THYROIDINE METABOLISM IN THE RAT: 
EFFECT OF VARYING DOSES OF EXOGENOUS THYROIDINE

By

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ABSTRACT

Experiments were performed to determine quantitatively the peripheral metabolism of exogenous thyroidine (T₄) in rats brought to isotopic equilibrium with doses of ¹³¹I-T₄ ranging from 1 to 20 μg/100 g body weight/day. It was found that, although the absolute amount of T₄ either deiodinated and excreted as iodide in urine or excreted as T₄ in faeces increased as the dose of T₄ increased, the percentage of hormone excreted by either pathway at each dose level was relatively constant. In other words, the fractional rate of deiodination is not greatly influenced by the amount of T₄ administered. A 20-fold increase in the dose of T₄ resulted in only a 4-fold increase in serum T₄ concentration measured 24 h after injection, but serum T₄ levels were elevated considerably more than this for several hours between injections. Nevertheless, the highest dose of T₄ was not greatly thyrotoxic. The implications of these findings in relation to the possible association between the metabolism and action of T₄ are discussed.

In recent years, many studies of thyroidine (T₄) metabolism in vivo have been performed, both in this laboratory (Galton 1968, 1969, 1972; Galton & Nisula 1969) and by others (van Middlesworth & Jones 1961; Escobar del Rey & Moreale de Escobar 1961) using exogenous radioactive T₄ of known specific activity. Endogenous T₄ secretion and thyroidal accumulation of the released radioactive iodide are prevented by thyroidectomy or by administering an antithyroid drug, generally potassium perchlorate (KClO₄). This technique

This investigation was supported by Grant No. HD 02516, and by The General Research Support Grant, RR 05392 from The National Institutes of Health, Bethesda, Maryland.
permits study of the metabolism of a known amount of $T_4$ uncomplicated by the variables imposed when an intact pituitary-thyroid secretory system is present. However, there are major drawbacks to this technique. Firstly, it is not always a simple matter to select the dose of $T_4$ which will mimic most closely the physiological situation. In this laboratory, a dose of 2 $\mu$g $T_4/100$ g body weight/day, administered to normal rats, is just sufficient to prevent the release of $^{131}$I from a prelabelled thyroid gland (Galton, unpublished data). Thus this dose has been used in most experiments. However, under certain conditions, for example, during exposure to cold or altitude, thyroidal activity is altered (Galton & Nisula 1969; Galton 1972), and then this dose of $T_4$ is no longer satisfactory.

Secondly, the daily dose of $T_4$ is generally administered as a single sc injection. This method is apparently adequate for maintenance of a euthyroid state. However, it is likely that marked, unphysiological changes in serum $T_4$ concentration occur between injections. These changes may influence the metabolic handling of the hormone.

Accordingly, experiments were carried out to assess the peripheral metabolism of exogenous $T_4$ over a range of doses from 1 to 20 $\mu$g/100 g body weight/day. In addition, serum $T_4$ concentrations were determined over the 24-h period following injection of the various doses of $T_4$.

**METHODS AND MATERIALS**

Male Sprague-Dawley rats (Carworth, Inc., New City, Rockland County, N.Y.), weighing approximately 150 g at the start of each experiment were employed. They were fed Purina Labena rat chow ad libitum and were given a solution of 1 % $\text{KClO}_4$ in place of drinking water to prevent thyroidal accumulation of iodide. The rats were injected daily (sc) with $^{131}$I-$T_4$\textsuperscript{1}) of known specific activity in amounts ranging from 1 to 30 $\mu$g/day. After 14 days, when the total body pool of $T_4$ was approaching $^{131}$I/$^{127}$I isotopic equilibrium, the animals were placed in individual metabolism cages and 24-h collections of urine were made. In all experiments, after 15 days the urinary excretion of $^{131}$I was relatively constant, indicating that approximate isotopic equilibrium had been achieved. At least three more 24-h urine collections were then made, and faeces were also collected. Blood was obtained from the tail at the end of each collection period. Excretion of $^{131}$I and the amount of trichloroacetic acid (TCA)-precipitable $^{131}$I in serum and faeces were measured by methods previously described (Galton 1969; Galton & Nisula 1969). From these values and the known specific activity of the injected $^{131}$I-$T_4$, the absolute concentration of $T_4$ in the serum, the quantity of hormone deiodinated and excreted in urine as iodide, and the amount of $T_4$ excreted

\textsuperscript{1} $^{131}$I-labelled $T_4$ was obtained from Abbott Laboratories, Oak Ridge, Tennessee. Upon arrival it was used at once to label the solutions of stable $T_4$ prepared for injection in 50 % propylene glycol. Chromatographic analysis revealed that at least 94 % of the $^{131}$I was in the form of $^{131}$I-$T_4$. 

