Dentinogenesis in the incisor teeth of adult rats after persisting daily overdosage of 1α-hydroxyvitamin D_3, with special reference to osteodentin formation

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Abstract : The dentinogenesis in the incisor teeth of adult rats that were orally administered with 0.1, 0.5 or 2.5μg/kg body weight (b.w.) /day of 1α-hydroxyvitamin D_3 (1αOHD_3) for 30 days was studied with special reference to osteodentin formation. The decalcified maxillary incisors were examined by light and electron microscopy. Conspicuous osteodentin formation was observed only in the highest dose group. Small defects appeared at some intervals on the pulpal side of dentin in the middle-third of the tooth. The dentinal defects were occupied by many regressive odontoblasts, some of which were embedded in the irregular dentin just above the defects. Moreover, in the middle-third of the pulp, predentinoid tissue was frequently observed in the subodontoblastic area except for the regions where dentinal defects could be seen. Ultrastructurally, predentinoid tissue was composed of dense collagenous matrix containing small round membrane-bound vesicles similar to matrix vesicles. One or two cytoplasmic projections, which were very akin to bonafide odontoblast processes during early dentinogenesis morphologically, were often extended from the proximal aspect of the odontoblasts in contact with the predentinoid matrix.
A mass of osteodentin was observed in the apical-third of the dentin. From these results, we suggest that at least two different processes participate in the formation of osteodentin after the daily overdosage of 1αOHD_3, i. e., the partial regression of odontoblast population and predentinoid formation in the subodontoblastic area.

Key words : dentinogenesis, osteodentin formation, rat incisor, 1α-hydroxyvitamin D_3

Previous studies[1-9] have shown that the overdosage of vitamin D affects the dentinogenesis in the developing teeth. Kubota[8] reported that the large daily dosage of dihydrotachysterol (DHT) interrupted the dentinogenesis in the continuously growing incisor teeth of rats and caused the pathologic changes in the pulp-dentin complex, including osteodentin formation. He also described that amorphous matrix containing collagen fibers was formed in the subodontoblastic area,

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speculating that amorphous matrix was initially produced by the pulp cells and subsequently collagen fibers were by the newly differentiated odontoblasts from the pulp cells. He also suggested that the original odontoblasts were eventually embedded in this dentinoid matrix, concluding that thus osteodentin was formed. However, no evidence for his speculation was demonstrated. More recently, Pitaru et al.[9] showed the osteodentin in rat incisors after daily injection with a toxic dose of 1, 25-dihydroxyvitamin D_3 [1, 25 $(OH)_2 D_3$], but did not describe as to how osteodentin was formed. In addition, little ultrastructural information has been presented to date concerning the dentinogenesis affected by overdosage of vitamin D. In the present study, we have attempted for the first time to use 1α-hydroxyvitamin D_3 (1αOHD_3)[10-14] in expectation of the formation of osteodentin in the incisors of rats given large daily doses of this synthetic vitamin D_3 analog.

Materials and Methods

Experimental Animals

Ten-week-old male CRJ : SD rats, weighing 320-350g, were divided into four groups with five in each. One of the groups was untreated, while the remainder were treated experimentally. All animals were housed in plastic cages individually at room temperature of 22 ± 1 °C, maintained on a standard laboratory diet(ORIENTAL YEAST CO., LTD., Japan) containing 800-units of vitamin D_3/kg, and given water *ad lib.* throughout the laboratory work.

Administration of Vitamin D_3 Analog

As the synthetic analog of vitamin D_3, ALFAROL soln® (CHUGAI PHARMACEUTICAL CO., LTD., Japan) was used for this study. The drug was prepared at a concentration of 0.5μg of 1αOHD_3/ml. The animals in three experimental groups were orally administered with 1α OHD_3 for 30 days at dose levels of 0.1, 0.5, and 2.5μg/kg body weight (b.w.) /day, respectively. The analog was given by glass microsyringe with an attached stomach tube to insure the volume of administration.

