EFFECT OF THYROXINE AND GROWTH HORMONE ON LONGITUDINAL BONE GROWTH IN THE HYPOPHYSECTOMIZED RAT

By
K.-G. Thorngren and L. I. Hansson

ABSTRACT

The effect of L-thyroxine and bovine growth hormone (NIH-GH-B16) on the growth in length from the proximal growth plate of the tibia in hypophysectomized female Sprague-Dawley rats was studied by the tetracycline method. The width of the growth plate was also determined, and the weight of the body and heart was registered. Completeness of the hypophysectomy was determined microscopically. Daily sc injections of 5, 10, 20 or 40 \( \mu \text{g/kg} \) L-thyroxine alone, or in combination with 25 or 100 \( \mu \text{g} \) NIH-GH-B16, were given for 20 days, starting 15 days after hypophysectomy which was performed when the rats were 60 days of age. Thyroxine alone resulted in stimulation of the longitudinal bone growth with an optimum effect at 10–20 \( \mu \text{g/kg} \). Further increase of the thyroxine dose did not increase the bone growth. Thyroxine given in association with growth hormone had a higher growing promoting effect than thyroxine or growth hormone alone. The growth stimulation of the two hormones was also significantly higher than the expected additive effect, indicating a potentiating synergism. When thyroxine and growth hormone were given in combination, the longitudinal bone growth reached an optimum for almost the same dose (20 \( \mu \text{g/kg} \)) of thyroxine as when it was given alone. At this optimum dose of thyroxine, the dose of growth hormone determined the longitudinal bone growth. The width of the growth plate was not influenced by thyroxine admini-
istration. The body weight decreased somewhat when thyroxine was given alone, and the combination with growth hormone seemed to compensate for this weight loss. The heart weight was found to increase with increasing doses of thyroxine both when given alone and in association with growth hormone.

It is well known that growth hormone given to hypophysectomized rats results in growth stimulation (for review see Asling & Evans 1956; Asling et al. 1965; Urist 1972; Thorngren et al. 1973b). This stimulation is known to be dose dependent and to increase with the period of administration (Thorngren et al. 1973b).

Thyroxine participates both in growth and differentiation of bones (Riekstniece & Asling 1966). However, the effect on growth in length by thyroxine given to hypophysectomized rats has been variable. In some studies, thyroxine has been shown to promote growth in length (Ray et al. 1950; Simpson et al. 1950; Scow 1954; Asling & Evans 1956; de Groot 1963; Meites & Krüdt 1964; Pannain & Galvao 1964); in other studies, this effect has not been found (Bielschowsky et al. 1962; Goodall & Gavin 1966). The simultaneous administration of growth hormone and thyroxine to hypophysectomized rats is known to give a higher rate of longitudinal bone growth than that of growth hormone alone (Simpson et al. 1950; Asling & Evans 1956; Baume et al. 1958; de Groot 1963); also a synergistic effect of growth hormone and thyroxine has been described (Evans et al. 1939; Marx et al. 1942; Asling & Evans 1956). A basal secretion of thyroxine from the thyroid gland in spite of the removal of the pituitary has been considered necessary for the growth stimulating effect of growth hormone in the hypophysectomized rat (Asling et al. 1965).

Growth hormone given to thyroidectomized rats promotes growth in length, but the animals are relatively insensitive to the hormone (Simpson et al. 1950), and the growth stimulation is sometimes negligible (Asling et al. 1965). The actions of thyroxine in enhancing body growth in thyroidectomized rats is known to be very sensitive, requiring only a low substitution dose for manifestation (Evans et al. 1960; Asling et al. 1968).

In the hypophysectomized-thyroidectomized rat, thyroxine alone gives a slight but significant increase in length (Simpson et al. 1950; Scow 1959). Growth hormone given alone results in minor growth stimulation, the animals respond in a similar manner to only thyroidectomized rats and are relatively insensitive to the hormone (Simpson et al. 1950; Scow 1959). The combined administration of growth hormone and thyroxine results in growth promotion that is more marked than either hormone given alone (Simpson et al. 1950; Scow 1959).