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as unchanged hormone in faeces were calculated. The data were then used to calculate plasma $^{131}\text{I}-T_4$ clearance rates: total, deiodinative (i.e., as iodide in urine) and faecal, as previously described (Galton 1968).

In two experiments, a study was also made of serum $^{131}\text{I}-T_4$ concentrations during a 24-h period following the daily injection. Small samples of blood were obtained from the tail immediately before and at 2, 4, 6, 9 and 24 h after injection of $T_4$. The serum TCA-precipitable $^{131}$I content was determined and the $T_4$ concentration was calculated.

After the final urine collection, the animals in some experiments were killed by exsanguination, and the $T_4$ concentration in aliquots of liver (homogenized in a known amount of human plasma) and serum was determined as described above. The liver/serum $T_4$ concentration ratios were then calculated.

![Graph](image)

**Fig. 1.**

$T_4$ iodine excreted in urine ($\mu g \ T_4/24 \ h$), $\bullet--\bullet$, and serum $T_4$ concentration ($\mu g/100 \ ml$), $\circ--\circ$, determined 24 h after a $T_4$ injection, in rats equilibrated with various amounts of $^{131}\text{I}-T_4$. Each point represents the mean of values obtained in 6 rats per group.

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2) Random samples of serum, urine and faeces (the latter made into a homogenate with human plasma and water) were subjected to analysis by paper chromatography solvent system: butanol–dioxane–$2 \times \text{NH}_4\text{OH}$, 4:1:5). The radioactive spots were located by autoradiographing the strips on No-Screen X-ray film. Only $^{131}$I-iodide was detected in urine. Serum and faeces both contained unchanged $T_4$ and $^{131}$-iodode. No other labelled products were detected.

3) Samples of liver homogenate were also subjected to chromatographic analysis. More than 96% of the total labelled organic iodine present was in the form of $T_4$. Small quantities of $T_4$-glucuronide and labelled “origin-material” were also detected.
Table 1.
Peripheral metabolism of thyroxine in rats equilibrated with different doses of $^{131}$I-T$_4$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Final weight g</th>
<th>Dose µg/day</th>
<th>Serum T$_4$ µg/100 ml</th>
<th>T$_4$ deiodinated (T$_4$ iodine in urine) µg T$_4$/24 h</th>
<th>T$_4$ in faeces µg/24 h</th>
<th>%/µg dose</th>
<th>Calc.</th>
<th>Measured</th>
<th>Urinary</th>
<th>Faecal**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>299 ± 6.6</td>
<td>3</td>
<td>3.13 ± 0.2</td>
<td>1.2 ± 0.08</td>
<td>39.8 ± 2.8</td>
<td>1.2</td>
<td>1.2</td>
<td>0.08</td>
<td>39 ± 4.1</td>
<td>57 ± 6.0</td>
<td>96 ± 8.8</td>
</tr>
<tr>
<td>B</td>
<td>290 ± 5.1</td>
<td>4.5</td>
<td>4.62 ± 0.3</td>
<td>2.1 ± 0.13</td>
<td>45.7 ± 2.9</td>
<td>2.4</td>
<td>1.9</td>
<td>0.10</td>
<td>45 ± 4.7</td>
<td>52 ± 5.0</td>
<td>98 ± 7.5</td>
</tr>
<tr>
<td>C</td>
<td>297 ± 5.9</td>
<td>6</td>
<td>6.25 ± 0.35</td>
<td>2.8 ± 0.16</td>
<td>45.8 ± 2.7</td>
<td>3.2</td>
<td>2.2</td>
<td>0.10</td>
<td>44 ± 4.9</td>
<td>51 ± 3.0</td>
<td>95 ± 6.1</td>
</tr>
</tbody>
</table>

1) 6 animals per group.
* Mean ± se.
** Derived from the total and urinary clearance values by difference.
RESULTS

Results of an experiment in which rats received T\(_4\) in doses ranging from 3 to 6 \(\mu g\) T\(_4\) per day are shown in Fig. 1 and Table 1. Based on the weight of the animals when the final measurements were made, these doses were 1, 1.5 and 2 \(\mu g\) T\(_4\)/100 g body weight/day and were thus considered to be close to physiological doses. The data shown represent the mean values of closely agreeing results obtained on 4 separate days. An increase in the amount of T\(_4\) administered was associated with linear increases in both the serum T\(_4\) concentration and the rate of excretion in urine of iodide derived from T\(_4\) (Fig. 1). Approximately the same percentage of each dose was excreted by this pathway. Since the animals were in isotopic equilibrium, this was the case also for the faecal excretion of T\(_4\); a value calculated as the difference between the \(\mu g\) T\(_4\) injected and the \(\mu g\) T\(_4\) deiodinated and excreted as iodide in urines. The measured values for the amount of T\(_4\) excreted in faeces were somewhat lower than the estimated values (Table 1), presumably because of the technical errors associated with the analytical procedures employed. At these low dose levels, the clearance of T\(_4\) from plasma was insignificantly influenced by the amount of hormone administered.

