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Morphometric Lightmicroscopic and Immunohistochemical Analyses of Differentiated Thyroid Carcinomas*

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Summary. Twenty-four differentiated human thyroid carcinomas were investigated light microscopically and immunohistochemically by morphometric methods. The study revealed that thyroglobulin synthesis in follicular and papillary carcinomas is independent of tumor type and histostructural differentiation. The immunohistochemical results suggest that a defect in thyroglobulin synthesis and thyroglobulin secretion in addition to iodine accumulation and organification defects impairs the effectiveness of postoperative radioiodine treatment of differentiated thyroid carcinomas.

Key words: Thyroid cancer – Thyroglobulin – Immunohistochemistry – Morphometry

The effectiveness of postoperative radioiodine treatment of differentiated thyroid carcinomas depends on the accumulation and organification of the isotope by the tumor. Biochemical (Valenta et al. 1968; Thomas-Morvan et al. 1974), immunohistochemical and electron microscopic studies (Böcker et al. 1981) have revealed that these and other organspecific processes like thyroglobulin synthesis do take place in differentiated human thyroid carcinomas, although at reduced levels. In vivo studies of radioiodine therapy have shown, that the concentration of radioiodine in the great majority of differentiated thyroid carcinomas occurrs independent from its follicular of papillary histological structure (Pochin 1969, 1971).

The aim of this investigation was to determine the immunohistochemical intensity of thyroglobulin synthesis in 24 differentiated thyroid carcinomas with regard to tumor type and their degree of differentiation. Histomorphological structures of known functional relevance in the thryoid gland were

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analysed in order to eveluate their significance in predicting the functional activity as indicated by the thyroglobulin synthesis.

Material and Methods

Of 234 differentiated thyroid carcinomas surgically treated in the University of Hamburg and the General Hospital of Hamburg-Harburg from 1964–1980, 24 (16 papillary carcinomas and 8 follicular carcinomas) were retrospectively selected for morphometric lightmicroscopic, thyroglobulin (TG) immunohistochemical and electron microscopic analysis (details of the ultrastructural results are in preparation). The cases were selected randomly, the only prerequisite being that at least three paraffin blocks were available. The grading of the follicular carcinomas was carried out according to the WHO classification (1974) that of the papillary carcinomas according to Tscholl-Ducommun and Hedinger (1982). Histological sections were stained with hematoxylin and eosin, PAS and Masson-Goldner. The immunohistochemical staining of TG was carried out using a modification of the method of Sternberger et al. (1970) (Böcker et al. 1981).

Morphometric Analyses

The morphometric analyses of the tumors were performed on 2–6 histological sections with a minimum cut surface of 150 mm^2 being examined in each case. The volumetric fractions of follicular epithelium, intraluminal colloid, follicular lumen, interstitium and TG-producing cells were determined with the point counting method (Delesse 1847; Chalkley 1943; Hennig 1958; Weibel 1963; Dunnill 1968). Using a Zeiss II integrating eyepiece, a square grid with 100 standard points was superimposed on the histological sections (Fig. 1). This method is based on the fact that the relative volumes of the various tissue components are numerically equal to the relative areas occupied by those components. As the distance between the points should be equivalent to the mean diameter of the structure under investigation, the sections were examined at 25 fold magnification.

