EFFECT OF ADMINISTRATION FREQUENCY OF GROWTH HORMONE ON LONGITUDINAL BONE GROWTH IN THE HYPOPHYSECTOMIZED RAT

By

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

ABSTRACT

The effect of the administration frequency of growth hormone on longitudinal bone growth was investigated with tetracycline as intravital marker of the bone growth of the proximal tibia in hypophysectomized rats. The total dose of growth hormone (NIH-GH-B16) and the administration period were the same in all compared experiments. It was possible to achieve an optimum growth response for a certain total dose of growth hormone by increasing the injection frequency. The period of hormone administration was 10 or 5 days followed by a 10 days withdrawal period. When the growth hormone was administered alone or in association with L-thyroxine for 10 days, the optimum injection frequency for growth hormone was found to be 1 inj./day in hypophysectomized rats and 2 inj./day in thyroxine-treated hypophysectomized rats.

When the administration period was 5 days for growth hormone given in association with L-thyroxine, the growth stimulation induced by one daily growth hormone injection was the same as that induced by two or four daily injections of the same total dose.

An increase in the administration frequency for a total daily dose of thyroxine from 1 to 2 inj./day did not increase the longitudinal bone growth either when thyroxine was given alone or in association with growth hormone.

There are few reports on the effect of administration frequency of growth hormone on bone growth.

In earlier investigations in hypophysectomized rats, the influence of growth
hormone on the longitudinal growth (Asling & Evans 1956; Urist 1972) and on the width of the growth plate (Greenspan et al. 1949; Geschwind & Li 1955; Papkoff & Li 1962) has generally been studied with daily injections of the hormone. Changes in the injection frequency have shown varying results on the width of the growth plate in the tibia test; no influence on the plate width was found by Greenspan et al. (1949) and Geschwind & Li (1955), whereas Papkoff & Li (1962) found significant differences when the same daily dose was divided into two equal injections instead of one daily injection.

The longitudinal bone growth induced by growth hormone in the hypophysectomized rat depends on the dose and the administration period (Asling & Evans 1956; Urist 1972; Thorngren et al. 1973b). Various factors influencing the dose-dependent growth response after administration of growth hormone have been investigated (Thorngren & Hansson 1975). Preliminary investigations have then shown the growth in length to be significantly influenced by the administration frequency.

In the present investigation, the effect of the administration frequency on the dose-dependent longitudinal bone growth, induced by growth hormone administration in hypophysectomized rats, has been determined. The influence of the administration frequency can be used to achieve maximum growth response in the bioassay of growth hormone (Thorngren & Hansson 1974b,c,d).

MATERIAL AND METHODS

Animals. – Hypophysectomized female rats were used. The treatment of the animals, the hypophysectomy and the checking of the hypophysectomy were performed as described earlier (Thorngren et al. 1973a; Thorngren & Hansson 1975). The rats were hypophysectomized at 60 days of age. After a postoperative control period of 15 days, the hormone administration was started. Of 410 animals, 211 survived the investigation period. Of these 174 had complete hypophysectomy.

Hormones. – Bovine growth hormone (NIH-GH-B16)1) was dissolved in saline and kept frozen.

A solution of 10 μg/ml of L-thyroxine2) was prepared twice a week with saline and kept under nitrogen (Thorngren & Hansson 1973a).

The administration period of the hormones was 10 days in Groups A, B and C; in Group D, 5 days. The administration period in all the groups was followed by a 10-day period without treatment in order to register the total growth stimulating effect of the given hormones (Thorngren & Hansson 1974a,b,c).

Group A. – To study the influence of changes in the administration frequency of a specific total dose of growth hormone, a total dose of 500 μg NIH-GH-B16 was

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1) Kindly supplied by the NIAMDD, National Institutes of Health, Bethesda, USA.
2) Kindly supplied by Nyegaard and Co., AB, Norway.
Table 1.

Effect of administration frequency on the growth stimulation of a specific total dose of growth hormone (500 μg NIH-GH-B16) given to female rats hypophysectomized at 60 days of age (Group A). Beginning 15 days post-operatively, growth hormone was administered for 10 days followed by a withdrawal period of 10 days. Determination of accumulated growth in length of proximal tibia (uncorrected and corrected) 15–35 days post-operatively. Width of proximal tibial growth plate 35 days post-operatively. Body weight at different post-operative intervals.

