RADIOACTIVE PHOSPHORUS AS INDICATOR OF THYROID STIMULATION IN THE RAT

By
B.-A. Lamberg, P. Tala and U. Uotila

The use of radioactive phosphorus as an indicator of thyroid function was originally suggested by Borell & Holmgren (1949) for the assay of thyroid stimulating hormone (TSH) in the guinea-pig. They observed that TSH increased the uptake of P³² by the thyroid when the hormone was given in two doses at intervals of 24 hours. A simultaneous increase in the mean acinar cell height was noted. The uptake of P³², however, seemed to be a more sensitive gauge of thyroid function than the cell height. The uptake of P³² by the thyroid gland was later used as an index of thyroid function in assays of TSH in young chicks by Besford, Crooke & Matthews (1952), Crooke & Matthews (1953) and Lamberg (1952, 1953 a, b). It was also demonstrated that there was a close relationship between the uptake of P³² and the histological changes in the thyroid gland (Lamberg, 1953 b, Olin-Lamberg & Lamberg, 1953) when the histological activity was measured by using the mean acinar cell height (Starr & Rawson, 1937) and the percentage of epithelium (Uotila & Kannas, 1952) as indices of thyroid function.

While a close relationship between the uptake of P³² and the histological changes in the thyroid following a single injection of TSH was clearly demonstrated in young chicks, very little was known about the conditions in the non-hypophysectomized rat. Brimblecomb, Haigh, Halkerston, Reiss & Warledge (1952) reported that the intact rat did not respond satisfactorily to prolonged stimulation with TSH when the uptake of P³² was used as an index.

The response was improved, however, after hypophysectomy. Dedman, Mason, Morris & Morris (1953) showed in hypophysectomized rats that the uptake of P³² by the thyroid also increased following the injection of a single dose of TSH. These investigators were also able to demonstrate a decrease in the uptake following treatment with thyroxine, and an increase with propylthiouracil
and potassium thiocyanate in intact rats. Recently Goldberg & Wolff (1954) reported a similar finding following treatment with propylthiouracil, but pointed out that the response was rather poor.

As there seemed to be some evidence in favour of the view that the behaviour of $^{32}$P might be different after an acute stimulation with a single dose of TSH and prolonged stimulation either with TSH or thyrostatic drugs (Dedman, Mason, Morris & Morris, 1953) and since the relationship between the uptake of $^{32}$P and the histological changes was not elucidated in the intact rat, it seemed to be of considerable interest to investigate this further in order to clarify the conditions in this animal.

**MATERIAL AND METHODS**

Seventy-eight white male rats of the Wistar strain were used. 8 animals formed a control group, the remainder being divided in 14 groups of five animals each. Before the start of the experiments the animals were kept in a room at a constant temperature of 25–26°C.

A commercial product (Ambinon, Organon) was used as the thyrotropic preparation. It was injected intraperitoneally. $^{32}$P was given intraperitoneally 1 hr. before sacrifice in doses of about 12 $\mu$C with added carrier (Lamberg, 1953, Isotalo, 1953). After the animals were killed the thyroid lobes were immediately dissected out and weighed on a torsion balance. The left lobe was used for measurement of the uptake of $^{32}$P, the right one for histological studies. The radioactivity in a thyroid lobe was measured with a Geiger-Müller counter after the lobe had been dried at room temperature for 24 hours (Lamberg, 1953). The lobe used for histological purposes was immediately fixed in Bouin's solution, rinsed with alcohol, embedded in paraffin and then cut into two halves, and 8 to 10 slices were cut at 3 $\mu$ from the surfaces. The slices were stained with the Koneff's modification (1938) of Mallory's azan, which was used by Tala in earlier investigations (1952, 1953). The histological activity was measured with the histoquantitative linear method of Uotila & Kannas (1952) by which the percentage of epithelium, colloid and stroma is determined.

**RESULTS**

1. **Effect of a single and repeated stimulation with TSH.**

Two experiments were performed. In the first one TSH was given in doses of 2 guinea-pig units (Junkmann & Schoeller, 1932), in the second in doses of 20 units. The results are shown in Table 1 and some of them are graphically presented in Fig. 1.

