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ACTION OF PARATHYROID HORMONE, WITH SPECIAL REFERENCE TO ITS ANABOLIC EFFECT ON DIFFERENT KINDS OF TISSUES IN RATS (I)

BY

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ABSTRACT

The effect of parathyroid hormone (PTH) on the spongiosa of the proximal tibia and the alveolar bone in immature rats was studied using a time marker by the injection of lead acetate.

1) When intact rats were fed on a low calcium diet, the promotion of the apposition of the alveolar bone and the longitudinal formation as well as the resorption of the spongiosa were observed, but no change was detected in the serum calcium level. The resorption was more remarkable than the formation. 2) By parathyroidectomy (PTX) or thyroparathyroidectomy (TPTX) the effect described in 1) disappeared completely, but there subsequently occurred a fall of the serum calcium level and a marked inhibition of the formation and resorption. The decrease in the appositional formation was stronger than in the longitudinal formation. 3) When PTH was injected into the rats having undergone PTX or TPTX, all of the effects in 2) were reversed with the recovery of the apposition being at an extremely high rate.

All the foregoing results indicate that PTH has an evident anabolic action in addition to the action of increasing the bone resorption and that the sensitivity to PTH is stronger in the periosteal bone than in the spongiosa.

INTRODUCTION

It is widely known that parathyroid hormone (PTH) is highly important for the regulation of calcium homeostasis in the blood and that it increases the bone resorption and decreases the bone formation to maintain the normal serum calcium level. Many investigators[1-4] studied the effect of parathyroidectomy (PTX) on the development of the bone and found an inhibitory effect, while some investigators[5,6] reported that the longitudinal growth of the long bone in the young growing rats was increased by PTH. Recently, Kalu *et al.*[7] studied the bone formation using isotopically labelled proline and suggested that PTH has an anabolic effect on the bone in the rats. Similar results were reported by Nguyen and Jowsey[8] (dog), Walker[9] (rat) and Labarrere and Mautalen[10] (human). However, there has been no report based on the quantitative observation of the effect of PTH on the resorption and formation of the bone, especially of the spongiosa in the long bones the resorption and formation of which are strongly correlated with each other.

The present paper deals quantitatively with the effect of PTH on the two kinds of bones, i.e., alveolar (periosteal) and spongiosa of the proximal tibia (endochondral) using lead acetate as a time marker.[11]

MATERIALS AND METHODS

Male Wistar rats, each weighing about

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60 g, were used in all experiments. The rats were divided into 8 groups (I–VIII) and then fed on a commercial diet (Oriental Yeast Co., Ltd., Japan) for three days. To make a comparison of the effect of the endogenous PTH between the normal calcium (0.51%: a modification of Shaw's diet[12]) (Group I—intact rats) and low calcium (0.03%: a modification of Shaw's diet[12]) (Group II—intact rats), the rats were fed on either diet for 13 days. Group III was fed on the normal calcium diet for three days, while the remaining five groups (IV–VIII) were fed on the low calcium diet for three days prior to the operation. Groups III and IV were sham-operated (SHAM) and fed on the normal calcium diet or the low calcium diet, respectively. The following are the processes used for examining the effects of PTX, thyro-parathyroidectomy (TPTX) and exogenous PTH. PTX or TPTX was performed by a hot-wire cautery under ether anaesthesia and a binocular dissecting microscope. The PTX (V and VI) and TPTX (VII and VIII) groups were divided into the saline-injected (V and VII) and the PTH-injected (VI and VIII) groups. The SHAM groups were injected only with saline. Saline or 40 USP units/100 g body weight of PTH (Lilly Co.) was injected subcutaneously once a day for three and six consecutive days beginning six days after the operation. The influence of calcium in the diet, removal of the glands and exogenous PTH was compared between the groups.

All the rats had intravenous injections of lead acetate in a dose of 3 mg/kg body weight to leave a time mark on the hard tissues. The injection was made into the femoral or the dorsal vein of the foot[13] every three days. The rats were killed by decapitation on the day following the last injection of lead acetate. To observe the variation in the level of serum calcium, the animals under ether anaesthesia were bled before decapitation 6 hours after the last injection of saline or PTH by the insertion of polyethylene cannula into the carotid artery. The serum calcium concentration was determined by the atomic absorption spectrophotometric method.

