THE EFFECT OF A SINGLE DOSE OF THYROTROPHIN ON THE UPTAKE OF $P^{32}$ BY THE GUINEA-PIG THYROID

By

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

The effect of thyrotrophin (TSH) on the uptake of $P^{32}$ by the thyroid in experimental animals has been investigated on a limited scale. Borell & Holmgren (1949) observed a marked increase in the uptake in guinea-pigs 24 hours after the second injection of TSH, when this was given at 24 hourly intervals. Borell (1945) demonstrated a maximal histological reaction at the same interval of time. Tala (1952), on the other hand, observed a maximal response 2 hours after a single dose of TSH, but the reaction did not increase when stimulation was repeated. In view of this discrepancy it seemed to be of interest to investigate more thoroughly the effect of a single dose of TSH on the uptake of $P^{32}$ by the guinea-pig thyroid. Furthermore it should be borne in mind that a maximal uptake of $P^{32}$ and a maximal histological reaction have been observed within 4–8 hours after the injection of a single dose of TSH in young chicks (Besford, Crooke & Matthews, 1952, Crooke & Matthews, 1953, Lamberg, 1952, 1953 a, b). The hypophysectomized rat also responds to a single dose of TSH (Dedman et al., 1953), whilst the intact rat responds only to prolonged treatment (Lamberg, Tala & Uotila, 1955). Since certain results seem to indicate, moreover, that the histological reaction and the increase in the uptake of $P^{32}$ do not run parallel in the young chick (Lamberg, 1953 b, Olin-Lamberg & Lamberg, 1953, Lamberg & Olin-Lamberg, 1955, Lamberg, Wahlberg & Olin-Lamberg, 1955) we wanted to ascertain whether such a difference was also noticeable in the guinea-pig.

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Ponse (1951) emphasized that cell division is initially amitotic after stimulation with TSH; this is followed by a mitotic phase, which is in turn followed by another amitotic phase. Lever (1950) observed a maximum of mitotic cells in the chick thyroid 24 hours after the first injection, when 100 guinea-pig units of TSH were injected at 24 hourly intervals. The maximal rate was 1.34 per cent. Aron (1936), Cutting (1939) and Kippen & Loeb (1935) also suggested the use of the increase in mitosis as an index of thyroid activity following stimulation with TSH. An increase in the number of mitotic cells has also been observed after treatment with thiourea derivatives (Rerábek & Rerábek, 1947, Lever, 1950). One of the present authors has suggested that the metabolism of nucleic acid and the rate of mitosis may in some way be associated with the increased uptake of P³² by the thyroid in response to stimulation with TSH (Lamberg, 1955). Hence we thought it would be of interest to investigate the rate of mitosis in the present material.

**MATERIAL AND METHODS**

The material includes 60 male guinea-pigs weighing from 200 to 240 gm. Two weeks prior to the experiments they were kept under controlled dietary conditions in a special room with a temperature of 20 ± 2°C. An Ambinon powder (Organon) was used as the TSH preparation. It was dissolved in physiological saline solution and given as a single dose subcutaneously, since Tala (1952) has shown that the effect is the same whether TSH is administered subcutaneously or intraperitoneally. P³² with a carrier added was given subcutaneously in doses of 10–15 μc. 60 min. before sacrifice. Both TSH and P³² were injected in a volume of 0.25 ml. throughout the experiments.

Two experiments were performed. In the first, 25 animals were injected with 1 Heyl-Laqueur unit (1934) and sacrificed in groups of 5 after 1, 2, 4, 8 or 24 hours respectively. Five animals were used as untreated controls. In the second experiment 30 animals were used in 5 groups of 6 each. One group was used as untreated controls. The other 4 were injected with 0.001, 0.01, 0.1 or 1 unit.

One lobe was used for histological examination. It was fixed in Bouin's solution and stained by Koneff's method as previously described (Tala, 1952). The histological activity was estimated by Uotila & Kannas's linear histoquantitative method (1952) involving calculation of the percentage of epithelium (E%) ; the change in activity is expressed as the difference in E% between treated animals and controls (dE%). In the other lobe the uptake of P³² was measured when the preparations had been dried for 24 hours at room temperature (Lamberg, 1953 b). The uptake was expressed either as counts per minute (CPM) / mg. of thyroid tissue wet weight or as CPM / whole thyroid. The difference in the uptake was expressed as the ratio treated/controls.

