EFFECT OF IODIDE AND THYROTROPHIN ON IN VITRO $^{14}$C-AMINO ACID INCORPORATION INTO RAT THYROID PROTEINS

By

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ABSTRACT

Thyroid lobes from rats on normal (NID) or low iodine (LID) intake were incubated for 4 hours in vitro in the presence of $^{14}$C-amino acids. The $^{14}$C-amino acid incorporation into thyroid protein was significantly higher in thyroids from LID than from NID fed rats, 7.82 ± 1.01 % (mean ± sd) of total radioactivity of the incubation mixture per 100 mg tissue compared to 3.74 ± 0.60 % respectively. Thyrotrophin (TSH) in vitro did not influence the $^{14}$C-amino acid incorporation. Iodide in concentration 10$^{-7}$ M and higher decreased $^{14}$C-radioactivity incorporation into protein by 19.40 ± 3.06 and 26.59 ± 4.06 % of the control value for NID and LID rats respectively. This effect of iodide did not depend on iodine organification and was not influenced by the changes of free amino acids pool. There were no significant differences in the relative concentration of $^{14}$C-labelled thyroglobulin and total $^{14}$C-thyroid protein. Differential fragility demonstrable by unfolding or dissociation was observed between different classes of thyroglobulin. The fragility was increasing from the old non-labelled molecules to newly iodinated and newly synthesized ones. It is concluded that iodide has a direct intrathyroidal blocking effect on thyroid protein synthesis which may contribute to its antigoitrogenic action. The lack of in vitro stimulation of protein synthesis by TSH remains unexplained.
Iodide prevents goitre formation, opposes goitrogenicity of antithyroid compounds in experimental animals and may lead to shrinkage of existing goitre. The effects of iodide were extensively reviewed by Wolff (1964, 1969). Since goitre occurs only in the presence of intact thyroid-pituitary relationships and is probably due to stimulating of thyroid growth by thyroid stimulating hormone (TSH), it is logical to suppose that iodide acts through increased formation of thyroid hormone which itself suppresses TSH secretion. However, Heywood (1966) has demonstrated that iodide blocks 14C-amino acid incorporation into thyroid protein in slices and an acellular system in vitro. We observed this phenomenon using whole rat thyroid lobes incubated in vitro. These findings suggest that in part, the antigoitrogenic effect of iodide may be due to its direct intrathyroidal effect on protein synthesis.

We also examined the effect of in vitro added TSH on 14C-amino acid incorporation into rat thyroid proteins and namely into thyroglobulin. In agreement with others (Raghupathy et al. 1964; Nataf et al. 1967; Seed & Goldberg 1965), we were unable to demonstrate any significant change.