In earlier investigations (Simpson et al. 1950; Asling & Evans 1956; Baume et al. 1958; de Groot 1963; Pannain & Galvao 1964; Asling et al. 1965) the
promoting effect of different hormones on the growth in length have been determined on the whole body or on a whole single bone, sometimes by means of callipers or X-ray. The width of the growth plate has also been widely used to evaluate the growth in length (Geschwind & Li 1952, 1955; Hulth & Nylander 1962; Papkoff & Li 1962; Riekstniece & Asling 1966; Asling et al. 1968). However, the widening of a growth cartilage is not always associated with stimulation of the longitudinal bone growth (Becks et al. 1948; Asling et al. 1965; Riekstniece & Asling 1966; Hansson 1967).

In order to determine the effect of thyroxine on the longitudinal bone growth from one growth plate of a long bone in the hypophysectomized rat, this investigation was performed with tetracycline as an intravital marker of bone growth from the proximal growth plate of the tibia. The effect of simultaneous administration of thyroxine and growth hormone on longitudinal bone growth was also determined.

MATERIAL AND METHODS

Female Sprague-Dawley rats with known date of birth (day of birth registered as day 1) were delivered from the breeding farm (Møllegaard, Denmark) one week before hypophysectomy. The animals were kept in clear plastic cages with a metal grill top in an air-conditioned room with daylight illumination. The temperature was 22–24°C and the relative humidity 40–50 %/o. On arrival and during the experiments, the general condition of the animals was controlled and the weight was registered. All the animals were given pellets (Anticimex 213) and water ad libitum.

420 female rats were hypophysectomized at exactly 60 days of age; 174 animals survived the period of investigation. Of these 138 rats had complete hypophysectomy when determined microscopically (see below).

The rats were hypophysectomized by the parapharyngeal approach (Smith 1930; Thorngren et al. 1973a), and after a post-operative control period of 15 days, hormone administration was started. Groups of animals were given thyroxine (5, 10, 20 or 40 μg/kg) alone or in association with growth hormone (25 or 100 μg/kg). In addition, two groups of animals were given 10 μg/kg thyroxine from the day of hypophysectomy, and in one of those groups, 25 μg growth hormone was added after the 15-day post-operative control period.

A solution of 10 μg/ml of synthetic L-thyroxine (Nyegaard, Norway) was made twice a week with 0.9 % NaCl and kept under nitrogen according to Jacobsohn (1960). Bovine growth hormone (NIH-GH-B16) from National Institutes of Health, USA was dissolved in saline every day. Thyroxine and growth hormone were injected separately sc once a day during 20 days in volumes of 0.1–0.6 ml according to the dose. The controls were not given any injections.

The completeness of the hypophysectomy was determined by microscopical examination of serial sections of sella turcica (Jacobsohn 1966; Thorngren et al. 1973a) stained with azan. In addition, the body weight and weight of the heart ventricles (in the following called heart weight) were registered, and the ovaries were examined. Of the operated animals 33 %/o (138/420) had complete hypophysectomy. If the animals
with incomplete hypophysectomy (36 animals) were excluded from the total of operated animals, the survival rate was 36% (138/384).

Histological sections of the left proximal tibia stained with haematoxylin and eosin (Hansson 1967) were investigated microscopically to study the morphology of the growth plate. In some cases, the brittleness of the bones made sectioning impossible, which is indicated as reduced number of animals in Table 2.

To determine the accumulated growth in length, the experimental animals were given consecutive doses (10 mg/kg) of oxytetracycline, OTC (Terramycin®), intraperitoneally. The rats were temporarily anaesthetized with ether to increase the accuracy of the injections (Hansson et al. 1972). The animals were killed with ether.

The proximal tibia of the right side was dissected out, and undecalcified sections were made according to an earlier described method (Hansson 1964, 1967) from the medial part of the proximal tibia (Hansson et al. 1972). Growth determination was made with the aid of a fluorescence microscope with an error of method calculated to about 5 µm (Hansson 1967; Hansson et al. 1972).

The animals were given two injections of OTC, the first at hypophysectomy and the second 15 days post-operatively. After the 20-day administration period of hormones, they were killed on the day after the last hormone injection. The measurements were made between the two fluorescent bands corresponding to the injections of OTC, and between the second fluorescent band and the erosion line between the metaphysis and growth plate (Fig. 1) (Thorngren et al. 1973b). The width of the second fluorescent band was also determined (Thorngren et al. 1973a).

Earlier investigations using intraperitoneal injection of OTC for the determination of bone growth have shown that in some instances fluorescent bands might be missing at the microscopical investigation (Ahlgren 1968; Hansson et al. 1972). In the present investigation, this was found in 3 animals (2 with complete and 1 with incomplete hypophysectomy), which made it impossible to determine the post-operative accumulated growth in length during all the intended periods. These animals were also excluded from the calculations of the other growth parameters, making the total of completely hypophysectomized rats in Table 2 to be 136 animals.

