Histochemical Characteristics of Parafollicular Cells and Medullary Thyroid Carcinoma

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Normal canine parafollicular cells share a common set of histochemical characteristics, which include masked metachromasia and argyrophilia, with human as well as rat medullary thyroid carcinomas. Masked metachromasia, a property of polypeptide hormone-producing cells, was demonstrated by staining with toluidine blue or coriophosphine O after acid hydrolysis. Argyrophilia was demonstrated both by the Grimelius silver nitrate technic and by the Azzopardi modification of the Bodian method. With these technics, parafollicular cells in the dog and medullary carcinoma of human and rat origin showed nearly identical staining reactions. Fixation in glutaraldehyde was superior to formaldehyde in demonstrating masked metachromasia of normal dog parafollicular cells, while formaldehyde fixation was superior for the demonstration of argyrophilia of granules. In general, the Grimelius method was superior to the Azzopardi modification of the Bodian technic for the demonstration of argyophilia. The results of this study provided further support for the parafollicular cell origin of human and rat medullary thyroid carcinomas and also provided a useful set of histochemical criteria for the diagnosis of this neoplasm (Am J Pathol 72:119–128, 1973).

MEDULLARY CARCINOMA of the thyroid is a distinctive neoplasm which was first described as a definite clinical and pathologic entity by Hazard *et al* in 1959.[1] The typical medullary carcinoma is composed of nests and cords of epithelial cells which are surrounded by an amyloid-containing stroma. The tumor may assume a wide spectrum of growth patterns, ranging from a predominant spindle cell configuration to areas where follicle formation is evident. Because of this variation in histologic appearance, it is important to characterize the biochemical, histochemical and ultrastructural features of these neoplasms to allow more precise classification.

Recent investigations have revealed that medullary carcinomas produce calcitonin both *in vivo* and *in vitro*.[2–4] In addition, the tumors may elaborate vaso-active peptides as well as 5-hydroxytryptophan and serotonin.[2] These neoplasms are thought to be derived from parafollicular or C-cells of the thyroid.[3–5]

A similar naturally occurring neoplasm has been noted in 12 to 45%

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of aged Long-Evans rats.[6] Most of these tumors are regarded as low grade malignancies, and it is of considerable interest that two of the more malignant tumors had focal areas of stromal amyloid deposition.[7] More recently, Boorman *et al* have noted that 37% of aged WAG/Rij rats developed medullary thyroid carcinomas.[8] Electron microscopic examination confirmed the presence of dense core secretory granules similar to those noted in the human tumors and normal parafollicular cells.[8] The purpose of the present investigation was to compare the histochemical characteristics of normal canine parafollicular cells with human and rat medullary carcinomas.

Materials and Methods

Fresh specimens of thyroid tissue were obtained from 2 male adult mixed breed dogs and from 2 patients with familial medullary thyroid carcinoma. Tissue blocks measuring 3 to 5 mm in thickness were fixed in 4% formaldehyde solution (10% neutral formalin) for 24 hours. Additional portions of dog thyroid were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 6 hours. After routine dehydration and embedding, 6-μ thick paraffin sections were prepared. Formalin-fixed paraffin-embedded rat thyroid tumor (WAG/Rij strain) was kindly supplied by Dr. C. F. Hollander and was processed in a similar fashion.

Tissue sections were stained with hematoxylin and eosin for evaluation of overall tissue architecture. Additional sections were stained according to each of the following methods: lead hematoxylin for 2 hours at 45 C (Solcia variant of MacConnaill's method);[9] Grimelius' argyrophil technic (variant 1, 24 hours at 37 C);[10] the Azzopardi modification of Bodian's argyrophil method for 36 hours at 37 C;[11] Solcia's toluidine blue (0.01% toluidine blue in 0.02 M McIlvaine buffer, pH 5.0, following hydrolysis in 0.2 N HCl at 60 C for 6 hours);[12] Bussolati's coriophosphine O (0.01% coriophosphine O in 0.2 M acetate buffer, pH 5.2, following hydrolysis in 0.2 N HCl at 60 C for 6 hours);[13] Ljungberg's 0.05% aqueous cresyl fast violet.[14]