MATERIAL AND METHODS

White male Wistar rats, initial weight approximately 120 g were kept for periods of 4 to 18 weeks on “normal” iodine diet (NID, Extralabo, France, Torresani et al. 1968), containing 300 to 400 µg iodine per 100 g, or on low iodine diet (LID) with only 1.0 to 1.5 µg iodine per 100 g as measured by neutron activation (Simon 1964). The animals were killed by exsanguination, thyroids were rapidly removed and put on ice. Hemithyroids from 6 to 10 rats were pooled, weighed and put into flasks with 1.0 or 2.0 ml Eagle’s minimum essential medium (Eagle 1959) pre-incubated at 37°C in a Dubnoff metabolic shaker in the presence of O2. The medium was enriched by 10%/v/v rat serum (in case of iodide experiments, from LID fed rats), and contained 20 µCi algal 14C-labelled protein hydrolysate. Thyroid lobes opposite to those which served as a control were incubated in the same conditions except for addition of bovine TSH (Thytopar Armour), 0.2 to 10.0 mU per ml, or iodide in concentration 10^-5, 10^-6, 10^-7, 10^-8 and 10^-9 M. Preliminary studies have shown that significant 14C-amino acid incorporation occurs in the presence of well preserved tissue at 4 h after incubation and this time interval was then routinely used. After incubation the flasks were chilled in ice, thyroids washed by phosphate buffered saline and homogenized in a Potter–Elvehjem homogenizer in 2 ml of 0.066 M phosphate buffer pH 6.8 with saline added to final ionic strength (I) 0.15 (PBS I 0.15). Aliquots of the homogenate and incubation medium were examined for total and trichloracetic acid (TCA) precipitable 14C-radioactivity. In some cases thyroids were homogenized in the incubation medium. The homogenates were centrifuged at 800 x g for 5 min to remove the tissue debris and nuclei. The 800 x g pellet was washed with PBS I 0.15. Both supernatants were recombined and centrifuged in rotor 40 of a Spinco L centrifuge at 105 000 x g for 2 h. The pellet was again once washed with PBS I 0.15 and then resuspended in the same buffer containing digitonin or Triton X-100, final concentration 0.5%/v, or 1%/v respec-
tively. The 105,000 x g supernatant was extensively dialyzed against PBS / 0.15. Ali-
quot; from all homogenates, 105,000 x g pellets and supernatants were examined for 
14C-radioactivity. All these measurements were done in the following manner: each 
sample was put on four Whatman 3MM paper discs and allowed to dry. Two paper 
circles were dipped into a big volume of 10% TCA which was exchanged 5 to 6 
times after an interval of 30 min. TCA was finally removed from the filter paper by 
a mixture of equal volumes of alcohol and ether. All paper discs were put into the 
bottom of counting flasks and covered with 10 ml of Bray’s solution (Bray 1960). They 
were counted for 14C-radioactivity in a Tricarb (Packard) liquid scintillation counter. 
14C-radioactivity of the incubation medium before and after incubation was also 
determined. TCA precipitable 14C-radioactivity per 100 mg thyroid tissue (wet weight) 
was expressed in per cent of total 14C-radioactivity of the incubation mixture before 
incubation. Changes in 14C uptake in the presence of TSH or iodide were then cal-
culated as A in per cent of the 14C uptake in the control lobes, the latter being 
asumed to be equal to 100%.

The following control measurements were done to find out whether TCA pre-
cipitation and dialysis yield reliable results: 1) 14C-amino acid mixture alone or mixed 
with thyroid homogenate just before extraction were put on filter paper and extracted 
with 10% TCA. No significant radioactivity remained on the filter paper. 2) TCA 
extraction was done on the same thyroid 14C-labelled homogenate before and after 
extensive dialysis. No difference of radioactivity was found between the material 
dialyzed only or dialyzed and precipitated with TCA. The difference between TCA 
precipitated non-dialyzed and dialyzed material was less than 5%.

In an alternate set of experiments individual thyroid lobes were incubated in Krebs-
Ringer 0.05 m phosphate buffer pH 7.5 with 0.01 m glucose (KRBG) containing 5 to 
10 μCi 14C-amino acid mixture and test substances as indicated in results in total 
volume 0.4 to 0.6 ml. Incubation was done in a Dubnoff metabolic shaker at 37°C 
under 95% O2 and 5% CO2 (v/v) for 4 h. At the end of the incubation period the 
content of the tubes was rapidly frozen at -70°C. Thyroids were then homogenized in 
the incubation medium or separately. The homogenate or incubation medium were 
extensively dialyzed against PBS. An aliquot of the homogenate or medium was di-
gested with Protosol and its 14C-radioactivity measured in a liquid scintillation counter.
Protein was determined by the Lowry method (Lowry et al. 1951). Radioactivity was 
compared with protein concentration and is referred as cpm per mg protein. Difference 
in 14C-protein bound radioactivity between two thyroid lobes of the same rat incubated 
in the same or different experimental conditions is expressed as A in % of the higher 
value of radioactivity.

Thyroid proteins were examined by sucrose gradient centrifugation. An aliquot of 
dialyzed 105,000 x g supernatant, and digitonin or Triton X-100 extract of the 
105,000 x g pellet was put on 5 to 20% linear sucrose gradient and centrifuged for 
15 to 16 h at 32,000 to 34,000 r.p.m. in the SW 40 rotor of a Spinco L65B centrifuge 
at 4°C. All samples were examined in sucrose gradients prepared in PBS / 0.15. In 
some cases, gradients of ionic strength 0.40 and 0.015 were also used as described 
previously (Valenta & Lissitzky 1971). Fractions of sucrose gradients (0.17-0.2 ml) were 
diluted with H2O and examined for absorbance at 280 nm, in a Zeiss spectropho-
tometer, then mixed with 10 ml of Bray’s solution and their 14C-radioactivity was 
measured in the Tricarb scintillation counter. Radioactivity of the identified peaks was 
expressed in per cent of total 14C-radioactivity of the gradient.