The effect of a single injection of 2 units of TSH is illustrated in Fig. 1. Although there seems to be a tendency towards a rise both in the uptake of $^{32}$P and in the percentage of epithelium the changes are, however, not statistically significant. The same holds true with regard to the effect of a single dose of 20 units of TSH (Table 1). Twenty four and 48 hours after the ad-
Table 1.
Response of the rat thyroid to treatment with TSH.

<table>
<thead>
<tr>
<th>Dosage JS units</th>
<th>Hours after start of treatment</th>
<th>Histological data</th>
<th>Thyroid weight mg.</th>
<th>Uptake of P\textsuperscript{32}</th>
<th>per mg.</th>
<th>per whole thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E %</td>
<td>C %</td>
<td>S %</td>
<td>dE %</td>
<td></td>
<td>CPM/mg.</td>
</tr>
<tr>
<td>0 (contr.)</td>
<td>0</td>
<td>84.1</td>
<td>13.1</td>
<td>2.8</td>
<td>0.0</td>
<td>23.9</td>
</tr>
<tr>
<td>2 u.</td>
<td>2</td>
<td>80.3</td>
<td>18.2</td>
<td>1.2</td>
<td>-3.8</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>88.0</td>
<td>10.7</td>
<td>1.3</td>
<td>+3.9</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>84.5</td>
<td>12.3</td>
<td>3.2</td>
<td>+0.4</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>87.3</td>
<td>11.6</td>
<td>1.1</td>
<td>+3.2</td>
<td>17.0</td>
</tr>
<tr>
<td>2 × 2 u.</td>
<td>48</td>
<td>81.2</td>
<td>16.7</td>
<td>2.1</td>
<td>-2.9</td>
<td>14.7</td>
</tr>
<tr>
<td>3 × 2 u.</td>
<td>72</td>
<td>86.0</td>
<td>11.3</td>
<td>2.7</td>
<td>+1.9</td>
<td>18.3\textsuperscript{*}</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>90.2</td>
<td>7.6</td>
<td>2.2</td>
<td>+6.1</td>
<td>18.6\textsuperscript{*}</td>
</tr>
<tr>
<td>20 u.</td>
<td>4</td>
<td>81.8</td>
<td>15.1</td>
<td>3.1</td>
<td>-2.3</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>86.4</td>
<td>11.2</td>
<td>2.4</td>
<td>+2.3</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>86.1</td>
<td>12.4</td>
<td>1.5</td>
<td>+2.0</td>
<td>12.2</td>
</tr>
</tbody>
</table>

E %, C % and S % = percentage of epithelium, colloid and stroma.

CPM = counts per minute.

\textsuperscript{*}) Difference from control values statistically significant (p < 0.05).
Reaction of the rat thyroid to a single and repeated stimulation with 2 JS units of TSH.

A.
- CPM/whole thyroid
- Thyroid weight

B.
- CPM/mg.
- dE %

Administration of 3 injections of 2 units there is a significant increase in the uptake of P³² while the dE % is not significantly altered. The thyroid weight of the animals in these two groups also indicates a higher level of stimulation. It is thus evident that the thyroid of the intact rat does not react to a single dose of TSH but only responds to stimulation over a prolonged treatment.

2. Effect of treatment with propylthiouracil.

The results of this experiment are presented in Table 2. There is a significant response in every instance both in the uptake of P³², the histological changes and the thyroid weight. The stimulation seems to be maximal even at the 25 mg./day-level of dosage. When the uptake is measured as CPM/mg. thyroid tissue the uptake of P³² is increased to double. When the uptake is expressed as CPM/whole thyroid the maximal increase is about three-fold.
Table 2.
Response of the rat thyroid to treatment with propylthiouracil during 5 days.

