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Immunohistochemistry of the Thyroid Gland

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The immunohistochemical detection of thyroid hormones and related compounds has been successfully applied to the analysis of the function of thyroid gland in normal and pathological conditions. In this review, a few examples of recent immunohistochemical study on the development and maturation of fetal rat thyroid gland and the expression of the endocrine function in human and experimental rat thyroid neoplasms were presented.

Key words: Immunohistochemistry, T3, T4, thyroglobulin, thyroid gland.

Introduction

The immunohistochemical technique has recently made rapid progress with ever growing range of its application in the fields of bio - medical research.[1]

Also, this technique has been usefully applied in the study on the thyroid gland, i.e., analysis of functional differentiation in the fetal period,[2,3] functional aspects of thyroid tumors,[4,5] endocrine function of cultured follicular cells,[6] Ig classes and subclasses which are synthesized in plasma cells and the subsets of lymphocytes infiltrating in the thyroid tissue in autoimmune thyroid diseases,[7] etc.

In this review, recent results of our studies will be summarized focussing on the development and maturation of fetal rat thyroid gland and expression of endocrine function in human and experimental rat thyroid tumors.

1. Immunohistochemical demonstration of thyroid hormone in thyroid tissues.

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Wilson et al.[8] reported the immunofluorescent method to localize the thyroid hormone in frozen sections of the rat thyroid gland. We attempted to localize thyroxine (T_4) and triiodothyronine (T_3) in the human thyroid tissues using the immunoperoxidase technique.[9]

Most of the materials including relatively normal thyroid tissues, hyperplastic glands, adenomas and carcinomas showed positive immune staining for both T_4 and T_3 in varieties of both intensity and distribution pattern. Non-pathologic thyroid tissues revealed weakly positive staining for T_4 in the colloid substance and occasionally on the luminal surface of the follicular epithelial cells (Fig.1). In the hyperplastic thyroids found in the specimens from the patients of Graves' disease an increased intensity in staining of T_4 was observed as compared with those of the normal tissue, where T_4 was localized in the colloid substance, on the luminal surfaces of the follicular epithelia, and in the cytoplasms of some epithelial cells (Fig. 2). The T_3 immunoreactivity was detected in the follicular epithelial cells as well, although it was mostly undetectable in the colloid substance. Both T_4 and T_3 were able to be localized in tumor tissues,

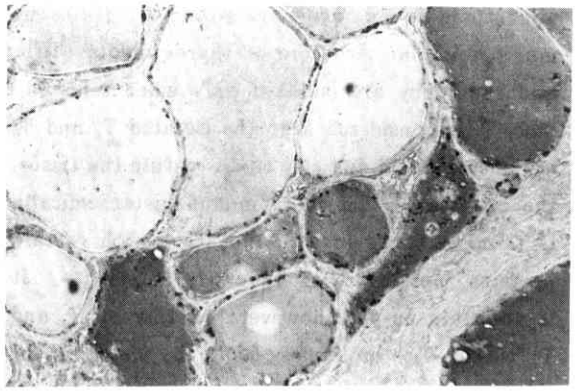

Fig. 1. Normal human thyroid tissue stained for T_4. x350

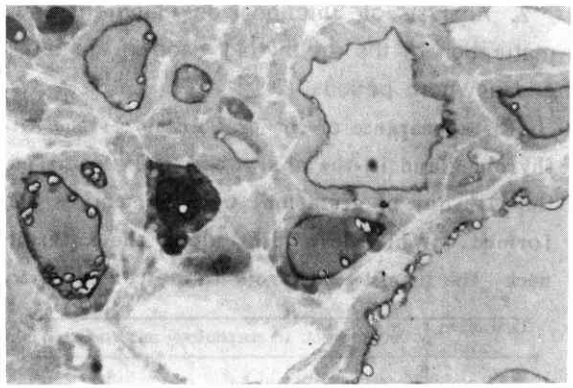

Fig. 2. Human thyroid gland with hyperplasia stained for T_4. x350

where T_4 was stained more frequently in adenomas than in the carcinomas. As was observed in non-neoplastic tissues, the colloid substance and the cytoplasm of the tumor cells lining the follicles were positive for T_4 immunoreactivity with considerable variety in staining pattern.