In several cases the fractions constituting 14C-radioactivity peaks were pooled and
the amount of $^{14}$C-thyroglobulin was determined by a double antibody radioimmunoassay as described by Roques et al. (1969).

Non-specific precipitation was done by mixing the sample with anti-human serum albumin serum. Rabbit anti-thyroglobulin serum was then added for 48 h at 4°C. Goat anti-rabbit gamma globulin serum was used as the second antibody. $^{14}$C-radioactivity was measured at all steps of immunoprecipitation.

Statistical significance of the results was examined by Student's $t$-test.

RESULTS

Non-dialyzable, TCA precipitable $^{14}$C-radioactivity uptake was $3.75 \pm 0.60\%$ (mean $\pm$ sd) per 100 mg thyroid tissue (wet weight) of normal iodine diet (NID) rats and $7.82 \pm 1.01\%$ per 100 mg of LID fed rats after 4 h of incubation in 8 and 18 experiments respectively (Table 1). The difference is statistically significant ($P = 0.01$).

When hemithyroids of the same glands were incubated in separate vials, the variance in their $^{14}$C-radioactivity incorporated into protein per 100 mg thyroid tissue was less than 5\%. This illustrates the reproducibility of the assay and minor importance of non-specific factors. TSH in vitro did not produce any significant change compared to the control lobes from which the incorporation differed by $-0.35 \pm 3.15$ and $3.44 \pm 8.82\%$ for NID and LID rats respectively when the $^{14}$C-amino acid incorporation into protein of the control lobes was assumed to be equal to 100\%.

Table 1.

$^{14}$C-amino acid incorporation into rat thyroid peptide, influence of TSH and iodide.

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>#</th>
<th>$^{14}$C-uptake$^b$ (%)</th>
<th>TSH effect</th>
<th>Iodide effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>$\Delta$ uptake</td>
<td>#</td>
<td>$\Delta$ uptake</td>
</tr>
<tr>
<td>NID</td>
<td>8</td>
<td>3.74 ± 0.60</td>
<td>4</td>
<td>-0.35 ± 3.15</td>
</tr>
<tr>
<td>LID</td>
<td>18</td>
<td>7.82 ± 1.01</td>
<td>7</td>
<td>3.44 ± 8.82</td>
</tr>
</tbody>
</table>

NID – normal iodine diet, LID – low iodine diet. # – number of experiments. $^b$ – $^{14}$C-uptake means TCA precipitable $^{14}$C-radioactivity per 100 mg thyroid tissue homogenate (wet weight) in per cent of total radioactivity of the incubation mixture. $\Delta$ – change of $^{14}$C uptake in experimental thyroid lobes when the $^{14}$C uptake in control lobes is assumed to be equal to 100\%. Conditions of assays see Methods. The figures represent mean $\pm$ sd.
Table 2.

*In vitro* ^14^C-amino acid incorporation into thyroid protein of rats on normal iodine diet, effect of graded doses of iodide.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>KI concentration (M)</th>
<th>cpm/mg protein</th>
<th>A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>4576</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4393</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>0</td>
<td>5818</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10^-9</td>
<td>5306</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0</td>
<td>4730</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>10^-8</td>
<td>4223</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>0</td>
<td>5964</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>10^-7</td>
<td>2856</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>0</td>
<td>6382</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>10^-6</td>
<td>3305</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>0</td>
<td>4573</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>2652</td>
<td></td>
</tr>
</tbody>
</table>

Individual thyroid lobes were incubated under O\(_2\) + CO\(_2\) (95:5 \%/v, v/v) at 37°C for 4 h in KRBG containing 5 µCi ^14^C-amino acid mixture and KI as indicated (molar concentration), in total volume 0.6 ml. The tissue was homogenized in the incubation medium and dialyzed against PBS. Protein and ^14^C-radioactivity were compared as cpm/mg protein. Difference (Δ) in cpm/mg protein between two thyroid lobes of the same animal is expressed in % of the higher value.

