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

BY

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ABSTRACT

The effect of parathyroid hormone (PTH) on mineralization and matrix formation was studied on the incisal depth of immature rats by using a time marker as well as the histochemical method. 1. When the hypersecretion of PTH was caused by a low calcium diet, mineralization and matrix formation were both accelerated. 2. Both mineralization and matrix formation were clearly inhibited by parathyroidectomy or thyro-parathyroidectomy which brought about a widening of the predentin (dентинoid) as a result of the conspicuous inhibition of mineralized matrix formation. The maturation of the matrix seemed to be inhibited also. 3. Demineralization was lower and slower in the labial than in the lingual dentin. 4. The inhibitory effect disappeared totally by the PTH injection, but the restoration of matrix formation was faster compared with that of mineralization. 5. The increase or decrease in mineralization did not necessarily occur in parallel with that in matrix formation. 6. Acid mucopolysaccharide formation in the dentin depended clearly on the quantity of PTH.

INTRODUCTION

In the preceding paper it was reported that parathyroidectomy (PTX) or thyro-parathyroidectomy (TPTX) brings about the widening of the predentin as well as the inhibition of dentin formation. This result may be interpreted that PTH has almost no effect on the matrix formation but has an effect mainly on the mineralized matrix formation. The present paper will be concerned with the results of the author’s experiments that lead us to the definite conclusion about this hypothesis.

MATERIALS AND METHODS

The materials were the same as in the previous studies (Papers I and II) except that the lower incisors were employed for the experiments on mineralization and the upper incisors for those on matrix formation. The direction of sectioning was longitudinal for the upper incisors and transverse for the lower incisors and the thickness was 10 to 15 μm. The effect of 20 USP units as well as 40 USP units of PTH was observed. Haematoxylin staining was used for the measurement of mineralization. In the demineralized section of the hard tissues, the strongly stained portion is believed to be well mineralized and the weakly stained portion to be less mineralized.1,2 For the purpose of observing the effect of PTH on the formation of acid mucopolysaccharides, part of the transverse sections of the lower incisors was stained with toluidine blue O (pH 2.6). It is known that the change of mineralization in the incisal dentin by drugs

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Received for publication, September 16, 1978.
or hormones can be observed more clearly in the lingual dentin than in the labial dentin, so that attention was directed mainly to the effect of PTH on mineralization in the lingual dentin. Mineralized matrix formation rate was measured by using the lead lines as described in the previous paper. A time marker appropriate for the measurement of the matrix formation has not been found. On the other hand, the rate of the dentin (or the matrix) formation varies depending on the age of the odontoblast, the age of the rat, individual animal differences and the location of the incisal dentin (labial, lingual, distal or medial). It is, therefore, difficult to grasp the effect of PTH on the matrix formation as accurately as in the case of the formation of the mineralized matrix. If, however, the function of the odontoblasts of a certain age at the same location in the incisors can be measured and compared with each other, the effect on the matrix formation should be known to the same extent. For this purpose it is important to select an appropriate location for the measurement. Such a location is the end points of the lead line time markers among which the end point (where the dentin formation begins) for the first lead line will be referred to as point A on the longitudinal section of the upper incisors (see Plate III). The reason why this portion was chosen is that the odontoblast at the point A is matured enough for the dentin formation to attain a steady state as described in the previous paper. The measuring method of matrix formation will be stated under the following section.

**RESULTS**

**Effect of Calcium in Diet, SHAM, PTX, TPTX and PTH on Mineralization**

1) **Effect of low calcium diet (endogenous PTH)**

Plate I and Figs. 1 and 2 show the lingual dentin among the transverse sections of the lower incisors. The dentin between lines A and B and outside of line A was formed during the period of administration of a commercial diet (Oriental Yeast Co., Ltd., Japan). The dentin between lines B (or mark NC) and G in the rat in Fig. 1 was formed during the administration of a normal calcium diet (0.51% Ca, a modification of Shaw's diet). The dentin of this rat stained homogeneously with haematoxylin, and no difference in staining was evident between the dentin formed before and after the administration of the normal calcium diet. In eight rats the result was confirmed that the normal calcium diet had almost no effect on mineralization. However, in the rat in Fig. 2, the dentin (between lines B or mark LC and G in Fig. 2) formed after giving a low calcium diet (0.08% Ca, a modification of Shaw's diet) stained darker with haematoxylin than that outside of line B (control dentin). The dentin in the rat fed on the normal calcium diet stained pink homogeneously with toluidine blue O. However, the dentin formed after giving the low calcium diet showed the tendency of staining pink strongly as compared with that formed before giving the low calcium diet or with the dentin in the rat fed the normal calcium diet. No difference in the width of the predentin, however, could be found between the normal and low calcium diet group.

