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Original Articles

Production of Thyroxine (T_4) and Triiodothyronine (T_3) in Nontoxic Thyroid Tumors

An Immunohistochemical Study

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Summary. Thyroid tissue specimens from 27 patients with thyroid tumors were examined for thyroxine (T_4) and triiodothyronine (T_3) by the peroxidase-labeled antibody method. The result revealed localization of T_4 in 12 of the 14 follicular adenomas, in all the 8 papillary carcinomas and in 1 of the 3 follicular carcinomas studied, and of T_3 in 13 of the 14 follicular adenomas, in all the 8 papillary carcinomas and in all the 3 follicular carcinomas.

In the tumor tissue, the thyroid hormones were demonstrated in the colloid substance, on the luminal surface of tumor cells and in their cytoplasm. Compared with nontumorous thyroid tissue, the tumor tissue showed localization of the hormones predominantly in the cytoplasm and to a lesser extent in the colloid substance, with conspicuous variations in tissue distribution of positive areas and intensity of staining. This tendency was more marked in thyroid carcinomas.

The demonstration of T_4 and T_3 in routine histological paraffin sections of formalin-fixed thyroid tissues in this investigation indicates potential usefulness of thyroid hormone detection by the peroxidase-labeled antibody technique. It is an effective diagnostic tool for evaluating the functional activity of thyroid tumors as well as for determining whether a malignant growth under examination originates from the thyroid.

Key words: Thyroid gland – Thyroid tumor – Immunohistochemistry – Thyroid hormone – Functioning tumor

Introduction

Most tumors of the thyroid are regarded as non-functioning since they rarely produce clinical manifestation of thyrotoxicosis, except in the case of Plummer's disease (Werner 1978). However, it is suggested from results of scintigraphical analysis of ^{131}I uptake (Lobo et al. 1965; Miller and Hamberger 1965), immuno-

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histochemical demonstration of thyroglobulin localization in tissues (Dralle and Böcker 1977; Böcker et al. 1978; Lo Gerfo et al. 1978; Böcker et al. 1980) and detection of various enzyme activities (Lindsay and Arico 1963; Harcourt-Webster and Stott 1966) that thyroid tumors are by no means literally nonfunctioning in respect of hormone production and maintain some, though not excessive, thyroid function (Valenta 1976; Valenta and Michel-Béchet 1977).

Recently, immunohistochemical demonstration of thyroid hormones in tissues from laboratory animals was reported (Wilson et al. 1978), and subsequently, we were successful in the detection of the hormones in paraffin sections of biopsy and surgical thyroid tissue using an immunoperoxidase technique (Kawaoi et al. 1981). The purpose of this study was to evaluate thyroid function in thyroid tumors clinically diagnosed as nontoxic using immunohistochemical detection of hormones. At the same time we intended to compare the findings with those noted for nontumorous thyroid tissues.

Materials and Methods

Materials. Biopsy or surgical specimens of thyroid tumors obtained from 27 patients were studied. These included 14 cases of follicular adenoma, 1 case of papillary adenoma, 8 cases of papillary carcinoma, 3 cases of follicular carcinoma and 1 case of anaplastic carcinoma. Eleven other specimens, colloid goiter (1 case), adenomatous goiter (2 cases) and goiter of Basedow's disease (8 cases) were also investigated as controls. All tissues were fixed in 10% neutral formalin, embedded in paraffin and cut into thin sections by the routine histological procedure.

Antisera and Peroxidase-Labeled Antibody. Anti-thyroxine (T_4) and anti-triiodothyronine (T_3) rabbit antisera were products of E.Y Laboratories, Inc. San Mateo, Cal., and Cappel Laboratories, Cochranville, PA. Both antisera, which had been prepared by the use of bovine serum albumin (BSA) as a carrier protein for the hapten, were completely deprived of anti-BSA activity by incubation with BSA prior to use for staining. Concomitantly, absorption tests of the antisera were also carried out with T_4 and T_3 to confirm specificity of the immune staining, of which the procedure was described elsewhere (Kawaoi et al. 1981). Peroxidase-labeled anti-rabbit IgG goat gammaglobulin was prepared in this laboratory by the method of Nakane and Kawaoi (Nakane and Kawaoi 1974).