Iodide concentration 10^-9 and 10^-8 M did not change the ^14^C-amino acid incorporation into thyroid protein. However, in concentration 10^-7 M and higher (10^-6 and 10^-5 tested) iodide caused decrease in ^14^C-amino acid incorporation. Compared to incorporation by control thyroid lobes assumed to be 100 %, the decrease was \(-19.40 \pm 3.06\) and \(-26.59 \pm 4.06\) % for NID and LID rats respectively. The difference between NID and LID groups was not significant. The iodide effect on ^14^C-amino acid incorporation was consistent only after 4 h incubation and occurred inconsistently at 1 and 2 h incubation period.

No clear dose response relationship was observed, and ^14^C-amino acid incorporation fell abruptly at iodide concentration 10^-7 to 10^-6. This is documented for thyroids from NID rats in Table 2. Therefore, Table 1 does not distinguish different iodide concentrations, which varied between 10^-5 and 10^-7 M.
Table 3.

*In vitro* 14C-amino acid incorporation into thyroid protein of LID fed rats, effect of iodide and antithyroid compounds.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Tested compound</th>
<th>cPM/mg protein</th>
<th>A (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>8542</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8689</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>0</td>
<td>13208</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>KI</td>
<td>7890</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0</td>
<td>11300</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>MMI</td>
<td>11670</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>MMI</td>
<td>10466</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>MMI + KI</td>
<td>7535</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>0</td>
<td>9119</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SCN</td>
<td>9260</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>SCN</td>
<td>12294</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>SCN + KI</td>
<td>11911</td>
<td></td>
</tr>
</tbody>
</table>

Individual thyroid lobes were incubated in total volume of 0.4 ml KRBG containing 5 µCi 14C-amino acid mixture and, where indicated, 10⁻⁶ M KI and 10⁻³ M methimazole (MMI) or KSCN. Values of cPM/mg protein and A were obtained in the same way as in Table 2.

When incubation was done in KRBG, and with individual lobes, greater variability was observed than with pool of thyroids incubated in Eagle’s medium. The difference in 14C-amino acid incorporation between two lobes of the same thyroid was occasionally as high as 15 ‰. However, the inhibitory effect of iodide was reproducible and mostly exceeded 20 ‰ suppression of 14C-amino acid incorporation into protein.

In order to establish whether iodine organification is necessary for the suppression effect on 14C-amino acid incorporation, the incubation medium was enriched by methimazole. As shown in Table 3, methimazole itself in concentration 10⁻³ M did not substantially change the 14C-amino acid incorporation into thyroid protein and did not prevent the effect of iodide. On the other hand, the iodide effect was abolished by thiocyanate (10⁻³ M) which is known to discharge non-organified iodide from thyroid cells.

A question arises whether iodide exerts its effect on 14C-amino acid incorporation directly or whether it changes the pool of available free amino acids by interference with their transport across the cell membrane. Several
Table 4.
In vitro $^{14}$C-amino acid uptake and incorporation into thyroid protein of LID fed rats, effect of iodide.

<table>
<thead>
<tr>
<th>Materials</th>
<th>KI concentration (M)</th>
<th>cpm/mg protein</th>
<th>$\Delta$ (%&lt;i&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid homogenate, non-dialyzed</td>
<td>0</td>
<td>54 615</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10$^{-6}$</td>
<td>5 610</td>
<td></td>
</tr>
<tr>
<td>Thyroid homogenate, dialyzed</td>
<td>0</td>
<td>1 3042</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>10$^{-6}$</td>
<td>9648</td>
<td></td>
</tr>
<tr>
<td>Incubation medium, non-dialyzed</td>
<td>0</td>
<td>1 254 040$^*$</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10$^{-6}$</td>
<td>1 194 400$^*$</td>
<td></td>
</tr>
<tr>
<td>Incubation medium, dialyzed</td>
<td>0</td>
<td>4 060$^*$</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>10$^{-6}$</td>
<td>2 316$^*$</td>
<td></td>
</tr>
</tbody>
</table>

$^*$ Total cpm of the incubation medium.

