THE UPTAKE RATIO BETWEEN THE THYROID AND THYMUS AS AN INDEX OF THYROID FUNCTION IN ASSAYS OF THYROTROPHIC HORMONE WITH P₃² AS INDICATOR

By

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Borell & Holmgren (1949) observed that thyrotrophic hormone (TSH) increased the uptake of radioactive phosphorus by the thyroid in guinea-pigs. TSH was given in two doses with an interval of 24 hours, and the animals were sacrificed on the third day, 40 min. after an intraperitoneal injection of 50 μc. P₃². These investigators assumed that P₃² would be a suitable indicator for the assay of TSH on guinea-pigs, but they did not elaborate any special method. Using radioactive phosphorus on young chicks Lamberg (1953) demonstrated that the uptake of radioactivity in the thyroid eight hours after the administration of a single dose of TSH can be used as an index of thyroid activity. He also elaborated a method for the assay of small amounts of TSH involving injection of P₃² subcutaneously 60 min. before sacrifice. In this way it was possible to detect doses of about 0.005 Junkmann-Schoeller unit (JS unit, Junkmann & Schoeller, 1932). The utilizable dosage range was found to include doses from about 0.005 to 0.05 JS unit. Whilst the paper concerned was in the press, Besford, Crooke & Matthews (1952) reported similar results from assays with chicks.

Some experiments, however, had given rise to the suspicion that the absorption of P₃² from the subcutaneous tissue in young chicks may be subject to individual variations. Investigations were therefore made to determine whether the uptake of P₃² by the thyroid could be replaced by a relative uptake as an index of thyroid activity. Variations in absorption are of course reflected in the uptake by the thyroid and other organs. The use of a relative uptake, i. e.

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the ratio between the uptake by the thyroid and some other organ, would per-
haps reduce to some extent the error caused by these variations even if it
would not eliminate it altogether. Owing to its anatomical position and glan-
dular structure the thymus seemed to be a suitable organ for comparison. In
spite of the fact that the relations between the thyroid, hypophysis and thymus
are not yet clarified, the thymus was chosen and the effect of TSH on this
organ investigated at the same time in assays of short duration.

MATERIAL AND METHODS

The material includes 1379 White Leghorn chicks from one to three days old. They
were all taken from the same hatchery in order to rule out the variations in activity
and sensitivity to TSH of the thyroid noticeable between broods from different hat-
tcheries (Bates, Riddle & Lahr, 1941). The chicks were taken a few hours after hatching
and transported in a heated car to the laboratory, where they were kept in open cages
in which the temperature was maintained at about 36° C. by means of ordinary in-
candescent lamps. During the first 24 hours the chicks were given nothing but tap
water; later they were fed with a commercial starting mash. The sex was determined
at autopsy. This was, however, not taken into consideration in the evaluation of the
results.

As thyrotrophic preparations two Aminon powders were used (Nordiska Organon
AB. and N. V. Organon, Oss). They were either dissolved in alkaline distilled water
or suspended in physiological saline and filtered; the powder did not dissolve in
distilled water or in physiological saline without the addition of alkali (NaOH). It
was shown that the potency of the solution was the same irrespective of the manner in
which it had been made. Throughout the investigation the preparation was injected
subcutaneously into the breast in a volume of 0.3 ml.

Radioactive phosphorus with a carrier added was injected subcutaneously into the
other side of the breast in a volume of 0.3 ml in doses of 2–12 μc.

The animals were killed by decapitation. One or two samples of the thymus were
taken; their weight varied between 2 and 10 mg. Both thyroid lobes were dissected
out and united into one preparation after weighing. The samples of firm tissue were
weighed on a torsion balance graduated from 0.02 mg. to 10 mg. They were placed
in small aluminium planchets belonging to the device for measurement of radioactivity,
and dried at room temperature for 24 hours. This method of drying had been found
suitable in investigations of this kind (Lamberg, 1953). Radioactivity was measured
with an end-Mica-window Geiger-Müller counter. The uptake of P³² by the organs
concerned was expressed as counts per minute (CPM)/mg. fresh tissue.