The tibiae and the lower jaws were removed from the rats, fixed in 10% (v/v) neutral formalin, decalcified in 0.2 N HCl continuously saturated with H_2S, cut into 10–15 μm frozen sections, stained with 0.1% (w/v) chloroauric acid to develop the lead lines, which appeared as brown lines, and then reduced by 5% (w/v) sodium thiosulfate. The sagittal sections were made from the proximal tibiae and the transverse sections from the lower jaw at the level of the first molar. The sections were further stained with haematoxylin and eosin.

The rates of bone formation and resorption were microscopically measured using the lead line as an index. The details of the measurements will be described in the subsequent sections.

Results

Effect of PTH With Low Calcium Diet on Serum Calcium Concentration

The relation between calcium concentration in the diet and that in the serum is shown in Table 1. Serum calcium level in the intact rats fed on the normal calcium diet (Group I) remained normal, and no variation was found in the rats fed on the low calcium diet (Group II). Also the SHAM groups (Groups III and IV) showed no effect, whereas it was confirmed, as has been indicated by many workers, that the serum calcium level decreased clearly after PTX or TPTX. The effect was more conspicuous in the former than in the latter. The saline injection to the PTX or TPTX group showed no effect, while the subcutane-

Table 1. Effect of PTH With Low Calcium Diet on Serum Calcium Level in Normal Rats

Group		Treatment	Normal calcium diet	Low calcium diet
			Serum calcium (mg/100 ml)	
I	II	Intact	11.44±0.18 (8)	10.95±0.20 (8)
III	IV	SHAM+saline	10.78±0.23 (7)	11.10±0.21 (8)
	V	PTX+saline	—	5.77±0.22 (8)
	VI	PTX+PTH (40 USP)	—	9.85±0.24 (8)
	VII	TPTX+saline	—	6.38±0.27 (7)
	VIII	TPTX+PTH (40 USP)	—	11.22±0.31 (8)

Numbers in parentheses indicate the number of rats sampled.
Data are presented as average±standard error.

ous injection of PTH clearly affected the recovery from the lowered serum calcium level. This effect was clearer in the TPTX group than in the PTX group.

Effect of Calcium in Diet, SHAM, PTX, TPTX, and PTH on Longitudinal Formation and Resorption of Spongiosa in Proximal Tibia

1) Effect of low calcium diet on intact and SHAM groups (endogenous PTH)

Plate 1 shows the mid-sagittal sections of the proximal tibiae. When 3 mg/kg of lead acetate were injected intravenously, the lead ions were deposited as a thin layer of lead phosphate in the metaphyseal bone (spongiosa) where the calcium ions are normally deposited. Each lead line (A, B, C, D, E, F and G in Plate 1) in the metaphyses and diaphyses of the proximal tibiae corresponds to one intravenous injection of lead acetate. The rate of longitudinal formation can be obtained precisely by measuring the distance between the two adjacent lead lines. Plate 1 and Fig. 1 show the proximal tibia of the rat fed on the normal calcium diet and Fig. 2 that of the rat fed on the low calcium diet. Feeding on both diets was begun at the marks NC and LC, respectively, lead acetate being injected intravenously every three days. The distance between A and B in Plate 1 and Figs. 1 and 2 is the rate of the longitudinal formation for the first three days in the rats fed on the commercial diet, and the portion between B and F is the bone formed for 12 days while giving the normal-calcium or low-calcium diet. The results of the measurement are shown in Table 2. The difference in the bone formation among the three sagittal sections from each rat was not statistically significant. All the calculations were based on the mean of the three sections from each rat. Almost no effect was seen by the replacement of the commercial diet with the normal calcium diet, and the longitudinal formation of spongiosa was continuous. The standard error of the longitudinal formation rate in Table 2 represents that for every three days in the rats composing each group. This method of calculating the error applies to all other tables used in this paper. In Table 1 and Group I, the standard error of the formation rate for every three days in the same individual is very small compared with that for the different individuals. On the other hand, in the group fed on the low calcium diet, the epiphyseal cartilage plate is wider (Plate 1 and Fig. 2) than in Group I. This widening is especially conspicuous in the layer of the hypertrophic cells. The longitudinal formation rate was also found to increase gradually by giving the low calcium diet.

