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Immunohistochemical Analysis of Thyroglobulin Synthesis in Thyroid Carcinomas *

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Summary. This immuno-histochemical description of thyroglobulin synthesis in human thyroid carcinomas is based on the analysis of 72 malignant thyroid neoplasms and about 100 cases of thyroid adenomas and other diseases of the thyroid gland.

In our experience immuno-histochemistry has been an invaluable diagnostic adjunct to light microscopy for three reasons: 1) as an approach to a functional classification of thyroid carcinomas, 2) as an aid in the differential diagnosis of thyroid carcinomas of follicle cell type from tumors of other origins, 3) as an aid in the functional classification of non-cancerous thyroid tissue.

In the field of metastasizing thyroid carcinoma this immuno-histochemical approach combined with a morphometrical method may enable accurate identification of patients for whom radioiodine therapy is appropriate.

Key words: Tyhroid carcinoma – Immuno-histochemistry – Thyroglobulin synthesis – Differential diagnosis.

Introduction

It is well known that thyroid tumors, derived from the follicular epithelium, still show some organ specific functions (Taylor, 1953; Leslie et al., 1960; Owen et al., 1960; Bay, 1965; Horst et al., 1967; Valenta et al., 1967; Valenta et al., 1968; Pochin and Thompson, 1969; Schneider et al., 1970; Arvy, 1971; Thomas-Morvan et al., 1974; Valenta et al., 1974; Heinze et al., 1975; Valenta, 1976; Johannessen et al., 1978). The most important of these is the unique biological

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ability to trap and organify iodides, which may be exploited in the management of approximately 70% of all organoid cancers (Schneider, 1973; Bürgi and Labhart, 1974; Ingbar and Woeber, 1974; Klein et al., 1976; Werner and Ingbar, 1978). Although the histologic classification of malignant thyroid tumors recommended by the WHO (1974) represents a morphological approach with therapeutic value (Hirabayashi and Lidsay, 1961; Russell et al., 1963; Woolner et al., 1968; Meissner and Warren, 1969; Bokelmann et al., 1970; Franssila, 1971; Neracher and Hedinger, 1975; Heitz et al., 1976; Droese, 1977; Georgii, 1977; Krisch et al., 1977; Löhrs et al., 1977) it still gives little information of the functional properties of the tumor tissue.

Our knowledge about the variety of factors influencing radioiodine uptake and organification in neoplastic thyroid cells is incomplete. From the cytophysiology of normal thyroid tissue the synthesis and secretion of thyroglobulin by neoplastic cells appears to have a most important role in this context. A high degree of correlation between the radioiodine uptake and thyroglobulin content of thyroid carcinoma is shown for example by a previous biochemical study of Valenta and Michel-Bichet (1977). The aim of the present study is to analyse by an immuno-histochemical method the content and distribution of thyroglobulin of thyroid carcinomas as compared to normal and non-cancerous thyroid tissue.

Material and Methods

Preparation of Thyroglobulin. Thyroglobulin was obtained from human thyroids which were removed for small papillary carcinomas or for benign diseases. The tissue was homogenized with twice its volume of 0.9% NaCl. Coarse debris was separated by centrifugation (4,000 × 15 min). The supernatant was dialysed against 0.2 M phosphate buffer, ph 7.2 and further purified on Sephadex G 200. The first peak which read at 280 nm was collected, lyophilized and used as antigen. This antigen was analysed by polyacrylamide gel electrophoresis according to the method of Ornstein and Davies (1962).

Immunisation Schedule. 5 mg antigen dissolved in 0.2 ml saline and emulsified in 0.2 ml complete Freund's adjuvant (Difco Labatories, Detroit) was injected into the footpads of white rabbits weighing about 2,500 g. After 3 and 5 weeks the animals were boostered in the same manner. The antisera obtained after 7 weeks showed only a single precipitation line against thyroid extracts and purified thyroglobulin.

Immuno-Peroxidase Staining. 5 samples of normal thyroid tissue, 11 diffuse or nodular goiters, 7 toxic diffuse goiters, 70 adenomas (Institute of Pathology of the University of Hamburg) and 72 thyroid cancers (Institute of Pathology, General hospital Hamburg-Harburg) were stained using the triple layer method. Usually 3–4 paraffin-embedded tissue blocks of these tumors were cut at 6 μm. After deparaffinization and rehydration the slides were treated in the following order: a) rabbit anti-human thyroglobulin, diluted 1:20 up to 1:1,000. b) goat anti-rabbit-gamma-globulin, dilution constantly 1:5, c) PAP-complex, diluted 1:5 up to 1:100 (Sternberger et al., 1970).

Histochemical staining was carried out according to Graham and Karnowsky (1960). Control slides were performed by replacing specific antisera in stead of normal rabbit antiserum. 10 breast carcinomas, 8 papillary serous ovarian carcinomas, 5 endometrial carcinomas, 3 carcinomas and 2 adenomas of the parotid gland and 3 papillary adenocarcinomas of the kidney were immunohistochemically analysed in the same manner.