In some experiments the activity in the serum was also measured. The samples were

2. This commercial starting mash (Punaheltta) is manufactured by Messrs. Vasa Ång-
kvarnsaktiebolags Foderkvarn, and consists, according to the manufacturers' de-
claration, of 12 % moisture, 5.2 % raw fat, 24.5 % raw protein, 6 % raw fibre,
45.4 % nitrogen-free extractable substances, and 6.9 % ash.
3. By the courtesy of Docent Fr. Paulsen, M. D. and H. de Jager, M. D., to whom
I wish to express my sincere gratitude.
4. The apparatus is owned by the Medicinska Understödfsöreningen Liv och Hälsa
and constructed at the Institute of Physics, Helsingfors University.
obtained by pipetting 0.02 ml. of serum on round discs of filter paper glued to the bottom of the aluminium planchets. Activity was expressed as CPM/0.02 ml. serum.

RESULTS

1. Absorption of $P^{32}$ from a subcutaneous store and uptake by the thyroid and thymus. Untreated, 1- to 3-day-old chicks were injected subcutaneously with $P^{32}$ and sacrificed at given intervals. The activity of the serum, the thyroid and the thymus was measured. The concentration curves for serum and the thyroid have also been published elsewhere (Lamberg, 1953). The assays consisted of a number of separate experiments. In order to obtain comparable results the number of impulses was reduced in each experiment so as to correspond to 1 $\mu$C., after which the different curves were combined. The results are presented in Fig. 1.

It is found that the serum activity reaches a maximum about 15 min. after injection, and then rapidly subsides. With regard to its general shape, this curve is in agreement with the activity curves for rabbits published by Hevesy & Hahn (1940), Palm (1948), and others. The uptake by the thyroid is also fairly rapid. Within 30 min. a maximal level is reached, which is maintained for at least 180 min. The slight decline is not statistically significant. The

![Fig. 1.](image)

Time-concentration curve on $P^{32}$ in the serum, thyroid and thymus after a subcutaneous injection.

- - - - serum    O-O thyroid    x-x thymus.

Ordinates: right: counts per minute/mg. tissue.

left: counts per minute/0.02 ml. serum.

Abscissa: time in minutes.
uptake by the thymus is found to be more than twice the uptake by the thyroid. The maximum of the concentration curve for the thymus is not reached until about 120 min. after injection.

2. The effect of a single dose of TSH on the uptake of P\textsuperscript{32} by the thyroid and thymus. Day-old chicks were injected with 0.5 JS units of TSH and sacrificed after 2, 4, 6, 8, 12, 24 or 48 hours. P\textsuperscript{32} was always given 60 min. before sacrifice. Activity was measured from the thyroid and thymus. The increase in uptake was expressed as the ratio between the uptake by the organ concerned in treated animals and in untreated controls, which were also given P\textsuperscript{32} 60 min. before sacrifice (CPM/mg\textsubscript{treated} : CPM/mg\textsubscript{controls}). Furthermore the ratio between the uptake by the thyroid and the thymus was calculated (CPM/mg\textsubscript{thyroid} : CPM/mg\textsubscript{thymus}). This ratio was compared with the corresponding ratio in untreated controls

\[
\frac{\text{CPM/mg}_{\text{thyroid}} : \text{CPM/mg}_{\text{thymus}} - \text{treated}}{\text{CPM/mg}_{\text{thyroid}} : \text{CPM/mg}_{\text{thymus}} - \text{controls}}.
\]

The results of three experiments are given in Fig. 2.

**Fig. 2.**

Time-action curve on the uptake of P\textsuperscript{32} by the thyroid and the thymus and on the uptake ratio between thyroid and thymus. Dose 0.5 JS unit. P\textsuperscript{32} was given 60 min. before sacrifice.

- O——O uptake by the thyroid
- x——x uptake by the thymus
- ◯——◯ uptake ratio.

Ordinate: ratio treated/controls.
Abscissa: time in hours.
The uptake by the thyroid increases rapidly, the increase being about 50 per cent two hours after injection of TSH. It continues linearly until 8 hours after injection, at which time the uptake has increased by about 450 per cent and a maximum has been reached. This level is maintained for at least 12 hours after injection, after which the concentration curve declines. After 48 hours the level of the control is again reached.

