IODINE METABOLISM OF THE THYROID GLAND*

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Because of the similarity in structure between diiodotyrosine and thyroxine, Harington and Barger (1) suggested that the former is the precursor of thyroxine in the thyroid gland. The formation of thyroxine from diiodotyrosine has been demonstrated lately in vitro (2, 3). Recent data (4, 5) obtained with radioiodine used without carrier indicate the rates of incorporation of iodine into the various thyroid fractions but do not indicate the sequence of formation nor the respective sources of the finished components.

In a series of preliminary experiments with subphysiological doses of radioiodine, I\(^{131}\)0 (without carrier), we have attempted to determine whether diiodotyrosine is a natural precursor of thyroxine, whether inorganic iodine is normally present in the normal non-iodized thyroid (6, 7), and whether this inorganic iodine in the thyroid gland is the source of the iodine in the diiodotyrosine molecule.

Radioiodine (I\(^{131}\)0) injected in subphysiological amounts as iodide and without carrier should behave like iodide already present in physiological amounts in the blood stream and thus is actually representative of the circulating iodide. The radioactivity found in the thyroid gland iodine fractions is the result of absorption and subsequent reactions in the gland which utilize newly arrived radioiodine obtained from circulating iodide.

The iodine fraction which is first formed or appears soon after

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the administration of the radioiodine should have the highest proportion of radioactivity. Later on, the proportion of radioactivity should rise in the other fractions as they are synthesized.

By the proportion of radioactivity we mean the percentage of administered radioiodine per microgram of $I^{127}$, which is called the specific radioactivity. Measuring the radioactive isotope of $I^{130}$ and determining the chemical iodine (stable isotope $I^{127}$) from $\frac{1}{2}$ to 48 hours after injection of $I^{130}$ without carrier, we can arrive at the specific radioactivities which are indicative of the normal relative rates of turnover of inorganic iodine, diiodotyrosine, and thyroxine in the thyroid gland. It is assumed in this type of experiment that the total combined iodine values (inorganic and organic) for both $I^{127}$ and $I^{130}$ remain constant for a particular animal under normal conditions, since this level should not be disturbed by the extremely small amount (subphysiological) of tracer radioactive iodine injected.

Method

Radioactive iodine was prepared by the proton bombardment of tellurium in the cyclotron, resulting in a product in which the isotope with a half life of 12.6 hours ($I^{130}$) was predominant. Radioactive iodine without carrier was separated from tellurium by oxidation with a chromic-sulfuric acid mixture, reduction with phosphorous acid, and distillation, according to Matthews, Curtis, and Brode's modification (8) of the Leipert procedure (9) for microdetermination of iodine. The radioactive iodine was collected in an alkaline medium, evaporated to a suitable volume, and partially neutralized with phosphoric acid to pH 7.5 and 8.0. The radioactive iodine was thus obtained as iodide presumably suitable for intravenous injection.

Six adult dogs, weighing between 20 and 40 kilos, received an injection of 10 cc. of radioactive iodine solution (containing practically no $I^{127}$) into the saphenous vein of the hind leg. At intervals of $\frac{1}{2}$, 8, and 48 hours after the injection, the animals were sacrificed by illuminating gas. The thyroid glands were trimmed, cut into small pieces, and dried in the chamber of a Fisher-Abderhalden drier at a temperature of 79° first for 1 hour, then removed and homogenized, and returned to the chamber which is then connected to a Hyvac pump until a constant weight of tissue was obtained.
Aliquots were then taken for the total inorganic and thyroxine iodine determinations (both $^{131}$ and $^{127}$). Inorganic iodine was separated by the water extraction method according to Gutman et al. (10). As an added precaution against the possibility of extracting small amounts of protein containing iodine, trichloroacetic acid was added to the solution obtained after centrifugation. Traces of precipitate were obtained which contained insignificant amounts of radioactivity. The solution was then ashed and distilled according to the method of Matthews, Curtis, and Brode (8). The distillate obtained was evaporated and made up to volumes of 10 or 25 cc., depending upon the radioactivity content of the sample. 2 cc. of this volume were taken for radioactivity determinations by means of the dipping Geiger-Müller counter according to the method of Bale et al. (11). The remainder of the solution was then analyzed as usual for the determination of $^{127}$ (8).

Thyroxine was separated by the butyl alcohol extraction procedure according to Blau (12). After reduced pressure distillation to remove the butyl alcohol, the residue obtained was ashed and treated in the same manner as was the inorganic iodine. Total iodine was determined by treating an aliquot of desiccated thyroid in the same manner as were the aliquots measured for inorganic iodine and thyroxine after their separation, ashing, etc. Harington (13) has demonstrated that diiodotyrosine and thyroxine account for all of the organic iodine. Therefore, the iodine remaining after the inorganic iodine and thyroxine iodine were subtracted from the total iodine was considered the iodine of diiodotyrosine and is so designated in the rest of this paper. The determinations were made in duplicate and the iodine determinations were performed on the residue from the two extractions.