Thus, the result of the immune staining proved that formalin fixed and paraffin embedded thyroid tissue sufficiently preserved the antigenic activity of the thyroid hormone. However, it still remains unknown how much of the initial immunoreactivity of the hormone was lost through tissue processing. A quantitative estimation of the effect on thyroid hormone antigenicity of various fixatives, incubation temperature and organic solvents should be conducted in future.

The localization of T_3 and T_4 commonly reveals divergent staining patterns, in accordance with the type of thyroid disease, or with different lesions in the same thyroid tissue, not showing uniformity even in the same follicle. The localization of T_3 and T_4 in the thyroid gland tissue of rats was examined by the immunoperoxidase method under various experimental conditions in order to determine the functional significance of such varied localization patterns.[10]

It was confirmed that T_4 and T_3 were localized in the colloid of the follicles of the thyroid gland, and sometimes in the cytoplasm of the follicular epithelium (Fig. 3). Both hormones revealed a generally similar stainabiltity and

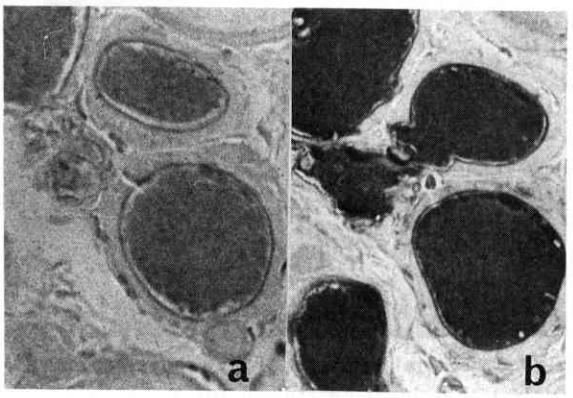

Fig. 3. Serial sections of normal rat thyroid tissue stained for T_4 (a) and Tg (b). x350

localization, and it was presumed that the synthesis of both was closely associated. It was found in thyroid stimulating hormone (TSH) - injected animals that the immunostainability was increased, and not only the follicular colloid but also absorption vacuoles formed on the interface with the epithelium as well as the clear and vacuolated cytoplasm of the epithelium, were stained in a coarse granular pattern (Fig. 4). On the other hand, in hypophysectomized and propylthiouracil (PTU) - administered animals, the stainability of the epithelium as well as the colloid was greatly reduced, and it finally disappeared

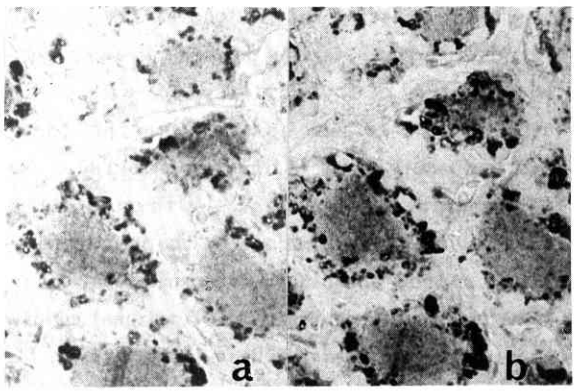

Fig. 4. Serial sections of TSH stimulated rat thyroid gland stained for T_4 (a) and Tg (b). x350

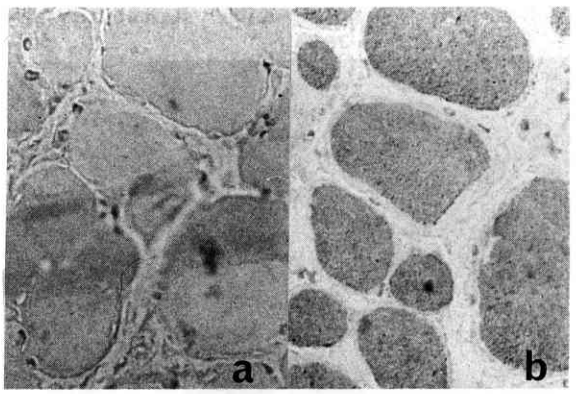

Fig. 5. Serial sections of hypophysectomized rat thyroid gland stained for T_4 (a) and Tg (b). x350

(Fig. 5). These findings support the view that the immunostainability of T_4 and T_3 is closely correlated with the functional state of the thyroid gland.

