Protein composition in single follicles, homogenates and fine-needle aspiration biopsies from normal and diseased human thyroid

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Abstract. The protein composition of the thyroid colloid was analysed by microgel electrophoresis and densitometry in 41 euthyroid patients. The colloid samples were obtained from single follicles by micropuncture, from homogenates of microbiopsies or from aspiration biopsies. Fourteen of the patients had morphologically normal thyroid tissue, 18 had atoxic nodular goitre and 9 of the patients had atoxic adenoma. Ten of the patients with nodular goitre had prior to the investigation received lithium therapy for psychiatric disorders. The main component of the thyroid colloid was 19S thyroglobulin (TG), but larger iodoproteins (S-TG) and smaller protein fractions, an albumin-like protein and a pre-albumin fraction, were also present in varying relative amounts. Analyses of homogenates of microbiopsies from normal thyroid tissue demonstrated the same protein composition as observed in single follicles. In colloid samples from atoxic nodular goitre the lighter protein fractions were absent in most of the samples. Analyses of homogenates or aspiration biopsies could not demonstrate this alteration in the protein composition in nodular goitre. Lithium therapy resulted in a significantly lower amount of the lighter protein fractions but unchanged amounts of the globulin fractions in atoxic nodular goitre.

In the atoxic adenomas the protein composition was heterogeneous. The major globulin fractions as well as the lighter protein fractions were present in the analyses of colloid and homogenates of microbiopsies. Aspiration biopsies from atoxic adenomas could not be used for analyses of the protein composition due to contamination with serum proteins.

The thyroid gland is structurally divided into three main compartments, the follicular lumina containing the colloid, the follicular epithelium and the interfollicular space with interstitial tissue and blood- and lymph-vessels. Analysis of colloid collected by micropuncture of single thyroid follicles has shown the presence of thyroglobulin, larger aggregates of thyroglobulin here designated S-TG and of albumin-like protein and pre-albumin in varying relative amounts (Smeds 1972b). Cell-fractionating studies in animal experiments have shown that the exocytotic vesicles in the follicle cells contain thyroglobulin-like molecules, the half-molecule of thyroglobulin (the 12S iodoprotein) and an albumin-like protein (Björkman et al. 1976). The dominant protein fraction in the extrafollicular tissue fluid is the albumin fraction (Smeds 1972a). In addition α₂-macroglobulin and a number of lighter proteins are present in a constant proportion as determined by microgel electrophoresis. The scope of interest in the present study was to analyse the protein composition of the colloid in thyroid follicles from normal and diseased human

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Materials and Methods

Peroperative thyroid biopsies were obtained from 22 patients operated on for hyperparathyroidism, atoxic nodular goitre or follicular adenoma. The patients were clinically euthyroid at the time of operation and had no previous thyroid medication. Fourteen of the patients had morphologically normal thyroid tissue in the punctured area. Five patients had nodular goitre and 3 patients had follicular adenomas. In the latter 3 patients colloid was collected from the adenomas. During the operation thyroid biopsies (1–3 g) were collected and stored in a solution containing four parts of phosphate buffer 10 mM, pH 7.0 with saline 150 mM and one part of sucrose (PBS, 20% w/w).

Collection of the colloid

Thirty min to 4 h after the operation the colloid collection started. A piece of the biopsy was mounted under a stereo microscope (× 32). Collection of the colloid was performed by puncture of the follicles with glass pipettes as previously described in detail (Smeds 1972a). In general 10–15 follicles were punctured in each specimen, but measurable amounts of colloid proteins were obtained from a smaller number of follicles due to either too small or too large amounts of protein in the samples. Immediately after extraction of the colloid the sample was transferred to the electrophoresis capillary.

Preparation of homogenates of thyroid biopsies

From the part of the gland where the puncture of the follicles was performed, a microbiopsy (100–200 mg) was cut out. This biopsy was homogenized at 20–22°C with a Potter-Elvehjem homogenizer in PBS (100 mg tissue per 5 ml buffer). The tissue was not perfused prior to homogenisation. A supernatant containing the soluble thyroid proteins was prepared by centrifugation at 10000 g for 30 min at 4°C (Sovall RC-5 Ultra centrifuge). The supernatant was diluted 1:10 with the buffer solution. Three to 5 samples (1 μl) of this solution were transferred to microgel electrophoresis capillaries.

