ABNORMAL THYROIDAL METABOLISM IN TWO PATIENTS WITH NON-TOXIC NODULAR GOITERS

By

Vas V. Row, MacAllister W. Johnston, Calvin Ezrin and Robert Volpé

ABSTRACT

In a study of iodine metabolism in patients with non-toxic nodular goiter (using $^{127}$I chromatographic techniques) two patients were shown to have unusual findings. Both patients were euthyroid by clinical examination and laboratory tests. Tetraiodothyroacetic acid (tetrac) was the major iodocompound produced by the nodular tissue of one patient. The monoiodotyrosine:diiodotyrosine ratio (0.625) in this gland was normal, indicating that there was no defect in the synthesis of the hormone precursors. The second patient showed the formation of an abnormal triiodothyronine complex in the nodule, partially identified by ultraviolet absorption, infrared spectrum and reaction with l-aminoacid oxidase of the eluted compound. There was no detectable thyroxine in the nodule. The monoiodotyrosine:diiodotyrosine ratio (6.3) was greatly elevated denoting defective halogenation of monoxidotyrosine.

In case one, it may be suggested that possible alterations in the enzyme systems viz., the presence of l-aminoacid oxidase or transaminase and decarboxylase systems, in the nodular tissue might have resulted in the formation of the acetic acid analog of thyroxine. The cause of the defect in case two is unclear.

In recent years, reports have appeared from different groups of workers on the production of abnormal iodinated compounds by the thyroid gland in various thyroid disorders. Rupp et al. (1959) have reported the presence of large amounts of circulating triiodothyronine in a patient with non-toxic

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goiter. Werner et al. (1960) have demonstrated triiodothyronine in abnormal concentrations in the plasma and as the major component of a thyroid nodule in a euthyroid person with a non-toxic nodular goiter. Shimaoka (1963) has reported a case of toxic adenoma of the thyroid, in which triiodothyronine was the principal circulating thyroid hormone. Kahn et al. (1962) and Greenspan et al. (1963) have observed abnormal circulating iodoproteins in patients with non-toxic nodular goiters. DeGroot et al. (1958) have also shown an abnormal circulating iodoprotein in certain adenomatous colloid goiters. Parker & Beierwaltes (1962) have noted an elevation of serum protein-bound iodine in patients taking iodine deficient diets and have related this to the presence of an abnormal iodoprotein in the circulation. The above observations indicate that abnormal pathways of iodine metabolism may occur in various diseases of the thyroid gland.

The purpose of the present report is to record two more examples of intra-thyroidal metabolic abnormalities in patients with non-toxic nodular goiter. The nodule of one of the patients produced a compound, chromatographically identified as tetraiodothyroacetic acid (tetrac), the acetic acid analog of thyroxine (Row et al. 1963). In the thyroid gland of the second patient, an abnormal iodothyronine, not completely identified, was demonstrated.

**CASE REPORTS**

Mrs. R. R., age 25 years. The clinical status of this patient and the histological appearance of the excised nodule has been reported in an earlier communication (Row et al. 1963). In summary, however, this woman had a longstanding multinodular goiter, which was surgically excised. She was clinically euthyroid, with normal PBI and 131I uptake. Histological appearance was that of a non-toxic nodular goiter with some degeneration.

Mrs. D. G. This 47 year old woman had been aware of a rightsided goiter for about seven years. In the last several months before her June 1962 hospital admission, she believed that the goiter was enlarging somewhat. There was no local pain, choking or dysphagia. She felt quite well otherwise and had no symptoms suggestive of either hyperthyroidism or hypothyroidism. There was no known family history of goiter.

On examination, a firm, smooth nodule was palpated in the right lobe of the thyroid gland, approximately 4.5 cm in diameter. It moved with swallowing and no bruit could be heard. The isthmus and left lobe of the gland were normal. The trachea was shifted slightly to the left. There was no cervical lymphadenopathy. The remainder of the physical examination was quite unremarkable.

The serum P. B. I. was 4.9 μg/100 ml and the 24 hour thyroidal uptake of 131I was 31%. A scan of the thyroid revealed the right sided nodule to be «cold». The serum anti-thyroglobulin titre (TRCA) was positive 1:1 594 323, while the anti-thyroid microsome test (CF) was non-reactive.

Despite the high anti-thyroglobulin titre, itself consistent with Hashimoto's struma, surgical extirpation of the nodule was advised and was performed June 15, 1962. Microscopic examination showed only a nodular goiter with considerable evidence of
Two-dimensional chromatogram of the extract of the nodular tissue from patient R. R. showing diiodotyrosine (DIT), monoiodotyrosine (MIT), iodide (I−) and tetraiodothyroacetic acid (TETRAC). Thyroxine (T4) is not revealed because of its low concentration.

Solvents: 2BuOH:NH4OH along the longer axis and Tert. Amyl: NH4OH along the shorter axis.

degeneration and some haemorrhage. There was no evidence of malignancy, nor was lymphocytic infiltration a predominant feature.

