Increase of whole-body calcium and skeletal mass in normal and osteoporotic adult rats treated with parathyroid hormone

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Summary

1. The effect of long-term administration of parathyroid hormone (PTH) on whole-body calcium and ash weight of individual bones has been studied in normal and osteoporotic adult female rats in order to examine whether such a treatment could induce a positive calcium balance.

2. Osteoporosis was induced by calcium restriction during pregnancy and lactation. Sequential measurements of whole-body calcium were made by neutron activation.

3. In non-osteoporotic intact and thyroparathyroidectomized rats a daily dose of 75 units of human PTH 1-34 given subcutaneously for 3 weeks increased whole-body calcium.

4. In osteoporotic animals 25-50 units of either bovine PTH 1-84 or human PTH 1-34 given subcutaneously twice daily for 6 weeks increased both whole-body calcium and ash weight of individual bones. Microradiographic examination of the tibiae indicates, however, that PTH administration does not result in the restoration of individual trabeculae lost during the development of osteoporosis.

5. The results show that PTH can enhance skeletal mass in both normal and osteoporotic rats. In osteoporotic animals the restoration of whole-body calcium and ash weight of individual bones is not accompanied by a return of the morphological structure of the tibia to normal.

Key words: bone mass, calcium neutron activation, osteoporosis, parathyroid hormone.

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Abbreviations: PTH, parathyroid hormone; Cl₂MDP, dichloromethanediphosphonate; 1,25-(OH)₂D₃, 1,25-dihydroxyvitamin D₃.

Introduction

Despite numerous investigations, the treatment of osteoporosis still remains an unsolved problem. The various therapies investigated have been based on two main modes of action, namely inhibition of bone resorption and acceleration of bone formation. The first includes substances like oestrogens, calcitonin, diphosphonates and calcium. The effect in low-turnover osteoporosis has, as could have been expected, been rather disappointing. Diphosphonates have been found to be effective in osteoporosis with a high bone resorption, such as that found after immobilization [1]. One of the problems of compounds which decrease bone resorption is the fact that resorption in vivo is tightly coupled with formation, so that usually only a decrease in turnover is obtained without an increase in net balance. In stimulating bone formation fluoride seems to have some effect [2], but its use is hampered by side-effects and by the fact that it is not known whether the new bone formed is of satisfactory quality. An association of calcitonin and inorganic phosphate has been found to enhance both formation and total bone mass in man [3]. Extensive references in the field of the treatment of osteoporosis can be found in a recent symposium [4].

Recently it has been suggested that parathyroid hormone (PTH) would lead both to an increased turnover and a positive calcium balance [5]. This seemed rather paradoxical since the
classical effect of PTH is to stimulate bone resorption. However, PTH has also a direct stimulatory effect on bone formation [6]. It has been claimed that according to the experimental or pathophysiological conditions this latter effect could be stronger than that on resorption, thus explaining the increase in bone mass [7]. The experimental data for this interesting suggestion are, however, as yet scanty. In very young rats PTH can induce an increase in bone mass [8, 9]. In humans, morphological data suggest that trabecular bone is increased after PTH administration [10]. This increase, which is accompanied by an elevation of bone formation measured with $^{47}$Ca kinetics, is, however, not always matched by an increase in calcium balance [10].

The aim of this study was to investigate whether PTH can increase whole-body calcium and the weight of bones in both normal and osteoporotic adult rats. The whole-body calcium was measured by neutron activation, thus allowing a time-course study on the influence of treatment in the same animal. Previous investigations in rats [11] have shown a very high degree of correlation between whole-body calcium determined by neutron activation and either total bone ash or total bone calcium.

**Materials and methods**

**Animals**

All animals were female Wistar rats from our own breeding colony. They were kept on a standard pellet diet (Altromin C1314) containing 0.9% calcium, 0.7% phosphorus and 600 i.u. of vitamin D$_3$/kg until they reached a weight of 200 ± 10 g. At this time they were put on a diet with the same content in calcium, phosphorus and vitamin D$_3$ but with less calories (Altromin C1324). The influence of PTH on whole-body calcium was studied in intact or thyro-parathyroidectomized (TPTX) non-osteoporotic adult rats. The whole-body calcium was measured by neutron activation, thus allowing a time-course study on the influence of treatment in the same animal. Previous investigations in rats [11] have shown a very high degree of correlation between whole-body calcium determined by neutron activation and either total bone ash or total bone calcium.

