

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

BY

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ABSTRACT

The effect of parathyroid hormone (PTH) on the formation of the incisor in the rats was investigated using a time marker by the injection of lead acetate. 1) When intact immature rats were fed on a low calcium diet, the rate of the longitudinal and appositional formation of the incisal dentin was increased. 2) Both formations were inhibited by parathyroidectomy (PTX) or thyro-parathyroidectomy (TPTX), the inhibition being stronger in the appositional than in the longitudinal formation. The mechanism generating the characteristic responses (thin dentin and irregularity of dentin formation) which appeared after the operations was made clear. 3) All of the effects in 2) were reversed by the injection of PTH. However, the restoration in the appositional formation was faster in appearance and higher in rate than in the longitudinal formation. The results of 1) to 3) indicate that PTH has an anabolic effect on the hard tissues. 4) The sensitivity of the tissues to PTH varies according to their embryological origin.

INTRODUCTION

A series of investigations were made by the author on the effect of parathyroid hormone (PTH) on the different kinds of tissues, i.e., the alveolar bone, proximal tibia and incisor of the rats, with special attention to whether PTH has an anabolic action and whether the different tissues have a different sensitivity to the hormone.

The present paper is concerned about the effect of PTH on the incisor. The effect of PTH on the bones was already described in the preceding paper and that on mineralization as well as on the matrix formation will be described in the following paper.

MATERIALS AND METHODS

The upper incisors of the immature rats

weighing about 60 g were used. The grouping of the animals and the dose and time schedule of the administration of PTH and lead acetate were the same as in the previous work. Also the histological procedures were the same except for sectioning which was made longitudinally at a thickness of 10 μ m. Only the mid-sagittal sections were subjected to the measurement of the longitudinal and appositional formation of the incisal dentin. The rate of these formations was measured microscopically by using lead lines formed in the dentin by the intravenous injection of 3 mg/kg of lead acetate every three days as time markers.¹⁾ The details of the measurements will be described in the respective sections.

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RESULTS

Effect of Calcium in Diet, SHAM, PTX, TPTX, and PTH on Longitudinal Formation of Incisor

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

Plate 1 and Figs. 1 and 2 show the mid-sagittal sections of the upper incisors. The thin black lines in the labial and lingual dentin and the alveolar bone are the lead lines formed by the intravenous injections of lead acetate every three days. These lead lines correspond to those mentioned in the preceding paper. Plate 1 and Fig. 5 show the schematic diagram of the labial dentin in the growing upper incisor. When 3 mg/kg of lead acetate were injected, the lead ions were deposited as a thin layer (LL 3 in Fig. 5) of lead phosphate at the dentin-predentin junction where calcium is normally deposited. By the injections of lead acetate at certain intervals the lead lines in the labial dentin appeared as shown in LL 1, 2 and 3 in Fig. 5. A, B and C in Fig. 5 and A, B, C, D, E, F and G in Figs. 1 and 2 represent the points (referred to as end-points), where the dentin formation begins. The longitudinal formation rate of the incisal dentin was determined by measuring the distance between the two adjacent end points. "A" is the end point of the lead line formed by the first injection and "G" by the last injection. 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 a commercial diet (Oriental Yeast Co., Ltd., Japan), and the portion between B and G is the incisor formed for 15 days while giving a normal calcium diet (0.51% Ca, a modification of Shaw's diet²⁾) or a low calcium diet (0.03% Ca, a modification of Shaw's

Table 1. Effect of Calcium in Diet on Longitudinal Formation of Incisal Dentin in Intact Rats

Days after treatment	Longitudinal formation (mm/3 days)	
	Group I (8)	Group II (8)
	Commercial diet	
0-3	1.235±0.049	1.330±0.068
	Normal calcium diet Low calcium diet	
0-3	1.230±0.064	1.389±0.059
3-6	1.225±0.082	1.452±0.094
6-9	1.230±0.058	1.505±0.071
9-12	1.255±0.054	1.556±0.099

Numbers in parentheses indicate the number of rats sampled.

Data are presented as average±standard error.

diet²⁾). The results of the measurement are shown in Table 1.

The formation rate of the incisor did not change even when the commercial diet was substituted (point B or mark NC in Fig. 1) by the normal calcium diet (Group I). When the commercial diet was changed (point B or mark LC in Fig. 2) to the low calcium diet the longitudinal formation was gradually accelerated (Group II).

