THYROXINE-LIKE PROPERTIES OF 3:5-DINITRO-L-THYRONINE

By

A. N. Kuusisto and Viljo Antila

Recent investigations have shown that, in addition to thyroxine, blood contains another organic iodine compound, which Gross & Pitt-Rivers (1951, 1952) found to be 3:5:3'-triiodothyronine. Contrary to the former belief according to which thyronine compounds with a smaller iodine content than that of thyroxine were considered biologically less active than thyroxine, it was found that 3:5:3'-triiodothyronine was 4–5 times more active. (For references, see Pitt-Rivers, 1954; Klein, 1954).

There has recently been an increasing interest in the derivatives of thyronine, and its halogen compounds particularly have been investigated.

In the present study the effect of a nitro-compound, 3:5-dinitro-l-thyronine, especially on the thyroid gland, has been investigated in the guinea-pig and the changes compared with those caused by l-thyroxine.

MATERIAL AND METHODS

The material consists of thirty male guinea-pigs of the same strain weighing 450–650 gm. Ten animals received 3:5-dinitro-l-thyronine in a daily dose of 0.5 mg./kg. of body-weight by intraperitoneal injection over a period of ten days and ten guinea-pigs received intraperitoneal injections of l-thyroxine in the same dose. Both preparations were dissolved in 0.049-N NaOH, from which a basic dilution was prepared with physiological saline. Ten animals served as controls and were injected daily with the solvent. After the experimental period, the test animals and their controls were killed by decapitation and bled. The thyroid, hypophysis, adrenals, thymus and testes were removed and weighed. After fixation in Bouin's fluid the thyroids were embedded in paraffin and stained by Mallory's azan method. For the determination of the percental proportion of the thyroid epithelium, colloid and stroma, the method of Uotila & Kannas (1952) was employed.

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RESULTS

At the end of the experimental period, no appreciable change in weight was observed in the animals which had been given dinitrothyronine, whereas those treated with thyroxine showed a marked loss of weight. In the control group the weight had slightly increased.

The average initial and final weights and the average percentage weight changes were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Initial body-weight (gm.)</th>
<th>Final body-weight (gm.)</th>
<th>Weight change (%/o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinitrothyronine group (n = 10)</td>
<td>528 ± 22</td>
<td>547 ± 25</td>
<td>+ 1.4 ± 4.3</td>
</tr>
<tr>
<td>Thyroxine group (n = 10)</td>
<td>535 ± 23</td>
<td>400 ± 22</td>
<td>—25.3 ± 3.2</td>
</tr>
<tr>
<td>Control group (n = 10)</td>
<td>559 ± 21</td>
<td>639 ± 23</td>
<td>+13.8 ± 1.1</td>
</tr>
</tbody>
</table>

In the animals treated with dinitrothyronine the weight did not increase as much as in the controls. It is possible that dinitrothyronine has a stimulating effect on metabolism, though this effect is less marked than that of thyroxine. If the effect of the thyroxine on the weight is indicated by 1, that of dinitrothyronine is approximately 0.3 as compared with the weight increase in the controls.

Histological examination of the thyroids showed increased colloid and low epithelium in the animals treated with dinitrothyronine as compared with the controls. In the thyroxine group the increase in colloid and the reduction in epithelium was even more marked. The mean absolute and relative weights (mg./100 gm. of body-weight) of the thyroids and the mean percentages of epithelium, colloid, and stroma, as measured according to the method of Uotila & Kannas, are given in Table 1.

Table 1.
The mean absolute and relative weights of the thyroids and the mean percentage of epithelium, colloid and stroma.

<table>
<thead>
<tr>
<th></th>
<th>Weight of the thyroid (mg.)</th>
<th>Epithelium (mg./100 gm.)</th>
<th>Colloid (%/o)</th>
<th>Stroma (%/o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinitrothyronine group (n = 10)</td>
<td>72.8 ± 4.4</td>
<td>13.4 ± 1.3</td>
<td>52.6 ± 2.2</td>
<td>32.0 ± 1.8</td>
</tr>
<tr>
<td>Thyroxine group (n = 10)</td>
<td>49.1 ± 3.5</td>
<td>12.4 ± 0.8</td>
<td>47.9 ± 2.1</td>
<td>38.9 ± 2.1</td>
</tr>
<tr>
<td>Control group (n = 10)</td>
<td>91.6 ± 6.0</td>
<td>14.3 ± 0.8</td>
<td>59.6 ± 1.8</td>
<td>26.8 ± 2.3</td>
</tr>
</tbody>
</table>

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The average weight of the thyroid was highest in the control group and lowest in the thyroxine group. The weight of the thyroid in the dinitrothyronine group was midway between these limits. The differences in the average weights of the thyroids in the three groups are statistically significant, whereas no statistically significant difference could be observed in the mean relative weights of the thyroids.

