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INDEXING WORDS

calcium-regulating hormones; bone cell activity; fracture healing

SYNOPSIS

The majority of calcium is stored in bone and transferred to blood. In order to maintain calcium blood levels within a normal range, three important hormones regulate calcium between bone and blood. These are parathyroid hormone, vitamin D and calcitonin. However, the exact relationship between these hormones and bone cell activities is yet unknown. In order to study the relationship between these hormones and bone cell activities, we did the experimental work using rats.

After transverse midshaft fractures were made at the femur of immature rats, either vitamin D (1α hydroxycholecalciferol) or calcitonin was given to rats.

During the process of fracture healing bone formation and bone resorption could be observed at the fracture site by using both decalcified and undecalcified sections. Blood levels of calcium, phosphate, alkaline-phosphatase and parathyroid hormones were carried out over the course of the study.

These experimental works resulted in the following conclusions : vitamin D may activate all bone cells and stimulate bone metabolism. Calcitonin may inhibit all bone cell activities and depress bone metabolism, particularly in bone remodeling. Parathyroid hormone may activate the osteoclasts and the osteocytes and stimulate bone resorption.

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INTRODUCTION

Serum calcium is controlled by many factors, since it is necessary to maintain calcium blood levels within a narrow range. The majority of calcium is stored in bone and transferred to blood.[13] There are three important hormones which regulate calcium between bone and blood. These are parathyroid hormone, vitamin D, and calcitonin. It has been shown that parathyroid hormone enhances bone resorption by stimulating the activities of osteoclasts, and that vitamin D also increases bone resorption by activating osteoclasts and osteocytes. Calcitonin, on the other hand, inhibits the transport of calcium from bone to blood by depressing the activity of osteoclasts. However, the exact relationship between these hormones and bone cell activities is yet unknown. There are some questions concerning calcium-regulating hormones and bone cell activities :

1. Does parathyroid hormone only influence bone resorption?
2. Does vitamin D only stimulate bone resorption?
3. Does calcitonin only act on the osteoclast?

In order to study the relationship between these hormones and bone cell activities, we did the following experimental work.

MATERIALS AND METHODS

Transverse midshaft fractures were made at the mid-femur of immature rats and immediately fixed with an intramedullary wire. The operated rats were divided into three groups. Group A rats were given vitamin D ($1\alpha OHD_3$) 0.25 μg per Kg every day, group B rats were given eal calcitonin 300 mU per 100 gm every day and group C rats were controls. Rats were sacrificed each week to observe the process of fracture healing. During the process of fracture healing bone formation and bone resorption can be observed at the fracture site by using both decalcified and undecalcified sections. On the decalcified sections, Hematoxilin-Eosin staining and Toluidin-blue staining were done. In undecalcified sections, microradiogram, tetracycline labeling and Paragon's staining were used. Blood levels of calcium, phosphate, alkaline-phosphatase and parathyroid hormones were carried out over the course of the study.

OBSERVATION AND DISCUSSION

1. Process of fracture healing in the untreated control rats.

a) Histological findings.

The healing process of the fractured bone is divided into four stages (Fig. 1). During the first stage the young mesenchymal tissue is seen in most areas at the fracture site. Fibroblasts and chondroblasts are abundant. During the second stage granulation tissues mature, and the enchondral ossification takes place in the bone marrow at the same time as periosteal proliferation occurs outside of the cortex. At this stage, the chondrocytes and chondroclasts are active, and the fibrous tissues with rich blood vessels penetrate the fracture site. As osteoblastic activity increases, the primary spongiosa is formed. During the third stage

