Separation of Thyroidal Iodoproteins and Purification of Thyroglobulin by Gel Filtration and Density Gradient Centrifugation

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Thyroglobulin, the major protein component of normal thyroid glands, contains more than 80% of the thyroidal iodine and has been considered the only source of thyroid hormones (1). Aqueous thyroid extracts contain, besides thyroglobulin, other proteins in variable amounts. Essentially, four methods have been used for the characterization of the various components in such extracts and for the purification of thyroglobulin: salting out, electrophoresis, column chromatography, and ultracentrifugation.

Salting out with ammonium sulfate or potassium phosphate (2) is the classical method for preparing thyroglobulin. However, salting out preparations are always contaminated with compounds that sediment faster than thyroglobulin, the 19 S ultracentrifugal component of thyroid extracts (3-5). Electrophoretic methods likewise yield preparations of thyroglobulin known to be heterogeneous when analyzed in the analytical ultracentrifuge (4, 6-9). Column chromatography on DEAE-cellulose gives highly purified thyroglobulin (4, 10) but the yield is rather poor. The main disadvantage of this method is that thyroglobulin itself is fractionated on these columns according to its iodine content. The ultracentrifugally homogeneous fraction obtained by this method has a considerably lower iodine content than the whole thyroglobulin (4, 11-13). Ultracentrifugation is one of the most useful analytical procedures, but preparative moving boundary ultracentrifugation (8) requires multiple runs to obtain good yields of highly purified thyroglobulin. All the methods now available for the purification of thyroglobulin and for the analysis of crude or partially purified thyroid extracts therefore present major shortcomings.

The need for suitable methods for the analysis of thyroid extracts, as well as for the preparation of highly purified thyroglobulin, led to the work to be reported here. Methods of fractionation based essentially on molecular size were found to be most useful. Filtration through granulated agar or dextran gel columns was used for the purification of thyroglobulin; ultracentrifugation in a linear density gradient was employed for both analytical and preparative purposes.

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EXPERIMENTAL PROCEDURE

Preparation of Thyroid Extracts—Thyroid glands of rats or of guinea pigs were removed after exsanguination of the animals and were then frozen and minced. Sheep and beef thyroids were removed and frozen 10 minutes after slaughter. A few hours later, they were thawed, freed from fat and connective tissue, then ground twice in a meat grinder. Human glands were chilled immediately after surgery, freed from extraneous tissue, and then frozen and sliced. The minced or sliced tissue was extracted for 12 to 16 hours at 4° with 0.1 M KCl (2 ml per g of fresh tissue) and, in the case of larger batches, the tissue debris was removed by filtration through gauze. The extract was clarified by centrifugation at 4° for 10 minutes at 30,000 x g. In a few instances, the clarified solution was again centrifuged for 30 minutes at 105,000 x g in order to remove subcellular particles. Between 50 and 150 mg of total protein per g of fresh gland were thus obtained. In several instances, radioactive glands (labeled with 125I or 131I or both in vitro or in vivo) were used. In this case, more than 95% of the original radioactivity was recovered in the extract. A crude lyophilized hog thyroid extract was kindly supplied by Dr. R. L. Kroc of the Warner Lambert Research Institute. A 5% aqueous suspension was clarified by centrifugation as described above.

The thyroid extracts were finally dialyzed at 4° against KCl-phosphate buffer. The composition of the dialyzed extract was determined by analytical ultracentrifugation (see below).

Buffer—Unless otherwise stated, a 0.1 M KCl-0.02 M sodium phosphate buffer, pH 7.4 (referred to as "KCl-phosphate buffer"), was used.

Sucrose Gradient Centrifugation—Sucrose gradient centrifugation, for analytical as well as preparative purposes, was performed essentially as described by Martin and Ames (14). Linear sucrose gradients were prepared at room temperature in 0.05 M sodium phosphate buffer, pH 7.4. The tubes containing the gradient, as well as the rotor, were allowed to equilibrate at 4° for several hours or overnight. Then the sample (containing 1% or less of total protein) was layered over the sucrose gradient. Other experimental conditions concerning the centrifugation are given in Table I.

