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Comparative Ultrastructure of Thyroid, Tongue and Eyelid Lesions in the Neuroma Phenotype of Medullary Carcinoma of the Thyroid

Association of Amyloid with Fibroblasts in Thyroid Tumor and in Mucosal Neuromas

J. A. Thliveris, W. J. Dubé, and R. Banerjee

Department of Anatomy, University of Manitoba, Winnipeg, Manitoba, Canada; Department of Medicine, University of Manitoba and Consultant in Endocrinology, Winnipeg Clinic, Winnipeg, Manitoba, Canada; Department of Pathology, University of Manitoba, Winnipeg, Manitoba, Canada

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Summary. Electron-microscopic and histochemical studies of thyroid tumor, tongue neuromas and eyelid neuromas from the lesions of a patient with medullary thyroid carcinoma were compared. In the thyroid tumor, a significant number of the C cells showed heterogeneity of granule types; no C cells, however, were identified in the tongue and eyelid neuromas. Amyloid was clearly shown by Congo red staining in the thyroid neoplasm and in the tongue neuromas. In all tissues, amyloid fibrils were found to be ultrastructurally closely associated with fibroblasts. These fingings suggest that the fibroblast rather than the C cell may have played the important role in the deposition of amyloid in this patient's thyroid carcinoma.

Key words: Ultrastructure — Thyroid — Mucosal neuromas.

Medullary carcinoma of the thyroid is considered to be a neoplasm of the parafollicular or C cells of the thyroid gland (Williams, 1966; Dubé *et al.*, 1969; Meyer and Abdel-Bari, 1968; Hill *et al.*, 1973). Calcitonin, which is secreted by these parafollicular cells, is elevated (Tashjian and Melvin, 1968; Cunliffe *et al.*, 1968; Aliapoulios *et al.*, 1969; Milhaud *et al.*, 1968) in patients with this neoplasm so that its measurement in the blood affords the best criterion for establishing the diagnosis (Melvin *et al.*, 1971; Baylin *et al.*, 1972). Clinically, medullary carcinoma of the thyroid usually presents as a normal phenotype (Dubé *et al.*, 1969; Meyer and Abdel-Bari, 1968; Hill *et al.*, 1973; Tashjian and Melvin, 1968) and is often associated with bilateral pheochromocytomas. It is also seen, less frequently, as a neuroma phenotype (Cunliffe *et al.*, 1968; Aliapoulios *et al.*, 1969; Williams and Pollock, 1966; Melvin *et al.*, 1972) with multiple mucosal neuromas, a marfanoid habitus and hyperparathyroidism. The clinical manifestations of medullary thyroid carcinoma are suggestive of a variant neuropolyendocrine syndrome (Schimke and Hartmenn, 1965; Weichert, 1970) and since the disorder sometimes has a familial occurrence (Hill *et al.*, 1973; Melvin *et al.*, 1972; Ljungberg *et al.*, 1967; Bartlett *et al.*, 1971), it can be considered to be the result of

an inherited genetic defect of neuroendocrine cells (Melvin *et al.*, 1972; Weichert, 1970; Schimke *et al.*, 1968).

Several morphological studies of medullary carcinoma of the thyroid have been reported. Light-microscopic findings of thyroid and other tissues have been described in patients where medullary carcinoma of the thyroid, pheochromocytoma and mucosal neuromata coexisted (Williams and Pollock, 1966; Schimke *et al.*, 1968); in patients where medullary carcinoma was found in association with only pheochromocytomas (Schimke and Hartmenn, 1965; Ljungberg *et al.*, 1967; Slavotinek *et al.*, 1968); and, in patients where the thyroid tumor alone was found (Williams *et al.*, 1966; Ibanez *et al.*, 1966). Electron microscopic studies, on the other hand, have been confined mainly to thyroid tissue (Meyer and Abdel-Bari, 1968; Hill *et al.*, 1973; Meyer, 1968; Gonzales-Licea *et al.*, 1968; Braunstein *et al.*, 1968; Meyer *et al.*, 1973), metastatic lymph nodes (Meyer and Abdel-Bari, 1968) and pulmonary (Gordon *et al.*, 1973) involvement, and all the studies have been examined in cases of the normal phenotype.

