ULTRACENTRIFUGAL PATTERNS OF THE
THYROID PROTEINS FROM HUMAN PATHOLOGICAL
THYROID GLANDS

By

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ABSTRACT

The ultracentrifugal pattern of the thyroid proteins from seven 'normal' human thyroid glands, 24 thyrotoxic glands, 28 cases of non-toxic, two cases of Hashimoto's disease, and three of anaplastic carcinoma of the thyroid have been examined. It has been demonstrated in thyrotoxicosis as compared to non-toxic goitre that there is a failure of formation of proteins > 19S. It is suggested that in the thyrotoxic gland there may be some defect in the manufacture of protein > 19S. In Hashimoto's disease an increased quantity of 6S protein was present in both cases studied. An increased quantity of lightweight protein was also found in cases of anaplastic carcinoma.

Over the last few years there has been a great deal of interest in both the in vitro and the in vivo biosynthesis of thyroglobulin. Although there are many reports in the literature of the electrophoretic patterns of the thyroid proteins from pathological glands, the available data on the ultracentrifugal patterns of the thyroid proteins from human pathological material is comparatively limited.

Most reports available have dealt with rather uncommon thyroid problems such as thyroid glands with an abnormal iodoprotein present (Lissitzky et al. 1967) either due to an inherited defect in thyroglobulin biosynthesis, or due to Hashimoto's thyroiditis (De Groot et al. 1962), or to changes found in malignant thyroid glands (Lupulescu et al. 1968; Valenta et al. 1968).

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There are few reports of the thyroid protein patterns from normal human material (Witebsky et al. 1956) and the only sizable series of human pathological glands studied have been by De Groot & Carvalho (1960), Stanley (1964) and Ramagopal et al. (1965).

The present paper reports our experience with a series of thyroid glands from patients with a variety of pathological states affecting the thyroid. Our results differ in certain respects from those of the authors referred to above.

**MATERIAL AND METHODS**

'Normal' human thyroid tissue was obtained from seven subjects undergoing neck exploration for possible parathyroid adenoma in whom there was no naked eye or histological abnormality of the thyroid gland.

Twenty-four patients who underwent thyroidecomy for thyrotoxicosis were studied. In 20 the thyrotoxicosis had been controlled preoperatively with carbimazole supplemented by either potassium iodide 60 mg b. d. or Lugol's iodine 5 drops t. i. d. for the 7–10 days before operation. In four the preoperative treatment had been with potassium perchlorate with of course no preoperative iodide medication.

Fourteen patients with diffuse or multinodular non-toxic goitre were studied as were 14 patients in whom a clinically single thyroid nodule in a euthyroid patient had been resected. Nine of these nodules were 'cold' nodules as judged by a preoperative thyroid scan, the remainder were 'hot'. In none of these patients with a non-toxic goitre or a single thyroid nodule was any neoplastic change found on histological examination of the resected specimen.

Tissue from two patients with the histological changes of Hashimoto's thyroiditis and from three patients in whom the pattern of an anaplastic carcinoma of the thyroid were found were also examined.

The tissue was collected from the operating theatre on ice. Some tissue was immediately homogenised in the cold (4°C) in ice cold phosphate buffered saline (PBS 0.15 M sodium chloride in 0.01 M potassium phosphate pH 6.8) and was prepared for ultracentrifugal study. Thin slices were cut by hand from the remaining tissue using a sharp razor for incubation studies. Approximately 300 mg of thyroid slices were added to 4 ml PBS in a conical flask to which was added either 20 μCi 125I (carrier free) or 20 μCi 3H-L-leucine (S. A. 250–1000 mCi/mm) obtained from the Radiochemical Centre, Amersham. These flasks were incubated for four hours under O2 at 37°C in a shaking water bath. This time of incubation was selected on the basis of previous published work (Seed & Goldberg 1965) as being a time at which amino acids in the incubation medium should have been incorporated into thyroglobulin. At the end of incubation the flask contents were homogenised as above. The homogenate from all the preparations were spun in a refrigerated centrifuge at 16 000 r. p. m. for 10 min to remove cellular debris. The supernatant was decanted and was taken to 50 % saturation with ammonium sulphate. The mixture was then kept on ice for one hour after which it was again spun in the refrigerated centrifuge at 16 000 r. p. m. for 10 min. The supernatant was then discarded. The centrifuge tube was drained of any remaining ammonium sulphate and the precipitate was then dissolved in PBS for immediate study or was in a few cases stored frozen until examined.