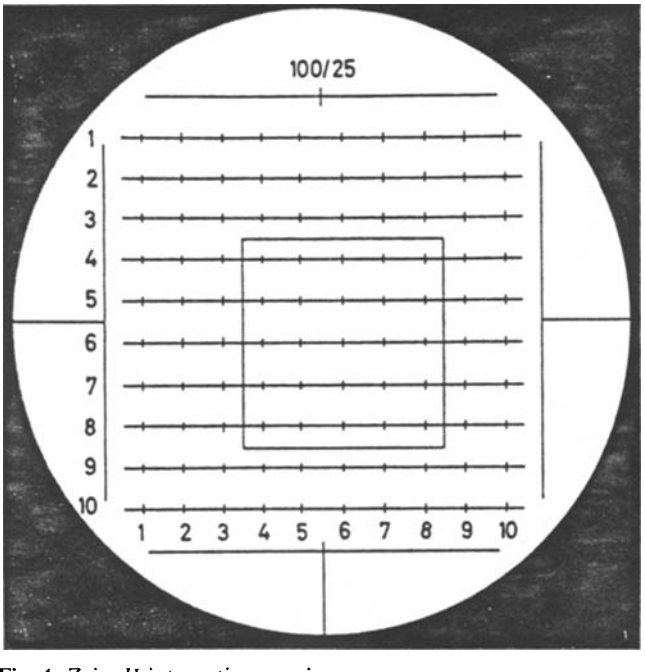
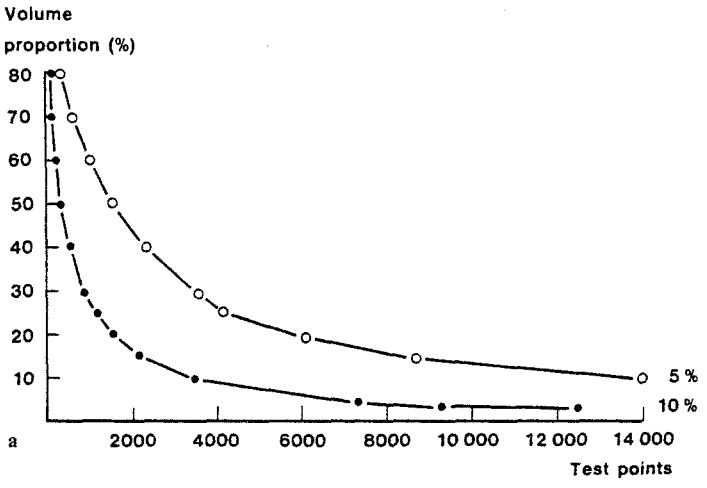


Fig. 1. Zeiss II integrating eyepiece



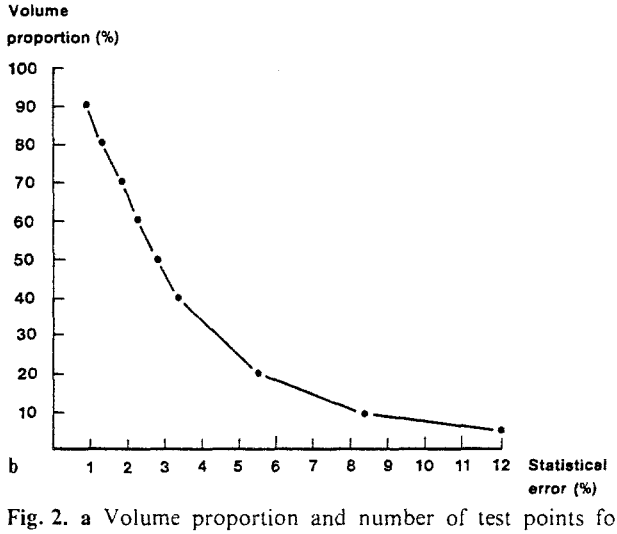


Fig. 2. a Volume proportion and number of test points for a given standard deviation. b Volume proportion and statistical error for a given number of test points ($N = 5{,}000$)

Statistical Analyses

a) Number of Points. The number of points (N) which have to be counted if the error is not to exceed 5% can be calculated using the normal approximation of the binomial distribution. With 95% confidence limits:

$$N = \frac{3.84 \times 400 \times q}{p}$$

were $q = 1\text{-}p$; $p =$ volumetric fraction

The equation reveals that the smaller the volumetric proportion to be estimated, the higher the number of test points that must be counted for a given standard deviation (Fig. 2a). In other words, with a given number of points, the statistical error is smaller the larger the size of the fraction to be estimated (Fig. 2b). We decided to count 50 fields in each histological

section. Thus there was a statistical error of about 10% in cases where the volumetric proportion was 10% or less.

b) Distribution of the test points within the tissue. As Weibel (1963) demonstrated, the accuracy of the morphometric method depends not only on the total number of points (see above) but also on their distribution within the histological section. It is necessary to encompass as large an area as possible. Therefore after determining the magnitude of the tumor area, we apportioned the points – 5,000 per section – homogeneously over the entire area. The percentage error with a homogeneous distribution is approximately ten times smaller.

