Intermittent treatment with parathyroid hormone (PTH) has an anabolic effect on both intact cancellous and cortical bone. Very little is known about the effect of the administration of PTH on the healing of fractures or the incorporation of orthopaedic implants. We have investigated the spontaneous ingrowth of callus and the formation of bone in a titanium chamber implanted at the medioproximal aspect of the tibial metaphysis of the rat. Four groups of ten male rats weighing approximately 350 g were injected with human PTH (1-34) in a dosage of 0, 15, 60 or 240 µg/kg/day, respectively, for 42 days from the day of implantation of the chamber.

During the observation period the chamber became only partly filled with callus and bone and no difference in ingrowth distance into the chamber was found between the groups. The cancellous density was increased by 90%, 132% and 173% in the groups given PTH in a dosage of 15, 60 or 240 µg/kg/day, respectively. There was a linear correlation between bone density and the log PTH doses (r² = 0.6).

Our findings suggest that treatment with PTH may have a potential for enhancement of the incorporation of orthopaedic implants as well as a beneficial effect on the healing of fractures when it is given in low dosages.

Parathyroid hormone (PTH) is a major regulator of bone metabolism. It increases calcium in the extracellular fluid by activating osteoclasts, increasing tubular reabsorption of calcium in the kidney and indirectly promoting intestinal absorption of calcium in the gut. These effects have dominated the views of the action of PTH for many years, but it also has anabolic effects on the skeletal metabolism. Whereas constant levels of PTH activate osteoclasts, intermittent PTH predominantly increases osteoblastic activity.

In recent years a number of animal studies have shown that intermittent administration of PTH increases bone mass in both cancellous and cortical bone and may therefore have some potential in the treatment of osteoporosis. A dose-related increase in the formation of femoral cortical bone was found in both old and young rats after treatment with PTH. It is thought to stimulate the differentiation of osteoblasts by direct interaction with receptors but it may also act by modulation of the action of cytokin.

There is little information on the effect of treatment with PTH on bone regenerative phenomena such as the incorporation of orthopaedic implants and the healing of fractures. In ovariectomised rats, PTH has been shown to inhibit the decrease in the mechanical strength of healing fractures induced by the ovariectomy. It is able to enhance the mechanical strength and volume of callus of healing fractures in normal adult animals in a dose of either 60 µg/kg/day or 200 µg/kg/day.

We have now applied a titanium bone-chamber model to normal rats and investigated how intermittent treatment with PTH in a dosage of 15, 60 or 240 µg/kg/body-weight/day can influence the formation of callus and bone inside the chamber.

In this model the formation of bone takes place exclusively by metaplastic (membranous) ossification, which is a specific component of the more complex process of the repair of fractures. Such formation also is responsible for the incorporation of stable orthopaedic implants. By standardising the shape of the newly formed bone, the chamber facilitates measurement of bone density, which should reflect the strength of the bone supporting an implant.

Materials and Methods

The bone conduction chamber (BCC) consists of a titanium screw with a cylindrical interior space. The interior of the
chamber has a diameter of 2 mm and it is 7 mm long. The outside diameter is 3.5 mm and the overall length 13 mm. One end of the chamber has holes for tissue ingrowth and is screwed into the bone. Thus, the ingrown bone-derived tissue can further invade the chamber without competition with other tissues. Because of its size, the chamber will not be completely filled with tissue. Soft-tissue ingrowth is followed by a frontier of ossification, advancing from the holes towards the other end of the chamber. The ingrowth distance can then be measured as an estimate of bone growth by metaplastic ossification (Fig. 1).

We used 40 male Sprague-Dawley rats each of which had one chamber implanted. They weighed between 320 and 350 g and were fed a standard laboratory diet. The animals were housed at 22°C with two rats to each cage and with free access to food and water. They had a 12-hour light-dark cycle.

After implantation of the chamber the rats were randomly divided in four groups of ten. Three groups had a subcutaneous injection of human PTH (1-34) (Bachem, Bubendorf, Switzerland) at a dose of either 15, 60 or 240 µg/kg/body-weight/day dissolved in a vehicle of 0.5M saline with 2% heat-inactivated rat serum. The fourth group was injected only with the vehicle. The injections were given once a day for 42 days between 8 am and 10 am. The animals were weighed once a week, and the doses were adjusted to body-weight.

Chamber implantation. The rats were anaesthetised with 0.6 to 0.7 ml of a mixture of pentobarbital (15 mg/ml) and diazepam (2.5 mg/ml). We exposed the medioproximal aspect of the left tibial metaphysis through a longitudinal incision using an aseptic technique. Before surgery 12.5 mg of streptomycin had been given intramuscularly. The periosteum was removed anterior to the insertion of the medial collateral ligament. The medial cortex was breached with a pinpointed 2.7 mm bone drill, which was carried up through the cortex. Each chamber was screwed into place so that the pointed end engaged the opposite cortex and the bone ingrowth holes were at the level of the cortical bone. The wound was closed with continuous subcutaneous stitches using a 4/0-monofilament nylon suture so that the entire chamber was subcutaneous.