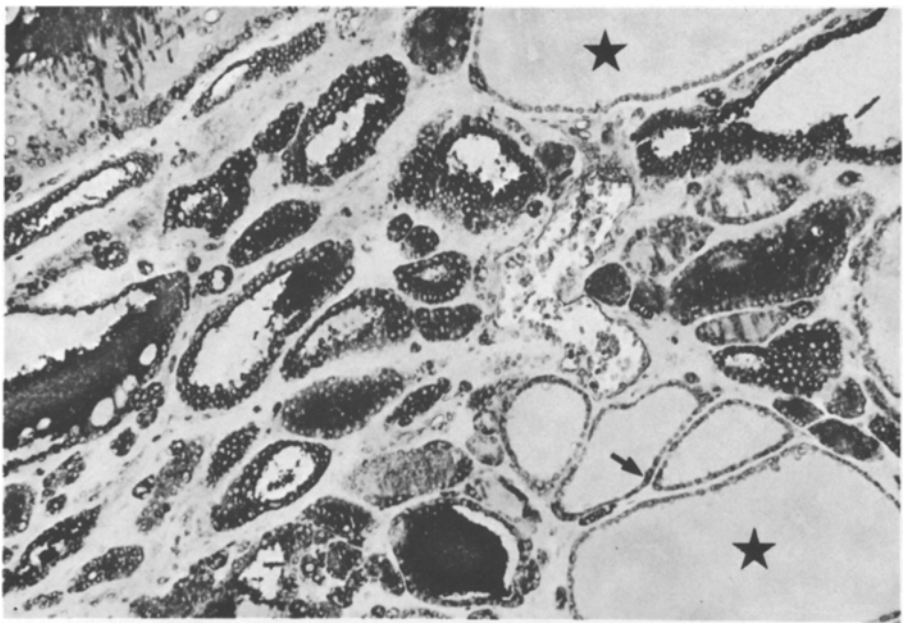


Fig. 1. Normal thyroid gland showing an intense immunohistochemical staining both in the cytoplasm of thyrocytes as well in the follicular lumina. Note feeble staining of the inactive follicular lining of "colloid follicles" *(arrow)* and negative colloid *(asterics)*. PAP-method. Weak counterstaining with heamtoxylin. × 100

Results

Before embarking on a description of thyroid carcinomas a brief account will first be given of immuno-histochemical findings of normal tissue and non-cancerous thyroid disorders.

The vast majority of follicles of normal thyroids are composed of cuboidal cells well outlined by specific reaction product (Fig. 1). Only the cytoplasm and to a lesser degree the colloid are endowed with thyroglobulin, the nuclei and the interstitium being consistently negative.

The histological and cytological changes of diffuse or nodular goiter vary greatly according to cause (iodine deficiency, inborn errors of metabolism and Graves' disease) and duration of the disease. From the point of the follicular unit these may be discussed under two headings: a) hyperplastic follicles with tall columnar epithelium and moderate to scant colloid, b) large atrophic follicles with flattened epithelium and extensive accumulation of colloid.

In a): the current study reveals an abundance of thyroglobulin in the cytoplasm of cells of hyperplastic follicles – independent of the cause of hyperplasia. On the other hand the follicular lumina usually contain much more specific reaction product in diffuse toxic goiters than do those of hyperplastic thyroids caused by inborn errors of metabolism or iodine deficiency (Fig. 2). As noted previously the pattern of distribution obtained for thyroglobulin in diffuse toxic goiters is identical with that in toxic autonomous adenomas (Dralle and Böcker, 1977).