c) Correlation of the histomorphometric with the Immuno-histomorphometric Data. The statistical analysis of the morphometric data included the calculation of the mean ($\bar{x}$), of the standard deviation (SD), the calculation of Pearson's correlation coefficient and the rank correlation coefficient of Spearman and the performance of the *t*-test for the following structural groups:

1. follicular carcinomas (FC)
2. papillary carcinomas (PC)
   a) papillary areas (pa) in PC
   b) follicular areas (fa) in PC

Results

Morphometry of follicular carcinomas (Tables 1 and 3a). The 8 follicular carcinomas (FC) examined had large epithelial fractions (79%) and very small colloid fractions (2%). The fraction containing thyroglobulin positive thyrocytes (TGpT) was on an average 9% but in fact two groups with significantly different volumetric proportions could be observed: a) FC with

Table 1. Follicular carcinomas: grade of tumor differentiation and volume proportions of epithelium, colloid, lumina, interstitium and thyroglobulin positive thyrocytes (TG p.T.)

J.-No.	Grade of diff.[a]	Volume proportion				
		epith.	colloid	lumen	interst.	TG p.T.
1 H 4246/64	+	82.5	1.9	3.7	13.2	4.9
2 E 17135/71	+ + +	79.4	2.1	5.1	12.6	17.1
3 E 7528/73	+/+ +	81.6	0.2	4.0	14.8	1.5
4 H 871/76	+ +	83.7	0.1	0.4	15.7	0.3
5 H 5914/76	+/+ +	72.9	0.0	1.2	25.8	28.0
6 E 4505/78	+ +/+ + +	83.2	3.8	6.9	6.1	9.5
7 H 7974/78	+ +/+ + +	73.2	0.4	10.9	15.5	2.7
8 E 28457/78	+ +/+ + +	74.0	5.1	2.6	18.3	9.8
		6)[b]	7)	8)	9)	10)
1–8	$\bar{x}$, s^{n-1}	78.8±4.7	1.7±1.9	4.4±3.4	15.3±5.6	9.2±9.4
		1)[b]	2)	3)	4)	5)
1, 3, 4, 6–8	$\bar{x}$, s^{n-1}	82.6±3.8	0.7±1.0	2.7±2.0	14.6±3.0	2.2±2.4
		1)[b]	2)	3)	4)	5)
2, 5	$\bar{x}$, s^{n-1}	76.2±4.6	1.1±1.5	3.2±2.8	19.2±9.3	22.6±7.7

[a] Grade of differentiation: + low; + + moderate; + + + high
[b] See Table 4

Table 2a. Papillary carcinomas (total): grade of tumor differentiation and volume proportions of epithelium, colloid, lumina, interstitium and thyroglobulin positive thyrocytes

J.-No.	Grade of diff.[a]	Volume proportion				
		epith.	colloid	lumen	interst.	TG p.T.
1 H 4828/66	+ + +	51.4	4.3	18.9	25.4	10.2
2 H 5463/68	+ + +	35.4	2.4	45.0	17.1	23.3
3 H 7375/70	+ + +	40.1	12.7	8.0	39.2	6.1
4 H 655/72	+ + +	53.7	2.0	24.8	20.2	14.8
5 H 7456/73	+ + +	49.2	25.3	5.5	20.1	9.6
6 H 1751/75	+ / + + / + + +	63.1	6.1	8.0	22.8	1.2
7 H 2010/75	+ + / + + +	62.9	2.3	15.7	19.1	4.4
8 H 2599/75	+ + / + + +	63.1	8.5	19.0	9.5	2.8
9 H 1405/76	+ / + + / + + +	68.0	5.7	6.1	20.3	0.6
10 H 4435/76	+ / + + +	85.6	0.0	4.3	10.1	3.4
11 H 4455/76	+ + / + + +	63.1	18.2	14.5	4.2	22.4
12 H 5243/76	+ + +	16.6	2.7	3.5	77.0	0.8
13 H 5559/77	+ + +	67.8	9.2	2.8	20.2	21.6
14 H 5560/77	+ + / + + +	67.7	16.2	7.8	8.3	3.1
15 H 10886/77	+ + +	46.8	0.6	44.0	8.6	0.7
16 E 36/80	+ + +	67.7	2.5	3.9	26.0	9.1
		6)[b]	7)	8)	9)	10)
1–16	$\bar{x}$, s^{n-1}	56.4 ± 16.4	7.8 ± 7.1	14.5 ± 13.4	21.8 ± 17.1	8.4 ± 8.0
		11)[b]	12)	13)	14)	15)
1, 3–10, 12, 16	$\bar{x}$, s^{n-1}	56.6 ± 16.8	6.8 ± 7.3	13.4 ± 11.6	23.6 ± 18.3	5.2 ± 4.5
		11)[b]	12)	13)	14)	15)
2, 11, 13	$\bar{x}$, s^{n-1}	55.4 ± 17.5	9.9 ± 7.9	20.8 ± 21.8	13.8 ± 8.5	22.4 ± 0.8

