THE RELATIVE ROLES OF IODINATION AND IODOTHYRONINE CONTENT ON THYROGLOBULIN STABILITY

By
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ABSTRACT

We have independently varied the degree of iodination and of iodothyronine formation over a wide range by acutely administering various doses of perchlorate and/or methimazole to severely iodine-deficient rats 30 min before giving \(^{131}\)I with graded quantities (1–100 \(\mu\)g of \(^{127}\)I). Thyroids were removed 4 h later and the soluble protein analyzed for labelled iodoamino acid composition and with sucrose density gradient ultracentrifugation. Since the total thyroid iodine content before administering \(^{127}\)I was less than 1 \(\mu\)g, calculation of the degree of iodination and iodothyronine content of the labelled Tgb could be made from the known specificity of the injected labelled iodide. Newly organified iodine ranged from \(<0.1\) to \(1.4\) \(\mu\)g/thyroid and labelled iodothyronines from \(<5\) to 962 pmoles/thyroid. Both the degree of iodination and iodothyronine content varied directly with Tgb stability in the absence of inhibitors. But when Tgb iodination was kept constant, Tgb stability at pH 10.1 varied directly with iodothyronine content. When iodothyronine content was kept constant, Tgb stability was independent of the degree of iodination. Correlation of stability with iodothyronine content was highly significant \((r=0.79, P < 0.001)\) but not of stability with iodine content \((r=0.49, P > 0.05)\). We conclude that the primary determinant of Tgb stability in mild alkali is the iodothyronine content and not the degree of iodination of the protein. The increased Tgb stability may be induced by coupling between ioddotyrosil residues of different 12 S subunits rather than between residues of the same 12 S subunit.

Thyroglobulin (Tgb) with a low iodine content unfolds or disaggregates into 12 S subunits more readily in the presence of mild dissociating agents than does normally iodinated Tgb (Lissitzky et al. 1964, 1966; Sellin & Goldberg...
injected Chagrin was each 2-8 Male the to iodination of peptide process venting whose preformed rats than the same residue is 1969, 1965; Simon et al. 1966; Ui & Tarutani 1968; Tarutani & Ui 1969a: Andreoli et al. 1969; Rolland & Lissitzky 1970; Sinadinovic et al. 1973). This decreased stability has been demonstrated in the thyroglobulin of intact iodine-deficient animals (Inoue & Taurog 1968b; Tarutani & Ui 1969b; Rossi et al. 1973) and in that of hypophysectomized iodine-replete animals (Rosenberg & Cavalieri 1969, 1971; Cavalieri et al. 1970) in which the capacity of the thyroid to iodinate Tgb substrate is limited. In both these conditions, the formation of iodothyronines is also depressed. Therefore, the relative roles of degree of iodination and of iodothyronine content in determining Tgb stability is uncertain.

Recent studies have indicated that increasing the iodothyronine content of preformed Tgb by giving TSH to iodine-deficient hypophysectomized rats whose intrathyroidal Tgb has been prelabelled with radioiodine, while preventing additional Tgb iodination through simultaneous administration of perchlorate, is associated with an increase in Tgb stability (Greer et al. 1971, 1974).

Our theory to explain these data is that during the coupling reaction there is a transfer of the phenolic group of one iodotyrosyl residue to an iodotyrosyl residue in an adjacent segment of peptide chain (which may be from either the same or a different chain as the donor chain) to form iodothyronine. This process leaves the alanyl residue of the donor iodotyrosine intact in the donor peptide segment. The phenolic translocation is associated with formation of covalent or non-covalent bonds between the adjacent peptide segments. This increase in bonding makes the Tgb molecule more stable if the two chain segments participating in the reaction are from different 12 S subunits of the same Tgb molecule so that a stronger union is formed between the subunits. The reaction does not involve any net increase in iodination.

In the present studies, we have examined this theory by studying the stability of Tgb acutely labelled in vivo with radioiodine in iodine-deficient rats. 127I was simultaneously injected in various concentrations so that the degree of iodination of the Tgb could be estimated from the specific activity of the injected material. Methimazole (MMI) and perchlorate were additionally used to control the degree of iodination and coupling. Our data indicate that under the conditions of our experiments iodothyronine content is more important than the degree of Tgb iodination in maintaining stability of this protein.