2) Effect of parathyroidectomy (PTX) or thyro-parathyroidectomy (TPTX)

Plate 2 and Fig. 1 show the longitudinal section of the upper incisor in the TPTX rat. Mark LC in this figure indicates the beginning of the low calcium diet administration and mark TPX the time of the operation, and point F the day before the autopsy. The labial dentin was elongated as far as the length between C and F for nine days after the operation. The results of the measurement are shown in Table 2.

In the SHAM group (Group IV) the effect of the operation could not be observed, while the effect of the low calcium diet was the same as in the case of Group II (Table 1). The effect of the diet disappeared definitely by TPTX. In addition the longitudinal

Table 2. Effect of SHAM, PTX, TPTX, and PTH on Longitudinal Formation of Incisal Dentin in Normal Rats

Days after treatment	Longitudinal formation (mm/3 days)				
	Group IV (8)	Group V (8)	Group VI (8)	Group VII (7)	Group VIII (8)
			Commercial diet		
0-3	1.421±0.079	1.506±0.066	1.588±0.064	1.560±0.052	1.481±0.071
			Low calcium diet		
0-3	1.465±0.052	1.530±0.073	1.630±0.051	1.635±0.053	1.512±0.078
	SHAM	PTX	PTX	TPTX	TPTX
0-3	1.512±0.055	1.320±0.086	1.342±0.050	1.425±0.068	1.348±0.053
3-6	1.575±0.085	1.286±0.051	1.204±0.067	1.340±0.085	1.334±0.070
	saline	saline	PTH (40 USP)	saline	PTH (40 USP)
6-9	1.603±0.049	1.249±0.064	1.305±0.058	1.302±0.045	1.502±0.049
9-12	1.663±0.058	1.205±0.055	1.372±0.069	1.255±0.047	1.554±0.049

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

formation was inhibited by about 20% of the preoperative formation rate as measured 12 days after the operation (Group VII). Similar results were obtained in the PTX rats (Group V), but the inhibitory effect was milder than in the TPTX rats.

3) Effect of exogenous PTH

The effect of exogenous PTH was examined with the rats whose longitudinal formation had been inhibited by PTX or TPTX. Plate 2 and Fig. 4 show the effect of the hormone on the TPTX rat, and the results obtained by the previously mentioned method are tabulated in Table 2. The longitudinal formation inhibited by TPTX was restored by the subcutaneous injection of 40 USP units/100 g body weight of PTH once a day for six days (between points D and F in Fig. 4) from the sixth postoperative day (point D or mark H). This recovery became conspicuous by the repeated injections, and the injections on the second three days (between points E and F in Plate 2 and Fig. 4) increased the formation rate by about 16% of that of preadministration (between points C and D). Effect of the hormone injection on the PTX group (Group VI) was almost

similar to that on the TPTX group (Group VIII), but the percentage of the increase was smaller.

Effect of Calcium in Diet, SHAM, PTX, TPTX, and PTH on Apposition of Incisal Dentin

1) Effect of low calcium diet (endogenous PTH)

The appositional formation rate of the incisal dentin was measured by dropping a perpendicular line from one end point to the next lead line.²⁾ In Plate 1 and Fig. 5 the lengths A-A' and B-B' show the appositional formation rate of the labial dentin for a certain period. Both lengths are the formation rate under the same condition because the end point is the portion where the dentin formation begins. The results of the measurement are shown in Table 3.

The apposition was hardly affected by the substitution of the commercial diet with the normal calcium diet (Group I) and the apposition per three days at each end point showed almost the same rate. However, when the commercial diet was changed to the low calcium diet, the formation rate increased gradually (Group II). Plate 1 and

Table 3. Effect of Calcium in Diet on Apposition of Incisal Dentin in Intact Rats

Days after treatment	Apposition (μ /3 days)	
	Group I (8)	Group II (8)
	Commercial diet	
0-3	32.51 \pm 1.33	29.51 \pm 1.23
	Normal calcium diet Low calcium diet	
0-3	31.87 \pm 1.45	31.75 \pm 1.44
3-6	32.05 \pm 1.36	32.52 \pm 1.38
6-9	33.14 \pm 1.35	33.24 \pm 1.58
9-12	32.21 \pm 1.25	35.31 \pm 1.24

Numbers in parentheses indicate the number of rats sampled.

Data are presented as average \pm standard error.

Figs. 3 and 4 are highly magnified photomicrographs of the area around the point A (coronal side) in Figs. 1 and 2, respectively. These pictures are shown to understand the results in Table 3 more easily. When the apposition rate is measured along the dentinal tubule using the lead lines at the same portion, a similar result to that in Table 3 can be obtained.