[a] Grade of tumor differentiation: + low; + + moderate; + + + high
[b] See Table 3

relatively large TGpT fractions (23%); and b) FC with small TGpT fractions (2%).

Both these groups of follicular carcinomas differed only with regard to the volumetric proportions of TGpT; there were no significant differences in the proportions of epithelium, colloid, lumen and interstitium. Moreover the structural differentiation of tumors from both groups did not differ (Fig. 3a, 3b).

Morphometry of papillary carcinomas (PC) and comparison with follicular carcinomas (Tables 2a and 3a). The epithelial fraction of the 16 papillary carcinomas analysed was significantly smaller (56%) than that of the FC. The colloid and lumen proportions on the other hand were clearly larger in the PC than in the FC. Among the PC two groups could also be distinguished on the basis of significant quantitative differences in the TGpT fraction: a) PC with a relatively large TGpT fraction (22%); and b) PC with a small TGpT fraction (5%).

Table 2b. Papillary carcinomas, papillary areas (pa) and follicular areas (fa): grade of tumor differentiation and volume proportions of epithelium, colloid, lumen, interstitium and thyroglobulin positive thyrocytes (TG p.T.)

	J.-No.	Grade of diff.[a]		Epithelium	
		fa	pa	fa	pa
1	H 655/72	+ + +	+ + +	66.1	41.2
2	H 1751/75	+/+ + +	+/+ +/+ + +	73.3	67.3
3	H 2010/75	+ +/+ + +	+ +	71.9	58.2
4	H 2599/75	+ + +	+ +/+ + +	55.9	50.5
5	H 1405/76	+/+ +/+ + +	+ + +	59.4	70.1
6	H 4435/76	+ + +	+	86.9	83.1
7	H 4455/76	+ +/+ + +	+ + +	64.2	60.9
8	H 5560/77	+ +/+ + +	+ +/+ + +	67.0	70.7
				16)[b]	16)
1–8		$\bar{x}$, s^{n-1}		68.1 ± 9.6	62.8 ± 13.4

[a] Grade of tumor differentiation: + low; + + moderate; + + + high
[b] See Table 3

As with the corresponding FC groups, both groups differed only with regard to the volumetric proportions of TGpT. The epithelial, colloid, lumen and interstitial proportions did not differ significantly between the two groups. The structural differentiation of the tumors again in no way correlated with the volumetric proportion of TGpT (Fig. 3c, 3d).

Morphometric comparison between papillary (pa) and follicular (fa) areas in papillary carcinomas (Tables 2b and 3a). In those papillary carcinomas which contained purely papillary and purely follicular tumor areas, additional tumor areas were examined. It was found that the colloid and lumen proportions clearly differed in the papillary and follicular tumor areas. The volumetric proportions of epithelium, interstitium and TGpT showed no significant statistical differences at the 5% level.