Statistical analysis (Student's t-test) of difference between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>NIH-GH-B16</th>
<th>Number of animals</th>
<th>Accumulated growth in length</th>
<th>Cartilage width (μm)</th>
<th>Body weight</th>
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<td></td>
<td>μg/inj.</td>
<td>Total number of injections</td>
<td>Uncorrected (μm)</td>
<td>Corrected (μm)</td>
<td>15 days post-op. (g)</td>
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<tr>
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<td>150 ± 11</td>
</tr>
<tr>
<td>IV</td>
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<td>5</td>
<td>211 ± 12</td>
<td>217 ± 24</td>
</tr>
<tr>
<td>V¹)</td>
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<td>10</td>
<td>8</td>
<td>419 ± 18</td>
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<td>9</td>
<td>443 ± 34</td>
<td>407 ± 21</td>
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Values = M ± SEM.

¹) From Thorngren & Hansson 1974b.
Table 2.

Effect of administration frequency on the growth stimulation of a specific total dose of growth hormone (500 μg NIH-GH-B16) given to female rats hypophysectomized at 60 days of age (Group B). Beginning 15 days post-operatively, growth hormone was administered for 10 days in association with a daily injection of L-thyroxine followed by a withdrawal period of 10 days. Determination of accumulated growth in length of proximal tibia (uncorrected and corrected) 15-35 days post-operatively. Width of proximal tibial growth plate 35 days post-operatively. Body weight at different post-operative intervals. Statistical analysis (Student’s t-test) of difference between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>NIH-GH-B16</th>
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<th>Accumulated growth in length</th>
<th>Cartilage width (μm)</th>
<th>Body weight</th>
</tr>
</thead>
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<td>Total number of injections</td>
<td>Uncorrected (μm)</td>
<td>Corrected (μm)</td>
<td>15 days post-op. (g)</td>
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<td>0</td>
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<td>289 ± 35</td>
</tr>
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<tr>
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<td>764 ± 33</td>
<td>752 ± 28</td>
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<tr>
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<td>$1031 \pm 29$</td>
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<td>VI</td>
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<td>$1133 \pm 48$</td>
<td>$1060 \pm 37$</td>
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<td></td>
<td>$1198 \pm 53$</td>
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<td>14</td>
<td>$1076 \pm 17$</td>
<td>$1048 \pm 16$</td>
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</table>

Values = m ± SEM.  

1) Values from Thorngren & Hansson 1974c.  
2) Injections 08.00, 11.00, 14.00 and 17.00.
given to each animal in sc injections for 10 days. The total dose of growth hormone was divided into different sets of equal doses, given over appropriate time-intervals during the 10-day administration period. The total number of injections ranged from 1–20 (Table 1).

**Group B.** – To study the influence of changes in the administration frequency of a specific total dose of growth hormone given in association with thyroxine administration, a total dose of 500 μg NIH-GH-B16 was divided into different sets of equal doses and administered as in Group A. In addition separate sc injections of 20 μg/kg L-thyroxine were given once daily during the 10-day administration period. The different groups of animals received from 1 to 80 injections of growth hormone in association with 1 daily injection of thyroxine (Table 2). Some animals were given 4 daily growth hormone injections between 08.00–17.00 (at 08.00, 11.00, 14.00 and 17.00) instead of evenly distributed growth hormone injections (Table 2).

**Group C.** – To study the effect of changes in the administration frequency of thyroxine, the animals were given 2 daily injections of 10 μg/kg L-thyroxine at equal time intervals for 10 days. Some animals were given only thyroxine; other animals were given in addition a total dose of 500 μg NIH-GH-B16 by means of separate sc injections. The total growth hormone dose was divided into 10 or 20 equal doses, given either once or twice daily at equal time intervals.

**Group D.** – To study the influence of the administration frequency in the most favourable bioassay model for growth hormone (Thorngren & Hansson 1974b,c), different total doses of NIH-GH-B16 were administered for 5 days in association with a daily sc injection of thyroxine (20 μg/kg). The growth hormone was given either twice daily at equal time intervals or four times daily during the usual working period of the laboratory 08.00–17.00 (at 08.00, 11.00, 14.00 and 17.00).

**Growth determination.** – The longitudinal bone growth from the proximal growth plate of the tibia was determined with oxytetracycline (OTC) as intravital marker a sprectively described (Hansson et al. 1972; Thorngren & Hansson 1974b,c,d). The animals were given 2 injections of OTC; the first at hypophysectomy and the second 15 days post-operatively. They were killed with ether at the end of the investigated period.

