THE RELATIVE ACTIVITIES OF TRIIODOTHYRONINE AND THYROXINE

By E. G. TOMICH AND E. A. WOOLLETT

From the Glaxo Laboratories Ltd., Greenford, Middlesex

(Received 26 January 1954)

SUMMARY

The biological activity of 3:5:3'-L-triiodothyronine (TIT) has been compared with that of L-thyroxine (THX) by three different methods.

1. The first compared increases in oxygen consumption rate in rats and showed that TIT had 3.7 and 7.5 times the activity of THX when administered subcutaneously and orally. Although the onset of action appeared to be more rapid with TIT, the overall effects of the two compounds on oxygen consumption in intact rats were qualitatively similar.

2. The second, a mouse anoxia method involving subcutaneous injection, gave a ratio of 4.5 in favour of TIT.

3. In the third, the effects of the two compounds in preventing goitre formation were studied in rats given thiouracil. The relative activity of TIT established by this method was 7.4 times that of THX.

The finding by Gross & Pitt-Rivers [1952] that 3:5:3'-L-triiodothyronine was 3 to 4 times as active as L-thyroxine aroused widespread interest and encouraged fresh speculation about the chemical nature of the thyroid hormone in the gland and the circulation. We have repeated this work, and also compared the activities of the two compounds by other means. Our earlier findings have been briefly reported [Tomich & Woollett, 1953]. The present communication describes the details both of those experiments and of later work.

MATERIALS AND METHODS

Hormones

L-Thyroxine and 3:5:3'-L-triiodothyronine were obtained as the pentahydrates of the sodium salts, and were dissolved in physiological saline containing 0.01M-Na₂CO₃. For the sake of brevity these hydrated sodium salts are referred to in the text simply as thyroxine (THX) and triiodothyronine (TIT).

Animals

Inbred Albino rats of Wistar origin (WAG strain) were used in Expts. 1-3 and 5, and inbred fawn mice (GFF strain) in Expt. 4. All animals were housed in a room kept thermostatically at 79° ± 1° F.

Diets

The rats were fed a dough made by mixing 30% of water with 70% of a powder (RBSS 10), consisting of wholemeal wheat flour 70%, full cream milk powder 25% and dried yeast 5%. The mice were maintained on a commercial cube diet (diet 41, Bruce [1950]).
Determination of oxygen consumption rate

The method used was a modification of that described by Maclagan & Sheahan [1950]. When large numbers of determinations are undertaken we prefer 1 l. wide-necked flasks to desiccators as they occupy less space, are easier to handle and clean, and replacements are relatively cheap. The 'warming up' effect described by Maclagan is almost negligible with thin-walled flasks. Carbon dioxide is absorbed by soda-lime contained in wire-mesh cylinders. After the rubber bung has been inserted in the neck of the flask, the joint is sealed with a few drops of water containing a trace of detergent.

Consumption studies on individual rats or groups of three mice can be made over periods of hours without apparent discomfort to the animals. The atmosphere inside the flask is fortified with oxygen at hourly intervals after each pressure recording. For a rat weighing 80 g, the fall in pressure over 1 hr is approximately 150 mm of mercury. Fig. 1 shows a group of twelve individual rats under test.

Goitre prevention assay

The technique used was that of Dempsey & Astwood [1943].

Mouse anoxia assay

The method used was that of Smith, Emmens & Parkes [1947], as modified by Basil, Somers & Woollett [1950].
EXPERIMENTAL PROCEDURE

Expt. 1

Oxygen consumption after subcutaneous administration

Seven groups of four male rats were used, with body weights ranging from 70 to 90 g. The oxygen consumption rate (OCR) for each rat was measured on 10 consecutive days. Group I served as controls, while groups II–IV received daily subcutaneous injections with graded doses of THX from the 3rd to the 9th day inclusive. Groups V–VII received TIT by the same route. OCR was measured also on the 2nd, 4th, 5th, 8th and 11th days after the final injection. In this way it was possible to compare the rates at which the OCR of the various groups returned to normal.