Correlation between the individual structural components of follicular and papillary carcinomas (Table 3b). Correlations between the functionally important structural components – epithelium, colloid and TGpT – were made using the Pearson and Spearman rank correlation coefficiant. A positive correlation was said to exist if the probability of error at the 5% level was significant. In the *follicular carcinomas* there was a significant negative correlation between the volumetric proportions of epithelium and TGpT, that is, large volumes of epithelium correlated with small volumes of TGpT and vice versa. Three of these tumors with large epithelial proportions (Table 1, case nos. 1, 3 and 4) showed in the main moderate to low degrees of differentiation. On the other hand FC with a moderate degree of differentiation and a small epithelial proportion (case no. 5) were also found. The

Table 2b (continued)

Colloid		Lumen		Interst.		TG p.T.	
fa	pa	fa	pa	fa	pa	fa	pa
3.5	0.4	16.9	31.6	13.5	26.9	29.6	0.1
6.5	3.7	5.6	11.6	14.7	17.3	5.6	1.0
0.8	3.4	4.2	18.3	23.2	20.1	3.2	4.6
17.1	7.6	15.1	29.4	11.9	12.5	4.3	0.2
19.4	2.3	2.2	7.2	19.0	20.6	2.4	0.3
0.0	0.0	3.1	6.8	10.1	10.1	2.4	5.2
23.9	6.8	8.5	26.6	3.4	5.7	34.6	2.4
18.7	6.0	7.7	8.3	15.3	15.3	3.2	7.6
17)	17)	18)	18)	19)	19)	20)	20)
11.2±9.5	3.8±2.9	7.9±5.4	17.5±10.4	12.8±6.4	16.1±6.7	10.6±13.4	2.7±2.8

Table 3a. Statistical analyses of follicular and papillary carcinomas by Students *t*-test (see Tables 1 and 2)

No. (see Table 1 and 2)	Tissue	*t*-test	Significance (5% level)
1	FC	2.53	−
2	FC	0.29	−
3	FC	0.22	−
4	FC	0.92	−
5	FC	4.58	+
6	FC/PC (total)	3.76	+
7	FC/PC (total)	2.17	+
8	FC/PC (total)	2.08	+
9	FC/PC (total)	1.04	−
10	FC/PC (total)	0.23	−
11	PC (total)	0.11	−
12	PC (total)	0.65	−
13	PC (total)	0.89	−
14	PC (total)	0.88	−
15	PC (total)	6.45	+
16	PC (pa/fa)	0.93	−
17	PC (pa/fa)	2.12	+
18	PC (pa/fa)	2.30	+
19	PC (pa/fa)	0.69	−
20	PC (pa/fa)	1.66	−

− = no significance; + = significant

Table 3b. Statistical analyses of follicular and papillary carcinomas by the calculation of Pearson's correlation coefficient and the rank correlation coefficient of Spearman

No. (cases in Table 1 and 2)	Tissue	Histological structure	Pearson	Spearman	Significance $p=0.05$
1–8	FC	epithelium/colloid	0.00	+0.1	no
		epithelium/TG p T	−0.49	−0.60	−
		colloid/TG p T	0.00	+0.26	no
1–16	PC	epithelium/colloid	0.00	+0.06	no
		epithelium/TG p T	0.00	−0.12	no
		colloid/TG p T	0.20	+0.17	no
1–8	PC – pa	epithelium/colloid	−0.17	−0.26	no
		epithelium/TG p T	+0.56	+0.74	+
		colloid/TG p T	0.00	−0.02	no
1–8	PC – fa	epithelium/colloid	−0.72	−0.69	−
		epithelium/TG p T	−0.20	−0.20	no
		colloid/TG p T	+0.22	+0.30	no

no = no correlation; − = negative correlation; + = positive correlation

comparison between the structural components epithelium/colloid and colloid/TGpT produced no significant correlation. In the *papillary carcinomas* there were also no significant correlation between these components.

In the *papillary areas of papillary carcinomas* (Tables 2b and 3b) there was a positive correlation between the epithelial and TGpT proportions. A definite relationship with stage of differentiation did not exist however, there were tumor areas with a high degree of differentiation, a relatively small epithelial fraction and a very small TGpT fraction (case no. 1) as well as areas with minimum differentiation, a large epithelial fraction and moderately large TGpT fractions (case no. 6). With regard to the structural components epitehlium/colloid and colloid/TGpT there was neither a positive nor a negative correlation. In the *follicular areas of papillary carcinomas* (Tables 2b and 3b) there was a negative correlation between the epithelial and colloid proportions. For the structural components epithelium/TGpT and colloid/TGpT there was neither a positive nor a negative correlation.