2) **Effect of PTX or TPTX**

Plate I and Fig. 3 show a higher magnification of the incisal dentin on the lingual side in the PTX rat, and Plate 2 and Figs. 1 and 2 that on the labial and lingual sides, respectively, in the TPTX rat. Mark PX or TPX indicates the time of PTX or TPTX. The dentin formed after PTX or
TPTX (between lines C and F in Plate 1 and Fig. 3, and Plate 2 and Figs. I and 2) stained lighter than the control dentin. However, the dentin formed after PTX or TPTX had two kinds of staining responses by haematoxylin on the lingual side. In the same rats the dentin stained very light, resembling the staining response of predentin (Plate 1 and Fig. 3) while in the other rats in the same group the dentin appeared splotchy and the lead lines (D, E and F) formed after the operations changed into distinct broken lines (Plate 2 and Fig. 2). The effect of PTX or TPTX on the staining response was then studied in detail. The characteristics of the rats whose staining resembled that of predentin were that the lead lines (D, E and F), formed by the injections of lead acetate every three days after the operations, were fused into a single wide band, that the dentinal tubules in the wide predentin portion curved gently similar to the shape of the letter S and that in making the sections this portion was easier to break than the control dentin. The characteristics of the rats whose dentin appeared splotchy were that the dentin, formed within three days after the operations, stained darker than the control dentin, although there appeared a clear inhibition of dentin formation and a sudden alteration of the staining response, and that an evident splotchy pattern was shown. The labial dentin also showed different kinds of staining responses. The responses were very weak compared with those of the lingual dentin and appeared gradually after the operations, forming unbroken lead lines (Plate 2 and Fig. 1) during our term of observation (about 12 days). However, in some PTX or TPTX rats dentin which stained darker than in the control dentin was formed during the above period, although the effect of the operations on the dentin formation was of almost the same degree of inhibition among all the rats employed. The results of the staining response with toluidine blue O were almost the same as in the case with haematoxylin.

5) Effect of exogenous PTH

Plate 1 and Fig. 4 show the effect of subcutaneous injections of PTH (40 USP units/100 g/day) for three days (between line E or mark H and line F) on the mineralization of the incisal dentin in the PTX rats, and Plate 2 and Figs. 3 and 4 show the effect of injecting PTH (20 USP units/100 g/day) to the TPTX rats for six days (between line E or mark H and line G). The time of starting the hormone injections was six days (line E or mark H in Plates 1 and 2) after the operations (line C or marks PX and TPX in Plates 1 and 2). The picture of the dentin, strongly stained with haematoxylin, appeared about three days after starting the hormone injections, and the disappearance of the splotchy pattern was also observed at this time point. When the injections were continued further, the dentin returned completely to the normal state (Plate 2 and Figs. 3 and 4). In some rats a marked correspondence between the staining and the time of hormone injection was observed. However, the relation between the amount of hormone injection and the degree of staining was difficult to decide, although the dentin formation was found to change by the amount of hormone injection. Decrease by PTX or TPTX of the substance that stained pink with toluidine blue O was distinctly restored by the PTH injection.

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

Plate 3 and Figs. 1, 2 and 3 show highly magnified pictures of the area around the end point A of the first lead line formed in the labial dentin in the longitudinal section
of the upper incisors, and Fig. 4 is a schematic diagram for Fig. 1.

1) Effect of endogenous PTH and SHAM

Plate 3 and Fig. 1 show the effect of the low calcium diet and SHAM on the dentin (mineralized matrix) as well as on the matrix formation. The rate of dentin formation served as an index for that of matrix formation before SHAM since in the normal dentin the latter is equal to the former. After the operation the rates of dentin and matrix formation were measured respectively. The results of the measurement are shown in Table 1. The preoperative rate (X) of the dentin or matrix formation per day is obtained by measuring the distance (W₁ in Fig. 4) between the lead lines B and C along the dentinal tubule of the odontoblast at point A in Plate 3 and Fig. 1 and then by dividing the above distance by the corresponding period. The postoperative rate (Y) of dentin formation per day is obtained by dividing the distance (W₂) between the lead lines C and G by the corresponding period. Likewise the postoperative rate (Z) of matrix formation is obtained by dividing the distance (W₃) between the lead line C and the pulpal surface of the predentin by the corresponding period.