Expt. 2

Four groups of ten male rats were used, with body weights ranging from 50 to 90 g. As in the previous experiment, OCR was measured on 10 consecutive days, but no measurements were made after the 10th day. Both hormones were administered subcutaneously at two dose levels.

Expt. 3

Oxygen consumption after oral administration

Four groups of ten male rats were used, their body weights ranging from 60 to 85 g. Both compounds were administered orally at two dose levels each day from the 3rd to the 9th day, inclusive. OCR was measured on all 10 days.

Expt. 4

Mouse anoxia assay

The experiment, performed in triplicate, employed eight groups of nine male mice. The initial body weights ranged from 16 to 22 g. Three groups of mice were injected subcutaneously with graded doses of THX, and the remaining five groups received TIT. The injections (in a vol. of 0.2 ml.) were given on the 1st, 3rd and 5th days of the experiment. On the 7th day, i.e. 2 days after the last injection, individual survival times were determined.

Expt. 5

Goitre prevention assay

Two experiments were performed. The first employed six groups of seven female rats, whose body weights ranged from 150 to 250 g. From the 1st day of the experiment, groups I–V drank tap water containing 0.1% thiouracil, while group VI, the control group, received plain tap water. All six groups were given subcutaneous injections for 10 days. Groups I and II received THX, and groups III and IV TIT. Groups V and VI were injected with physiological saline. On the 11th day all the animals were killed and their thyroids were dissected free of connective tissue and weighed.

In the second experiment, four groups of eight female rats were used, with body weights ranging from 100 to 200 g. The procedure was similar to that used in the first experiment, except for the omission of groups V and VI.
RESULTS

Unless otherwise stated, the activity ratios quoted below were calculated on a weight basis, and refer to the pentahydrates of the sodium salts. In our earlier communication the values recorded were calculated on a molar basis. All fiducial limits were calculated to include \( P = 0.95 \).

Expt. 1

The effects of OCR produced by subcutaneous administration of THX and TIT at three dose levels are shown in Figs. 2 and 3 respectively. Each point in these graphs is the mean for four rats.

The relative activity of the two hormones was calculated in several ways.

(a) The mean OCR from days 1 to 4 \((A)\), and from days 5 to 10 \((B)\), was calculated for each rat in each of the treated groups. The percentage increase in OCR during the period of treatment is given by \((B - A) \times 100/A\). The activity ratio, calculated from these individual values, was 5.9 in favour of TIT, the fiducial limits being 3.0–15. When the hydrated sodium salts were compared on a molar basis, the activity ratio was reduced to 5.1 (fiducial limits 2.6–13).

(b) In the second method, \((B - A)\) was employed as the measure of effect, and the activity ratio, calculated on these individual values, was 6.8, with a fiducial range of 3.3–13.7.
In the third method, the areas under the curves (Figs. 2, 3) between days 4 and 14, measured with a planimeter, were employed as the measure of effect. Calculated in this way, a ratio of 8.0 was obtained.

Expts. 2 and 3

The effects on OCR produced by the subcutaneous and oral administration of THX and TIT at two dose levels are shown in Figs. 4 and 5 respectively.

The activity ratios were calculated on (B – A) values as described above under (b) in Expt. 1. For the subcutaneous route the ratio obtained was 4.3, with fiducial limits of 2.3–7.6. After oral administration the ratio was 8.7, with limits of 5.5–16.

Daily activity ratios were calculated also from the OCR for each day from the 5th to the 10th day inclusive, no reference being made to pretreatment OCR. On the 4th day the responses were insufficient to provide an estimate. The calculated relative activities and fiducial limits are given in Table 1.

