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Histometry of thyroids containing few and multiple nodules

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SUMMARY Histometric measurements were made on individual nodules and the internodular thyroid on glands from 15 patients without evidence of endocrine thyroid disease: some of the thyroids had multiple nodules. Measurements of the surviving internodular thyroid tissue showed that the various components were each within the normal range for thyroids from patients with no evidence of thyroid disease, but there were considerable deviations within a few glands in the colloid:epithelium ratio that were associated with histological appearances of dilated or collapsed acini. The interpretation of these appearances presented difficulties, but it was concluded that the balance of evidence favoured the inference that the internodular part of the gland was under physiological growth control at the time of study. The nodules varied in size: most contained proportionately less epithelium and more colloid than the internodular thyroid. It was concluded that the nodules were unlikely to be secreting autonomously in these patients.

Sporadic non-toxic goitre is the commonest category of disease found in the thyroid of clinically euthyroid patients.[1] During the earlier stages in the development of this disease the thyroid is thought to have the appearance of a colloid goitre, but, as the disease runs its natural course the thyroid is changed progressively into a nodular goitre.[2] The aetiology of this process is controversial and still incompletely understood, but it seems to be a response to low availability of iodine to the individual thyroid acini.[3]

There has been very active interest in the structure and function of the nodules in the advanced disease, and it is now clear that such nodules are usually not neoplastic. The nodules are sometimes autonomously functioning—"hot"—and sometimes metabolically inactive—"cold"—with respect to avidity of uptake of iodine from the blood and responsiveness to adenohypophyseal control.[4]

By contrast, there has been relatively little interest in the intervening compressed residual non-nodular thyroid tissue filling the space between the nodules. This paper reports the histometric measurements of the internodular portions of "normal" thyroid tissue within nodular thyroids and contrasts these with comparable measurements on the individual nodules in the nodular glands and the whole thyroids of "normal" controls.

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Material and methods

This study was performed on 15 thyroids: 14 were obtained at necropsy from three men and eleven women (aged 52–88 years) and one was an operation specimen from a man aged 58. Table 1 shows the details of the patients and their thyroids. The glands all contained rounded encapsulated nodules that were not obviously neoplastic on histological examination. Four of the glands contained multiple nodules (10, 36, 50, and 145, respectively), and the others contained seven nodules (two cases), four nodules (two), three nodules (two), two nodules (one), and a single nodule (four). Six of the glands had a volume greater than 40 ml and these were regarded as goitrous.

Each gland was weighed and fixed: its volume was determined immediately after removal from the patient and at intervals during processing for histological examination with the water displacement can and by applying Simpson's rule[5] to measurements of the surface area of 1 cm slices of the gland with methods described previously.[6] The surface of each fixed slice was examined for exposed nodules and then palpated: where a hidden nodule was suspected an incision was made perpendicular to the surface to allow inspection. When a nodule was identified the region of the gland was sliced at 1–2 mm intervals to expose the maximum diameter for measurement. The first three glands in the series were examined exhaustively

Table 1 *Details of patients*

Code	Sex	Age (year)	Body height (cm)	Body weight (kg)	Body surface area (m^2)	Total thyroid volume (ml)	Interstitial thyroid tissue									No of nodules
							Calculated volume (ml)	Epithelium %	Epithelium ml	Colloid %	Colloid ml	"Lumen"* %	"Lumen"* ml	Stroma including vessels %	Stroma including vessels ml	
1	F	65	155	57	1·54	264	29·0	12	3·3	53	14·7	8	2·2	27	7·4	50
2	F	82	163	61	1·67	24·0	14·6	15	2·1	46	6·6	7	1·0	33	4·6	10
3	F	52	155	65	1·66	32·5	26·8	10	2·7	73	18·7	3	0·8	14	3·6	7
4	F	88	148	46	1·38	12·2	9·2	15	1·3	39	3·4	5	0·5	40	3·5	4
5	F	55	153	55	1·51	12·5	12·0	21	2·5	31	3·6	10	1·1	39	4·6	3
6	F	85	155	45	1·43	7·5	7·0	16	1·1	44	3·0	8	0·6	33	2·2	3
7	F	78	153	53	1·49	57·0	16·5	16	2·4	44	6·8	6	0·9	35	5·2	1
8	F	63	147	65	1·53	64·2	43·8	14	6·2	60	25·7	5	2·1	21	8·7	1
9	M	76	168	54	1·65	42·0	19·3	16	2·9	48	8·6	8	1·4	28	5·1	1
10	F	77	160	64	1·66	16·5	16·0	18	2·8	45	7·1	12	1·9	26	4·0	1
11	M	72	168	71	1·81	33·0	30·6	9	2·7	38	10·9	11	3·1	43	12·4	2
12	F	73	158	77	1·75	31·0	18·4	19	3·5	30	5·3	5	1·0	45	8·1	7
13	M	70	167	70	1·80	19·0	18·8	10	1·9	45	8·0	5	0·9	40	7·1	4
14	F	82	157	42	1·42	105	10·8	11	1·1	40	4·3	3	0·3	47	5·0	36
15	M	58	160	78	1·78	558†	25·0	13	3·1	56	13·7	2	0·5	30	7·1	145