Table 2. Effect of Calcium in Diet on Longitudinal Formation and Resorption of Spongiosa in Proximal Tibia

	Days after treatment	Group I (8)	Group II (8)
		Commercial diet	
	0—3	710.1±36.0	692.5±30.9
		Normal calcium diet	Low calcium diet
Longitudinal formation (μ/3 days)	0—3	704.3±41.3	717.2±34.8
	3—6	708.7±46.1	735.1±36.1
	6—9	710.5±38.2	790.4±49.7
	9—12 (X)	708.4±34.8	825.0±36.4
	X/3 (Y)	236.1	275.0
Length of spongiosa (μ)	(Z)	711.5±44.4	93.5±21.4
	Z/Y (days)	3.02	0.34
Width of cart. plate (μ)		380.3±18.3	458.1±14.8

Numbers in parentheses indicate the number of rats sampled.
Data are presented as average±standard error.

The effect of the low calcium diet on bone resorption was also obtained using the lead line index. In Plate 1 and Fig. 1 the distance between the junction of the epiphyseal cartilage plate with the spongiosa and the F line produced by the last injection of lead acetate corresponds approximately to the daily rate of the formation of the spongiosa, since the rats were killed about 24 hours after the last injection of lead acetate. Also, the E line formed by the lead acetate injected four days before decapitation was almost complete. However, the D line formed by the lead acetate injection seven days before autopsy almost disappeared. The C, B and A lines formed by the injection 10, 13 and 16 days before autopsy, respectively, disappeared almost completely except in the cortex. The disappearance of the spongiosa at the mid-part of the proximal tibiae in the Group I rats was confirmed about four days after the cells in the epiphyseal cartilage had been transformed into spongiosa.

In order to obtain an accurate value for the time of the longitudinal resorption of the spongiosa, a measurement was made of the distance (the length of the spongiosa) between the lead line (F line) about 24 hours before autopsy and the diaphyseal ends of five spongy bones at the mid-part of the proximal tibia. The results of the measurement are shown in Table 2. The value obtained by dividing the length (Z) of the spongiosa by the rate (Y) of the longitudinal formation is represented by Z/Y. The length of the spongiosa in each animal was obtained by calculating the lengths measured from five spongy bones. The mean value of Z/Y from eight rats fed on the normal calcium diet was 3.02 days. The beginning of the longitudinal resorption of the spongiosa was confirmed to be about four days after the cartilage cells have been transformed into spongiosa since the rats were killed about 24 hours after the last injection of lead acetate (F line). The effect of the low calcium diet on the bone resorption in the Group II rats is shown in Plate 1 and Fig. 2. The line E formed by the lead acetate injected four days before autopsy disappeared almost completely and the length of the spongiosa in Group II decreased significantly as shown in Table 2. The value of Z/Y in Group II (0.34 days) was much smaller than in Group I (3.02

Table 3. Effect of SHAM, PTX, TPTX, and PTH on Longitudinal Formation and Resorption of Spongiosa in Proximal Tibia

	Days after treatment	Group IV (8)	Group V (8)	Group VI (8)	Group VII (7)	Group VIII (8)
				Commercial diet		
	0—3	630.2±30.4	642.5±28.3	659.7±34.1	575.8±21.4	708.1±35.1
				Low calcium diet		
	0—3	645.5±36.8	650.0±29.1	675.1±34.0	597.0±25.9	720.0±40.2
Longitudinal		SHAM	PTX	PTX	TPTX	TPTX
formation	0—3	664.2±34.0	474.8±24.5	471.3±23.1	367.5±19.4	545.5±30.3
(μ/3 days)	3—6	693.3±40.7	318.4±16.8	310.2±15.6	272.5±20.9	417.5±25.5
		saline	saline	PTH (40 USP)	saline	PTH (40 USP)
	6—9	728.5±23.4	287.5±26.4	380.5±39.9	225.1±15.9	535.2±32.2
	9—12(X)	755.2±49.2	258.0±15.3	425.1±34.9	204.0± 6.9	595.5±28.3
	X/3 (Y)	251.3	86.0	141.7	68.0	198.5
Length of spongiosa (μ)	(Z)	90.5±26.8	299.5±33.1	430.5±46.8	228.3±28.4	589.1±40.8
	Z/Y (days)	0.36	3.48	2.93	3.36	3.02
Width of cart. plate (μ)		429.1±18.0	273.0±32.8	280.6±24.5	257.6±29.1	283.8±33.4