RESULTS

Growth in length during control period (0–15 days post-operatively)

The determination of accumulated growth in length and width of the second fluorescent band after the post-operative control period of 15 days allowed a separation of the animals with complete and incomplete hypophysectomy, if no hormone administration was given during this period. Animals with incomplete hypophysectomy had both a higher accumulated growth in length and a wider fluorescent band than those with complete hypophysectomy (Fig. 2, Table 1).

If 10 µg/kg thyroxine was given during the post-operative 15-day control period, both the accumulated growth in length and the width of the second fluorescent band for animals with complete hypophysectomy were higher ($P < 0.1\%$, Student's $t$-test) than without thyroxine (Fig. 2, Table 1).
Growth in length of proximal tibia during the control 0–15 days post-operatively. Width of second fluorescent band (15 days post-operatively) measured in tenths of microns (ordinate) plotted to accumulated growth in length (abscissa). No treatment (○) or 10 µg/kg thyroxine daily (△) during control period. Individual values for complete (○,△) and incomplete (●,▲) hypophysectomy.

**Growth in length during administration period of thyroxine and growth hormone (15–35 days post-operatively)**

1. **Controls.** – Animals not given any injections of hormone had a very low accumulated growth in length (43 ± 33 µm) during the period 15–35 days post-operatively.

2. **Thyroxine.** – The daily injection of various doses (5–40 µg/kg) of thyroxine for 20 days, to animals not given any thyroxine during the pre-

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**Fig. 1.**

Fluorescence micrographs of proximal tibia (undecalcified sections) representing various doses of thyroxine and growth hormone administered daily for 20 days to female rats hypophysectomized at age 60 days starting 15 days post-operatively. Control (a), 20 µg/kg thyroxine (b), 20 µg/kg thyroxine and 25 µg NIH-GH-B16 (c), 20 µg/kg thyroxine and 100 µg NIH-GH-B16 (d). Growth plate (φ) and part of bony epiphysis at top of figure, metaphysis below. Two injections of oxytetracycline: the first, at hypophysectomy (0); the second, 15 days post-operatively (+15). The fluorescent bands (arrows) correspond to endochondral calcification at the time of injection. Erosion line (+35) between metaphysis and growth plate. (× 45).
Table 1.
Accumulated growth in length of proximal tibia during control period 0–15 days after hypophysectomy, body weight at operation and 15 days post-operatively. Values for animals with complete hypophysectomy (C. H.) and incomplete hypophysectomy (I. H.) including statistical analysis of difference (Student's t-test).

<table>
<thead>
<tr>
<th>Dose L-thyroxine daily during 0–15 days post-op. (µg/kg)</th>
<th>Number of animals</th>
<th>Accumulated growth in length (µm)</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>At operation (g)</td>
</tr>
<tr>
<td>C. H.</td>
<td>116</td>
<td>594 ± 105 *&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>176 ± 11</td>
</tr>
<tr>
<td>I. H.</td>
<td>30</td>
<td>961 ± 304 *&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>175 ± 12</td>
</tr>
<tr>
<td>C. H.</td>
<td>20</td>
<td>729 ± 171 *&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>177 ± 12</td>
</tr>
<tr>
<td>I. H.</td>
<td>5</td>
<td>1113 ± 279 *&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>169 ± 10</td>
</tr>
</tbody>
</table>

Values = means ± standard deviations.
The statistical analysis and the calculation of values for animals with I. H. were made with the assumption that these values are normally distributed.

*<sup>+++</sup> P < 0.1%<sup>*</sup>  *<sup>++</sup> 0.1% < P < 1.0%  *<sup>+</sup> 1.0% < P < 5%  (-) P > 5%

ceeding control period, resulted in significant stimulation of the longitudinal bone growth (Fig. 3 and Tables 2 and 3). The accumulated growth in length increased with the administration of 5 and 10 µg/kg and then remained constant at about 300 µm following the administration of 20 and 40 µg/kg thyroxine (Fig. 3, Tables 2 and 3).

In animals given 10 µg/kg thyroxine during the preceding 15-day control period, the continued administration of this dose for the following 20 days resulted in a longitudinal bone growth during the 20-day administration period which was almost the same as in animals not treated with thyroxine during the control period (Fig. 3).

