The predictive value of histometry of thyroid tissue in anticipating hypothyroidism after subtotal thyroidectomy for primary thyrotoxicosis

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SYNOPSIS Thyroid tissue removed at partial thyroidectomy from primary thyrotoxicosis patients, after preparation for operation with carbimazole and Lugol's iodine, was studied with the histometric technique. In patients with little or no evidence of autoimmunity before operation, the chance of developing postoperative hypothyroidism was approximately 1 in 2 in those who had volume percentage of epithelium greater than 40, but only approximately 1 in 10 in those who had volume percentage of epithelium less than 40. This approach may ultimately prove of predictive value in a substantial proportion of patients for anticipating hypothyroidism after subtotal thyroidectomy for primary thyrotoxicosis.

It is not known why subtotal thyroidectomy effects a permanent cure in a substantial proportion of patients with primary thyrotoxicosis, nor is it clear which patients are most likely to benefit from the operation. Recent reports of the incidence of hypothyroidism after partial thyroidectomy range from 35% (Hedley, Flemming, Chesters, Michie, and Crooks, 1970) to 48% (Beahrs and Sakulsky, 1968). Previously it has been shown that patients with low remnant weight (Michie, Pegg, and Bewsher, 1972), high preoperative antithyroid antibody titres (Irvine and Stewart, 1967), and abundant lymphoid infiltration of the thyroid (Greene, 1950; Levitt, 1951; Greene, 1953) are predisposed to the development of postoperative hypothyroidism.

The present histometric study was undertaken to determine whether the structure of the glands at the time of operation bore any relation to the outcome of treatment.

Materials and Methods

This study was undertaken on 69 female primary thyrotoxic patients for whom subtotal thyroidectomy was regarded as the treatment of choice. The diagnosis of thyrotoxicosis was based on the clinical diagnostic index (Crooks, Murray, and

Wayne, 1959) and serum protein-bound iodine (PB¹²⁷I) estimations. Diagnosis was confirmed by a radioactive iodine uptake test, supplemented where necessary by the response to triiodothyronine (Hobbs, Bayliss, and MacLagan, 1963). All of the patients were prepared for operation with carbimazole and had received potassium iodide (5 minims of Lugol's iodine three times daily) for 10 days before operation. Postoperative hypothyroidism was assessed by clinical examination based on the standard diagnostic index (Billewicz, Chapman, Crooks, Day, Gossage, Wayne, and Young, 1969), on electrocardiographic evidence, and on serial serum PB¹²⁷I and cholesterol estimations. The final arbiter was a therapeutic trial with thyroxine (Werner and Spooner, 1955). Patients were examined at one, four, and 12 months after operation and thereafter on a yearly basis until the services of a computer-assisted register (Hedley, Scott, Weir, and Crooks, 1970) became available.

At the time of operation, the size of the thyroid remnant was estimated visually (Michie *et al*, 1972). The weight of the thyroid was then deduced as the sum of the remnant and resected specimen weights. The resected tissue was fixed in 4% neutral buffered formaldehyde; thereafter the thyroids were cut into 5 mm slices. Blocks were taken from any areas of unusual naked-eye appearance, in addition to a minimum of two representative blocks from each lobe. Sections (5 μ m) were prepared from paraffin blocks and stained with haematoxylin and

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Hypothesis that Observation A greater if Condition B Satisfied		No. of Patients Satisfying Condition B	U Statistic	P
A	B			
%E	%L $\geq$ 1.0	16	436	NS ¹
Total E	%L $\geq$ 1.0	16	462	NS
%L	ACA positive	28	379	<0.01
Total L	ACA positive	28	237	<0.001
%L	TRC positive	19	246	<0.001
Total L	TRC positive	19	379	<0.01
%E	ACA positive	28	460	NS
Total E	ACA positive	28	479	NS
%E	TRC positive	19	314	<0.05
Total E	TRC positive	19	279.5	<0.01

Table I Mann Whitney ranking tests on relation of histometric and serological findings

¹NS = not significant at 5% level.

eosin with standardized techniques. For histometry, sections were examined with a $\times 40$ objective and a $\times 10$ focusing eyepiece containing a 36-intersection graticule. The volume percentages of epithelium (% E), colloid (% C), lymphoid tissue infiltrate (% L), and other tissues (blood vessels and stroma) were measured with the point-counting technique (Anderson and Dunnill, 1965) by one observer who did not have prior knowledge of preoperative treatment until the completion of the study. From the product of these measurements and the weights of the total gland and the remnant respectively, the total epithelial cell volume (total E), remnant epithelial volume (remnant E), and total glandular colloid (total C), remnant colloid (remnant C), total glandular lymphoid tissue (total L), and remnant lymphoid tissue (remnant L) were calculated. In preliminary studies, it was shown (1) that the standard error of the observations was less than 3% when 20 fields were selected for counting using random numbers to define the coordinates of the vernier scale of the mechanical stage, and (2) that examination of one section with this method gave

results closely comparable to those obtained from examination of several sections from the same gland.