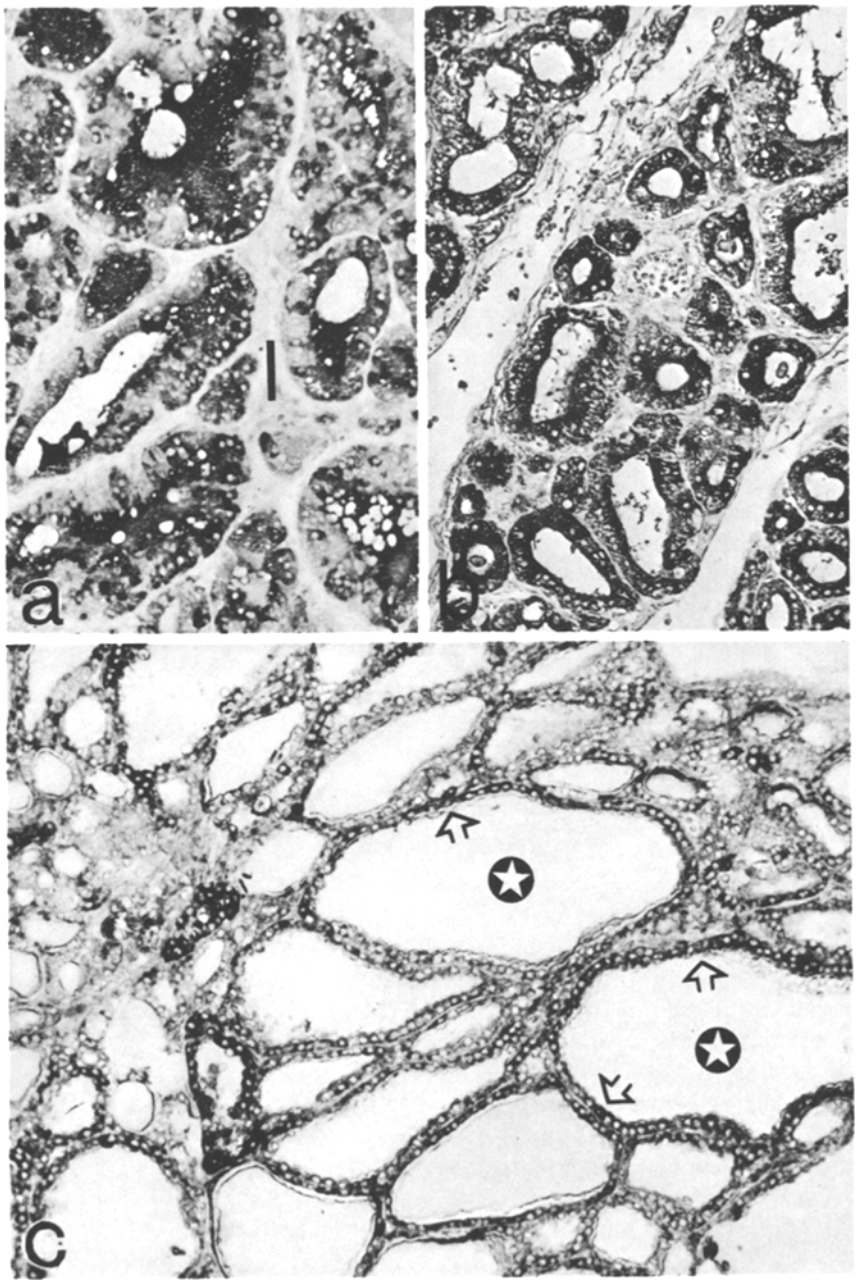


Fig. 2a–c. Diffuse hyperplastic goiters. **a** Graves' disease (M. Basedow). The hyperplastic follicles contain much cytoplasmic *and* luminal thyroglobulin. The interstitium (*I*) and the nuclei are completely devoid of thyroglobulin. ×400. **b** Dyshormonogenetic goiter of a 14-year-old female child without hypothyroidism. The thyroglobulin is confined to the cytoplasm of the hyperplastic follicles. The lumina show only weakly immunohistochemical reaction product. ×400. **c** Thyroid in Graves' disease after preoperative treatment with iodine. Note the replenishment of the colloid store which gives a completely negative immunohistochemical reaction (*asterics*), while the thyrocytes still contain thyroglobulin (*arrows*). ×100

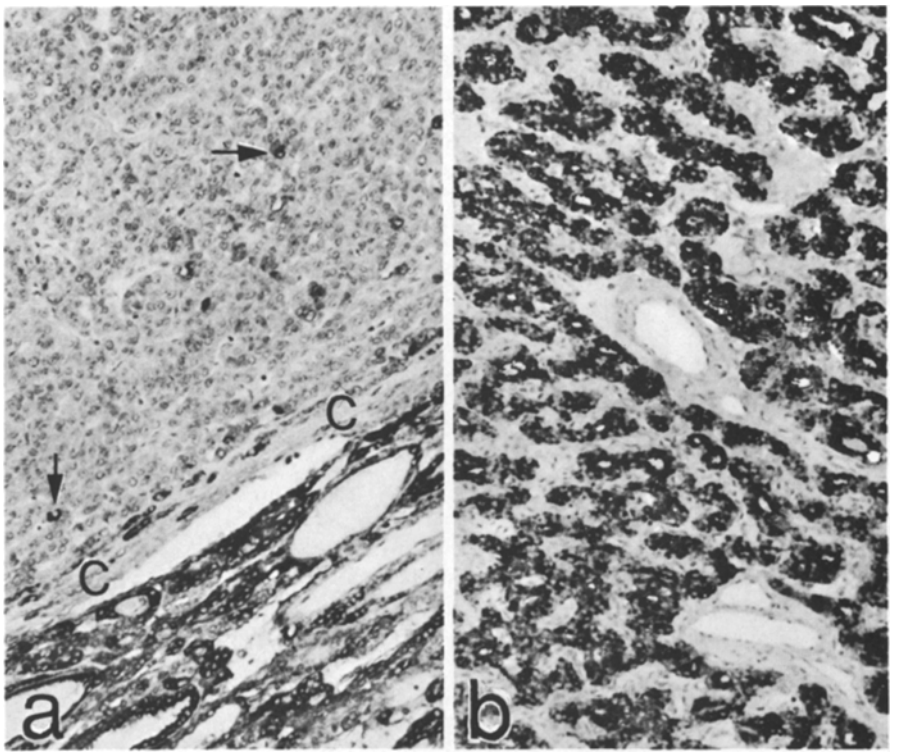


Fig. 3. a Oxyphile adenoma with only occasional positive cells *(arrows)*. Note intense staining of paranodular tissue. *C*=capsule. ×100. **b** Toxic autonomous adenoma (hot nodule) of trabecular type. Intense immunocytochemical staining of the cytoplasm of thyrocytes and occasionally of lumina. ×100

In b): disappearance of thyroglobulin has been found, regularly associated with morphological advancement of involution exemplified by the changes following preoperative therapy of diffuse toxic goiters with iodine (Fig. 2).