Results

Hematoxylin and eosin-stained sections of normal dog thyroid revealed typical clusters of polyhedral cells with faintly basophilic cytoplasm between the follicles and occasionally within the basement membrane of the follicle. The results of the histochemical reactions of normal canine parafollicular cells and human and rat medullary carcinomas are summarized in Table 1. Coriophosphine O staining after acid hydrolysis imparted an intense orange-red metachromasia to the cytoplasm of parafollicular cells. The intensity of the staining reaction was greater in sections which had been fixed in glutaraldehyde than those fixed in formaldehyde. Staining with toluidine blue after acid hydrolysis revealed an identical cell population characterized by red-purple cytoplasmic metachromasia (Figure 1). The results with toluidine blue were similar to those obtained with coriophosphine, in that the intensity of the metachromasia was greater in

Table 1—Histochemical Characteristics of Normal Canine Parafollicular Cells and Human and Rat Medullary Thyroid Carcinomas

	Normal dog thyroid		Human medullary carcinoma	Rat medullary carcinoma
Fixation	G	F	F	F
Stain				
Coriophosphine O*	+++	+ to ++	+ to ++	+ to ++
Toluidine blue*	+++	+ to ++	++	+ to ++
Cresyl fast violet	+	— to ±	—	—
Lead hematoxylin	++	++	++	++
Grimelius	+ to ++	+++	+++	+ to ++
Azzopardi	+	+ to ++	± to +	± to +

* stained after hydrolysis in 0.2 N HCl at 60 C for 6 hours
G = glutaraldehyde fixation; F = formaldehyde fixation; intensity of staining designated as 0 to +++

glutaraldehyde-fixed tissues. It is of interest that gastric argyrophil cells in some species and adrenal medullary cells in all species have been reported to give a positive toludine blue-induced metachromasia without previous acid hydrolysis.[15]

Formaldehyde-fixed dog thyroid tissue which was stained according to the Grimelius technic revealed a finely granular yellow-brown precipitate within the cytoplasm of parafollicular cells (Figure 2). This reaction, although present in glutaraldehyde-fixed tissues, was considerably less distinct and intense than in formaldehyde-fixed material. Tissues stained with the Azzopardi modification of the Bodian technic gave a positive but weak reaction. Primary fixation in formaldehyde was superior to glutaraldehyde in the visualization of argyrophil granules with the Azzopardi technic. Cresyl fast violet stained the cytoplasm of parafollicular cells weakly in sections which had not been subjected to previous acid hydrolysis. Lead hematoxylin, on the other hand, stained the cytoplasm of parafollicular cells equally well after glutaraldehyde or formaldehyde fixation.

The histopathologic features of both the human and rat thyroid carcinomas are similar to those which have been described previously.[1,8] Coriophosphine O staining after acid hydrolysis imparted a pale orange-red metachromasia to the cytoplasm of medullary carcinoma cells both in the human and in the rat (Figures 3 and 4). In both cases, the intensity of the staining reaction was less than that noted in glutaraldehyde-fixed normal canine thyroid but comparable to that noted after formaldehyde fixation. Toluidine blue staining after acid hydrolysis revealed an intense red-purple granular cyto-

plasmic metachromasia in the 2 cases of human medullary carcinoma (Figure 5). In the rat tumor, many of the component cells revealed a faint cytoplasmic metachromasia with scattered strongly stained individual cells (Figure 6). Cresyl fast violet failed to stain either the human or rat thyroid tumor cells selectively.

Lead hematoxylin stained the component cells of both the human and rat tumors (Figure 7). The two cases of human medullary carcinoma gave a strongly positive Grimelius reaction, while the rat tumor gave a somewhat less intense reaction (Figures 8 and 9). The Azzopardi reaction was weakly positive, both in the human and in the rat tumors (Figure 10).

Discussion

Human medullary thyroid carcinomas may exhibit a wide range of histologic appearances. Areas within the primary tumor and foci of metastatic tumor may lack the characteristic stromal amyloid deposits. Because of the prognostic implications of the diagnosis of medullary carcinoma and the high familial incidence, it is imperative to establish an unequivocal pathologic diagnosis. While ultrastructural studies may not be possible on an individual case because of the small size of the biopsy or improper fixation, histochemical studies may aid in the delineation of histogenesis of the tumor.