Individual thyroid lobes were incubated under conditions described in Table 2, in the presence of KI as indicated. Thyroids then were washed three times with PBS and homogenized in 2 ml PBS. Protein and $^{14}$C-radioactivity of the homogenate and the medium were measured before and after dialysis. Protein of the medium was non-measurable. Expressions cpm/mg protein and $\Delta$ have the same meaning as in Table 2.

approaches were used to resolve this question: 1) $^{14}$C-amino acid uptake was measured before dialysis of the homogenate. The results illustrated in Table 4 indicate that dialysable $^{14}$C-amino acids corresponding most probably to free amino acids, accumulated in the thyroid tissue, exceeded several times $^{14}$C-amino acid incorporation into protein, and their uptake was about the same in the presence or absence of iodide. Therefore, iodide could not act through limiting the amino acid uptake. This experiment also illustrates that decreased $^{14}$C-labelled thyroid protein induced by iodide does not result from increased loss of the protein into the incubation medium, since the amount of the protein-bound $^{14}$C of the medium was also lower in the presence of iodide. 2) The incubation medium was enriched by non-labelled amino acids (a mixture of 18 amino acids) in concentration 0.5, 0.25, or 0.05 mM each. As indicated in Table 5, the iodide effect was demonstrable in the presence of non-labelled amino acids as high as 0.05 mM. Substantial reduction of $^{14}$C-amino acid incorporation into protein occurred with 0.25 and 0.5 mM non-labelled amino acids, which made the iodide effect inapparent. 3) The homogenate was precipitated by 10% TCA, precipitate removed by centrifugation and supernatant recovered for measurement of amino acids and TCA soluble peptides using
In vitro $^{14}$C-amino acid incorporation into thyroid protein of LID fed rats, effect of KI and non-labelled amino acids.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Non-labelled amino acids (mM)</th>
<th>KI (m)</th>
<th>cpm/mg protein</th>
<th>$\Delta$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0</td>
<td>4573</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>4800</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>0.25</td>
<td>0</td>
<td>2024</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>$10^{-6}$</td>
<td>2277</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0.05</td>
<td>0</td>
<td>4675</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>$10^{-6}$</td>
<td>2960</td>
<td></td>
</tr>
</tbody>
</table>

Conditions of incubation the same as in Table 2. Non-labelled amino acids and KI were added in concentration (molar) as indicated. Values of cpm/mg protein and $\Delta$ were obtained in the same way as in Table 2.

ninhydrin reagent (Hirs 1967). No difference was detectable between thyroids incubated in the presence and absence of iodide and between thyroids of rats on NID of LID.