**MATERIALS AND METHODS**

Male Sprague-Dawley rats weighing 200–250 g were fed a low-iodine diet (LID) for 2–8 weeks which contained approximately 30 μg 127I/kg (General Biochemicals, Inc., Chagrin Falls, Ohio). There were 3–6 animals per group in each experiment. The rats in each group in individual experiments were all fed LID for the same period. Each experiment was performed 2–5 times, always with similar results. Three mg
sodium perchlorate and/or 0.03–0.3 mg MMI were separately injected ip in a volume of 0.5 ml of 0.9 % sodium chloride. Thirty min later, 0.3–100 pg 125I labeled with 25–150 μCi 125I were injected ip in 0.5 ml of 0.9 % saline. The animals were killed with chloroform 4 h after administration of iodine. The thyroids were carefully dissected, weighed and placed in 0.3 ml of phosphate buffer-saline (PBS, pH 6.8, ionic strength 0.15) containing 0.02 M MMI and maintained at 4°C. All subsequent processing and storage of the thyroid protein were at this temperature. The radioactivity of each thyroid was measured at the top of a well-type scintillation counter and compared with an appropriate standard. The glands were then individually homogenized with a glass homogenizer and centrifuged at 2500 r. p. m. (1500 g) for 15 min. The supernatant containing soluble thyroid protein was removed. A portion of each thyroid supernatant was individually digested for 24 h at 37°C with 1 % pancreatin (Vio-kase-4 X) in Tris-HCl buffer (pH 8.6) containing 0.02 M MMI and chromatographed in butanol-acetic acid-water (BAW) or butanol-ethanol-ammonia (BEA) (Shimoda & Greer 1968). The chromatograms were scanned with a strip scanner and the distribution of total radioactivity in each labelled substance determined with a digital integrator. In some experiments, pools were made of the undigested supernatant from all thyroids in each group. Each individual homogenate contributed an equal amount of radioactivity to the pool. Reference Tgb which was assumed to have a sedimentation coefficient of 19 S was obtained in the same manner from thyroid glands of rats which had been fed a high-iodine diet (Purina Laboratory Chow, 3 mg 125I/kg) for several weeks and labelled with 20 μCi 125I in vivo for 24 h. Ten to 50 μl aliquots of the experimental supernatant and 10 μl of reference Tgb solution were mixed in a total volume of 0.3 ml of PBS or 0.1 M Tris (pH 10.1) and kept at 4°C for 10–15 min before ultracentrifugation.

Sucrose density gradient centrifugation (5–20 % w/v) was performed in 11 ml of PBS or Tris at 29 000 r. p. m. (105 000 g) for 15 h at 4°C in the SW 65 rotor of an International Ultracentrifuge. After centrifugation, 50–52 fractions were collected from each tube with a peristaltic pump, the first fraction starting from the bottom. The 125I and 131I content of each fraction were measured simultaneously in a dual channel automatic well-type gamma counter. Sedimentation coefficients of the various labelled iodoprotein peaks were roughly calculated by comparison with the location of the major peak of the reference 125I labelled Tgb which was assumed to have a sedimentation coefficient of 19 S.

Relative stability of the experimental Tgb in each gradient tube was calculated by the following formula:

\[
\frac{(\text{cpm in 18-19 S experimental Tgb})}{(\text{cpm in 10-12 S experimental Tgb})} \quad \frac{(\text{cpm in 18-19 S reference Tgb})}{(\text{cpm in 10-12 S reference Tgb})}
\]

**RESULTS**

**Effect of prior administration of perchlorate on the effect of subsequent injections of labelled 125I on iodoamino acid synthesis in iodine-deficient rats**

It is well-known that small doses of stable iodide (5 μg or less) can inhibit iodoamino acid synthesis in iodine-deficient rats through the Wolff-Chaikoff effect. This inhibition is produced by a high concentration of intrathyroidal
iodide and can be prevented by inhibiting the iodide pump with certain monovalent anions such as perchlorate. A reduction of iodide substrate below a critical level also decreases iodothyronine formation. Perchlorate itself, in a very high concentration, can apparently inhibit organic iodination independently of the reduction of iodide substrate. Since our aim was to have very iodine-deficient Tgb in which the quantity of pre-existing iodine would be negligible in relation to the dose of $^{127}$I injected, it was necessary to determine appropriate amounts of perchlorate and $^{127}$I for our experiments.