**Induction of osteoporosis**

At various ages, starting from 5 months, osteoporosis was induced by subjecting the rats to pregnancy and lactation under a diet with a rather low content of calcium [12, 13]. When pregnant, the animals were put into individual cages. During the pregnancy and lactation, i.e. 6 weeks, they received *ad libitum* a diet made from a stock diet (Altromin C1034) containing 0.3% calcium and to which phosphorus and vitamin D$_3$ were added so that it contained 0.8% phosphorus and 1300 i.u. of vitamin D$_3$/kg. At the end of lactation they were either kept on the same diet or switched to a diet containing the same amount of phosphorus and vitamin D$_3$ but 1.1% calcium. Some animals were also subjected to pregnancy and lactation but received the diet with 1.1% calcium during the whole period.

From the onset of PTH administration all animals consumed the same amount of food. Demineralized water was given *ad libitum* throughout breeding and the experimental period.

Since it was found that the whole-body calcium of the mother decreased when the litter size went up to six pups, but was not further affected by larger litters, only mothers with seven or more pups were used.

The exact duration of the various phases as well as the time of the investigations is given in the legends of the Tables and Figures.

**Hormones and drugs**

Bovine PTH 1-84 (b-PTH 1-84) was obtained from Inolex Corp., Chicago, IL, U.S.A. Human PTH 1-34 (h-PTH 1-34) was from Armour Pharmaceutical Co., Kankakee, IL, U.S.A. The potency of the h-PTH 1-34 which has been used in the present work (lot no. K844-126) was determined in the 'subcutaneous chick' assay. The mean potency was found to be 6900 units/mg (Dr J. P. Dailey, Armour Pharmaceutical Co., Chicago, personal communication). The dose of h-PTH 1-34 administered in the present study was calculated according to this mean value of potency. Both b-PTH 1-84 and h-PTH 1-34 were injected subcutaneously, dissolved in a solution of NaCl (0.15 mol/l) containing HCl (0.01 mol/l), glutathione (21 mmol/l) and bovine albumin (1 mg/ml). 1,25(OH)$_2$D$_3$ was a generous gift from F. Hoffmann-La Roche and Co. AG, Basle, Switzerland. It was injected intraperitoneally in ethanol solution. Cl$_2$MDP was administered subcutaneously in isotonic sodium chloride solution (0.15 mol/l). NaF was dissolved in the drinking (distilled) water.

**Bone analyses**

For gravimetric measurements the skeleton was cleaned with dermestid beetles [14]. Exposure of skinned and eviscerated animals to these beetles for 24–48 h results in an efficient
cleaning from all soft tissues. The bones were then dried at 85°C for 3 days and the dry weight was recorded, after which they were ashed for another 3 days at 700°C and reweighed.

**Chemical determination of calcium**

Calcium determinations were by EGTA titration with a Corning Calcium Analyzer (model 940).

**Determination of whole-body calcium in vivo**

Whole-body calcium was measured by neutron activation analysis with thermal neutrons [11]. The precision of calcium determination by this method was found to be 3-9% by analysis of variance [11]. The determination of calcium by neutron activation analysis gives a good estimate for the skeletal calcium, since in fasting animals the latter comprises about 99% of the whole-body calcium.

**Statistics**

The results presented in the Figures and in Tables are given as mean values ± SEM. Significance between groups was expressed by the two-sided Student’s t-test.

**Results**

**Effect of PTH in non-osteoporotic rats**

Six months old females, not pregnant, were given human PTH 1-34 subcutaneously, 75 units/day for 3 weeks. As depicted in Fig. 1, a significant increase in whole-body calcium was observed during the 3 weeks of treatment. No further effect was seen during the post-therapy period. However, the difference was maintained over the 9 weeks of observation. The administration of this amount of PTH had no observable effect on the 24 h pattern of plasma calcium when tested every 4 h.

In order to assess whether the effect could also be elicited in the absence of the thyroparathyroid glands, intact and thyroparathyroidectomized female rats of 7 months of age were given human PTH 1-34 at a dose of 75 units subcutaneously daily for 3 weeks. As shown in Fig. 2, both groups displayed a similar increase in whole-body calcium.

**Induction of osteoporosis**

Under our experimental conditions pregnancy and lactation on a restricted calcium intake induced a loss of about 35% in whole-body calcium (Fig. 3).

