INTRATHYROIDAL IODINE METABOLISM IN HASHIMOTO'S DISEASE

By

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ABSTRACT

The thyroid of Hashimoto's disease is unique in its susceptibility to exogenous iodide and the positive perchlorate test. It would be of interest to know if there is any alteration of iodine metabolism in the thyroid. The results obtained in 9 cases with untreated Hashimoto's disease were compared with those in 9 normal thyroids; 5 cases were investigated for iodine metabolism and 4 cases for the peroxidase study.

The thyroglobulin content in thyroid wet weight showed lower values 1.6 ± 1.2% compared to 8.0 ± 1.5% in normal. Thyroglobulin represented 18.9 ± 9.3% of total soluble protein and was significantly lower than normal, 66.9 ± 5.4%. Iodine content of thyroglobulin was extremely low, 0.10 ± 0.05% compared to 0.49 ± 0.03% in normal. The thyroglobulin with low degree of iodination showed a low sedimentation coefficient of 18.1 ± 0.1. Distribution of iodo-amino acid-iodine was 8.1 ± 2.8% for thyroxine (T₄) and 14.4 ± 9.7% for triiodothyronine (T₃), resulting in a much higher T₃/T₄ ratio than in normal thyroids. Monoiodotyrosine/diiodotyrosine (MIT/DIT) ratio was higher and T₄/DIT ratio was apparently lower than normal. These results resemble the pattern of that found in iodine deficiency. T₃/T₄ ratio was increased in the blood of patients with Hashimoto's disease. Consequently the alteration of distribution of iodo-amino acids in the thyroid may contribute to the increase in T₃/T₄ ratio in the blood of this disease.

Peroxidase activity showed 103 ± 51 U/mg particulate protein and was significantly lower than values in normal thyroids, 299 ± 85 U/mg protein.
On the other hand, the total amount of thyroglobulin was not significantly decreased in this disease. The total amount of organified iodine, however, was apparently lower than normal. These results suggest that the low degree of iodination of thyroglobulin is an essential alteration in the thyroid of Hashimoto's disease.

With the progress of immunology, diagnosis of Hashimoto's disease, a typical autoimmune disease, has been greatly facilitated. At the same time several questions regarding the physiopathology of this disease are still unsolved: cause of positive test for perchlorate discharge, probably by some iodide organization defect in the thyroid (Morgans & Trotter 1957; Nilsson & Berne 1964) and the possibility of subsequent hypothyroidism (Blizzard et al. 1960; Braverman et al. 1971), sometimes by exogenous iodide, and origin for the increase in T₃/T₄ ratio in the peripheral blood (Gharib et al. 1972).

Morgans & Trotter (1957) suggested a defective organic binding of iodine by the thyroid in Hashimoto’s thyroiditis and later on Nilsson & Berne (1964) also described juvenile auto-immune thyroiditis showed positive perchlorate test. In addition, there is another suggestion in the literature concerning the disturbance of thyroid hormone synthesis, provoked by exogenous iodide; Braverman et al. (1971) described enhanced susceptibility to iodide myxoedema in patients with Hashimoto’s disease.

Gharib et al. (1972) reported an increase in T₃/T₄ ratio in the peripheral blood of Hashimoto’s disease patients maybe influenced by TSH stimulation. They also pointed out that the increase in T₃/T₄ ratio could be assumed because of a decreased intrathyroidal iodide pool.

Therefore, it is important to know which phase of iodide metabolism is affected in the thyroid with Hashimoto’s disease. In order to get some ideas about iodide organification defect, possible cause of the defect and origin of the peripheral increase in T₃/T₄ ratio, the present experiment was designed. Hence intrathyroidal metabolism of stable iodine in Hashimoto's disease was compared with that in normal thyroid.

**MATERIALS AND METHODS**

The materials analysed consisted of 9 normal thyroid tissues obtained at autopsy from subjects with non-endocrine disease or surgery of thyroid adenoma and 9 thyroid tissues from untreated patients with Hashimoto’s disease obtained by open biopsy; 5 cases were tested for the study of iodine metabolism and 4 cases for peroxidase assay. Iodine content of thyroglobulin was also measured in the cases of the peroxidase study to demonstrate if there is a disturbance of iodide organification or not.

Biopsy test was performed with the consent of patients concerning the purpose and the procedure, which were informed to the patients verbally. The amount of biopsied
tissue was limited to less than 1.0% of estimated thyroid weight; the patients did not show any complications after the test.

