Original Articles

Production of Thyroxine (T₄) and Triiodothyronine (T₃) 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 (T₄) and triiodothyronine (T₃) by the peroxidase-labeled antibody method. The result revealed localization of T₄ in 12 of the 14 follicular adenomas, in all the 8 papillary carcinomas and in 1 of the 3 follicular carcinomas studied, and of T₃ 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 T₄ and T₃ 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 ¹³¹I uptake (Lobo et al. 1965; Miller and Hamberger 1965), immuno-
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 (T4) and anti-triiodothyronine (T3) 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 T4 and T3 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 0.01M phosphate buffered saline, pH 7.2 (PBS), for 10–15 min, and incubated with anti-T4 or anti-T3 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 T4 and T3 are summarized in Table 1. Of the 15 cases of adenoma, 12 were positive for T4 and the remaining 3 (papillary adenoma, Hürthle cell adenoma and tubular adenoma) negative. Thirteen cases of adenoma were positive for T3 and one case of Hürthle cell adenoma showed negative staining.