**Effect of bovine PTH 1-84 on osteoporotic rats**

Fig. 3 shows the effect of administration of bovine PTH 1-84 on whole-body calcium in 9-10 months old rats made osteoporotic by pregnancy and lactation during calcium restriction. When the animals were returned to a 1.1% calcium diet, they recuperated some of their loss but did not reach the level of rats which were subjected to a 1-1% calcium diet during the whole period of pregnancy and lactation. The recovery was particularly prominent during the 2 weeks after the return to the high calcium diet. b-PTH 1-84 (50 units given twice daily for 5½ weeks) induced an increased recovery. The effect of PTH was observed between the second and sixth weeks after starting the high calcium regimen, the hormone appearing to prolong the period of recovery under the high calcium supply. Whole-body calcium of osteoporotic rats treated with PTH actually reached that of the animals which had not been restricted in calcium. The gain induced by PTH was maintained at least 4 weeks after discontinuation of the hormone.

Table 1 shows the effect of bovine PTH 1-84 on the individual bones of the animals shown in Fig. 3. The rats were killed after the last whole-body calcium treatment made at the end of
FIG. 2. Effect of 3 weeks' administration of 75 units of human PTH 1-34 subcutaneously daily on whole-body calcium in 7 months old intact and thyroparathyroidectomized (TPTX) female rats.

- O, Control rats; •, PTH. Vertical bars indicate ± SEM.

The tenth week of observation (see Fig. 3). All types of bones taken from the group of 'osteoporotic' rats having received the PTH solvent have a smaller ash weight than that of the normal animals. In 'osteoporotic' animals given PTH, the ash weight of most individual bones tended to reach the values observed in those taken from normal rats. The results were similar when calcium was determined instead of ash weight (not shown).

**Effect of bovine PTH 1-84, human PTH 1-34 and 1,25(OH)$_2$D$_3$ on osteoporotic rats**

Table 2 shows that human PTH 1-34 (2 × 50 units daily subcutaneously) has a positive effect on whole-body calcium, very similar to that obtained with the same dose of bovine PTH 1-84. Human PTH 1-34 given in doses of 2 × 25 units/day was still effective, but doses of 2 × 5 units were not. 1,25(OH)$_2$D$_3$ given in doses of 13 and 26 pmol daily intraperitoneally did not influence the whole-body calcium recovery of osteoporotic rats fed on a high calcium diet.

Table 3 shows the effect of these various treatments on the individual bones. As for the experiment presented in Fig. 3 and Table 1, the results corroborate those obtained with the whole-body calcium measurement. Bovine PTH 1-84 and human PTH 1-34 had similar effects and human PTH 1-34 was no longer effective at the dose 2 × 5 units daily. 1,25(OH)$_2$D$_3$ had no effect.

In order to investigate whether PTH would also restore bone structure, microradiographs were made. As shown in Fig. 4, PTH did not restore normal bone structure in the tibia despite the fact that it normalized whole-body calcium and bone ash. Therefore some shift in the location of calcium must have occurred.

PTH at the doses shown to be effective on whole-body calcium and ash weight of individual bones has little effect on plasma calcium. Thus, after 5 weeks of treatment, no difference in plasma calcium concentration between animals receiving 2 × 50 units of PTH 1-84 or 1-34/day subcutaneously and those receiving the PTH solvent was detected at 3 h before or at 2 and 3 h after injection.
Parathyroid hormone increases bone mass

FIG. 3. Effect of bovine PTH (b-PTH) 1-84 on whole-body calcium in osteoporotic rats.

All rats were subjected to 6 weeks of pregnancy and lactation at the age of 6–7 months and then kept for 3 months on the same diet up to the measurement period (10 weeks). From the start to the end of this latter period, all animals ate 16 ± 0.2 g of food/day. O, □, Normal rats (n = 6) given a 1.1% calcium diet throughout the experiment. △, ■, Osteoporotic rats (n = 6) given a 0.3% calcium diet up to the measurement period, then a 1.1% calcium diet and 2 × 0.2 ml subcutaneously daily of PTH solvent. ●, □, Osteoporotic rats (n = 6) given the same diets as the previous group but with 2 × 50 units of bovine PTH subcutaneously daily for the first 5¼ weeks. *P < 0.05, ***P < 0.01, ****P < 0.001, as compared with the osteoporotic rats with no PTH. Vertical bars indicate ± SEM.