Other experiments were performed in which the rats received T\(_4\) in doses ranging from 1.5 to 30 \(\mu g\)/day (approximately 1 to 20 \(\mu g\)/100 g body weight). Results in all these experiments were similar and data from one are presented in Table 2. It is evident that even the highest dose of T\(_4\) (30 \(\mu g\)/day), given under these conditions for the period of 3 weeks, was not highly toxic; the animals continued to gain weight although at a slower rate than did those given the low doses of T\(_4\). Again, an increase in the daily dose of T\(_4\) was associated with an increase in serum T\(_4\) concentration. However, as the dose of T\(_4\) increased above 2 \(\mu g\)/100 g body weight/day, the increase ceased to be linear. A 20-fold increase in the amount of T\(_4\) injected resulted in only a 4 to 5-fold elevation of serum T\(_4\) concentration. Nevertheless, both the amount of T\(_4\) eliminated in faeces and the amount deiodinated and excreted in urine continued to rise with the dose and the relative proportion of each dose eliminated by the two pathways remained approximately constant. Accordingly, the clearance of T\(_4\) from plasma by both the deiodinative (i.e. as iodide in urine) and faecal pathways was considerably increased over this dose range (4-fold).

The liver-serum T\(_4\) concentration ratios determined 24 h after the final injection of T\(_4\) are also shown in Table 2. A 20-fold difference in the dose of T\(_4\) resulted in an increase in the ratio from 0.95 to 1.53. Clearly, at this point, the liver was not concentrating a very large proportion of the administered T\(_4\).

The variability of serum T\(_4\) concentration during the 24-h period following
### Table 2.
Peripheral metabolism of thyroxine in rats equilibrated with different doses of $^{131}$I-T$_4$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Final weight g</th>
<th>Dose $\mu$g/day</th>
<th>Serum T$_4$ $\mu$g/100 ml</th>
<th>T$_4$ deiodinated (T$_4$ iodine in urine) $\mu$g T$_4$/24 h</th>
<th>% dose</th>
<th>T$_4$ in faeces $\mu$g/24 h</th>
<th>Clearances ml plasma/24 h</th>
<th>Liver/serum T$_4$ conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>181 ± 9.1*</td>
<td>1.5</td>
<td>1.81 ± 0.03</td>
<td>0.58 ± 0.04</td>
<td>38 ± 2.6</td>
<td>0.92</td>
<td>0.59 ± 0.06</td>
<td>34 ± 4.1</td>
</tr>
<tr>
<td>B</td>
<td>186 ± 6.7</td>
<td>3.0</td>
<td>2.42 ± 0.15</td>
<td>1.19 ± 0.10</td>
<td>39 ± 3.3</td>
<td>1.81</td>
<td>1.43 ± 0.09</td>
<td>50 ± 0.7</td>
</tr>
<tr>
<td>C</td>
<td>183 ± 6.7</td>
<td>6.0</td>
<td>4.08 ± 0.32</td>
<td>2.51 ± 0.07</td>
<td>41 ± 1.3</td>
<td>3.49</td>
<td>2.15 ± 0.22</td>
<td>63 ± 5.4</td>
</tr>
<tr>
<td>D</td>
<td>169 ± 3.0</td>
<td>15.0</td>
<td>4.40 ± 0.34</td>
<td>4.70 ± 0.24</td>
<td>31 ± 1.6</td>
<td>10.30</td>
<td>5.24 ± 0.34</td>
<td>110 ± 9.4</td>
</tr>
<tr>
<td>E</td>
<td>162 ± 4.0</td>
<td>30.0</td>
<td>9.20 ± 0.70</td>
<td>10.50 ± 0.37</td>
<td>34 ± 1.3</td>
<td>10.50</td>
<td>21.70 ± 2.53</td>
<td>116 ± 7.4</td>
</tr>
</tbody>
</table>

1) 6 animals per group.
* Mean ± se.
** Derived from the total and urinary clearance values by difference.
Serum $T_4$ concentration ($\mu g/100$ ml) in rats equilibrated with various amounts of $^{131}$I-$T_4$ and measured immediately before and at 2, 4, 6, 9 and 24 h after the daily sc injection of hormone. Each point represents the mean of the values obtained in 6 rats per group.