The present communication is the first report of comparative ultrastructural studies from three different tissue sites in the same individual with the neuroma phenotype of medullary thyroid carcinoma. The electron microscopic findings of the thyroid, tongue neuromas and eyelid neuromas from this patient are described in detail. Unlike previous reports (Meyer, 1968; Gonzalez-Licea *et al.*, 1968; Meyer *et al.*, 1973) a significant number of C cells were found to have heterogeneity of granule types. More importantly, in the mucosal neuromas of the tongue and eyelids, there was ultrastructural and histochemical evidence of amyloid which, because of its close relationship with the fibroblasts present, suggests that the amyloid was associated with the fibroblasts rather than with the C cells.

Methods

The tissues studied were the surgically-excised thyroid tumor and biopsies of neuromas form the dorsum of the tongue and the margins of the eyelid of the same patient. Full clinical details will be reported elsewhere.

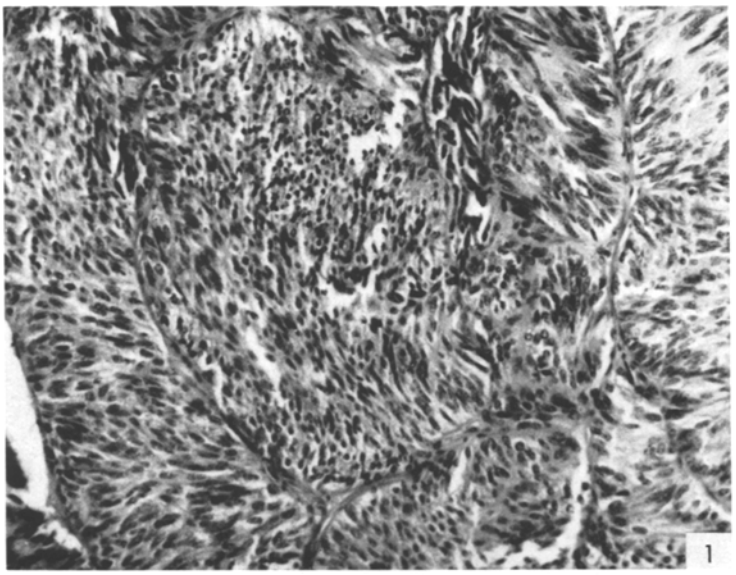
For light microscopy, specimens of the thyroid and tongue were fixed in 10% buffered formalin. Five micron sections were stained with hematoxylin and eosin for routine examination, and with Congo red for determination of the presence of amyloid.

For electron microscopy, portions of the thyroid, tongue and eyelid lesions were initially fixed in 3% glutaraldehyde buffered in 0.1 M phosphate (pH 7.4) for 2 hours at 4°C. The specimens were rinsed overnight in 0.1 M phosphate buffer (pH 7.4) containing 0.2 M sucrose and subsequently post-fixed in 1% osmium tetroxide buffered in 0.1 M phosphate (pH 7.4) for 2 hours at 4°C. After rapid dehydration in ascending concentrations of ethanol, the specimens were embedded in Epon 812 according to the method of Luft (1961). Thin sections were stained with uranyl acetate and lead citrate, viewed, and photographed in a Philips EM 201 electron microscope.

Results

Light Microscopy

Histological examination of the thyroid lesion revealed the presence of alveolar masses and sheets of polygonal and spindle-shaped cells (Fig. 1), the latter predominating. The cells were separated by a hyaline stroma (Fig. 2) containing foci of calcification and areas giving a positive reaction for amyloid (green birefringence with Congo red).



