The Effect of Propylthiouracil on the Intrathyroid Metabolism of Iodine in Rats*

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It has long been known that treatment with propylthiouracil and other antithyroid drugs inhibits organic binding of iodine and leads to a reduction in total iodine in the thyroid (1). The work of Franklin et al. (2-4) showed that these agents also prevent incorporation of radiiodine into organic compounds by thyroid slices in vitro. Pitt-Rivers (5), finding that acetyldiiodotyrosine did not form acetylthyroxine when incubated with these compounds, suggested that they may act by inhibiting coupling of diiodotyrosines to form thyroxine in vivo. However, Slingerland (6) was unable to obtain evidence in support of this possibility.

Recent investigations have suggested that although propylthiouracil blocks the iodination of tyrosyl groups in thyroglobulin, it inhibits the formation of diiodotyrosine more strongly than the formation of monoiodotyrosine. Slingerland et al. (7) felt that this block favored the synthesis of triiodothyronine over the formation of organic iodine compounds. However, Slingerland (8) found no triiodothyronine and a reduced quantity of thyroxine in the glands of rats treated with small amounts of propylthiouracil.

All of the studies on the effect of propylthiouracil on the intrathyroid metabolism of radiiodine have been conducted by administration of radiiodine after treatment with graded doses of the drug. In the studies to be described we thought it might be informative to "label" the thyroids of animals before drug treatment and to follow subsequently the radioactivity in the several iodine-containing compounds in the glands after instituting antithyroid treatment.

**EXPERIMENTAL PROCEDURE**

The animals used were female Sprague-Dawley rats weighing 150 to 250 g which had been fed a diet† containing 0.25 μg of iodine per g for 1 to 5 weeks. In one group each animal was treated by intraperitoneal injection with 50 μc of carrier-free NaI131, and uptake by the thyroid was determined 24 hours later. Each day for 7 days the thyroid glands from four animals in this group, which served as a control group, were removed for analysis. Two other groups were similarly studied. Twenty-four hours after the injection of I131, one group was injected subcutaneously with 200 μg of potassium perchlorate in 20% gelatin and thereafter a diet supplemented with 20 μg of iodide as potassium iodide and thereafter a diet supplemented with 2 μg of iodide as potassium iodide per gram.

The rate of release of I131 from the thyroid (Fig. 1) was determined in another group of animals which had been fed the control diet for 1 week. These rats then received, intraperitoneally, 50 μc of I131, and the 24 hour uptake by the thyroid was determined. Five rats each then were treated as in the previous four groups, i.e., control, propylthiouracil, potassium perchlorate, and iodide; however, animals were not killed each day, and the radioactivity in the thyroid was determined by external counting.

The thyroid glands were hydrolyzed by the method of Tong and Chaikoff (9). Each gland was ground in a glass homogenizer with 1.0 ml of cold 0.11 N sodium chloride-0.04 N sodium bicarbonate solution. The suspension was centrifuged, and 0.3 ml of the supernatant was added 20 μl of a 1.0% suspension of propylthiouracil, 10 μl of 0.1 N manganous sulfate, and 2 drops of toluene. This preparation was then incubated for 24 hours at 38°.

Each hydrolysate, in 20 to 100 μl portions, was applied across each of two 1.5-inch strips of either No. 1 or No. 3 Whatman paper and developed by ascending chromatography in n-butanol-absolute ethanol-0.5 N ammonium hydroxide (8:1:2) and n-butanol-glacial acetic acid-water (15:2:3) solvent systems, respectively, for about 16 hours. Each strip was analyzed for radioactivity by an automatic strip scanner and Brown recorder and then radioautographed. The radioactive iodine components were identified by comparison of the Rf values of both the radioautographed and strip-scan records to those of simultaneously chromatographed samples of authentic thyroxine, triiodothyronine, diiodotyrosine, monoiodotyrosine, and iodide. The chromatograms of these standards were visualized by the ceric sulfate method of Kono and Astwood (10). The relative proportions of the I131 components were determined from the scan-
The mean thyroid uptake of radioiodine in 24 hours for all of the animals used in this study was 13.3 ± 4.8% (mean ± S. D.). The animals fed the control diet, relatively low in iodine, for 7 days before the injection of I\textsuperscript{131} had a mean uptake of 12.4% of the administered dose; 14 days, 15.8%; 30 days, 16.1%; and 34 days, 14.7%. Thus, over a 5-week period the low iodine diet had its maximum effect on thyroid I\textsuperscript{131} uptake within the first 7 days and reached a peak effect within 14 days.