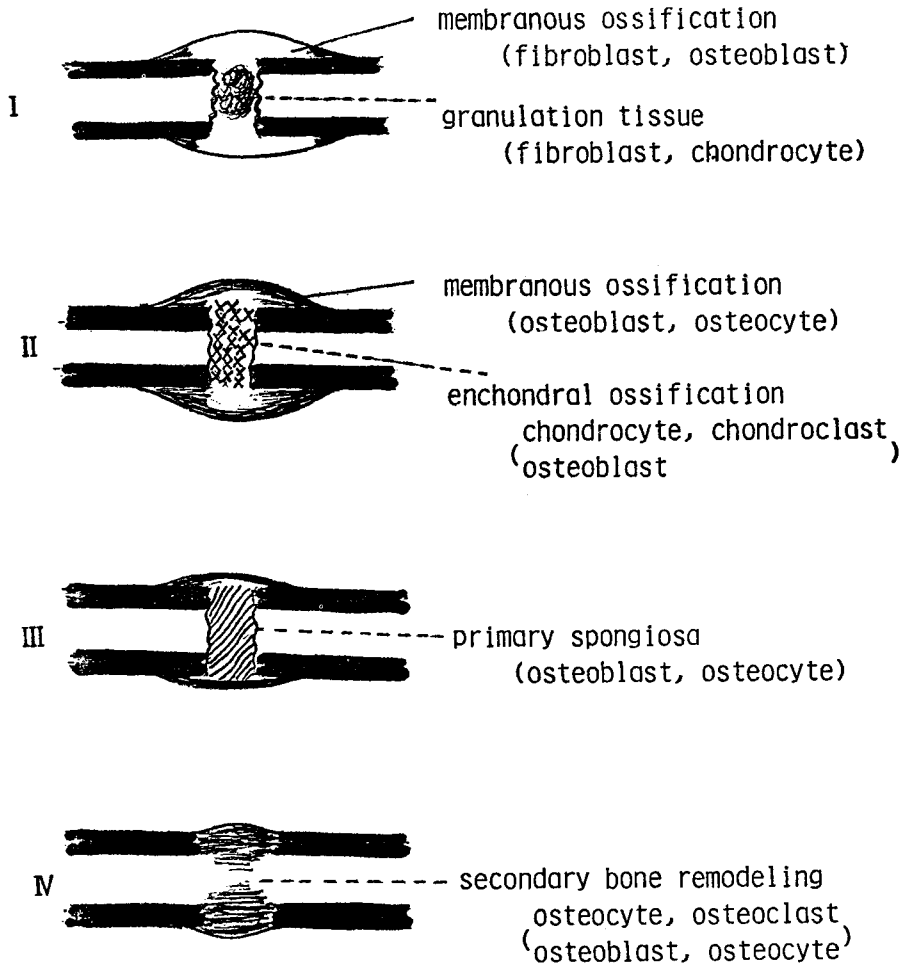


Fig. 1 Process of normal fracture healing. Healing process is divided into four stages.

primary spongiosa is seen at a wide area of the fracture site, bridging the bone ends. Osteoblastic activity is significantly high, producing many osteoid seams. The osteoblasts become surrounded by calcified tissues and are changed to osteocytes. In parts of the bone, secondary bone remodeling occurs by the osteoclasts and the osteocytes. During the fourth stage the secondary bone remodeling is remarkable and primary woven bone is converted to lamellar bone. Both osteoblastic and osteocytic activities are high during this stage.

b) Serum calcium level.

Serum calcium level changes throughout the process of fracture healing corresponding closely with the histological changes (Fig. 2). Serum calcium levels drop immediately after the fracture occurs and continues into the second stage. At this stage, histologically, the young growing cells are prominent at the fracture site, but also osteoclasts and osteocytes can be seen in the distal femur away from the fracture site where bone resorption takes place.

In the second stage in which chondroclasts and osteoblasts are present for enchondral ossification, the serum calcium level is still lower. However, it gradually rises as soon as the osteoblasts become active during calcification.