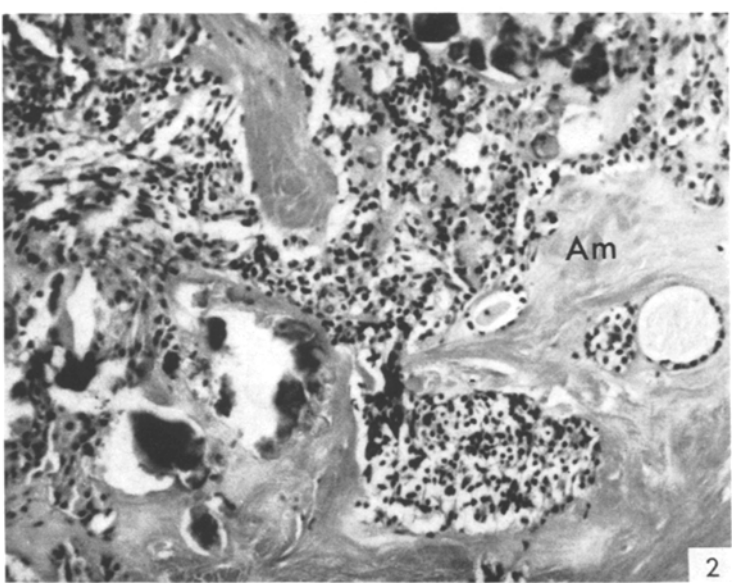
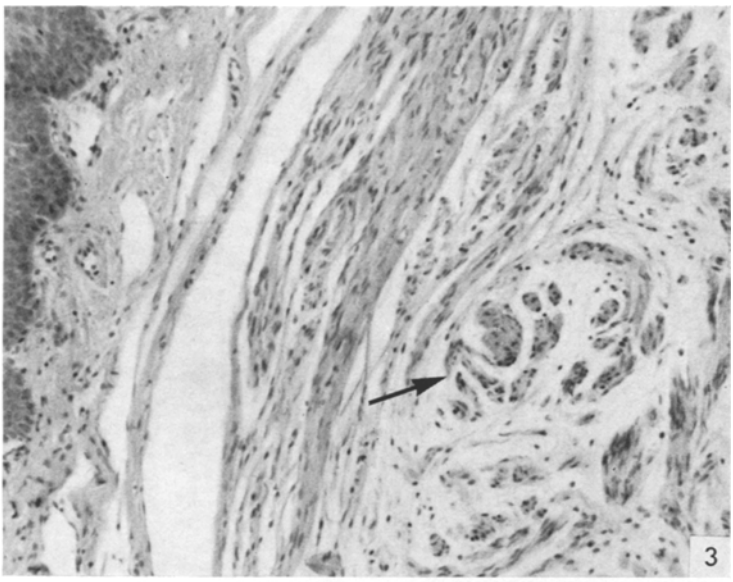


Fig. 1. Light photomicrograph of the typical pattern of medullary carcinoma of the thyroid showing solid sheets of polygonal and spindle shaped cells (Hematoxylina and Eosin ×520)

Fig. 2. Light photomicrograph of medullary carcinoma of the thyroid with deposits of amyloid (*Am*) within the stroma of the neoplasm. Darkly stained areas are foci of calcification (Hematoxylin and Eosin ×520)

Biopsies of the tongue lesions showed clusters of nerve fibers scattered in a haphazard manner throughout a loose connective tissue matrix (Fig. 3). Amyloid was clearly demonstrated by Congo red staining of the tissue.