In an initial study, 3 mg sodium perchlorate given 30 min before administration of carrier-free $^{131}$I was very effective in inhibiting the 4-h thyroid uptake of $^{131}$I. Accordingly, this dose of perchlorate was given ip 30 min before administration of graded doses of $^{127}$I (1–100 µg) labelled with 150 µCi $^{131}$I to rats fed LID 13 days. One group was given 1 µg $^{127}$I labelled with 20 µCi $^{131}$I without prior injection of perchlorate; the 4-h $^{131}$I uptake of this group was 52% (Table 1). Prior injection of perchlorate reduced the radioiodine uptake to 10% of this value. Thyroid radioiodine uptake was progressively further diminished by increasing doses of carrier $^{127}$I. Perchlorate pre-treatment reduced the quantity of $^{131}$I into iodothyronines when only 1 µg of carrier $^{127}$I was used, as expected (Inoue & Taurog 1968a) (Group B). This effect was not seen when 10 µg $^{127}$I or more was used (Groups C–E).

### Table 1.

Effect of perchlorate and iodide on thyroidal labelled iodoamino acid synthesis in LID-fed rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Thyroid $^{131}$I content (% of dose)</th>
<th>O</th>
<th>I-</th>
<th>MIT</th>
<th>DIT</th>
<th>T4</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>51.8±1.1°</td>
<td>3.1±0.1</td>
<td>5.0±0.3</td>
<td>32.3±1.8</td>
<td>23.9±0.6</td>
<td>25.8±1.5</td>
<td>3.4±0.3</td>
</tr>
<tr>
<td>B</td>
<td>4.9±0.9</td>
<td>1.5±0.3</td>
<td>4.1±0.5</td>
<td>49.6±0.5</td>
<td>26.8±1.4</td>
<td>5.5±0.7</td>
<td>2.3±0.6</td>
</tr>
<tr>
<td>C</td>
<td>3.9±0.4</td>
<td>3.5±0.2</td>
<td>6.2±0.6</td>
<td>32.4±1.9</td>
<td>24.6±2.1</td>
<td>22.2±3.0</td>
<td>3.9±0.8</td>
</tr>
<tr>
<td>D</td>
<td>3.3±0.3</td>
<td>4.0±0.1</td>
<td>6.1±0.6</td>
<td>30.6±0.2</td>
<td>25.9±0.4</td>
<td>21.3±1.2</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>E</td>
<td>1.4±0.2</td>
<td>2.7±0.1</td>
<td>8.5±1.2</td>
<td>27.5±2.3</td>
<td>31.9±0.8</td>
<td>19.5±1.0</td>
<td>2.4±0.2</td>
</tr>
</tbody>
</table>

Four rats fed LID for 13 days were used in each group. Group A was given 1 µg of iodide with 20 µCi of $^{131}$I. Groups B through E were given 3 mg of perchlorate 30 min before various doses of iodide, 1, 10, 30 and 100 µg, respectively, with 150 µCi of $^{131}$I. The rats were killed 4 h after $^{131}$I injection. Origin (O), I, MIT and DIT were calculated from BAW chromatography, T4 and T3 from BEA.

* Mean and standard error.
Table 2.

Content of thyroidal organified iodine and iodothyronines, sedimentation coefficient in PBS and relative stability of Tgb after treatment with perchlorate and iodide.