Immune Staining. The indirect peroxidase-labeled antibody technique was employed. After deparaffinization, tissue sections were immersed in 0.01 M phosphate buffered saline, pH 7.2 (PBS), for 10–15 min, and incubated with anti-T_4 or anti-T_3 rabbit antiserum (diluted to 1:40 in PBS containing 1% BSA) at room temperature for 60 min. After thoroughly rinsing with sufficient PBS, the sections were incubated with a 1:40 dilution of peroxidase-labeled antibody at room temperature for 30 min, and finally incubated with 3,3′ diaminobenzidine (DAB) containing 0.005% hydrogen peroxide (Graham and Karnovsky 1966) for 10–15 min, followed by dehydration and sealing for microscopic observation. Tissue sections treated by using antisera specifically absorbed with the antigens or nonimmune rabbit serum in place of the antisera, or incubated merely with the substrate solution were observed as controls for staining. None of these control tissues proved positive except for reactivity due to endogenous enzyme activities of the erythrocytes. No thyroid peroxidase activity in follicular epithelium was demonstrated in any of the controls studied.

Results

The results of immune staining for T_4 and T_3 are summarized in Table 1. Of the 15 cases of adenoma, 12 were positive for T_4 and the remaining 3 (papillary adenoma, Hürthle cell adenoma and tubular adenoma) negative. Thirteen cases of adenoma were positive for T_3 and one case of Hürthle cell adenoma showed negative staining.

Table 1. Result of Immune Staining of Nontoxic Thyroid Tumors for T_4 and T_3

Tumors	Cases	Positive for	
		T_4	T_3
Follicular adenoma	14	12	13
Trabecular adenoma	1	1	1
Tubular adenoma	10	9	10
Colloid adenoma	2	2	2
Hürthle cell adenoma	1	0	0
Papillary adenoma	1	0	n.d.
Papillary carcinoma	8	8	8
Follicular carcinoma	3	1	3
Anaplastic carcinoma	1	0	n.d.
Total	27	21	24

n.d.: not done

All the eight papillary carcinomas gave positive reactions for both T_4 and T_3, and the three follicular carcinomas for T_3. The anaplastic carcinoma showed no appreciable reaction for T_4.

Localization in Normal and Hyperplastic Thyroid Tissues. In normal and hyperplastic thyroid tissues, T_4 was demonstrated chiefly in the colloid substance of follicles although the intensity of staining varied from follicle to follicle, some being completely negative (Fig. 1). The luminal surface of follicular epithelium was often intensely stained and, in cases of hyperplasia, some follicular epithelial cells were found stained within the cytoplasm.

T_3 tended to be localized not so much in the colloid substance as in the epithelial cytoplasm, the staining being generally less conspicuous than that for T_4 (Fig. 2).

Localization in Thyroid Adenoma. Both T_4 and T_3 were demonstrable in the majority of cases as described above though the intensity of staining differed markedly among the cases. Localization of T_4 was not limited to the colloid substance in follicles but, frequently, follicles with negative reactivity of colloid substance showed positive staining in the epithelial cells (Fig. 3a). Occasional follicular epithelia had a linear positive staining along their inner aspect while epithelial cells uniformly positive in the entire cytoplasm were not infrequently encountered (Fig. 4).

The adenomas with well differentiated follicular structures showed remarkable staining for T_4 (Fig. 3a). T_4 specific staining was evident, nevertheless, even in tissues with poorly developed follicles.