Experimental conditions for the sampling and the analysis of fractions after sucrose gradient centrifugation are reported in Table II. When labeled material was present, the radioactivity...
Experimental conditions for sucrose gradient centrifugation of thyroid extracts

<table>
<thead>
<tr>
<th>Spinc model I rotor</th>
<th>Range of sucrose concentration in the gradient</th>
<th>Maximum speed</th>
<th>Vacuum chamber</th>
<th>Temperature</th>
<th>Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW 39</td>
<td>0-20%</td>
<td>36,000</td>
<td>-5</td>
<td>200 μg/ml</td>
<td>6 hrs</td>
</tr>
<tr>
<td>SW 25</td>
<td>5-25%</td>
<td>23,000</td>
<td>-5</td>
<td>&lt;20 μg/ml</td>
<td>22 hrs</td>
</tr>
</tbody>
</table>

* Calculated according to Martin and Ames (14).

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of each collected fraction was measured (see below). The total protein and hemoglobin content of each fraction was then determined spectrophotometrically after appropriate dilution (Table I). Most commercial preparations of sucrose contain impurities which absorb light at 280 nm and even more at 210 nm. For this reason, absorbance measurements were made with the use of a sucrose solution at a concentration corresponding to 1 mg (SW 39) and 10 mg (SW 25) of protein.

For preparative purposes, appropriate fractions were pooled, concentrated, and dialyzed against KCl-phosphate buffer. The purity of the pooled fractions and the yield of TG1 were determined as described below.

Gel Filtration—Sephadex G-200 (Pharmacia, Uppsala), a dextran gel, was suspended in water and allowed to swell for 7 days at 4°C. Granulated agar gels of various concentrations (2.5, 3.5, 5.7, 9, and 11%) were prepared from a purified grade of agar ("purified agar," Difco Laboratories, Inc., Detroit) according to Andrews (15). In order to prevent softening of the gel, especially at the lower concentrations, crushed ice was added prior to disintegration in a Waring Blender. The blending time varied from less than 1 minute for the 2.5% gel to about 20 minutes for the 11% gel.

Both the Sephadex and the agar gel suspensions were sieved through stainless steel United States Standard sieves. A strong tap water jet was used to force the granules through the sieves; in this way, deformation of the granules was avoided. The following fractions were collected: 44 to 74 μ (325 to 200 mesh), 74 to 149 μ (200 to 100 mesh), and 149 to 250 μ (100 to 60 mesh). After sieving, the granules were washed on the sieve successively with distilled water and with KCl-phosphate buffer, then allowed to equilibrate at 4° in the same buffer. Preliminary trials showed that 74 to 140 μ granules were most suitable, except in the case of 2.5% agar gels, for which 149- to 250-μ granules were used.

Columns with bed volumes ranging from 18 ml to 7.5 liters were used. The ratio of the diameter to the height of the columns varied between 1:10 and 1:30. In order to avoid zone deformation due to wall effects, the column tubes were treated with dimethyldichlorosilane (Applied Science Laboratories, Inc., State College, Pennsylvania) and then with methanol. This treatment makes the surface of the glass permanently hydrophobic. After this treatment, the column tubes were equipped near the tip with a sintered glass disk having a maximum pore size of 10 to 15 μ (Corning, medium). A suspension of the gel in KCl-phosphate buffer was used for filling the tube. Uniform packing was facilitated by gently moving a perforated stainless steel disk in the portion of the columns which had not yet settled.

Uniform layering of the sample on top of the column, which had been equilibrated overnight at 4°, was achieved by application of the sample through the same perforated disk, which was held slightly above the packed gel. The protein concentration of the sample was kept below 5% for Sephadex columns and below 10% for agar gel columns; the sample volume was kept as small as possible and never exceeded 3/5 of the bed volume. Ratios of total protein to bed volume ranged from 0.2 to 3 g per liter. Ratios below 2 g per liter were found to be satisfactory.

Elution was carried out with KCl-phosphate buffer at 4° and at a flow rate adjusted to about 1 ml per cm2 per hour by means of a Mariotte bottle.

Fractions were collected in an automatic fraction collector and assayed for protein concentration and for radioactivity. The purity of selected fractions was assessed by ultracentrifugal analysis. Appropriate fractions were then pooled and concentrated.