The protein pattern of the precipitate so obtained was examined by layering an
appropriate amount of the solution on top of a 5–20% sucrose gradient and spinning the protein in a model L or model L2-65B Beckman ultracentrifuge using the SW 39 rotor at 24,000 r.p.m. for 16 h or the SW 41 rotor at 28,000 r.p.m. for 16 h at 4°C. After ultracentrifugation the optical density (OD) pattern was recorded by aspiration from the bottom of the gradient and passage through a Beckman DB recording spectrophotometer. Fractions, each of 10 drops, were collected and the radioactivity assayed after the addition of 10 ml Bray's solution in a Nuclear Chicago liquid scintillation spectrometer.

In all runs a specimen of sheep thyroglobulin was spun in the rotor and the position of the main OD peak at 280 nm was taken as 19S for reference purposes. In some experiments the position of the peaks was verified by including a small amount of 125I labelled 19S protein from a normal rat thyroid in the bucket with unlabelled human material. The S values shown in the figures are calculated by either of these means although it is appreciated that neither method is exact and differences of fractions of an S unit are probably not significant.

RESULTS

The OD pattern of the 'normal' thyroid tissue is shown in Fig. 1 along with that of sheep thyroglobulin. The OD patterns are very similar with the main

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**Fig. 1.**

Ultracentrifugal pattern of sheep and human thyroglobulin (SW 39 rotor at 24,000 r.p.m. for 16 h).
OD peak at 19S but with definite 6S and 27S peaks present. In one of seven 'normal' human glands a trace amount of a 32S protein was seen. This was usually but not invariably present in our sheep thyroglobulin preparations.

In the 20 patients with thyrotoxicosis treated preoperatively with carbimazole and iodide the OD pattern, a representative example of which is shown in Fig. 2, is of a main OD peak in the 19S region with a smaller peak in the 6S region. Except in two where a trace amount of a 27S protein was present there was no peak heavier than 19S. This was verified by spinning the material at a slower speed (in SW 39 rotor at 21 000 r. p. m. for 16 h (Fig. 3). As would be expected $^{125}$I was not incorporated into thyroglobulin but $^3$H leucine was well incorporated into thyroglobulin and its sub-units.

Likewise in those thyrotoxic patients treated postoperatively with KCIO$_4$ without iodine (Fig. 4) although the OD pattern was different from the carbimazole and iodine treated patients in that the amount of 6S protein was

![Fig. 2](https://via.placeholder.com/150)

**Fig. 2.**

Ultracentrifugal pattern of control sheep thyroglobulin and of the thyroid proteins from a thyrotoxic patient pretreated with carbimazole and iodine showing the incorporation of $^{125}$I and $^3$H leucine (SW 39 rotor at 24 000 r. p. m. for 16 h).
increased as compared to the 19S protein, both groups were similar in the absence of a protein $> 19S$. Both $^{125}$I and $^3$H leucine were well incorporated into the 19S protein in the KClO$_4$ treated gland.

The tissues from non-toxic diffuse or multinodular goitre, from single nodules, either ‘hot’ or ‘cold’, gave basically similar results. In all but two of 14 non-toxic goitres and all but one of 14 single adenomas the pattern of results is as shown in Fig. 5. A 19S protein is present as the predominant protein with a small amount of 6S and 27S protein present. In the three exceptions mentioned above (one ‘hot’ nodule, one ‘cold’ cystic nodule and one multinodular goitre) no 27S peak was discernible. $^{125}$I and $^3$H leucine were both well incorporated with the $^3$H leucine incorporated into a protein of S value slightly lower than the $^{125}$I labelled protein.

In the two cases of Hashimoto’s disease there was a relative increase in the quantity of 6S protein; a 19S protein was present in both (Fig. 6); no 27S peak could be seen in either gland but in one a 32S peak was present. Iodine was incorporated in the thyroglobulin region and lighter proteins but leucine was very poorly incorporated.

Fig. 3.
Ultracentrifugal pattern of control sheep thyroglobulin and of the thyroid proteins from a thyrotoxic patient pretreated with carbimazole and iodine (SW 39 rotor at 21 000 r.p.m. for 16 h).
Ultracentrifugal pattern of control sheep thyroglobulin and of the thyroid proteins from a thyrotoxic patient pretreated with potassium perchlorate showing the incorporation of $^{125}$I and $^3$H leucine (SW 39 rotor at 24 000 r. p. m. for 16 h).