The total of epithelium, colloid and stroma was designated by 100. The percentage of epithelium was lower in the dinitrothyronine and the thyroxine group than in the control group. In both experimental groups the values for colloid were higher than in the controls. As compared with the control group, the differences in both epithelium and colloid are statistically significant. In this experiment thyroxine caused definite alterations, thus indicating an inactivation of the thyroid. The results correspond to those obtained by Tala (1952) with thyroxine. In our experiment the effect of dinitrothyronine on the thyroid was similar to that of thyroxine, although it was less marked. If the effect of thyroxine on the epithelium is indicated by 1, the effect of dinitrothyronine is about 0.6.

The mean absolute and relative weights (mg./100 gm. of body-weight) of the other endocrines in the three groups are given in Table 2.

In the dinitrothyronine group a slight involution of the thymus was observed, whereas the thymus glands of the guinea-pigs treated with thyroxine were almost completely atrophied. The differences between the mean absolute and relative weights of the thymus in the three groups are statistically significant. The relative weight of the adrenal gland was almost the same in the dinitrothyronine group as in the control group. In the thyroxine group the relative weight of the adrenal gland was somewhat greater than in the control group. The difference is statistically significant. The relative weight of the hypophysis was somewhat greater in the experimental groups than in the controls. The differences are not, however, statistically significant. The mean absolute weight of the testes was almost the same in the three groups. The relative weight was greatest in the thyroxine group, and somewhat higher in the dinitrothyronine than in the control group.

**Discussion**

Our experiments show that 3:5-dinitrothyronine has thyroxine-like effects on experimental guinea-pigs. Dinitrothyronine prevented an increase in the weights of the animals, which may be due to a higher metabolic rate. In the group treated with dinitrothyronine and in that treated with thyroxine the weight of the thyroid was, however, smaller than in the controls. The thyroids showed signs of inactivation. The epithelium was low and there was an increased
Table 2.
The mean absolute and relative weights of thymus, adrenals, hypophysis and testes.

<table>
<thead>
<tr>
<th></th>
<th>Thymus</th>
<th></th>
<th>Adrenals</th>
<th></th>
<th>Hypophysis</th>
<th></th>
<th>Testes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>mg./100 gm.</td>
<td>mg.</td>
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<td>mg.</td>
<td>mg./100 gm.</td>
</tr>
<tr>
<td>Dinitrothyronine group (n = 10)</td>
<td>136 ± 16.7</td>
<td>24.4 ± 2.7</td>
<td>286 ± 27.4</td>
<td>52.7 ± 5.2</td>
<td>16.2 ± 1.2</td>
<td>3.1 ± 0.6</td>
<td>2480 ± 16</td>
<td>455 ± 16.3</td>
</tr>
<tr>
<td>Thyroxine group (n = 10)</td>
<td>29 ± 4.8</td>
<td>7.0 ± 0.9</td>
<td>256 ± 13.7</td>
<td>66.0 ± 6.5</td>
<td>13.2 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>2540 ± 17</td>
<td>402 ± 14.0</td>
</tr>
<tr>
<td>Control group (n = 10)</td>
<td>241 ± 16.5</td>
<td>37.6 ± 2.8</td>
<td>353 ± 44.0</td>
<td>54.2 ± 5.1</td>
<td>14.5 ± 0.6</td>
<td>2.3 ± 0.7</td>
<td>2590 ± 19</td>
<td>640 ± 23.8</td>
</tr>
</tbody>
</table>
amount of colloid. The percentage of epithelium was lower, whereas that of colloid was higher than in the controls. In the dinitrothyronine and thyroxine groups the thymus glands were smaller than in the controls. The effect of dinitrothyronine was clearly weaker than that of thyroxine. If we take the control group as the basis of comparison and indicate the changes caused by thyroxine by 1, the effect of an equal dose of dinitrothyronine on the weight of the body is approximately 0.3, on the epithelium percentage 0.6, and on the weight of the thymus 0.2.

On the basis of previous experiments and their own observations, Lerman et al. (1952) concluded that thyroxine analogues of this type have a depressing effect on the hypophysis and thyroid gland but only in relation to their calorigenic activity. This is probably also true of dinitrothyronine.

**SUMMARY**

In the present investigation the effect of 3:5-dinitrothyronine on guinea-pigs, especially on the histological picture of the thyroid, has been studied. The results suggest that dinitrothyronine probably has thyroxine-like effects. Dinitrothyronine prevented an increase in the weight of the body. It had a depressing effect on the thyroid, and caused involution of the thymus. As compared with thyroxine, the effect was weak. If the corresponding effect of thyroxine is indicated by 1, the effect of dinitrothyronine on the weight of the test animals was about 0.3, on the percentage of epithelium 0.6, and on the weight of the thymus 0.2.

**ACKNOWLEDGMENT**

We wish to express our gratitude to Mr. Niilo Seppäläinen, the chemist of Orion, for the synthesis of the 3:5-dinitro-l-thyronine used in our experiments.

**REFERENCES**

Tala, P.: Acta endocrinol. 10, Suppl. 9, 1952.