During the third stage the serum calcium level is elevated because of the osteoclastic, osteocytic, and osteoblastic activities which are in-

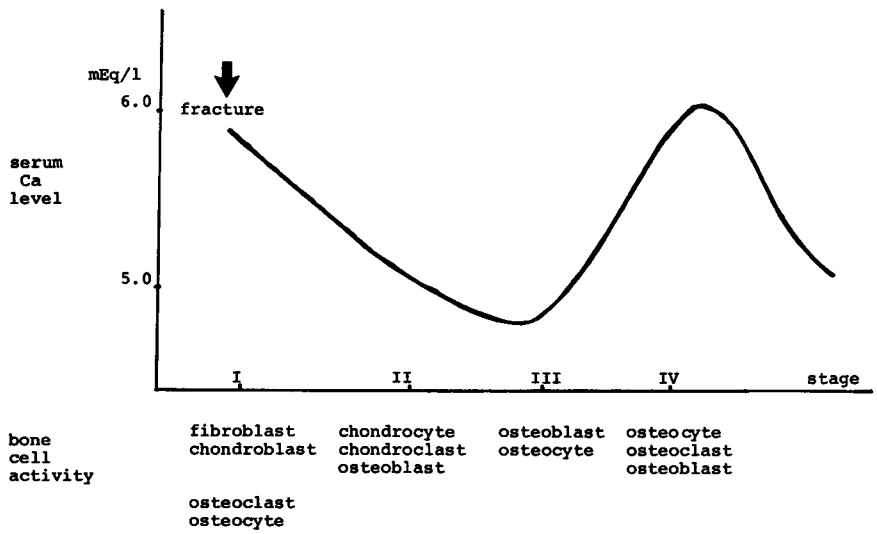


Fig. 2 Relationship between serum calcium level and histological findings during normal fracture healing.

volved in secondary bone remodeling. Calcium metabolism is most significant at this stage. As the secondary bone remodeling is completed during the fourth stage, serum calcium comes back to the initial level and bone cell activities become quiescent.

c) Other biochemical changes.

Serum phosphate is higher at the stage of enchondral ossification and also with secondary bone remodeling. After the secondary bone remodeling, it becomes normal. Alkaline-phosphatase also becomes higher at the stage of enchondral ossification and during secondary bone remodeling. Serum parathyroid hormone was measured by radioimmunoassay techniques, although this method proved to be difficult. Parathyroid hormone was higher in the serum in the early stages of bone healing, and then became lower as calcification increased at the area of the fracture site. These findings suggest that parathyroid hormone is produced in order to raise serum calcium levels for calcium deposition at the fracture site.

2. Bone cell activity during the process of fracture healing in treated rats.

a) Bone cell activity in the 1αOHD_3-treated rats.

Serum calcium levels drop immediately after fracture, and return to the initial levels sooner when compared with non-treated rats as described above, remaining at a higher level until the stage of secondary bone remodeling. Then it returns to the initial level. The calcium level is variable during the early stages of healing after fracture.

Histological changes are also characteristic. Although no particular difference can be seen at the fracture site, which is occupied by the young granulation tissues, bone resorption takes place on the distal part of the femur during the first stage of fracture healing in the 1αOHD_3-treated rats. This bone resorption may be caused by osteoclastic and osteocytic osteolysis,[2, 12] as revealed by microradiograms which also show subperiosteal bone resorption.

At the second stage, osteoblastic activities increase and enchondral ossification starts. This phase goes on for a longer period. The secondary bone remodeling follows at a later stage of the enchondral ossification. In the secondary bone remodeling, osteoclastic bone resorption, osteoblastic bone formation, as well as osteocytic osteolysis are intermingled. These findings continue until the fourth stage when the solid lamellar bone has been completed. The healing time of the fractured

bones in the 1αOHD$_3$-treated rats is almost the same as in the control rats, but the character of bone union at the fracture site is completely different from the non-treated rats. In the 1αOHD$_3$-treated rats the fracture site shows that the remainder of bone union has resulted from extensive remodeling of the original bone cortex and both internal and external callus formation. These findings are evident on the undecalcified sections labeled with tetracycline. Extensive remodeling of the fracture site may be caused by osteoclasts, osteoblasts, and osteocytes whose activities were stimulated by 1αOHD$_3$.[4]