The rat thyroid glands used as a marker for iodinated components was obtained 24 h after the injection of 500 μCi of carrier-free 131I given intraperitoneally to male Sprague-Dawley rats fed on Oriental MF pellets, which are iodine sufficient.

Thyroid tissue was homogenized with a glass homogenizer in 4 ml of cold 0.1 M phosphate buffered saline (pH 6.8). The homogenate was centrifuged at 2000 r. p. m. for 10 min and the supernatant obtained was further centrifuged at 105,000 x g for 60 min. The supernatant was used as a marker for various iodinated components.

1. Analysis of thyroid soluble protein

After weighing 0.2-0.3 g of human thyroid tissue, the tissue was chopped finely and treated in the same way as the rat thyroid. Protein concentration in 105,000 x g supernatant was determined by the method of Lowry et al. (1951). Approximate iodine content of homogenate was measured by the method of Pileggi & Kessler (1968). The actual procedure of iodine analysis is described in the following section of this paper. These measurements were necessary as a preliminary test.

The concentration of protein in supernatant was adjusted with phosphate buffered saline to 1.2-1.5 mg in 0.8 ml, a marker was added, and this was applied on the top of 4.6 ml of a 5-20% linear sucrose density gradient in phosphate buffered saline. Three tubes were simultaneously centrifuged at 25,000 r. p. m. for 13 h using a rotor (Hitachi, model 40 rps) and the bottom of each tubes was punctured to collect 10 drops for each fraction consecutively.

131I-activity was counted in a well-type scintillation counter and protein for each fraction was determined by the method of Lowry et al. (1951). From the remaining two other tubes, three fractions corresponding to thyroglobulin peak, based on the results of preliminary protein measurement, were pooled and placed in a Visking tube and then dialyzed against 2000 ml of cold buffered saline for 12 h, twice. After dialysis, protein and iodine in the sample were measured and the degree of iodination of thyroglobulin was calculated.

Thyroglobulin content in the thyroid wet weight and per cent distribution of thyroglobulin in thyroid soluble protein were calculated based on these results.

Sedimentation coefficient of human thyroglobulin was measured by the method of Inoue & Taurog (1968) assuming that of marker rat thyroglobulin is 19 S.

Total amount of thyroglobulin and organified iodine in the thyroid were calculated from the results mentioned above and the estimated thyroid weight by means of a scintigram.

2. Iodine analysis of iodoamino acids and thyroglobulin

131I-labelled rat thyroid soluble protein of about 1.5 x 10^5 CPM was added to homogenate of human thyroid containing approximately 1.5 μg of iodine. The mixture was adjusted to contain 0.05 M methimazole and 0.3 M Tris buffered saline (pH 8.6). Anaerobic hydrolysis was performed at 37°C for 16 h after adding 1/10 vol. of a pronase solution (25 mg/0.5 ml Tris) by the method of Inoue & Taurog (1967). 60-80 μl of hydrolysate was delivered on Whatmann 3MM filter paper and chromatographed with n-butanol:ethanol:0.5 N ammonia (5:1:2) solvent system for 14 h. Dried filter paper was exposed to X-ray film for 24 h and a radioautogram was prepared. The separations of iodo-amino acids were always good. 131I-activity was counted. The paper fraction was further cut up and placed in an extraction tube with a glass stopper of 30 ml
capacity. Twelve ml of 50% acetic acid, 4 ml of 2 N hydrochloric acid and 4 ml of 0.01 M sodium hypochlorite solution were added to this tube and it was incubated at 37°C for 30 min. After centrifugation at 2000 r. p. m. for 10 min, 5 ml of supernatant was added with 0.5 ml of 0.1 M arsenious acid and 1.0 ml of 0.02 M ceric ammonium reagent. This mixture was incubated at 25°C for 20 min. At the end of the incubation period, the reaction was stopped by adding 2 drops of 0.2 N HgNO₃. Transmittance per cent at 420 nm was read in a spectrophotometer and results were obtained based on a reading from standard curves of iodide, iodotyrosine and iodothyronine. Thyroglobulin was purified as mentioned above. Three ml of 50% acetic acid, 1 ml of 2 N hydrochloric acid and 1 ml of 0.01 M sodium hypochlorite were added to 1 ml of thyroglobulin solution with a fixed amount of protein. This mixture was dealt with in the same way as iodo-amino acids. Iodide was used as a standard. Determination of iodine content of thyroglobulin by this method was lowered by thyroglobulin protein itself into the small extent, 5–15% in present range of assay. Hence, this technique was initially calibrated by the ashing method of Barker (1964).