Table 1: Daily activity ratios (TIT/THX) based on increases in oxygen consumption in rats (Expts. 2 and 3)

<table>
<thead>
<tr>
<th>Day</th>
<th>Subcutaneous route</th>
<th>Oral route</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>8.3 (2.4–remote)</td>
<td>8.9 (both limits remote)</td>
</tr>
<tr>
<td>6</td>
<td>3.1 (1.3–5.9)</td>
<td>6.9 (3.4–18)</td>
</tr>
<tr>
<td>7</td>
<td>5.1 (1.7–17)</td>
<td>7.6 (3.5–24)</td>
</tr>
<tr>
<td>8</td>
<td>2.8 (0.4–7.5)</td>
<td>7.2 (4.3–14)</td>
</tr>
<tr>
<td>9</td>
<td>4.0 (1.5–9.4)</td>
<td>8.0 (5.0–15)</td>
</tr>
<tr>
<td>10</td>
<td>4.8 (1.1–21)</td>
<td>5.7 (3.7–9.1)</td>
</tr>
</tbody>
</table>
Expt. 4

Group mean survival times, together with their standard errors, are given in Table 2. The doses are expressed as µg injected per mouse on each of three occasions. Each mean survival time represents twenty-seven mice. Statistical analysis of these figures gave an activity ratio of 5.2, with a fiducial range of 3.9–6.7. This figure is reduced to 4.5 (3.4–5.8) when calculated on a molar basis.

Table 2. Mouse anoxia assay (Expt. 4)

<table>
<thead>
<tr>
<th>Group</th>
<th>Single subcutaneous dose in µg/mouse repeated 3 times</th>
<th>Group mean survival time (min) ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-THX Na.5H2O</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2.5</td>
<td>114.9 ± 4.3</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>90.6 ± 2.8</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>76.7 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>L-TIT Na.5H2O</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0.15625</td>
<td>119.5 ± 3.1</td>
</tr>
<tr>
<td>V</td>
<td>0.3125</td>
<td>111.6 ± 3.4</td>
</tr>
<tr>
<td>VI</td>
<td>0.625</td>
<td>104.3 ± 3.4</td>
</tr>
<tr>
<td>VII</td>
<td>1.25</td>
<td>94.1 ± 3.3</td>
</tr>
<tr>
<td>VIII</td>
<td>2.5</td>
<td>78.5 ± 2.3</td>
</tr>
</tbody>
</table>

Expt. 5

Details of dosage and final thyroid gland weights for the combined experiments are recorded in Table 3. The combined results were analysed and gave a relative activity of 8.6. The fiducial range was 6.4–10.0. Expressed on a molar basis, this figure is reduced to 7.4 (5.5–8.7). In the calculation, the group mean gland weights for groups V and VI were ignored; for this reason, it was not considered necessary to correct the thyroid weights of the other groups to allow for variations in body weight.

Table 3. Goitre-prevention assay (Expt. 5)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats in group</th>
<th>Thio^uracil in drinking water (µg/rat/day)</th>
<th>Mean thyroid weight (mg/rat) ± s.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Subcutaneous dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-THX Na.5H2O</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>15</td>
<td>+</td>
<td>14.09 ± 0.78</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>+</td>
<td>9.86 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>L-TIT Na.5H2O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>+</td>
<td>21.59 ± 1.34</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>+</td>
<td>12.05 ± 0.95</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>+</td>
<td>28.99 ± 0.97</td>
</tr>
<tr>
<td>VI</td>
<td>7</td>
<td>−</td>
<td>10.33 ± 0.60</td>
</tr>
</tbody>
</table>