*Retraction space between colloid and acinar epithelium; †operation specimen.

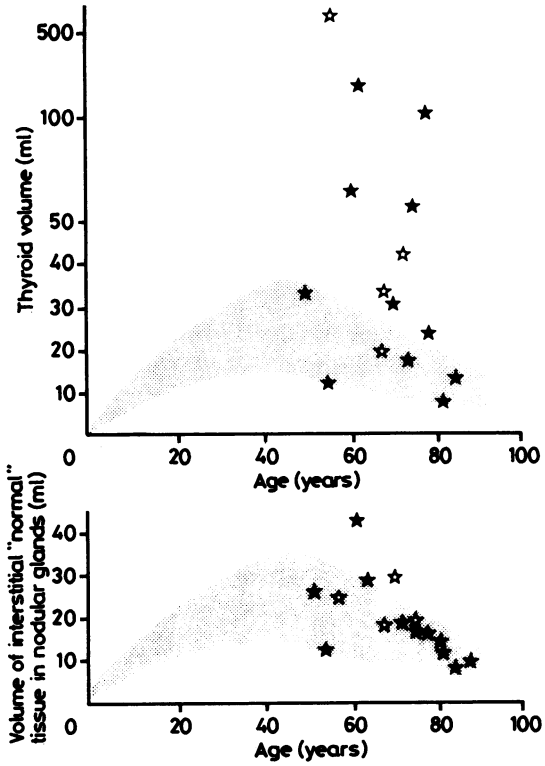

Fig. 1 *(a) Volume of nodular thyroids measured before fixation. Solid symbols indicate female patients and open symbols results on male patients. "Hatched" area includes all observations from previous survey of local patients without any evidence of thyroid disease.*[6]
(b) Volumes of interstitial portions of thyroid parenchyma between nodules. Measurements were made after fixation and slicing of glands, and these were corrected for shinkage. Most observations lie within "normal" range.

to ensure that this sampling procedure did not miss small nodules. The diameter of the individual nodules was measured with a ruler.

The volume of each nodule was calculated on the assumption that it was spherical. Histometric measurements were made on sections of every nodule and on the internodular thyroid tissue (assessing each anatomical unit as if it were a whole thyroid), as described previously[6]: these results were then used to calculate absolute values for volumes of histological components, number of acini/unit volume, and internal and external surface areas of the acini. We appreciated that these methods would probably result in a small overestimate of the numbers of acini and their surface areas in nodules, because the basic assumption that acini are roughly spherical is only partly valid due to the occasional occurrence of partly fused adjacent acini.

Results

The total volumes of the nodular thyroids were substantially greater than those of the normal glands studied under similar conditions (Fig. 1a).[6] When, however, the volume of the internodular part of each of the nodular glands was calculated it became clear that the values for this residual "normal" component were comparable with those of the whole volume of the normal glands studied previously (Fig. 1b).

Accordingly, the first stage of this investigation determined the extent to which the internodular portions of the nodular glands corresponded to the whole glands of control subjects who did not have endocrine disease. The primary measurements were obtained by point counting (Table 1). Virtually all of the measurements in each of the categories fell within the range observed in control patients from Tayside[6] of