Histological and Ultrastructural Examinations

The animals were anesthetized by ether inhalation, and their blood was collected by intracardiac puncture for serum calcium analysis on the next day after the termination of the experimental period. They were then perfused through the left ventricle with a modified Karnovsky's fixative containing 2.0% paraformaldehyde and 1.25% glutaraldehyde in 0.2M cacodylate buffer (pH 7.2) for about 20 min at room temperature. After perfusion, the maxillary incisors were carefully dissected out from each animal and additionally immersed overnight in the same fixative at 4 °C. The teeth from the rightside jaws were decalcified with 10% EDTA at 37°C for about a month, washed in running water, dehydrated through graded ethanols, and embedded in "acrytron E" (resin embedding medium, MITSUBISHI RAYON CO., LTD., Japan). Series of 3.0 μm thin sections were cut in a sagittal plane and stained with hematoxylin-eosin. Only the sections containing the entire pulp from the basal to apical end were used for the light microscopic examination. Teeth from the left-

side were decalcified by suspending them in 2.5% EDTA (pH 7.4) containing 0.2M sucrose at 4°C for about two months[15], divided into 10 serial segments transversely to their long axis, and washed in 0.2 M cacodylate buffer. The tissue blocks were then postfixed with 1% OsO_4 in the same buffer for 1.0 h and embedded in Epon 812 after dehydration with graded ethanols. Semi-thin cross sections were cut on an LKB Ultrotome using glass knives and stained with toluidine blue for light microscopy. After optical observation, the specimens were trimmed and cut into ultrathin sections on the same ultramicrotome equipped with glass or diamond knives. The sections were then mounted on formvar-covered grids, double-stained with uranyl acetate and lead citrate, and examined in a JEM-100B electron microscope.

Results

The pulp-dentin complex of the maxillary incisors in the highest dose group (2.5 μg/kg b.w./day of 1αOHD$_3$) revealed various histological abnormalities. However, in the lower dose groups (0.1 and 0.5 μg/kg b.w./day of 1αOHD$_3$) it was found to be normal and indistinguishable from that of the untreated animals histologically. Therefore, the results from the highest dose group will be represented and explained in detail.

Light Microscopy

In the following, findings on the labial-side of the pulp-dentin complex were used (Figs. 1-6), since no major difference was observed between the labial and palatal aspect of the sagittal sections of the incisor.

The pulp-dentin complex of the teeth of the untreated animals showed normal appearances (Fig. 1). On the contrary, that of the animals treated with 1αOHD$_3$ presented severely disturbed features (Figs. 2-6). A small dentinal defect in the pulpal aspect emerged first at the point of the basal third of the dentin and was accompanied with slightly atrophic odontoblasts and moderately irregular dentin (Fig. 2a). The life cycle of most odontoblasts in this region was already in the stage of "old odontoblasts"[16], because these cells attained a maximum length and small blood vessels had penetrated deep into the odontoblastic layer. It is known that in the rat incisor, this stage is continuous apically except for the necrotic position at the incisal tip[17]. The width of predentin layer in the defective portion was a little narrower than that of the unaffected area.

In the region of the middle-third of the dentin, the dentinal defects appeared at some intervals, increasing in depth and width (Fig. 2b). The defective portions were occupied by many atrophic odontoblasts in disordered arrangement, some of which lost their cell polarity (Fig. 3). No predentin was observed on the distal side of these cells, and the predentin layer observed between the dentinal defects was apparently narrower than that observed in the middle-third of the normal pulp-dentin complex (Figs. 1 and 2b). The odontoblasts which lost their polarity were about to be embedded in the dentin matrix (Fig. 3), and some inclusions of pyknotic odontoblasts could be seen in the irregular dentin matrix just above the dentinal defects (Figs. 2b and