Discussion

Up till now histomorphometric studies of the human thyroid gland have only been performed on normal and ageing glands (Roberts 1974; Forstreuter et al. 1976; Rother 1979) but not on malignant thyroid tumors. We therefore made histomorphometric analyses of organoid thyroid carcinomas of different degrees of differentiation and evaluated their function by thryoglobulin immunohistochemistry. In 8 follicular and 16 papillary thryoid carcinomas quantitative evaluations using the point counting method (Delesse 1847; Chalkley 1943; Hennig 1958; Weibel 1963; Dunnill 1968) were per-

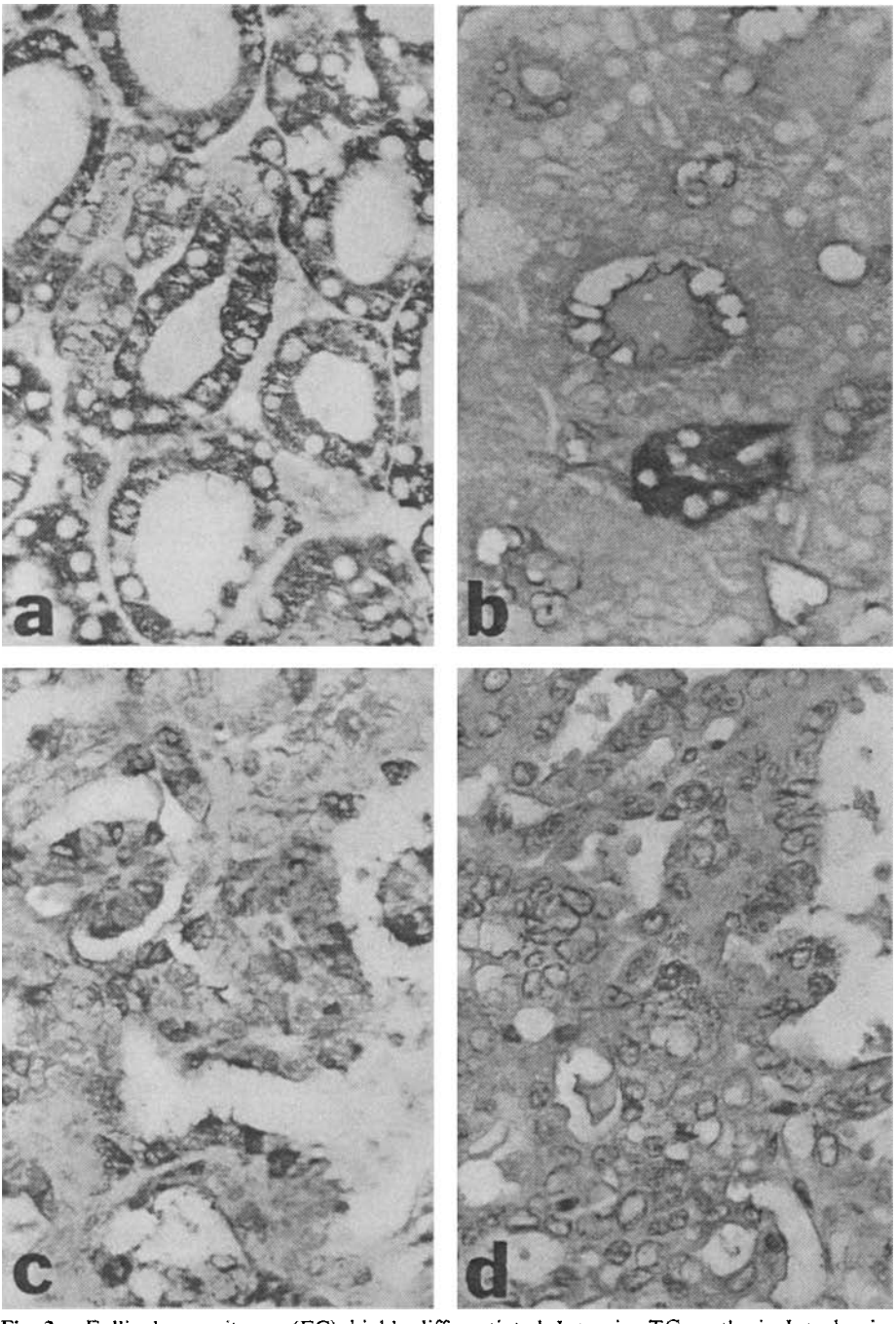