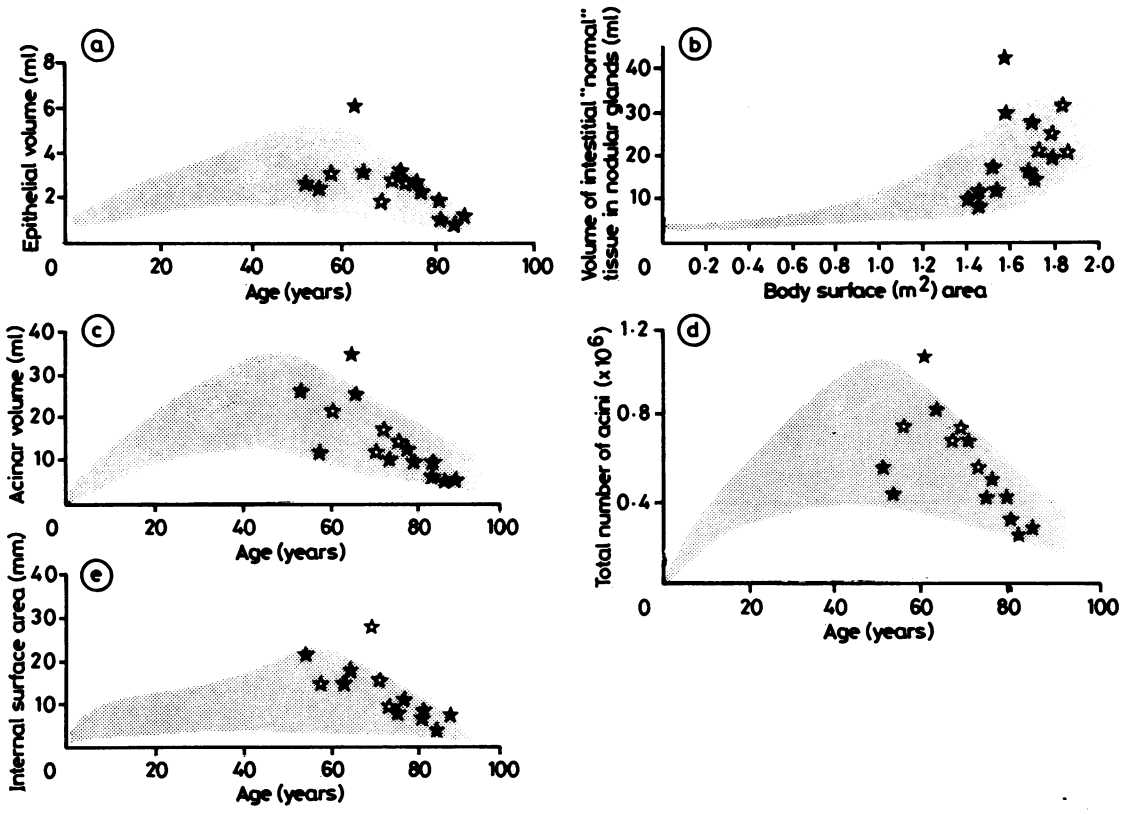

Fig. 2 *Histometric measurements on interstitial surviving "normal" portions of nodular thyroids.*
(a) Volume of epithelium related to age;
(b) volume of interstitial thyroid related to body surface area;
(c) volume of acini related to age;
(d) internal surface area of acini related to age;
(e) total number of acini related to age.

comparable age (Fig. 2a) and also lay within the 95% confidence limits described by the mean standard deviation. The measurements of the internodular parts in the nodular glands bore a similar correlation to body weight, height, and surface area as the whole glands of the control patients (Fig. 2b). Similarly, the total volume of the acini (Fig. 2c) and their internal surface area (Fig. 2d), and the total number of acini in the internodular gland (Fig. 2e) were similar to the corresponding measurement for the control glands.

Although the primary measurements fell within the normal range, there was some imbalance between them in certain specimens. In case 3 the colloid: epithelium ratio was about 4:1, corresponding to a histological appearance of dilated acini of the type seen in colloid goitre; the total volume of epithelium in this gland, however, was 2·7 ml (not reduced), but the colloid volume was considerably increased (18·7 ml, near the higher limit of normal). Case 8 was generally similar, with a high epithelium content (6·2 ml) and proportionately more colloid (25·7 ml). By contrast, cases 5 and 12 had relatively more epithelium (2·5 and 3·5 ml, respectively), compared with a colloid content towards the lower limit of normal (3·6 and 5·3 ml, respectively), and presented a histological appearance of smaller relatively collapsed acini with more prominent epithelial lining.

By contrast, there was much more variability in the measurements on the thyroid nodules. Table 2 summarises the findings on glands containing fewer than seven nodules. Figs. 3a and b show the measurements on two multinodular glands: the results of histometry on the four other multinodular glands were generally similar to these examples.