3. Growth hormone. – The daily injection of 25 or 100 µg NIH-GH-B16 for 20 days (during 15–35 days post-operatively) resulted in an accumulated growth in length of about 580 µm or 800 µm, respectively (Table 2).

4. Thyroxine and growth hormone. – The daily injection of 25 or 100 µg growth hormone given simultaneously with various doses (5–40 µg/kg) of thyroxine, to animals not given any thyroxine during the preceding control period, resulted in a higher longitudinal bone growth than that of growth hormone alone (Fig. 3, Tables 2 and 3). The thyroxine dose 20 µg/kg was
Accumulated growth in length of proximal tibia after daily administration for 20 days of thyroxine and NIH-GH-B16 in various doses starting 15 days post-operatively. Thyroxine alone (—o—), thyroxine and 25 µg NIH-GH-B16 (——o——), thyroxine and 100 µg NIH-GH-B16 (—— o ——) given to animals not pretreated during preceding control period. Animals pretreated with 10 µg/kg thyroxine daily (△).

optimum showing an accumulated growth in length of about 1100 µm and 1900 µm for 25 and 100 µg NIH-GH-B16, respectively. No further growth promotion was achieved with 40 µg/kg thyroxine. Furthermore, the simultaneous administration of thyroxine and growth hormone at all dose levels gave a higher accumulated growth than the expected additive effect of the two hormones (Table 4). This difference was statistically highly significant for the higher growth hormone dose (100 µg) and for animals pretreated with thyroxine. The 20 µg/kg dose of thyroxine and 25 µg growth hormone also resulted in a significant difference in accumulated growth in length between simultaneous administration and the expected additive effect (Table 4). Furthermore, the higher values for combined administration at all dose levels also

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Table 2.
Effect of L-thyroxine and growth hormone (NIH-GH-B16) given daily for 20 days (15-35 days post-operatively) to female rats hypophysectomized at 60 days of age, complete hypophysectomy. Accumulated growth in length and width of growth cartilage of proximal tibia, weight of body and heart.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>No. of animals</th>
<th>Accumulated growth in length (μm)</th>
<th>Cartilage width</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-thyroxine (μg/kg)</td>
<td>NIH-GH-B16 (μg)</td>
<td>Un-differentiated (μm)</td>
<td>Columnar zone (μm)</td>
<td>Total (μm)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>43 ± 33</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>128 ± 45</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0</td>
<td>8</td>
<td>276 ± 91</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0</td>
<td>7</td>
<td>338 ± 145</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>0</td>
<td>9</td>
<td>304 ± 109</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>25</td>
<td>16</td>
<td>578 ± 134</td>
<td>12 ± 4 (15)</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>25</td>
<td>8</td>
<td>772 ± 94</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>25</td>
<td>8</td>
<td>960 ± 156</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>25</td>
<td>6</td>
<td>1144 ± 135</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>25</td>
<td>11</td>
<td>1075 ± 271</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>100</td>
<td>6</td>
<td>795 ± 133</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>100</td>
<td>7</td>
<td>1903 ± 214</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>100</td>
<td>9</td>
<td>1750 ± 179</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>14</td>
<td>10¹</td>
<td>0</td>
<td>12</td>
<td>295 ± 129</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>10¹</td>
<td>25</td>
<td>8</td>
<td>1287 ± 84</td>
<td>12 ± 2</td>
</tr>
</tbody>
</table>

Values = means ± standard deviations. Reduced number of animals in brackets.
1) Pretreatment with L-thyroxine 10 μg/kg daily during control period 0-15 days after hypophysectomy.
indicate that the combined administration of thyroxine and growth hormone gives a potentiating synergistic stimulation of longitudinal bone growth.

In animals given 10 µg/kg thyroxine during the preceding 15-day control period, the continued administration of thyroxine in combination with 25 µg growth hormone for the following 20 days, resulted in an accumulated growth higher (1287 ± 84 µm) than that in the animals given the same combination for 20 days, but not previously treated with thyroxine during the control period (960 ± 156 µm) (Fig. 3, Tables 2 and 3).

**Cartilage width**

Administration of various doses of thyroxine alone resulted in minor changes in the total cartilage width as compared with the controls (Fig. 4, Tables 2 and 3). The animals daily given 25 µg or 100 µg growth hormone alone had a higher total width of growth cartilage than the controls. The simultaneous administration of 5 µg/kg thyroxine and 25 µg growth hormone resulted in a decrease in cartilage width. A further increase of the thyroxine dose did not change the cartilage width (Fig. 4). Animals given the simultaneous administration of thyroxine and 100 µg/kg growth hormone showed no change in the cartilage width as compared with animals given only growth hormone.

