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EFFECT OF PARATHYROID HORMONE ON TOOTH MOVEMENT IN RATS

BY

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ABSTRACT

The orthodontic tooth movement may be considered to be affected by hormones. An attempt was made to examine the relation between parathyroid hormone and osteoclast activity during tooth movement.

One hundred and thirty-four Wistar strain rats were divided into three groups; sham-operated, parathyroidectomized, and parathyroidectomized plus parathyroid extract treated. Then the experimental tooth movement was made on the upper right first molar. Observations were done histologically on the interradicular septum of the first molar, and osteoclasts were counted for the purpose of estimating the degree of bone resorption quantitatively.

The findings are summarized as follows:

1. Incident to the experimental tooth movement, the decrease of parathyroid hormone results in the inhibition of the appearance of osteoclasts and bone resorption on the compression side, as well as the appearance of osteoblasts on the tension side.

2. The increase of parathyroid hormone results in the accelation of the appearance of osteoclasts and bone resorption on the compression side, and the inhibition of the appearance of osteoblasts on the tension side.

INTRODUCTION

The orthodontic tooth movement is being considered as a result of a biological response of the periodontal tissues to mechanical force. From this point of view, the tissue changes incident to experimental tooth movement such as bone resorption, bone formation, and degenerative and reformative changes of the periodontal ligament have been discussed by many investigators[1–8].

Recently, Deguchi[9] reported the histochemical changes incident to tooth movement in normal, cortisone-treated, and parathyroid extract (PTE)-treated rats. Davidovitch *et al.* also reported the increase of osteoclasts in PTE-treated cats during tooth movement. From these findings, it should

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be evident that the orthodontic tooth movement is affected by hormones.

It is well known that parathyroid hormone affects the bone cells directly, and, especially, it increases osteoclast activity and promotes bone resorption[11-18]. From these facts, it is easily expected that the change of parathyroid activity should influence the rate of tooth movement.

The present investigation was undertaken to examine the effect of parathyroidectomy and administration of parathyroid extract on osteoclast activity during tooth movement.

Materials and Methods

One hundred and thirty-four Wistar strain male healthy rats, weighing about 80 g, were divided into three groups. The first group received sham-operation (sham-operated group), and the second group was parathyroidectomized, its successful operation being proved by serum calcium level (PTX group). The parathyroid glands of these animals were ectomized by the electrocautery method, and the level of serum calcium was measured by the atomic absorption spectrophotometer (Model 303, Perkin-Elmer Ltd.,

Table 1. Schedule of tooth movement

	No. of rats	Operation	Days after operation				Time after elastic insertion (hr)			
			1	5	6	7	6	12	24	48
Sham-operated group	5	S	B			E	D			
	5	S	B			E		D		
	5	S	B			E			D	
	10	S	B			E				D
PTX group	5	PTX	B			E	D			
	5	PTX	B			E		D		
	5	PTX	B			E			D	
	10	PTX	B			E				D
PTX+PTE group	5	PTX	B	PTE	PTE	PTE E	D			
	5	PTX	B	PTE	PTE	PTE E		D		
	5	PTX	B	PTE	PTE	PTE E			D	
	9	PTX	B	PTE	PTE	PTE E			PTE	D

S: sham-operation
B: blood drawn
E: elastic insertion
D: decapitation
PTX: parathyroidectomy
PTE: injection of parathyroid extract