2) Effect of PTX or TPTX

The apposition of the incisal dentin was not affected by the sham-operation although the effect of the low calcium diet was observed in the formation (Table 4 and Group IV). However, the apposition was remarkably inhibited by PTX or TPTX, and 12 days later the apposition rate was less than 30% of the preoperative rate. This result brought about a thinning of the dentin (Plate 2 and Figs. 1, 2 and 3). The inhibitory effect of the operation was stronger in the TPTX (Group VII) than in the PTX (Group V) rats. Similar results to those in Table 4 can also be obtained by measuring the apposition rate along the dentinal tubule in Plate 2 and Figs. 2 and 3, which are highly magnified photomicrographs of the area around the end points B and A, re-

spectively, in Plate 2 and Fig. 1.

When the mid-sagittal sections of the incisors in the rats which received PTX or TPTX were examined microscopically, there were observed the characteristic responses of the irregularity of the dentin-predentin junction and thinning of the dentin or thickening of the predentin or dentinoid (Plate 2 and Fig. 2). The lead lines (D, E and F in Plate 2 and Figs. 1 and 2) produced after the operations curved in a wave-like shape and this pattern became more evident as the days progressed (lines E and F in Fig. 2). At the extremely curved portion of the lead lines remarkable histological changes in the odontoblasts as well as extreme inhibition of apposition were observed, and the vascular inclusion into the dentin was frequently observed as has been reported by Schour and Massler⁴⁾ and Bernick.⁵⁾ The irregularity of the dentin-predentin junction and the vascular inclusion into the dentin appeared markedly in the dentin (Plate 2 and Fig. 2) formed by the odontoblasts which had been immature at the time of the operation and by the functionally differentiated cells after the operation (Plate 2 and Fig. 1). However, the above responses hardly appeared in the dentin (Plate 2 and Fig. 3) formed by the odontoblasts which had been mature and in high activity at the time of the operation.

3) Effect of exogenous PTH

The effect of exogenous PTH on the apposition of the incisal dentin was observed on the PTX and TPTX rats by the aforementioned method. The results of the measurement are shown in Table 4.

The apposition which had been inhibited by these operations was obviously restored by the injection of PTH, and six days after the injections the formation rate became more than 150% of the rate before the in-

Table 4. Effect of SHAM, PTX, TPTX and PTH on Apposition of Incisal Dentin in Normal Rats

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	25.53 \pm 1.32	33.08 \pm 1.18	34.62 \pm 1.12	30.24 \pm 1.43	29.21 \pm 1.21
			Low calcium diet		
0-3	26.21 \pm 1.51	34.55 \pm 1.24	35.14 \pm 1.19	32.51 \pm 1.31	30.34 \pm 1.35
	SHAM	PTX	PTX	TPTX	TPTX
0-3	26.92 \pm 1.29	14.59 \pm 1.16	13.25 \pm 1.03	15.03 \pm 1.06	15.52 \pm 0.94
3-6	27.79 \pm 1.37	11.28 \pm 0.81	10.04 \pm 0.78	11.56 \pm 0.91	8.44 \pm 0.51
	saline	saline	PTH (40 USP)	saline	PTH (40 USP)
6-9	29.05 \pm 1.52	10.23 \pm 0.46	24.58 \pm 2.45	7.51 \pm 0.82	22.52 \pm 2.38
9-12	30.21 \pm 1.38	10.18 \pm 0.47	27.02 \pm 2.26	7.25 \pm 0.77	24.59 \pm 2.78

Numbers in parentheses indicate the number of rats sampled.

Data are presented as average \pm standard error.

jections. The effect was much stronger in the appositional than in the longitudinal formation as described already (Table 2). The effect of PTH appeared rapidly after the injection, but the formation rate during the second three days tended to be higher than that during the first three days. The effect of the hormone injection was more evident in the TPTX (Group VIII) than in the PTX (Group VI) rats. Atrophy of the odontoblasts and the irregularity in the dentin-predentin junction which appeared after the operations showed a tendency of restoration by the continuous injections of PTH. Plate 2 and Fig. 5 are the highly magnified photomicrograph of the area close to the end point A in the labial dentin. A result similar to that in Table 4 can also be obtained when the apposition was measured along the dentinal tubule in this area.