During a corresponding period of time the uptake by the thymus does not change much. The deviation from the control level discernible in the figure is statistically significant (95 per cent fiducial limits) only after 4 hours, but the number of animals used for this point was no more than six and only one experiment is represented. The significance of this individual deviation is therefore very questionable, and it cannot, without further evidence, be regarded as typical of the effect of TSH on the thymus.

As was to be expected, the time-concentration curve obtained by noting the uptake ratio between the thyroid and thymus corresponds well with the concentration curve for the thyroid, with one exception: the 4-hour-period. This deviation is, of course, due to the corresponding deviation in the curve for the thymus and must be regarded with the same reservation.

3. Use of the uptake ratio between the thyroid and thymus as an index in the assay of TSH. A total of 14 dose-response curves were made on 1- to 3-day-old chicks. The animals were injected with various doses of TSH, and P\textsuperscript{32} was given 60 min. before sacrifice, which took place 8 hours after the injection of TSH since the effect on the uptake by the thyroid has reached a maximum at this time. The results appear in Fig. 3. Each point of the curves represents the mean value of six to eight animals. Activity is expressed as uptake ratio. It is found from these dose-response curves that there is a direct relation between the increase in the uptake ratio and the log dose within certain dosage limits. The linear portion of the dose-response curves lies approximately between 0.005 and 0.05 JS unit. The slope and absolute level of the curves varies in the different experiments. These variations do not seem to be due to seasonal factors. Exp. 8 was performed in February, Exp. 9, 10, 14, 20 in March, Exp. 11, 12, 13, 15, 16, 17, 21 in May and Exp. 18 and 19 in June of the same year. As a rule the level of the curves from February to March seems to be somewhat higher, and their slope seems to be somewhat steeper, than that of the curves obtained in May and June, but exceptions are found. It does not seem justifiable, therefore, to ascribe this variation to seasonal factors. It seems rather to be contingent on the type of response to TSH peculiar to each brood. The same appears to hold good with regard to the uptake by the thyroid alone (Lamberg, 1953).

In three series of experiments the influence of age on the response of the thyroid to TSH was investigated. Essentially symmetrical dose-response curves
Fig. 3.
Dose-response curves on 1-, 2- and 3-day-old chicks. Uptake ratio between thyroid and thymus after a subcutaneous injection of various doses of TSH. Experimental period 8 hours. P32 was given 60 min. before sacrifice.
Ordinate: ratio treated/controls.
Abscissa: dose in JS units.
were made on two or three successive days. The results appear in Fig. 4. The curves are of the same type irrespective of the age of the experimental animals, which implies that either 1-, 2- or 3-day-old chicks may be used, without any difference in the response to TSH.

Since Burn, Finney & Goodwin (1950) stated that parallel-line assays, using some standard preparation for comparison, are necessary in the test of unknown preparations, two parallel-line determinations were performed.

A 4-point design was used for investigating the effect of the solvent on the potency of TSH. Ambinon powder was suspended in distilled water and

\[ \text{Ordinates: ratio treated/controls.} \]

\[ \text{Abscissae: dose in JS units.} \]

**Fig. 4.**

Influence of age on the uptake ratio between thyroid and thymus after a subcutaneous injection of various doses of TSH. Dose-response curves made on 1-, 2- and 3-day-old chicks of the same brood. Experimental period 8 hours. P³² was given 60 min. before sacrifice.

- • 1-day-old chicks.
- ••••••••• 2-day-old chicks.
- •---••• 3-day-old chicks.
made slightly alkaline by the addition, drop by drop, of sodium hydroxide. Thus a clear solution was obtained, which was injected into 16 animals, 0.01 JS unit into eight and 0.05 JS unit into the other eight. The potency of this solution was compared with the potency of another solution, prepared by suspending Ambinon powder in physiological saline and filtering off the insoluble portion. The solutions were prepared so as to be equally potent provided that no loss occurred. The latter solution was also injected into 16 animals, of which half were given 0.01 JS unit and the other half 0.05 JS unit. The result of the assay is represented graphically in Fig. 5a. It is seen that the slope of the two curves is about the same, and a statistical analysis showed that there is no significant difference between them. It is therefore justifiable to conclude that the solutions concerned are equally potent although differently prepared.