DISCUSSION

The results (Table I) indicate a general agreement with the values of $^{131}$ reported by Perlman et al. (4) for the sheep. The total quantity of radioactive iodine fixed in the gland rose throughout the three periods of observation to the highest level at 48 hours.

In order to study the rates of formation or turnover of inorganic and organic iodine it is necessary to compare the values for $^{131}$ with the values for $^{127}$ for each of the compounds studied. The
per cent of injected $^{131}$I divided by the amount of $^{127}$I present in the same iodine fraction gives a ratio which is called the "specific radioactivity" shown in Table I. This permits the comparison of the rate of formation of the different fractions in the same animal but does not permit direct comparisons with similar ratios obtained in other normal animals.

### Table I

**Rates of Turnover of Inorganic Iodine, Diiodotyrosine, and Thyroxine in Thyroid Gland**

All determinations were made in duplicate. The figures in bold-faced type represent the relative specific radioactivities.

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of dogs</th>
<th>Radiative iodine</th>
<th>Chemical iodine</th>
<th>Specific radioactivity $\times 10^4$</th>
<th>Radiative iodine</th>
<th>Chemical iodine</th>
<th>Specific radioactivity $\times 10^4$</th>
<th>Radiative iodine</th>
<th>Chemical iodine</th>
<th>Specific radioactivity $\times 10^4$</th>
<th>Radiative iodine</th>
<th>Chemical iodine</th>
<th>Specific radioactivity $\times 10^4$</th>
</tr>
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<tr>
<td>hrs.</td>
<td></td>
<td>Total per cent</td>
<td></td>
<td></td>
<td>Total per cent</td>
<td></td>
<td></td>
<td>Total per cent</td>
<td></td>
<td></td>
<td>Total per cent</td>
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<tr>
<td>2</td>
<td>15</td>
<td>8.2</td>
<td>86.3</td>
<td>0.96</td>
<td>3.00</td>
<td>3.00</td>
<td>0.62</td>
<td>7.66</td>
<td>1.17</td>
<td>0.30</td>
<td>166.6</td>
<td>0.18</td>
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<td></td>
<td>14</td>
<td>15.2</td>
<td>420.5</td>
<td>3.56</td>
<td>3.30</td>
<td>54.0</td>
<td>0.61</td>
<td>11.33</td>
<td>265.0</td>
<td>5.30</td>
<td>96.0</td>
<td>0.47</td>
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<td>8</td>
<td>12.2</td>
<td>1906.0</td>
<td>0.62</td>
<td>0.82</td>
<td>165.0</td>
<td>0.50</td>
<td>10.32</td>
<td>115.0</td>
<td>0.89</td>
<td>1.00</td>
<td>643.0</td>
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<td>1366.0</td>
<td>1.29</td>
<td>1.30</td>
<td>145.0</td>
<td>0.87</td>
<td>15.33</td>
<td>807.0</td>
<td>1.90</td>
<td>1.00</td>
<td>364.0</td>
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<td>48</td>
<td>12</td>
<td>1637.0</td>
<td>2.87</td>
<td>1.50</td>
<td>232.0</td>
<td>0.69</td>
<td>43.2</td>
<td>1290.0</td>
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<td>106.0</td>
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<td>21.9</td>
<td>676.00</td>
<td>3.19</td>
<td>1.00</td>
<td>24.0</td>
<td>0.24</td>
<td>2.20</td>
<td>65.6</td>
<td>3.42</td>
<td>1.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Per cent of total dose.
† Stable isotope $^{127}$I.

Since the proportion of radioactivity in the total iodine fraction is not constant from animal to animal, supposedly due to variations in blood iodide ($^{127}$I) concentrations and in the thyroid gland iodine concentrations of the different fractions among the animals, the specific radioactivity of the total thyroid iodine is made equal to unity in every experiment, and by proportion, the specific radioactivity of the iodine fractions in each individual thyroid is...
adjusted accordingly. This new value is designated as the "relative specific radioactivity" and direct comparison may now be made among the values obtained in the case of one animal or animals with others, and permits graphing of the values. The "relative specific radioactivity" is recorded below the "specific radioactivity" in Table I. The "relative specific radioactivities" have been plotted in Fig. 1.

If what we titrate as inorganic iodine was produced during the extraction procedure by splitting off from thyroxine or diiodotyrosine or both, the specific radioactivity of the iodide at any time interval should be equal to either one, or lie somewhere in between the specific radioactivities of thyroxine and diiodotyrosine.

However, at 48 hours, the specific radioactivity of inorganic iodide is considerably below that of either thyroxine or diiodotyrosine. Likewise, any losses of I$^{127}$ in the chemical extraction would be accompanied by proportional losses of I$^{129}$, thus leaving the ratios or specific radioactivity values undisturbed. Therefore, thyroid inorganic iodine is a true entity even in non-iodized glands (6, 7).