<table>
<thead>
<tr>
<th>Group</th>
<th>Newly organified iodine (µg/gland)</th>
<th>Newly formed iodothyronines (pmol/gland)</th>
<th>Sedimentation coefficient in PBS</th>
<th>Relative Tgb stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.492 ± 0.010</td>
<td>289.4 ± 14.9</td>
<td>19.0</td>
<td>0.78</td>
</tr>
<tr>
<td>B</td>
<td>0.047 ± 0.008</td>
<td>8.2 ± 2.4</td>
<td>18.4</td>
<td>0.44</td>
</tr>
<tr>
<td>C</td>
<td>0.362 ± 0.041</td>
<td>197.8 ± 12.6</td>
<td>19.0</td>
<td>0.80</td>
</tr>
<tr>
<td>D</td>
<td>0.980 ± 0.090</td>
<td>483.8 ± 51.5</td>
<td>19.6</td>
<td>1.11</td>
</tr>
<tr>
<td>E</td>
<td>1.261 ± 0.206</td>
<td>611.5 ± 91.6</td>
<td>19.6</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Data were obtained from the same experiment as in Table 1; groups A through E correspond with those in Table 1.

1) Calculated from $^{127}$I administered, thyroidal $^{131}$I uptake and chromatography of thyroid digest.
2) Calculated by comparison with the location of the major peak of $^{125}$I-labelled reference Tgb, assumed to be 19 S.
3) Relative Tgb stability in Tris was obtained according to the formula:

$$\frac{^{131}I (18-19 S) / ^{131}I (10-12 S)}{^{125}I (18-19 S) / ^{125}I (10-12 S)}$$

where $^{131}$I-labelled Tgb was from the experimental rats and $^{125}$I-labelled Tgb was from Purina-fed rats.
4) Pooled samples of each group were examined.

The $T_3^*/T_4^*$ ratio was relatively low under all conditions but was highest in Group B, given perchlorate plus the lowest dose of iodide. The $MIT^*/DIT^*$ ratio decreased progressively with increasing doses of $^{127}$I in the groups given prior perchlorate. This is consistent with the previously observed rapid depression of $MIT^*/DIT^*$ and $T_3^*/T_4^*$ ratios with acute injection of relatively small doses of $^{127}$I in iodine-deficient rats and the observation that $T_3^*/T_4^*$ ratios are depressed more readily by small amounts of $^{127}$I than $MIT^*/DIT^*$ ratios (Studer & Greer 1968).

In this and all subsequent experiments, the sucrose density gradients in PBS did not show any striking difference between the experimental and high-iodine reference Tgb. Various parameters are shown in Table 2. With increasing quantities of $^{127}$I incorporated into the experimental Tgb, there was a gradual shift of the major iodoprotein peak from 18.4 S to 19 S or higher.

1) An asterisk indicates that only the radioiodinated component is being considered.
The relative Tgβ stability was positively correlated with both total organic $^{127}$I content and iodothyronine content.

**Combined use of perchlorate, MMI, and stable iodide**

These experiments were designed to provide thyroglobulin with independently variable degrees of iodination and iodothyronine content. Small doses of MMI given 30 min before carrier-free $^{131}$I$^-$ inhibited coupling more than organic iodination. However, when stable iodide was administered 30 min after the methimazole, the combination produced appreciable inhibition of organic iodination with very small doses of $^{127}$I$^-$, sometimes in the submicrogram range. This may have been because of an additive effect of the $^{127}$I$^-$ and MMI. Since it is a high intrathyroidal iodide concentration which inhibits organic iodination, perchlorate was given simultaneously with MMI to keep intrathyroidal iodide concentration low even when $^{127}$I$^-$ was administered.

A representative experiment is shown in Tables 3 and 4. As little as 0.03 mg MMI reduced formation of iodothyronines approximately 90% (cf. C and D in Table 4). As compared with B, D had only one third the iodothyronines (275.0 vs. 91.9 pmoles/gland) but an identical content of newly organified iodine. Thus, dissociation between the degree of iodination and iodothyronine formation could be obtained by the combined administration of perchlorate,

**Table 3.**

Effect of combined administration of MMI, perchlorate and iodide on thyroidal labelled iodoamino acid synthesis in LID-fed rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Thyroid $^{131}$I content (% of dose)</th>
<th>% of total chromatograph radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>I$^-$</td>
</tr>
<tr>
<td>A</td>
<td>67.2 ± 4.5</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td>4.5 ± 0.3</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>C</td>
<td>5.2 ± 1.2</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>D</td>
<td>1.7 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>E</td>
<td>0.95 ± 0.12</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>F</td>
<td>0.50 ± 0.14</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