The other assay, in which a 6-point design was used, involved a comparison of the Heyl-Laqueur unit (HL unit, Heyl & Laqueur, 1934) and the Andersen unit (A unit, Andersen, 1943). According to the manufacturers, 1 HL unit corresponds to 0.25 A unit. Alkaline aqueous solutions were made so as to be equally concentrated on the basis of the manufacturers' statement. Twenty-one animals, seven for each point, were injected with one of the solutions in doses

![Graph A](attachment:image1.png)

![Graph B](attachment:image2.png)

Fig. 5.

4- and 6-point assays.


A: Comparison between an alkaline and a saline solution of the same TSH preparation.
B: Comparison between A unit and HL unit.
Table 1.

Uptake of P32 by the thymus in day-old chicks after a subcutaneous injection of various doses of gelatine and ovalbumin. Experimental period 8 hours.

<table>
<thead>
<tr>
<th>Series No</th>
<th>Preparation</th>
<th>Dose µg.</th>
<th>No. of animals</th>
<th>CPM/mg. ± e</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gelatine</td>
<td>0 (Contr.)</td>
<td>8</td>
<td>60.5</td>
<td>3.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>7</td>
<td>72.0</td>
<td>3.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>8</td>
<td>72.0</td>
<td>4.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>4</td>
<td>74.2</td>
<td>7.10</td>
</tr>
<tr>
<td>2</td>
<td>Gelatine</td>
<td>0 (Contr.)</td>
<td>10</td>
<td>86.5</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>7</td>
<td>98.2</td>
<td>6.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>8</td>
<td>95.0</td>
<td>4.21</td>
</tr>
<tr>
<td></td>
<td>Ovalbumin</td>
<td>6</td>
<td>10</td>
<td>109.5</td>
<td>5.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>8</td>
<td>97.8</td>
<td>6.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>10</td>
<td>91.4</td>
<td>5.14</td>
</tr>
</tbody>
</table>

of 0.01, 0.02 and 0.04 HL unit, whilst the same number of animals per point were injected with the other solution in doses of 0.0025, 0.005 and 0.01 A unit. The result of this assay appears from Fig. 5 b. It is seen that the slope of the curves is about the same; no significant difference between the two dose-response curves was demonstrable. It may therefore be concluded that 1 HL unit corresponds to 0.25 A unit.

4. The effect of ovalbumin and gelatine on the uptake of P32 by the thymus.

For the purpose of testing the specificity of the increase in the uptake ratio, gelatine and ovalbumin solutions were injected into day-old chicks in doses corresponding to the amounts in µg. of Ambinon used in the previous assays (6 µg. = 0.01 JS unit). The results appear in Table 1. In 3 cases of 8 the p value obtained in Student's test was less than 0.05; in 2 it was very near the 0.05 level. Using 95 per cent fiducial limits these three cases differ significantly from the control material, which would imply that small amounts of indifferent protein cause a slight increase in the uptake by the thymus. However, it was found that a statistically significant increase was an occasional occurrence not at all comparable with the change in the uptake ratio by TSH. It would not, therefore, be justifiable to generalize the slight change in the uptake by the thymus that did perhaps occur in these assays. It seems more reasonable to ascribe this change to unknown variations in the animal material. In any case it is no contraindication to the use of the uptake ratio between the thyroid and the thymus as an index of thyroid activity.

Lamber (1953) demonstrated that the uptake of P32 by the thyroid is at least as specific a reaction to TSH as the histological changes in the gland. He
emphasized, however, that in general the question of specificity of the reaction cannot be regarded as settled by his results; further investigations are required for the individual problems to which P³² method may be applied. The same holds good with regard to the present study.