Immuno-histochemical studies on thyroglobulin in thyroid adenomas have recently been published (Dralle and Böcker, 1977; Böcker et al., 1978). From these immuno-histochemical and electron microscope studies it is evident that the content and pattern of distribution of thyroglobulin in these tumors depends mainly on the cytoplasmic differentiation of the tumor cells. Tumors of special cell type like the oxyphilic and clear cell adenomas are nearly devoid of thyroglobulin (Fig. 3a) while toxic adenomas and adenomas of ergastoplasm-rich cells regularly contain much specific reaction product. Compared with nodular goiters and most thyroid carcinomas the pattern of distribution of thyroglobulin is much more homogeneous in adenomas.

Thyroid Carcinomas

The immuno-histochemical results of 72 cases of thyroid neoplasms are recorded in Table 1.

Table 1. Immuno-histochemical analysis of 72 thyroid tumors

Microscopic diagnosis	Total number of cases	Cases with positive thyroglobulin (percentage in parentheses)	Mean values of a Semiquantitative analysis of PAP-positive cells within the tumors
Papillary carcinoma	37	35 (95%)	30%
Follicular carcinoma	9	9 (100%)	40%
Anaplastic carcinoma	10	4[a] (40%)	1%
Medullary carcinoma	9	0 (0%)	–
Other carcinomas[b]	7	0 (0%)	–

[a] In these cases the thyroglobulin was exclusively found in organoid structures (compare text)

[b] Most of these represented metastases of bronchial, mammary or renal carcinomas

The follicular carcinomas are devisible into two distinct clinicopathological entities: slightly invasive follicular carcinoma, which is encepsulated and typically invades capsular vessels; and overtly invasive follicular carcinoma. All tumors of these groups investigated showed a cytoplasmic synthesis of thyroglobulin immuno-histochemically (Fig. 4a and b). The follicular lumina often seemed to be devoid of this antigen although in routinely stained sections colloid is often found. Usually the pattern of distribution is quite patchy involving larger islands of tumor cells in the immediate neighbourhood of negative neoplastic cells.

With the exception of two cases all papillary carcinomas are characterized, at least focally, by specific cytoplasmic staining. This is often feeble but is well brough out by the three layer enzyme method (Fig. 5). In pure papillary carcinomas three immuno-histochemically different cell types can be distinguished, often intermingled with each other (compare Fig. 6): a) tumor cells devoid of thyroglobulin, b) tumor cells with focal thyroglobulin synthesis, often in the apical cytoplasm, c) tumor cells with an intense immuno-histochemical staining throughout the cytoplasm. Independent of the degree of thyroglobulin synthesis within the tumor cells the interpapillary spaces are nearly always devoid of this glycoprotein or at least contain only small amounts. Similarly PAS-positive material was frequently missing. Occasionally thyroglobulin can be visualized as a small rim at the apical cell corder. Epidermoid differentiation in papillary carcinomas was correlated with decreasing thyroglobulin production. Psammoma bodies are free of thyroglobulin. Follicular structures of papillary carcinomas show identical thyroglobulin contents to those described under follicular carcinomas. Graham – tumors and encapsulated papillary carcinomas showed the same thyroglobulin-pattern. Immunohistochemically there were usually no differences between primary tumors and metastases of papillary carcinomas. The diagnostic value of this special aspect is substantiated by the following case report.