The intensity of epithelial staining for T_4 varied not only from one follicle to another but also among the cells within the same follicles (Fig. 4). Markedly

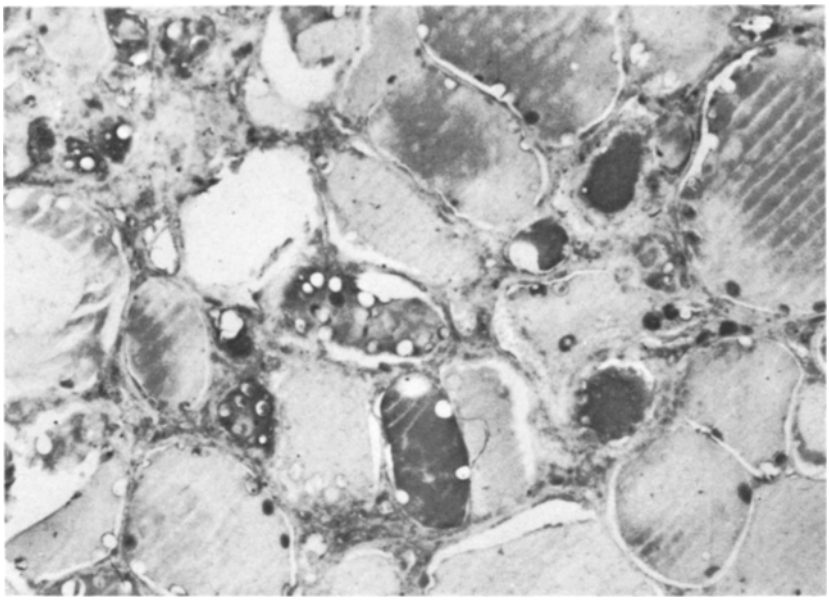

Fig. 1. Normal thyroid tissue. T_4 immune staining is localized mainly in colloid substance with variable intensity. × 185

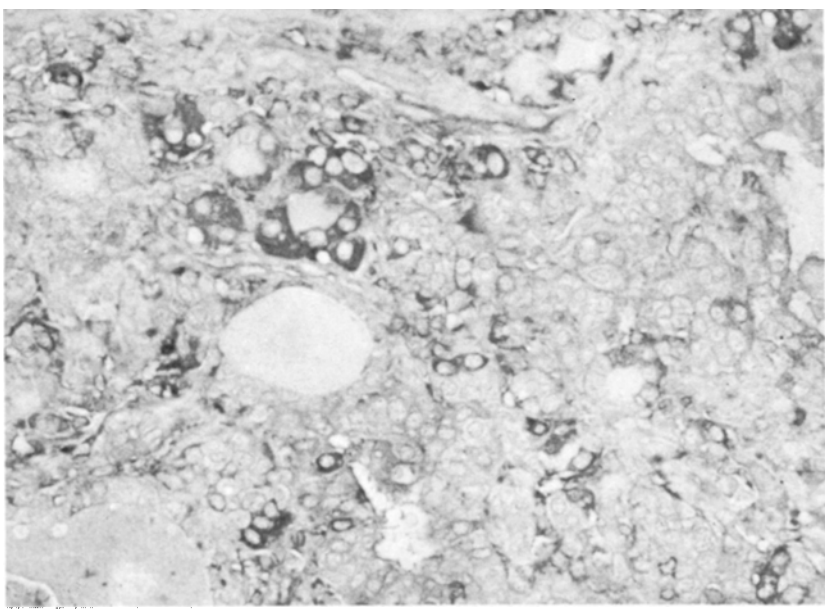

Fig. 2. Diffuse hyperplasia. T_3 positive cells are irregularly distributed. The lumina of the colloid follicles are negative. × 370