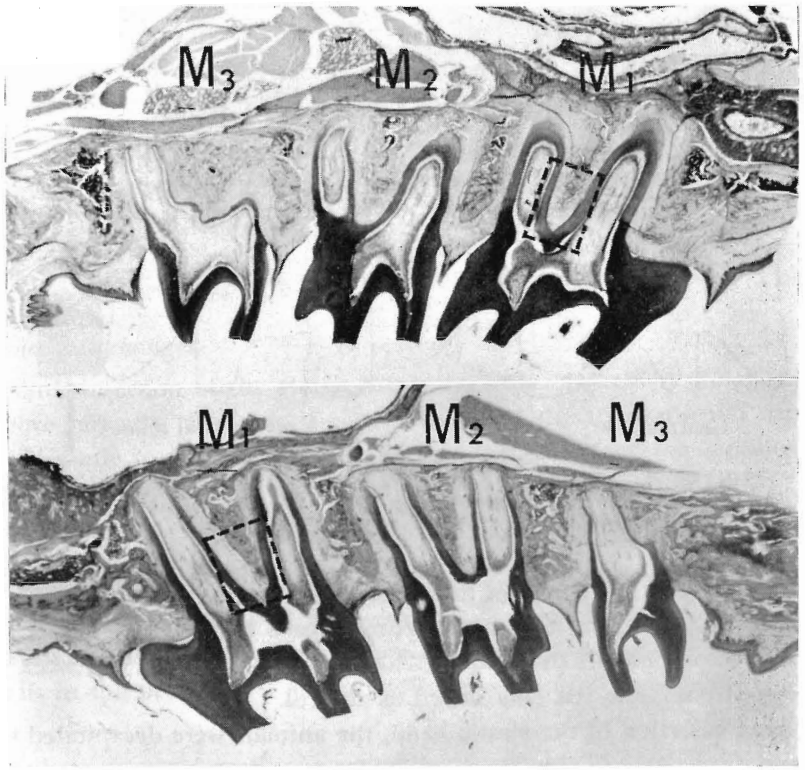

Fig. 1. Photomicrographs of mesio-distal section of the upper molars of rat. (H.E. ×18).
(a) Control side. The molars are under physiologic distal movement. The examination is focussed on the interradicular septum of the first molar outlined by a dotted rectangle.
(b) Experimental side. The first molar is being tipped mesially, reverse of the physiologic movement. The elastic band was inserted between M_1 and M_2.

U.S.A.) according to the method of Willis[19], one day after the operation (Table 1). The third group was parathyroidectomized and treated daily with a subcutaneous injection of 50 U.S.P. units of parathyroid extract (Lilly) per 100 g body weight (PTX+PTE group). This injection was begun 5 days after the operation (Table 1).

The serum calcium levels at the time of decapitation in PTX and PTX+PTE groups were checked to confirm the parathyroid hormone activity. All the animals were fed on the stock diet and tap water was available freely.

Mechanical force was applied to the upper molars of all the animals

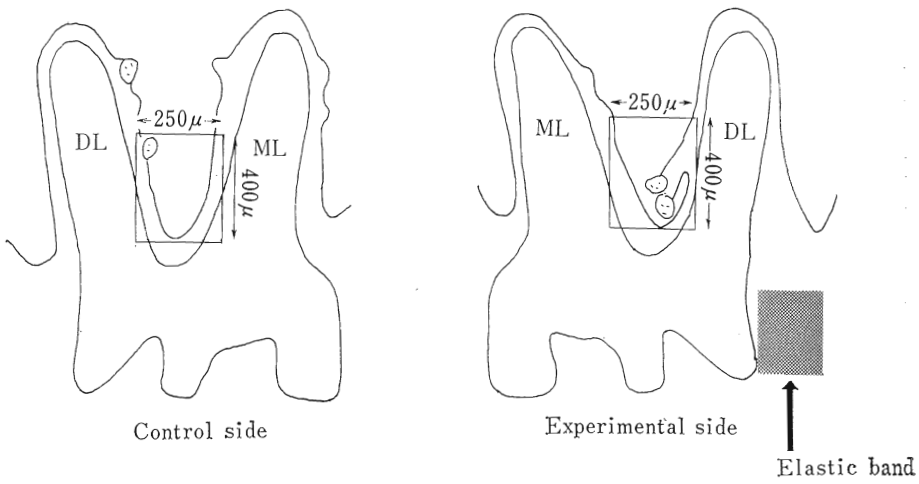

Fig. 2. The field for osteoclast counting. ML: mesiolingual root, DL: distolingual root.

of three groups. A segment of the orthodontic elastic band (0.25 mm thickness) was inserted into the interproximal space between the upper right first and second molars (method of Waldo and Rothblatt[20]) one week after the operation. The left side served as control.