Table 2 *Summary of histometric findings on nodules in thyroids with four or fewer nodules*

	No of nodules	Volume (ml)	Acinar volume (ml)	No of follicles ($\times 10^6$)	Internal surface area (m^2)	External surface area (m^2)	Epithelium (%)	Colloid space (%)	Stroma (%)
Case 4	1	0·119	0·089	0·05	0·0033	0·0035	8	68	25
	2	0·036	0·027	0·02	0·001	0·0011	8	68	24
	3	0·036	0·021	0·03	0·0009	0·0011	20	39	36
	4	3·3	2·58	0·3	0·055	0·059	10	70	19
Case 5	1	0·034	0·03	0·006	0·0008	0·0009	13	75	9
	2	0·034	0·024	0·01	0·0008	0·0001	21	50	22
	3	0·53	0·43	0·12	0·013	0·014	10	72	13
Case 6	1	0·037	0·023	0·04	0·0011	0·0013	18	41	37
	2	0·122	0·099	0·02	0·0025	0·0026	8	74	16
	3	0·037	0·029	0·007	0·0008	0·0009	9	70	18
Case 3	1	41·5	18·4	27·8	0·84	1·01	14	32	48
Case 8	1	21·2	12	18·9	0·54	0·57	19	37	38
Case 9	1	24·2	13·4	11·8	0·53	0·62	15	41	40
Case 10	1	0·55	0·48	0·12	0·013	0·015	15	72	11
Case 11	1	0·4	0·27	0·2	0·01	0·11	10	58	26
	2	3·26	1·19	2·5	0·06	0·07	13	24	56
Case 13	1	0·118	0·081	0·09	0·004	0·0041	9	60	25
	2	0·118	0·077	0·08	0·0035	0·0030	11	54	28
	3	0·55	0·49	0·09	0·013	0·013	7	84	7
	4	0·28	0·178	0·05	0·005	0·006	11	52	29

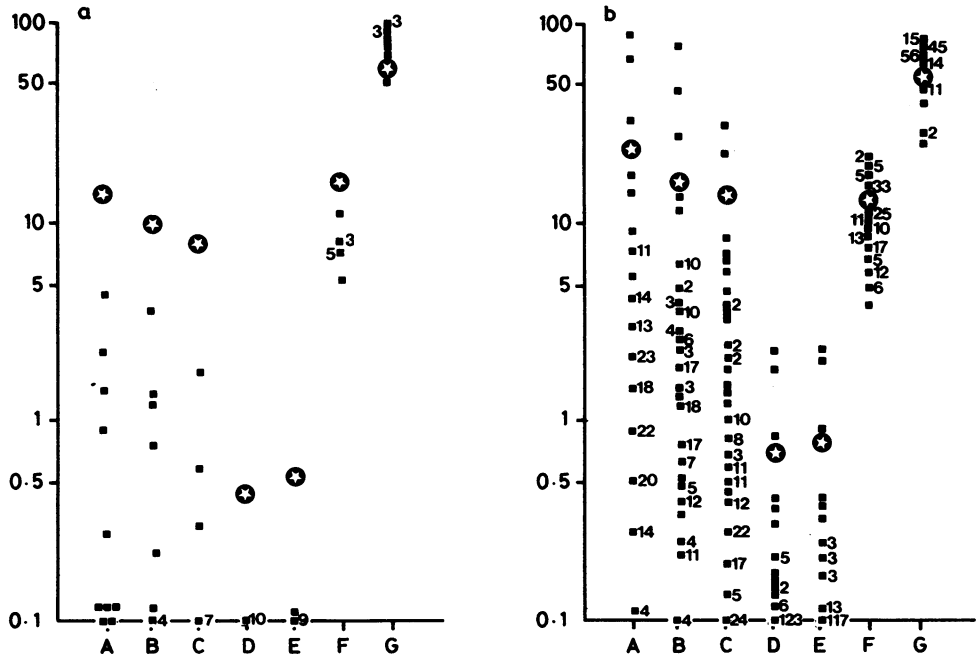

Fig. 3 *Diagram summarising findings in individual nodules (solid squares) and in interstitial "normal" thyroid (solid circle with central open star) from (a) case 2 containing 10 nodules and (b) case 15 containing 145 nodules. Labels for columns indicate:*
A = total volume (ml);
B = acinar volume (ml);
C = total follicular number ($\times 10^6$);
D = internal surface area of acini (m^2);
E = external surface area of acini (m^2);
F = epithelial volume as % of total volume;
G = colloid space volume as % of total volume.