Numbers in parentheses indicate the number of rats sampled.
Data are presented as average±standard error.

days) since the rate (Y) of the longitudinal formation was increased by feeding on the low calcium diet as described above. Increase in the longitudinal resorption of the spongiosa in the proximal tibiae was eight times greater in the rats fed the low calcium diet than in those fed the normal calcium diet, and the increasing effect of calcium deficiency on the longitudinal resorption was stronger than on the longitudinal formation.

The increase in bone resorption due to feeding the low calcium diet was also recognized in the cortex (compact bone) by using the lead line index, but it was difficult to show the results quantitatively because the bone formation did not always occur at the same site of the cortex.

2) Effect of PTX or TPTX

Plate 1 and Fig. 3 show the effect of TPTX and mark TPX indicates the time of the operation. Compared with Fig. 1 (the normal calcium diet group), the epiphyseal cartilage plate is wider in the SHAM group but narrower in the PTX or TPTX group. To further investigate the PTX or TPTX effect, some of the rats were killed every three days after PTX or TPTX. The width of the cartilage plate began to decrease three days after both operations and this became clearer as time elapsed. A shortening in the length of the spongiosa was observed as a characteristic response. To disclose the mechanism of the shortening of the spongiosa, the rate of the longitudinal formation was measured by the aforementioned method. The results of the measurement are shown in Table 3. The longitudinal formation in the SHAM group increased gradually after feeding on the low calcium diet. However, the formation was clearly inhibited by PTX or TPTX and this effect was greater in the TPTX group than in the PTX group, though the difference was slight.

The rate of bone resorption was obtained by the method mentioned in the above section. The results are shown by Z/Y in Table 3. Compared with Group I (Table 2) the value of Z/Y is smaller in Group IV (SHAM) and larger in Groups V and VII. In other words, the effect of the low calcium diet was

influenced by PTX or TPTX but not by the sham-operation, the bone resorption being inhibited by the removal of the glands. The inhibitory effect of PTX or TPTX on the bone resorption was also confirmed by the fact that the lead line (F in Plate 1 and Fig. 3) formed four days before autopsy was almost complete. The inhibitory effect was lesser on the bone resorption than on the bone formation.

3) Effect of exogenous PTH

Plate 1 and Fig. 4 show the mid-sagittal section of the proximal tibia in the TPTX rats which received subcutaneous injections of 40 USP units/100 g of PTH once a day for six consecutive days (from line E to G) from the sixth day (line E or mark H) after the operation. As a result of the hormone injections the width of the epiphyseal cartilage plate became wider in Group VIII than in the saline-injected group (Plate 1 and Fig. 3). A widening of the hypertrophic cell layer was conspicuous. The spongiosa increased in length. To elucidate the mechanism of the elongation of the spongiosa, the rate of the longitudinal formation and the length of the spongiosa were measured and the rate of the longitudinal resorption of the spongiosa was calculated by the aforementioned methods. The results are shown in Table 3.

Increase in the longitudinal formation of the spongiosa by PTH was clear but not in the case of the longitudinal resorption. The longitudinal formation inhibited by TPTX was definitely restored by the injection of PTH, and this restorative effect was observed soon after the hormone injection. The bone formation rate during the second three days was higher than that during the first three days. The effect of PTH injected after PTX was similar to that found in the TPTX rats, but the effect in the former tended to decline compared with that in the latter (Table 3).

