

## Histological Detection of Thyroid Medullary Carcinoma by Selective Stainings

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The histological demonstration of human thyroid calcitonin or C cells in normal conditions is not very easy under light microscopy (1-6). Among the methods proposed for such a purpose there are: A) staining of C cell granules by toluidine blue, or pseudoisocyanin, or coriphosphine O after acid hydrolysis with dilute hydrochloric acid; B) simple staining with lead haematoxylin; C) acid hydrolysis followed by lead haematoxylin; D) silver impregnation according to Grimelius; E) immunohistochemistry.

Among these methods procedure A) is commonly working, provided a suitable fixation is adopted (the best being glutaraldehyde), but the preparations are labile; staining B) is also satisfactory in several vertebrates, but it fails to work in man (at least in our hands); method C) gives fairly good results and stable preparations, but it greatly prefers glutaraldehyde mixtures for fixation; impregnation D) seems to be decidedly preferable to the previous techniques, because it works on formaldehyde fixed material, too, and the slides are permanent; immunohistochemistry, of course, is very brilliant, but its use is practically limited to scientific investigations.

Within the family of thyroid neoplasms, the medullary carcinoma, at first identified as a separate histopathologic entity (7), has been recently shown to produce calcitonin as well as a peculiar clinical syndrome (8) and to reproduce histological, histochemical and ultrastructural features of C cells (4; 9-14).

On this basis we planned to demonstrate the neoplastic C cells by using techniques reliable for normal C cells.

By revising the series of primary thyroid carcinomas diagnosed in our Department (mainly surgical specimens, generally fixed in formalin and embedded in paraffin), 12 cases of medullary carcinoma out of 136 thyroid tumours were detected. The silver impregnation was applied in our 12 cases and the results were as follows: silver positive reaction in 9 cases; negative reaction in 3 cases. The positive cases showed various amounts of silver impregnated granules in their cell population, some cells being apparently devoid of reactive secretory granules; when present, the cytoplasmic granulations were far less

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numerous than in normal human C cells. In two cases a good deal of silver impregnated granules was detected in all tumour cells; these cases also reacted consistently with basic dyes or lead-haematoxylin following HCl treatment, while other cases showed scarce reactivity to these techniques. The clinical histories of the first 7 cases were silent from the endocrine point of view, while the other two had mild hypocalcaemia, symptoms of latent tetany, hypophosphoraemia, severe watery diarrhea, hypokalaemia and paresis. One of these, besides bilateral multiple medullary carcinomata of the thyroid, had multiple mucosal neuromas too, with special location in the tongue, lips and eyelids, this association having already been noticed with or without concomitant pheochromocytoma (15). The simultaneous growth of thyroid C cells and cells known to originate from neural crests (as adrenal chromaffin cells, nerve cells and carotid body cells) fits in perfectly with the recently demonstrated origin of thyroid C cells from neural crests (16).

The comparison between clinical and morphological data achieved underlines: A) some possible parallelism existing between the amount of secretory granules and the endocrine function, even in tumour cells; B) like for other polypeptide-producing endocrine tumours, the neoplastic element fails either to produce or just to store high amounts of hormone in its cytoplasmic granulations. The question seems to remain open whether the non-argyrophil tumours are true medullary carcinomas, deriving from the C cells, but functionally atypical as to have lost secretory properties (undifferentiated medullary carcinomas), or are pseudo-medullary tumours, somewhat related with the principal thyroid cells.

Whenever a thyroid tumour shows a solid architecture we stress the pathological and even clinical importance of checking its possible silver impregnation by the Grimelius' technique (17, 18).

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Fig. 1 shows proliferation of medullary carcinoma adjacent to normal thyroid structures. Haem.—eos.

Fig. 2 evidences the spindle-shaped pattern with giant cells sometimes displayed by the neoplasia. Haem.—eos.

Fig. 3 illustrates the rich stromal amyloid deposition very often present between tumoral cords. Haem.—eos.

Fig. 4. Strong silver impregnation of cytoplasmic granulations in all tumour elements. Grimelius' silver method.