Four rats fed LID for 41 days were used in each group. Group A was given 0.3 µg of $^{127}$I$^-$ with 25 µCi of $^{131}$I$^-$. Groups B and C were given 3 mg of perchlorate 30 min before 10 and 30 µg of $^{127}$I$^-$, respectively, with 150 µCi of $^{131}$I$^-$. Groups D, E, and F were given 0.03, 0.1 and 0.3 mg of MMI with 3 mg of perchlorate, respectively. 30 min before 30 µg $^{127}$I$^-$ with 150 µCi of $^{131}$I. Origin, I$^-$, MIT and DIT were calculated from BAW, T$_4$ and T$_3$ from BEA.

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Table 4.
Thyroid content of organified iodine and iodothyronines and relative stability of Tgb with combined use of MMI, perchlorate and iodide.

<table>
<thead>
<tr>
<th>Group</th>
<th>Newly organified iodine (µg/gland)</th>
<th>Newly formed iodothyronine (pmol/gland)</th>
<th>Relative Tgb stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.192 ± 0.014</td>
<td>124.1 ± 11.8</td>
<td>0.56</td>
</tr>
<tr>
<td>B</td>
<td>0.411 ± 0.026</td>
<td>275.0 ± 28.0</td>
<td>1.1</td>
</tr>
<tr>
<td>C</td>
<td>1.439 ± 0.342</td>
<td>962.7 ± 27.0</td>
<td>0.92</td>
</tr>
<tr>
<td>D</td>
<td>0.454 ± 0.116</td>
<td>91.9 ± 24.3</td>
<td>0.43</td>
</tr>
<tr>
<td>E</td>
<td>0.220 ± 0.028</td>
<td>17.2 ± 4.0</td>
<td>0.37</td>
</tr>
<tr>
<td>F</td>
<td>0.102 ± 0.025</td>
<td>–</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Data were obtained from the same experiment as shown in Table 3. Details for calculations are given in the footnote of Table 2.

MMI and stable iodide. Inhibition of coupling without a decrease of iodination could be demonstrated under these conditions. Although the relative stability of the pooled thyroglobulin of each group was roughly proportional to both the degree of iodination and iodothyronine formation, Group D (Tables 3 and 4), which had significantly less iodothyronines than Group B, had less than half the relative Tgb stability of Group B despite having the same content of organic iodine. Subsequent experiments were therefore performed using the same basic experimental design.

Relation of iodothyronine content to thyroglobulin stability

As shown in Fig. 1, administration of 10 µg of stable iodide 30 min after perchlorate greatly increased formation of organic iodine and iodothyronines. By increasing the quantity of stable iodide to 30 or 60 µg and adding MMI to the perchlorate pre-treatment, total organic iodination was kept at about the same level but iodothyronine formation decreased significantly. These thyroglobulins, which had independently variable degrees of iodination and iodothyronine formation, were submitted to ultracentrifugal analyses.

Two thyroglobulins from this experiment with an identical organic iodine content but a 4-fold variation in iodothyronine content, were analyzed in sucrose density gradients using PBS (pH 6.8) and Tris (pH 10.1) (Fig. 2). There was no appreciable difference in centrifugal pattern at pH 6.8 between the two experimental thyroglobulins, both having a major peak at 18.4 S. At pH 10.1, both dissociated into 10–12 S subunits more readily than the highly
iodinated reference Tgb. However, the Tgb with the lowest iodothyronine content dissociated more completely. The reverse was seen with thyroglobulins with a similar iodothyronine content but different degrees of iodination (Fig. 3). Although both had the same gradient pattern in PBS, relative Tgb stability at pH 10.1 was greater in the more poorly iodinated Tgb with a slightly higher iodothyronine content than in the highly iodinated thyroglobulin with slightly lower iodothyronine content.

Utilizing data from 2 separate experiments, relative Tgb stability at pH 10.1 was calculated as a function of newly organified iodine per gland. A non-significant correlation coefficient of 0.49 ($P > 0.05$) was obtained. The data from the same experiments using newly formed iodothyronines instead of newly organified iodine gave a significant correlation coefficient of 0.79 ($P < 0.001$) between relative Tgb and iodothyronine content.