As is immediately seen from Table 1, in SHAM (Group IV, corresponds closely with that in Papers I and II) the value of Y is larger than that of X and Z is larger than Y. The former relation can be interpreted as the effect of the hypersecretion of PTH due to the administration of the low calcium diet and not as the effect of SHAM. The interpretation of the latter relation is that the matrix as well as the dentin formation is accelerated by the hypersecretion of PTH. For comparison of the degree of acceleration between both formations it is necessary to make some correction on the value of Z, and this corrected value is to be obtained by subtracting the width of the normal predentin from the value of Z in Table 1, since lead acetate was used as a time marker.

Using ten normal rats such measurements were made on the width of the normal predentin formed at the same position of the incisal dentin, and there was obtained a mean value of the width of 22.5 μm, which was equivalent to the apposition of dentin for about 1.16 days. Thus, when the value of Z was recalculated, a value of 14.42 μm/day was obtained, which was almost the same as the value of Y.

Table 1. Effect of PTH on Mineralized Matrix and Matrix Formation in Incisal Dentin

<table>
<thead>
<tr>
<th>Group IV (8)</th>
<th>Group V (8)</th>
<th>Group VI (8)</th>
<th>Group VII (7)</th>
<th>Group VIII (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>13.69±0.64</td>
<td>17.27±0.50</td>
<td>17.50±0.62</td>
<td>16.21±0.42</td>
</tr>
<tr>
<td>SHAM PTX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Saline</td>
<td>+ Saline</td>
<td>PTH (40 USP)</td>
<td>Saline</td>
<td>PTH (40 USP)</td>
</tr>
<tr>
<td>Y</td>
<td>14.50±0.81</td>
<td>0.58±0.42</td>
<td>14.32±0.91</td>
<td>5.89±0.81</td>
</tr>
<tr>
<td>Y/X</td>
<td>1.07</td>
<td>0.38</td>
<td>0.82</td>
<td>0.36</td>
</tr>
<tr>
<td>Matrix formation (μ/day)</td>
<td>10.12±0.69</td>
<td>16.68±1.52</td>
<td>9.45±0.49</td>
<td>15.05±0.75</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of rats sampled.
Data are presented as average±standard error.
Group number corresponds to that in the preceding papers (I and II).
2) Effect of PTX or TPTX

Plate 3 and Fig. 2 and Table 1 show the effect of PTX or TPTX on the dentin and matrix formations. From the table it is seen that the operations definitely inhibit both formations (Groups V and VII). The same correction as in the case of Group IV was made on the value of Z, and the degree of inhibition was compared between the dentin and matrix formations; when the width of the predentin is supposed to be always invariable, the inhibitory effect of the operations on the matrix formation in the labial dentin is evaluated to be about 10.7% lower in Group V and about 11.6% lower in Group VII than the inhibitory effect of the operations on the dentin (mineralized matrix) formations. The predentin-like portion which appeared in the dentin after the operations was wider in the lingual than in the labial dentin as shown in Plate 1 and Fig. 3.

3) Effect of exogenous PTH

In Plate 3 and Fig. 3 is shown the effect of daily injections (40 USP units/100 g/day for six days) of PTH on the dentin and matrix formations, the injection being started six days (line E or mark H) after PTX (line G). The rats were killed about 24 hours after the final lead-acetate injection (line G). The results in Table 1 and Groups VI and VIII were obtained by the same method as that mentioned already.

The dentin and the matrix formation which had been inhibited by PTX or TPTX were confirmed to be restored by the PTH injection. The recovery effect for the mineralized matrix formation, which was confirmed by the staining response to haematoxylin, began to appear about three days after starting the injections, while for the matrix formation it appeared immediately after the first injection.

Discussion

There have been many papers on the effect of PTH on mineralization using the incisal dentin of the rodents. These authors have obtained results that PTX or TPTX inhibits the mineralization of the incisal dentin and that the mineralization is recovered from the inhibition by the injection of PTH or transplantation of the parathyroid glands. Among the investigators, however, there is no agreement as to the time and degree of the appearance of decalcification as well as the recovery by PTH. Such a disagreement is thought to result from the fact that their experiments were carried out without appropriate time markers on different portions of different incisal dentins. In the present experiment, by using lead acetate as a time marker, the difference in the responses between the labial and the lingual dentin was confirmed. This is considered to be due to the difference in the nature of the odontoblasts on both sides.