Injection of $T_4$ is illustrated in Fig. 2. At all dose levels, $T_4$ concentration was highest about 4 h after an injection. At this time there was a 10-fold difference in the serum $T_4$ concentration between the rats receiving 1.5 $\mu g$ and those receiving 30 $\mu g$ $T_4$. Furthermore, it was evident that serum $T_4$ concentration in rats given the highest doses of $T_4$ were considerably elevated for several hours of the 24-h period.

**DISCUSSION**

This study was performed in part to determine whether or not marked differences occur in the metabolic handling of $T_4$ when rats are equilibrated with various amounts of $T_4$. It was found that, although the absolute amount
of T₄ deiodinated increased as the dose of T₄ increased, the percentage of the hormone deiodinated at each dose level was relatively constant; in no experiment was there a statistically significant difference between the percentage deiodination of the highest and lowest doses employed.

Since the animals were in isotopic equilibrium (as indicated by a constant rate of excretion of ¹³¹I-iodide in urine and faeces), it follows that the calculated percentage of the hormone excreted in faeces at each dose level was also constant. Although the measured values for faecal T₄ excretion were generally somewhat lower than those derived by calculation, the technique was still used routinely to ascertain that the daily excretion of T₄ had become constant. Thus, it was concluded that, over a dose range of 1–20 μg T₄/100 g body weight/day, the relative rates of excretion of T₄ in faeces, or following deiodination as iodide in urine are not greatly influenced by the amount of T₄ administered.

It was notable that in all experiments a linear relationship existed between serum T₄ concentration (measured 24 h after an injection) and the dose of T₄, up to doses of 2 μg/100 g body weight/day. This linearity ceased as the dose was further increased and a 20-fold increase in dose only produced a 4-fold increase in serum T₄ concentration. However, it is evident from Fig. 2 that serum T₄ concentrations were much higher after 4 h than after 24 h following an injection. Indeed, 4 h after injection a linear relationship between dose and serum T₄ concentration was approximated at all dose levels. Moreover, although serum T₄ concentration varied less than 2-fold with the low dose, it was increased almost 10-fold with the high dose during a 24-h period. Clearly the 24-h value was a poor indication of an average serum T₄ concentration especially with high doses of T₄.

Plasma T₄ clearance rates were all calculated using the value for serum T₄ concentration obtained at 24 h. This method has often been used in the past (Galton & Ingbar 1966; Galton 1968, 1972; Galton & Nisula 1969; Cullen et al. 1973) and can furnish meaningful information in studies employing groups of rats maintained with the same dose of T₄. However, its use is clearly limited in experiments in which a range of dose levels is employed. Possibly, the mean serum T₄ concentration experienced over a 24-h period should be used to calculate the T₄ clearances. The present method of calculation results in clearance values that are too high, and the degree of error may not be constant from dose to dose. Studies are currently in progress to explore in more detail the relationship between the dose of T₄, the plasma T₄ concentration and the rate of clearance of T₄ from plasma.

The highest dose of T₄ was somewhat thyrotoxic. The animals in this group did not gain weight as rapidly as did those on the small doses. Although the maximum increase in serum T₄ concentration 24 h after an injection was only 4-fold, the levels were elevated considerably more than this for several hours.

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between injections. Thus a 20-fold increase in the amount of T₄ administered did in fact result in approximately a 20-fold increase in the amount of T₄ deiodinated in the tissues. The role of deiodination in the physiological action of T₄ is uncertain, but there is considerable evidence to support the view that T₄ metabolism and action are in some way directly related. The subject has been reviewed (Galton 1969). If this is so, then the highest dose employed in the present study should have been about 20 times as effective as the lowest dose. Two possible indices of T₄ action in the whole body are basal metabolic rate (BMR) and heart rate. BMR can be increased by T₄ administration and if sufficient hormone is given, the animals do lose weight. Although such measurements were not made in the present study, it has been found previously in this laboratory that doses of T₄ of 30 μg/100 g body weight/day, given for at least two weeks, result in a loss of body weight; doses less than this only retard weight gain. However, it was evident that this dose did not induce even a 10-fold increase in BMR; oxygen consumption and heart rate were merely doubled (Galton, unpublished observations). Very large doses of T₄ (100–200 μg/day) induced only small additional increases in BMR. It is quite possible that there are other factors which may limit the extent to which BMR and heart rate can be elevated by T₄ and thus effects of T₄ cannot necessarily be used as a quantitative index of T₄ action. Nevertheless, these observations do raise again the question of whether or not at least some of the T₄ which undergoes deiodination in vivo does so by a mechanism which is merely degradative and is not associated with the physiological action of the hormone.

REFERENCES

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Received on May 15th, 1974.