**Comparison of iodoamino acid composition of 18–19 S and 10–12 S iodoprotein components after dissociation in Tris**

In some experiments employing sucrose gradient centrifugation at pH 10.1, the fractions comprising the 18–19 S and 10–12 S components were separately

![Graph](image-url)  
**Fig. 1.** Effect of perchlorate, MMI and graded doses of iodide on thyroid iodine metabolism. Four rats fed LID for 48 days were used in each group. Three mg perchlorate with or without 0.03 mg of MMI was given 30 min before various doses of $^{127}$I labeled with $^{131}$I. Each value was calculated from the specific activity of the iodide administered and the labelled iodoamino acid composition of the thyroid digest. The vertical lines indicate SE.

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Comparison of sedimentation pattern of Tgb with similar total organic iodine but different iodothyronine contents. Left panel: 3 mg perchlorate was given 30 min before 10 μg of iodide with 150 μCi of $^{137}$I to LID-fed rats. Right panel: 3 mg perchlorate with 0.03 mg of MMI was given 30 min before 30 μg of iodide with 150 μCi of $^{137}$I. Soluble iodoprotein from each animal was subjected to 5–20% sucrose density ultracentrifugation in pH 6.8 PBS (upper) and pH 10.1 Tris (lower) with $^{125}$I-labelled reference Tgb from a Purina-fed rat. Collection fractions are shown on the abscissa starting from the bottom of the tube on the left. Each tic indicates 5 tubes. The ordinate shows cpm normalized to show the highest radioactivity in each fraction at the top of the graph. The solid line is experimental $^{131}$I-Tgb and the dotted line reference $^{125}$I-Tgb. Values for total organic iodine and $T_4 + T_3$ are shown as content per gland. R. S. = relative stability.

pooled and dialyzed against pH 8.6 Tris-HCl buffer containing 0.02 M MMI for 24 h at 4°C. The dialyzed material was then digested with 1% pancreatin and chromatographed. The iodothyronine content of the 18–19 S fraction was invariably twice as high as that of the 10–12 S fraction (Table 5).

DISCUSSION

The pre-existing iodine content of the thyroglobulin of the LID rats was considered to be negligible in relation to the quantity of $^{127}$I administered in these experiments. Tarutani & Ui (1969b) reported that the average iodine
Comparison of sedimentation pattern of Tgb with similar iodothyronine but different total organic iodine content. Upper panel: 0.3 μg of $^{127}$I labelled with 20 μCi $^{131}$I was given. Lower panel: 5 mg perchlorate and 0.03 mg MMI was given 30 min before 60 μg $^{127}$I labelled with 150 μCi $^{131}$I. Sucrose density ultracentrifugation was carried out in pH 10.1 Tris. The ordinate represents normalized cpm and the abscissa the number of fractions as in Fig. 2.

content per Tgb molecule was much less than unity in goitrogen-treated hogs. Our iodine-deficient Tgb labelled with carrier-free $^{131}$I totally dissociated into subunits at pH 10.1 and 18–19 S stability was significantly increased by addition of very small amounts of carrier $^{127}$I.

Since we made sucrose density gradient separations of very small aliquots of soluble thyroid protein, there was insufficient protein in the 18–19 S fraction to be measured accurately. However, we assume that the total quantity of Tgb in each thyroid of the same experiment was approximately equal.
All rats in each experiment were of the same age and received the same diet until the time of study. All experiments were only of 4 h duration, making it unlikely that significant differences in intrathyroidal Tgb concentration would occur between groups during this time. The thyroid weight in the rats of each experiment was approximately the same.

The present data support the theory that stability of Tgb under mildly adverse conditions is primarily dependent upon the iodothyronine content rather than upon the degree of iodination of the protein. There was a significant correlation of Tgb stability in mild alkali with iodothyronine content but no significant correlation of Tgb stability with total iodine content.