Fig. 1.
Sucrose gradient centrifugation of thyroid soluble proteins (two upper panels) and particulate proteins solubilized by digitonin (lower panel). Material obtained from rats fed LID, thyroid lobes were incubated for 4 h in vitro under conditions described in Methods, except for addition of 1 $\mu$Ci $^{131}$I to the incubation mixture. Sedimentation is from right to left. Fractions 0.18–0.20 ml were diluted with 0.20 ml H$_2$O and their absorbance at 220 nm measured in a microcuvette of the Zeiss spectrophotometer. Radioactivity $^{131}$I was measured subsequently in a Pacard Spectrogammameter. After the decay of $^{131}$I-radioactivity, $^{14}$C-radioactivity was measured in a liquid scintillation counter as usual. The peaks are labelled by sedimentation coefficients obtained in a separate run by comparison of the $^{131}$I – 18.5S peak with 19S rat $^{125}$I thyroglobulin used as an internal sedimentation marker. PBS-phosphate buffered saline, PB-phosphate buffer. Open circles represent absorbance at 220 nm, closed circles and lower figures on the right axis are $^{14}$C-radioactivity, small open circles and upper figures on the right vertical axis represent $^{131}$I-radioactivity. Note symmetrical peaks in PBS I 0.40 with only small shift of $^{14}$C-labelled newly synthesized thyroglobulin to about 18S and small amount of 12S, and heterogeneity of thyroglobulin at low ionic strength (middle panel). The heterogeneity is relatively smaller for the oldest thyroglobulin molecules represented by protein patterns than for $^{131}$I- (newly iodinated) and $^{14}$C- (newly synthesized) species which shift to 16.5 and 15.5S respectively and dissociate into approximately 11S. Note also relatively high concentration of 27 and 18S species in the particulate fraction (lower panel).
On sucrose gradient centrifugation, the $^{14}$C label was found in the region of about 18S and 3–8S, when centrifugation was done in the buffer of ionic strength 0.15 and higher (Fig. 1). In these conditions sedimentation coefficient of 18S, $^{14}$C-labelled and ergo newly synthesized thyroglobulin, was only slightly lower than that of newly iodinated thyroglobulin or thyroglobulin previously synthesized and stored in the tissue. The sedimentation coefficient of the latter was about 18.5S in LiD fed rats, when compared with thyroglobulin from rats on iodine sufficient diet, which was considered to be 19S. A number of investigators reported that the sedimentation coefficient of $^{14}$C-newly synthesized thyroglobulin may be as low as 13 to 16S (Nunez et al. 1965; Inoue & Taurog 1968; Vecchio et al. 1972). This was not observed in our study, unless the sucrose gradient analysis was done at low ionic strength such as 0.01 M phosphate buffer, as illustrated in Fig. 1. At low ionic strength, heterogeneity of thyroglobulin was observed with marked quantitative differences between $^{14}$C-newly synthesized, $^{131}$I-newly iodinated, and stored thyroglobulin (represented by the protein pattern). There was decrease of the sedimentation coefficient to 14–16S and dissociation into 11–12S species. This was most pronounced with $^{14}$C-labelled protein and least with the protein represented by protein pattern.

Proteins solubilized from the 105 000 $\times$ g pellet displayed pattern quantitatively different from the soluble phase. There was less 3–8S protein and often the $^{14}$C, but namely $^{131}$I label occurred in the region of 27S in markedly in-

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Experimental conditions} & \# \text{ assays} & 27+18+12S \ ^{14}\text{C-radioactivity, \% total} & \\
& & \text{Soluble proteins} & \text{Particulate proteins} \\
\hline
\text{TSH} & 7 & 45.1 \pm 2.7 & 62.2 \pm 2.9 \\
\text{Controls to TSH} & 7 & 40.6 \pm 2.9 & 53.5 \pm 4.8 \\
\text{Iodide} & 8 & 31.1 \pm 6.5 & 51.3 \pm 5.0 \\
\text{Controls to iodide} & 8 & 38.8 \pm 4.5 & 50.3 \pm 4.1 \\
\hline
\end{array}
\]

Conditions of assay see Methods. The sum of the $^{14}$C-radioactivity sedimenting as 27, 18 and 12S peak on sucrose gradient centrifugation is expressed in per cent of total radioactivity of the sucrose gradient for both soluble and solubilized particulate proteins. The figures are mean ± sd. The observed differences are not statistically significant.

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increased proportion as compared with the soluble phase (Fig. 1). The amount of $^{14}$C-labelled thyroglobulin ($27 + 18 + 12S$) was relatively higher than in the soluble phase, accounting for more than 50% of total protein as opposed to about 40% in the 105,000 x g supernatant.

The data on immunoreactive thyroglobulin are available only for the soluble phase in 6 cases. The 18 and 12S protein reacted quantitatively with the antithyroglobulin serum. Only 20 to 30% of the 3–8S $^{14}$C-labelled protein reacted with the antithyroglobulin serum.

The 105,000 x g supernatants and solubilized particulate proteins from homogenates obtained from both control and experimental thyroid lobes were analyzed by sucrose gradient centrifugation in 7 TSH and 8 iodide experiments. The sum of $27S + 18S + 12S$ $^{14}$C-radioactivity peaks expressed in percent of total radioactivity of the gradient is given in Table 6. The differences in thyroglobulin concentration between all studied groups were statistically not significant although there was some increase in TSH and decrease in iodide experiments.

**DISCUSSION**

*Heywood* (1966) has demonstrated that iodide and thyroxine suppress $^{14}$C-amino acid incorporation into protein specifically in the thyroid and are more efficient in iodine deficient than normal rats. Thyroid slices and microsomes were shown to respond in a similar fashion.