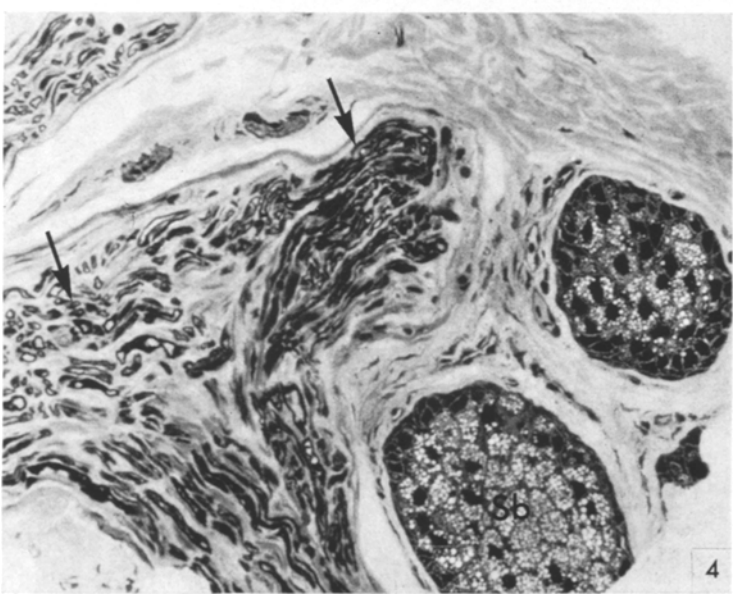


Fig. 3. Light photomicrograph of biopsied tongue neuroma from medullary carcinoma of the thyroid. Clusters of nerve fibers (arrows) are seen scattered throughout the loose connective tissue (Hematoxylin and Eosin ×520)

Fig. 4. Thick Epon sections (1 μ) of biopsied eye neuroma from medullary carcinoma of the thyroid showing numerous myelinated nerve fibers (arrows). Sebaceous glands (*Sb*) are also shown. (Toluidine blue stain ×850)

The small eyelid neuroma contained numerous myelinated nerve fibers and sebaceous glands in a loose connective tissue matrix (Fig. 4). There was insufficient material available for Congo red staining.

Electron Microscopy

Thyroid. The tumor cells of the thyroid tissue were tightly packed and contained numerous membrane-bounded granules that varied in density and size (2,000–4,000 angstroms in diameter). The cells exhibited moderate numbers of polymorphic mitochondria and well-developed Golgi membranes and associated vesicles (Fig. 5). Rough-surfaced endoplasmic reticulum was present and arranged as tubular profiles.

Fibroblasts were seen as elongated cells with long, narrow cytoplasmic processes. They had an irregular nucleus which occupied a very large area of the cell body; tubular profiles of rough-surfaced endoplasmic reticulum, numerous free ribosomes and polymorphic mitochondria were present (Fig. 6).

Numerous collagen fibers, with their characteristic periodicity, as well as short thin fibrils similar to amyloid, were seen scattered throughout the stroma of the tumor tissue. The amyloid fibrils, approximately 150 angstroms in diameter, were not associated in any way with the granule-containing cells described above but appeared to be in contact with the plasmalemma of the fibroblasts (Fig. 7). Plasma cells and lymphocytes were not seen, nor were there any nerve elements such as Schwann cells, myelinated and non-myelinated fibers.

Tongue and Eyelid Lesions. The ultrastructural findings of the lesions of the tongue and eyelid were similar (Figs. 8—10). The surface epithelia of the neuromata appeared normal. The underlying dermis, on the other hand, exhibited numerous clusters of Schwann cells, myelinated and non-myelinated nerve fibers (Fig. 8). Fibroblasts (Fig. 9) were frequent and mast cells were occasionally seen within the stroma. There were no cells in the stroma that had any ultrastructural resemblances to C cells, plasma cells or lymphocytes.

The Schwann cell cytoplasm (Figs. 8, 9) exhibited an oblong nucleus, numerous ribosomes, glycogen and short profiles of rough-surfaced endoplasmic reticulum. Moderate numbers of polymorphic mitochondria and well-developed Golgi complexes were also observed in these cells. A prominent basal lamina surrounded each cell (Fig. 8).

The myelin sheath of the medullated fibers was well developed. A thin but prominent basal lamina surrounded the axons of both myelinated and non-myelinated fibers. The axoplasm of myelinated and non-myelinated nerve fibers contained numerous neurofilaments and few mitochondria.

Collagen fibers were abundant and scattered throughout the nervous tissue elements. The characteristic fibrils of amyloid (Fig. 10) were seen interspersed with the collagen fibers and often in direct contact with the plasma membrane of the fibroblasts. This amyloid was not seen to be associated with the nervous tissue components.