Determination of Protein Concentrations—Total protein and hemoglobin concentrations were estimated spectrophotometrically in a Beckman model DU instrument. The absorbance of hemoglobin was read at 414 μm (oxyhemoglobin) and a specific extinction coefficient of 18.7 was used. Total protein was determined at 210 or 280 μm, or both. At 210 μm an ultracentrifugally homogeneous preparation of hog TG has a specific extinction coefficient of 207. At 280 μm, values ranging from 9.7 to 11.0 (3, 4, 9, 12) have been reported for TG of various species and various degrees of purity. In the present investigation, values of 10 and 207 for the specific extinction coefficients at 280 and 210 μm, respectively, were used for all proteins. Determinations at 210 μm are more accurate for crude extracts, since at this wave length the specific extinction coefficients are nearly the same (about 205) for most proteins (16). In the case...
of extracts of normal thyroid, in which TG is the major protein component, measurement at 280 nm does not introduce an appreciable error in the calculated proportion of TG.

**Radioactivity Measurements**—Radioactive fractions were counted in a well-type scintillation counter equipped with a pulse height analyzer. In the case of doubly labeled fractions (\(^{125}\)I and \(^{131}\)I) a double channel spectrometer was used. All measurements were accomplished with a counting error of less than 2%.

**Ultracentrifugal Analysis (Moving Boundary)**—Analytical ultracentrifugation was carried out in a Spinco model E ultracentrifuge with the use of standard procedures. Double sector cells were employed at a maximum nominal speed of 52,640 or 56,100 r.p.m. The proportion of the proteins accounting for each peak was determined from the relative areas under the boundaries in the schlieren diagram. No corrections were made for the Johnston-Ogston effect or for the radial dilution of the protein. A protein fraction was considered ultracentrifugally homogeneous when a single, symmetrical boundary was observed at a protein concentration of at least 1%.

**Concentration and Storage of Protein Fractions**—Various methods for the concentration of protein solutions were examined. The formation of some insoluble material as well as the appearance of a breakdown product (about 13 S) was occasionally observed when TG solutions were lyophylized or concentrated by transevaporation through Visking tubing. In the present investigation, protein solutions were concentrated, when necessary, by vacuum filtration through a dialysis bag at 4°C. In this method, the salt concentration of the solution remains constant. No denaturation or breakdown of TG was observed. TG was stored at -10°C after precipitation with ammonium sulfate as suggested by Ui and Tarutani (4) or as a quickly frozen solution (2 to 4%). After 2 years of storage of a 4% TG solution in the frozen state, no change in the ultracentrifugal pattern was observed. A partial breakdown, however, occurs after repeated freezing and thawing, especially of dilute solutions.

**Yields**—Since the ratio of TG to total protein in a crude thyroid extract is quite variable, yields of TG were calculated on the basis of the amount of TG present in the original protein mixture.

**RESULTS**

**Analysis of Protein Components of Aqueous Thyroid Extracts**—Analyses of a large number of crude thyroid extracts of several species were carried out by sucrose gradient centrifugation, and the results were compared with those obtained in the analytical ultracentrifuge. Typical examples are shown in Fig. 1. Three major absorbance peaks were observed in all sucrose gradient patterns. The first one, closest to the meniscus, is not symmetrical and comprises a variable proportion of the total protein. This fraction contains albumin-like thyroidal proteins, serum proteins, and variable amounts of hemoglobin as determined by the ratio of the absorbances at 280 nm and 414 nm. The second peak, found under our experimental conditions between one-half and two-thirds of the distance between the meniscus and the

\[
s = \frac{\text{fraction number of peak} \times 19}{\text{fraction number of TG peak}}
\]

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![Graph](image-url)
The protein composition of crude extracts obtained from normal glands of several species (man, ox, hog, sheep, guinea pig, rat) was found to be quite similar, except for the variable amount of contaminating blood proteins, such as hemoglobin in the slowest sedimenting fraction. Such a contamination was particularly pronounced when thyroidec- tomy had been performed without previous bleeding, as in the case of human thyroid (cf. Fig. 1).

When extracts of labeled glands were analyzed, the distribution of radioactivity was established together with the absorbance pattern. An example is given in Fig. 2, in which the patterns obtained with extracts from a normal and an adenomatous portion of the same human gland are compared. Although very little TG was present in the adenomatous portion, almost the entire radioactivity of the gland migrated with the 19 S fraction. A similar observation was made when extracts from iodine-deficient rat thyroids were analyzed. Radioiodine was present in the 27 S peak, thus establishing its iodoprotein nature. A broad zone of radioiodine in the slowly sedimenting protein (corresponding to about 4 S iodoprotein) was also seen in some instances.