Table 4. Effect of Calcium in Diet on Apposition of Alveolar Bone

Days after treatment	Apposition (μ/3 days) Group I (8)	Group II (8)
	Commercial diet	
0—3	16.43±1.07	19.18±1.64
	Normal calcium diet	Low calcium diet
0—3	15.65±1.72	19.98±1.90
3—6	14.65±1.89	21.87±1.98
6—9	14.33±1.56	22.71±1.23
9—12	14.22±0.99	23.94±1.50

Numbers in parentheses indicate the number of rats sampled.
Data are presented as average±standard error.

Effect of Calcium in Diet, SHAM, PTX, TPTX, and PTH on Periosteal Formation (Apposition) of Alveolar Bone

1) Effect of low calcium diet (endogenous PTH)

The effect of PTH on periosteal formation was studied using an area of the alveolar bone, since the apposition of the alveolar bone is comparatively regular in the area of the first molar in the lower jaw compared with the other areas of the alveolar bone and other compact bones.

Plate 2 and Fig. 1 show the apposition of the alveolar bone of the rat fed on the normal calcium diet. In the figure, the distance between the lead lines A and B is the apposition rate of the alveolar bone for three days in the rat fed the commercial diet, and the portion between line B or mark NC and line F is the bone formed for 12 days after giving the normal calcium diet. The results of the measurements are shown in Table 4.

The apposition rate of the alveolar bone was lower than the longitudinal formation rate of the proximal tibia. After feeding on the normal calcium diet the apposition rate tended to decrease gradually, which response

Table 5. Effect of SHAM, PTX, TPTX, and PTH on Apposition of Alveolar Bone

Days after treatment	Apposition (μ/3 days)				
	Group IV (8)	Group V (8)	Group VI (8)	Group VII (7)	Group VIII (8)
			Commercial diet		
0—3	15.85±1.18	25·84±1.33	30.00±2.04	32.05±2.22	17.15±0.98
			Low calcium diet		
0—3	16.23±1.32	27.22±2.05	31.88±2.13	33.13±2.08	17.53±1.56
	SHAM	PTX	PTX	TPTX	TPTX
0—3	17.05±2.14	9.33±1.93	13.75±1.23	12.53±1.33	7.52±1.24
3—6	17.98±0.99	6.58±1.77	7.33±0.77	7.21±0.68	3.55±1.01
	saline	saline	PTH (40 USP)	saline	PTH (40 USP)
6—9	18.75±1.44	4.94±0.72	22.39±2.24	4.55±0.84	12.80±2.94
9—12	19.21±1.09	4.58±0.99	29.12±1.98	4.42±0.79	16.42±2.71

Numbers in parentheses indicate the number of rats sampled.
Data are presented as average±standard error.

was also observed in the rats fed the commercial diet. However, after the replacement (lead line B or mark LC in Plate 2 and Fig. 2) of the commercial diet by the low calcium diet, the apposition increased gradually (Table 4 and Group II).

2) Effect of TPX or TPTX

Plate 2 and Fig. 3 show the effect of PTX. Twelve days after the operation the formation rate was 80% less than before the operation, the effect being stronger than in the longitudinal formation of the proximal tibia. The lead lines (D, E and F in Plate 2 and Fig. 3) in the bone formed after the operation and the lead line (C or mark PX) indicating the time of the operation are too close to each other and thus form a wide single lead band. This means that there occurred an extreme inhibition of the apposition. The effect of TPTX was similar to that of PTX except that the degree of the inhibition tended to be stronger after TPTX than after PTX (Table 5).

3) Effect of exogenous PTH

Plate 2 and Fig. 4 show the results for TPTX rats which received subcutaneous injections of 40 USP units/100 g of PTH once a day for six consecutive days (between D and F) from the sixth day after the operation (line D). In this case as well, the same situation is seen as in the case of PTX, i.e., a wide single lead band is composed of lead lines (C and D) in the bone formed after the operation as well as the lead line (B or mark TPX) indicating the time of the operation, as shown in Plate 2 and Fig. 3. The effect of the operation was modified by the injection of PTH (Table 5 and Group VIII and the lines D–F in Plate 2 and Fig. 4), the formation during the second three days being greater than that during the first three days. The same effect of the exogenous hormone was also observed in the PTX rats (Table 5 and Group VI) but the effect was milder than in the case of TPTX. The inhibiting effect of PTH on the decrease in the bone formation caused by PTX or TPTX was stronger in the appositional formation of the periosteal bone than in the longitudinal formation of the long bone.