In each of the experimental groups, the patterns or degrees of staining of T_4 and T_3 were not always uniform in spite of their being in the same thyroid gland tissue, and some differences were noted even between different follicles. This may reflect variations in the functional state of each follicle.

When thyroid hormones are detected immunohistochemically, the problem arises that, unlike other hormones in general, they are synthesized and stored in an incorporated state within the macromolecular structure of thyroglobulin (Tg), and that they are isolated only when released. Since it is considered that the isolated T_4 and T_3 are unstable and difficult to fix within the tissue, the substance detected immunohistochemically is thought to be in a bound state with certain proteins, possibly with Tg or its fragments. It is uncertain as yet, however, whether all T_4 and T_3 within Tg molecules could be detected by reaction and combination with immunoglobulin molecules.

2. Analysis of functional development and maturation of the rat thyroid gland in the fetal and newborn periods.

The appearance of the primordium of the rat thyroid gland in the pharyngeal wall occurs at the 11th day of gestation, when 25 somites are formed. While descending along the anterior neck, the primitive thyroid tissue continues to proliferate and is located at the anterior portion of the trachea at the 15th day (Table 1).

Gestation (days)	Development in morphology and function
9	one-somite embryo
11	25-somite embryo, primordium of thyroid
12	40-somite embryo, growth of thyroid primordium
15	60-somite embryo, cartilage formation, thyroid primordium with two lobes and isthmus in front of trachea
16	primitive follicles of thyroid, TSH-sensitive (in vitro)
17	TSH cells in anterior pituitary, iodine metabolism in thyroid, synthesis of DIT, T3, T4 (in vitro)
18	effect of hypophysectomy -- a crucial turning point in thyroid development
19	definitive follicular structure
21	secretion of T4

Table 1. Maturation of rat fetal thyroid.

It has been established by various experimen-

tal techniques that the thyroid glands thereafter show a rapid development in both structural and functional characteristics until the end of foetal life. Ultrastructural studies have demonstrated remarkable development of rough surfaced endoplasmic reticulum, mitochondria, and Golgi apparatus, and follicular spaces which, especially from the 17th to 18th day, appear as a primitive follicular structure formed between neighbouring epithelia.[11] Autoradiographic experiments have indicated that the beginning of organification of iodine by the follicular epithelia occurs at the 17th day,[11] and in vitro analysis has demonstrated the production of monoiodotyrosine (MIT) and diiodotyrosine (DIT) by cultured thyroid tissues from the rat foetus at the 17th day of gestation.[12]

It was also found by enzyme histochemical techniques that the thyroid peroxidase which is essential to iodine organification in the colloid spaces was detected first on the 17th day in the nuclear envelope and rough surfaced endoplasmic reticulum, and was finally transferred to the apical surface of the epithelial cells where iodination of Tg is thought to take place.[13]

According to the immunofluorescence observations of Feldman et al.,[14] Tg immunoreactivity first appeared in the follicular spaces of the foetal rat thyroid on the 20th day of gestation.

However, a highly sensitive immunoperoxidase technique succeeded in demonstrating Tg in the cytoplasm of the immature thyroid epithelium on as early as the 15th day when no follicular structure had developed, and both T_4 and T_3 were still negative, providing evidence that the production of Tg precedes the synthesis of T_4 and T_3 in the foetal thyroid gland (Fig. 6).[2]

T_4 and T_3 were found to occur on the 17th day of gestation, i.e., 2 days after the detection of Tg, when primitive follicles were observed by electron microscopic studies. The localization of the thyroid hormones was limited only to the follicular lumen throughout foetal life, and failed to be demonstrated in the epithelial cytoplasm like Tg which was detected in the follicular space as well (Fig. 7).[2]

