Congenital goitre due to 
"thyroid peroxidase-iodinase defect"

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Abstract. A 16-year-old male cretin with congenital goitrous hypothyroidism and 95% discharge in the perchlorate test underwent thyroidectomy. Thyroid studies disclosed negligible peroxidase (TPO) activity in the tyrosine iodinase assay, 6 nmoles 1\(^{-}\) inc./g (normals: 220–410). Using the same particulate preparations, a high activity was obtained in the guaiacol assay, 485 U/mg vs. 176 U/mg of a control gland. Goitre TPO was solubilized by treating the thyroid pellets with deoxycholate, trypsin and acetone. Soluble goitre TPO was further purified on Sephadex G-200. By this procedure we obtained a single peak of enzyme activity for oxidizing guaiacol, although no activity was found for iodinating tyrosine. I\(_2\) formation, as measured by the triiodide assay, was only 28% of that expected for normal TPO when compared for guaiacol oxidation. It is concluded that this abnormal TPO was the cause of the congenital hypothyroidism of the patient. We suggest the term "thyroid peroxidase-iodinase defect" for defining this newly found inborn error.

Among the inborn errors of thyroid hormonogenesis there is a group whose main characteristic is a failure of binding of iodine to the tyrosil residues of thyroglobulin (Stanbury 1972). This organification defect might theoretically be caused by a number of mechanisms which are related to the four major components of the iodination process: a) thyroid peroxidase, b) preformed thyroglobulin, c) I\(_2\), and d) iodide. Since normal organification of iodine takes place at the cell-colloid interface, through a peculiar subcellular mechanism that involves a "rendezvous" between peroxidase, thyroglobulin, H\(_2\)O\(_2\), and iodide (DeGroot & Niepomniszcze 1977), it is possible that another cause of organification defect, the so-called cytostructural defect (Niepomniszcze et al. 1972), would be located at the subcellular level of iodination. Recently, it was postulated that the thyroidal failure of Pendred's syndrome would be due to this mechanism (Niepomniszcze et al. 1978). Most of the patients with organification block have been shown to have peroxidase defects (Niepomniszcze 1977). A qualitative enzyme failure, the apoenzyme-prosthetic group defect, was described by Niepomniszcze et al. (1972, 1975). A probable quantitative defect, in which there are cases with an almost complete absence of peroxidase (Niepomniszcze et al. 1973; Valenta et al. 1973; Pommier et al. 1976) or very low levels of an apparently normal enzyme have also been described (Niepomniszcze et al. 1975, 1976). Moreover, a patient whose peroxidase was inactive in crude preparations, but normal after true solubilization, was studied by Pommier et al. (1974). Other cases, with impaired organification of iodine, were reported as having abnormal thyroglobulin (Kusakabe 1972, 1973; Niepomniszcze et al. 1977) or defective H\(_2\)O\(_2\) supply (Kusakabe 1975; Niepomniszcze 1976).

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In this report we present biochemical studies performed on the thyroid of a patient with congenital goitreous cretinism with an almost complete discharge of radioiodine during the perchlorate test. The main finding was a qualitative abnormal thyroid peroxidase that was unable to iodinate tyrosine.

Materials and Methods

Case report

Patient R.A., a 16-year-old male, weighed 4200 g at birth. When he was seen at the Children's Hospital at the age of 11 months he was a sluggish, clinically hypothyroid, mentally retarded goitreous cretin. Medication with triiodothyronine (T3), 20 μg daily, started immediately. The size of the goitre decreased markedly, and an improvement of clinical findings was promptly noticed. However, the mental retardation remained unchanged. The dose of T3 was gradually augmented, reaching 60 μg daily at the age of 3 years. When he was 5 years old T3 medication was stopped to allow thyroid studies. At that time his I.Q. was 38, and his bone age 4 years. The serum PBI was 0.4 μg/100ml and the BEI 0.3 μg/100ml. Thiocyanate test showed a 73% discharge of thyroidal radioactivity within the first 15 min after drug administration. His parents and brothers were also studied, and all of them were normal. Patient R.A. continued with thyroid medication until the age of 16 years, when he was referred to us because of the permanence of his goitre. Five weeks after stopping treatment with l-thyroxine a study of thyroid function was carried out (Table 1). The day before total thyroidectomy he received a tracer dose of 500 μCi 123I in order to carry out biochemical studies on the operated tissue. The excised gland weighed 140 g, was multinodular, and histologically it showed cellular hyperplasia and compact follicles with little colloid. No evidence of Hashimoto's thyroiditis was observed.