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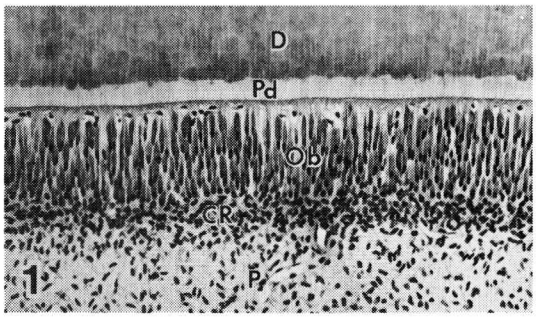
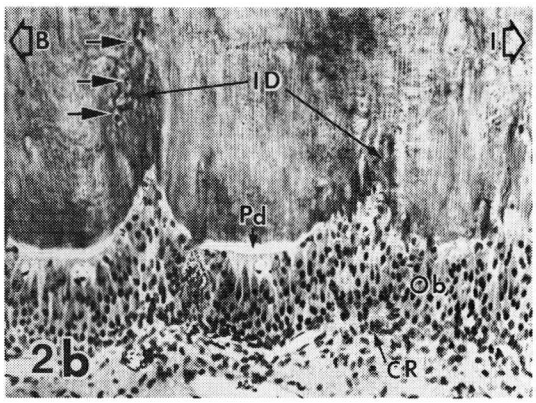
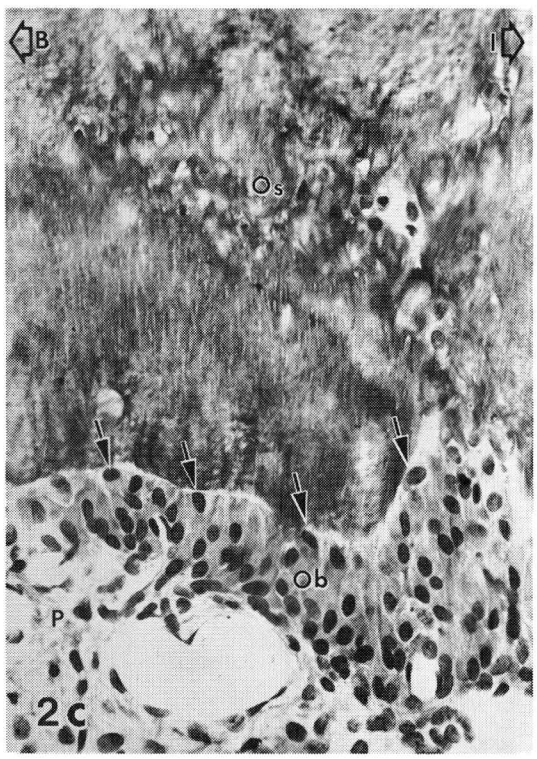
3）. Additionally, small blood vessels were frequently found penetrated into these defects (Fig. 3). These derangements were progressively accentuated







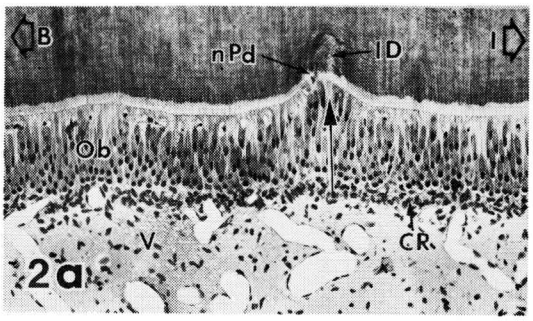


Fig. 1. Pulp-dentin complex in the middle-thirds of the maxillary incisor of the untreated rat showing normal appearance. D, dentin; Pd, predentin; Ob, odontoblasts; CR, cell-rich zone; P, pulp. ×165