**Table 3.**

Statistical analysis (Student’s t-test) of difference in growth between various groups (Table 2) of treatment with L-thyroxine and growth hormone (NIH-GH-B16).

<table>
<thead>
<tr>
<th>Group v. Group</th>
<th>Accumulated growth in length</th>
<th>Total cartilage width</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Body</td>
</tr>
<tr>
<td>2 v. 1</td>
<td>85***</td>
<td>-10(-)</td>
<td>-13*</td>
</tr>
<tr>
<td>3 v. 2</td>
<td>148**</td>
<td>20**</td>
<td>-11*</td>
</tr>
<tr>
<td>4 v. 3</td>
<td>57(-)</td>
<td>-4(-)</td>
<td>5(-)</td>
</tr>
<tr>
<td>5 v. 4</td>
<td>-29(-)</td>
<td>4(-)</td>
<td>-8(-)</td>
</tr>
<tr>
<td>7 v. 6</td>
<td>194**</td>
<td>-20**</td>
<td>1(-)</td>
</tr>
<tr>
<td>8 v. 7</td>
<td>188*</td>
<td>-26(-)</td>
<td>5(-)</td>
</tr>
<tr>
<td>9 v. 8</td>
<td>184*</td>
<td>23(-)</td>
<td>4(-)</td>
</tr>
<tr>
<td>10 v. 9</td>
<td>-60(-)</td>
<td>-5(-)</td>
<td>-10(-)</td>
</tr>
<tr>
<td>12 v. 11</td>
<td>1108***</td>
<td>-9(-)</td>
<td>11*</td>
</tr>
<tr>
<td>13 v. 12</td>
<td>-153(-)</td>
<td>-8(-)</td>
<td>-16**</td>
</tr>
<tr>
<td>14 v. 3</td>
<td>19(-)</td>
<td>-7(-)</td>
<td>14**</td>
</tr>
<tr>
<td>15 v. 8</td>
<td>327***</td>
<td>25(-)</td>
<td>10*</td>
</tr>
</tbody>
</table>

**P < 0.1%**  **0.1% < P < 1.0%**  **1.0% < P < 5%**  **(P > 5%)**
Table 4.
Accumulated growth in length of proximal tibia after simultaneous administration of various doses of L-thyroxine and growth hormone (NIH-GH-B16) for 20 days (Table 2) compared with the calculated additive effect of the two hormones' growth stimulating effect when given alone. Statistical analysis of difference in growth stimulation (Student's t-test).

<table>
<thead>
<tr>
<th>Dose</th>
<th>Simultaneous administration (µm)</th>
<th>Additive effect (µm)</th>
<th>Difference (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-thyroxine (µg/kg)</td>
<td>NIH-GH-B16 (µg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>772 ± 94 (8)</td>
<td>706 ± 45 (7)</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>960 ± 156 (8)</td>
<td>854 ± 91 (8)</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>1144 ± 135 (6)</td>
<td>911 ± 145 (7)</td>
</tr>
<tr>
<td>40</td>
<td>25</td>
<td>1075 ± 271 (11)</td>
<td>822 ± 109 (9)</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>1903 ± 214 (7)</td>
<td>1128 ± 145 (7)</td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>1750 ± 179 (9)</td>
<td>1099 ± 109 (9)</td>
</tr>
<tr>
<td>10¹</td>
<td>25</td>
<td>1287 ± 84 (8)</td>
<td>873 ± 129 (12)</td>
</tr>
</tbody>
</table>

Values = means ± standard deviations. Number of animals in parenthesis.

¹) Pretreatment with L-thyroxine 10 µg/kg daily during control period 0–15 days after hypophysectomy.

***) P < 0.1 %  ** P < 1.0 %  * P < 5 %  (−) P > 5 %

Within the growth plate, the undifferentiated cartilage zone had almost the same width in all the groups. The columnar zone shows the changes found for total cartilage (Table 2).

**Body weight**

After the post-operative control period of 15 days, animals with incomplete hypophysectomy had a considerably higher body weight than those with complete hypophysectomy, whereas there was no difference in body weight at operation (Table 1). Animals given 10 µg/kg thyroxine during the control period had almost the same body weight at the end of this period as animals not given any thyroxine (Table 1).