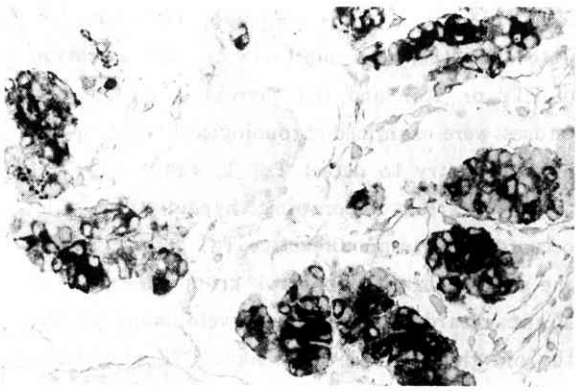

Fig. 6. Fetal rat thyroid gland at the 16th gestational day stained for Tg. x700

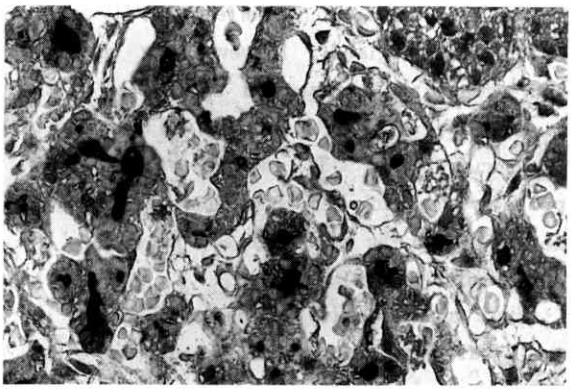

Fig. 7. Fetal rat thyroid gland at the 18th gestational day stained for T_3. x700

A good coincidence in the appearance of T_4 and T_3 in the thyroid follicles and that of thyrotrophs in the anterior pituitary of the foetal rat, appears to suggest that foetal TSH affects the initiation of the thyroid hormone synthesis in the foetal thyroid.[15] However, the occurrence of immunoreactive Tg production would not depend on the foetal TSH, since Tg is detected in the thyroid epithelium prior to the appearance of thyrotrophs.

In order to elucidate the maternal factors influencing the functional development of the

fetal rat thyroid gland, pregnant rats were subjected to either thyroidectomy or administration of PTU or TSH and the thyroid glands of the fetuses were examined chronologically by immunohistochemistry to detect Tg, T_4 and T_3.[16]

In the group undergoing thyroidectomy, the occurrence of immunoreactive Tg, T_4 and T_3 was the same as in the control group in spite of slight retardation of the development of the thyroid gland. On the other hand, PTU administration caused remarkable degeneration of the hyperplastic epithelium of the follicles, where immunoreactivity of T_4 and T_3 was barely detectable, suggesting a transplacental effect of PTU on the fetal thyroid gland. However, Tg remained unaffected and was stained as well as in the controls (Fig.8). Injection of TSH led to a delay in the occurrence of T_4 and T_3 by one day, probably due to increased levels of thyroid hormone from the stimulated thyroid gland of the motherrats (Table 2).[16]

Since a stimulative action of TSH on the proliferation and functional maturation of the

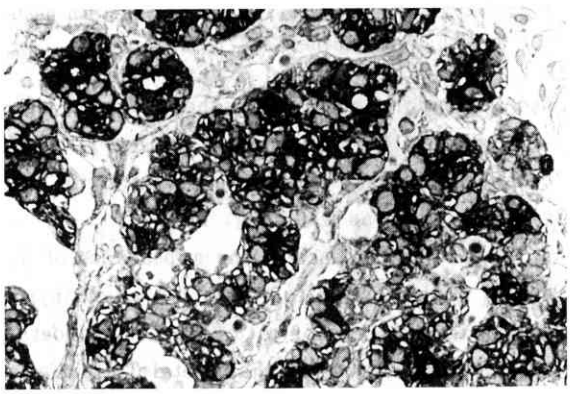

Fig. 8. Thyroid gland of the rat fetus at the 18th gestational day born by TSH administered mother stained for Tg. x700

thyroid epithelium has been observed in an in vitro study,[17] proof of decreased TSH in the fetal blood of this group could provide evidence that

Day of gestation		15	16	17	18	19	20	21	22
Tx	T_3:	-	-	±	±	+	→	→	→
	T_4:	-	-	±	±	+	→	→	→
	Tg:	±	+	→	→	→	→	→	→
PTU	T_3:	-	-	-	-	-	-	-	-
	T_4:	-	-	-	(±)	-	-	-	-
	Tg:	±	+	→	→	→	→	→	→
TSH	T_3:	-	-	-	±	+	→	→	→
	T_4:	-	-	-	+	→	→	→	→
	Tg:	±	+	→	→	→	→	→	→
Control	T_3:	-	-	±	±	+	→	→	→
	T_4:	-	-	±	+	→	→	→	→
	Tg:	±	+	→	→	→	→	→	→

