IODINATED PARTICLES IN THE RAT THYROID
I. A RAPID SEPARATION METHOD

By
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ABSTRACT

In a first step, the quantitative preparation of the whole population of iodinated particles was reinvestigated in thyroids obtained from rats maintained in an isotopic equilibrium with $^{125}$I. The maximum yield in the iodinated particles was obtained for a 0.15 M sucrose solution in 0.1 M Tris-HCl buffer pH 7. This yield was 3.5% and 1.7% of the total iodine content of the thyroid for rats adapted to receive 50 (group 50) and 5 $\mu$g (group 5) of iodine daily respectively. The iodinated particles of group 50 were found to be more sensitive to pH variations than those of group 5.

In a second step, a rapid method was developed to separate the whole population of iodinated particles into physiological populations (tentatively colloid droplets and secondary lysosomes). By centrifuging onto a discontinuous Ficoll gradient, three distinct iodine peaks were regularly obtained for rats of group 5 and 50 and also for other rats receiving a diet rich in iodine. For group 50, the iodine pools ($^{127}$I) as measured by isotopic equilibrium were found to represent about 16% (peak 1), 25% (peak 2) and 59% (peak 3) of the total particulate iodine content of the gland.

The possible nature of these three populations of iodinated particles is discussed.

The thyroid follicles contain in their luminal colloid large amounts of thyroglobulin in which are bound by peptide linkage the thyroid hormones. Histochemical and radioautographic studies (Ponse 1951; Nadler et al. 1962; Wollman et al. 1964; Bauer & Meyer 1964) have clearly demonstrated that luminal...
colloid may enter the follicular cells by pinocytosis in the form of colloid droplets. It has also been demonstrated by histochemistry (Wetzel et al. 1965) that fusion of colloid droplets with lysosomes resulted in the formation of «secondary lysosomes» (de Duve 1964) in which proteolysis of thyroglobulin probably occurred (Wollman et al. 1964).

In fact, these two kinds of iodinated particles were only differentiated by electronmicroscopic cytochemistry (Wetzel et al. 1965) while the proteolytic activity was only demonstrated after homogenization on a «mitochondrial fraction» which probably contained both kinds of iodinated particles (Balasubramaniam & Deiss 1965; Balasubramaniam et al. 1965; Deiss et al. 1966; Ahn & Rosenberg 1967).

In an effort to separate the whole population of iodinated particles into physiological populations (tentatively colloid droplets and secondary lysosomes) a rapid method of separation was developed. The present investigation is concerned with the results obtained on the thyroid gland of the rat.

MATERIAL AND METHODS

Three groups of male Wistar rats weighing about 300 g were housed at 23°C ± 1°C with constant hygrometry (55% ± 5%) and regular lightning (light from 8 a.m. to 8 p.m.). They were adapted to receive daily, 5 μg of iodide (group 5), 50 μg of iodide (group 50) or a rich iodine diet (RID group) respectively. In addition, rats of group 5 and 50 were maintained in an isotopic equilibrium with 125I as previously described (Simon 1964).

In a first set of experiments, each rat received a tracer dose of 131I intraperitoneally 15 hours before death. The dose was usually 100 μCi (group 5), 200 μCi (group 50) and 200 μCi (RID group) respectively. In a second set of experiments, the tracer dose of 131I (120–200 μCi) was administered to rats of group 50, 8 to 24 hours before sacrifice. Immediately after sacrifice, the thyroid lobes were excised as quickly as possible and kept in the homogenization medium at 0–4°C until use. All the following steps of preparation were performed at +4°C. In all cases six lobes were homogenized in 1.5 ml of medium. After trying three different media (see Results) it was found that a 0.15 M sucrose solution buffered at pH 7.0 with 0.1 M Tris-HCl gave the best results. Homogenization was performed with an Ultra-Turrax homogenizer during 3 × 10 seconds at one third the value of the maximum speed. The homogenate was centrifuged at 600 × g during 10 min and the pellet discarded. The 600 × g supernatant was centrifuged at 34 000 × g during 15 min with the MSE High Speed 18 centrifuge (n° 69,181 rotor). The 34 000 × g pellet was used either unwashed or washed three times with the same medium.