Inspection of Table I also shows that in the dogs sacrificed 30 minutes after the administration of radioactive iodide, the relative specific radioactivity of diiodotyrosine is much greater than that of inorganic iodide.

Since the radioactive iodine is administered intravenously in the form of iodide, and very soon penetrates into the extracellular fluid of the thyroid gland, a situation must exist soon after ad-
ministration wherein the specific radioactivity of iodide in the extracellular fluid of the thyroid gland is very high in relation to the specific radioactivity of the iodide inside of the thyroid cell. Since we measure the sum of the iodide contained in both the extracellular space and the thyroid cells, a high value in the relative specific radioactivity of iodide in the extracellular space might well be hidden. If we assume that all of the diiodotyrosine containing I^{131} has been newly manufactured (no exchange) \(14, 4\), then the findings of the high values at \(1\) hour for diiodotyrosine (3 times that of iodide) indicates that the recently arrived inorganic I^{131} in the thyroid cell apparently could not have contributed in any great part to the iodine (I^{131}) which is part of the diiodotyrosine molecule, for if it did the specific radioactivity of the iodide should be higher or at least as high as that in the diiodotyrosine fraction.

Another possibility arises. Previous to \(1\) hour, the specific radioactivity of iodide might have been very high and was decreasing at \(1\) hour, and more subsequently. Inspection of Fig. 1 shows that this possibility is incompatible with the findings, for the relative specific radioactivity of iodide is increasing between \(1\) and 8 hours. It is rather inconceivable that the relative specific radioactivity of iodide was very high previous to \(1\) hour, then fell rapidly, and then rose again. It is also possible that some of the iodide present in the thyroid gland may have its origin from the breakdown of the newly formed diiodotyrosine molecule. Therefore most of the inorganic iodide coming from the interstitial fluid, and part of which may be destined to enter the thyroid cell as iodide, must be transformed immediately into diiodotyrosine before it is incorporated into the cells. Most likely this transformation takes place at the level of the cell membrane. Two other alternative explanations may be examined: (1) The reaction might take place in the extracellular fluid of the thyroid. This is unlikely because of the lack of specificity of extracellular fluids in general. (2) Diiodotyrosine might come to the thyroid already made by some other tissue or organs. This seems highly improbable in view of previous results indicating that diiodotyrosine as such does not enter the thyroid gland \(14\).

The fact that the relative specific radioactivities of diiodotyrosine in the animals sacrificed in \(1\) hour are many times greater...
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than those found for thyroxine indicates that diiodotyrosine synthesis precedes thyroxine synthesis but does not necessarily mean that diiodotyrosine is a thyroxine precursor. That diiodotyrosine is a thyroxine precursor is demonstrated, however, by the additional evidence that there is practically a linear increase in the relative specific radioactivity of thyroxine throughout the period of time studied and to a magnitude which finally is relatively high. This means that the thyroxine originated from a compound having a high relative specific activity which has been maintained practically constant during this time. Inspection of Table I and Fig. 1 indicates that the relative specific radioactivity values of diiodotyrosine fulfil this requirement although the possibility that a small fraction of the total thyroxine formed may arise in another manner is not excluded.

From these data it is possible also to obtain some information concerning the absolute rate of formation or turnover of thyroxine. Hevesy and Hahn (15) have pointed out that the rate of formation of a substance can only be measured by the ratio of its specific radioactivity to the specific radioactivity of its precursor, and that the specific radioactivity of the latter must be kept constant. This ratio times 100 is equal to the per cent of the substance formed. An additional condition must be fulfilled, however, in order that the value be valid; namely, an insignificant amount, or none, of the radioactive compound must be removed. Since the relative specific radioactivity of diiodotyrosine apparently has remained more or less constant for most of the 48 hour period, and since not much "radioactive" thyroxine has been removed from the thyroid (otherwise the thyroxine curve would have a curvature which would be concave facing the abscissa), an approximate value for the rate of formation of thyroxine can be obtained. Using an average relative specific radioactivity of 1.3 for diiodotyrosine, we estimate that 1.55 per cent of the thyroxine is formed per hour.

SUMMARY

1. Inorganic iodine does exist as such in the normal, non-iodized thyroid gland of the dog.
2. The level of the relative specific radioactivity of the inorganic iodide in the thyroid gland does not rise high enough at any time
during the period of observations (½ to 48 hours) to indicate that it is the major source for the iodine of diiodotyrosine. Therefore, the conversion of the injected radioactive iodide into diiodotyrosine must take place at the level of the cell membrane. The results do not permit us to eliminate the improbable (14) hypothesis that diiodotyrosine arises elsewhere in the body and is secondarily fixed as such in the thyroid cell.

3. The diiodotyrosine fraction (obtained by subtracting the inorganic iodine and the thyroxine iodine from the total iodine) appears to be the natural precursor of thyroxine.

4. In the six dogs studied, it is estimated that 1.55 per cent of the thyroxine contained in the thyroid gland is formed per hour.

BIBLIOGRAPHY

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