Thyroid fine-needle aspiration biopsies

Nineteen patients with atoxic thyroid diseases were studied by fine-needle aspiration biopsies. Thirteen of the patients had a general thyroid enlargement. At the time of the study 10 of the latter patients had received lithium therapy for psychiatric disorders for 1 to 10 years. The cytological diagnosis for the lithium treated group was moderate colloid rich goitre (n = 9) and thyroiditis (n = 1), that of the remaining three glands was atoxic nodular goitre. Six patients had benign tumours, their cytological diagnosis being follicular adenomas (n = 4) microfolicular adenoma (n = 1) and Hürthle-cell adenoma (n = 1). The biopsies were collected from enlarged and easily palpable parts of the thyroid glands. Two fine-needle aspiration biopsies for biochemical analysis were obtained from each patient using a Franzén syringe. The aspirated material was immediately ejected into 150 μl of PBS, homogenized and centrifuged at 10000 g for 30 min at 4°C. The protein composition of the supernatant was analysed by microgel electrophoresis in duplicate after three and ten times dilution.

Table 1.

Relative amount (standard deviation and number of samples in parentheses) of the thyroid protein fractions in colloid and micro-biopsies of normal human thyroid tissue and atoxic nodular goitre.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sample</th>
<th>Protein fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-TG (sd, n)</td>
<td>TG (sd, n)</td>
</tr>
<tr>
<td>Normal thyroid</td>
<td>Colloid</td>
<td>22 (8.2, 27)</td>
</tr>
<tr>
<td></td>
<td>Homogenate</td>
<td>21 (7.3, 51)</td>
</tr>
<tr>
<td>Atoxic nodular goitre</td>
<td>Colloid</td>
<td>21 (9.3, 15)</td>
</tr>
<tr>
<td></td>
<td>Homogenate</td>
<td>17 (7.1, 11)</td>
</tr>
<tr>
<td></td>
<td>Aspiration biopsy (no lithium)</td>
<td>10 (4.6, 12)</td>
</tr>
<tr>
<td></td>
<td>Aspiration biopsy (lithium)</td>
<td>18 (8.3, 30)</td>
</tr>
<tr>
<td>Rat thyroid*</td>
<td>Colloid</td>
<td>16 (7.0, 70)</td>
</tr>
</tbody>
</table>

* The results from previously reported protein composition of the thyroid colloid in the rat is included for comparison (Smeds 1972b).
Gel electrophoresis

Separation of the proteins in the samples of colloid, in the homogenates of the microbiopsies and in the fine-needle aspiration biopsies was performed at room temperature by micro-gel electrophoresis on 10 or 12 per cent polyacrylamide gels, as previously described in detail (Smeds 1969, 1972a). Protein patterns were recorded by microdensitometry and the total and relative amount of the proteins was calculated as described in the same papers.

Morphology

Tissue from the nodular goitre and follicular adenomas was sent to routine microscopic examination at the pathological department to verify the clinical and cytologic diagnosis. Macroscopically normal tissue adjacent to the punctured area from the patients operated on for hyperparathyroidism was histologically defined as normal (n = 7) or nodular.

Results

Normal thyroid

The protein composition in normal thyroid tissue was analysed in the colloid and in homogenates of the corresponding microbiopsies. Fine-needle aspiration biopsies were not obtained from normal thyroid tissue.

A measurable amount of colloid was obtained from 2–6 follicles in 7 out of 14 biopsies and totally 27 samples were analysed (Table 1). In all the samples, the 19S thyroglobulin fraction and the more slowly migrating aggregates of thyroglobulin molecules, i.e. the 27S iodoprotein and larger molecules (S-TG) were observed (Fig. 1). Lighter protein fractions were observed in 17 colloids (63%). These fractions represent the albumin-like protein and the pre-albumin fraction (PA) (Fig. 2). The 12S fraction was observed in two colloid samples.

The relative amount of the different colloid protein fractions varied between follicles in the same gland. In the whole material the 19S thyroglobulin fraction comprised between 37 and 87% of the total amount of protein. The S-TG fraction varied between 10 and 48% and the lighter protein fractions varied between 0 and 24%.

The soluble proteins in the homogenates of the microbiopsies at the punctured area had the same protein composition and equal relative amounts as found in the colloid from single follicles (Table 1).

Atoxic nodular goitre

The protein composition in the nodular thyroid tissue from 5 glands was analysed in 15 samples of colloid and in 11 homogenates of the corresponding microbiopsies. Six fine-needle aspiration biopsies were analysed from 3 glands.

It was observed that the relative amount of the globulin fractions in the colloid samples was the same in this tissue as that observed in the normal thyroid tissue (Table 1). The lighter protein fractions were detected in 2 of the 15 samples of colloid. In one of these samples 1% of the protein was pre-albumin, in the other, 22% of the protein was the albumin-like protein (19%) and pre-albumin (3%).

The relative amount of the soluble proteins in the homogenates of the corresponding microbiop-
Fig. 2.
Full length densitometric recording also illustrating the lighter protein fractions (Alb-PA) in a colloid sample collected from a patient with atoxic nodular goitre.