DISCUSSION

I. Anabolic action of PTH

There have been literatures on the relationship between the PTH and the incisor growth.^{6,7)} According to these authors growth was inhibited by TPTX, the growth-retardation was rather stronger in the length

than in the width of the incisor, including the jaw, and the effect of PTH on the restoration of the growth from the inhibited state was not clear. This was probably due to the fact that it is difficult to explore the effect of PTH on the growth by the changes of eruption, weight and radiograph of the incisor because the incisor and alveolar bone growth fluctuated among the animals as shown in the tables of the present and preceding papers.

The present results that the hypersecretion of PTH by the low calcium diet increased the appositional and longitudinal formation rate and that the decrease in the rate of these formations due to the PTX or TPTX was clearly restored by the PTH injection lead us to the conclusion that PTH has the action of increasing the tooth growth. In this case there remains the possibility that the inhibition of the incisor growth is brought about by the decrease in body weight. However, it was found in the previous studies that, although starvation inhibited the body weight gain of the rats, the incisor growth was almost normal during one week after the beginning of the experiment.⁸⁾ Also it was found that, in regard

to the effect of hypophysectomy on the growth of the hard tissues, the decrease in the body weight gain by hypophysectomy was stronger than that by PTX or TPTX and that the inhibitory effect of hypophysectomy on the incisor growth was much stronger on the longitudinal than on the appositional formation.⁹⁾ Therefore, it is thought that the results in this experiment are the effect of PTH. The results in this and the preceding paper showed that PTH had an anabolic action on the hard tissues. The reports on the effect of PTH on the mitotic activity in the rat bone marrow¹⁰⁾ and also on the liver regeneration^{11,12)} will support our hypothesis.

The mechanism of the occurrence of the thin dentin after PTX or TPTX is understood as the result of the difference in the degree of inhibition between the appositional and longitudinal formation rate. Since the apposition is closely related with the mineralization of dentin, the effect of PTH on the mineralization was also examined, but this will be brought out separately in the following paper.

II. Relationship between the tissues and their sensitivity to PTH

PTH showed different anabolic actions to the embryologically different kinds of tissues, the most sensitive being the apposition of the tooth (ectodermal- as well as mesodermal origin) or the bone (mesodermal origin). Because the action was clearer than that on the serum calcium level, it will be possible to devise a new method for the biological assay of PTH using the appositional formation as an excellent indicator.

When the incisor was demineralized by a special method using lactic acid (unpublished), the lead lines remained not only in the immature enamel but also in the mature enamel. By using these lead lines, the effect

of PTH on the longitudinal and appositional formation of the incisal enamel (ectodermal origin) was examined, and it was found that the change of the longitudinal formation rate in the incisal enamel was exactly the same as that of the longitudinal formation in the incisal dentin (mesodermal origin) and that the change of the apposition of the incisal enamel was definitely lower than that of the incisal dentin. Thus, it is thought that the sensitivity to PTH of the tissues of mesodermal origin is higher than that of the ectodermal origin.

It is of interest that in the bone and tooth the apposition which is almost not involved in the proliferation of the germ cells was more sensitively affected by PTH than the longitudinal formation which is involved in the proliferation of the germ cells. As to the generating mechanism of the irregularity in the dentin-predentin junction and the vascular inclusion into the dentin, the following conclusion is attained: PTX or TPTX rapidly induces the hypofunction of the odontoblasts and the remarkable changes of the cellular structure with the advance of postoperative days, but the degree of the hypofunction and histological changes of the odontoblasts is not uniform among the cells. The cells recovered from the irregularity of dentin formation by the injection of PTH but not by the injection of calcium gluconate.¹³⁾ This indicates that PTH is highly important for the maturation of the odontoblasts.

III. Effect of thyroid hormone and calcitonin on the action of PTH

It is thought that the difference in the response between the PTX and TPTX groups is the effect of the thyroid hormone. However, as shown in Tables 2 and 4, the effect of thyroid hormone was not so strong, and the effect of PTH could be clearly ob-

served even in the TPTX rats. Therefore, almost all the results in the present study can be ascribed to the effect of PTH.

The difference in the response to PTH between the intact groups (Group II and Tables 1 and 3) and the [TPTX+PTH] groups (Group VIII, Tables 2 and 4) and between the [PTX+PTH] groups (Group VI, Tables 2 and 4) and the [TPTX+PTH] groups may be due to the effect of calcitonin. The effect of this hormone will be reported in detail in a separate paper. (The preliminary results of this study were presented at the Regional Meeting of the International Union of Physiological Sciences, in Sydney, Australia in 1972).