Histological and histomorphometric evaluation. The rats were killed after six weeks with an overdose of pentobarbital. Sections of decalcified specimens were then taken parallel to the long axis of the chamber. Three parallel sections from the middle of the specimens, each 300 µm apart, were stained with haematoxylin and eosin. Histological and histomorphometric assessments were performed with blinded specimens examined in random order. We used two methods to measure the compartment of new ingrown bone which includes marrow cavities and trabecular bone. First, it was circumscribed on a digitising table using the Videoplan equipment (Kontron Bildanalyse GmbBH, Esching, Germany) at a magnification of 40. The bone ingrowth distance was calculated by dividing the bone area by the width of the specimen. In the second method the Merz grid was used for point counting of the entire specimen to measure total area, the area of the bone compartment including marrow, and the bone density within this compartment. The last was expressed as the percentage of points covering bone tissue in relation to the total number of points covering the bone compartment. From each specimen the three different sections were measured and their mean value was used for analysis. Point counting was repeated by an independent person in order to estimate the measuring error.

Results

All the implants were clinically stable with no sign of discolouration or swelling. Treatment with PTH did not affect the body-weight of the rats. In the control rats the mean body-weight increased by 39 ± 4% during the injection period. In the different PTH groups (15, 60, 240 µg/kg/day) the increase was 40 ± 6%, 38 ± 3% and 43 ± 5%, respectively.

Morphological findings. In all cases, soft tissue had reached the largest distance into the chamber, followed by an advancing frontier of metaplastic bone formation. Behind this frontier the bone was resorbed to make place for a marrow cavity with only a few trabeculae. With intermittent treatment with PTH a dense network of bone trabeculae filled this cavity. Otherwise, there was no difference (Fig. 2).

Histomorphometric findings. The bone ingrowth distance was unchanged by treatment with PTH (Fig. 3), but there was a substantial increase in bone density in all three groups (Fig. 3; ANOVA p = 0.0001). In the vehicle group, the bone density, as defined above, was 25 ± 10%. This figure is derived from the entire bone compartment and is highly influenced by the amount of bone surrounding the
marrow cavity in which the bone density appeared to be almost zero. With a dosage of 240 µg PTH/kg/day, however, the density was 70 ± 10%, which reflects an almost homogeneous distribution of bone within the bone compartment. There was a linear correlation between the bone density and the log PTH dose ($r^2 = 0.6; p = 0.0001$). The measuring error (sd) in point counting was 5 percent units. There was a correlation between the ingrowth distance as measured by computer-assisted analysis and by point counting ($r^2 = 0.9$).

**Discussion**

Our study has shown that intermittent treatment with PTH in a dose-dependent manner increased the amount of bone in the chambers whereas the bone-ingrowth distance was unchanged. There have been few studies on the healing of fractures after administration of PTH. Kim et al$^{11}$ showed that the decreased mechanical strength seen in fractures in ovariectomised rats could partly be prevented by giving the animals 175 µg of PTH (1 to 84)/kg/day. This corresponds to the 60 µg of PTH (1 to 34)/kg/day which we used. Fukuhara and Mizuno$^{14}$ studied parathyroidectomised rats with and without administration of PTH during the first five weeks of fracture healing. Using histological techniques, they found that in the early stage of healing, administration of PTH enhanced the formation of bone. Later in the healing period resorption was decreased. Administration of PTH to normal adult rats at a dose of 60 µg and 200 µg/kg/day, respectively, increased the amount of callus and the mechanical strength of the fractures after 40 days of healing.$^{12}$

PTH stimulates the formation of bone at the endosteum and to a much less degree at the periosteum.$^{3,15}$ The lack of an effect of treatment with PTH on the distance of bone ingrowth in our study may result from the relatively weak effect of the peptide on the periosteal envelope. Although
the cells in the chamber are of endosteal origin, the appearance of the metaphlastic ossification frontier has some similarity to the periosteal modelling drifts.

The mechanism behind the anabolic effect of treatment with PTH is not fully understood. It seems to increase the bone-forming activity of the individual osteoblast and may increase the maturation rate of preosteoblasts into osteoblasts. PTH binds to receptors on osteoblasts. The regulation of immediate early genes by PTH has important functional consequences in the downstream regulation of bone matrix genes via the differentiation of bone cells and in the anabolic action of PTH on bone. It may also enhance the IGF-I synthesis as well as the secretion of IGF-1-binding proteins in osteoblast-like cells. These factors are then thought to increase bone formation further. PTH also seems to have a vasodilatory effect in bone and may therefore be important in facilitating the early stages of the healing of fractures.

In a series of 32 patients with isolated tibial fractures Hardy et al showed that there was increased serum levels of PTH 24 hours after the fracture which progressively declined within the next six weeks. It was concluded that PTH is likely to play a role in the early stages of fracture healing.

Our results indicate that treatment with PTH may increase bone density within a fracture callus and may therefore be considered as a possible drug to enhance incorporation of orthopaedic implants as well as being used in fracture healing.

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