The stains employed in the present study have been used previously to study the distribution of polypeptide hormone-producing cells (APUD system).[16] The various cell types within this system have the capacity to concentrate and decarboxylate precursors of biogenic amines and, in addition, are capable of synthesizing a specific polypeptide hormone. These cells share a common set of histochemical characteristics which include masked metachromasia,[12,13] argyrophilia,[10] high levels of acid phosphatase, and esterase and/or cholinesterase activity.[16]

The property of masked metachromasia after acid hydrolysis and staining with toluidine blue or coriophosphine O is thought to be dependent on the presence of a random coil polypeptide with a high concentration of side chain carboxyl groups. Acid hydrolysis not only removes basophilic substances such as RNA and DNA but also may convert side chain carboxamido groups into carboxyls.[15] Lead hematoxylin may also reflect the presence of side chain carboxyl groups.[9] The characteristic argyrophilia, as demonstrated by the Grimelius and Azzopardi methods, probably reflects the presence of catecholamines and/or indolylethylamines. However, the contribution of the

protein content of the granules to argyrophilia in these cells may also be significant.

Comparison of normal canine parafollicular cells with human and rat medullary thyroid carcinomas reveals nearly identical staining reactions. These results provide further support for the idea that both the human and rat tumors are of parafollicular cell origin. It is also apparent that the intensity of the staining reaction is dependent on the initial fixation. In the case of masked metachromasia with toluidine blue or coriophosphine O, fixation in glutaraldehyde gives a more intense and uniform staining reaction. Lead hematoxylin appears to give equally good results with formaldehyde or glutaraldehyde fixation. The argyrophil reactions appear more intense in sections which had been fixed initially in formaldehyde. In contrast to the studies of Ljungberg,[14] we have not found cresyl fast violet to be of value in the study of normal or neoplastic parafollicular cells.

Previous investigators have studied the distribution of argyrophilic substances in medullary thyroid carcinomas. Using the Bodian reaction in an ultrastructural study of human medullary carcinoma, Tateishi *et al* were able to demonstrate deposits of reduced silver within the secretory granules of the tumor cells.[17] We have found that the argyrophilia of normal canine parafollicular cells and of human and rat medullary carcinomas can be demonstrated more regularly and uniformly with the Grimelius stain than with the Azzopardi modification of the Bodian reaction.

Ljungberg has recently described two types of cells in human medullary thyroid carcinomas.[18] The more numerous cell type, similar to that noted in the present report, was characterized by argyrophilia and basic dye metachromasia. The less numerous cell, also referred to as "spider cell," gave a positive argentaffin reaction with the Masson-Fontana technic and also contained orthochromatic granules.[19,20] Although we noted similar spider cells in human and rat medullary thyroid carcinomas, these cells gave a distinctive metachromasia after acid hydrolysis with toluidine blue and coriophosphine O.

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[*Illustrations follow*]

Fig 1—Normal dog thyroid, fixed in glutaraldehyde and stained with toluidine blue after acid hydrolysis. Groups of parafollicular cells *(arrow)* are characterized by an intense red-purple cytoplasmic metachromasia which appears black in this reproduction (× 250).

Fig 2—Normal dog thyroid, fixed in formaldehyde and stained according to the Grimelius technic. The parafollicular cells contain a finely granular yellow-brown cytoplasmic precipitate which appears black in this reproduction *(arrow)* (× 250).

Fig 3—Human medullary carcinoma stained with coriophosphine O after acid hydrolysis. Individual tumor cells contain pale orange-red metachromatic cytoplasmic granules which appear white in this reproduction (× 400).

Fig 4—Rat medullary carcinoma stained with coriophospine O after acid hydrolysis. The cells show a staining pattern similar to the human medullary carcinoma (× 250).

Fig 5—Human medullary carcinoma stained with toluidine blue after acid hydrolysis. The tumor cells in this area are spindle shaped and show a red-purple cytoplasmic metachromasia (× 315).

Fig 6—Rat medullary carcinoma stained with toluidine blue after acid hydrolysis. Most of the cells show a pale cytoplasmic metachromasia while occasional cells are strongly stained *(arrow)* (× 315).