Although our data differ in several details, they confirm the results of this investigator and extend them namely in describing the patterns of soluble and particulate thyroid proteins.

In our system, the iodide effect up to 2 h incubation was inconsistent whereas 90 min incubation used by Heywood gave reproducible results. This is probably due to methodologic differences. Tissue slices were used by Heywood, while we incubated whole thyroid lobes and there was probably a difference in the rapidity of amino acid uptake. This seems to be confirmed by the fact that when the factor of tissue penetration is excluded by incubation of microsomes (*Heywood* 1966), the iodide effect may be demonstrated during the first few minutes.

We obtained the same response to iodide in thyroids of rats on NID as on LID using the same or only slightly higher iodide concentration. In the experiments of Heywood iodide effect was not observed when thyroid microsomes from NID rats were studied. It was demonstrable in thyroid slices, but using iodide concentration much higher ($10^{-4}$ M) than that effective in LID rat thyroids ($10^{-6}$ M). We do not have any explanation for these variations but again, we suspect methodologic differences.

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The fact that iodide inhibits $^{14}$C-amino acid incorporation in an acellular system (Heywood 1966) makes unlikely the possibility of primary effect in diminishing the intracellular pool of free amino acids. As estimated by ninhydrin reaction, there was no difference in free amino acids or peptides between glands treated with iodide and control lobes and between thyroids from LID or NID fed animals. Free $^{14}$C-amino acid uptake was not affected by iodide and iodide effect was not abolished by increasing the amino acid mixture of the incubation medium to concentration as high as 0.05 mM. All these data speak against changes of free amino acid pool as the cause of the iodide effect.

Experiments using MMI and SCN$^-$ point to iodide as the species responsible for suppression of amino acid incorporation into thyroid protein. Whereas this effect was abolished by SCN$^-$, known to discharge the inorganic form of iodine from the thyroid tissue, it was not apparently changed by MMI, a drug interfering with iodine binding to thyroid protein.

The inhibitory effect of iodide on thyroid protein synthesis may be one of the important factors involved in the antigoitrogenic action of iodide. Mechanism of this action is obscure. According to our preliminary results (Valenta & Greer, unpublished) it occurs even in hypophysectomized rats and in the presence of TSH in vitro. Therefore, it is unlikely that iodide interferes directly with TSH.

As a number of others (Raghupathy et al. 1964; Nataf et al. 1967; Seed & Goldberg 1965) we were unable to obtain any significant stimulation of $^{14}$C-amino acid incorporation into rat thyroid protein by TSH added in vitro contrary to the effect in vivo (Raghupathy et al. 1963; Bradley & Wissig 1966) or in isolated thyroid cells cultured in vitro (Tong 1967). Several speculations could be made about these discrepancies: 1) TSH stimulation of protein synthesis needs more time to become manifest in vitro than is adequate survival of thyroid tissue in vitro. 2) Since one of the earliest effects of TSH is stimulation of thyroglobulin hydrolysis, "cold" amino acids from pre-existing thyroglobulin enter the intrathyroidal pool and incubation medium and dilute the radioactive amino acids, while in vivo they are removed by circulation. 3) An inhibitor accumulates in the incubation medium, which in vivo is removed by circulation. Such an inhibitor could be e.g. exogenous iodide present in the incubation medium or iodide accumulating from deiodinated iodo-amino acids. There is an experimental evidence, that thyroid tissue of iodine-depleted rats is more sensitive to the effect of TSH than when iodine intake is normal (Bray 1968; Chapman 1941; Halmi 1954; Halmi & Spirtos 1955). 4) The first change may be of qualitative rather than quantitative value such as increased synthesis of thyroglobulin at the expense of sedentary proteins. At least some increase in concentration of thyroglobulin following TSH was observed in our study (Table 2), although it did not occur consistently enough to be statistically significant. Also, in hypophysectomized rats we observed that there was a
decrease of thyroid protein with antigenic properties of thyroglobulin to 10–15% of the 3–8S component compared to usual 20–30% (Valenta & Greer, unpublished). This would agree with the findings of Pavlovic-Hournac et al. (1971) who reported selective decrease of thyroglobulin in hypophysectomized rats.

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