Fig. 3. **a** Follicular carcinoma (FC), highly differentiated. Intensive TG synthesis. Intraluminar negative TG staining reaction. PAP, ×400. **b** FC, highly differentiated. Only weak TG synthesis. PAP, ×400. **c** Papillary carcinomas (PC), highly differentiated. Intensive TG staining reaction. PAP, ×400. **d** PC, highly differentiated. Weak TG synthesis. PAP, ×400

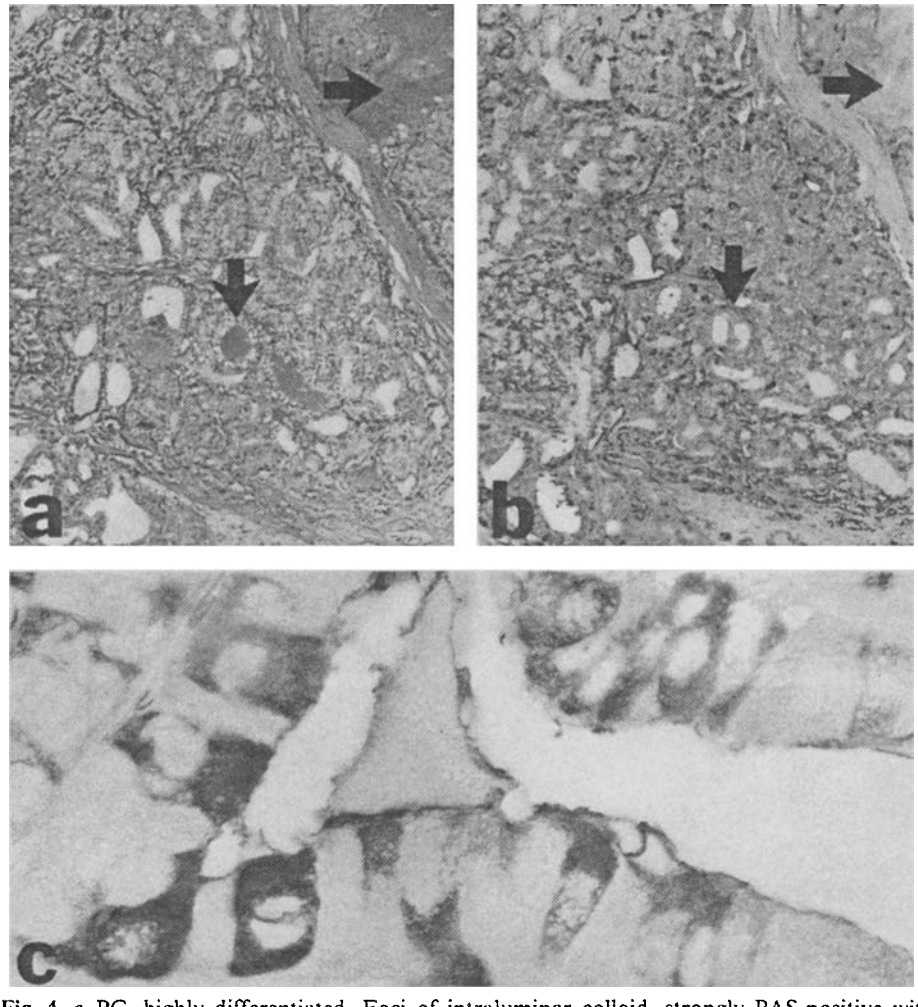


Fig. 4. **a** PC, highly differentiated. Foci of intraluminar colloid, strongly PAS-positive with a negative TG staining reaction (*arrows*); see Fig. 4b. PAS, ×250. **b** PC, same section as Fig. 4a. PAP, ×250. **c** PC, highly differeniated. Great variation of the TG reaction within the tumor cells. PAP, ×1,000

formed on the structural components epithelium, colloid, lumen, interstitium and TG positive tumor cells.