Fig. 3.
Densitometric recording of the microgel electrophoretic separation of proteins in an aspiration biopsy from a follicular adenoma. Note the larger amount of albumin (Alb) and the presence of haemoglobin (H) and another protein fraction (\(\downarrow\)) in addition to thyroglobulin (TG) and the 27S iodoprotein (S-TG).

Sies did not differ from that observed in the normal thyroid tissue (Table 1).

Several samples of the aspiration biopsies were directly discarded due to heavy contamination with blood. Only a few samples were clear and most of the samples contained haemoglobin as observed in the separation patterns (Fig. 3). Compared to the protein composition in the microbiopsies it was observed that in the aspiration biopsies from patients without lithium therapy the relative amount of the lighter fractions was increased \((P < 0.001)\) whereas that of the 19S thyroglobulin as well as the proteins in the S-TG region of the gels was decreased \((P < 0.001)\) (Table 1). In patients with lithium the aspiration biopsies also contained a significantly increased amount of the lighter protein fractions \((P < 0.01)\) and a reduced amount of 19S thyroglobulin \((P < 0.01)\) whereas no difference was observed in the amount of S-TG fractions (Table 1).

Follicular adenoma
Samples of colloid were collected from three follicular adenomas. The S-TG fractions, 19S thyroglobulin and the lighter fractions were all observed in the samples. There was a pronounced quantitative discrepancy between the adenomas. A normal relative amount of the globulin and albumin fractions was observed in the samples from two adenomas whereas the third had a reduced relative amount of the globulin fractions and an increased amount of the lighter protein fractions (Table 2).
Aspiration biopsies were obtained from tumours in 6 patients of whom 4 were classified as follicular thyroid adenomas, one as a micro-follicular adenoma and one as a Hürte-cell adenoma. In 4 aspiration biopsies from 2 of the follicular adenomas globulin fractions were recovered in the electrophoretic separation patterns. In these samples more than 50% of the protein was albumin and lighter fractions (Table 1). The separation patterns of the samples from the other adenomas were dominated by albumin and resembled the tissue fluid pattern. Haemoglobin was occasionally observed.

Total amount of protein in the colloid samples
The total amount of protein in the colloid in all of the samples varied between 21 and 545 ng. The mean amount in the normal group was 123 ng, in the nodular group 119 ng and in the adenoma group 101 ng. This difference between the groups is not statistically significant. Thus, the observed variation of the protein composition between the colloid in the normal tissue and in the nodular tissue can probably not be due to a difference in the total amount of protein in the samples.

Discussion
The present investigation of the protein composition of the thyroid colloid was performed with well established micromethods used in this laboratory for several investigations concerning the protein composition of the colloid in single rat thyroid follicles both in vivo and in vitro (Smeds 1972a,b; Smeds & Anderberg 1977, 1978). Adoption of these methods to human thyroid tissue has for the first time made it possible to directly analyse the protein composition of the colloid in single human thyroid follicles.

It is interesting to note that the qualitative protein composition in the colloid from histologically normal thyroid tissue is the same as that observed in the rat thyroid colloid (Smeds 1972a). Although it cannot be excluded that small amounts of other proteins cannot be detected in the present system these observations indicate that the colloid generating mechanisms are basically the same in these two species.

19S thyroglobulin was the dominating protein in all of the colloid samples. Larger protein fractions (S-TG) were also present in varying relative

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**Table 2.**
Protein composition of colloid and corresponding micro-biopsies in 3 follicular adenomas (FA) and in aspiration biopsies from 4 follicular adenomas, one microfollicular adenoma (MFA) and one Hürte-cell adenoma (HCA).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sample</th>
<th>Protein fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S-TG</td>
</tr>
<tr>
<td>FA</td>
<td>Colloid</td>
<td>21</td>
</tr>
<tr>
<td>FA</td>
<td>Colloid</td>
<td>19 (14.7, 3)</td>
</tr>
<tr>
<td>FA</td>
<td>Colloid</td>
<td>14 (4.3, 5)</td>
</tr>
<tr>
<td></td>
<td>Homogenate</td>
<td>10 (3.1, 3)</td>
</tr>
<tr>
<td>FA</td>
<td>Aspiration biops</td>
<td>7 (4.5, 3)</td>
</tr>
<tr>
<td>FA</td>
<td>Aspiration biops</td>
<td>10</td>
</tr>
<tr>
<td>FA</td>
<td>Aspiration biops</td>
<td>-</td>
</tr>
<tr>
<td>FA</td>
<td>Aspiration biops</td>
<td>-</td>
</tr>
<tr>
<td>MFA</td>
<td>Aspiration biops</td>
<td>-</td>
</tr>
<tr>
<td>HCA</td>
<td>Aspiration biops</td>
<td>-</td>
</tr>
</tbody>
</table>

* Haemoglobin and other serum protein fractions are excluded from the calculations.