After insertion of the elastic band, the animals were decapitated 6, 12, 24, and 48 hr later (Table 1). The upper maxillary bones were dissected immediately, fixed in 10% neutral Formalin, decalcified in 5% formic acid, and embeded in paraffin. They were cut into mesio-distal serial sections of 7 μ in thickness. The sections which included the mesial and distal roots of three molars were selected and stained with Hematoxylin and Eosin, and silver stain (Watanabe's method). Histological observation was focussed on the interradicular septum of the first molar (Fig. la, 1b).

For the purpose of estimating the degree of bone resorption, osteoclasts were counted by using representative four sections stained with Hematoxylin and Eosin, selected from control and experimental sides of each animal 48 hr after insertion of the elastic band. The field for osteoclast count was limited to a smaller area of the interradicular septum shown in Fig. 2.

Results

The results obtained were as follows:

Changes in serum calcium level

The mean serum calcium level and the standard deviation in each

Table 2. Serum calcium level in the rats after various treatments.

Group	No. of rats	One day after operation	At the time of decapitation
Sham-operated	25	9.98±0.40	
PTX	25	6.12±0.49	6.77±0.73
PTX+PTE	24	6.14±0.56	10.75±1.15

The values (mg%) indicate mean ± standard deviation.

group are shown in Table 2.

Histological changes

With insertion of the elastic band, the upper right first molar started to move mesially, changing the mesial side of the interradicular septum of this tooth to a tension area and the distal side of the septum also became a compression area, reverse of the physiologic distal drift (Figs. 1a, 1b). Histological findings in each period of experiment were as follows:

At 6 hours:

In all the three groups, the periodontal space of the tension side was enlarged and the principal fibers were markedly stretched. The long axis of cells in the periodontal membrane became parallel to the fiber bundles

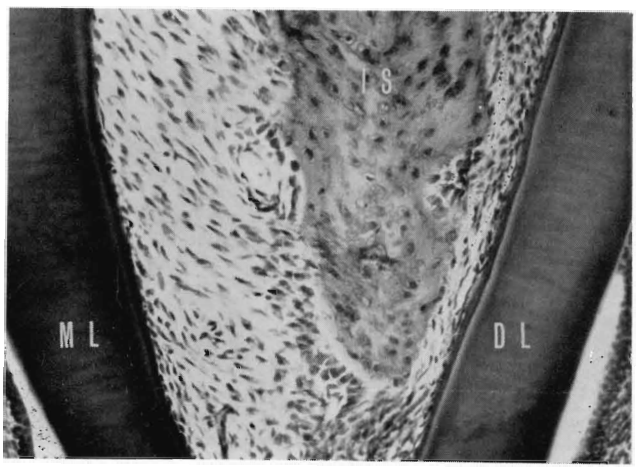

Fig. 3. Sham-operated group, 6 hr. (H.E. ×180).
The periodontal space is enlarged in the mesial side of the interradicular septum, and is strongly compressed in the distal side.
ML: mesiolingual root. DL: distolingual root.
IS: interradicular septum.

and blood vessels were elongated along the direction of strain. Osteoblasts were found only on the crest surface (Fig. 3). These cells might be the remains of osteoblasts which had been observed during physiologic condition.

On the compression side, the periodontal space was strongly compressed resulting in the disorganized and irregularly arranged fibers, and the nuclei of the cells were pyknotic or had disappeared. No blood vessels were visible. Osteoblasts, usually observed in the physiologic tooth movement, were diminished (Fig. 3).

At this period of 6 hr, no significant difference in histological changes was observed among these three groups.

At 12 hours:

In both sham-operated and PTX groups, the principal fibers on the tension side were still stretched (Fig. 4), and a few osteoblasts were observed along the crest surface. The fibers on the compression side were disorganized and destroyed, and small masses of necrotic tissue were found on the crest area. The changes in these two groups at 12hr were essentially the same as those at 6 hr, showing no dominant difference between the two groups.