REFERENCES

- 1) Okada, M. and Mimura, T.: Zur Physiologic und pharmakologie der Hartgewebe. 1. Mitteilung: Ein Vitalfärbungsmethode mit Bleisalzen und ihre Anwendung bei den Untersuchungen über die rhythmische Streifenbildung und der harten Zahngewebe. *Jap. J. Med. Sci., IV. Pharmacology*, 11: 166*-170*, 1938.
- 2) Shaw, J. H.: Carious lesions in cotton rat molars. 1. The role of mechanical factors studied by the extraction of antagonistic molars. *J. Dent. Res.*, 26: 47-51, 1947.
- 3) Yonaga, T.: Growth of hair, teeth and bones and the endocrine glands. 1. Growth of hair, teeth and bones and the relationship between them in normal rats (in Japanese, English abstract). *Ochanomizu Med. J.*, 7: 1947-1978, 1959.
- 4) Schour, I. and Massler, M.: The teeth. *In The rat in laboratory investigation*, edited by Farris, E. J. and Griffith, J. Q. Hafner Publishing Co., New York, 1962, pp. 104-165.
- 5) Bernik, S.: Histochemical study of dentin in parathyroidectomized rats. *J. Dent. Res.*, 48: 1251-1257, 1969.
- 6) Ziskin, D. E. *et al.*: Effect on skeletal and dental development of rat thyro-parathyroidectomized at birth. *J. Dent. Res.*, 18: 251-252, 1939.
- 7) Ziskin, D. E. *et al.*: The effect of thyro-parathyroidectomy at birth and at 7 days on dental and skeletal development on rats. *J. Dent. Res.*, 19: 93-102, 1940.
- 8) Okada, M. and Yonaga, T.: The effect of hypophysectomy and sialoadenectomy on the growth of hard tissues and the action of parotin (in Japanese). *Hormon to Rinsho (Clinical Endocrinology)*, 6: 192-200, 1958.
- 9) Yonaga, T.: Quantitative studies of the effect of hypophysectomy on the growth of bone, teeth and hair and the relationship to each other. *Bull. Tokyo Med. Dent. Univ.*, 16: 73-84, 1969.
- 10) Perris, A. D. *et al.*: Parathyroid glands and mitotic stimulation in rat bone marrow after hemorrhage. *Am. J. Physiol.*, 220: 773-778, 1971.
- 11) Rixon, R. H. and Whitfield, J. F.: Parathyroid hormone: A possible initiator of liver regeneration. *Proc. Soc. Exper. Biol., Med.*, 141: 93-97, 1972.
- 12) Rixon, R. H. and Whitfield, J. F.: Parathyroid hormone and liver regeneration. *Proc. Soc. Exper. Biol. Med.*, 146: 926-930, 1974.
- 13) Okada, M. *et al.*: The effect of dietary calcium content on the action of parathyroid hormone on the dentin growth (in Japanese). *Folia Endocrinol. Japon.*, 39: 280, 1963.

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. These lines correspond to those described in the preceding paper, because the rats used in this experiment were exactly the same as those used in the previous work. 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, H=beginning of injections of PTH, P=pulp, LD=labial dentin, Od=odontoblasts.

Plate 1.

Figs. 1-4. Photomicrographs of the mid-sagittal (longitudinal) section of the dentin in the upper incisors.

Fig. 1. The incisor in the rat fed on a normal calcium diet.

Fig. 2. The incisor in the rat fed on a low calcium diet. Marks A, B, C, D, E, F and G are the end points of each lead line in the labial dentin.

Fig. 3. A higher magnification of the area close to the end-point A of the labial dentin in Fig. 1.

Fig. 4. A higher magnification of the area close to the end-point A of labial dentin in Fig. 2.

Fig. 5. Schematic diagram of the labial side in an incisor.

Figs. 1-5. Photomicrographs of the mid-sagittal (longitudinal) section of the dentin in the upper incisors.

Plate 2.

Fig. 1. The incisor in the TPTX rat. Marks A, B, C, D, E and F are the end points of the lead lines.

Fig. 2. A higher magnification of the area close to the end point B of the labial dentin in Fig. 1.

Fig. 3. A higher magnification of the area close to the end point A of the labial dentin in Fig. 1.

Fig. 4. The incisor in the TPTX rat, which received a subcutaneous injection of 40 USP units/100 g of PTH once a day for six consecutive days, on the sixth day after the operation (point D or mark H).

Fig. 5. A higher magnification of the area close to the end point A on the labial dentin in Fig. 4.