Fig. 2. Demonstrations of the pulp-dentin complex of the maxillary incisor of the rat administrated. 2.5μg/kg b.w./day of 1αOHD$_3$ for 30 days. [a] Region at the basal third of the tooth showing the initial dentinal defect (arrow) accompanied with slightly atrophic odontoblasts and moderately irregular dentin (ID). Pulp tissue contains many vacuoles (V) in the subodontoblastic area with fewer cells than the normal pulp (Fig. 1) and a cell-rich zone (CR) is also scarce of cells. Note somewhat narrower predentin (npd) than that of basal (B) and incisal (I) to it. Ob, odontoblasts. ×200 [b] The middle-third of the tooth showing the appearance of dentinal defects with some intervals. The defects were wider and deeper than that of Fig. 2a. No predentin was observed on the distal side of the atrophic odontoblasts in the defects. Note the inclusion of pyknotic cells (arrows) in the irregular dentin (ID) just above the defect. The width of predentin layer (Pd) is narrower than that of the untreated tooth (Fig. 1). A cell-rich zone (CR) is becoming more scanty than that of Fig. 2a. Ob, odontoblasts; B, basally; I, incisally. ×200 [c] The apical-thirds of the pulp-dentin complex showing the embedding of many odontoblasts, i. e., osteodentin (Os). Odontoblasts (Ob) are atrophic in disordered arrangement. Note the cells (arrows) losing their polarity. Predentin layer and a cell-rich zone are unrecognizable. P, pulp; B, basally; I, incisally. ×620

toward the apical direction. In the apical-third of the dentin, the dentin with many inclusions of pyknotic odontoblasts, i.e., osteodentin was observed (Fig. 2c).

In the basal region of the apical-half of the pulp-dentin complex, the odontoblasts between the dentinal defects were also very atrophic separating from each other and some of them were about to be left in the dentin matrix losing their