The term "Bone Multicellular Unit" (BMU) was first used by Frost. BMU means bone remodeling by the osteoclasts, the osteocytes, and the osteoblasts within one unit of the enlarged lacunae.[6] Bone resorption takes place on a lacuna and bone formation follows it in the same enlarged lacuna.[2] In our experiment it is possible that active vitamin D might activate BMU in the process of fracture healing. Fig. 3 shows the active behavior of each cell. The osteoclasts are seen along the inner surface of the lacunae; the resultant bone resorption causes the uniting of enlarged lacunae. The osteocytes are seen within the lacunae. Their activities are significant such that each lacuna becomes enlarged (and stains well with Paragon's method), and is called osteocytic osteolysis. The osteoblasts are easily found on the sections. Because of their activity thick osteoid seams are formed. 1αOHD$_3$ acts on all bone cells, (not only on the osteoclasts but also on the osteocytes and the osteoblasts), activating the bone multicellular units.[10, 11]

b) Bone cell activity in the calcitonin-treated rats.

Serum calcium level is not significantly changed, and is maintained at almost the same level in this group. Probably, calcium transport is low and therefore bone cell activity may also be low.[3] No significant difference between the calcitonin-treated rats and the control rats can be seen at the fracture site during the first stage. However, during the second stage, much cartilage tissue is present at the fracture gap in the calcitonin-treated rats.[5] This cartilaginous mass gets larger in volume until the third week after operation. In contrast, the primary bone formation consists of woven bone in the control group with only a small volume of cartilage (Fig. 4). It is possible that chondroclastic activity is depressed by the influence of calcitonin.[8, 9]

During the third to fourth stage when bone remodeling is active in the control rats, the activities of osteoclasts and osteocytes in the calcitonin-treated rats are not in much evidence, since bone remodeling is

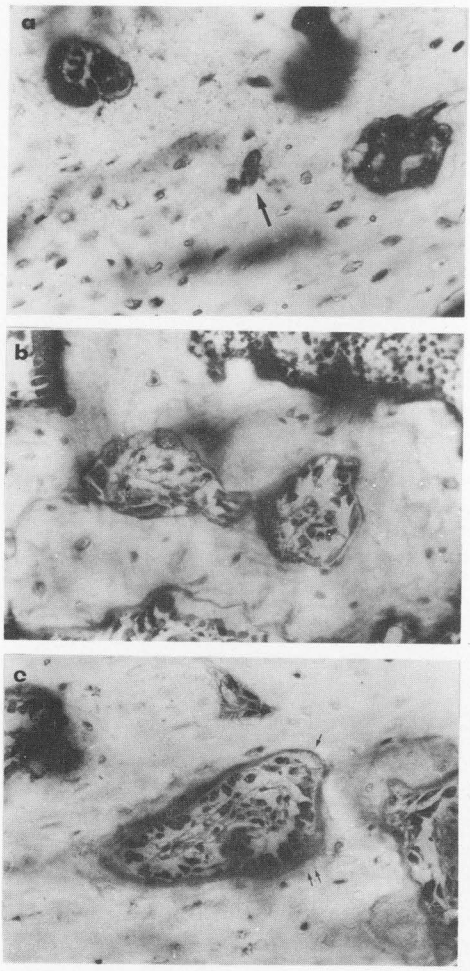


Fig. 3 Bone cell activity in the $1\alpha OHD_3$-treated rats.

(a) Osteocytic osteolysis and the enlarged lacunae due to activities of the osteocytes. (X400)

(b) Osteoclastic osteolysis due to activity of the osteoclasts seen along the inner surface of the uniting enlarged lacunae. (X400)

(c) A bone multicellular unit consisting of osteoclasts causing bone resorption (arrow) and osteoblasts with osteoid seams with bone formation (double arrow). (X400)