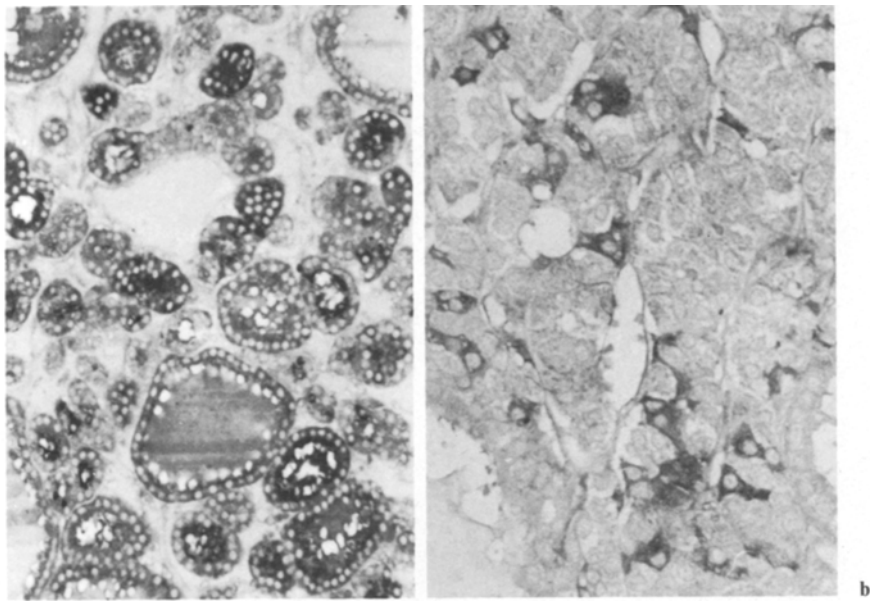

Fig. 3. a Tubular adenoma. Most of the follicles show positive staining for T_4 in colloid substance as well as epithelial cytoplasm. ×185. **b** Tubular adenoma. T_3 positive epithelial cells are scattered among the negative ones. The colloid substance is not stained for T_3. ×370

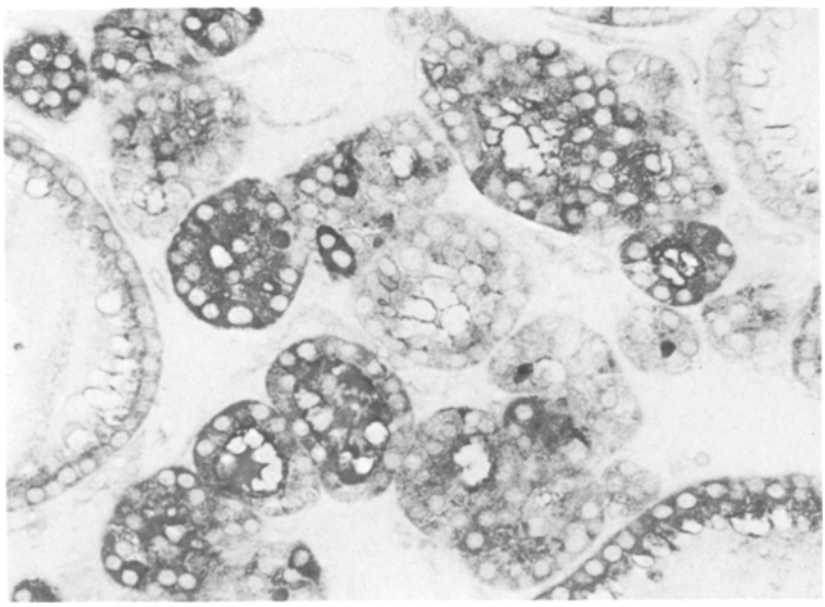

Fig. 4. Tubular adenoma. The intensity of T_4 staining varies among the epithelial cells within the same follicles. ×370