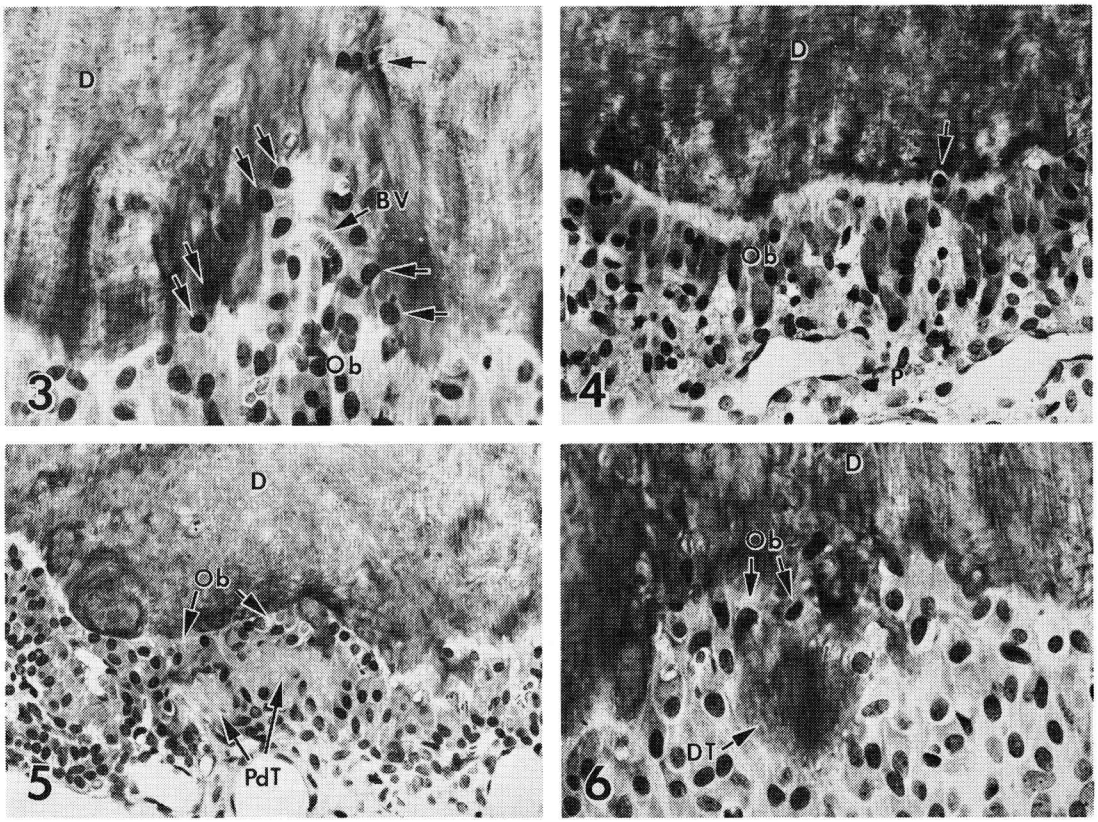


Fig. 3. Atrophic odontoblasts occuping the dentinal defect. The cells losing their polarity (arrows) are about to be left or embedded in the dentin matrix. Blood vessel (BV) penetrated into the defect can be seen. Ob, odontoblasts; D, dentin. ×650

Fig. 4. Atrophic odontoblasts (Ob) between the dentinal defects. These cells are uneven in length and separating from each other. Note the cell (arrow) that is about to be left in the dentin matrix. Pulp tissue (P) demonstrates the reticular atrophy in the subodontoblastic area. A cell-rich zone is unobservable. D, dentin. ×480

Fig. 5. Predentinoid tissue (PdT) formed in the subodontoblastic area. The atrophic odontoblasts (Ob) are sandwiched between dentin (D) and this fibrous predentinoid tissue. Many pulp cells are gathering on the pulpal side of this tissue. These cells are interpreted to be fibroblasts or undifferentiated mesenchymal cells by their pale and ovoidal nucleus with one or two distinct nucleoli in a poor cytoplasm. ×400

Fig. 6. Dentinoid tissue (DT) in the subodontoblastic area. This region is filled with atrophic odontoblasts and many small cells. This tissue seemed to be calcified from its hematoxylin affinity. Some of the atrophic odontoblasts (Ob) are sandwiched between dentin (D) and this tissue. The tubular structures similar to the dentinal tubules can be seen in the dentinoid tissue. ×720

polarity (Fig. 4). Partial dentinal defects were no longer observed in the incisal region of the apical-half.

The dentinal tubules in the irregularly formed dentin were twisted without normal parallel arrangement, or sometimes they were unrecognizable (Figs. 2a, 2b, 2c, and 3).

On the other hand, an accumulation of predentinoid tissue was found just beneath the odontoblastic layer at various places in the middle-third of the pulp except for the dentinal defective regions (Fig. 5). This tissue was formed in contact with the pulpal aspect of the odontoblasts and stained well with eosin, showing fibrous structure at the light microscopic level. It could also be demonstrated that many pulp cells assembled at the pulpal side of the large mass of the predentinoid tissue (Fig. 5), though no gathering of such cells was observed just beneath the small mass of this tissue. These cells were interpreted to be fibroblasts or undifferentiated mesenchymal cells, since they possessed a pale and ovoidal nucleus with one or two distinct nucleoli and poor cytoplasm. Furthermore, masses of dentinoid tissue were observed just beneath the very atrohpic odontoblasts in the apical region of the middle-third of the pulp and frequently covered with many small cells (Fig. 6). The dentinoid tissue was densely stained with hematoxylin or toluidine blue as well as dentin, giving a suggestion of calcified tissue. The atrophic odontoblasts were sandwiched between the dentin and this dentinoid tissue, which often included tubular structures similar to those of the dentinal tubules in the normal dentin.

As in reticular atrophy, the pulp tissue contained fewer cells than the normal pulp and numerous spaces or vacuoles especially in the subodontoblastic area (Figs. 1, 2a, and 4). The cell density of a "cell-rich zone" was also lower than that of the normal pulp and became more scarce toward the apical direction (Figs. 1, 2a, 2b, anb 4). In addition, such