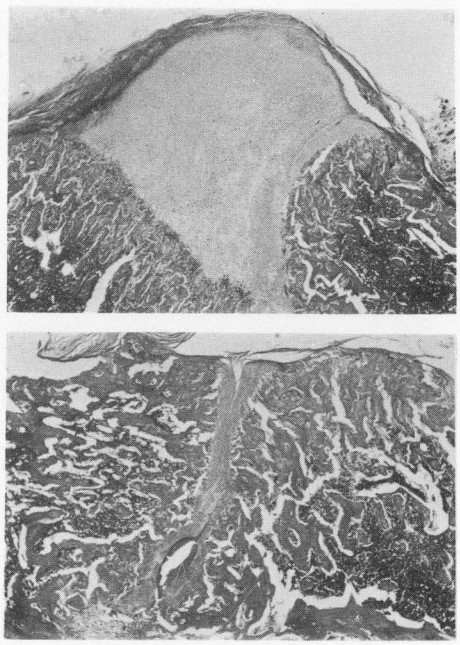


Fig. 4
Large cartilaginous mass in the calcitonin-treated rats (above) compared with the control (below).

seen only in a small area. These findings are well demonstrated in the Toluidine-blue section, which shows a large mass of cartilage, and also in the tetracycline-labeled slides. Paragon's staining also demonstrates activity of the osteoclast and the osteocyte.[7] Internal remodeling on the calcitonin-treated rats is less than in the control rats (Paragon's staining shows fewer osteoclasts than in the calcitonin-treated rats (Fig. 5)).

These findings indicate that calcitonin inhibits bone cell activity, particularly in the stage of bone remodeling after a primary bone formation from the cartilage by enchondral ossification and also when the lamellar bone forms from the woven bone by secondary bone remodeling.

Calcitonin inhibits the activities of all bone cells, not only osteoclasts[8] but also osteocytes and osteoblasts, particularly at the time of bone remodeling.

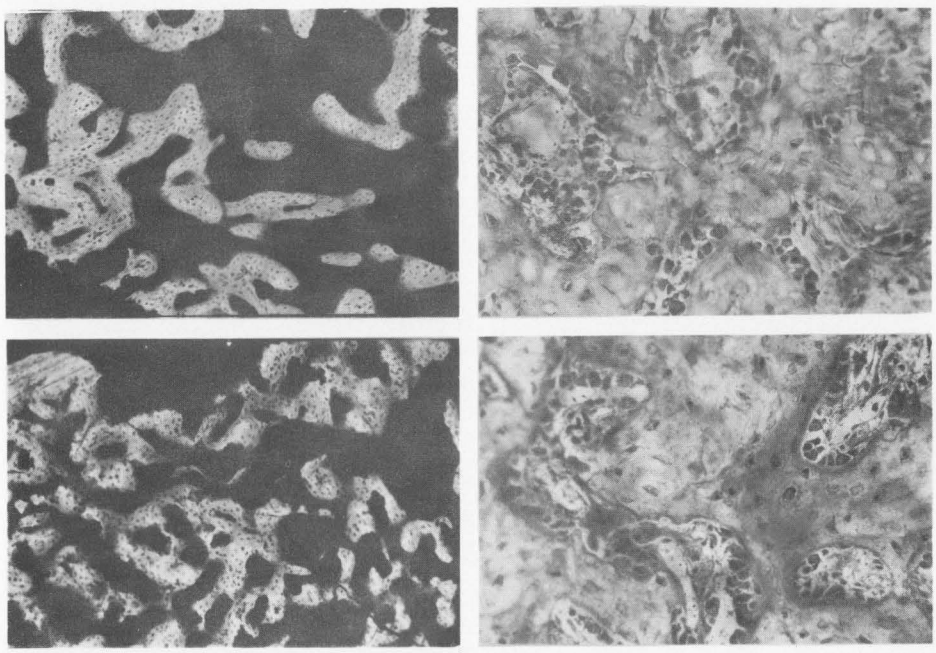
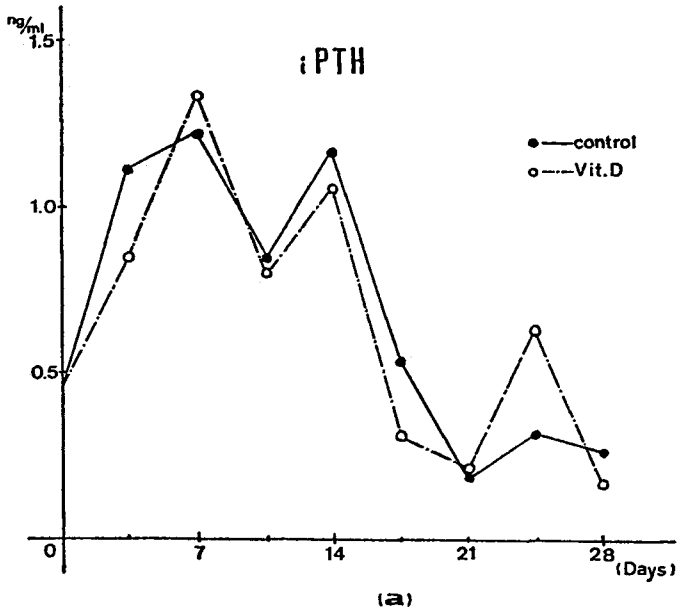