Discussion

Medullary carcinoma of the thyroid is a unique neoplasm because of its specific histological features and ability to synthesize and secrete calcitonin. The cells elaborating calcitonin, referred to as C cells or parafollicular cells, are markedly more numerous in tumor tissue than in normal thyroid tissue (Wolfe *et al.*, 1973). The C cells described in this report, while morphologically similar to tumor C cells described in previous studies (Meyer, 1968; Gonzalez-Licea *et al.*, 1968; Braunstein *et al.*, 1968; Meyer *et al.*, 1973), had a significant pattern of

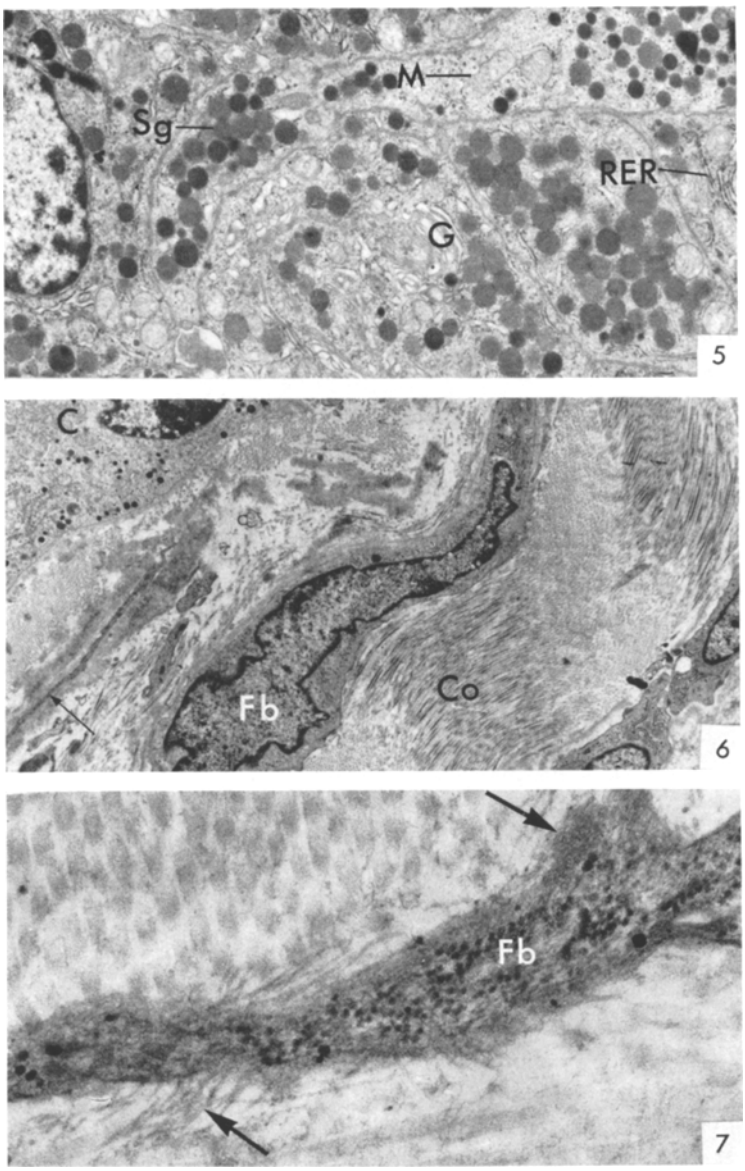


Fig. 5. Electron photomicrograph of tumor cells of the thyroid in medullary carcinoma of the thyroid. These cells are characterized by the presence of numerous secretory granules (*Sg*) varying in density and ranging between 2,000–4,000 A in diameter. Also present are polymorphic mitochondria (*M*), Golgi membranes (*G*), and tubules of rough-surfaced endoplasmic reticulum (*RER*). (×10,900)

Fig. 6. Electron photomicrograph of fibroblasts (*Fb*) from the thyroid carcinoma. Fibroblasts are typically elongated with attenuated cytoplasmic processes (arrow). Collagen fibrils (*Co*) and a tumor cell (*C*) are also shown (×5,400)