<table>
<thead>
<tr>
<th>Daily dose mg.</th>
<th>Histological data</th>
<th>Uptake of P³²</th>
<th>per mg.</th>
<th>per whole thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E °/o  C °/o  S °/o</td>
<td>mg.</td>
<td>Ratio treated/ controls</td>
<td>mg.</td>
</tr>
<tr>
<td>0 (contr.)</td>
<td>84.1  13.1  2.8</td>
<td>0.0</td>
<td>14.2</td>
<td>23.9</td>
</tr>
<tr>
<td>25</td>
<td>97.0  1.6  1.4</td>
<td>12.9</td>
<td>20.9</td>
<td>48.7</td>
</tr>
<tr>
<td>50</td>
<td>96.7  2.2  1.1</td>
<td>12.6</td>
<td>25.3</td>
<td>42.2</td>
</tr>
<tr>
<td>75</td>
<td>95.9  2.3  1.8</td>
<td>11.8</td>
<td>19.1</td>
<td>47.9</td>
</tr>
<tr>
<td>100</td>
<td>97.2  2.0  0.8</td>
<td>13.1</td>
<td>24.4</td>
<td>45.9</td>
</tr>
</tbody>
</table>

E °/o, C °/o and S °/o = percentage of epithelium, colloid and stroma.
CPM = counts per minute.

3. Correlation between the uptake of P³² and the percentage of epithelium.

From the correlation diagram in Fig. 2 it is evident that there is some correlation between the uptake of P³² and the percentage of epithelium in the thyroid gland.

Fig. 2.
Correlation between uptake of P³² (CPM/mg.) and dE °/o.
A. Propylthiouracil treated animals.
Ordinate: Uptake of P³², ratio treated/controls.
Abscissa: dE °/o.
DISCUSSION

The results presented here confirm previous observations on the high resistance of the rat thyroid to stimulation with TSH (Aron, 1930, 1932, Brimblecomb et al., 1952, Janssen & Loeser, 1932, Junkmann & Schoeller, 1932, Loeb & Basset, 1930). The rat thyroid seems to be functioning at an almost maximal rate which is clearly illustrated by the behaviour of I$^{131}$ in intact and in hypophysectomized TSH-treated animals (Ghosh, Woodbury & Sayers, 1952). In our experiments a maximal stimulation of the thyroid gland with propylthiouracil brought about a two-fold increase in the uptake of P$^{32}$ which was in good agreement with other observations (Dedman et al., 1953, Goldberg & Wolff, 1954). Simultaneously the percentage of epithelium rose from 84 to 97 $\%$, i.e. dE $\%$ increased to about 13.

No increase either in the uptake of P$^{32}$ or in E $\%$ could be demonstrated after the injection of a single dose of TSH. Thus the question of a possible difference between an acute and prolonged stimulation of the thyroid gland could not be settled by the present experiments as far as the uptake of P$^{32}$ is concerned. Results obtained in the hypophysectomized rats treated with TSH (Dedman et al., 1952) indicate a more rapid turnover of P$^{32}$ than in the control animals; this rapid turnover evidently also includes an increased release of phosphorylated compounds from the thyroid gland. Thus the measurable uptake will probably be equal to the difference between the binding of P$^{32}$ by the thyroids and the simultaneous release of labelled compounds. If these two functions acted independently of each other i.e. the binding function being more active in the acute phase of stimulation compared with the release function than after prolonged stimulation this might explain the difference in response obtained in hypophysectomized rats (Dedman et al., 1952) and young chicks (Wahlberg, 1954) when given single and multiple doses of TSH. This hypothesis is worthy of further investigation.

SUMMARY

Studies were performed on the uptake of P$^{32}$ by the thyroid of the intact white male rat both after a single and repeated stimulation with thyrotrophic hormone and after prolonged treatment with propylthiouracil. It was shown that the thyroid reacted neither histologically nor by an increased uptake of P$^{32}$ to a single injection of 2 or 20 Junkman-Schoeller units. Prolonged treatment with TSH brought about a small increase in both end reactions and the treatment with propylthiouracil caused arise in the uptake to about double. This increase seemed to be maximal. It is concluded that the intact rat is not a suitable animal for P$^{32}$-studies on the stimulation of the thyroid gland.
REFERENCES

Tala, P.: Acta endocrinol. suppl. 9, 1952.
Tala, P.: Endocrinology 53, 474, 1953.