In the present investigation, fluorescent bands were missing in 13 animals making it impossible to determine the post-operative accumulated growth in length during all the intended periods. These animals were excluded from the calculations of the other growth parameters.

Corrected values of longitudinal bone growth were calculated as previously described (Thorngren & Hansson 1974b,c). The equations used for correction were:

- **Group A:** corrected response \( y' = y - 0.6 \) (v-550)
- **Groups B and C:** corrected response \( y' = y - 0.9 \) (v-550)
- **Group D:** corrected response \( y' = y - 0.3 \) (v-550),

\( y \) being the measured growth during the experimental period and \( v \) during the control period.

**Morphology of growth plate and body weight.** – The width of the growth plate and the body weight were determined as earlier described (Thorngren & Hansson 1973b,c).
**Significance levels.** - The following levels were used in statistical tests of significance:

- ******* \( P < 0.001 \)
- **** \( 0.001 < P < 0.01 \)
- \( * \) \( 0.01 < P < 0.05 \)
- \( (-) \) or **NS** \( P > 0.05 \).

**RESULTS**

**Effect of administration frequency on the growth stimulation of a specific total dose of growth hormone** (Groups A and B)

**Longitudinal bone growth.** - When a total dose of 500 \( \mu g \) NIH-GH-B16 was divided into different sets of equal fractions, the injections being evenly distributed during the administration period, the growth in length was found to increase with increasing injection frequency up to injections once daily (10 injections) in Group A and twice daily (20 injections) in Group B (Tables 1 and 2, Figs. 1 and 2). A further increase in the administration frequency did not increase the growth in length (Figs. 1 and 2). Group B at the optimum administration frequency showed no difference in longitudinal bone growth between evenly distributed injections and injections given during day-time (Table 2).

Group A, when the administration frequency was increased from 1 injection to 10 injections during the administration period (up to 1 injection per

![Graph](image.png)

**Fig. 1.**

Effect of administration frequency of a total dose of growth hormone (500 \( \mu g \) NIH-GH-B16) on accumulated longitudinal bone growth (corrected) of proximal tibia in hypophysectomized rats (Group A, Table 1). (Logarithmic scale on abscissa).
Fig. 2.
Effect of administration frequency of a total dose of growth hormone (500 µg NIH-GH-B16) on accumulated longitudinal bone growth (corrected) of proximal tibia in thyroxine-treated hypophysectomized rats (Group B, Table 2). (Logarithmic scale on abscissa).

day), showed a 5-fold net increase in the corrected longitudinal bone growth (growth hormone-induced minus control value) (Table 1). Group B, when the total number of injections was increased from 1 to 20, showed a 9-fold net increase in the corrected longitudinal bone growth (Table 2).

*Width of growth plate and body weight.* – In both Groups A and B, the total width of the growth plate of the proximal tibia at the end of the investigation period showed minor changes obviously unrelated to the number of injections given, which shows that the hormone-induced growth stimulation had ceased (Tables 1 and 2). The body weight seemed to be somewhat higher with the higher number of injections than with the lower ones (Tables 1 and 2). For the change in body weight, the net increase was 6-fold in Group A and 3-fold in Group B at corresponding injections per day as for the longitudinal bone growth, but the body weight showed greater variation for the different injection frequencies.

*Effect of administration frequency of thyroxine* (Group C)
L-thyroxine (10 µg/kg) given twice daily alone or in association with growth hormone showed no difference in the longitudinal bone growth from that of animals given one daily injection (20 µg/kg) of thyroxine (Table 3). The width of the growth plate and the body weight also seemed uninfluenced by the change in administration frequency of thyroxine (Table 3).
Table 3.
Effect of administration frequency of a specific total dose of thyroxine (20 μg/kg L-thyroxine daily) given to female rats hypophysectomized at 60 days of age (Group C). Beginning 15 days post-operatively, L-thyroxine (10 μg/kg) was administered twice daily for 10 days alone or in association with growth hormone (NIH-GH-B16) followed by a withdrawal period of 10 days. Determination of accumulated growth in length of proximal tibia (uncorrected and corrected) 15–35 days post-operatively. Width of proximal tibial growth plate 35 days post-operatively. Body weight at different post-operative intervals. Statistical analysis (Student's t-test) of difference between groups.