3. Peroxidase assay

Thyroid peroxidase was prepared according to the method described by Niepmińskie et al. (1969). For the peroxidase study, all specimens were obtained by surgery. Twenty % thyroid homogenates were prepared in chilled 0.15 M KCl with a glass homogenizer. The homogenate was centrifuged at 600 x g for 10 min and the supernatant was further centrifuged at 15 000 x g for 30 min. The pellet was washed twice with KCl solution. The pellet was re-suspended in KCl solution and an aliquot was subjected to protein measurement by the method of Lowry et al. (1951). One ml of 1% digitonin solution in 0.05 M phosphate buffer (pH 7.0) was added to the pellet including 7.5 mg of protein. After 30 min of continuous shaking incubation at 0°C, the mixture was centrifuged at 40 000 x g for 20 min and the supernatant was collected for peroxidase assay. Thyroid peroxidase activity was measured by a modification of the triiodide method of Alexander (1962) described by Niepmińskie et al. (1969).

The entire process of the present experiment was performed without producing any freezing effect on the sample.

RESULTS

All cases of Hashimoto's disease had positive tests for thyroglobulin antibody and thyroid microsome antibody in sera. However, final diagnosis was made by histopathological findings.

The estimated thyroid weight in Hashimoto's disease was 79.5 ± 20.5 g and was significantly heavier than that of the normal thyroid gland 20.3 ± 3.5 g.

As shown in Fig. 1, the sedimentation pattern of thyroid soluble protein from Hashimoto's disease was quite abnormal. The fraction of thyroglobulin in thyroid soluble protein was decreased to 18.9 ± 9.3% in Hashimoto's disease with low sedimentation coefficient of 18.1 ± 0.1 compared to the results from normal, 66.9 ± 5.4% and 18.5 ± 0.2, respectively, as shown in Table 1. Thus the thyroglobulin of Hashimoto's disease was abnormal in terms of a low sedimentation coefficient.
Fig. 1.
The upper figure is a sedimentation pattern of soluble protein of normal thyroid and lower one is that of Hashimoto's disease. Broken line connecting open triangles indicates per cent distribution of total protein. Solid line connecting solid circles indicate per cent distribution of $^{131}$I-labelled rat thyroid soluble protein, as a marker. Distribution of thyroid protein in normal thyroid shows from the left side of upper figure a small peak at 27 S, the highest peak at 19 S and then a broad peak at 4 S. The first two peaks, iodinated by $^{131}$I in rat samples are known to be thyroglobulin. Distribution of thyroglobulin in thyroid soluble protein was significantly decreased in Hashimoto's disease with a low sedimentation coefficient.
Table 1.
The study of normal and Hashimoto’s thyroids.

<table>
<thead>
<tr>
<th></th>
<th>Tg content (%)</th>
<th>I content of Tg (%)</th>
<th>Tg (S)</th>
<th>Distribution of iodoamino acid (％)***</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>***</td>
<td>***</td>
<td></td>
<td>DIT***</td>
</tr>
<tr>
<td>Normal (5)*</td>
<td>8.0 ± 1.5</td>
<td>0.49 ± 0.03</td>
<td>18.5 ± 0.2</td>
<td>42.1 ± 1.8</td>
</tr>
<tr>
<td>Hashimoto (5)*</td>
<td>1.6 ± 1.2</td>
<td>0.10 ± 0.05</td>
<td>18.1 ± 0.1</td>
<td>39.6 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &gt; 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total Tg (g)</th>
<th>Total organified iodine (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (5)*</td>
<td>1.59 ± 0.31</td>
<td>7.7 ± 1.7</td>
</tr>
<tr>
<td>Hashimoto (5)*</td>
<td>1.26 ± 0.95</td>
<td>1.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.1</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

* Number of cases.
** Due to limited amount of biopsied specimen, only 3 cases of Hashimoto’s disease were examined.
*** Abbreviations; thyroglobulin (Tg), diiodotyrosine (DIT), monoiodotyrosine (MIT), thyroxine (T4) and triiodothyronine (T3).
The thyroglobulin content in thyroid wet weight in Hashimoto’s disease showed a low value of $1.6 \pm 1.2\%$ compared to the normal, $8.0 \pm 1.5\%$. Furthermore iodine content of thyroglobulin, namely the degree of iodination of thyroglobulin in Hashimoto’s disease showed extremely low values of $0.10 \pm 0.05\%$ compared to $0.49 \pm 0.03\%$ in normal. Thyroid soluble protein in Hashimoto’s disease consisted mainly of $4\text{S}$ protein, which did not include organified iodine.