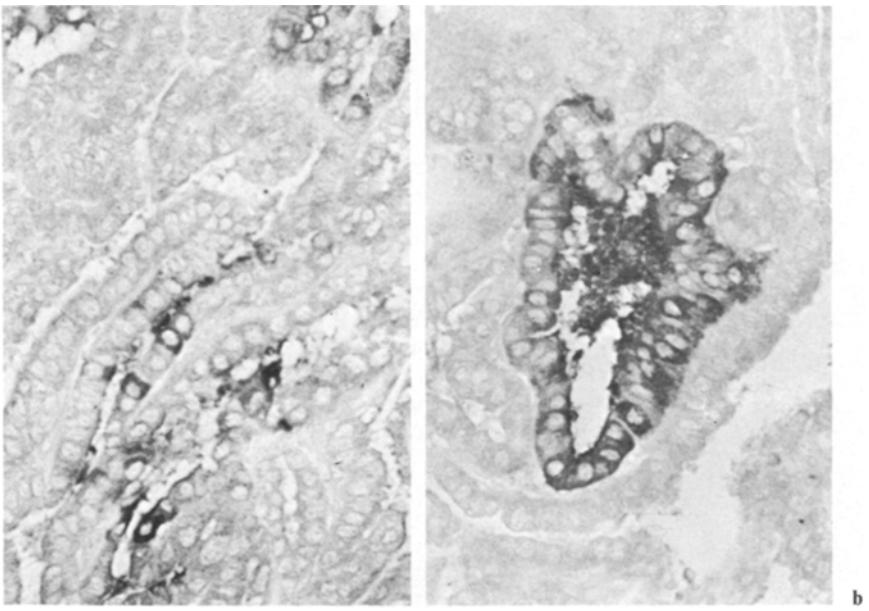

Fig. 5. a Papillary carcinoma. T_4 positive cells disperse in the tissue. ×370. **b** Papillary carcinoma. One glandular structure composed of T_4 positive cells is seen surrounded by negative area. The content of the gland is also stained. ×370

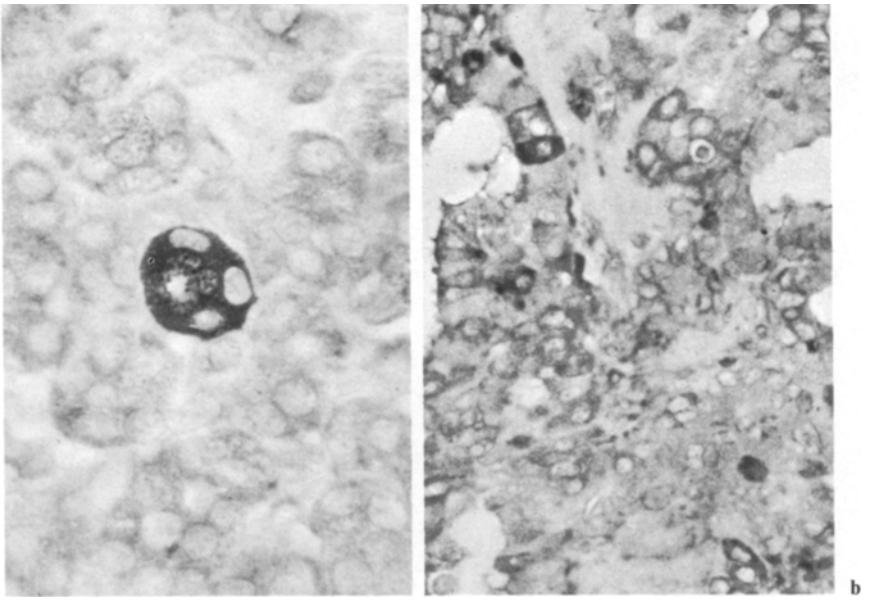

Fig. 6. a Papillary carcinoma. Cytoplasmic staining for T_4 is evident, leaving negative nuclear site. ×740. **b** Papillary carcinoma. T_3 positive cells are scarce and scattered in the tumor tissue. ×370

positive cells were intermixed with completely negative cells in occasional follicles. Moreover, not infrequently even the epithelium of a follicle with a narrow lumen, almost devoid of colloid substance, was stained for T_4.