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<th>Accumulated growth in length</th>
<th>Cartilage width (μm)</th>
<th>Body weight</th>
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<td></td>
<td>μg/inj.</td>
<td>Total number of injections</td>
<td></td>
<td>Uncorrected (μm)</td>
<td>Corrected (μm)</td>
<td>15 days post-op. (g)</td>
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<td>186 ± 23</td>
<td>167 ± 25</td>
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</tr>
<tr>
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<td>841 ± 34</td>
<td>764 ± 32</td>
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<td>1048 ± 46</td>
<td>979 ± 24</td>
<td>174 ± 5</td>
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</table>

Values = M ± SEM.

1) Group value from Table 2.
Effect of administration frequency on the bioassay of growth hormone (Group D, Table 4). Accumulated longitudinal bone growth (corrected) of proximal tibia for various total doses of growth hormone (NIH-GH-B16). The values in the curve with 1 daily injection of growth hormone were taken from Thorngren & Hansson (1974c).

Statistical analysis (Student’s t-test) of difference between the groups.

Effect of administration frequency on the bioassay of growth hormone (Group D)

Different total daily doses of growth hormone were administered to thyroxine-treated hypophysectomized rats with injections given twice or four times daily, and the longitudinal bone growth was compared with the growth stimulating effect of the injections given once daily of these total doses (values from Thorngren & Hansson 1974c) (Fig. 3 and Table 4). The administration period was shorter than in Groups A, B and C. As Fig. 3 and Table 4 show, no significant difference was found between the same total doses of growth hormone when the injections were given once, twice or four times daily.

The width of the growth plate and the body weight also seemed uninfluenced by the studied change in administration frequency (Table 4).

DISCUSSION

In earlier investigations using other methods for growth determination, growth hormone has mainly been given once daily (Asling & Evans 1956; Urist 1972). Few investigations have been performed with changes in the administration
Table 4.
Effect of administration frequency on the bioassay of growth hormone (Group D). In female rats hypophysectomized at 60 days of age, beginning 15 days post-operatively, growth hormone was administered for 5 days in association with L-thyroxine (20 μg/kg daily in one injection) followed by a withdrawal period of 10 days. Determination of accumulated growth in length of proximal tibia (uncorrected and corrected) 15–30 days postoperatively. Width of proximal tibial growth plate 30 days post-operatively. Body weight at different post-operative intervals.

<table>
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<th>Number of injections per day</th>
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<th>Cartilage width (μm)</th>
<th>Body weight</th>
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<tr>
<td></td>
<td>μg/inj.</td>
<td>μg total</td>
<td>Uncorrected (μm)</td>
<td>Corrected (μm)</td>
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<td>–</td>
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<td>139 ± 2</td>
<td>165 ± 2</td>
<td>155 ± 1</td>
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Values = M ± SEM.

1) From Thorngren & Hansson 1974c.
2) Injections 08.00, 11.00, 14.00 and 17.00.
frequency. With the tibia test (the width of the cartilage growth plate in hypophysectomized rats) contradictory results have been obtained: Greenspan et al. (1949) and Geschwind & Li (1955) found no difference; Papkoff & Li (1962) found a significantly increased width of the growth plate for 2 injections per day compared with one daily injection.

In hypophysectomized mice, Lostroh & Li (1957) also found a significantly increased width of the growth plate with 2 injections per day. McShan (1971) used a modified tibia test injecting twice daily for 3 days, but no comparison as against one daily injection was presented.

A comparison of the metabolic effects in human subjects of human growth hormone (HGH) administered once daily as against the same total daily amount given in divided doses showed no difference in effectiveness of the HGH (Korner et al. 1959; Wright et al. 1965). In the clinical administration of HGH to hypopituitary patients, 2 or 3 injections/week are usually given, but administration with 1 injection/week also results in growth promotion (Frasier et al. 1969).

As the present investigation shows, the injection frequency has an influence on the stimulation of longitudinal bone growth by growth hormone when a long administration period is used (Groups A and B). Thus, when only one single injection with the total dose was given at the beginning of the 10-day administration period, the longitudinal bone growth was only somewhat higher than that found for controls given no growth hormone injection (Figs. 1 and 2, Table 1 and 2). The bone growth was increased with the injection frequency up to an optimum, both in hypophysectomized rats (5-fold increase) and in thyroxine-treated hypophysectomized rats (9-fold increase). With the longer administration period (10 days) in thyroxine-treated animals (Group B), 2 daily injections resulted in a significantly higher growth response than one daily growth hormone injection (Table 2). Thus, it is possible to achieve an optimum growth response for a certain total dose of hormone by increasing the injection frequency.