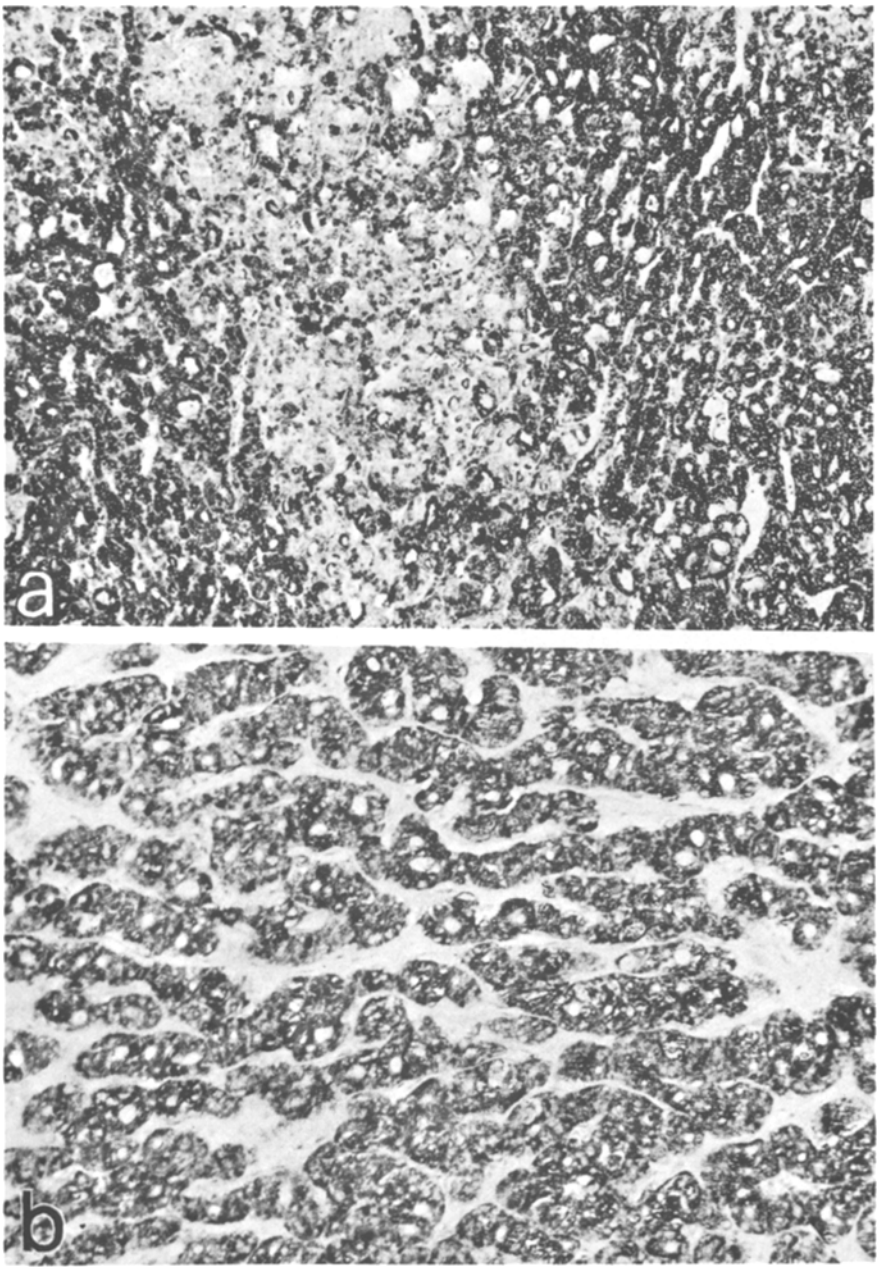


Fig. 4. Follicular carcinoma. **a** Prominent cytoplasmic staining in the left and right area, which shows a follicular differentiation. ×100. **b** Predominantly tubular type of carcinoma. Moderate to intense staining of nearly all tumor cells. ×250