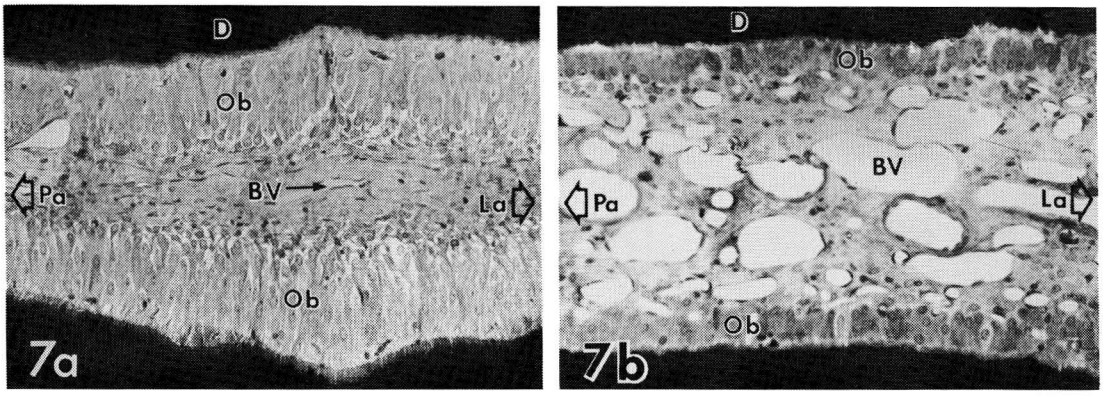


Fig. 7. Cross-sections at the apical third of the maxillary incisors of rats. [a] The pulp of the untreated animal. The odontoblasts (Ob) are tall columnar in a maximum length of about 40μm, belonging to "old odontoblasts". [b] The pulp of the 1αOHD$_3$ treated animal. An increase in vascular supply with dilatation is notable. The odontoblasts (Ob) are extremely shorter in length than that of the untreated tooth (Fig. 7a). BV, blood vessel; D, dentin; La, labially; Pa, Palatally. ×320