Table 1. Effect of bovine PTH 1–84 on ash weight of various bones in osteoporotic rats

The animals were those of Fig. 3, killed after the 10 weeks of observation. All values are means ± SEM. *P < 0.05, as compared with the osteoporotic group, which received only the PTH solvent. Group 3 received twice daily 50 units of b-PTH 1–84 subcutaneously for 5¼ weeks. See the legend of Fig. 3 for further details.

<table>
<thead>
<tr>
<th>Ash weight (mg)</th>
<th>Cervical spine</th>
<th>Thoracic spine</th>
<th>Lumbar spine</th>
<th>Tail</th>
<th>Femur</th>
<th>Tibia</th>
<th>Humerus</th>
<th>Radius</th>
<th>Ulna</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>302</td>
<td>560</td>
<td>847</td>
<td>1365</td>
<td>451</td>
<td>311</td>
<td>218</td>
<td>66</td>
<td>102</td>
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<tr>
<td>Normal</td>
<td>± 66</td>
<td>± 24</td>
<td>± 42</td>
<td>± 57</td>
<td>± 28</td>
<td>± 10</td>
<td>± 6.4</td>
<td>± 1.7</td>
<td>± 3.1</td>
</tr>
<tr>
<td>Osteoporotic</td>
<td>2305</td>
<td>504</td>
<td>779</td>
<td>1014</td>
<td>388</td>
<td>271</td>
<td>192</td>
<td>58</td>
<td>93</td>
</tr>
<tr>
<td>+ solvent</td>
<td>± 48</td>
<td>± 24</td>
<td>± 52</td>
<td>± 130</td>
<td>± 28</td>
<td>± 13</td>
<td>± 12</td>
<td>± 1.5</td>
<td>± 3.2</td>
</tr>
<tr>
<td>Osteoporotic</td>
<td>2550</td>
<td>559</td>
<td>855</td>
<td>1171</td>
<td>436</td>
<td>299</td>
<td>210</td>
<td>63</td>
<td>99</td>
</tr>
<tr>
<td>+ b-PTH 1–84, 2 × 50 units s.c. daily</td>
<td>± 27*</td>
<td>± 8*</td>
<td>± 38</td>
<td>± 24</td>
<td>± 6</td>
<td>± 1.8</td>
<td>± 3.7</td>
<td>± 9.9*</td>
<td>± 1.5</td>
</tr>
</tbody>
</table>

Effect of Cl₂MDP and NaF on osteoporotic rats

Cl₂MDP (1 mg of phosphorus/kg subcutaneously daily for 10 weeks) and NaF (3.3 and 33 p.p.m. in the drinking water for 5¼ weeks) did not increase the whole-body calcium recovery of osteoporotic rats fed on a high calcium diet. As depicted in Fig. 3, in untreated osteoporotic rats the mean whole-body calcium increased from 2422 ± SEM 157 to 3177 ± 112 mg (n = 6) during the 10 week recovery period when the animals received the high calcium diet. The change in whole-body calcium was about the same in osteoporotic rats treated with either Cl₂MDP (from 2424 ± 124 to 3170 ± 68 mg; n = 4) or NaF (3.3 p.p.m.: from 2512 ± 90 to 3125 ± 107 mg, n = 6; 33 p.p.m.: from 2633 ± 86 to 3265 ± 104 mg, n = 6). Finally Cl₂MDP (1 mg of phosphorus/kg subcutaneously for 10 weeks) did not enhance the effect of bovine PTH 1-84 (2 × 50 units subcutaneously daily for 5¼ weeks) when given concomitantly. Thus, with PTH alone, the whole-body calcium increased from 2579 ± 64 to 3618 ± 118 mg (n = 6, see Fig. 3) during the 10 week recovery period. Addition of Cl₂MDP to the administration of PTH did not enhance this recovery since it rose from 2398 ± 199 to 3312 ± 189 mg (n = 6).

Discussion

The results reported above show that PTH at doses which do not induce hypercalcaemia can induce calcium retention in the rat. Both bovine and human PTH are able to promote such an increased retention in normal as well as in osteoporotic rats.