Discussion

Since the resorption of a compact bone can be observed clearly, this bone is frequently used for studying the effects of PTH and drugs on bone resorption. However,

our experiments showed the results that the pattern and the rate of bone resorption were not necessarily identical at the same site of the bone and that the bone formation and resorption did not always occur systematically, unlike the spongiosa in the long bones whose resorption and formation are strongly correlated with each other. Although it is difficult to observe the resorption of the spongiosa, as already described in the results, the use of the lead lines provides an accurate information on the resorption of the spongiosa. By the method, the generating process of osteoporosis and the effect of hormones and drugs on the generation can be obtained quantitively.

The results of the present studies on the effect of PTH on the bones, using the proximal tibia and alveolar bone, suggest that PTH possesses an anabolic action, as reported by Kalu *et al.*[7]. This effect was noted only on the bone growth. This should be further confirmed by studies on the incisor in which resorption is not observed and the embryological origin differs from the bone. The effect of the hormone on the incisor will be included in the paper to be published soon.

Interesting in the present experiments is the difference in the reaction to PTH between the periosteal bone formation and the endochondral bone formation. On the 12th postoperative day after PTX or TPTX, the magnitude of the decrease of the endochondral bone formation was approximately 60% while that of the periosteal bone formation was more than 80%. When the hormone was administered to the PTX or TPTX rats, the rate of recovery of the periosteal bone was extremely higher than that of the endochondral. The sensitivity to PTH is higher in the formation of the compact bone. Furthermore, this hormone widened the layer of the hypertrophic cells in the epiphyseal cartilage plate. From these results, it is thought that the mechanism of the anabolic action of PTH is different from the action of the growth hormone[14,15] and more detailed studies will be made on the mechanism of the anabolic action of PTH in the near future.

The effect of the thyroid hormone, widely known as a hormone with anabolic action, should be taken into consideration. However, based on the difference in the reaction between the PTX group and the TPTX group, as noted in Tables 3 and 5, it is possible that the effect of the thyroid hormone is not so strong. From the result in the present experiment that the effect of PTH could be clearly observed even in the TPTX rats, the anabolic action seems to be intrinsic to PTH. Furthermore, the effect of blood calcium on the action of PTH should be considered and this subject will be discussed in detail in a separate paper.

It is suggested from the increased bone resorption shown in Plate 1, Fig. 2 and Table 2 that the breeding of the intact rats by a low calcium diet results in the considerable secretion of PTH. The fact that the serum calcium level was scarcely affected by this hormone (Table 1) and that the effect of endogenous PTH in the intact rats was lesser than that of exogenous PTH in the TPTX rats (tables) is probably due to the effect of calcitonin (CT) secreted mainly from the thyroid glands. Detailed studies on CT will be reported in a paper to follow. (Preliminary results of this study were presented at the Regional Meeting of the International Union of Physiological Sciences in Sydney, Australia, in 1972).

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Description of Plates

Black lines (A, B, C, D, E, F and G) in the plates are the lead lines as a time marker formed by the intravenous injection of lead acetate every three days. All the rats were killed by decapitation about 24 hours after the last injection of lead acetate (F or G line). The sections were stained by haematoxylin. NC=beginning of a normal calcium diet administration, LC=beginning of a low calcium diet administration, TPX=time of TPTX, PX=time of PTX, S=beginning of injections of saline, H=beginning of injections of PTH, Pe=periosteum.

Plate 1.

Figs. 1–4. Photomicrographs of the mid-sagittal section of the proximal tibiae.

Fig. 1. The proximal tibia in the rat fed on the normal calcium diet.

Fig. 2. The proximal tibia in the rat fed on the low calcium diet.

Fig. 3. The proximal tibia in the TPTX rat which was killed 13 days after the operation.

Fig. 4. Proximal tibia in the TPTX rat which received subcutaneous injections of 40 USP units/100 g of PTH once a day for six consecutive days.

Plate 2.