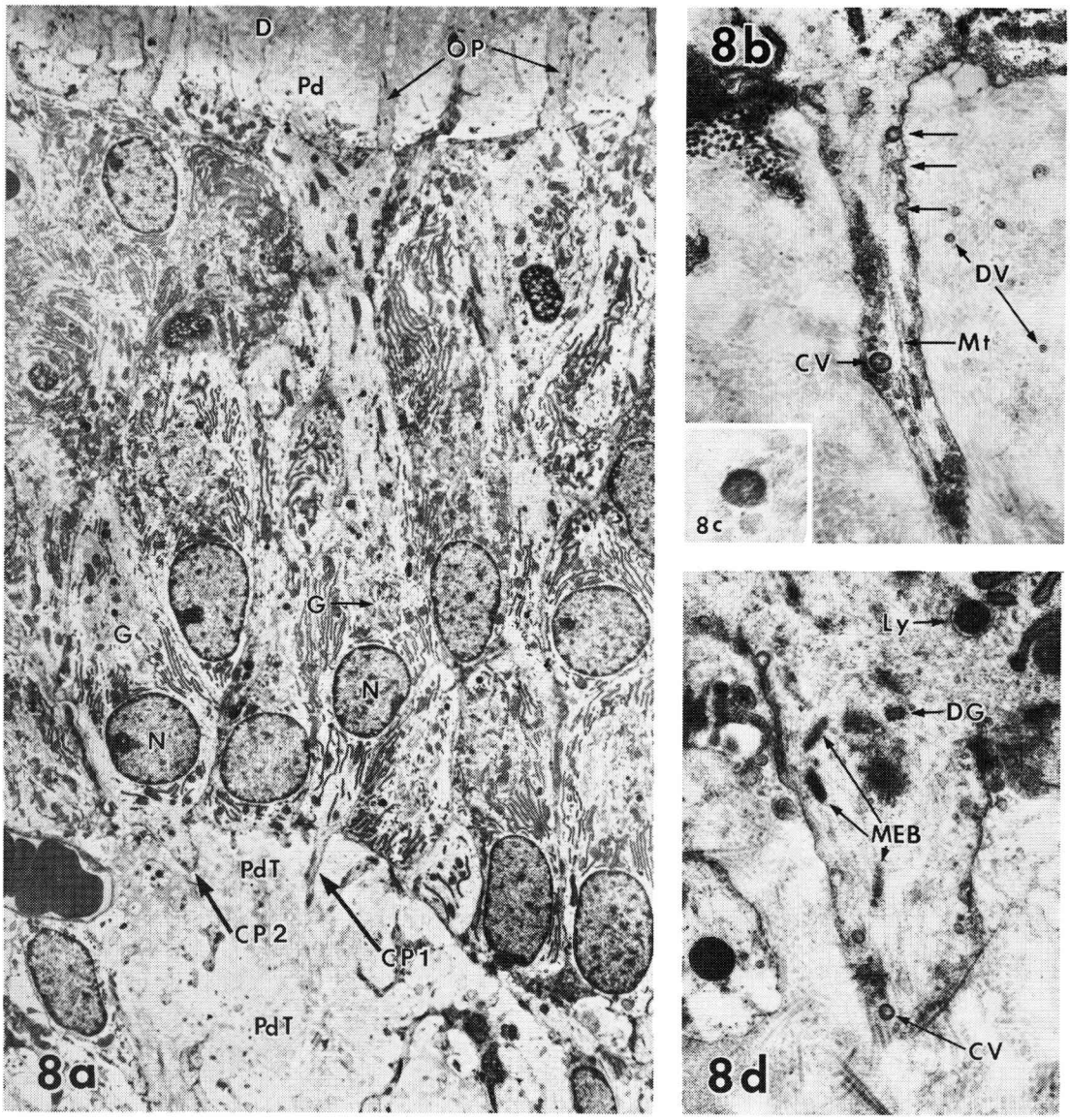


Fig. 8. Ultrastrastructure of the odontoblasts accompanied with small masse of predentinoid tissue on the pulpal side, and their enlargements. [a] The short cytoplasmic projections (CP 1 and CP 2) extending from the proximal aspect of the odontoblasts into the predentinoid tissue (PdT) containing dense collagen fibers. The localities of nucleus (N) and Golgi area (G) in such odontoblasts are normal. Both granular endoplasmic reticula and mitochondria can be seen in the proximal zone of these nuclei (N). D, dentin; Pd, predentin; OP, odontoblast process. ×2,000 [b] A higher magnification of CP 1 in Fig. 8a. The projection contains microfilaments, microtubules (Mt), and coated vesicle (CV). Pinocytotic invaginations (arrow) can be seen. The projection is surrounded with abundant collagen fibers. Note the small round dense vesicles (DV). ×11,700 [c] A higher magnification of the dense vesicle in Fig. 8b. The vesicle is membrane-bound. ×54,000 [d] A higher magnification of CP 2 in Fig. 8a. The projection contains membrane-limited elongated bodies (MEB), dense granule (DG), coated vesicles (CV), and lysosomal body (Ly). The typical banding pattern of collagen fiber is observed. ×11,700

pulp showed a striking increase in vascular supply, and moreover, the blood vessels were expanded (Fig. 7 b). Although the perfusion must have forced the vessels open, the vascular dilatation was evident when compared with those in the normal pulp (Fig. 7 a).

In spite of these pathologic changes in the apical two-thirds of the pulp-dentin complex, little alteration was observed in the basal-third.

Electron Microscopy

The odontoblasts accompanied with a small mass of predentinoid tissue in the adjacent subodontoblastic area often extended one or rarely two cytoplasmic projections from the proximal aspect into this tissue (Figs. 8 a and 9 a). The projections were devoid of major cell organelles but exhibited many microfilaments and microtubules running along the process. They also contained membrane-limited elongated bodies and dense granules similar to the secretory granules of odontoblasts[18-20], lysosomal large round membrane-bound bodies, and coated vesicles which were close to the cell membrane and on occasion fused with it, cre-