**Purification of Thyroglobulin**—Several preparations of ultracentrifugally homogeneous TG were obtained from crude or partially purified thyroid extracts, either by sucrase gradient ultracentrifugation or by gel filtration. The method was usually chosen on the basis of the amount of starting material available. Table III gives some typical examples of the methods used and of the yields obtained.

It can be seen that sucrase gradient centrifugation gave better yields than gel filtration. However, this procedure is not suita-

### Table III

Typical yields obtained during purification of thyroglobulin

<table>
<thead>
<tr>
<th>Total Protein</th>
<th>Thyroid Extract</th>
<th>TG Content</th>
<th>Method Used</th>
<th>TG Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg</td>
<td>Rat, crude</td>
<td>80</td>
<td>SG 39</td>
<td>%</td>
</tr>
<tr>
<td>4 mg</td>
<td>Guinea pig, crude</td>
<td>78</td>
<td>SG 25</td>
<td>68</td>
</tr>
<tr>
<td>10 mg</td>
<td>Human, crude</td>
<td>62</td>
<td>SG 25</td>
<td>75</td>
</tr>
<tr>
<td>18 mg</td>
<td>Hog, crude</td>
<td>45</td>
<td>Agar gel 5%</td>
<td>40</td>
</tr>
<tr>
<td>18 mg</td>
<td>Hog, crude</td>
<td>45</td>
<td>G-200</td>
<td>45</td>
</tr>
<tr>
<td>30 mg</td>
<td>Human, crude</td>
<td>55</td>
<td>SG 25</td>
<td>75</td>
</tr>
<tr>
<td>80 mg</td>
<td>Human, purified</td>
<td>55</td>
<td>Agar gel 5%</td>
<td>82</td>
</tr>
<tr>
<td>180 mg</td>
<td>Beef, purified</td>
<td>05</td>
<td>Agar gel 3.5%</td>
<td>70</td>
</tr>
<tr>
<td>475 mg</td>
<td>Sheep, crude</td>
<td>60</td>
<td>G-200</td>
<td>54</td>
</tr>
<tr>
<td>1.3 g</td>
<td>Hog, crude</td>
<td>45</td>
<td>Agar gel 5%</td>
<td>60</td>
</tr>
<tr>
<td>3.7 g</td>
<td>Human, crude</td>
<td>65</td>
<td>Agar gel 7%</td>
<td>68</td>
</tr>
<tr>
<td>6.0 g</td>
<td>Beef, purified</td>
<td>85</td>
<td>Agar gel 7%</td>
<td>70</td>
</tr>
<tr>
<td>15.0 g</td>
<td>Beef, purified</td>
<td>90</td>
<td>Agar gel 5%</td>
<td>78</td>
</tr>
</tbody>
</table>

* In the purified extracts the bulk of the proteins sedimenting more slowly than TG had been previously removed by differential centrifugation (3) or by prior passage through an agar column.
* Total protein, 100%.
* SG 39 and SG 25, sucrase gradient centrifugation in rotor SW 39 and SW 25, respectively; agar gel, filtration through granulated agar gels of the indicated concentrations; G-200, filtration through Sephadex G-200.
* Based on a single run and calculated as percentage of TG in the starting material.
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Fig. 3. Fractionation of crude thyroid extracts by gel filtration.  
A, hog thyroid extract, Sephadex G-200, 100 to 200 mesh; bed volume, 80 ml; fraction volume, 2 ml; total protein, 18 mg.  
B, human thyroid extract, agar gel 5%, 100 to 200 mesh; bed volume, 3.4 liters; fraction volume, 5 ml; total protein, 3.4 g.  
C, human thyroid extract; agar gel 7%, 100 to 200 mesh; bed volume, 18 ml; fraction volume, 0.5 ml; total protein, 3.4 mg.  
For other experimental conditions, see the text.

Recovery of total protein from both types of columns was virtually complete.

If a partially purified extract which is essentially free from lighter components (e.g. a salting out preparation) is used, a 5% agar gel seems most suitable, since in this case a better separation of TG from the heavier components is achieved than with 7% agar. The latter agar concentration is preferable if TG is to be prepared from a crude extract, however, because in this situation a complete separation of TG from lighter components, as well as a partial separation from heavier ones, is achieved.