The release of I\textsuperscript{131} from the thyroids of the control animals and of those treated with propylthiouracil, potassium perchlorate, and iodide is shown in Fig. 1. After 6 days, 53% of the 24-hour thyroid I\textsuperscript{131} had been lost in the control group, whereas 92% of that present in the propylthiouracil-treated rats had been released. The values for iodide-fed and potassium perchlorate-fed groups were intermediate between these extremes. The biological half-life of the I\textsuperscript{131} in the thyroid was 5.5 days in the controls, 4.6 days in the group given iodide, 3.1 days in the perchlorate group, and 1.65 in those given propylthiouracil.

Twenty-four hours after the injection of I\textsuperscript{131}, the total radioactivity in the thyroid hydrolysates of animals fed the control diet was distributed among the components analyzed as shown in Table I, Group 0. As shown by the other figures in Table I, daily determinations on animals receiving the control diet over the next 6 days showed no significant variation in the relative proportions of these components, whereas 53% of the total I\textsuperscript{131} initially present left the thyroid. Triiodothyronine was detectable in all hydrolysates until 48% of the total I\textsuperscript{131} activity was lost, and in none thereafter.

In the propylthiouracil-treated animals (Table II), the proportion of thyroxine diminished progressively, so that thyroxine accounted for only 8% of the residual radioactivity on the 6th day of treatment. After 48 hours of propylthiouracil treatment, i.e. 72 hours after administration of I\textsuperscript{131}, triiodothyronine was detectable in 3 of 4 hydrolysates and accounted for only 1.3% of the I\textsuperscript{131} activity; thereafter, no triiodothyronine activity was found. The high value for the I\textsuperscript{131} activity found at the origin in Group 3 and for the large S. D. is due to the value for one of the four animals in this group. There were no significant changes in the proportions of the other components or in the moniodotyrosine-diiodotyrosine ratio, whereas 92% of the total activity left the gland.

The relative proportions of the radioiodine-labeled components in the animals treated with potassium perchlorate (Table III) did not vary significantly from those of the control group (Table I), although about 1.5 times the total amount of I\textsuperscript{131} activity had left the glands by the end of 6 days. Triiodothyronine was detected in some of the hydrolysates through 72 hours of perchlorate treatment, at the end of which 48% of the total I\textsuperscript{131} activity had been lost.

In the thyroids of the iodide-supplemented animals (Table IV), the relative proportions of radioiodinated constituents differed from those in the control thyroids only in that moniodotyrosine decreased from 25% to 15% of the total I\textsuperscript{131} activity over the 6 days of treatment. Triiodothyronine was found in diminishing proportions in some of the hydrolysates for 72 hours of treatment, but in none thereafter.