The separation of the iodinated particles was obtained as follows. The 34 000 × g pellet was re-suspended in 0.5 ml of the same medium and layered on the top of a discontinuous gradient of Ficoll the concentrations of which were successively: 0.5% (1.25 ml), 1.5% (2.0 ml), 3% (2.5 ml), 10% (3.0 ml), 12% (2.5 ml) and 14% (4.1 ml). All solutions of Ficoll were made 0.15 M in sucrose and buffered at pH 7.0 with 0.1 M Tris-HCl. The centrifugation was run at 95 000 × g during 18 min with the MSE Super Speed 65 Ultracentrifuge (n° 59,590 rotor). Routinely 105–110 fractions.
of four drops (about 0.15 ml) were automatically collected with a Gilson fraction collector. The radioactivity content of each fraction (125I and/or 131I) was detected with a Packard Autogamma Spectrometer.

Iodine pools (127I) were calculated from 125I measurements for glands in isotopic equilibrium as previously described (Simon 1964). The specific radioactivity (SRA) of each fraction was expressed as % of injected dose of 131I/µg of 127I.

When necessary, Triton X 100 was used to destroy particles. The desired final concentration of detergent was obtained by adding to the suspension of particles a tenth its volume of a ten-fold concentrated solution of Triton X 100. After mixing, incubation was performed at room temperature for 15 min and the radioiodine content analyzed.

RESULTS

Total yield and pH sensitivity of iodinated particles

The total iodine content of the particles was determined for group 5 and 50 (isotopic equilibrium) with three-time washed 34 000 × g pellets.

With 0.1 M Tris buffer pH 7.0 the maximum yield of particulate iodine was obtained when this buffer was made 0.15 M in sucrose. Using 15 rats for experiment (a) and 18 rats for experiment (b) of group 5 distributed at random, it was observed that this medium gave better results than the unbuffered 0.25 M sucrose solution commonly used by other investigators and than the 0.15 M sucrose solution buffered at pH 7.0 with 0.1 M MOPS-NaOH (Table 1). Because of the insufficient number of measurements in each experiment no statistical study was performed. Nevertheless, it is clear from Table 1 that the sucrose solution buffered with Tris has consistently given the higher results for both the 127I content of particles and the 131I content, after labelling for 15 hours. From this, the relationship between yield and pH was studied with isotopically equilibrated rats of group 5 and 50 (Fig. 1). The same optimal pH of 7.0 was observed for both groups of rats. In this condition the yield in particles expressed as a percentage of total iodine content of the 600 × g supernatant was 3.5% and 1.7% for group 50 and 5 respectively. In addition, the iodinated particles of group 50 were found to be more sensitive to pH variations than those of group 5 (Fig. 1).

Particles separation

By centrifuging the 34 000 × g pellet (re-suspended in 0.5 ml of medium) on a discontinuous Ficoll gradient a rapid separation of the iodinated particles was obtained. As shown in Fig. 2, the sample was resolved into four radioactive peaks. It was observed that peak 4 (near the top of the gradient) was significant with an unwashed pellet but disappeared almost completely with a pellet previously washed three times. In addition, when 0.5 ml of the 34 000 × g supernatant was centrifuged in the same conditions, only peak 4
Table 1.
Sedimentation at 34,000 × g after three washings of iodinated particles from isotopically equilibrated (125I) thyroids. Each figure was given from a group of three rats of group 5 taken at random out of 15 rats for experiment (a) and 18 rats for experiment (b).

<table>
<thead>
<tr>
<th>Group 5</th>
<th>Total particulate iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>¹²⁷I content (°/oo 600 × g supernatant)</td>
</tr>
<tr>
<td>Homogenization medium</td>
<td>Experiment (a)</td>
</tr>
<tr>
<td>0.15 m Sucrose</td>
<td>2.70</td>
</tr>
<tr>
<td>in 0.1 m Tris-HCl</td>
<td>2.59</td>
</tr>
<tr>
<td>0.25 m Sucrose</td>
<td>1.82</td>
</tr>
<tr>
<td>in H₂O</td>
<td>1.29</td>
</tr>
<tr>
<td>0.15 m Sucrose</td>
<td>1.84</td>
</tr>
<tr>
<td>in 0.1 m MOPS-NaOH</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Tris: Tris(hydroxymethyl)aminomethane. MOPS: Morpholinopropanesulfonic acid.

was observed (Fig. 2). Furthermore, only peak 4 was also observed when 34,000 × g pellet was centrifuged after treatment with Triton X 100 (Fig. 3). From this, it was concluded that the total population of iodinated particles resolved into three distinct populations.