DISCUSSION

The relative biological activity of TIT and THX was first investigated by Gross & Pitt-Rivers [1952], who compared the effects of the two compounds in preventing the goitrogenic action of thio^uracil. They found TIT 3 to 4 times as active as THX. Later the same workers carried out a similar but more elaborate experiment and reported a relative activity of 5 [Gross & Pitt-Rivers, 1953]. The comparative effect on oxygen
consumption in mice was first reported by Maclagan, Sprott & Wilkinson [1952], who showed that a single subcutaneous dose of TIT produced a greater increase in oxygen consumption than THX, administered in an equimolar dose. Gross, Pitt-Rivers & Thibault [1953], investigating the effects on metabolic rate in thyroidectomized rats, obtained an activity ratio of 5. On the other hand, Gemmill [1953], comparing the two compounds by the mouse anoxia test, found that TIT was slightly more active than THX. In normal rats, TIT gave the same rise in metabolism at THX. Gemmill concluded: 'Nothing has come out of these tests which would indicate that triiodothyronine is very superior to thyroxine in biological activity, or that triiodothyronine is the essential hormone from the thyroid gland.' Heming & Holtkamp [1953] reported that TIT was 3-5 times as active as THX in the goitre-prevention assay, and not less than 3-5 times in raising the metabolic rate of either thyroidectomized or intact rats. Colville & Bonnycastle [1953], injecting the two compounds subcutaneously into normal rats, obtained values of 2-6 to 2-9 in metabolism experiments and of 6-2 in the goitre-prevention assay. Roth [1953] showed that the accelerating effect of L-TIT on the metamorphosis of Rana temporaria was 3 times that of D,L-THX. When studying galactopoiesis in lactating cows, Bartlett, Burt, Folley & Rowland [1953] found that subcutaneously TIT was more active than THX, but considerably less than 5 times as much. It should be noted that oral TIT was only slightly active in lactating cows, compared with oral THX.

Gross, Pitt-Rivers & Trotter [1952] compared the two compounds on myxoe- dematous patients, and concluded that TIT was the more active. Other studies in human myxoedema have given activity ratios of 4 to 5 [Lerman, 1953] and 5 to 10 [Asper, Selenkow & Plamondon, 1953]. TIT is about 10 times as effective as THX in depressing the uptake of $^{131}I$ by the normal human thyroid [Starr & Liebold-Schneck, 1953].

In our earlier communication [Tomich & Woollett, 1953], we gave values of 5-1 and 5-3 for the ratio of activities of the two compounds on oxygen consumption when administered subcutaneously and orally. The first figure was derived from Expt. 1 above, in which groups of four rats were used. The second figure was based on an experiment with groups of six rats. These estimates of activity were calculated on percentage increases in OCR. After the above report was published, the two experiments were repeated with larger groups. These experiments (2 and 3 above), both with ten rats per group, gave values of 3-7 and 7-5 (4-3 and 8-7 on a weight basis), with considerably narrower fiducial limits. A slightly different method of estimating the activity was used, $(B - A)$ values being substituted for percentage increases in OCR. Analysis of the results from these two experiments showed that THX is approximately twice as effective by the subcutaneous as by the oral route, whereas TIT is equally effective by either route. Gross & Pitt-Rivers [1953] stated that, given orally, TIT exhibits 85% of THX, only about 40%, of its parenteral activity. Table 1 demonstrates the day-to-day variation in activity ratio obtained in this type of experiment. Although the activity figures for the 5th day indicate that the onset of action may be somewhat more rapid with TIT, Figs. 2 and 3 show that the overall effects of the two compounds on OCR of intact rats are qualitatively similar. All groups reached their peak OCR simultaneously, and all had returned to normal levels by the 14th day.
The results of our anoxia test differ from those of Gemmill [1953], who, although stating that he used the method of Smith et al. [1947], measured survival times 20 hr after administering single injections, whereas Smith et al. recommended that three injections be given on alternate days and that survival times be measured on the 2nd or 3rd day after the last injection. These differences in techniques might explain the difference in results between Gemmill’s and the present study.

The results of the goitre prevention assay are in reasonable agreement with those reported by Gross & Pitt-Rivers [1953], and Colville & Bonncastle [1953].

We gratefully wish to acknowledge the help of Mr B. Basil, who carried out the statistical calculations.

REFERENCES

Gemmill, C. L. [1953]. Amer. J. Physiol. 172, 286.