In PTX+PTE group, the histological change was similar to other two

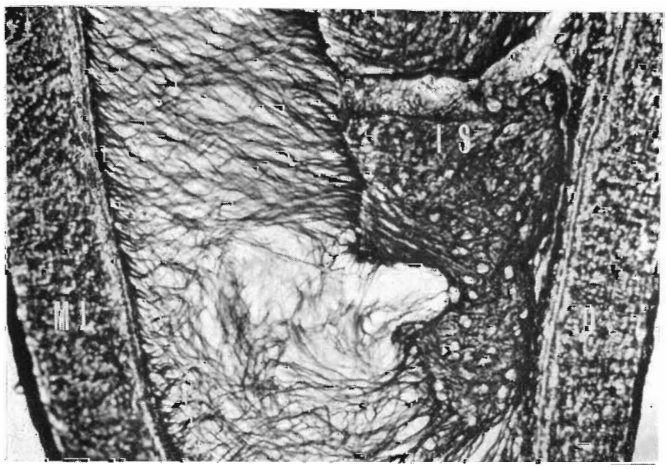

Fig. 4. PTX group, 12 hr. (Silver stain. ×180).
Note the stretched periodontal fibers on the tension side (ML side), and the disorganized and destroyed fibers on the compression side (DL side).
ML: mesiolingual root. DL: distolingual root.
IS: interradicular septum.

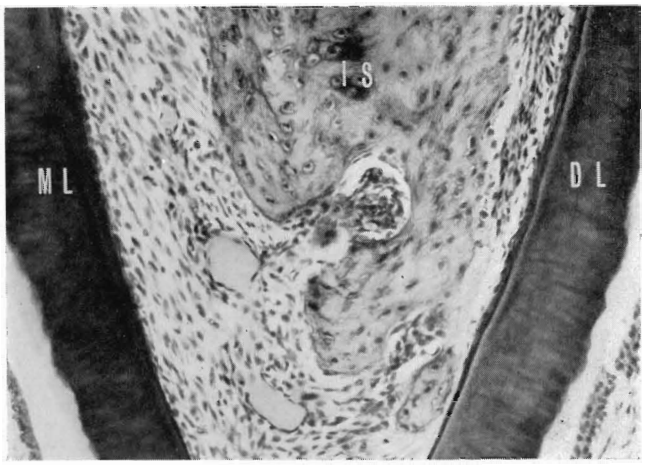

Fig. 5. PTX+PTE group, 12 hr. (H.E. ×180).
Note the absence of osteoblasts on the tension side (ML side), and necrotic tissue on the compression side (DL side).
ML: mesiolingual root. DL: distolingual root.
IS: interradicular septum.

groups except the following facts. No osteoblasts were observed on the crest area (Fig. 5) and elongated cells and fine argyrophilic fibrils were found in their stead. These facts seem to indicate the characteristic influence of parathyroid extract. On the compression side, clusters of mesenchymal cells were often observed in the periodontal membrane adjacent to a relative degenerated area (Fig. 5).

At 24 hours:

In sham-operated group, a layer of osteoblasts was observed and mesenchymal cells were proliferating along the bone surface on the tension side (Fig. 6). In some cases of experiments, osteoclasts were found in the lacunae neighboring the necrotic tissue on the compression side (Fig. 6).

In PTX group, osteoblasts and proliferation of the mesenchymal cells were hardly confirmed on the tension side. Compared to the situation at 12 hr, a more extensive necrotic tissue region was observed on the compression side. No osteoclasts were seen.

In PTX+PTE group, a few osteoclasts were found on the surface of the crest, and proliferation of the mesenchymal cells was seen on the tension side. Adjacent to the necrotic tissue on the compression side, osteoclasts were observed in several cases.