For counting the mitotic cells in Exp. 1 the sections were stained with Mayer's hemalum and eosin. When the material was scanned, the rate of mitosis was found to be about 1–2 cells per section, and hence only one section per group of test animals

2. This preparation was obtained from Organon, Oss, Holland, through the courtesy of H. de Jager, M.D., to whom we wish to express our gratitude.
was thoroughly investigated, as the occurrence of mitotic cells being recorded in a total of 2000 cells.

Immediately after dissection the thyroid gland was weighed on a torsion balance.

RESULTS

1. Time-response relations. The results are shown in Fig. 1 and Table 1. Within 8 hours dE % reaches a maximal level, which is maintained for at least 24 hours after the injection of TSH. This is surprising in view of Tala's previous investigations (1952), in which a maximum was observed within 2 hours. When expressed as CPM/thyroid, the uptake of P32 reaches a maximal level within one hour; this is maintained for 24 hours after the injection. When, on the other hand, the uptake is expressed as CPM/mg., a maximum is also found to be reached within one hour, after which there is a tendency towards a decrease. The 4-hour level does not differ significantly either from the controls (t = 1.31, f = 6) or from the 1-hour level (t = 1.50, f = 7); it seems, however, to lie closer to the level of the controls. In view of the relatively small number of animals in the groups it is not possible to say exactly
Table 1.
Percentages of epithelium and uptake of $^{32}$P in response to a single dose of TSH.

<table>
<thead>
<tr>
<th>Time hrs.</th>
<th>E $%$</th>
<th>dE $%$</th>
<th>CPM/mg.</th>
<th>t/c</th>
<th>CPM/thyroid</th>
<th>t/c</th>
<th>Thyroid weight, mg.</th>
<th>No. of mitoses in 2000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Contr.)</td>
<td>76.7</td>
<td>0</td>
<td>23.6</td>
<td>1.00</td>
<td>579</td>
<td>1.00</td>
<td>31.4</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>71.7</td>
<td>-5.0</td>
<td>31.4</td>
<td>1.33</td>
<td>852</td>
<td>1.47</td>
<td>30.3</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>79.5</td>
<td>2.8</td>
<td>29.3</td>
<td>1.24</td>
<td>872</td>
<td>1.51</td>
<td>29.8</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>82.5</td>
<td>5.8</td>
<td>26.9</td>
<td>1.13</td>
<td>855</td>
<td>1.48</td>
<td>30.9</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>85.5</td>
<td>8.8</td>
<td>30.5</td>
<td>1.29</td>
<td>856</td>
<td>1.48</td>
<td>28.2</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>86.0</td>
<td>9.3</td>
<td>33.1</td>
<td>1.40</td>
<td>948</td>
<td>1.64</td>
<td>29.1</td>
<td>1</td>
</tr>
</tbody>
</table>

CPM = counts per minute.

$ t/c = \text{ratio treated/controls.} $

where the maximum lies, nor whether there is an actual decrease. After 4 hours the histological and the uptake reaction appear to run parallel.

![Graph](https://via.placeholder.com/150)

**Fig. 2.**
The effect of various doses of TSH on the percentage of epithelium and the uptake of $^{32}$P in the thyroid gland.

- $\circ$ $dE\%$
- $\bullet$ $^{32}$P, CPM/mg.
- $\bigcirc$ $^{32}$P, CPM/whole thyroid.

Ordinates: left, uptake of $^{32}$P, ratio treated/controls; right, $dE\%$.

Abscissa: dose in Heyl-Laqueur units.
Table 2.

Percentage of epithelium and uptake of P³² in response to a single injection of various doses of TSH.