Fig. 5 Bone cell activity in the calcitonin-treated rats.

(a) Internal remodeling is less in calcitonin-treated rats (above) than in the control (below). (X100)

(b) Less activity of the osteoclasts and the osteoblasts in the calcitonin-treated rats (above) than in the control (below). (X400)



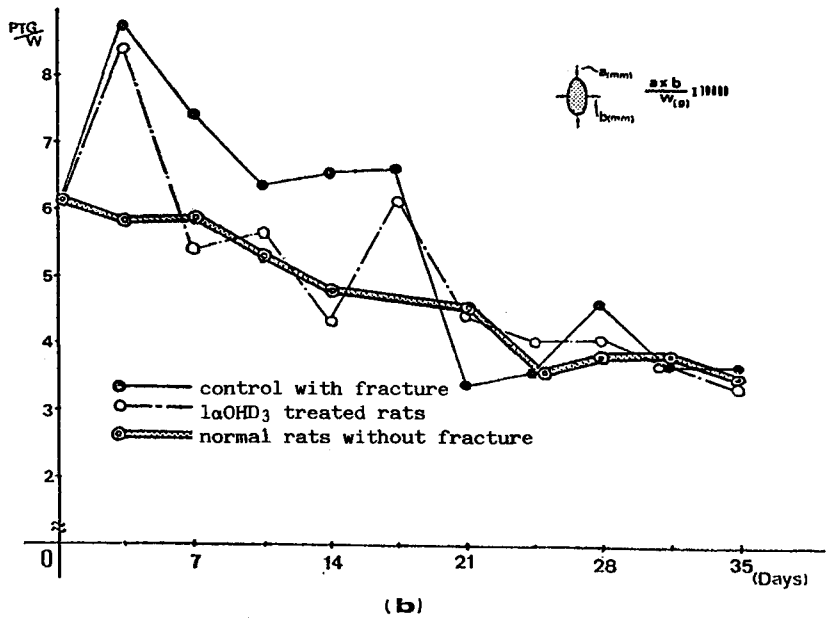


Fig. 6 Changes in serum level of the parathyroid hormone (above) and in size of parathyroid glands (below) during fracture healing.

c) Effects of parathyroid hormone on bone cell activity.

We have not given parathyroid hormone to these animals, so that we do not know if it acts directly on the bone in this experimental work. However, we have measured serum parathyroid hormones during these experiments using the 1αOHD$_3$-treated and the calcitonin-treated rats. Serum parathyroid hormone is elevated when serum calcium is lower, particularly in the first stage of the process of fracture healing when bone resorption is taking place. Also, parathyroid hormone is slightly elevated during secondary bone remodeling. However, there is no elevation of serum parathyroid hormone when osteoblastic activity is higher. It suggests to us that activities of parathyroid hormone are related to bone resorption only, and parathyroid hormone may act on the osteocytes and the osteoclasts but not on the osteoblasts. Of course, the osteoblastic activities are increased in patients with hyperparathyroidism.[1)] But it seems to us that this is not a direct action of parathyroid hormone on the bones but reactive bone formation following bone resorption by parathyroid hormone (Fig. 6).

The experimental results indicate that parathyroid glands are influenced by the process of fracture healing, and that fracture healing goes on under the influence of calcium-regulating hormones and bone cell activities.

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