the diagnosis of adrenal medullary disease has been made, Carney *et al.*[130] recommend bilateral total adrenalectomy and excision of any extraadrenal paraganglioma.[139]

Treatment and Prognosis of Medullary Thyroid Carcinoma

The only effective treatment known at present is surgery; the tumor has a poor response to irradiation and no proved chemotherapeutic agent is yet available. Factors of importance in consideration of a surgical approach to the thyroid are the occasional multiplicity of neoplastic components in the thyroid, especially in the familial type. The presence of cervical lymph node metastases in at least half of the patients and the penetration of lymph node capsules by some tumors are other important considerations.

Total thyroidectomy or, as a minimal procedure, total removal of the involved thyroid lobe and the isthmus and subtotal removal of the contralateral lobe,[140] have been recommended; the former is most often employed.[45,75,78,141] Exploration of lymph nodes of the cervical areas and biopsy of those enlarged has been advised for assessment of metastases.[140] Hill *et al.*[74] reported that the majority of patients having total thyroidectomy had a standard radical or modified neck dissection. Chong *et al.*[75] found a slightly higher incidence of recurrence in patients with the modified neck dissection, with a 10% incidence of incomplete clearance of involved nodes, and favor radical neck dissection when there has been spread to the regional nodes.

In a review of 82 cases of medullary thyroid carcinoma, Ibanez[78] reported 32 deaths due to the neoplasm; in 60 patients treated 3 or more years before the study, 32 (53%) were living; 13 for 10 years or more. Chong *et al.*[75] reported the results of adequate follow-up of 137 patients, with a median of 7.4 years (range of 3 months to 34 years); 78 were living, 27 (19%) had died of their disease; the 5-year and 10-year survival were 80 and 67%, respectively.

Woolner[142] in a report of 77 cases, found a marked variation in survival rate in association with the presence or absence of cervical lymph node involvement. In the absence of such metastases, the 5-year and 10-year survivorships closely approximated normal; however, for patients with lymph node involvement, the 10-year survival was 42 percentage points below the normal rate. Chong *et al.*,[76] in a review of the larger series (137 patients), reported a similar relation of survivorship to lymph node involvement: 65 patients without nodal involvement had a nearly normal survivorship of 86% at 10 years and those with nodal involvement had

46%. A decreased survival time was found in patients over 50 years of age, even after correction for a shorter life-span.

The survivorship in patients in the familial group has been better than the nonfamilial type. Chong *et al.*[75] reported only 3 of 29 patients dead as a result of the neoplasm (a range of 4 to 17 years of follow-up), despite the presence of bilateral thyroidal involvement and the incidence of cervical lymph node metastases similar to the nonfamilial type. They suggested that the neoplasia in the familial disease might be less aggressive. Another factor, of course, is the earlier recognition of the neoplasm in the familial group through suspicion of its occurrence and its more frequent demonstration in the preclinical phase.

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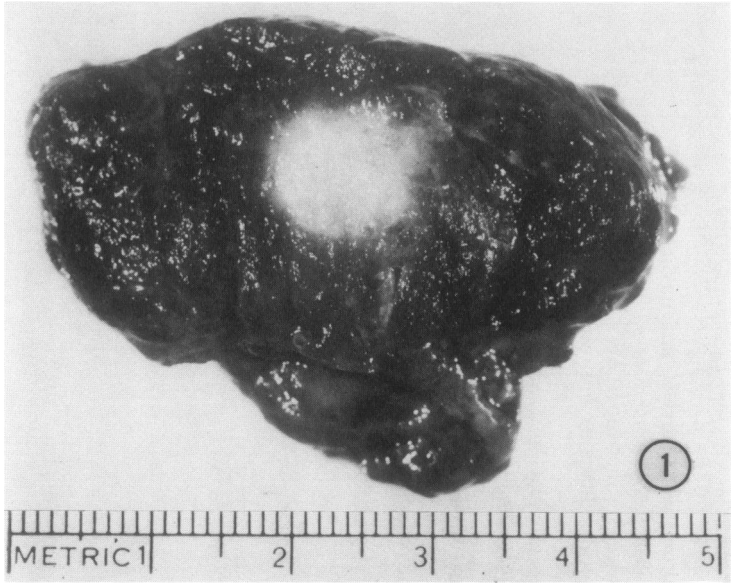