The pattern of Fig. 2 obtained with rats of the RID group was also obtained with group 5 and 50 (Fig. 4). It is noteworthy that for these three groups of rats, the pattern obtained for a 15 hour-labelling is similar but not identical, the distribution of the marker being dependent on the iodine diet.

Iodine pools

Group 50 was used to determine the respective iodine (¹²⁷I) pools of the three particulate peaks as measured with ¹²⁵I isotopic equilibrium. Table 2 gives the results for six experiments (18 rats) performed at different times of the year. For this group, the three corresponding iodine pools were found
Total yield in particulate iodine as a percentage of the iodine content of the 600 × g supernatant (left) and the same results expressed as a percentage of the maximum value for each group to show more clearly the pH sensitivity (right). Each group of rats was isotopically equilibrated with $^{125}$I as described by Simon (1964). For each pH, a three-times washed 34 000 × g pellet obtained from the thyroids of three rats was used. The medium of homogenization was a 0.15 M sucrose solution buffered with Tris plus HCl according to the pH.

to represent about 16% (peak 1), 25% (peak 2), and 59% (peak 3) of the total particulate iodine content of the gland.

As a preliminary experiment, group 50 was also used to test the labelling of the three peaks between 8 to 24 hours after $^{131}$I injection. Fig. 5 shows an example of such a labelling 15 hours after the injection. For all the intervals of time studied, it was consistently found that the SRA of peak 2 has an intermediate value between the SRA of peak 3 and peak 1. This and the intermediate position of peak 2 after centrifugation onto the gradient would appear to indicate that peak 2 is a mixture or an aggregate of particles of type 1 and type 3 (see Discussion).

**DISCUSSION**

In this preliminary work, we were only concerned with iodinated particles and no attention was paid to non iodinated particles such as primary lysosomes and mitochondria.

The total iodine content of the particles was found to represent 3.5% and 1.7% of the total iodine content of the gland (as represented by the 600 × g supernatant) for rats receiving 50 μg of iodine daily (group 50) and 5 μg of
Separation of iodinated particles (peaks 1, 2 and 3) and iodinated molecules (peak 4) obtained by centrifuging a 34 000 × g pellet on a discontinuous Ficoll gradient as described in the text. Three rats of the RID group were used after a 15 hour-injection of carrier free 131I (200 μCi each). The unwashed 34 000 × g pellet gave rise to the four peaks (+) but the 34 000 × g supernatant (●) gave only iodinated molecules (peak 4).

iodine daily (group 5) respectively. This result is in agreement with that of Balasubramaniam et al. (1965) who demonstrated that the total iodine content of particles accounts for about 2% of the total iodine content of the thyroid of the dog. This result also indicates that for group 50 the total particulate iodine pool is about twice as great as that of group 5. Similar results were previously obtained for the same groups of rats for total cellular iodine pool by Simon & Droz (1968). The results obtained in the present paper and by Simon & Droz (1968) lead to the conclusion that in the rat, the total particulate iodine pool and the total cellular iodine pool are both dependent on the iodine diet.

The whole population of iodinated particles (34 000 × g pellet) resolved consistently into three individual populations (peak 1, 2 and 3) after ultracentrifugation onto the Ficoll gradient. The particulate nature of the three corresponding peaks is well demonstrated by the action of Triton X 100
Radioiodine patterns obtained with three washed 34 000 x g pellets either untreated (full line) or treated with Triton X 100 at final concentrations of 0.2% (dashed line) or 0.4% (dotted line). Each pellet was prepared from the thyroids of three rats of the RID group after a 15 hour-injection of carrier free ¹³¹I (200 µCi each) and was centrifuged on the gradient as in Fig. 2.

Radioiodine patterns obtained with three washed 34 000 x g pellets prepared from the thyroids of three rats of group 5 (●), group 50 (●) and the Rid group (+) after they had received 100 µCi (group 5), 200 µCi (group 50) and 200 µCi (RID group) respectively, 15 h before sacrifice. Each pellet was centrifuged on the gradient as in Fig. 2.

(Fig. 3). Three populations of iodinated particles with the same position in the gradient were also found in the rabbit 24 hours after the ¹³¹I injection (unpublished data). It would be interesting to study other species in this way.

Normal rats were used and it seems reasonable to assume a steady state
Table 2.