Postoperatively, the patient recovered well and has been taking thyroxine 0.2 mg daily to attempt to prevent new goiter formation.

METHODS AND RESULTS

Parts of the thyroid nodule from each of the two patients were obtained at operation, quick frozen in dry ice and removed to the laboratory. The chemical extraction and chromatographic procedures followed have been described earlier (Row et al. 1963).

In the case of patient R. R., an iodinated compound was detected between the positions of thyroxine and triiodothyronine on the chromatogram. By co-chromatography with radioactive tetraiodothyroacetic acid of high specific activity, the compound was identified as the acetic acid analog of thyroxine (Fig. 1). An aliquot of the extract of the nodular tissue was chromatographed in redistilled secondary butanol:3.2 N ammonium hydroxide (2BuOH:NH4OH, 3:1 v/v) solvent system and the dry chromatogram cut into 4 × 1.4 cm strips. The strips were individually eluted with a mixture of 5 N sulfuric acid and 0.3 N arsenious acid. The eluates were reacted with 0.1 N ceric sulfate in 5 N sulfuric acid and the reduction of ceric ions read in a Bausch & Lomb spectronic colorimeter (Mandl & Block 1959). The relative distribution of the 127I compounds obtained is shown in Table 1.
Table 1.
Patient R.R.: Distribution of iodocompounds in the nodular tissue.

<table>
<thead>
<tr>
<th>Iodocompound</th>
<th>Percentage</th>
<th>MIT/DIT Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diiodotyrosine</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Monoiodotyrosine</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Iodide</td>
<td>11</td>
<td>0.625</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Tetraiodothyroacetic acid</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Iodohistidines</td>
<td>10 $\div$ 7</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2.
Two-dimensional chromatogram of the extract of the thyroid nodule from patient D.G., showing DIT, MIT, $I^-$ and Compound X. Solvents: 2BuOH:NH$_4$OH along the longer axis and Tert. Amyl: NH$_4$OH along the shorter axis.

In the case of patient D.G., initial chromatographic analysis showed an iodinated compound migrating above the position of triiodothyronine. Two-dimensional chromatography with 2BuOH:NH$_4$OH as the first solvent and Tertiary Amylalcohol saturated with 2 $\times$ NH$_4$OH as the second, showed the compound (X) as a single spot (Fig. 2). Co-chromatography with pure samples of thyroxine and triiodothyronine showed compound X to be separate from either (Fig. 3).

Six hundred $\mu$l of the extract of the nodule were chromatographed as a 12 cm strip and the paper developed in 2BuOH:NH$_4$OH solvent. After locating the position of compound X on the paper by spraying guide strips with ceric sulfate:arsenious acid:methylene blue reagent, the compound was eluted with
Fig. 3.
Extract of nodule from patient D. G., co-chromatographed with known thyroxine (T₄) and triiodothyronine (T₃).

Fig. 4.
Compound X chromatographed with triiodothyroacetic acid (TRIAC).
  a. Compound X.
  b. Compound X plus Triac.
Solvent: Tertiary amylalcohol saturated with 2 N NH₄OH.
water-saturated butanol. The eluate was concentrated to dryness in vacuo, the residue taken in 1 ml of methanol:NH₄OH (99:1 v/v) and used for further studies.

An aliquot of the extract of compound X was chromatographed with an authentic sample of triiodothyroacetic acid (triac) in tert. amylalcohol:NH₄OH solvent. On spraying the chromatogram for iodocompounds, it was seen that compound X appeared above triac (Fig. 4).

Assuming that the compound might be a peptide, an aliquot of the extract was evaporated to dryness, the residue taken in Borate Buffer (0.005 M at pH 8.5) and reacted with purified trypsin for 24 h, at 37° C. The incubation mixture was re-extracted with n-butanol and re-chromatographed and the chromatogram sprayed for iodocompounds. Compound X again appeared at the original position without having been hydrolysed.

Ultra-violet absorption spectrum

An aliquot of the eluate of compound X was read in a Beckman D. U., spectrophotometer. The maximum absorption was at 270 mμ which is similar to the Ultra-violet absorption spectrum of the iodothyronines (Pitt-Rivers & Tata 1959).

Infra-red analysis

A sample of compound X was subjected to Infra-red analysis using a Perkin-Elmer Infra-red spectrophotometer. The »finger print« region of the infra-red spectrum especially between 8 and 9 μ shows a distinct aromatic ether group which is the basic structure of the thyronine molecule (Fig. 5).

In addition to being ceric sulfate-positive, compound X was also ninhydrin-positive, indicating the presence of amino groups.