Table 2. Occurrence of immunostainable T_3, T_4 and Tg in the fetal rat thyroid glands in each experimental group. Tx: Thyroidectomy. PTU: Administration of PTU. TSH: Administration of TSH. +: Positive, ±: weakly or partially positive, − : negative.

fetel TSH plays a key role in the functional maturation of the thyroid gland.

Immunoelectron microscopy was applied for the detection of Tg, T_4 and T_3 in the fetal rat thyroid gland during the early stages of synthesis of these compounds in order to elucidate the subcellular aspects of the onset of their production.[3] Tg was detected in the perinuclear space, rER and Golgi apparatus as early as the 16th day of gestation when no follicle had yet formed. When primitive follicles had formed after the 17th day of gestation, both T_4 and T_3 appeared on the surface of microvilli and occasionally in their core poritons as well as Tg (Fig. 9). These findings provide evidence to suggest that follicles are essential to the extracellular synthesis of T_4 and T_3 in the fetal rat thyroid gland.

3. Analysis of functional aspects of human and experimental rat thyroid tumors.

Except for medullary carcinoma, most primary tumors of the thyroid are clinically diagnosed as nonfunctional regardless of their benign or malignant nature and histologic type, and functional tumors with toxic symptoms are con-

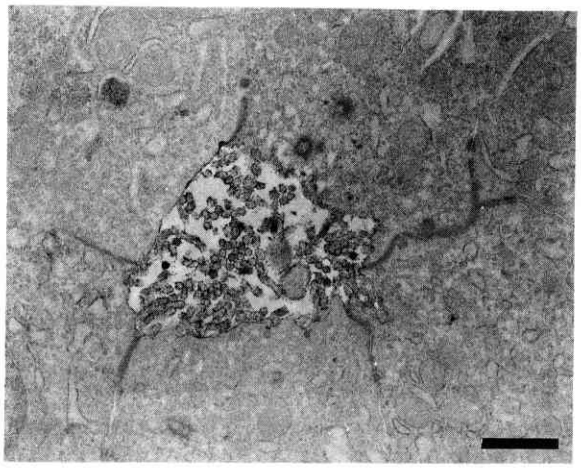

Fig. 9. Immunoelectron micrograph showing the localization of T_4 in the fetal rat thyroid gland at the 19th day of gestaion. x10,000 Bar=1 μ m

Tumor	No. of cases	Positive for T_4	Positive for T_3
Follicular adenoma	45	37	41
Trabecular adenoma	2	2	2
Tubular adenoma	24	20	23
Colloid adenoma	14	13	14
Oxyphilic adenoma	5	2	2
Papillary adenoma	3	1	2
Papillary carcinoma	21	14	20
Follicular carcinoma	11	6	9
Anaplastic carcinoma	10	0	4
Squamous cell carcinoma	2	0	0
Medullary carcinoma	3	0	0
Malignant lymphoma	2	0	0
Total	97	58	76

Table 3. Results for the immunostaining of thyroid tumors.

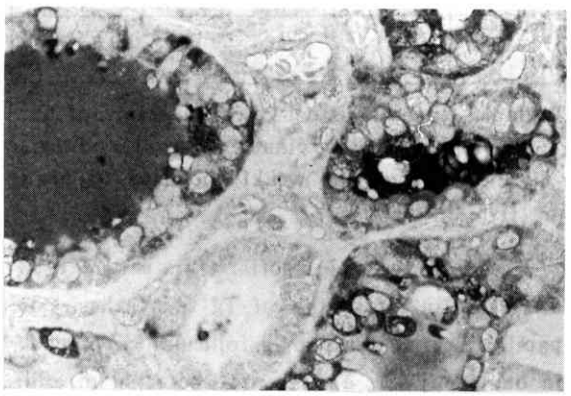

Fig. 10. Follicular adenoma of human thyroid stained for T_4. x700

sidered to be rare. Toxic symptoms are generally absent because these tumors fail to secrete excess thyroid hormones, T_4 or T_3. On the basis of examinations of each step of thyroid hormone biosynthesis — such as immunohistochemical detection of the localization of Tg as a site of hormone biosynthesis, histochemical detection of thyroidal peroxidase necessary for the oxidation of iodne, biosynthesis of iodotyrosine and its coupling and also detection of various hydrolytic enzymes required for the hydrolysis of reabsorbed colloid droplet, together with the results of electron microscopic studies — these tumors do not seem to be nonfunctioning but appear to have partial or incomplete ability to synthesize hormones, or be functional.[18]