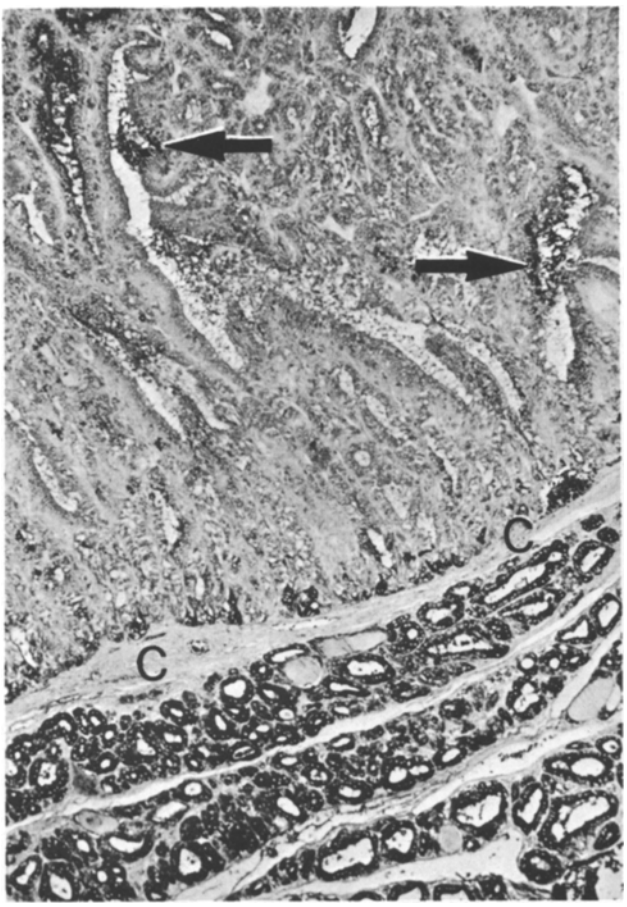
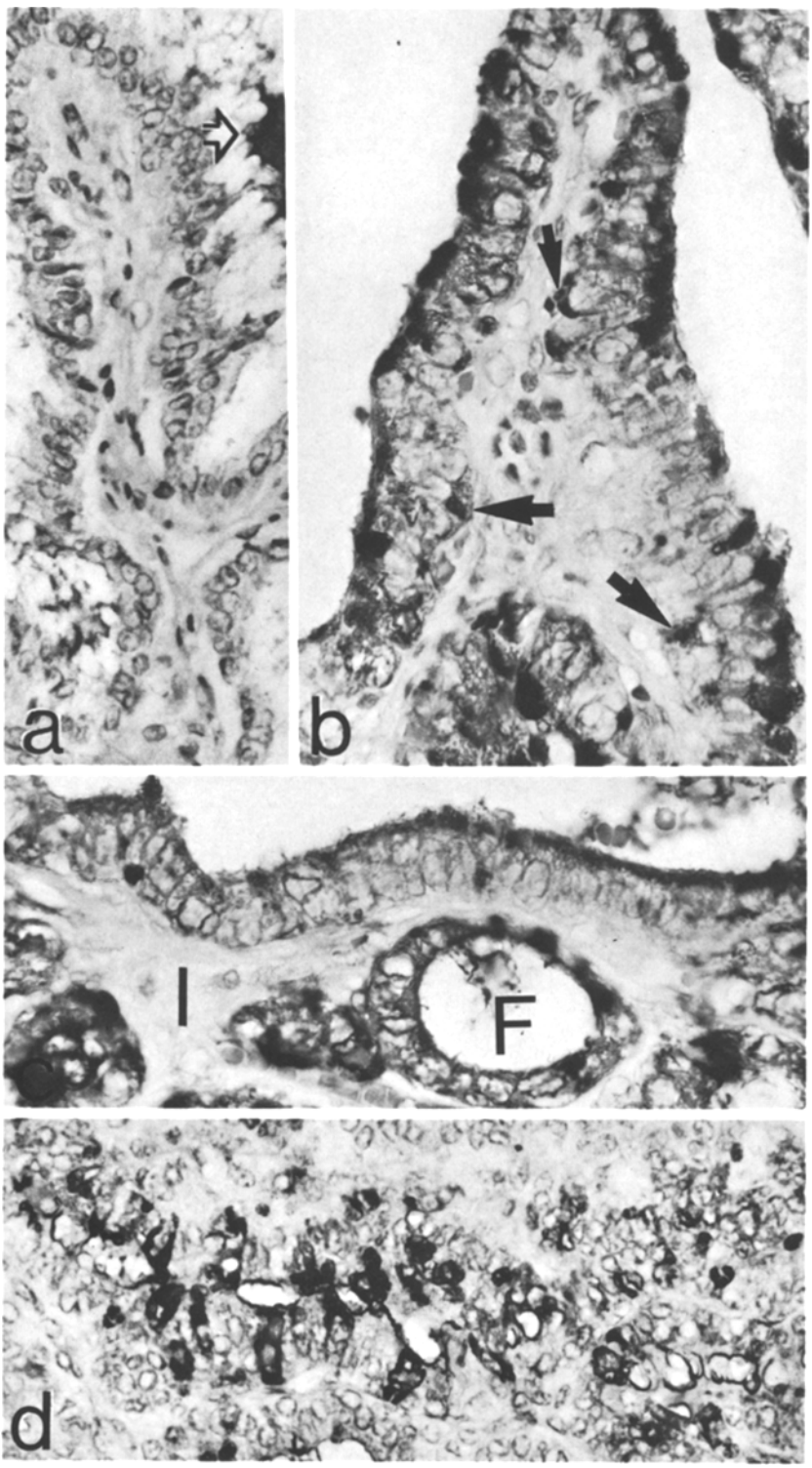


Fig. 5. Encapsulated papillary thyroid carcinoma. Compared to the intense immunohistochemical reaction of the normal thyroid there is only a patchy thyroglobulin distribution within the tumor *(arrows)*. *C*=capsule. ×250

Case Report

A man of 61 years of age without a known history of thyroid disease had noticed a gradual development of a very hard, painless, mammary swelling without any other general sign. In April, 1977, he underwent surgical removal of the tumor mass 6 cm in diameter. Histologically a papillary adenocarcinoma was found without psammoma bodies and containing only very few follicle-like