**Table I**

<table>
<thead>
<tr>
<th>Group No. per group</th>
<th>Time after</th>
<th>( T_1 )</th>
<th>( T_4 )</th>
<th>I-</th>
<th>MIT</th>
<th>DIT</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hours</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>( 3 \pm 0.8 )</td>
<td>( 21 \pm 3 )</td>
<td>( 23 \pm 1 )</td>
<td>( 25 \pm 2 )</td>
<td>( 42 \pm 4 )</td>
<td>( 46 \pm 2 )</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>( 3 \pm 0.4 )</td>
<td>( 23 \pm 3 )</td>
<td>( 33 \pm 1 )</td>
<td>( 24 \pm 3 )</td>
<td>( 41 \pm 35 )</td>
<td>( 35 \pm 0.7 )</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>( 2e )</td>
<td>( 18 \pm 2 )</td>
<td>( 6 \pm 0.5 )</td>
<td>( 23 \pm 3 )</td>
<td>( 43 \pm 28 )</td>
<td>( 38 \pm 1 )</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>( 0.9e )</td>
<td>( 24 \pm 3 )</td>
<td>( 2 \pm 1 )</td>
<td>( 25 \pm 3 )</td>
<td>( 41 \pm 37 )</td>
<td>( 37 \pm 1 )</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>( 0.3e )</td>
<td>( 26 \pm 1 )</td>
<td>( 4 \pm 0.9 )</td>
<td>( 23 \pm 3 )</td>
<td>( 39 \pm 36 )</td>
<td>( 32 \pm 2 )</td>
</tr>
<tr>
<td>5</td>
<td>144</td>
<td>( 3e )</td>
<td>( 22 \pm 2 )</td>
<td>( 4 \pm 0.6 )</td>
<td>( 20 \pm 4 )</td>
<td>( 42 \pm 37 )</td>
<td>( 37 \pm 3 )</td>
</tr>
<tr>
<td>6</td>
<td>168</td>
<td>0</td>
<td>25 ( \pm 0.8 )</td>
<td>0.8 ( \pm 0.8 )</td>
<td>20 ( \pm 2 )</td>
<td>48 ( \pm 37 )</td>
<td>37 ( \pm 1 )</td>
</tr>
</tbody>
</table>

* The abbreviations used are: \( T_1 \), triiodothyronine; \( T_4 \), thyroxine; I-, iodide; MIT, moniodotyrosine; DIT, diiodotyrosine; and Origin, material remaining at the origin of the chromatogram.
* Mean ± S. D. (after method of Dean and Dixon (11)).
* \( T_1 \) not detectable in all hydrolysates.

**Table II**

<table>
<thead>
<tr>
<th>Group No. per group</th>
<th>Treatment time</th>
<th>( I^{131} ) activity in components of thyroid hydrolysates (after 24-hour uptake of 60-( \mu )C dose of I\textsuperscript{131})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hours</td>
<td>( T_1 )</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>( 0^e )</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>( 1.4e )</td>
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<td>( 1.5e )</td>
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<td>5</td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>144</td>
<td>0</td>
</tr>
</tbody>
</table>

* Abbreviations as in Table I.
* Mean ± S. D. (after method of Dean and Dixon (11)).
* Equivalent to 24 hours after I\textsuperscript{131} injection.
* \( T_1 \) not detectable in all hydrolysates.
Since in all of these experiments the radioiodine was given before treatment with the various agents, it was important to determine the level of the block to radioiodine uptake throughout the 6-day period in order to know to what extent recycling of iodine had been inhibited. In another experiment, therefore, animals were given the control diet containing 0.25 µg of iodide per g for 15 days; four rats were used as untreated controls and groups of four were given, subcutaneously, 10 mg of propylthiouracil suspended in 20% gelatin, or 200 µg of potassium perchlorate in 20% gelatin, or 20 µg of iodide as potassium iodide in 0.5 ml of water. After 75 minutes, each of the animals in the four groups was treated by intraperitoneal injection with 25 µc of I131. The animals were then fed the diet corresponding to the similarly treated groups in the previous experiments. After 24 hours, the I131 uptake of the controls as determined by external counting was 9.2%, whereas the iodide group accumulated 7.2% of the administered dose of I131. In the animals treated with propylthiouracil or perchlorate, there was no uptake of I131. In the iodide-treated group, the increase in availability of the similarly treated groups in the previous experiments. After 24 hours, the I131 uptake of the controls as determined by external counting was 9.2%, whereas the iodide group accumulated 7.2% of the administered dose of I131. In the animals treated with propylthiouracil or perchlorate, there was no uptake of I131. In the iodide-treated group, the increase in availability of

**DISCUSSION**

In normal (i.e. untreated) animals, the relative proportions of the labeled compounds in hydrolysates of the thyroid remained remarkably constant over the 7-day period after the injection of I131. The findings agree well with those of Tong and Chaikoff (9), but it is not at all clear why these relative proportions did not change during the period when the total I131 content of the glands was decreasing. One explanation would be that the rates of conversion of monoiodothyrosine to diiodothyrosine and of the two of these compounds to triiodothyronine and thyroxine were exactly the same as the rate of secretion of triiodothyronine and thyroxine. In any event, the observed constancy strongly suggests that during 24 hours after giving I131, there was uniform labeling of all of the iodine “compartments” within the gland.