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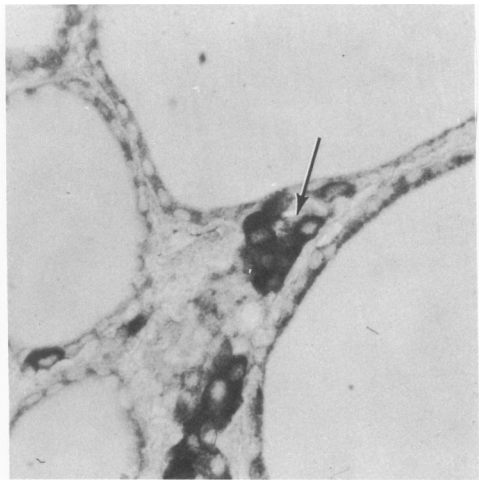

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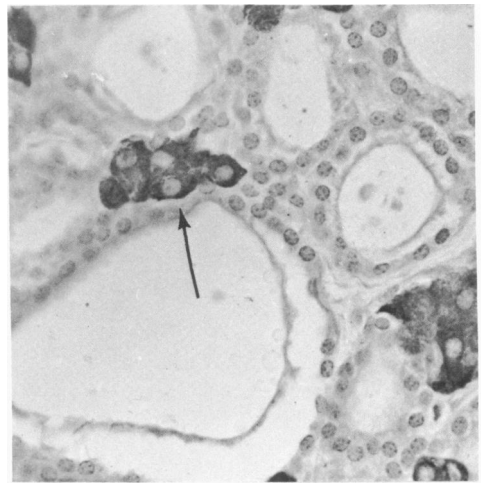

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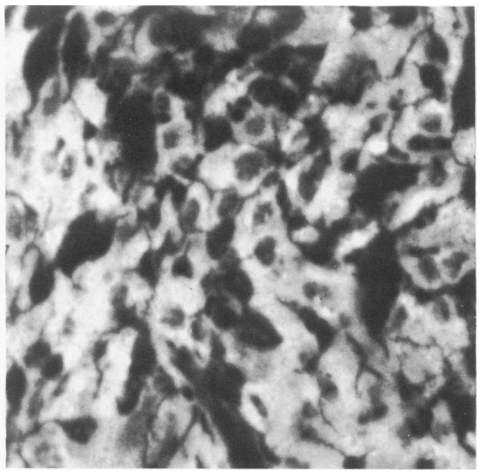

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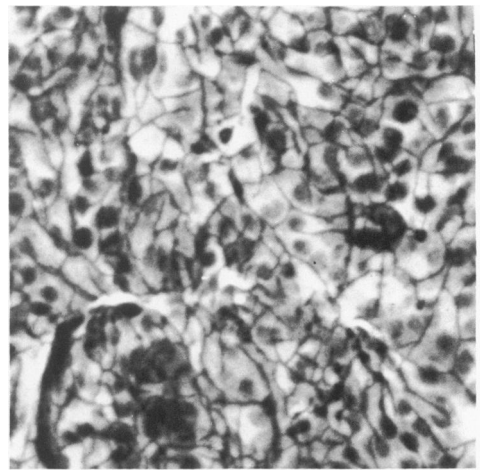

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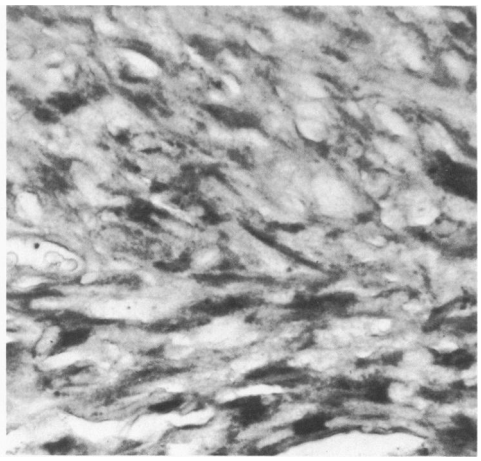