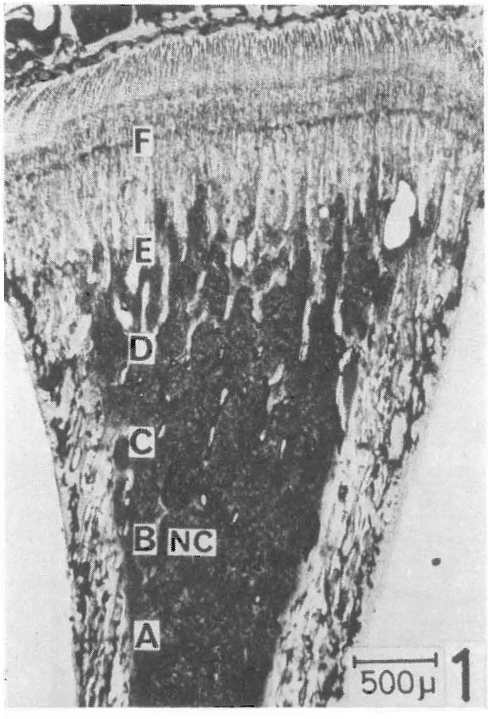
Figs. 1–4. Photomicrographs of the frontal section of the alveolar bone in the area of the first molar in the left lower jaw.

Fig. 1. Alveolar bone in the rat fed on the normal calcium diet.

Fig. 2. Alveolar bone in the rat fed on the low calcium diet.

Fig. 3. Alveolar bone in the PTX rat which was killed 10 days after the operation.