Particulate iodine pools in rats of group 50 as determined by isotopic equilibrium with $^{125}$I after centrifugation on the Ficoll gradient and expressed as % of total particulate iodine content of the gland. The mean values and their standard deviations were calculated from six experiments (18 rats) performed throughout the year.

<table>
<thead>
<tr>
<th>Group 50</th>
<th>Peak 1</th>
<th>Peak 2</th>
<th>Peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.4</td>
<td>20.4</td>
<td>64.2</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>22.6</td>
<td>65.9</td>
</tr>
<tr>
<td></td>
<td>13.2</td>
<td>25.7</td>
<td>61.0</td>
</tr>
<tr>
<td></td>
<td>16.7</td>
<td>27.3</td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td>19.4</td>
<td>31.3</td>
<td>49.2</td>
</tr>
<tr>
<td></td>
<td>16.6</td>
<td>26.1</td>
<td>57.3</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>16.1</td>
<td>25.6</td>
<td>58.9</td>
</tr>
<tr>
<td>± 3.0</td>
<td>± 3.8</td>
<td>± 6.0</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5.

Separation of iodinated particles and iodinated molecules obtained by centrifuging a washed 34000 × g pellet on a discontinuous gradient of Ficoll. The pellet was prepared from the thyroids of three rats of group 50. Rats were maintained in an isotopic equilibrium with $^{125}$I and received 200 µCi of $^{131}$I (+) 15 h before sacrifice. The $^{127}$I content (•) of each peak was calculated from $^{125}$I measurements as described by Simon (1964).

for all the iodine pools of the thyroid (Simon 1964). Table 2 shows that such was the case in our conditions. The relative standard deviation (as expressed as % of the mean) was about 10 % for the greatest pool (peak 3) and about 18 % for the smallest pool (peak 1). From this, it is concluded that the re-
producibility obtained was good. Furthermore, the results of Table 2 were obtained throughout the year, and from this, it is also concluded that carefully controlled conditions of animal husbandry are required for such a study. It is noteworthy that the iodine content of peak 1 is low, averaging 0.2 µg 127I or less for group 50. For group 5, such a determination was impossible because the content of 125I of peak 1 was too low after isotopic equilibrium was established.

For a 15 hour-injection of 131I, the pattern of particulate 131I was similar but not identical for three groups of rats which were fed three different iodine diets (Fig. 4). This preliminary result indicates that the three populations of iodinated particles participate in the iodine turnover of the gland at a rate which is dependent on the daily iodine intake to which the rats were adapted.

Assuming a steady state (Table 2), if peak 2 were a mixing of peak 1 and peak 3, the fraction of each peak which participates in the presence of peak 2 would be constant. In this case, the SRA of peak 2 would be the weighted mean of each initial SRA of peak 1 and peak 3 at each time of labelling. Let y be the fraction of peak 2 which was contributed by peak 1. For each time of labelling this fraction may be calculated by solving the equation:

\[
\text{SRA (peak 2)} = [y \cdot \text{SRA (peak 1)}] + [(1 - y) \cdot \text{SRA (peak 3)}]
\]

As shown in Fig. 6 for group 50, this fraction is not constant but decreases

![Graph](image)

Fig. 6. Evolution with time of labelling of the theoretical participation y of peak 1 in the presence of peak 2 for rats of group 50 in isotopic equilibrium with 125I from 8 to 24 hours after injection of 131I (200 µCi). Each point represents a group of three rats for which separation of iodinated particles of the thyroid was obtained as in Fig. 5. The SRA of each peak was calculated and expressed as % 131I of the injected dose per µg 127I. The value of y was derived from the equation described in the text and plotted with time of labelling x. The slope of the regression line (-0.025) was highly significant (P < 0.005). This result is incompatible with a constant value of y with time of labelling.
with time from 8 to 24 hours after labelling. The slope of the regression line is highly significant ($P < 0.005$). From this, it is concluded that particles of peak 2 are not a mixture or an aggregate of two types of particles corresponding to peak 1 and peak 3.

Our preliminary results do not demonstrate a separation of colloid droplets and secondary lysosomes but support the hypothesis that more than two distinct populations of iodinated particles are present in the thyroid of the rat. These distinct populations of iodinated particles remain to be characterized by other methods such as electronmicroscopy and determinations of enzymic activities.

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REFERENCES


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