As shown in Table 1, the distribution of iodo-amino acid-iodine in the thyroid of Hashimoto’s disease showed $8.1 \pm 2.8\%$ for $T_4$ and $14.4 \pm 9.7\%$ for $T_3$, resulting in much higher ratio for $T_3/T_4$. This was compatible with the increase in $T_3/T_4$ ratio in the peripheral blood shown in Table 2. MIT/DIT was also above normal and the $T_4$/DIT ratio was apparently below normal, $0.22 \pm 0.14$ vs. $0.49 \pm 0.05$. These results are very similar in pattern to that of iodine deficient rats (Inoue & Taurog 1968).

As shown in Table 1, the total amount of thyroglobulin in Hashimoto’s disease was not significantly decreased. However, the total amount of organified iodine was much lower than normal, in all cases. These results suggest that low degree of iodination of thyroglobulin is an essential alteration in Hashimoto’s disease. In addition, the high TSH level observed in serum in Hashimoto’s disease seems to support this assumption. Thyroidal $^{131}$I-uptake was high in Hashimoto’s disease as shown in Table 2.

As shown in Table 3, particulate protein was not significantly decreased in Hashimoto’s disease. On the other hand, peroxidase activity in Hashimoto’s disease was also above normal and the $T_4$/DIT ratio was apparently below normal, $0.22 \pm 0.14$ vs. $0.49 \pm 0.05$. These results are very similar in pattern to that of iodine deficient rats (Inoue & Taurog 1968).

### Table 2.

Hormones in blood and 24 h $^{131}$I-uptake.

<table>
<thead>
<tr>
<th></th>
<th>$T_4$ (μg/100 ml)</th>
<th>$T_3$ (ng/100 ml)</th>
<th>TSH (μU/ml)</th>
<th>$24\text{ h}^{131}$I-uptake (%)</th>
<th>$T_3/T_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.3 ± 1.6</td>
<td>127.6 ± 30.9</td>
<td>10.1 ± 7.9</td>
<td>$48.3 \pm 13.3$</td>
<td>0.0110 ± 0.0015</td>
</tr>
<tr>
<td>Hashimoto</td>
<td>8.4 ± 2.0</td>
<td>95.6 ± 28.5</td>
<td>3.8 ± 0.6</td>
<td>$(25 \pm 15)^*$</td>
<td>0.0256 ± 0.0087</td>
</tr>
<tr>
<td></td>
<td>$P &gt; 0.05$</td>
<td>$P &gt; 0.1$</td>
<td>$P &gt; 0.1$</td>
<td></td>
<td>$P &lt; 0.05$</td>
</tr>
</tbody>
</table>

* $^{131}$I-uptake value is not available for present normal cases.
  
Values in brackets indicate normal value of $^{131}$I-uptake in our laboratory.
  
$T_3/T_4$ ratio in blood is significantly high in Hashimoto’s disease compared to normal.
### Table 3.
Degree of iodination of thyroglobulin and peroxidase activity in normal and Hashimoto's thyroids.

<table>
<thead>
<tr>
<th></th>
<th>Degree of iodination of Tg (%)</th>
<th>U/mg of particulate protein</th>
<th>U/g tissue</th>
<th>Particulate protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (4)</td>
<td>0.40 ± 0.06</td>
<td>299 ± 85</td>
<td>3155 ± 1150</td>
<td>1.07 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>0.49 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (5)</td>
<td>0.14 ± 0.07</td>
<td>103 ± 51</td>
<td>1488 ± 505</td>
<td>1.52 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hashimoto (4)</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
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<tr>
<td>Hashimoto (5)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*P < 0.01  P < 0.05  P > 0.1*

disease was 103 ± 51 U/mg particulate protein and was significantly lower than normal, 299 ± 85 U/mg protein. The low activity of thyroid peroxidase might be a possible contributing factor in the development a disturbance of iodide organification in Hashimoto's disease.

**DISCUSSION**

In our present study, we found that the per cent distribution of thyroglobulin in thyroid soluble protein was significantly decreased in Hashimoto's disease. The content of thyroglobulin in thyroid wet weight was also low. This finding seems to correlate with the decrease in colloid of thyroid follicles usually observed in histological specimens from Hashimoto's disease. The present cases also showed a decrease in PAS staining substance in thyroid follicles.