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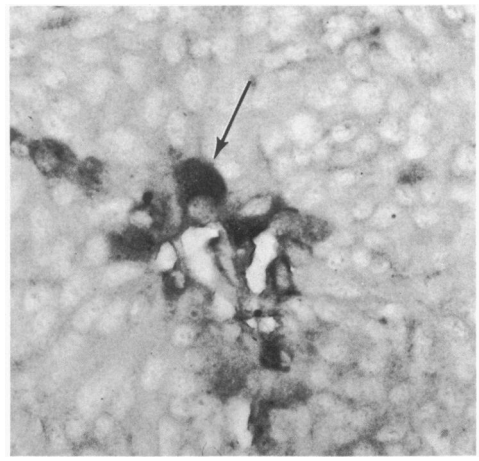
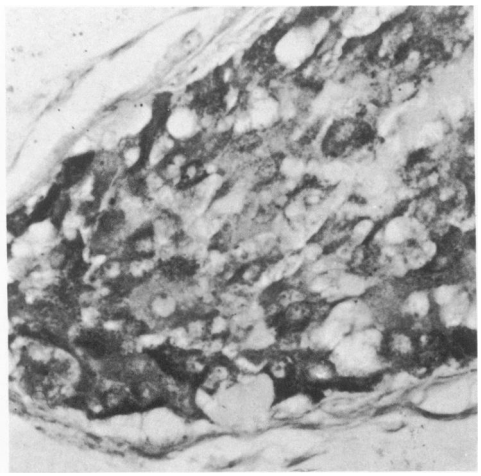




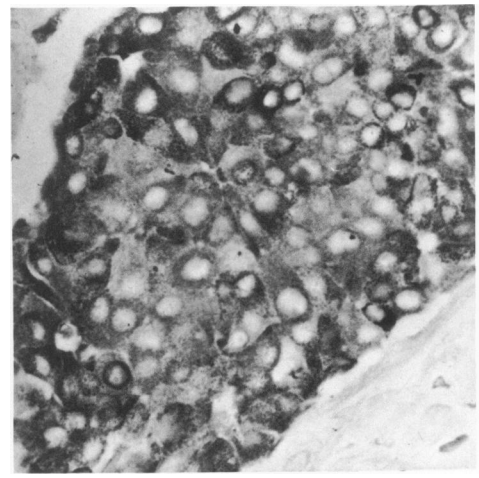
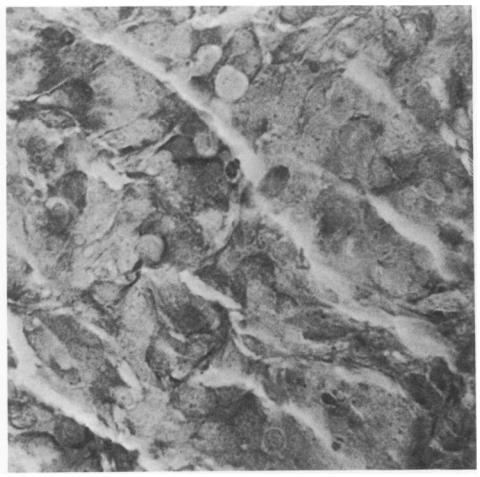


Fig 7—Human medullary carcinoma stained with lead hematoxylin. Most of the tumor cells contain blue-black cytoplasmic granules (× 250). **Fig 8**—Human medullary carcinoma stained according to the Grimelius technic. The individual tumor cells contain abundant brown-black cytoplasmic granules (× 250).



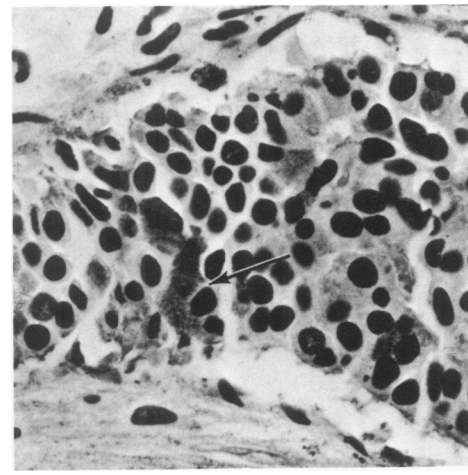


Fig 9—Rat medullary carcinoma stained according to the Grimelius technic. Although less intensely reactive than the human tumor, the rat tumor cells show an unequivocally positive reaction (× 250). **Fig 10**—Human medullary carcinoma stained according to the Azzopardi modification of the Bodian method. Occasional cells contain finely granular black intracytoplasmic deposits (*arrow*) (× 250).