Tissue sources

Thyroid tissue from patient R.A. was obtained at thyroidectomy. Two other human thyroids from patients who underwent subtotal thyroidectomy, and hog thyroids obtained from a local abattoir, were used as control glands. The control human thyroids were known to have normal peroxidase activity. For enzymatic studies all glands were handled simultaneously.

Enzyme preparations

Thyroid tissues were homogenized in a glass Potter-Elvehjem homogenizer with 4 vols. of Krebs Ringer phosphate (KRP) buffer. Crude preparations for peroxidase activity were obtained by ultracentrifugation at 700 g, 30 000 g, and 105 000 g (Niepomnischte et al. 1973). Solubilized thyroid peroxidase (Nagasaka & DeGroot 1971) was further purified by gel-filtration on Sephadex G-200, followed by extensive dialysis against 10 000 vols. of 0.1 m phosphate buffer, pH 7, during 24 h at 4°C.

Column chromatography

Chromatography was performed at 10°C on Sephadex G-200, equilibrated with 0.1 m phosphate buffer, pH 7, using a column of 85 cm height and 0.8 cm diameter. For peroxidase purification we utilized preparations solubilized by deoxycholate, trypsin and acetone treatment (Nagasaka & DeGroot 1971). For molecular weight studies the most purified enzymes were used. Markers were dextran blue, glucose oxidase, ovalbumin and cytochrome c.

Studies on 125I labelled tissue.

The distribution of radioiodine, in particulate and soluble fractions, was studied by means of a well scintillation gamma counter.

Table 1.

Parameters of thyroid function.

<table>
<thead>
<tr>
<th>Test</th>
<th>units</th>
<th>Patient R.A.</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum T4 (by CPB)</td>
<td>μg/100 ml</td>
<td>0.8</td>
<td>5-13</td>
</tr>
<tr>
<td>Serum TSH (by RIA)</td>
<td>μU/ml</td>
<td>193</td>
<td>1-8</td>
</tr>
<tr>
<td>Antithyroglobulin antibodies (by TRC test)</td>
<td>titer</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>Thyroid estimated maximum 131I-uptake (U-max.)</td>
<td>%</td>
<td>46</td>
<td>20-40</td>
</tr>
<tr>
<td>Perchlorate wash-out test (131I-discharge test)</td>
<td>%</td>
<td>95</td>
<td>0-9</td>
</tr>
<tr>
<td>Thyroidal iodide clearance (Ct)</td>
<td>ml/min</td>
<td>43.8</td>
<td>16</td>
</tr>
<tr>
<td>Renal iodide clearance (Cr)</td>
<td>ml/min</td>
<td>41.1</td>
<td>30</td>
</tr>
<tr>
<td>Thyroid iodine release slope (Kn , 2)</td>
<td>day⁻¹</td>
<td>0.630</td>
<td>0.012</td>
</tr>
<tr>
<td>Total thyroid iodine (kinetic analysis) Qt</td>
<td>μg</td>
<td>143</td>
<td>15 000</td>
</tr>
<tr>
<td>Extrathyroidal pool of iodine-T4 (Qt)</td>
<td>μg</td>
<td>3</td>
<td>480</td>
</tr>
<tr>
<td>Daily degradation of iodine-T4</td>
<td>μg</td>
<td>0.8</td>
<td>60</td>
</tr>
</tbody>
</table>
**Thyroglobulin studies**

Thyroglobulin was obtained from the supernatant at 105,000 g followed by ammonium sulphate precipitation at 45% (Rapoport et al. 1972). Immuno-electrophoresis of goitre thyroglobulin was carried out in agar plates. For anti-human thyroglobulin antibody we used the serum of a patient with Hashimoto's thyroiditis, whose antithyroglobulin antibody titer was higher than 1:25,000 in the TRC test. Normal human thyroglobulin was used as control.