Fig. 7. Portion of a fibroblast (*Fb*) from the thyroid lesion of medullary thyroid carcinoma. Note the close association between the cell membrane and small thin fibrils (150 A in diameter) that are morphologically similar to amyloid fibrils (arrows). Collagen fibrils, much larger in size and exhibiting periodicity, are readily apparent (×65,000)

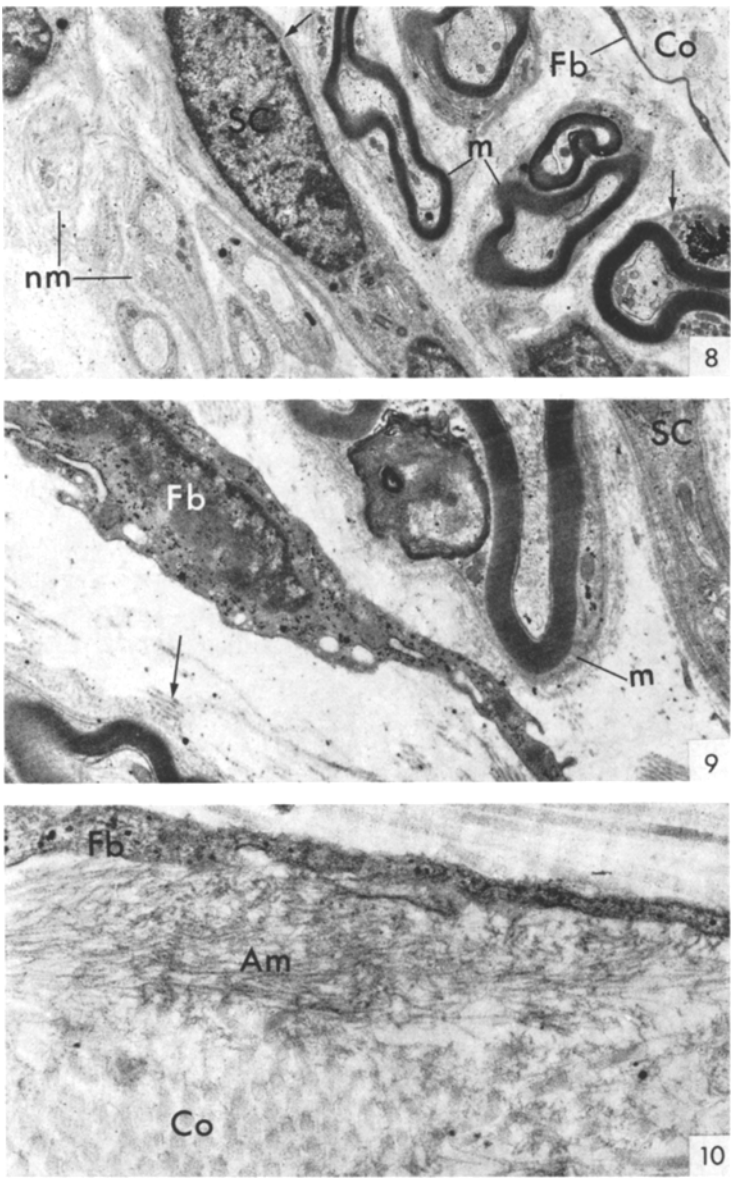


Fig. 8. Electron photomicrograph of the dermis of a tongue neuroma. Neuronal elements shown are Schwann cells (*SC*), myelinated fibers (*m*), and non-myelinated (*nm*) fibers. Note the presence of a well defined basal lamina (arrows) that surrounds the neuronal elements. Collagen fibrils (*Co*) and a portion of a fibroblast (*Fb*) are also shown (×4,700). Similar morphological features were seen in the eyelid neuromas

Fig. 9. Fibroblasts, such as the one seen here (*Fb*) in a tongue neuroma, are frequently observed in both tongue and eyelid neuromata. Also, shown are a myelinated fiber (*m*) and a portion of a Schwann cell (*Sc*). Collagen fibers (arrows) are dispersed throughout the area (×8,300)

Fig. 10. Electron photomicrograph of an eyelid neuroma. Frequently detected in the lesion are numerous thin amyloid fibrils (*Am*) interspersed among the larger collagen fibrils (*Co*), and in close association with a fibroblast's (*Fb*) cellular membrane (×47,500)

granule distribution. A large number of these cells exhibited a heterogeneity of granule types.