Antithyroglobulin antibody and anticytoplasmic antibody (ACA) were estimated by the tanned-red-cell-haemagglutination (TRC) (Fulthorpe, Roitt, Doniach, and Couchman, 1961) and immunofluorescence (IF) (Beck, 1971) techniques respectively. For the purpose of analysis of results, a TRC titre $\geq 1/250$ was regarded as being TRC-positive and an IF result of ++ or +++ was similarly regarded as being ACA-positive: this accords with the system used previously (Hedley, Ross, Beck, Donald, Albert-Recht, Michie, and Crooks, 1971).

Results

Scatter diagrams of the %E, %L, and total L did not show any relationship between these measurements and the age of the patient at the time of operation or the duration of preoperative treatment with carbimazole. Table I shows the results of Mann Whitney ranking tests which established that

Hypothesis that Observation A Greater if Condition B Satisfied			No. of Patients Satisfying Condition B	U Statistic	P
A	B				
	Preoperative Serology	Postoperative Thyroid Status			
Remnant weight	. . . ¹	Euthyroid	50	318	<0.05
Duration of therapy	. . .	Hypothyroid	19	218	<0.05
Duration of therapy	ACA negative	Hypothyroid	10	117	NS ²
Duration of therapy	ACA positive	Hypothyroid	9	34	<0.05
Duration of therapy	TRC negative	Hypothyroid	10	154	NS
Duration of therapy	TRC positive	Hypothyroid	9	11	<0.01

Table II Mann Whitney ranking tests on relation of remnant weight and duration of preoperative drug therapy to serological findings and postoperative status

¹. . . = not relevant²NS = not significant at 5% level

Fig 1 The volume percentage of epithelium in the fixed thyroids of 69 primary thyrotoxicosis patients, treated by partial thyroidectomy, distributed according to postoperative thyroid status (euthyroid ●; hypothyroid ▲) and the presence or absence of each of three manifestations of thyroid autoimmunity. ACA + = IF staining ++ or +++ ; TRC + = $\geq 1/250$; %L + = $\geq 1\%$.

the lymphoid tissue infiltrate is more abundant in patients who have anticytoplasmic antibody or antithyroglobulin antibody: these findings confirm and extend the work of Irvine and Stewart (1967). Furthermore, percentage E and total E are higher in those patients who have antithyroglobulin antibody, but not in those who have anticytoplasmic antibody: this result suggests that there may be a connexion between hyperplasia and the breakdown of tolerance to thyroglobulin, possibly from increased colloid spillage. There does not appear to be any relation between the extent of epithelial hyperplasia (deduced from percentage E and total E measurements) and the presence or absence of lymphoid infiltrate.

In this series of 69 primary thyrotoxicosis patients treated by partial thyroidectomy, 50 became euthyroid and 19 developed hypothyroidism after the operation. In an attempt to determine which of the factors studied are related to the occurrence of postoperative hypothyroidism, Mann Whitney ranking tests have shown (table II) that in patients who become hypothyroid after operation the remnant weights are smaller, thus confirming the findings of Michie *et al* (1972). Furthermore, in those patients who had antithyroid antibodies before operation, postoperative hypothyroidism is associated with longer preoperative drug therapy, but this does not apply to those in whom the autoantibody tests were negative (table II); Michie *et al* (1972) failed to

demonstrate any relationship between duration of drug treatment and postoperative hypothyroidism. No association is found between the remnant weight and the incidence of postoperative hypothyroidism in the smaller subgroup of patients with and without antithyroid antibodies. Total thyroid weight, age at operation, percentage and remnant E, total E, percentage and remnant C, total C, percentage and remnant L, and total L, taken individually, are not related to the incidence of postoperative hypothyroidism. The relation between %E and the three manifestations of thyroid autoimmunity in the patients who remained euthyroid after operation is contrasted with that for those who became hypothyroid in figure 1. The general distribution suggests that in those patients with little or no evidence of thyroid autoimmunity postoperative hypothyroidism is more frequent in those with a %E value greater than 40, whereas postoperative euthyroidism is almost the rule in those with a %E value less than 40. This trend is not apparent in patients with appreciable evidence of thyroid autoimmunity before operation. The validity of this arbitrary separation of %E above and below 40 was established with χ^2 tests (table III).