**DISCUSSION**

The relations between the hypophysis and thyroid, on the one hand, and the thymus, on the other, have attracted a great deal of interest. Nevertheless they are not yet completely understood. There is no doubt, however, that the thymus is influenced by the thyroid hormone. Thyroxin treatment of experimental animals causes involution of the thymus (Schockaert, 1931, Gregoire, 1941). Furthermore phosphorus metabolism and the uptake of radioactive phosphorus by the thymus are also reduced (Fraenkel-Conrat & Li, 1949, Östling & Lamberg, 1951), which seems to indicate that some biochemical processes, at least, are connected with involution. On the other hand, quite contrary effects have also been observed after the administration of TSH or thyroid hormone (Schockaert, 1931, Gregoire, 1941). Gregoire (1941) held that the administration of small doses of TSH increases the function of the thymus, whilst involution results from doses exceeding a certain level. The same observation has been made on the rest of the lymphocyte-producing apparatus (Kleine & Paal, 1934, Gregoire, 1941). The lymphatic reaction in thyrotoxic conditions is also well-known (Crotti, 1936, Gregoire, 1942, Biström, 1946, Means, 1948).

Thyroidectomy and treatment with antithyroid drugs are accompanied by involution of the thymus, whilst thymectomy seems to cause thyroid hyper trophy (Chiodi, 1940, Comsa, 1948, 1951 a, c, Gregoire, 1949, Sterba, 1951). Thymectomy seems, moreover, to result in an increased excretion of TSH in the urine of experimental animals (Comsa, 1951 b, c), and the thymus appears to be capable of inactivating TSH in vitro, though not to the same extent as thyroid tissue (Rawson, Sterne & Aub, 1942, Rawson, 1949).

Under the experimental conditions of the present investigation, the uptake of P³² by the thymus was not influenced by the doses used. The present results show, too, that the relations between the hypophysis and thyroid, on the one hand, and the thymus, on the other, cannot be studied by the simple P³² method employed here. It is apparently necessary to examine radioactivity in the different phosphorus fractions in the thymus, as has been done by Fraenkel-Conrat & Li (1949) and Borell & Diezfalusy (1952).

On comparing the present results, with those obtained on the same material using the uptake by the thyroid alone as an index of thyroid activity (Lamberg, 1953), it is found that the dose-response curves of the present study are less dispersed. Their course is also somewhat more even. The first series of ex-
experiments on the influence of age, demonstrated here in Fig. 4 a, is particularly interesting in this connection. The different dose-response curves are here essentially identical. On the other hand, when the uptake of $\text{P}^{32}$ by the thyroid alone was used as index, the curve for the 2-day-old animals differed markedly from the rest (see Lamberg, 1953, part IX, Fig. 13). This fact seems to indicate that disturbances of absorption are involved, which influenced the form of the curve when the uptake by the thyroid alone was used as an index.

With regard to sensitivity and the utilizable dosage range, the results of the two investigations are in complete agreement. In the present study the response to 0.001 JS unit differed significantly from the control level, which was not the case when the uptake by the thyroid alone was examined. It is not possible to decide on the basis of a single observation whether there is any difference in this respect between the two methods concerned. The results presented here seem, however, to argue in favour of the use of the uptake ratio between the thyroid and thymus as an index of thyroid activity in assays of small amounts of TSH as well as the uptake of $\text{P}^{32}$ by the thyroid alone.

**SUMMARY**

The effect of thyrotrophin (TSH) on the uptake of $\text{P}^{32}$ by the thyroid and thymus was assayed using young chicks as experimental animals. The following deductions are drawn:

1. Treatment with TSH in the doses employed did not influence the uptake of $\text{P}^{32}$ by the thymus.

2. Indifferent protein in the doses employed did not seem to influence the uptake of $\text{P}^{32}$ by the thymus.

3. The degree of thyroid activity caused by TSH treatment may equally well be estimated from the uptake ratio between the thyroid and thymus as from the uptake by the thyroid alone. The level and course of the dose-response curves is the same in both cases. It is found that the error caused by certain variations in absorption is apparently eliminated when the uptake ratio is used as index, and that the course of the dose-response curves is therefore more uniform. The lower limit of sensitivity in both cases is about 0.001 JS unit, and the utilizable dosage range is the same, or between about 0.005 and 0.05 JS unit.

4. The uptake ratio between the thyroid and thymus in young chicks is a suitable index of thyroid activity in the assay of small amounts of TSH and may be used in addition to the observation of the uptake by the thyroid alone.

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