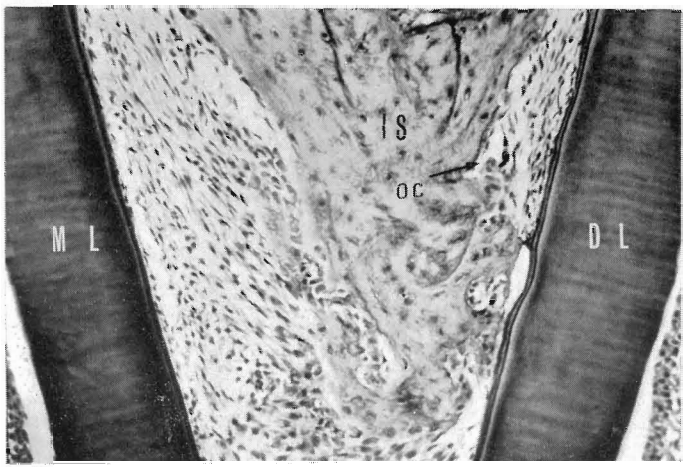

Fig. 6. Sham-operated group, 24 hr, (H.E. ×180).
Note proliferating mesenchymal cells on the tension side (ML side) and a osteoclast on the compression side (DL side).
ML: mesiolingual side. DL: distolingual side.
IS: interradicular septum. oc: osteoclast (arrows).

At 48 hours:

In sham-operated group, an osteoblast layer was observed covering the thin layer of pale-staining osteoid along the bone surface on the tension side (Fig. 7a). Proliferation of the cells in the periodontal membrane was dominant. On the compression side, undermining bone resportion was observed. The resorbed area was filled with granulation tissue accompanied by osteoclasts (Fig. 7a).

In PTX group, periodontal fibers were still stretched on the tension side and a few osteoblasts were seen on the bone surface (Fig. 7b). The cellularity of the periodontal membrane was lower than that in the sham-operated group. On the compression side, a large extent of necrotic tissue was observed and osteoclasts were hardly found in all the animals (Fig. 7b).

Fig. 7. At 48 hr. (H.E. ×180) →
(a) Sham-operated group. Note bone apposition on the tension side (ML side), and undermining bone resorption on the compression side (DL side).
(b) PTX group. Few osteoblasts on the tension side, and large nocrotic tissues on the compression side.
(c) PTX+PTE group. Spindle-shaped cells along the bone surface on the tension side, and many osteoclasts on the compression side.
ML: mesiolingual root. DL: distolingual root.
IS: interradicular septum. oc: osteoclasts (arrow).