For the purpose of further analysis of the data, a TRC titre $\geq 1/250$, ACA $\geq ++$, %L ≥ 1.0 were each regarded as distinct manifestations of thyroid autoimmunity. Figure 2 shows the relation between %E and the number of manifestations of

Patients with	Number with Postoperative				χ^2	Probability that Hypothyroidism Commoner in Patients with %E $\geq$ 40
	Hypothyroidism		Euthyroidism			
	%E $\geq$ 40	%E < 40	%E $\geq$ 40	%E < 40		
ACA negative	7	3	8	23	7.07	<0.005
ACA positive	4	5	8	11	0.04	NS
TRC negative	9	4	9	31	9.55	<0.0025
TRC positive	2	4	7	38	2.04	NS
Lymphoid <1%	7	6	12	28	2.43	NS
Lymphoid $\geq$ 1%	4	2	4	6	1.07	NS
No evidence of autoimmunity ¹	5	2	5	19	6.35	<0.0125
One manifestation of autoimmunity ¹	3	1	5	10	2.25	NS
Two manifestations of autoimmunity ²	2	5	4	5	0.42	NS
None or one manifestation of autoimmunity ²	8	3	10	29	8.26	<0.0025
One, two, or three manifestations of autoimmunity ³	6	6	11	15	0.19	NS

Table III χ^2 tests on incidence of postoperative hypothyroidism comparing glands with $\geq$ 40% or < 40% epithelium

¹NS = not significant at 5% level.

²For the purpose of this analysis, manifestations of autoimmunity are defined as lymphoid tissue $\geq$ 1%, ACA $\geq$ ++, TRC $\geq$ 1/250.

Fig 2 The volume percentage of epithelium in the fixed thyroids of 69 primary thyrotoxicosis patients, treated by partial thyroidectomy, distributed according to postoperative thyroid status (euthyroid ●; hypothyroid ▲) and the number of positive manifestations of autoimmunity.

autoimmunity in patients who remained euthyroid or became hypothyroid after operation. This grouping of the findings confirms that, for those patients with no manifestations of autoimmunity at least, the %E value of 40 forms a true watershed since in this group postoperative hypothyroidism developed in two of the 21 patients (9.5%) with %E < 40, whereas it was found in five of the 10 patients (50.0%) who had %E $\geq$ 40 ($\chi^2 = 6.35$; df = 1.0; $P < 0.0125$). Cumulation of the data does not increase the predictive value of the observations (table III). Figure 2 also shows that in the patients who remained euthyroid after operation the mean %E increases with increasing number of manifestations of autoimmunity ($\chi^2 = 6.28$; df = 3.0; $P < 0.05$): an opposite trend is seen in the patients who became hypothyroid ($\chi^2 = 4.20$; df = 3.0; $P < 0.15$). No relationship is found between the %C or total C and the various factors studied above.

Discussion

Quantitative histology is more discriminating than visual observation in distinguishing differences in microscopic structure. The histometric technique has shown marked differences, not obvious in conventional microscopy, in the extent of lymphoid infiltrate in the thyroid of primary thyrotoxicosis patients prepared for operation with different drug treatment regimes (Beck, Young, Simpson, Gray, Nicol, Pegg, and Michie, 1973). With this technique we have now demonstrated that the volume percentage of epithelium in the fixed resected gland specimen is related to the development of postoperative hypothyroidism.

The overall incidence of hypothyroidism in our series is 28%. However, this study has revealed a group of patients in whom the incidence of postoperative hypothyroidism is substantially lower. These patients did not have detectable serological evidence of thyroid autoimmunity before operation and, at the time of operation, glandular epithelium formed less than 40% of the volume of the fixed thyroid: in this group the hypothyroid rate is 12%. Similar hypothyroid rates are found in the groups with no evidence of lymphoid infiltrate or with none of the three manifestations of autoimmunity: this reflects the positive correlation between these parameters. Conversely, in patients with one or more manifestations of autoimmunity, those remaining euthyroid postoperatively had a significantly greater proportion of epithelium at operation (fig 2).

It therefore seems possible that histometric measurement of thyroid epithelium may play a part, together with serological data and estimation of remnant size, in predicting the occurrence of postoperative hypothyroidism: further work will be required before it is possible to determine whether these approaches can be used to choose the optimum manner of treatment of a patient with primary thyrotoxicosis.

It is probable that postoperative hypothyroidism results from progressive destructive autoimmunity. We (Hedley *et al*, 1971) have suggested that this may be stimulated by release of thyroid epithelial cell antigens into the operative bed that contains dead and dying thyroid tissue and blood clot and in which organization and suture granulomas develop. It follows that the intensity of antigenic stimulation will be related to the amount of damaged thyroid epithelium exposed and hence to the volume percentage of epithelium in the gland. Of those patients who had little or no evidence of destructive autoimmunity before operation, those who developed postoperative hypothyroidism had a larger mean volume percentage of epithelium and this suggests that the more intense antigen stimulation broke down immunological tolerance with respect to thyroid antigens and so started the process of destructive autoimmunity leading progressively to hypothyroidism. By contrast, of those patients who showed two or more manifestations of destructive autoimmunity before operation, those who became hypothyroid had a much smaller mean volume percentage of epithelium and we suggest that in these patients, a smaller dose of antigen has stimulated further destructive autoimmunity. We cannot explain on immunological grounds why the

mean volume percentage of epithelium rises with increasing number of manifestations of thyroid autoimmunity in those patients who remained euthyroid after operation: perhaps it is related to the regenerative capacity of the thyroid epithelium.

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