The differences in the effect of the administration frequency found when adding thyroxine seems to depend on a change in the basal metabolism which increases the sensitivity of the animals to the growth hormone.

A change in the injection frequency for thyroxine from 1 to 2 injections/day did not change the longitudinal bone growth when the thyroxine was given alone or in association with growth hormone given at different intervals (Table 3). Thus, the experimental model with one daily thyroxine injection in association with various injection frequencies for the growth hormone, seems efficient in this respect. The 10-day withdrawal period was used in the present investigation to obtain the total effect of the hormone administration as shown previously (Thorngren & Hansson 1974a).

For the bioassay of growth hormone, earlier investigations (Thorngren &
Hansson 1974b,c) have shown that a model with administration of growth hormone for 5 days in association with thyroxine followed by a 10 days withdrawal period is the most favourable. This model is best for sensitivity, precision, and administration period. Thus, the experiences with increased growth response for an increased injection frequency of growth hormone when administered for 10 days are expected to be also effective with a 5 days administration period. However, as Fig. 3 and Table 4 shows, neither 2 nor 4 daily injections increase the growth response compared to one daily injection for 5 days of the same total dose of growth hormone. The 5-day period seems too short to achieve the effect of increased growth response with the increased administration frequency. Thus, it is not possible to use the increased injection frequency as a means of increasing the sensitivity in the bioassay of growth hormone. The earlier used model for testing different growth hormone preparations (Thorngren & Hansson 1974d), seems to be the most effective and also the most simple for the bioassay of growth hormone using tetracycline as intravital marker of the longitudinal bone growth of the proximal tibia in thyroxine-treated hypophysectomized rats.

The increased longitudinal bone growth found for an increased injection frequency of a certain total amount of growth hormone might depend on several factors, e. g., the absorption time of the growth hormone from the injection site, the biological half-life of growth hormone, the possible production of an intermediary substance, and the responsiveness of the target organ (the cartilage cell system in the growth plate) to constant or intermittent stimulation.

In recent years, it has been proposed that growth hormone exerts its peripheral effects through the production of intermediary hormones called somatomedins (for review, see Daughaday & Garland 1972; Luft & Hall 1975). As found in the present investigation, the eventual somatomedin production would be affected not only by various doses of growth hormone but also by the injection frequency of a specific total dose of growth hormone. The optimum injection frequencies found in the present investigation might reflect the time for production or function of somatomedin.

The administration of growth hormone in association with thyroxine might give a higher somatomedin concentration or increase its action, as found by the higher longitudinal bone growth for thyroxine-treated animals in the present investigation. Experiments with purified somatomedins, when these substances become available, would possibly resolve this question.

The cellular mechanisms involved in the acute effects of growth hormone are to a large extent unknown (Ahrén et al. 1976). A stimulatory effect of growth hormone on the monosaccharide and amino acid transport in the diaphragm muscle of hypophysectomized rats is seen both after in vivo and in vitro administration. This is, however, a transitory phenomenon (2–3 h)

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followed by a period (24-48 h) when the diaphragm muscle can no longer be stimulated again by growth hormone (Ahrén et al. 1976). The optimum injection interval found in the present investigation might represent a similar phenomenon in the cartilage cells of the growth plate. With the highest number of injections the growth hormone dose per injection also might be too low to start the optimum growth process.

The optimum injection frequency found in the present investigation might also reflect the optimum cycle time for the proliferation of cartilage cells. As shown previously (see Thorngren & Hansson 1973b,c) growth hormone stimulates longitudinal bone growth through the production of an increased number of new cells in the growth plate. The duration of the DNA synthesis phase for cartilage cells in the growth plate of the proximal tibia of a 6-week old normal rat is known to be about 7 h (Kember 1972), and the time from the beginning of the DNA synthesis to the end of the mitosis has been calculated at about 11 h, then being followed by the interphase (Kember 1971). The optimum 12-hours interval between the growth hormone injections found for the thyroxine-treated hypophysectomized rats in the present investigation seems to agree with the results of these earlier investigations.

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