The fact that perchlorate did not cause a change from the normal amount in the proportion of the labeled iodoamino acids could be quoted in favor of either of two hypotheses of hormone secretion. The first, assuming uniform labeling, is the current concept that thyroglobulin is completely broken down to its component amino acids and that the thyronines are secreted and interconverted equal the rate of secretion. The more rapid loss of monoiodotyrosine caused by giving small doses of iodide is difficult to explain. The total uptake of I131 was only moderately reduced by the doses given; the half-time of loss was reduced only from 5.5 to 4.6 days, and consequently, recycling of I131 was probably only modestly inhibited. However, it may be presumed that the uptake of I131 was greatly augmented, because the intake of I131 was increased about 10-fold, whereas uptake of I131 was reduced by only about 25%. It is not easy to discern, however, why the lowered specific activity of the recycled I131 over the 7-day period or the augmented uptake of I131 should lower selectively the proportion of labeled monoiodothyrosine.

The rapid reduction in the proportion of “labeled” thyroxine in the animals given propylthiouracil contrasts sharply with the nearly constant proportion throughout in the other three groups of animals. It cannot be explained on the basis of inhibited binding of iodine or of a block to recycling of I131, because perchlorate had no such effect, although completely inhibiting uptake and recycling.

Whether the gradual reduction in the proportion of I131-triiodothyronine in the thyroids of control animals is artifactual or otherwise significant is not clear. Tong and Chaikoff (9), using a more sensitive method of counting, found 2 to 4% tri-
iodothyronine in the thyroids for 9 days after radioiodine administration. Richards and Ingbar (8), also using an automatic strip counter, found measurable triiodothyronine in only half of the animals 24 hours after I\textsuperscript{131} was given. Therefore, the loss of "labeled" triiodothyronine in our study may be a function of the low sensitivity setting of the strip scanner which had to be used in order to record the large quantities of monoiodotyrosine and diiodotyrosine. Thus, we cannot make valid comparison of the loss of triiodothyronine from control and treated animals, although the rapid disappearance when propylthiouracil was given probably has significance.

No block between monoiodotyrosine and diiodotyrosine was found in this study, although by the design of the experiments it is not certain that a block would be detectable. Pitt-Rivers et al. (12) have called attention to the relatively increased monoiodotyrosine and diminished proportions of diiodotyrosine and of the thyronines in almost every study (13-18) where normal thyroid function is altered, and concluded that the organic binding of iodine to form monoiodothyrosine is a primitive process. Moreover, large doses of iodine (19), hypophysectomy (20), diets deficient in iodine (21-23), and treatment with propylthiouracil (7, 8) have now been found to induce this so-called block between monoiodotyrosine and diiodotyrosine. Some monoiodotyrosine is found even in thyroids of rats treated with enormous doses of propylthiouracil (24).

The finding in the present study that propylthiouracil produces a relative reduction in the thyroxine component of thyroid hydrolysates may be interpreted to mean that propylthiouracil not only interferes with the initial iodination of tyrosine within thyroglobulin but also inhibits the coupling of iodotyrosines to form iodothyronines, as suggested by Pitt-Rivers (5). The loss of the iodothyronines during treatment with propylthiouracil, when interconversion is presumably completely blocked, indicates that, under these conditions, they can be released from thyroglobulin and deiodinated. However, it would appear that iodothyronines can be released from the thyroid at a rate faster than the iodotyrosines, a finding possibly incompatible with the theory that secretion of thyroid hormone requires complete hydrolysis of thyroglobulin.

**REFERENCES**

The Effect of Propylthiouracil on the Intrathyroid Metabolism of Iodine in Rats
W. E. Mayberry and E. B. Astwood