The body weights of animals given various doses of growth hormone and thyroxine daily for 20 days (15–35 days post-operatively) are presented in Fig. 4 and Tables 2 and 3. Animals given various doses of thyroxine alone had a lower body weight than the controls. The body weight for animals given both thyroxine and growth hormone was higher than for those given thyroxine only. This was most obvious for doses of thyroxine 10 µg/kg and
higher (Fig. 4). When given in association with thyroxine, the dose of growth hormone given determined the body weight.

**Heart weight**

When increasing doses of thyroxine were given alone for 20 days, the heart weight increased and reached an almost constant value with the two higher doses of thyroxine (Fig. 4, Tables 2 and 3). For animals given simultaneous administration of thyroxine and growth hormone, the heart weight was greater than for animals given thyroxine or growth hormone alone. It increased with the increased dose of thyroxine.

The ratio heart weight/body weight was calculated and was found to increase with the dose of thyroxine, both for animals given thyroxine alone and for those given thyroxine and growth hormone in combination at all dose levels (Tables 2 and 3).
DISCUSSION

Of the different growth parameters tested in this investigation, the accumulated growth in length and the heart weight responded best when increasing doses of thyroxine were given alone or in combination with growth hormone (Figs. 3 and 4, Tables 2 and 3). Earlier investigations (Thorngren et al. 1973b) have shown that the accumulated growth in length is the most sensitive growth parameter for growth hormone among those tested in this investigation. The growth stimulation by growth hormone is known to be dependent both on the dose and the length of the administration period (Thorngren et al. 1973b). In the present investigation, it was found that the accumulated growth in length is also sensitive to thyroxine. This is in agreement with some earlier investigations (Ray et al. 1950; Simpson et al. 1950; Asling et al. 1954; Asling & Evans 1956; de Groot 1963; Meites & Kragt 1964; Pannin & Galvao 1964). The negative results found by others (Bielschowsky et al. 1962; Goodall & Gavin 1966) might be due either to the thyroxine used or the determined growth parameter.

In this investigation, an optimum growth stimulating dose of thyroxine was found. The longitudinal growth increased with increasing doses of thyroxine alone up to 10–20 µg/kg. A further increase in the thyroxine dose did not result in any further increase in the longitudinal bone growth. Thus the dose dependent stimulation of growth in length is different between thyroxine and growth hormone. Moreover, Asling et al. (1954) have found that if hypophysectomized rats are given thyroxine in prolonged experiments for several months, the growth in length is only stimulated initially (Asling & Evans 1956).

In earlier investigations, different preparations of thyroxine have been given to rats in various doses. Jacobsohn (1960) used synthetic DL-thyroxine in increasing doses in the same animal from 2.5–10 µg/rat, corresponding to about 25–100 µg/kg. Scow (1954, 1959) used 40 µg/kg DL-thyroxine. Hulth & Nylander (1962) used L-thyroxine in doses corresponding to about 50 or 200 µg/kg, which they estimated at 5–20 times the maintenance dose. Riekstniec & Asling (1966), using L-thyroxine to study the growth in length and weight of the body, increased the dose from 0.25 µg/day to 0.5 µg/day and found twice as great an effect. Further increase to 1.0 µg/day did not give any further increase in the growth. Thus a daily dose corresponding to about 5 µg/kg had an optimum effect in their experiments. Asling et al. (1968) used a dose of L-thyroxine corresponding to 2.5 µg/kg. This dose is among the lowest reported in which thyroxine evoked significant in vivo biological effects (Asling et al. 1968). Thus the L-thyroxine doses tested in this investigation (5–40 µg/kg) are comparable to those used by other investigators to study the effects of thyroxine on growth. In this investigation, an optimum stimulation
of the growth in length was found for 10–20 μg/kg thyroxine when given alone or in association with growth hormone.

The simultaneous administration of thyroxine and growth hormone resulted in a higher accumulated growth in length than with thyroxine or growth hormone given alone. When the dose of growth hormone was kept constant, an increase in the thyroxine dose resulted in an increase in longitudinal bone growth up to an optimum thyroxine dose. Further increase in the thyroxine dose did not result in any further growth stimulation. This was almost the same optimum dose level as that when thyroxine was given alone.