In contrast, 1,25-dihydroxyvitamin D₃ (13 and 26 pmol intraperitoneally daily for 6 weeks)
TABLE 2. Effect of PTH and 1,25(OH)2D3 on whole-body calcium in osteoporotic rats

All animals were 4–5 months old when pregnancy commenced and 7–8 months old at the beginning of the various treatments. All ate 17 g of food/day from weeks 0 to 6. All received a 0.3% calcium diet throughout pregnancy, lactation and until the beginning of the treatments, when they were switched to a 1.1% calcium diet for another 6 weeks. All values are means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001, for groups 2–8 vs 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (weeks)</th>
<th>Whole-body calcium (mg)</th>
<th>Increase in whole-body calcium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PTH solvent)</td>
<td>n = 8</td>
<td>1855 ± 60</td>
<td>2530 ± 46</td>
</tr>
<tr>
<td>n-PTH</td>
<td></td>
<td>2015 ± 77</td>
<td>2837 ± 49***</td>
</tr>
<tr>
<td>weeks 0–6</td>
<td>n = 5</td>
<td>1918 ± 26</td>
<td>2735 ± 41**</td>
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<td></td>
<td>weeks 0–6</td>
<td>1923 ± 71</td>
<td>2752 ± 69*</td>
</tr>
<tr>
<td>n-PTH</td>
<td></td>
<td>1902 ± 73</td>
<td>2532 ± 55</td>
</tr>
<tr>
<td>weeks 0–6</td>
<td>n = 5</td>
<td>1795 ± 91</td>
<td>2512 ± 96</td>
</tr>
<tr>
<td></td>
<td>weeks 3–6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)2D3,14 pmol i.p. daily</td>
<td>n = 8</td>
<td>1891 ± 116</td>
<td>2590 ± 115</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td>1889 ± 116</td>
<td>2546 ± 72</td>
</tr>
</tbody>
</table>

TABLE 3. Effect of PTH and 1,25(OH)2D3 on the ash weights of various bones in osteoporotic rats

Conditions were the same as described in Table 2, the animals being killed after the 6 weeks of treatment. All values are means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001, for groups 2–8 vs 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (weeks)</th>
<th>Skull</th>
<th>Cervical spine</th>
<th>Thoracic spine</th>
<th>Lumbar spine</th>
<th>Femur</th>
<th>Tibia</th>
<th>Humerus</th>
<th>Ulna + radius</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td></td>
<td>2092</td>
<td>254</td>
<td>419</td>
<td>634</td>
<td>346</td>
<td>248</td>
<td>159</td>
<td>135</td>
</tr>
<tr>
<td>(PTH solvent)</td>
<td>n = 8</td>
<td>± 33</td>
<td>± 4.3</td>
<td>± 9</td>
<td>± 21</td>
<td>± 18</td>
<td>± 6.6</td>
<td>± 3.5</td>
<td>± 2.2</td>
</tr>
<tr>
<td>n-PTH</td>
<td></td>
<td>2265</td>
<td>284</td>
<td>496</td>
<td>749</td>
<td>399</td>
<td>288</td>
<td>194</td>
<td>153</td>
</tr>
<tr>
<td>weeks 0–6</td>
<td>n = 5</td>
<td>± 88*</td>
<td>± 11*</td>
<td>± 19***</td>
<td>± 42*</td>
<td>± 12**</td>
<td>± 8.5***</td>
<td>± 7.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weeks 0–6</td>
<td>2323</td>
<td>294</td>
<td>507</td>
<td>777</td>
<td>391</td>
<td>276</td>
<td>191</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
<td>± 45***</td>
<td>± 6.0***</td>
<td>± 7.5***</td>
<td>± 6.4***</td>
<td>± 10.8</td>
<td>± 7.8*</td>
<td>± 3.4***</td>
<td>± 3.3***</td>
</tr>
<tr>
<td></td>
<td>weeks 0–6</td>
<td>2254</td>
<td>285</td>
<td>456</td>
<td>750</td>
<td>382</td>
<td>268</td>
<td>193</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
<td>± 40**</td>
<td>± 5.8***</td>
<td>± 18*</td>
<td>± 14***</td>
<td>± 10</td>
<td>± 9</td>
<td>± 4.5***</td>
<td>± 3.6***</td>
</tr>
<tr>
<td></td>
<td>weeks 0–6</td>
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<td>268</td>
<td>436</td>
<td>624</td>
<td>342</td>
<td>243</td>
<td>166</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td>± 83</td>
<td>± 37</td>
<td>± 22</td>
<td>± 16</td>
<td>± 13</td>
<td>± 7.2</td>
<td>± 3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weeks 3–6</td>
<td>2194</td>
<td>278</td>
<td>463</td>
<td>713</td>
<td>360</td>
<td>263</td>
<td>176</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td>± 80</td>
<td>± 10.2</td>
<td>± 19-6</td>
<td>± 36</td>
<td>± 20</td>
<td>± 11.8</td>
<td>± 5.3*</td>
<td>± 5.4</td>
</tr>
<tr>
<td>1,25(OH)2D3,13 pmol i.p. daily</td>
<td></td>
<td>1981</td>
<td>246</td>
<td>422</td>
<td>630</td>
<td>330</td>
<td>239</td>
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<td>± 40</td>
<td>± 11.2</td>
<td>± 13</td>
<td>± 6.9</td>
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<td>1,25(OH)2D3,26 pmol i.p. daily</td>
<td></td>
<td>2071</td>
<td>246</td>
<td>425</td>
<td>589</td>
<td>335</td>
<td>251</td>
<td>168</td>
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<td>± 13</td>
<td>± 6</td>
<td>± 8.2</td>
<td>± 5.2</td>
</tr>
</tbody>
</table>
Parathyroid hormone increases bone mass