Fig. 6a–d. Papillary carcinoma. **a** Neoplastic papilla with completely negative tumor cells with ill-defined cell borders. The interpapillary space contains thyroglobulin *(double arrow)*. ×400. **b** Neoplastic papilla with intensely staining tumor cells. The thyroglobulin is mostly localized in the apical cytoplasm and only occasionally in the basal portion of the tumor cells *(arrows)*. ×400. **c** Basal portion of a papilla and adjoining neoplastic follicles. The cytoplasm of the follicular structures (*F*) shows a slightly more intense reaction. *I*, interstitium. ×400. **d** Portion of predominantly solid area with few heavily staining cells in the near neighbourhood of negative cells. ×400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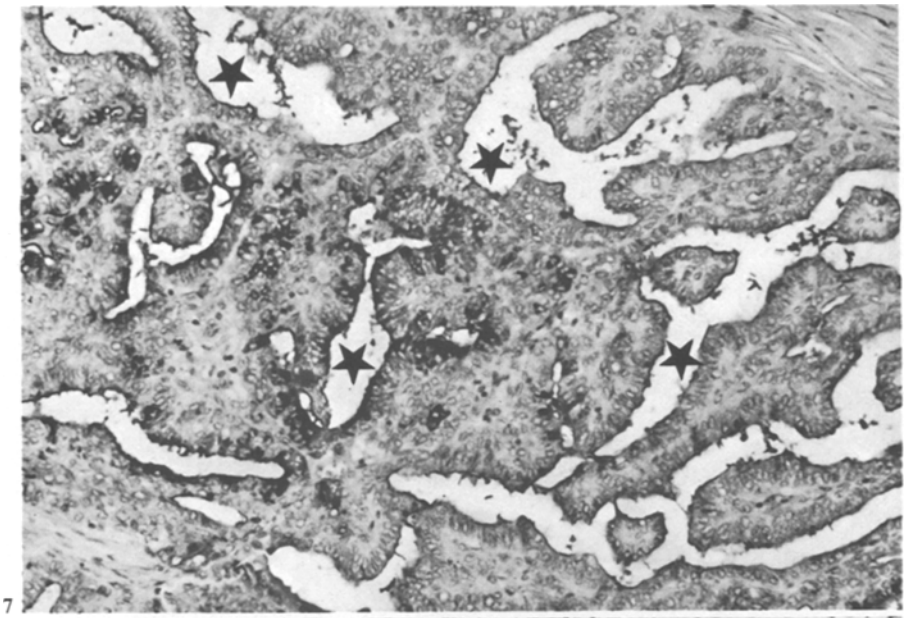

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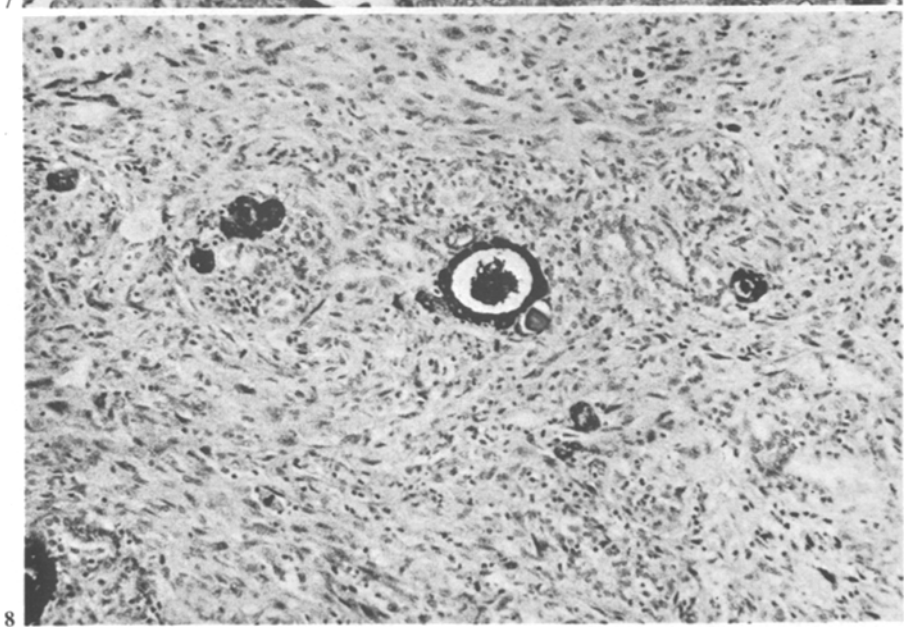

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Fig. 7. Papillary thyroid carcinoma. Area with a typical thyroglobulin content and distribution. There is only sparse thyroglobulin within interpapillary spaces *(asterics)*. Occasional areas with many positive cells are found. ×250

Fig. 8. Anaplastic thyroid carcinoma of spindle cell type. The anaplastic parts are completely devoid of thyroglobulin. Included are positive staining follicles (normal ? – neoplastic ?). ×100

structures (J.-No. 12714/77). The tumor was thought to represent a metastasis of a thyroid carcinoma. Immunohistochemical studies were therefore performed. As the tumor showed a patchy but well defined thyroglobulin synthesis (Fig. 7) it was interpreted as a metastasis of a papillary thyroid carcinoma. Surgical resection of the thyroid gland (May, 1977) revealed a nodular goiter weighing 120 g with a region of white induration in the left lobe of about 2.5 cm in diameter (J.-No. 15536/77). The histological examination showed a predominantly papillary thyroid carcinoma with occasional follicular structures and epidermoid differentiation. In June and August 1977 lymph nodes were excised containing further metastases of a papillary thyroid carcinoma with occasional anaplastic variants (J.-No. 16637/77; 31350/77; 491/78). The immuno-histochemical findings of these tumors were in accordance with those found in the previous specimen.