Reaction with l-aminoacid oxidase

Compound X was further reacted with l-aminoacid oxidase (LAAO, Snake

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venom) by a modification of the method of Nakano et al. (1959). The compound was added to 200 µl of a solution of l-aminooacid oxidase (10 mg of LAAO in 10 ml of 0.005 M Borate Buffer at pH 8.5) and incubated for 60 min at 37°C. At the end of the time, a drop of NH₄OH was added to the incubation mixture to bring the pH to 12 in order to inactivate the enzyme and the reaction mixture chromatographed as a single spot on Whatman No. 3 chromatography paper. Pure samples of tetrac and triac* and an aliquot of the solution of LAAO were also spotted alongside on the same paper. The paper was developed in tert. amylalcohol saturated with 2 N NH₄OH and the chromatogram sprayed to reveal the iodocompounds. The reaction mixture showed part of compound X converted to triiodothyroacetic acid. All the unreacted material migrated above triac. No iodocompounds were present in the enzyme itself (Fig. 6).

* Triac and tetrac were kindly provided by Warner-Lambert Research Institute, Morris Plains, New Jersey, research affiliate of Warner-Chilecott Laboratories.
Table 2.
Patient D. G.: Distribution of iodocompounds in the thyroid nodule.

<table>
<thead>
<tr>
<th>Iodocompound</th>
<th>Percentage</th>
<th>MIT/DIT Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diiodotyrosine</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Monoiodotyrosine</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Iodide</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Thyroxine</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Compound X</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Iodohistidines</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

An aliquot of the eluate of compound X was chromatographed and the individual iodocompounds estimated by colorimetry as described earlier. The relative distribution of the iodocompounds is given in Table 2.

**DISCUSSION**

In patient R. R., the thyroid nodular tissue was apparently producing iodo-tyrosines in normal proportions so as to show an MIT:DIT ratio of 0.625 which is close to normal (normal ratio is 0.7). Tetrac was found to be the largest component synthesized by this thyroid tissue. It is well known that the parent compound thyroxine has to undergo oxidative deamination followed by oxidative decarboxylation to form the acetic acid analog (Lissitzky 1959). This biochemical step is enzyme-mediated in the presence of l-aminoacid oxidase. The finding of tetrac in the thyroid gland of this patient indicates that enzyme systems may be altered in the nodular tissue of the gland. The nodular tissue might well have contained an aminoacid oxidase or transaminase and decarboxylase systems capable of transforming thyroxine to its acetic acid analog.

Though less calorigenic than thyroxine, tetrac has been shown to cause a drop in the level of serum cholesterol, weight loss and creatinuria without raising basal metabolic rate (Lerman 1960). It is therefore of interest whether the formation of tetrac in this patient had anything to do with the maintenance of the euthyroid condition of the patient. Since the blood of the patient was unfortunately not analysed, it can only be a conjecture. As an alternative, it is also tempting to speculate that a possible excess of thyroxine produced by the nodular tissue might have been metabolized into a less active form, that of tetrac in an amount sufficient to maintain a euthyroid state in the patient. Until more such data become available and possible changes in enzyme systems are thoroughly understood, all explanations would be only in the nature of speculation.
In regard to patient D. G., it can be seen that the monoiodotyrosine to di-
iodotyrosine ratio is elevated far above normal. The accumulation of MIT
with little DIT being formed, appears to denote defective halogenation of
monoiodotyrosine.

The iodocompound detected above triiodothyronine in the chromatograms
was neither a known type of iodothyronine nor its analog, as seen from the
results of the various tests conducted. Compound X was not a peptide either
since it did not yield to enzyme hydrolysis. During one of the tests, the com-
pound became spontaneously deiodinated, with the result that only iodide
showed up on the chromatogram after spraying, while a second chromatogram
of the same sample treated with ninhydrin, showed the amino groups of com-
pound X above the position of triiodothyronine. Though fortuitous, it proved
beyond doubt that compound X was an iodo-compound. The Ultra-violet ab-
sorption spectrum gave an indication that it might be a thyrone. This ob-
servation was further substantiated by the Infra-red analysis which showed
the presence of a distinct aromatic ether group.

In case compound X was an iodothyronine, it was thought worthwhile to
react it with 1-aminoacid oxidase, since according to Nakano et al. (1959), the
respective acetic acid analogs are formed by the enzyme. The reaction mixture
showed part of the compound transformed into triiodothyroacetic acid. The
above findings therefore lead us to believe that the iodocompound was a tri-
iodothyronine complex.

The thyroid nodule chromatograms of patient D. G. show two abnormalities:
(a) a markedly elevated MIT/DIT ratio and (b) an abnormal iodothyronine.
Since only the nodule of the thyroid was examined chromatographically, it
cannot be stated whether these alterations were confined to the nodule or were
present in the apparently normal thyroid tissue. It is, however, quite possible
that the findings are true only for the nodule.

At any rate, the nodular tissue is apparently incapable of adequately halo-
genating MIT to DIT. Its metabolic pathways are further altered to produce
the iodothyronine as mentioned. Since the nodule was «cold» by scanning and
thus unable to concentrate much iodide, it would seem that the absolute
amount of iodocompounds produced must be quite limited. This is perhaps
further suggested by the inability to demonstrate the abnormal iodothyronine
in the circulation. The changes noted are quite possibly a result of the injury
to thyroid cells (since there was considerable degeneration and haemorrhage),
rather than due to congenital enzymatic defects. although no proof can be
offered for this statement.
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