In the example shown in Fig. 3C, the individual fractions, 400 to 450, contained mainly the 27 S component and gradually increasing amounts of TG. Fractions 450 to 500 contained ultracentrifugally homogeneous TG. All fractions above tube 525 contained slower sedimenting material (4 to 7 S) which separated into two peaks. The first one (Fractions 525 to 580) included iodinated albumin-like proteins as shown by the presence of radioactivity, and contained a reddish brown protein with an absorbance maximum at 404 μm. The second peak (Fractions 580 to 614) consisted essentially of hemoglobin as indicated by the ratio of the absorbances at 414 and 280 μm.

Fig. 4 presents the sucrose gradient and moving boundary ultracentrifugation patterns of two pooled fractions collected from this column. The upper patterns were obtained with the combined Fractions 400 to 450, and contained mainly 27 S iodoprotein. Lower patterns give the results obtained with the combined Fractions 451 to 500 and reveal a single peak characteristic of TG.

**DISCUSSION**

Analysis of Thyroid Extracts—Moving boundary ultracentrifugation has been especially useful in the analysis of thyroid extracts because the major iodine-containing constituent, thyroglobulin, forms a distinctive, rapidly sedimenting boundary. Ultracentrifugation in a continuous sucrose gradient, while providing a similar degree of resolution of thyroidal proteins has, in addition, certain advantages which derive from the fact that a zonal, rather than a moving boundary, separation is involved. Since the zones are separated from each other in the course of the analysis, each fraction can be analyzed for several...
FRACTION NUMBER

Fig. 4. Sucrose gradient and moving boundary ultracentrifugation patterns of human thyroid extract fractions obtained by agar gel filtration. A, combined Fractions 400 to 450 of Fig. 3C; B, combined Fractions 451 to 500 of Fig. 3C. Left (A and B): sucrose gradient, 5 to 28%o; rotor, SW 25; equivalent time of centrifugation, 22 hours at 23,000 r.p.m.; total protein, 9 mg (A) and 4 mg (B). T and B on the abscissa signify top and bottom fractions, respectively. Right (A in “wedge” cell, B in standard cell): moving boundary ultracentrifugation from left to right; bar angle, 60°; 20 minutes at 56,100 r.p.m.; protein concentration, about 0.9%; wedge cell (top), 27 S-enriched fraction; standard cell (bottom), thyroglobulin. The minor peak in A and the single peak in B have a sedimentation coefficient of about 19 S and represent thyroglobulin. The major peak in A has a sedimentation coefficient of about 27 S.

Density gradient centrifugation also offers several advantages over other methods used to analyze thyroid extracts. Although the salting out method (2) can separate thyroglobulin from the albumin-like proteins which precipitate at higher salt concentration, (5, 18), it does not discriminate between TG and the faster sedimenting components (e.g., References 3, 4, 12). Crude thyroid extracts analyzed by moving boundary electrophoresis exhibited four boundaries (6), but the major peak contained a greater proportion of the total protein than did the 19 S component in the ultracentrifugation pattern obtained with the same extracts (6, 7), thus indicating its heterogeneity. In zone electrophoresis on starch columns (7) or on filter paper (7-9), TG prepared by salting out, and known to be heterogeneous, migrated as a single zone. Furthermore, the same phenomenon has been observed with a thyroid protein fraction containing about 40% of TG and 60% of the 27 S component (4).

In starch gel electrophoresis (7), on the other hand, three protein zones have been found when the starting material was a salting out preparation of thyroglobulin, or a more highly
purified 19 S fraction. Although the use of fractions prepared
by a discontinuous sucrose gradient indicated that the middle
fraction corresponded to 19 S TG, and the slower and faster
migrating bands corresponded to heavier and lighter components,
respectively, Spiro suggested that these components were in
ready equilibrium with each other. This is contrary to our
experience with the 19 S and 27 S components, which are stable
under the conditions reported here (cf. Fig. 4). The exact
identity of the zones seen in starch gel requires clarification.

Analysis of crude thyroid extracts by DEAE-cellulose chro-
matography (4, 10, 19) is complicated by the fact that serum
proteins have been shown to contaminate the iodoprotein
fractions (20). On the other hand, this method is a useful
analytical tool since it is able to distinguish between thyro-
globulin molecules having different degrees of iodination (11,
13, 19, 21). The most highly iodinated fraction, however, is
contaminated with the 27 S protein. Another subfractionation
of TG into three closely related fractions has been described in
salting out curves (2), but the significance of this heterogeneity
is uncertain.