The simultaneous administration of thyroxine and growth hormone at all dose levels tested resulted in a higher accumulated growth in length than the expected additive single effects of the two hormones. Thus, the combination of the two hormones resulted in a potentiating synergistic action on the growth in length (Table 4).

Other investigators (Simpson et al. 1950; Asling & Evans 1956; Baume et al. 1958; de Groot 1963) have studied the effect of thyroxine and growth hormone when given simultaneously and found that the growth stimulation by the two hormones in combination is higher than for growth hormone alone. The synergistic action of thyroxine on the growth hormone-induced longitudinal bone growth has been proposed by some investigators (Marx et al. 1942; Asling & Evans 1956). In the present investigation, it was possible to demonstrate a statistically significant potentiating synergism between thyroxine and growth hormone on the longitudinal bone growth (Table 4).

Pretreatment with thyroxine during the 15-day post-operative control period, did not significantly increase the following thyroxine-induced longitudinal bone growth when thyroxine was given alone at an optimum dosage (Tables 2 and 3). Thus, it seems as if thyroxine does not accumulate. However, in animals given thyroxine during the preceding control period, the continued administration of thyroxine, in association with growth hormone for the following 20 days, resulted in an accumulated growth in length that was higher than that of animals given the same combination for 20 days but not previously treated with thyroxine (Tables 2 and 3). Thus pretreatment with thyroxine increased the combined growth hormone- and thyroxine-induced longitudinal bone growth. It seems as if the growth hormone-induced bone growth is further potentiated by the previous effect of thyroxine on the animals; so that the growth in length achieved by the administration of growth hormone starts at a metabolically higher level.

Earlier investigators using the tibia test have shown that thyroxine alone does not induce any sustained plate widening, whereas thyroxine and growth hormone in association results in marked widening of the growth plate, i.e. higher than that of growth hormone alone (Asling et al. 1968). More pro-
longed administration periods and the higher thyroxine doses used might explain the different results found here.

In the present investigation when increasing doses of thyroxine were administered alone, the width of the growth plate of proximal tibia was almost constant as compared with that of the controls not given any injections (Fig. 4, Tables 2 and 3). The animals given growth hormone alone showed the highest width of the growth plate. This decreased somewhat when thyroxine was added, even if the accumulated longitudinal bone growth increased considerably (Figs. 3 and 4). The results indicate that thyroxine acts as a stimulator and enhancer of the process involved in endochondral growth, since it was found that there was considerable growth stimulation even if the growth plate was narrow. This indicates that there is no direct correlation between the growth rate and the width of the growth plate under experimental conditions, which has been claimed previously (Rang 1969).

Thyroxine alone decreased the body weight as compared with the controls not given any injections (Fig. 4, Table 2 and 3). The body weight was almost the same for controls and animals given growth hormone alone. The simultaneous administration of thyroxine and growth hormone did not change the body weight. Thus it seems as though thyroxine decreases the body weight and that the combination with growth hormone compensates for this weight loss. Asling et al. (1968) when studying the growth plate also tried to study other growth parameters. They found that the detection of somatic growth, except for body weight, was difficult under their assay conditions. The body weight increased after the administration of growth hormone, and a further increase in body weight was achieved with simultaneous administration of thyroxine and growth hormone, whereas thyroxine alone did not increase the body weight (Asling et al. 1968).

The heart weight is normally known to be related to the body weight and also after hypophysectomy (Beznák 1954). The heart weight is not influenced selectively by administration of growth hormone after hypophysectomy (Beznák 1967; Tipton & Tcheng 1971; Thorngren et al. 1973b). In the present investigation, the heart weight was found to increase but not the body weight after the administration of increasing doses of thyroxine, indicating a specific growth promoting effect of thyroxine on the heart ventricles. This is in accordance with earlier investigations (Scow 1954; Jacobsohn 1960; Beznák 1967). The increased metabolism induced by the thyroxine administration might increase the demands on the heart. As shown in earlier investigations with use of aortic constriction and DOCA injections, cardiac enlargement can occur in hypophysectomized rats only when the work demands on the heart have been increased (Beznák 1967; Tipton & Tcheng 1971).

The longitudinal bone growth can be used for bioassay of growth hormone (Thorngren et al. 1973b). As shown in this investigation, thyroxine potentiates
the growth stimulating effect of growth hormone. By means of the tetracycline method, it is possible to determine the biological effect of various doses of growth hormone in combination with an optimum dose of thyroxine, thus allowing the determination of small doses of growth hormone during short periods.

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