Three cases of anaplastic thyroid carcinoma were studied. Two patterns were seen. In two cases the pattern was that obtained in the upper portion of Fig. 7 in which there was practically no 19S protein present, the only readily discernible peak being in the 6S region. In the other an appreciable amount of 19S protein was present. Histological examination of this gland showed occasional survival islets of normal thyroid acini in the middle of sheets of neoplastic cells. In this last instance there was some $^{125}$I incorporation into the 19S protein but apart from this neither $^3$H leucine nor $^{125}$I were significantly incorporated into the 19S region.

**DISCUSSION**

This paper presents our experience of the ultracentrifugal patterns of the thyroid proteins of normal and pathological human thyroid glands. The
Ultracentrifugal pattern of control sheep thyroglobulin and of the thyroid proteins from a multinodular non-toxic goitre showing the pattern of incorporation of $^{125}$I and $^3$H leucine (SW 39 rotor at 24 000 r.p.m. for 16 h).

Fig. 5.

Thyrotoxic glands differed from the normal or from a variety of non-toxic goitres in that in the thyrotoxic gland a protein > 19S was not found. This was the case no matter which of two regimes of preoperative drug preparation was used. This was also found in the series of Stanley (1964) where 2 of 3 thyrotoxic glands had no protein > 19S and by Ramagopal et al. (1965) although neither author commented upon this finding.

It is possible that in a thyrotoxic gland the turnover of the thyroid proteins is such that the 27S protein normally present, which has been postulated to be a store of highly iodinated thyroid proteins with a slow turnover rate, is used up. This would be an attractive hypothesis in a hyperplastic gland such as that treated by $\text{KClO}_4$ in which colloid can be shown histologically to be virtually absent. The finding of a similar result in a gland pretreated with carbimazole and iodine is, however, against this. In this latter case colloid can be shown...
Ultracentrifugal pattern of control sheep thyroglobulin and of the thyroid proteins from a patient with Hashimoto’s thyroiditis showing the pattern of incorporation of \(^{125}\)I and \(^{3}H\) leucine (SW 39 rotor at 24 000 r. p. m. for 16 h).

Histologically to have reaccumulated and as can be seen by comparing Figs. 2 and 4, a greatly increased concentration of 19S protein is present. It may be that the failure of formation of proteins \(>19S\) is a feature of the thyrotoxic process itself. It would obviously have been of great interest to have studied the pattern of an untreated thyrotoxic gland but is was considered that thyroid biopsy for this purpose was not ethically justifiable.

In a wide variety of goitres from euthyroid subjects, a relatively normal ultracentrifugal pattern of thyroid proteins was obtained. This is in keeping with the histological finding that all these glands contained abundant colloid. De Groot & Carvalho (1960) found similar results in patients with colloid goitres and thyroid adenomas. In the series of Stanley (1964) a large amount of a 12S protein was present. In part at least the reason for this difference would appear to be due to differences in methodology. The glands in our series were dealt with immediately after resection whereas in the series of
Ultracentrifugal pattern of control sheep thyroglobulin and of the thyroid proteins from two patients with anaplastic carcinoma of the thyroid (SW 39 rotor at 24 000 r. p. m. for 16 h).

Stanley (1964) the glands were frozen and then thawed before slicing; furthermore before ultracentrifugal analysis was performed the samples were freeze-dried and reconstituted at a later date. Recent work by Simon et al. (1966) and Inoue & Taurog (1968) has shown that freezing and thawing of thyroid tissue results in the breakdown of thyroglobulin especially where this is poorly iodinated.

In Hashimoto's disease there was an increase in lightweight protein. This has also been found by Stanley (1964) and by De Groot et al. (1962). It is interesting that in this disease there was a discrepancy between the incorporation of $^{125}$I and $^3$H leucine. This probably reflects the small amount of functioning thyroid epithelial cells which are capable of incorporating $^3$H leucine into thyroglobulin although it is still possible to iodinate preformed thyroglobulin. The iodination of thyroid protein other than thyroglobulin is consistent with the known finding of abnormal iodinated peptides in this condition (Murray &
The exact relationship between the 32S peak, found in one of two patients with this condition who were studied, and thyroglobulin has yet to be defined.

The protein patterns found in anaplastic carcinoma are similar to those found in a larger series by Valenta et al. (1968) and as stated in that paper are obviously dependent on the number of surviving thyroid follicles which happen to be included in the part of the gland under examination.

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REFERENCES

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