On application of immunohistochemistry to a small number of cases with a diagnosis of nonfunctioning thyroid tumor, localization of T_4 and/or T_3 was demonstrated in most (Table 3).[4] Interestingly, the localizations of T_4 and T_3 in the tumor tissue did not always agree.

In 97 of the tumors, the immunostaining method was applied to serial or semiserial sections to study the correlation among the localization of Tg, T_4, and T_3.[5] The localization of T_4 agreed relatively well with that of Tg, and part of the Tg - positive structure frequently revealed simultaneous positive staining for T_4. The localization of T_3, however, did not always correspond with that of T_4 or Tg. T_4 and T_3 with localizations identical to that of Tg may be considered to be bound to Tg, but the mode of existence of T_3 without correspondence in localization to Tg remains unknown (Table 4).

Immunostainablity of Tg, T_4, and T_3 was found to be correlated with the histologic types of thyroid tumor to some extent. In follicular

adenoma, no remarkable difference in immunostaining was observed between the tubular type and colloid type (Fig. 10). In trabecular adenoma, the stainability for Tg was low. In some specimens of oxyphilic cell adenoma, T_4 and T_3 were detected in addition to Tg. The areas with positive reaction for these compounds were found only in a very limited variety of cells, and their stainability was weak.

	Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tubular adenoma	T_3	+	+	+	+	+	+	±	±	±	±	±	+	±	-
	T_4	+	+	+	±	±	-	+	±	±	±	±	±	±	-
	Tg	+	+	+	+	+	+	+	+	+	+	+	±	±	-
Colloid adenoma	T_3	+	+	+	+	+	+	+	+	+	+	+	±		
	T_4	+	+	+	+	+	+	+	+	±	±	+	±		
	Tg	+	+	+	+	+	+	+	+	+	+	+	+		
Papillary carcinoma	T_3	+	+	+	+	+	+	+	+	+	+	±	+	-	
	T_4	±	±	±	±	±	-	-	-	-	-	-	±	-	
	Tg	+	+	+	+	+	+	+	+	+	+	+	±	±	
Pollicular carcinoma	T_3	+	+	+	+	+	-	±	-						
	T_4	+	+	±	±	-	-	±	±						
	Tg	+	+	+	+	+	+	±	±						

Table 4. Immunohistochemical detection of T_3, T_4 and Tg in neoplastic thyroid nodules. +: Positive, ±: weakly positive, -: negative staining.

According to some reports, there is very little difference in the ability of Tg to synthesize in papillary carcinoma and in follicular carcinoma. In our study, T_4 and T_3 were detected in addition to Tg, although the stainability for T_3 was more distinct than that for T_4 in both kinds of carcinoma (Fig. 11). In anaplastic carcinoma, on the other hand, no T_4 was demonstrated at all, and the numbers of cells with positive reaction for Tg and T_3 were very small even in specimens with positive reactions for these (Fig.12). The functional level thus appeared to be much lower than that in the various types described above.

In the immunostaining of the tumor tissue, sites of positive reaction for T_4 and T_3 were rather sporadic, compared with those of Tg. Whether this reflects a functional heterogeneity or difference in the functional phase among various follicles observed in nontumorous thyroid