The study also revealed localization of T_3 in adenomas, with a less conspicuous degree of staining for T_4 than in normal thyroid tissues. T_3 was likely to be localized more in the epithelium than in the colloid substance (Fig. 3).

Neither T_4 nor T_3 was demonstrated in the case of Hürthle cell adenoma.

Localization in Thyroid Carcinoma. Both T_4 and T_3 were demonstrated in thyroid carcinomas with essentially the same frequencies as in the adenomas. However, generally, areas positive for these hormones were considerably limited and sparse in the tumor (Figs. 5, 6). The mode of their localization in carcinomas was comparable to that observed in the adenomas, but the malignant tissues displayed a distinct trend for localization in the epithelial cytoplasm and more pronounced varieties in intensity of staining within the same tissue (Fig. 5b). In most instances, the hormones were distinctly demonstrated in relatively small areas of tumor tissue, most of which was negative (Fig. 6).

Discussion

Histological evaluation of the function of the thyroid gland is usually achieved by such techniques as autoradiographic study of ^{125}I uptake (Fujita 1969), histochemical detection of various enzymes (Lindsay and Arico 1963; Harcourt-Webster and Stott 1966; Strum and Karnovsky 1970) and immunohistochemical demonstration of thyroglobulin (Pelletier et al. 1976; Paiement and Leblond 1977), which have been generally regarded as effective diagnostic tools for detecting latent function in thyroid tumors (Dralle and Böcker 1977; Böcker et al. 1978; Böcker and Dralle 1980).

Immunohistochemical demonstration of thyroid hormones which are the final products of thyroid function is considered to be the most direct means for evaluating the thyroid hormone production, but it has only recently been reported that T_4 and T_3 can be detected by immunofluorescence in frozen sections of the rat thyroid gland (Wilson et al. 1978). We have successfully demonstrated T_4 and T_3 by immunoperoxidase method in formalin-fixed, paraffin-embedded tissue sections of human thyroids (Kawaoi et al. 1981).

In the present investigation, an attempt was made to detect these thyroid hormones by the use of peroxidase-labeled antibody method in series of nontoxic thyroid tumors. The hormones were demonstrated in the majority of the cases studied, regardless of whether the tumors being benign or malignant. The results indicate that many of the so-called nonfunctioning thyroid tumors have potential ability for hormone synthesis.

Compared with nontumorous thyroid tissues including hyperplasia, the tumor tissues showed a marked tendency for intracytoplasmic localization of T_4, with conspicuous inter-follicular and inter-epithelial variation. These immunohistochemical features of the thyroid tumors might suggest some disturbance in mechanism of hormone secretion in tumor cells as well as functional hetero-

geneity of the tumor tissue (Thomas-Morvan et al. 1974). While there have been reports of marked inter-cellular differences in thyroglobulin localization in tumors (Böcker et al. 1980), the tendency observed in this study for localization of cells producing T_4 was marked, from which it may be postulated that these cells represent only a part of the tumor cell population that are functionally differentiated to an extent that permits thyroglobulin production.

The similarity of the localization of T_4 and T_3 to that of thyroglobulin (Dralle and Böcker 1977; Böcker et al. 1980) suggests that these hormones mostly occur bound to thyroglobulin in tumor tissues. It cannot be determined, except by a detailed immunoelectron microscopic analysis, which stage in the process from biosynthesis in the thyroglobulin molecule to retention in the follicular space, reabsorption into the epithelium and final hydrolytic breakdown the T_4 and T_3 immunoreactivities in thyroid tissue might be.

The immunohistochemical demonstration of thyroid hormones in the routine histopathological preparations of thyroid tumors in the present study indicates the potential usefulness of the technique in pathology of the thyroid tumors. It permits the diagnosis of latent hormone producing activity of thyroid tumor, confirming whether the neoplasm under examination originates from the thyroid gland, and helping to classify thyroid tumors on a functional basis.

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