<table>
<thead>
<tr>
<th>Dose unit *</th>
<th>E %/0</th>
<th>dE %/0</th>
<th>CPM/mg.</th>
<th>t/c</th>
<th>CPM/thyroid</th>
<th>t/c</th>
<th>Thyroid weight, mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Contr.)</td>
<td>72.8</td>
<td>0</td>
<td>12.5</td>
<td>1.00</td>
<td>357</td>
<td>1.00</td>
<td>29.2</td>
</tr>
<tr>
<td>0.001</td>
<td>72.2</td>
<td>-0.6</td>
<td>12.3</td>
<td>0.99</td>
<td>361</td>
<td>1.01</td>
<td>29.5</td>
</tr>
<tr>
<td>0.1</td>
<td>75.9</td>
<td>3.1</td>
<td>12.6</td>
<td>1.00</td>
<td>375</td>
<td>1.05</td>
<td>29.5</td>
</tr>
<tr>
<td>0.1</td>
<td>78.8</td>
<td>6.0**</td>
<td>12.6</td>
<td>1.00</td>
<td>331</td>
<td>1.00</td>
<td>27.7</td>
</tr>
<tr>
<td>1.0</td>
<td>84.0</td>
<td>11.2**</td>
<td>18.2**</td>
<td>1.46</td>
<td>492**</td>
<td>1.38</td>
<td>27.4</td>
</tr>
</tbody>
</table>

CPM = counts per minute.
t/c = ratio treated/controls.
* = Heyl-Laqueur unit.
** = difference from controls statistically significant (P < 0.05).

The difference between the histological and the P³² uptake reaction prior to the 4-hour period, particularly at the 1-hour interval, where dE% shows a non-significant decrease despite a maximal increase in the uptake of P³², is interesting and will be discussed.

2. Dose-response relations. In view of Tala's (1952) previous results, 2 hours was chosen as the experimental period, the histological results of the former experiment not being measured. Hence the dose-response was not investigated at the optimal point of time. The results are demonstrated in Fig. 2 and Table 2. There is a linear rise in dE% but the increase does not become significant until after 0.1 unit. The uptake of P³² does not show a significant increase until after 1.0 unit.

3. Occurrence of mitotic cells. As previously mentioned, one section from each of the 6 groups of test animals was investigated. The results appear in Table 1.

DISCUSSION

The present results raise some interesting problems.

1. In the same laboratory Tala (1952) previously demonstrated a maximal histological reaction 2 hours after injection of another Ambinon powder. The occurrence of a maximum at 8 hours in the present experiments was therefore quite unexpected. In chicks, a maximal response in the uptake of P³² at 8 hours, and in the percentage of epithelium at 4 hours, was simultaneously obtained by Wahlberg (1955) in another laboratory, with the same Ambinon powder as
was used in the present investigation. Olin-Lamberg & Lamberg (1953) showed that the mean acinar cell height and the uptake of P\(^{32}\) ran parallel in chicks. The fact that the maximal histological reaction occurred at 2 hours in Tala's previous investigations (1952), and at 8 hours in the present experiments may, perhaps, be referred to a difference between the TSH preparations employed. The same factor may also be responsible for the different localization of the maximum figures in Borell's (1952) and Tala's (1952) investigations. It should also be remembered, however, that markedly different responses have been obtained when the test animals have been of the same species, but of different strains and broods, and when there have been differences in environment. This question is discussed in greater detail elsewhere (Lamberg, 1955).

2. The uptake of P\(^{32}\) reached a maximal level within one hour of the injection of TSH. At 2 and 4 hours a tendency towards a decrease was observable; an increase then set in, and the maximal level was again reached 8 hours after injection. Obviously the response in the uptake was immediate, whilst the percentage of epithelium did not begin to increase until after 2 hours. After the 4-hour interval the uptake of P\(^{32}\) and E \(\%\) seem to run parallel.

From investigations on hypophysectomized rats Dedman et al. (1953) concluded that the increased uptake of P\(^{32}\) by the thyroid in response to stimulation with TSH depends on two separate reactions: an initial reaction, which rapidly reaches a maximum and is independent of thyroid growth, and a late reaction, in which thyroid growth plays a part. This view is not supported by the present results, if thyroid growth is taken to mean an increase in thyroid weight. If, on the other hand, this expresses growth of the epithelium, then the present investigation lends support to this statement. It is possible that the time-response curve for the uptake of P\(^{32}\) consists of two partial curves: an initial curve and a later curve, which follows the growth of the epithelium.