Abnormal thyroglobulin in terms of low sedimentation coefficient was observed in Hashimoto's disease. Furthermore, the degree of iodination of thyroglobulin was very low. These findings were very similar to that for iodine deficient animals (Nunez et al. 1965; Simon et al. 1966; Inoue & Taurog 1968). A decrease in iodine content of thyroglobulin in Hashimoto's disease was originally reported by Rolland et al. (1972). It could be assumed that lowered sedimentation coefficient of thyroglobulin in Hashimoto's disease is a consequence of the decrease in the degree of iodination of thyroglobulin which might cause a change in tertiary structure of thyroglobulin.

In the case of Hashimoto's disease, the pattern of intrathyroidal iodine metabolism is quite similar to that of iodine deficient animals. It should be emphasized that there was an obvious decrease in total organified iodine in
all the cases, which provide further evidence to support the view that the
decrease in the degree of iodination of thyroglobulin is an essential alteration
in Hashimoto’s disease of the thyroid. MIT/DIT ratio was increased in the
present cases. The same results were reported by Rolland et al. (1972) and
Volpé et al. (1965). The present data agreed well with previous reports in this
respect. However, \( T_3/T_4 \) ratio of their data was quite different from ours;
Rolland et al. (1972) found that \( T_4 \) values were normal or above normal but
that there were trace amounts, if any, \( T_3 \). Volpé et al. (1965) found extremely
low values for \( T_3 \) and \( T_4 \). Neither of the investigators found an increase in
\( T_3/T_4 \) ratio. On the other hand, we found an increase in \( T_3/T_4 \) ratio in the
thyroid of this disease. Such a difference between their data and our own might
arise from a difference in the severity of disease because the cases of Volpé et al.
(1965), for instance, seemed to have more severe hypothyroidism than our own,
which still maintained serum \( T_4 \) concentration in subnormal level. Although
we can not generalize about these findings as an essential finding in Hashimoto’s
disease because of the limited number of cases, some of cases with this disease
having a high \( T_3/T_4 \) ratio in the thyroid at least at some stage of this disease.
Thus, alteration of distribution of iodo-amino acids in the thyroid of this disease
may greatly contribute to the increase in \( T_3/T_4 \) ratio in the peripheral blood,
as was reported by Gharib et al. (1972).

As mentioned above, disturbance of iodide organification was strongly in-
dicated in the thyroid gland of this disease. On the other hand, peroxidase
activity of Hashimoto’s disease was one third of the normal. One might think
iodination of thyroglobulin would be the most physiological function for peroxi-
dase enzyme. On the other hand, the present data were determined only by
\( I_3 \) assay. The authors recently found the activity of \( I_3 \) assay of solubilized
enzyme highly correlated with thyroglobulin-iodination activity (Nakashima &
Taurog 1978). Therefore, a decrease in peroxidase activity by \( I_3 \) assay might
show decrease in iodinating activity in Hashimoto’s disease. Subsequently the
decrease in peroxidase activity might be a possible contributing factor in
developing the disturbance of iodide organification. However, the presence of
\( \text{H}_2\text{O}_2 \) is also essential for iodination of thyroglobulin by peroxidase. We can
not ascribe the cause of the disturbance of iodide organification entirely to a
decrease in peroxidase activity since we have not determined if there is any
deficiency in the \( \text{H}_2\text{O}_2 \) generation.

As mentioned above, intrathyroidal iodine metabolism of Hashimoto’s disease
showed an iodine deficient pattern. Iodine deficiency from dietary source in
patients with Hashimoto’s disease living in Japan is very unlikely because the
people in Japan are generally eating a lot of sea weed. There might be a possi-
bility of iodine deficiency in the thyroid of Hashimoto’s disease due to a
decrease in active transport of iodide into the thyroid. However, this possibility
also seems unlikely because \( ^{131}\text{I} \)-uptake was apparently high in the present
case, although this test was performed by the administration of carrier-free $^{131}$I. Thus, Boyle et al. (1965) actually observed high iodide uptake in patients with Hashimoto’s disease using $^{131}$I with 2 mg of carrier iodide. Another possibility is that iodide deficiency based on a decrease of iodide from an intra¬thyroidal source due to a lack of dehalogenase. However, data on this point are not available at present. If there is an iodine deficiency in the thyroid of Hashimoto’s disease, is still uncertain. Further studies into this problem are necessary.

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