The individual nodules were mostly smaller than the internodular portion of the gland: in two of the nine glands with few nodules, however, and in three of the six multinodular glands a nodule was larger than the internodular thyroid tissue, but the nodule usually had proportionately fewer more distended acini. Most nodules, whether large or small, had proportionately (v/v) more colloid and less epithelium than the interstitial thyroid gland. These measurements correspond to the generally macrofollicular histological appearance seen in 228 (82·8%) of the 275 nodules: most of the nodules had acini lined by flattened epithelium, but many showed scattered small areas in which part of the lining of individual acini was composed of taller epithelium. There were a few colloid cysts.

Discussion

In the past clinical and pathological interest in nodular thyroids has concentrated on the nature of the nodules, in particular, distinguishing them from neoplasms and determining whether individual nodules are autonomous in relation to the pathogenesis of thyrotoxicosis. These aspects of nodular thyroids have been emphasised in the most recent review[7]: there are no published reports of histometric measurement.

Two main methods were used to identify nodules in the thyroids examined in this study. The primary method was inspection of the cut surface of the 1·0 cm slices, and this has the classical disadvantages of the "cherry cake" problem—namely, that a nodule with a diameter less than the thickness of the slice has a certain probability of escaping detection. Due to the random selection of the plane of sectioning there is an equal likelihood that the centre of the nodule will lie at any point on an imaginary line passing directly through the slice, and a nodule will be visible on the cut surface when its centre lies at a distance less than its radius from the cut surface: consequently, the probability that a nodule of a given size will be visible is $2r/t$, where r = radius and t = slice thickness. Thus all nodules with a diameter greater than 1·0 cm will be exposed at the surface: the probability that a small nodule will be exposed is linearly related to its diameter, and, with 1·0 cm slices, the probability that an 8 mm nodule will be exposed is 0·8 and for a 3 mm nodule it is 0·3. Nodules of 3–4 mm diameter, however, were readily detected by palpation, and so the overall probability that nodules of 3 mm diameter, or greater, would be detected in this study must approach 1·0. We considered that the error introduced into this study by failing to detect smaller nodules would be insignificant in relation to either the total thyroid volume (range 7·5–558 ml) or the calculated internodular thyroid volume (range 7·0–43·8 ml), as a 3 mm diameter spherical nodule has a volume of only 0·01 ml. We concluded that the calculated volume and histometric indices for the internodular thyroid tissue would not be unduly biased by the manner of detection and measurement of the nodules.

The cases selected for this study were deliberately chosen because the patients had not had evidence suggestive of metabolic effects of thyroid disease, and it would be reasonable to assume that they had been euthyroid. The interpretation of the histometric results for the internodular thyroid presents certain difficulties. On the one hand, the various measurements for the internodular parts of the glands all lay within the "normal" range. On the other hand, the colloid:epithelium ratios for the internodular parts of some glands were apparently abnormal due to deviation of the component values in opposite directions while still within the normal range. Thus a gland can adopt a histological appearance suggestive of colloid goitre while still retaining the normal content of epithelium. When a patient with Graves' disease is treated preoperatively with inorganic iodide the histological appearance of the thyroid changes from the extreme hyperplasia typical of the untreated disease into a much more normal looking structure, with cuboidal epithelium lining acini distended with colloid. This change has been described as "involution". It has been shown by histometric measurement that the change induced by iodide is associated with an increase in colloid content without any modification in the epithelium content,[8] and it was suggested that the lowering of the epithelium is a consequence of distension of the acini and a stretching of the epithelium over a much larger surface area. This experience suggests that epithelial volume may be a more useful indicator of hyperplasia, hypoplasia, or normality in the thyroid than the histological appearance. We therefore suggest that the internodular tissue in nodular thyroids was in a state of normal growth control at the time of our examination. This conclusion cannot, however, be extrapolated to an earlier stage of the patient's life. It is quite possible that the thyroid had been exposed to excessive thyroid stimulating hormone or TSH stimulation for a shorter or longer period and that this induced the focal overgrowth which culminated in nodule formation. It is clearly not possible to differentiate between the various mechanisms that have been suggested for the pathogenesis of the nodular thyroid from the appearance of the gland in middle or old age.

It is unlikely that any of the nodules in the glands studied here were "hot". It is relevant, therefore, that none of the nodules showed histopathological or his-

tometric evidence of hyperplasia. If a nodule had been autonomous it is likely that there would have been suppression of TSH secretion, and so the interstitial gland would have undergone atrophy through loss of trophic influence: whether this will prove to be the case will soon be determined by direct measurement.

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