**Assay of peroxidase activity**

Peroxidase activities were measured by the tyrosine-iodinase, triiodide and guaiacol methods, as previously described (Niepomnischce et al. 1972). The effects of haematin on the enzyme activity were studied as indicated in an earlier report (Niepomnischce et al. 1975).

Particulate fractions of both, congenital goitre and hog thyroids, were incubated with lactoperoxidase to search for inhibitors. These enzyme activities were measured by the tyrosine-iodinase assay.

**Other assays**

NADPH-cytochrome c reductase in the thyroid tissues was assayed as described (Niepomnischce et al. 1973).

Protein concentration was measured by the method of Lowry et al. (1951).

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**Results**

**Protein distribution**

Goitre tissue disclosed a particulate protein content of 73.3 mg/g of fresh thyroid. However, the soluble proteins were only 47 mg/g. These findings are in agreement with the histologic pattern showing a marked hypertrophy and hyperplasia and follicles with little colloid.

**Thyroglobulin studies**

Goitre thyroglobulin content was 45 mg/g of fresh tissue, comprising 95.7% of the total thyroidal soluble proteins. No 125I was found in thyroglobulin. All the thyroid radioactivity appeared in the form of inorganic iodide. By immuno-electrophoresis it was observed that goitre thyroglobulin reacted normally with human antithyroglobulin serum.

**NADPH-cytochrome c reductase activity**

Enzyme activity of goitrous tissue was 74 nEq/min/mg. Normal values are 17 ± 3 nEq/min/mg (Niepomnischce et al. 1977).

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Peroxidase activity in the tyrosine iodinase assay of goitre and control thyroid particulate preparations obtained by ultracentrifugation at 700 × g, 30,000 × g, and 105,000 × g. Enzyme activities are expressed as nmoles of I⁻ incorporated to tyrosine for each one of the different subcellular fractions contained in 1 g of fresh tissue.

**Studies of possible inhibitors**

Thyroid preparations of both, congenital goitre and hog thyroids had identical effects on a commercially purchased bovine lactoperoxidase (LPO).
Up to 0.5 mg of particulate protein, per ml of the incubation mixture, did not affect the LPO activity in the tyrosine-iodinase assay. Higher concentrations of thyroid pellets gradually inhibited the enzyme, reaching a maximum at 4 mg/ml. When the amounts of particulate proteins are high such inhibition is commonly observed in this assay. However, such inhibition is not dependent on any particular thyroid or non-thyroid tissue preparation.

**Thyroid peroxidase studies**

Crude preparations of congenital goitre showed negligible peroxidase activity in the tyrosine-iodinase assay (Fig. 1). Only 6 nmoles I'inc./g of tissue were found in the goitrous gland. A control human thyroid, which was handled simultaneously, showed a low normal enzyme activity, 232 nmoles I'inc./g. Normal values range from 220 to 410 nmoles/g (Niepomniszcze et al. 1977). Pre-incubation with haematin did not modify the enzyme activity of the congenital goitre. On the other hand, the same goitre preparations were very active when assayed in the guaiacol method, as compared with the control gland. In Fig. 2 it can be seen that peroxidase activities had a linear correlation with the amount of protein of the enzymatic preparations. The peroxidase activities of the particles obtained at 30 000 g are 485 U/mg and 176 U/mg, for the congenital goitre and the control thyroid, respectively. The entire enzyme activity for oxidizing guaiacol was found in the particulate fractions of the goitrous tissue; 65% was in the pellets obtained between 700 and 30 000 g, and 35% in the particles sedimented between 30 000 and 105 000 g.

**Table 2.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total guaiacol activity per gram of tissue *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>700–105 000 g crude particles</td>
</tr>
<tr>
<td>Goitre</td>
<td>5396</td>
</tr>
<tr>
<td>Control</td>
<td>4600</td>
</tr>
</tbody>
</table>

* Enzyme activity is expressed as the change of OD at 470 nm per 15 seconds at initial rate of the reaction, multiplied by a factor of 1000, and the result extrapolated to 1 g of fresh tissue.