Past investigators5,83 who studied the effect of the low calcium diet on mineralization have reported that deficient mineralization was observed in the hard tissues. This is considered to be due to the relatively long period of feeding animals on a low calcium diet (more than 20 days). Such a long-term use of a low calcium diet may cause fatigue of the parathyroid glands, leading to the hyposecretion of PTH. If the past investigators had adopted an appropriate period of low calcium feeding along with a proper time marker, they would have also been able to confirm the hypermineralization in the hard tissues which appears in the comparatively early stage of feeding.

By the method which was devised to obtain quantitatively the matrix formation
rate, the generating mechanism of thin dentin or thick predentin appearing after PTX or TPTX was investigated. And it was found that by the operations both the mineralization and the matrix formation are definitely inhibited but the inhibition of the former is extremely. However, the mineral in the matrix formed after the operations was deposited in the area very far from the odontoblasts and that by the PTH injection the normal pattern of mineral deposition was restored, to leading to the maturation of the matrix. The accelerating effect of PTH on the maturation of the matrix was also observed in the alveolar bone in the previous study (Plate 2 and Figs. 3 and 4 in Paper 1).

It is generally believed that the effect of PTH on the mineralization and the growth of teeth is a secondary action resulting from the elevation of the serum calcium level. This current opinion would, however, be difficult to accept in view of the present results in which the increase or decrease of mineralization did not necessarily occur in parallel with that of matrix formation and in which the formation of the acid mucopolysaccharides in the dentin clearly changed depending on the increase or decrease of PTH. The author's conclusion that the anabolic action of PTH on the teeth is the primary action on the matrix formation may also be supported by the fact that the inhibition of the matrix formation and mineralization after PTX or TPTX does not disappear even by the elevation of the serum calcium level due to the calcium gluconate injections.9)

REFERENCES
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 exactly to those described in the preceding papers (Papers I and II). All the sections were stained by haematoxylin. P = pulp, NC = beginning of a normal calcium diet administration, LC = beginning of a low calcium diet administration, TPX = time of TPX, PX = time of PTX, H = beginning of PTH injections, S = beginning of saline injections, SH = sham operation, ES = enamel space, Od = odontoblasts.

Plate 1.

Figs. 1–4. Photomicrographs of the transverse sections of the dentin in the lower incisors.

Fig. 1. Higher magnification of the lingual side of incisal dentin in a rat fed on the low calcium diet.

Fig. 2. Higher magnification of the lingual side of incisal dentin in a rat fed on the low calcium diet.

Fig. 3. Higher magnification of the lingual side of incisal dentin in the PTX rat.

Fig. 4. Higher magnification of the lingual side of incisal dentin in the PTX rat which received subcutaneous injections of 40 USP units/100 g of PTH once a day for three consecutive days (between line E or mark H and line G) from the sixth day after the operation (line E).

Plate 2.

Figs. 1–4. Photomicrographs of the transverse section of the dentin in the lower incisors.

Figs. 1 and 2. Higher magnifications of the labial (Fig. 1) and the lingual side (Fig. 2) of the incisal dentin in the TPX rat.

Fig. 3. Lower magnification of the transverse section of the lower incisor in the TPX rat which received subcutaneous injections of 20 USP units/100 g of PTH once a day for six consecutive days (between line E or mark H and line G) from the sixth day after the operation (line E).

Fig. 4. Higher magnification of the lingual side in Fig. 3.

Plate 3.

Figs. 1–3. Photomicrographs of the dentin which was formed by the odontoblasts of the same age at the labial side in the longitudinal section of the upper incisors.

Fig. 1. Higher magnification of the area around the terminal (end point A) of the first lead line in the labial dentin of the SHAM rat fed on the low calcium diet.

Fig. 2. Higher magnification of the area around the end point A in the labial dentin of the PTX rat.

Fig. 3. Higher magnification of the area around the end point A in the labial dentin of the PTX rat which received the subcutaneous injection of PTH (40 USP units/100 g/day) for six days from the sixth day after the operation.

Fig. 4. Schematic diagram of Fig. 1.
MINERALIZATION AND MATRIX FORMATION

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Plate 3

Enamel Space

Dental tubule

W1

W2

W3

Predentin

Odontoblasts

X = \frac{W_1}{3}

Y = \frac{W_2}{12}

Z = \frac{W_3}{13}