Preparation of Thyroglobulin—It is possible to obtain ultra-
centrifugally homogeneous thyroglobulin from a crude thyroid
extract, in a single step, by either gel filtration or sucrose gradient
centrifugation. It should be noted that, since the methods
depend only on separation of molecules according to size and
shape, it is possible that the thyroglobulin would be contami-
nated by trace amounts of serum macroglobulins which might be
present in thyroid extract (cf. Reference 22). The yield in both
methods is about 60 to 70% of the total 19 S component initially
present. Quantities of thyroglobulin ranging from less than 1
mg to about 8 g, and possibly more, can be obtained in one
operation by one or the other of these two methods

As little as 1 mg of a crude mixture of thyroidal proteins
(equivalent to one lobe of a rat thyroid) can be fractionated by
sucrose gradient centrifugation in the SW 39 rotor. In this
rotor, a maximum of 3 mg of protein (1 mg for each gradient
tube) can be worked up in one ultracentrifuge run. The use of
the SW 25 rotor allows the purification of 4 to 10 mg of protein
for one gradient tube (corresponding to a maximum of 30 mg
per run). From 30 to 500 mg of soluble thyroidal proteins are
best purified by filtration through a small or medium size column
of Sephadex G-200 or 7% agar gel. On a large scale, agar gel
filtration is the method of choice. Amounts of up to 15 g of
thyroidal proteins per column have been satisfactorily processed
in this laboratory. In a recent study (23), published since com-
pletion of the present work, Sephadex G-200 was used to prepare
TG in 11% yield from crude thyroidal extract containing 50 mg
of protein.

Other methods now available for the purification of thyro-
globulin have certain disadvantages which are largely avoided
by the procedures described above. From the data summarized
in Table IV it can be seen that the previous methods give either
impure TG in fairly good yield (salting out) or highly purified
TG in limited yield (differential centrifugation or DEAE-
cellulose chromatography). Furthermore, the ultracentrifuga-
lily homogeneous fraction of TG obtained by chromatography
on DEAE-cellulose is not representative of the whole TG present
in the thyroid extract (see above). The fractions which are eluted at lower ionic strength, which are the only ones not con-
taminated by faster sedimenting material, contain less iodine
than the whole thyroglobulin.

<table>
<thead>
<tr>
<th>Method</th>
<th>Range*</th>
<th>Degree of purity of TG</th>
<th>Approximate yield</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salting out</td>
<td>100 mg-50 g</td>
<td>%</td>
<td>90-95</td>
<td>50</td>
</tr>
</tbody>
</table>
| DEAE-cellulose chromatogra-
| phy                     | 50 mg-1.7 g | %                     | 99-100           | 9          | 4, 10 |
| Differential centri-
| trifugation             | 200 mg-3.6 g | ~100                 | ~100              | ~3         |
| Filtration through Sepha-
| dex                   | 30-500 mg | ~100                 | 50                | Present work |
| Filtration through agar gel | 30 mg-15 g | ~100                 | 60                | Present work |
| Sucrose gradient ultracentri-
| fugation             | 1-30 mg | ~100                 | 70                | Present work |

* Amount of thyroidal protein processed.
* As measured by analytical ultracentrifugation.
* Calculated on the basis of the amount of TG present in the
  crude extract.
* Only the most pertinent references are listed.
* Not stated.

SUMMARY

Improved methods for the analysis of the protein components
of a crude or purified thyroid extract, and for the preparation of
highly purified thyroglobulin, have been developed.

Experimental conditions suitable for the analysis of thyroidal
iodoproteins in a linear sucrose gradient were determined. This
technique gives results very similar to those obtained by analyti-
cal ultracentrifugation, but is more versatile.

Ultracentrifugally homogeneous preparations of thyroglobulin
are obtained in a single step either by gel filtration (agar or
dextran) or by density gradient ultracentrifugation. The yields obtained by either technique are considerably higher
(60 to 70% of the thyroglobulin initially present in the thyroid
extract) than those previously attained. The methods are
applicable on a micro or macro scale ranging from 1 mg to more
than 15 g of thyroid protein.

REFERENCES
2. DERRENN, Y., MICHEL, R., AND ROCHE, J., Biochim. et Biophys.
Separation of Thyroidal Iodoproteins and Purification of Thyroglobulin by Gel Filtration and Density Gradient Centrifugation

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