The granules of C cells in thyroid tissue have been reported to be associated with the amyloid fibrils seen histologically and it has been suggested (Meyer, 1968; Meyer *et al.*, 1973) that the C cells are the source of this amyloid in the thyroid. If this were the case, then one would expect to see amyloid in mucosal neuromas, which contain few C cells if any. However, our case showed unquestionable ultrastructural evidence of amyloid fibrils in both tongue and eyelid neuromas studied and there was, in addition, histochemical confirmation of amyloid in the tongue lesion. Since our electron microscopic findings suggest that the mucosal neuromata associated with phenotypic medullary carcinoma of the thyroid do not contain C cells, this is strong evidence in favor of a fibroblastic origin for the amyloid observed. Further to that, the amyloid fibrils observed were in direct contact with the plasma membranes of both mucosal neuroma fibroblasts and thyroid tumor fibroblasts.

Amyloid associated with fibroblasts is not a new phenomenon. The presence of amyloid in some non-endocrine diseases—macular cutaneous amyloidosis (Brownstein and Hashimoto, 1972; Hashimoto and Brownstein, 1972), actinic keratosis (Hashimoto and King, 1973), and amyloidosis of the eyelid and conjuctiva (Halasa, 1965; Richlin and Kuwabara, 1962)—is confined to dermal layers and there is evidence (Brownstein and Hashimoto, 1972; Hashimoto and Brownstein, 1972; Hashimoto and King, 1973) that fibroblasts are the source of the amyloid produced in these disorders. Also, in cases of peripheral polyneuropathy with amyloid deposition, amyloid has been seen not only in contact with fibroblast cell membranes but also within the cytoplasm of the fibroblasts (Coimbra and Andrade, 1971).

The finding of fibroblasts within a tumor of neural crest origin is not embryologically unusual. Since phenotypic medullary carcinoma of the thyroid is probably a result of gene-environment interaction (Schimke and Hartmenn, 1965; Schimke *et al.*, 1968), it is likely that the lesions of the thyroid, tongue and eyelids are embryologically related. The concept of the neural crest origin of C cells is well supported (Pearse and Carvalheira, 1967; Pearse and Polak, 1971) so that both C cells and neuromas can be considered to be of neuroectodermal origin. The possibility exists that a common neuroectodermal cell, or closely related cell-type, destined to develop into tumorous C cells or abberrant nervous tissue migrated during embryonic development from the neural crest to the anlage of the thyroid, tongue and eyelids. Such a possibility does not exclude the fibroblast which is primarily derived from the mesenchyme, for it has been pointed out (Arey, 1965) that some embryonic mesenchyme is derived from ectoderm giving rise to mesectoderm and indirectly to neural crest tissue. Also, since the mesenchyme is an extraordinarily versatile tissue with the potential for a variety of expression under differing conditions during its course of development, it is not unlikely that mesectodermal elements of the neural crest are the ones which could selectively migrate to different tissue sites in medullary thyroid carcinoma and subsequently produce amyloid.

The presence of amyloid appeared to be closely related to the fibroblasts in all of the three sites where it was seen in our case of the neuroma phenotype

of medullary thyroid carcinoma. There is no evidence to suggest that the fibroblast per se is an immunologically active cell so that this cannot be considered as representative of an immune phenomenon. The function of this amyloid, if any, is not known but it is interesting to speculate that the fibroblast, if it is the cell that is secreting amyloid, may be attempting to retard tumor growth.

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Dr. J. A. Thliveris
Department of Anatomy
University of Manitoba
730 William Avenue
Winnipeg, Manitoba
Canada R3E OW3