**Fig. 3.**

Studies on the molecular weight of both goitre (GPO) and control (TPO) solubilized peroxidases. The volume of the effluent corresponding with the elution peak of dextran blue was taken as “zero” ml.

During tryptic digestion of the peroxidase particulate preparation of the congenital goitre 90% of enzyme activity was destroyed, although this method does not significantly affect normal thyroid peroxidases (Nagasaka & DeGroot 1971). From the remaining 10% of activity only 40% could be solubilized, while 60% remained attached to the cell particles. With the same procedure it was possible to solubilize 80% of the activity of the control thyroid, and the peroxidase was unaltered by the solubilization technique. All these calcula-
tions were done on the basis of the guaiacol activities of the crude and solubilized enzymatic preparations (Table 2).

Solubilized goitre thyroid peroxidase (GPO) was partially purified by column chromatography on Sephadex G-200. A single peak of enzyme activity was obtained. This activity was observed by guaiacol and triiodide assay, but no activity was detected by the tyrosine-iodinase assay, even when GPO was further dialyzed. Control thyroid peroxidase (TPO) was normally active in the three assays. Goitre peroxidase activity in the triiodide assay was only 28% of that obtained for control TPO when preparations were compared with identical guaiacol oxidation activity.

Gel-filtration on Sephadex G-200 revealed similar molecular weights for GPO and TPO (Fig. 3). The value of 47 000 agreed with those reported by Ljunggren & Åkeson (1968), and by Yip (1966), although they are considerably different from the values obtained by other investigators (Tauberg et al. 1970; Pommier et al. 1972).

Fig. 4 shows an enzyme-kinetic study comparing goitre and control soluble thyroid peroxidases. As measured by the guaiacol assay, a similar $K_{m, app}$ was observed toward hydrogen peroxide for both solubilized enzymes. GPO also shows some inhibition, at very high $H_2O_2$ concentrations, which is commonly observed with normal thyroid peroxidase preparations (Niepomnisszczc et al. 1975).

Discussion

We have presented a severely hypothyroid patient who had an inborn error of thyroid metabolism producing iodine organification block. Thyroid biochemical studies have disclosed peroxidase activity in the goitrous tissue, although almost no iodination of tyrosine was achieved either in vivo or in vitro. Peroxidase activity of both particulate and solubilized enzyme preparations was only observed in the guaiacol and triiodide assays. The tyrosine-iodinase assay, which uses the glucose-glucose oxidase system as $H_2O_2$ source, showed an almost complete lack of peroxidase activity in all the enzymatic preparations. Activities in the triiodide assay were lower than those expected for normal enzymes when the preparations were compared with identical guaiacol oxidation activity. Solubilization of goitre peroxidase destroyed 90% of the guaiacol oxidizing activity; however, the solubilized enzyme, which showed a normal $K_{m, app}$ for $H_2O_2$, had a behaviour like crude preparations in the three peroxidase assays since it presented high guaiacol activity, poor I$^3$ formation, and negligible tyrosine iodination. These results would suggest that active solubilized GPO, despite the fact that 90% of the total activity was destroyed during tryptic digestion, retained the characteristics of crude enzyme.

Recently, Neary et al. (1978) pointed out that thyroid peroxidase from carcinomatous glands may not be solubilized from its microsomal membranes due to the hydrophobic nature of the enzyme. However, they have found a good recovery of peroxidase activity after the solubilization procedure, despite the very small yield of formation of soluble TPO. Our patient disclosed a real loss of TPO activity during tryptic digestion, but he also showed a low percentage of solubilized-enzymatically active thyroid peroxidase.

Thyroglobulin was not iodinated in vivo with $^{125}$I, but appeared normal by immunoelectrophoresis. NADPH-cytochrome $c$ reductase, an enzyme possibly involved in the generation of $H_2O_2$ (DeGroot & Niepomnissczc 1977) showed an increment of activity.