FIG. 4. Microradiographs of the tibia of (a) rat without pregnancy and lactation, (b) osteoporotic rat given a 1.1% calcium diet and (c) osteoporotic rat given a 1-1% calcium diet and human PTH 1-34. The conditions were those described in Fig. 3.

did not enhance whole-body calcium or ash weight of individual bones of osteoporotic animals. Furthermore no positive effect on whole-body calcium was observed with dichloromethanediphosphonate (1 mg of phosphorus/kg subcutaneously daily for 10 weeks) or sodium fluoride (3.3 and 33 p.p.m. in drinking water for 5.4 weeks).

The mechanism of action of PTH on calcium balance cannot be deduced from the present investigation. It is unlikely to involve a stimulation of calcitonin, since PTH is active in thyroparathyroidectomized rats. It has been suggested that the plasma calcium level per se could have a positive effect on bone formation, assessed either histologically [15] or by 42Ca kinetics [16]. Furthermore, from data obtained in the greyhound it has been suggested that chronic administration of h-PTH 1-34 could be more effective in stimulating intestinal calcium absorption, osteoid formation and bone accretion when the hormone is given subcutaneously by single injection than by continuous infusion [17]. In this study the subcutaneous injection, but not the infusion, of h-PTH resulted in a significant rise of plasma calcium. This observation suggests that the transient increment in plasma calcium might be critical for enhancing bone accretion in these experimental conditions [17]. In our study PTH was also given in single subcutaneous injections. However, we have not been able to detect a significant change in the plasma calcium level of non-osteoporotic or osteoporotic intact rats treated with PTH in doses which enhanced whole-body calcium. It is possible that PTH would act directly on bone to increase mineral retention through a mechanism which remains to be elucidated at the cellular and molecular levels. The finding that bovine PTH 1-84 can increase the number of active osteoblasts of cultured embryonic bone [6] may be particularly relevant in this respect.

From our study it appears that the effect of PTH is not only a temporary uncoupling of bone formation and bone resorption, since the difference induced was maintained after discontinuation of the hormonal treatment.

The results of our experiment would support the earlier suggestion [5, 7, 10] that PTH might be of therapeutic value in human osteoporosis. However, in view of the microradiographic examination of the tibias, the location of the PTH-induced increase in bone calcium remains a puzzling problem. Indeed, whereas the positive effect of PTH on ash weight is present in the various bones examined, the hormone administration does not seem to have the capability of reversing the loss of trabecular bone in this osteoporosis model. It ensues that PTH must increase bone mass at another site, possibly on the surface of already pre-existing trabecular or cortical bone. The effect of PTH is unlikely to be due to metastatic calcification, since the kidney, which is very sensitive to PTH-induced ectopic calcification, did not show an increased calcium content (results not shown). Furthermore the increase in the ash weight of individual bones is in the same proportion as that in the whole-body calcium. Therefore, further studies with this model of osteoporosis should be aimed at determining the exact localization of the positive effect of PTH on bone mass in order to investigate the mechanism whereby the hormone exerts this specific action.
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