Presumably coupling of iodothyrosyl residues can occur either within the same 10–12 S subunit or between such subunits. Separate digestion and analysis of the 18–19 S Tgb and 10–12 S subunits obtained by sucrose density gradient centrifugation at pH 10.1 revealed an iodothyronine content approximately twice as high in the 18–19 S Tgb as in the 10–12 S subunits, agreeing with the observations of Gavaret et al. (1972) on Tgb dissociated with a different technique, the use of sodium dodecylsulphate. If coupling occurred only between 10–12 S subunits, the disproportion in iodothyronine content between 18–19 S and 10–12 S material would be expected to be even greater, since the increased stability which coupling induced in the 18–19 S Tgb, presumably through formation of covalent or non-covalent bonds, might

### Table 5.
Comparison of labelled iodoamino acid composition of 18–19 S thyroglobulin and 10–12 S subunits after dissociation in pH 10.1 Tris.

<table>
<thead>
<tr>
<th></th>
<th>18–19 S</th>
<th>10–12 S</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIT</td>
<td>0.87 ± 0.03</td>
<td>1.06 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DIT</td>
<td>0.87 ± 0.03</td>
<td>1.09 ± 0.05</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>T₄ + T₂</td>
<td>1.47 ± 0.12</td>
<td>0.81 ± 0.03</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MIT/DIT</td>
<td>1.01 ± 0.06</td>
<td>1.01 ± 0.05</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

Pooled Tgb from each group of 4 rats was obtained from the same experiment as shown in Fig. 1. Data from four different groups were combined. Soluble thyroid protein was dialysed against pH 8.6 Tris-HCl buffer containing 0.02 M MMI for 24 h and digested with pancreatin. Two fractions, 18–19 S (undissociated) and 10–12 S (dissociated), separated by sucrose density gradient centrifugation in pH 10.1 Tris, were dialysed in the same buffer and analysed independently. Values represent ratios (mean ± se) of per cent of total digest radioactivity as the various labelled iodoamino acids in 18–19 S or 10–12 S fractions compared to those in digests of soluble thyroglobulin before gradient centrifugation.
prevent dissociation under our conditions. Conversely, Tgb in which coupling did not occur might dissociate completely.

Other forces than the iodothyronine coupling reaction undoubtedly influence Tgb stability. Tgb with no detectable iodothyronine content does not dissociate under appropriate conditions of sucrose density ultracentrifugation, such as in PBS. With more severe conditions than the mild alkali we employed, even 18–19 S Tgb with a high iodothyronine content will dissociate completely. However, since a two-fold higher iodothyronine content in the 18–19 S than in the 10–12 S protein under conditions producing mild dissociation has been found in independent observations by Gavaret et al. (1972), Valenta (1974) and in the present studies, the evidence strongly suggests that coupling per se has some important influence on 18–19 S Tgb stability. Our current working theory is that coupling occurring within a 10–12 S subunit does not increase the strength of the bond between the two subunits in 18–19 S Tgb. It is only when coupling occurs between the subunits that this bond is increased, giving increased stability to the Tgb. The iodothyronines present in the 10–12 S subunits obtained by sucrose density gradient separation in Tris thus would primarily be those in which iodothyronine formation had occurred by coupling within 10–12 S subunits, while the 18–19 S Tgb would primarily represent material in which coupling had occurred between the subunits.

The present data are consistent with the often-reported observation that the sedimentation coefficient of Tgb is positively correlated with the degree of iodination (Inoue & Taurog 1968a; Nunez et al. 1966; DeCrombrugghe et al. 1967; Thomson & Goldberg 1968; Valenta 1974). Poorly iodinated Tgb consistently had a sedimentation coefficient of 18–18.5 S in PBS in our experiments. The “27 S” and “12 S” components, when present, of the poorly iodinated Tgb correspondingly sedimented less rapidly than those of the iodine-replete reference Tgb. In experiments in which a high degree of iodination of Tgb was attained, a “super thyroglobulin” was sometimes produced with a sedimentation coefficient > 19 S. Presumably this is because a greater degree of iodination occurred in vivo under the special conditions of our experiments than normally occurs even in iodine-replete animals. Tgb with a sedimentation coefficient > 19 S can also be produced by direct chemical iodination of Tgb with chloramine T or by in vitro enzymatic iodination with peroxidase (unpublished observations).

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