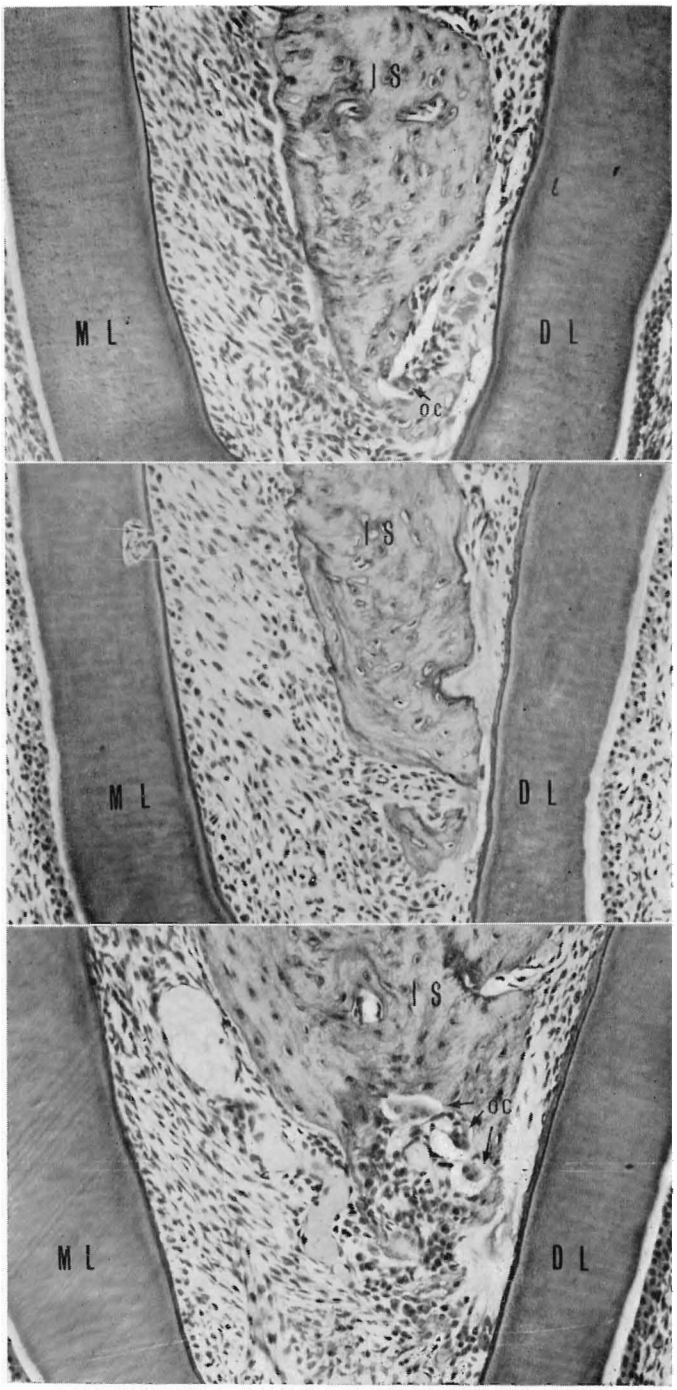

Fig. 7

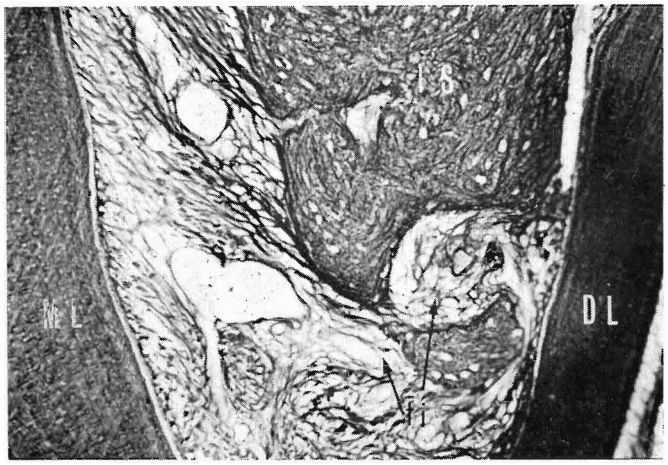

Fig. 8. PTX+PTE group, 48 hr. (Silver stain. ×180).
Note fine fibrils along the crest area on the tension side (ML side) and in the resorbed area on the compression side (DL side).
ML: mesiolingual root. DL: distolingual root.
Is: interradicular septum. fi: fibrils. (arrows).

In PTX+PTE group, many spindle-shaped cells (Fig. 7c) and fine fibrils were seen along the crest surface (Fig. 8) on the tension side, and a few osteoblasts were also observed. The cellularity and the vascularity in the periodontal membrane were increased. On the compression side, the bone adjacent to the necrotic tissue was markedly resorbed (Fig. 7c). The resorbed area was filled with many osteoclasts (Fig. 9), blood vessels, mesenchymal cells, and fine fibrils (Fig. 8).

Osteoclast count

The number of osteoclasts was counted on both control and experimental sides 48 hr after insertion of the elastic band (Fig. 2). The result obtained is shown in Fig. 10. Each number is the average count of osteoclasts in total section of each group.

The *t*-test supported the conclusion of no significant difference among the sham-operated, PTX, and PTX+PTE groups on the control side ($P>0.05$). Between control and experimental sides, significant differences were seen in sham-operated and PTX+PTE groups ($P<0.01$), but not in PTX group ($P>0.05$). And significant differences were found among these three groups ($P<0.01$) on the experimental side.