For the follicular carcinomas, a larger volumetric proportion of tumor epithelium than in the papillary carcinomas was characteristic, together with smaller colloid and lumen proportions. This finding is not surprising as one would expect a higher proportion of lumen and a lower proportion of epithelium in papillary carcinomas on the bases of their microstructure.

Regarding the degree of differentiation and the amount of TG no definite correlation could be found (Fig. 4a, 4b) neither in papillary nor in follicular carcinomas. The negative correlation found in follicular carcinomas between the content of epithelium and TG positive thyrocytes is possibly due to

the greater cell content of moderately to slightly differentiated follicular carcinomas. It appears to indicate that the degree of differentiation is relevant for the intensity of TG synthesis. For the individual tumor however a prediction on the bases of the histostructure is not possible. For example follicular carcinomas with a solid structure and intensive thyroglobulin synthesis were also found. Thus the morphometric studies reveal that the analyses of the histostructure of differentiated thyroid carcinomas alone does not allow a prediction to be made on the intensity of thyroglobulin synthesis by the tumor. These results are in accordance with biochemical studies on the thyroglobulin contents of differentiated thyroid carcinomas by Valenta et al. (1968), Thomas-Morvan et al. (1974) and Valenta and Michel-Bechet (1977). For both the follicular and papillary carcinomas, there proved to be two tumor groups with regard to TG – immunostaining; a larger group of follicular and papillary carcinomas with a relatively weak thyroglobulin synthesis (2–5% volume proportion) and a smaller group of follicular and papillary carcinomas with a very intensive thyroglobulin synthesis (about 20% volume proportion). Of a total of 260 thyroid carcinomas investigated by TG immunohistochemistry 2 papillary carcinomas even showed a negative thyroglobulin reaction.

For the overall assessment of TG synthesis with TG immunohistochemistry it is not only the number of tumor cells synthesising TG which is important but also the intensity and above all the localisation of the reaction product. Accordingly these aspects will also be discussed here.

In a number of tumor cells there is on the one hand (1) a noticeable absence of TG immunostaining. On the other hand (2) a positive TG reaction is only seldom seen in the glandular lumina of papillary and follicular carcinomas. These findings can be best explained (1) by a defect in TG synthesis due to a lack of cellular differentiation at the ultrastructural level. Corresponding biochemical studies which revealed a reduction in TG synthesis (Lupulescu et al. 1968; Valenta et al. 1968; Thomas-Morvan et al. 1974, 1977; Valenta and Michel-Bechet 1977) are in agreement with this observation.

With regard to the second observation (2) a defect in TG transport or secretion at the apical cell membrane can be hypothesised. As intraluminar TG is only seldom found, it must be assumed that there has been a breakdown in the functional interaction between the epithelial cell and the intraluminar colloid found in normal thyroid glands. The direct incretion of TG into the circulation, bypassing the glandular lumen, can thus be postulated. In contrast the basal cell membrane of differentiated carcinomas seems to be intact and responsive to TSH stimulation (Uller et al. 1973; Unger et al. 1980; Schlumberger et al. 1980). Studies on serum TG levels in thyroidectomised patients with differentiated thyroid carcinomas revealed a positive correlation with the TG synthesis demonstrated with immunohistomorphometry (Dralle et al. 1982).

With regard to the immunological specifity of the TG molecule, van Herle and Uller (1975) were able to show on the bases of physicochemical characteristics and immunological properties that TG from carcinomatous

tissue behaved identically to TG derived from normal thyroid tissue. Similarly the TG antiserum used in this study showed only one precipitation line in double diffusion tests against thyroid extract in agar (Böcker, unpublished data). Thus with this method there is immunostaining of the 19 S TG only; atypical iodoproteins or those of lower molecular weight give no positive reaction product.

To summarise the results presented here reveal that quantitative analyses with TG immunohistochemistry is a suitable method of obtaining a functional characterisation of differentiated thyroid carcinomas above and beyond the WHO classification. A prognostically useful correlation between radioiodine uptake and TG synthesis probably only exists for a few differentiated carcinomas however. TG immunohistomorphometry can thus only be regarded as an indirect assessment of the probable effectiveness of postoperative radioiodine therapy.

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