Three theoretical possibilities might be con-
sidered for an explanation of our findings: a) there was a complete lack of thyroid peroxidase in the goitrous gland. The activity detected in the guaiacol and triiodide assays could be due to a pseudo-peroxidase contaminant; b) normal peroxidase enzyme was inhibited in its capacity for iodinating tyrosine by an unknown compound, and c) a genetically abnormal thyroid peroxidase was unable to perform tyrosine iodination. Against the first possibility are the very high enzyme activities toward guaiacol, obtained with the crude preparations, and the normal $K_{\text{m,app}}$ for H$_2$O$_2$ of the solubilized enzyme. It is known that pseudoperoxidases have low enzyme activity (Niepomniszcze et al. 1972; Hosoya & Morrison 1967) and a very poor affinity toward H$_2$O$_2$ (Niepomniszcze & Altshuler 1972). The second possibility also seems unlikely, since an inhibitor able to perform such an effect must be: 1) produced specifically by the patient, 2) attached very firmly to a portion of the enzyme, 3) specific inhibitor for thyroid peroxidase, and 4) of a very low molecular weight. These requirements are based on the following facts: 1) no exogenous inhibitors can be postulated since the patient had the thyroidal defect since birth, and his relatives eating the same diet were normal; 2) since the tyrosine-iodinase activity remained inhibited, even though the enzymatic preparations were solubilized by trypsin and deoxycholate, further purified by Sephadex G-200, and subjected to extensive dialysis, and since guaiacol activity was always present, only an inhibitor which should be firmly bound to the portion of the enzyme involved in tyrosine iodination might be taken into account; 3) since no inhibition of lactoperoxidase was obtained with goitre preparations, a hypothetical inhibitor should be specific for the thyroid enzyme; 4) the molecular weights of both goitre and control thyroid peroxidases were identical, thus only a very small size inhibitor might be considered.

The third explanation appears to be the likely one. The high guaiacol activity, the normal $K_{\text{m,app}}$ toward H$_2$O$_2$, and the same molecular weight as the control enzyme are three important factors indicating that goitre peroxidase is a true thyroid enzyme. However, lack of iodinating activity, poor I$_2$ formation, and excessive lability to trypsin digestion strongly suggest that the enzyme is abnormal.

Thyroid peroxidase defects are a common cause of congenital goitre due to iodine organification block (Niepomniszcze 1976). Previous cases have disclosed qualitative and quantitative defects in this thyroid enzyme (Niepomniszcze et al. 1975). Since haematin was unable to restore iodinating activity of goitre peroxidase of patient R.A., it is possible to rule out an “apoenzyme-prosthetic group defect” (Niepomniszcze et al. 1972) as the cause of this peroxidase abnormality. Pommier et al. (1976) have reported a euthyroid woman with a partial discharge of radioiodide during the perchlorate test. They found an abnormal thyroid peroxidase that was inactive for iodinating thyroglobulin and poorly efficient in catalyzing the oxidation of guaiacol to tetraguaiacol. Iodide peroxidase ($\text{I}^-\rightarrow\text{I}_2$) was present but also abnormal. They have emphasized that none of their data obtained in vitro were able to explain the clinical euthyroidism and partial positive perchlorate test of their patient. In contrast to their findings, our case had a good correlation between the clinical parameters of thyroid function and the biochemical observations in the goitre tissue. For this reason we have concluded that this abnormal thyroid peroxidase was the cause of the congenital hypothyroidism of our patient R.A.

Since 1948, when Pauling et al. (1949) discovered that the haemoglobin of sickle-cell anaemia differed slightly from normal haemoglobin, and suggested that proteins might be altered in structure and function by a single amino acid substitution dictated by an inherited error in the genotype, many congenital defects have been described where the metabolic failures were due to genetic mutations (Stanbury 1972). We think that a genetic mutation producing an altered amino acid sequence at the site of apoenzyme, in which tyrosine can bind as a substrate, may explain the congenital defect of our patient R.A. We suggest the term “thyroid peroxidase-iodinase defect” for defining this newly found inborn error.

References


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