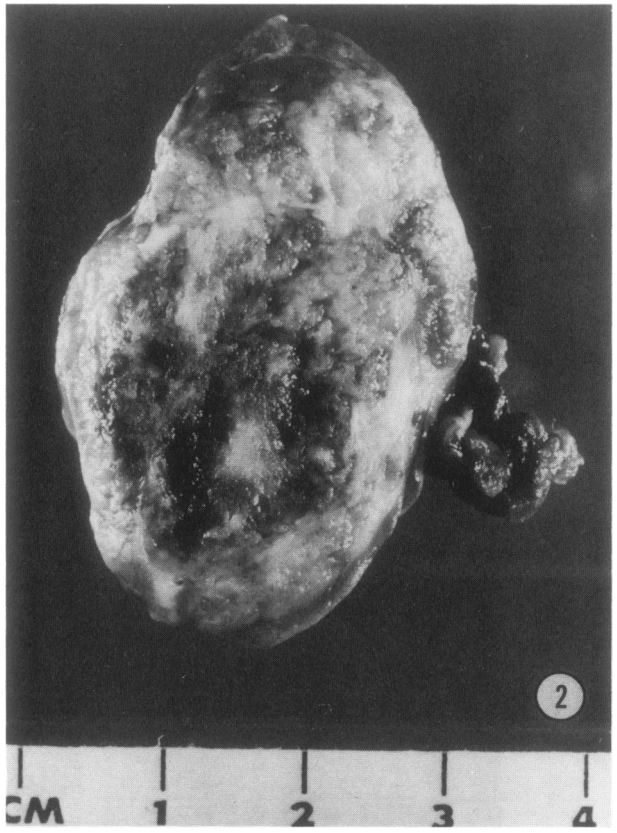

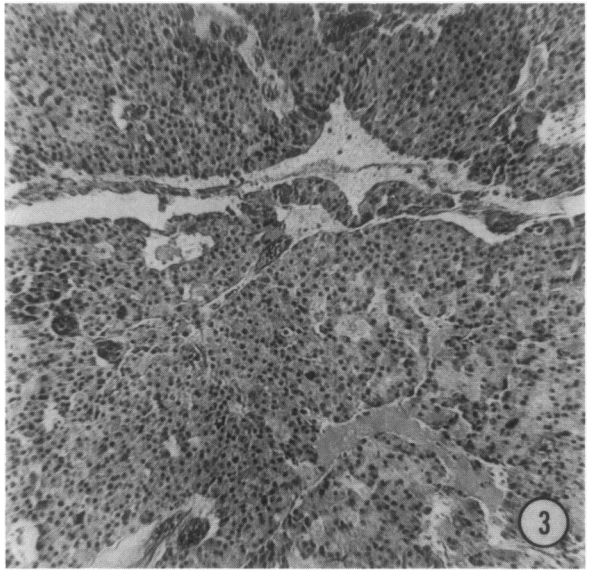

Figure 1—Neoplasm sharply circumscribed in part with other areas of local invasion of thyroid tissue. The tumor was of hard consistence, with a flat cut surface, and white, with a few light tan areas.

Figure 2—Medullary carcinoma of larger than usual size, replacing most of the thyroid lobe but with retention of thyroid capsule; the cut surface was generally slightly bulging. Tissue was of firm consistence, gray-white or light tan to white color, with purplish and dark reddish purple areas.

Figure 3—Sheets and lobules of neoplastic cells, with homogeneous stromal patches (amyloid). Tumor cells of round or polygonal shape; uniform distribution of nuclei and low nuclear/cytoplasmic ration. Most nuclei were of similar size but with a few larger and darker staining. (× 100)