Fig. 4. Alveolar bone in the TPTX rat which received subcutaneous injections of 40 USP units/100 g of PTH once a day for six consecutive days.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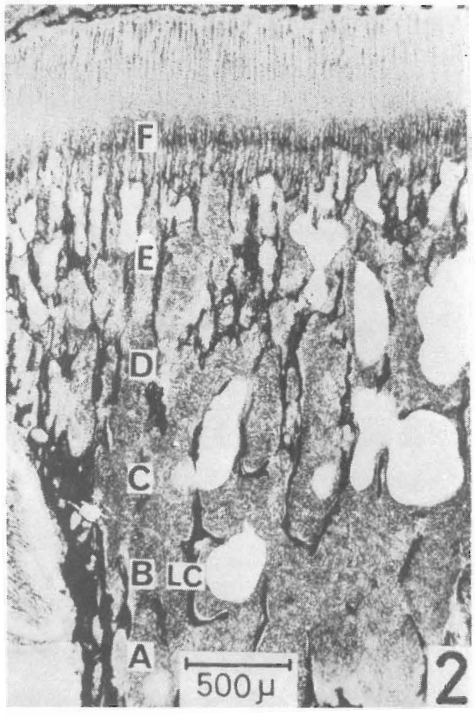
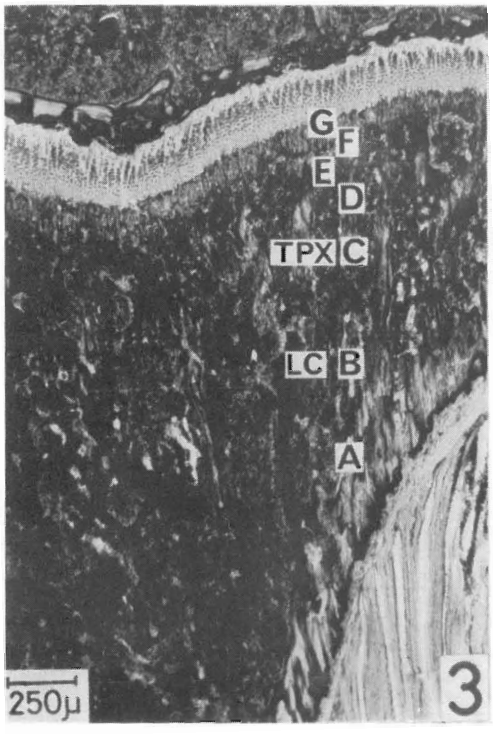
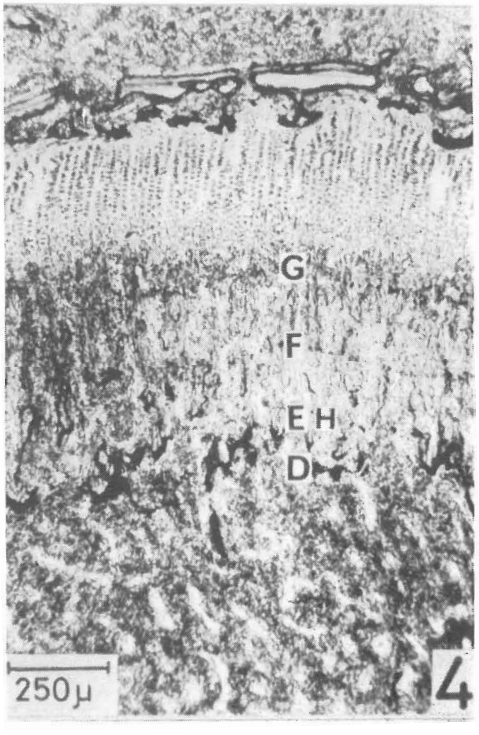
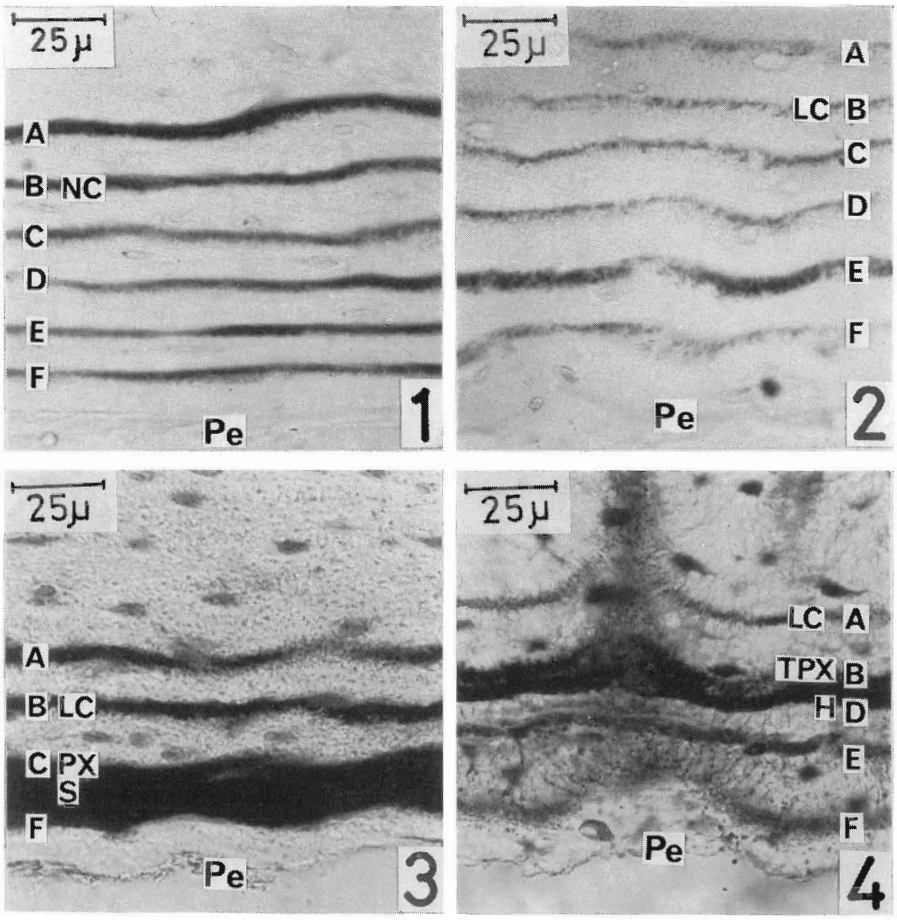

F
E
D
C
B LC
A
500μ
2


G
F
E
D
TPX C
LC B
A
250μ
3


G
F
E H
D
250μ
4


25μ
A
B NC
C
D
E
F
Pe
1
25μ
A
LC B
C
D
E
F
Pe
2
25μ
A
B LC
C PX
S
F
Pe
3
25μ
LC A
TPX B
H D
E
F
Pe
4