The relation between the time-response curves for the uptake of P\(^{32}\) and the histological reaction seems to depend on the species used. In the guinea-pig there is an immediate increase in the uptake; the histological curve runs parallel with the P\(^{32}\) curve only at a later phase. In young chicks, on the other hand, the percentage of epithelium reaches a maximum earlier than the uptake of P\(^{32}\), and after 24 hours the uptake has decreased considerably, whilst only a tendency towards a decrease is observed in the histological activity (Lamberg, 1953 b, Olin-Lamberg & Lamberg, 1953). The present results seem, moreover, to indicate that there is a fundamental difference between the histological reaction and the P\(^{32}\) uptake reaction, as has previously been emphasized (Olin-Lamberg & Lamberg, 1953, Lamberg & Olin-Lamberg, 1955, Lamberg, Wahlberg & Olin-Lamberg, 1955).

3. In the present investigation no marked correlation was demonstrable between E \(\%\) and the uptake of P\(^{32}\) with regard to the dose-response, such as was seen in chicks (Lamberg, 1953 b). Although the dose-response was in-
vestigated at a period which was not optimal with regard to the histological reaction, as was emphasized above, a linear rise in response to different doses is discernible in this curve (Fig. 2). Owing to the unfortunate choice of an experimental period of 2 hours the results do not, however, afford any clue about the magnitude of the optimal reaction obtainable with the doses of TSH in question. To some extent the results nevertheless confirm Tala’s (1952, 1953) views concerning the usefulness of the guinea-pig as a test animal for assay.

In the present experiments the response in the uptake of P³² was not as sensitive as the histological response. It is possible, however, that the time of investigation was also not advantageous in this respect, a slight decline being observed at the 2-hour period in the time-response curve in Fig. 1. The transient character of the maximum at one hour is a disadvantage as far as assay methods are concerned. It is possible that more sensitive results would have been obtained by using an 8-hour experimental period, since the maximum at this point remained at the same level for some time. The relative inertia of the histological response is an advantage both from a practical and from a theoretical point of view.

4. The fact that the rate of mitosis did not increase in the present experiments may, perhaps, be referred either to the time of treatment or to the dose. Kippen & Loeb (1935) and Cutting (1939) did not observe any marked increase until after treatment with TSH for 2–5 days; the dosage, however, is not indicated in their publications. Lever (1950) observed a maximum 24 hours after the administration of a single dose of TSH; the dose, however, was 100 guinea-pig units which is 100 times more than the dose employed in the present investigation. Obviously the change in mitotic activity is slower than the other histological reactions. The suggestion (Lamberg, 1955) that there is an increased rate of mitosis with increased uptake of P³² is not substantiated by the present results.

SUMMARY

The effect of a single dose of 1.0 Heyl-Laqueur unit on the percentage of epithelium, the rate of mitosis, and the uptake of P³² in the thyroid was investigated on guinea-pigs. The histological reaction reached a maximum within 8 hours after injection of TSH; in a previous investigation Tala (1952) observed a maximum at 2 hours using another TSH preparation. The difference between these results is discussed. The uptake of P³² increased maximally within one hour after injection; it then diminished only to reach another maximum at 8 hours. After the 4-hour point the histological curve and the uptake curve ran parallel. The results seem to indicate that there is a fundamental difference between these two end reactions.
Owing to the unexpected localization of the maximum on the time-action curve, the dose-response investigations were not entirely convincing. The results obtained 2 hours after stimulation showed, however, that there was a sensitive histological response in guinea-pigs which increased steadily with increased doses of TSH.

The rate of mitosis did not increase during the experiment; only a few mitotic cells were observed per 2000 cells. Hence it may be concluded that the effect of TSH on the uptake of P32 is not due to any increase in the mitotic activity of the thyroid.

REFERENCES

Crooke, A. C. & Matthews, J. D.: Ciba Foundation Colloquia on endocrinology 5, 25, 1953.
Tala, P.: Endocrinology 53, 474, 1953.