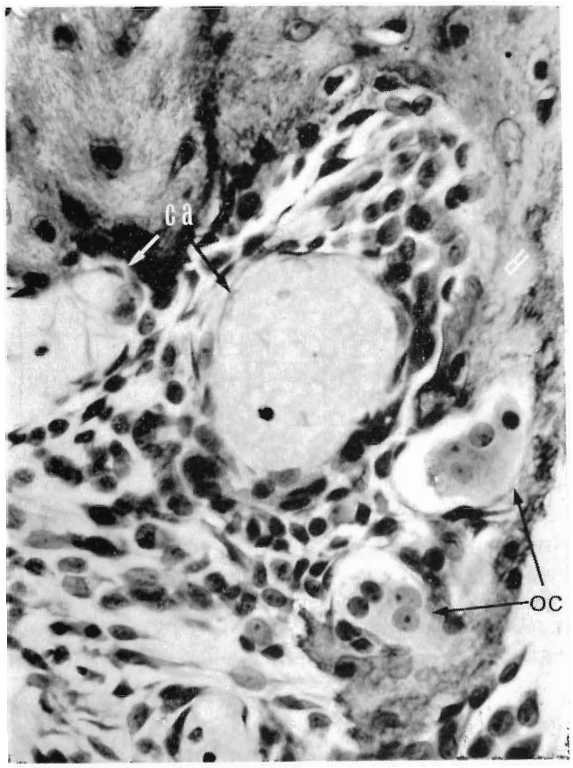

Fig. 9. PTX+PTE group, 48 hr. (H.E. ×400)
Higher magnification of the resorbed area. Note dominant osteoclasts and capillaries.
oc: osteoclast. (arrows). ca: capillary. (arrows).

Discussion

The effect of parathyroid hormone on tooth movement in rats is discussed from the histological point of view.

Histological changes

In the present experiment, difference in histological changes among the three groups became maximum 48 hr after insertion of the elastic band. The following discussion, therefore, will be focussed on the findings at 48 hr of experimental period.

Histological changes in the sham-operated group at this period were characterized by the osteoblast layer, and increase of cellularity in the periodontal membrane on the tension side. These facts correspond to the findings of Macapanpan *et al.*[4] and Baumrind and Buck[6]. The under-

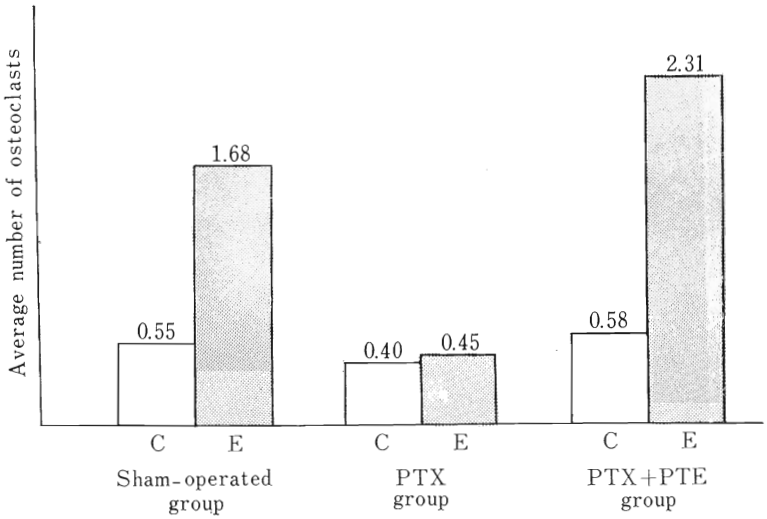

Fig. 10. Histogram showing the effect of parathyroid hormone on the number of osteoclasts during tooth movement. Numbers are the average osteoclast counts in total sections of each group. C: control side. E: experimental side. PTX: parathyroidectomized. PTX+PTE: parathyroidectomized plus parathyroid extract treated.

mining bone resorption was also common on the compression side, being accompanied with osteoclasts and capillaries. These capillaries might have an important role for the appearance of osteoclasts[5].