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amounts. Lighter protein fractions (albumin-like protein and pre-albumin) were present in minor amounts in about 60% of the colloid samples.

The S-TG fraction in the thyroid colloid represents the 27S iodoprotein and larger aggregates of well iodinated 19S thyroglobulin (Berg & Björkman 1975). Although we found a mean relative amount of S-TG similar to that earlier observed in normal rat thyroid glands (Smeds 1972b), the relative amount of S-TG in individual follicles demonstrated a large variation ranging from 10–48% of the total protein amount. The physiological importance of the S-TG fraction and its variation between individual follicles is not known, but earlier investigations clearly show, that the iodination of thyroglobulin is crucial for the formation of aggregates of thyroglobulin in the follicle lumen (Sinadinović et al. 1973; Frati et al. 1974; Smeds & Anderberg 1977, 1978).

The presence of the lighter protein fractions, which means the albumin-like protein and the pre-albumin fraction, are also of unknown physiological importance. There are two hypotheses concerning the origin of thyroid albumin since its discovery by Shulman et al. (1957). According to the identity hypothesis serum albumin diffuses into the follicular lumen, where it is iodinated and the presence of iodinated albumin in the blood is explained by back diffusion to the plasma. The other hypothesis implies not only iodination but also synthesis of the albumin-like protein in thyroid tissue. The latter hypothesis is supported by the observed difference in amino acid composition between serum albumin and thyroid albumin (Jonckheer & Karcher 1971) and by the observation of a synthesis of thyroid albumin in slices of human thyroid tissue (Otten et al. 1971). The lighter protein fractions have been demonstrated in thyroid colloid from rats in the same relative amount both with and without perfusion of the glands (Smeds 1972a,b). Furthermore the presence of albumin-like protein in exocytotic vesicles in the follicle cells is in favour of the hypothesis of a thyroid origin of this protein fraction (Björkman et al. 1976). The presence of the pre-albumin fraction as the single light protein in addition to 19S TG and S-TG has previously been observed in follicle lumina with high protein concentration (Smeds 1972a,b). The electrophoretic migration is very similar to a dissociation product of 19S TG and S-TG (Smeds & Ekholm 1972) and it cannot be excluded that PA represents unassembled subunits of thyroglobulin which are accumulated in the colloid. There is no evidence for PA to be identical with the thyroxine binding pre-albumin fraction in serum. The presented results also demonstrate a variation in the protein composition between follicles in the same biopsy. The mean relative amount of the different protein fractions in colloid from single follicles in normal thyroid tissue was identical to that obtained from analyses of homogenates of microbiopsies adjacent to the punctured area, despite the relatively small number of punctured follicles in each biopsy. This finding shows that the protein composition in homogenates of normal thyroid tissue is representative for the colloid proteins.

In the colloids from atoxic nodular goitres the amount of the lighter protein fractions was below the level of detection in all but two of the colloids. A similar reduction was, however, neither observed in the homogenates of the microbiopsies nor in the aspiration biopsies. This demonstrates in general that the samples of the colloid are not contaminated by proteins from extracellular sources and specifically that diffusion of serum albumin or synthesis of the albumin-like protein are inhibited in atoxic nodular tissue. In contrast to normal thyroid tissue, homogenates of atoxic nodular tissue are not representative for the qualitative protein composition of the colloid.

The significantly higher relative amount of the lighter protein fractions in the aspiration biopsies compared to the homogenates are probably due to the technique when taking aspiration biopsies, a technique which means that heavy suction is applied to the syringe and also that the needle is moved up and down in the tissue. Despite the evident contamination with serum and tissue proteins in the aspiration biopsies a significant decrease in the lighter protein fractions was observed in atoxic nodular tissue when the patients had received lithium therapy. The difference may be explained by the relatively high proportion of colloid in the latter biopsies due to colloid retention in the follicular lumen after lithium therapy, a hypothesis in accordance with the observations of Leppäluoto et al. (1973) and Radvila et al. (1976), who showed that lithium retards the secretion of thyroid hormones and augments the accumulation of thyroglobulin in the gland. The normal relative amount of S-TG and TG-fractions in the aspiration biopsies both without lithium and after lithium therapy are in agreement with earlier studies show-
ing that lithium does not interfere with the mechanism for aggregation of thyroglobulin, i.e. the iodine organization (Berens et al. 1979).

In the group of investigated follicular adenoma the analyses of colloid and the corresponding microbiopsies were similar. The aspiration biopsies of follicular adenomas were contaminated by serum protein fractions and no globulin fractions were observed in samples from the microfollicular and Hürthle-cell adenomas. The aspiration biopsy technique can therefore not be used in studies of follicle proteins in adenomas whereas biopsies of the gland are more reliable.

References

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