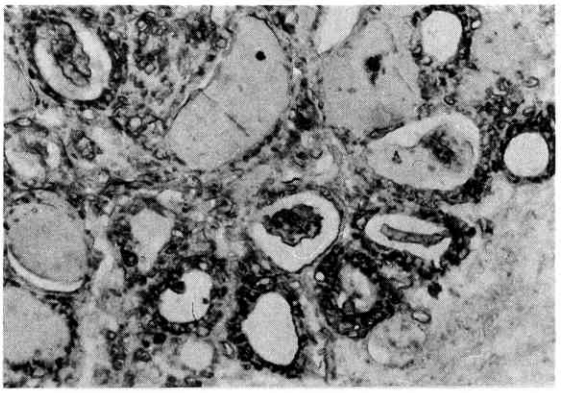

Fig. 11. Follicular carcinoma of human thyroid stained for T_3. x350

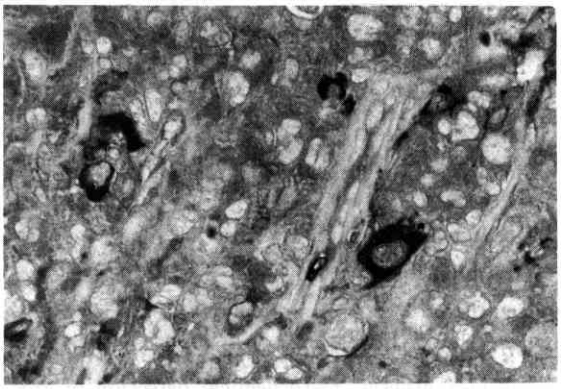

Fig. 12. Anaplastic carcinoma of human thyroid stained for T_3. x700

tissue or simply indicates functional differentiation of this part of the tissue, remains unclear. It is, however, impossible to discern such positive sites in sections stained with ordinary H&E and PAS stains. The immunohistochemical methods, therefore, certainly appear to be useful for evaluating the functional level of the tumor and for assessing the thyroidal origin of metastatic tumors.

A variety of focal lesions appear in the diisopropanolnitrosamine (DIPN) treated rat thyroid gland on a background lesion of diffuse hyperplasia of the follicular epithelium.[19] These lesions were classified into three main types: type 1, foci of cellular alteration; type 2, proliferative nodules; and type 3, carcinomas. The

type 2 lesions were further subtyped into 2a (follicular), 2b (papillary) and 2c (mixed), and the type 3 lesions into 3a (follicular), 3b (papillary), 3c (mixed) and 3d (anaplastic), respectively (Table 5).[19]

These lesions of various histological types were analyzed immunohistochemically in order to clarify the endocrinological activity, if any, of the individual foci in the glands. The localization of Tg, T_4 and T_3 was examined by the immunoperoxidase technique focussing on focal and nodular lesions which are considered to represent putative background lesions of tumorigenesis in the thyroid gland.[20] The results indicated that the immunostainability of Tg, T_4 and T_3 is intimately related to the histological patterns of thyroid lesions. The type 1 lesions showed immunostaining which was similar to that of the surrounding hyperplastic follicles and the tissue of normal control rats (Fig.13), whereas type 3 lesions were significantly decreased in their degree of staining, and the staining intensity of the type 2 lesions was in the range between those of type 1 and type 3 (Figs. 14,15). These findings suggested that the more the lesions are regarded as neoplastic or malignant, the more their endocrine function appears to be retarded, since type 3 lesions correspond to carcinomas (Table 6).

Type 1.	Foci of cellular alteration, AF.
Type 2.	Nodular Proliferation, NP. (hyperplasia or adenoma)
	a) follicular
	b) papillary
	c) mixed
Type 3.	Carcinoma, Ca.
	a) follicular
	b) papillary
	c) mixed
	d) anaplastic

Table 5. Histological classification of DIPN induced focal lesions of rat thyroid gland.