Comparison of histological changes in PTX group with those in sham-operated group, indicated that the bone remodeling is significantly retarded. These facts agreed with the findings of Jowsey *et al.*[21] who observed that parathyroidectomy reduces Haversian remodeling of diaphysial and metaphysial bone in dogs. Inhibition of the appearance of osteoblasts on the tension side might also correspond to Bernick's observation[22] that the decrease in cellularity and disorganization of the cellular arrangement followed parathyroidectomy in rats. Inhibition of the appearance of osteoclasts in PTX group was also in accord with the findings of Toft and Talmage[16], who reported the decrease of osteoclasts in the femurs of rats following parathyroidectomy.

In PTX+PTE group, many spindle-shaped cells and fine argyrophilic fibrils were observed at 48 hr on the tension side, but there were only a few of osteoblasts. That is, compared to the sham-operated group, osteoblasts were reduced and argyrophilic fibrils were increased. These findings are consistent with the reports of Selye[11], Weinman and Schour[12], Heller

et al.[13] Burrows[14] and Young[17] that osteoblasts are transformed into fusiform or spindle-shaped cells, or disappear at first and reappear after a while under the influence of parathyroid extract. Kroon[15] reported that osteoblasts change in morphological form and produce argyrophilic fibrils along the bone surface under the influence of parathyroid extract. Recently, using the autoradiographic method, Bingham *et al.*[18] reported that the depression of RNA synthesis in osteoblasts was observed after the insertion of parathyroid extract, but a stimulation of RNA synthesis, which was probably associated with the recovery phase, was found at 25 hr. Since parathyroid extract was daily injected in the present experiment, only depressed stage of RNA in osteoblasts continued without any recovery phase, and the appearance of osteoblasts might be disturbed on the tension side. Bone formation was, therefore, inhibited in this group.

On the compression side of PTX+PTE group, bone resorption was further advanced compared to the PTX group, and resorbed area was filled with many osteoclasts. Inversion of capillaries and proliferation of mesenchymal cells were also marked.

Many investigators[11–18] reported that osteoclasts increase immediately after the injection of parathyroid extract or stimulation of parathyroid excretion by low-calcium challenge. The result of the present investigation corresponded to the findings of these previous authors quite well, and was similar to the report of Davidovitch *et al.*[10], who observed histological changes in periodontal tissues following tooth movement under the influence of hyperparathyroid condition in cats. Recently, Bingham *et al.*[18] mentioned that parathyroid extract does not increase the number of osteoclasts immediately, but stimulates the activity of existing osteoclasts, and at the same time also stimulates the mesenchymal cells which are osteoclast precursor. These facts appeared to be related to the proliferation of mesenchymal cells in the resorbed area on the compression side during tooth movement.

Osteoclast count

Myers and Reeve[23], and Myers *et al.*[24] attempted quantitative examination of osteoclast concentrations as an index of bone resorption on the mandibular condyle in guinea pigs. Toft and Talmage[16] modified this method to present the index of parathyroid activity. They used the osteoclast count in the femurs of rats, and found that there was a significant difference in the number of osteoclasts per unit area between normal and parathyroidectomized groups. After the administration of parathyroid extract to parathyroidectomized rats, the number of osteoclast count returned

to a normal level.

In the present investigation, this method was employed for the estimation of the degree of bone resorption. On the control side, as shown in Fig. 10, no significant difference was seen among the three groups. These results were not sufficient to support the result of Toft and Talmage[16]. The main reason might be that the size of the field for counting in the present study was smaller than of previous authors.

Comparison of the experimental and control sides showed that osteoclast count was significantly larger on the experimental side in sham-operated and PTX+PTE group, but not in PTX group. From these findings, it seems that the osteoclasts increase during tooth movement only when parathyroid hormone exists.

On the experimental side, osteoclast count in the PTX group was lower than that in the sham-operated group. The count of osteoclasts in PTX+PTE group was higher than that in the PTX group, as well as in the sham-operated group.

These facts seem to indicate that number of osteoclasts, appearing during tooth movement, is related to the function of parathyroid activity.

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