Anaplastic thyroid carcinomas manifest thyroglobulin synthesis exclusively in incorporated organoid structures – which may represent either tumor invaded normal thyroid tissue of highly differentiated areas of the carcinoma (Fig. 8). The anaplastic tumor parts were all characterized by negative thyroglobulin staining.

Medullary carcinomas as well as those carcinomas which could not clearly be identified as primary thyroid carcinomas did not show any specific staining.

Discussion

Thyroglobulin is a glycoprotein with a molecular weight of approximately 670,000. It is found in normal thyroid tissue, thyroiditis, goiter and thyroid neoplasia (Valenta and Michel-Bechet, 1977; Thomas-Morvan et al., 1974). From our present immuno-histochemical study the role of this marker can be seen to be valuable in 3 areas of thyroid pathology:

1) as an approach to a more functional classification of thyroid carcinomas and 2) as an aid in the differential diagnosis of thyroid carcinoma from tumors of other origin and 3) as an aid in the functional classification of non-cancerous thyroid tissue.

In our own material nearly all organoid thyroid carcinomas do synthesize at least small amount of thyroglobulin. Slightly lower figures were obtained in the series studied by Lo Gerfo et al. (1978) with 38 out of 41 positive cases. This discrepancy may perhaps be due to the number of sections analysed in the two studies. The thyroglobulin is usually localized in the cytoplasm of the tumor cells and to a far lesser degree in the interpapillary spaces and in glandular lumina. Carcinomas with follicular differentiation on the average contained more thyroglobulin than papillary ones.

An interesting finding is the predominantly cellular localisation of thyroglobulin in thyroid carcinomas. Obviously this glycoprotein which has been shown to have the same physiological characteristics and immunological properties as that derived from normal thyroid tissue (Lo Gerfo et al., 1978) cannot be secreted (exocytosed) by tumor cells. Although the mechanism of concentration of radioiodine in carcinoma cells is presently unknown this secretion abnormality might well interfere with the organification of iodine into thyroglobulin and could therefore be responsible for the low degree of stable iodine in thyroglobulin as shown by Thomas-Morvan et al. (1974). Further studies will be needed to clarify this point.

The immuno-histochemical studies of follicular adenoma of main cell type (Dralle and Böcker, 1977) and follicular carcinoma may be very similar and thus confirm previous biochemical studies. The differences consisted only in a more heterogeneous thyroglobulin pattern and less luminal staining in follicular carcinomas.

Recently Valenta and Michel-Bechet (1977) found a high degree of correlation between clinical radioiodine uptake and biochemically analysed thyroglobulin content of thyroid carcinomas, which was regarded as indication that thyroglobulin synthesis seems to be linked to iodine trapping ability.

Occasionally papillary tumors of other organs may very closely simulate primary thyroid carcinoma. We have observed this with metastatic papillary tumors of the ovary and salivary gland, the kidney and once of the breast. In these cases the immuno-histochemical demonstration of cytoplasmic thyroglobulin represents a method which in our experience is highly characteristic and gives evidence of thyroid neoplasia of follicle cell type. On the other hand negative staining for thyroglobulin is suggestive of a non-thyroid tumor or of a C-cell neoplasm.

Although there are so far no serological methods of importance in the preclinical phase of thyroid carcinoma there must be little doubt that sensitive thyroglobulin-radioimmunoassays are very useful in the assessment of metastasizing organoid tumors (Owen et al., 1960; Hagemann, 1978). Thus sequential assays with rising titres may be indicative of tumor progression. It must, however, be kept in mind that progression of the disease may also occur without any changes or even decline of the plasma levels if there is a change to anaplastic thyroid carcinoma. Van Herle and Uhler (1975) in their study on serum thyroglobulin in differentiated thyroid carcinomas clearly demonstrated that there is a positive correlation between serum thyroglobulin level and the presence of organoid thyroid carcinoma, either primary or metastatic. Thus these authors found in the course of the disease elevated levels in those cases in which local recurrences or metastases after thyroidectomy were observed.

We have shown in previous studies (Böcker et al., 1978; Dralle and Böcker, 1977) that immuno-histochemistry represents an appropriate method of differentiating the various types of thyroid adenomas, especially those of functional activity. Similarly the thyroglobulin pattern is often quite distinct in toxic and nontoxic goiter so that a clear cut separation is possible. Consequently, it appears at present that thyroglobulin may have a useful diagnostic role in relation to the function of thyroid tissue. Hence it may be concluded that the immuno-histochemical demonstration of thyroglobulin seems to represent a significant addition to the methods available to the pathologist for the functional classification of thyroid disease and especially for the differential diagnosis of thyroid cancer. We hope that this approach combined with a morphometrical method may perhaps enable an accurate forecasting of those patients with metastasizing organoid carcinomas for whom radioiodine therapy is appropriate.

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