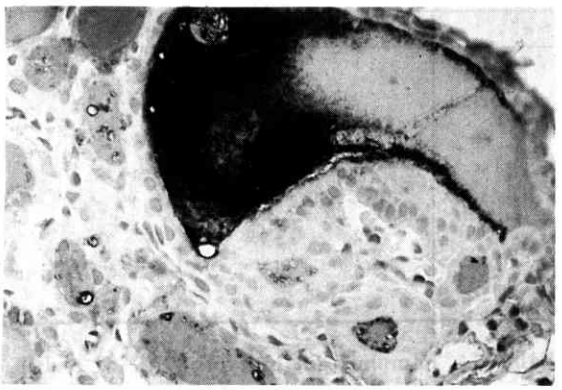

Fig. 13. Type 1 lesion of DIPN treated rat thyroid gland stained for T_3.x350

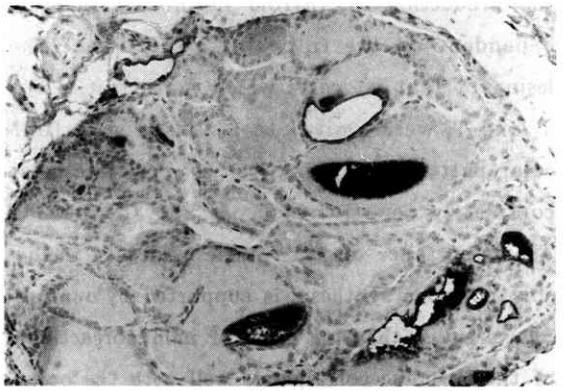

Fig. 14. Type 2a lesion of DIPN treated rat thyroid gland stained for T_3.x350

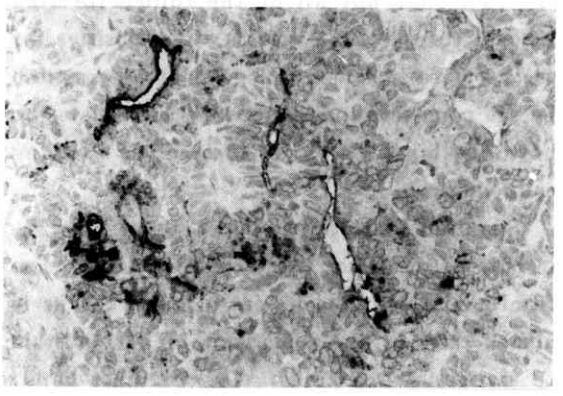

Fig. 15. Type 3b lesion of DIPN treated rat thyroid gland stained for Tg.x700

It was, further, clearly demonstrated that

	Tg	T_4	T_3
Type 1. (AF)	+	+	+
Type 2a. (NP)	+	(±) ~ +	(±) ~ +
2b.	(±) ~ +	- ~ (±)	-
2c.	(±) ~ +	- ~ +	- ~ +
Type 3a. (Ca)	± ~ +	- ~ ±	- ~ ±
3b.	±	-	-
3c.	- ~ +	- ~ (±)	- ~ (±)
3d.	- ~ ±	-	-

Table 6. Immunostainability for Tg, T_4 and T_3 of each type of thyroid lesions of rats treated with DIPN. (): infrequent.

the production of thyroid hormone is largely dependent on the follicular structure of the lesions. That is, cases which were positive for T_4 and T_3 were limited to lesions with a follicular pattern, while both of them were almost completely negative in types 2b, 3b and 3d. The importance of the follicle structure in thyroid hormone synthesis is supported by our previous finding that the onset of immunoreactivity of the hormone corresponds well with the occurrence of primitive follicles in the fetal thyroid gland at the 17th gestational day[2]. However, the fact that not all of the follicle-forming lesions (for instance, type 3a) were positive for the hormone immunostaining might be explained by the poor development of organelles related to hormone biosynthesis in the follicle forming epithelium[21].

Simultaneous localization of Tg, T_4 and T_3, insofar as the latter two were positive was confirmed in the thyroid lesions in our study as in human cases by the application of immunostain to serial tissue sections. On the other hand, positive staining for Tg was not necessarily accompanied by positivity for T_4 or T_3 as shown in the case of cancerous lesions. These discrepancies among the stainability of these compounds appear to be important when analyzing the mechanism of impaired function of the thyroid epithelium in neoplasms. It is postulated that in cases of negative T_4 or T_3 with positive Tg, the disturbance of hormone synthesis could be caused by a failure in the incorporation of iodide by the follicular epithelium, the process of organification, coupling of iodinated tyrosine etc., rather than by a failure in Tg synthesis.

Conclusion

A few examples of the fruits of immunohistochemical studies on the thyroid gland were present ed briefly. Although not mentioned in this article, immunohistochemical approaches to the expression of oncogenes or demonstration of their products in thyroid tumors, and localization of the receptors for TSH or thyroid stimulating immunoglobulins in the patients of autoimmune thyroid diseases could be another promising subjects in the near future.

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