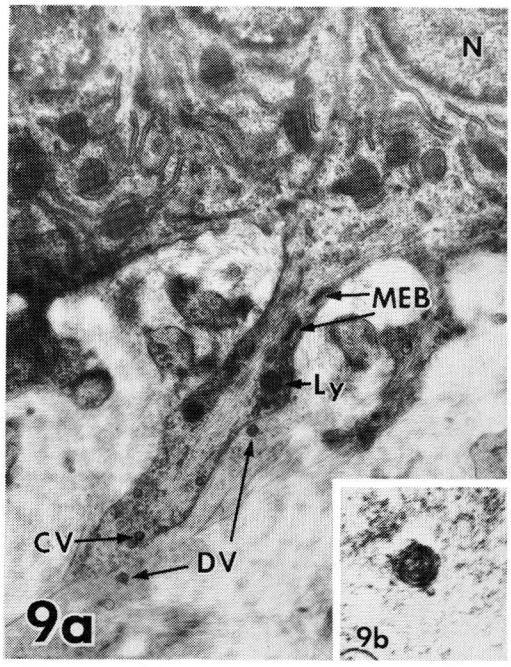


Fig. 9. [a] An odontoblast extending two proximal cytoplasmic projections into the predentinoid tissue composed of collagenous matrix containing small round dense vesicles (DV). N, nucleus; MEB, membrane-limited elongated bodies; Ly, lysosomal bodies; CV, coated vesicles. ×6,700 [b] A higher magnification of the dense vesicle shown in Fig. 9 a. The vesicle is membrane-bound. ×47,000

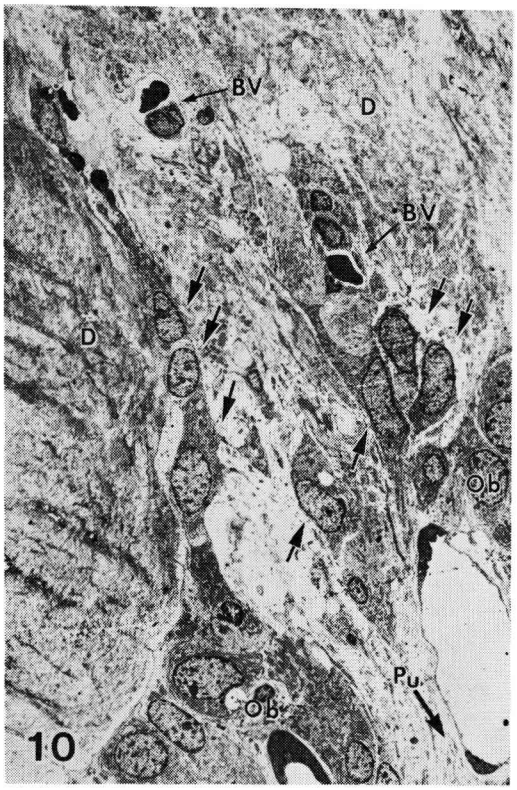


Fig. 10. Ultrastructure of the dentinal defect occupied by atrophic odontoblasts. The odontoblasts (arrows) are about to be left and embedded in the dentin matrix with separating from each other. Small blood vessels (BV) are also penetrated into the defect and embedded in the dentin matrix. Erythrocyte can be seen in these vessels. D, dentin; Ob, odontoblasts; Pu, pulpally. ×1,300

ating pinocytotic invaginations (Figs. 8b, 8d, and 9a). Ultrastructural feature of these cytoplasmic projections was very akin to the odontoblast process in early dentinogenesis[16, 21]. The predentinoid tissue was composed of collagenous matrix containing small round membrane-bound vesicles (Figs. 8a, 8b, 9a, and 9b), which were often found near the projections and whose configuration was about the same as decalcified extracellular matrix vesicles[22-26]. Longitudinal profiles of collagen fibers in this matrix displayed the typical banding patterns of native collagen (Fig. 8d). The locality of the nucleous and Golgi area in the odontoblasts that protruded the proximal cytoplasmic projection was not different from that of the normal odontoblasts, i. e., the nucleus was located on the proximal side of cell body and the Golgi area on the distal side of nucleus. In the proximal area of nucleus in such cells, there was a small number of granular endoplasmic reticulum and mitochondria.

On the other hand, the odontoblasts

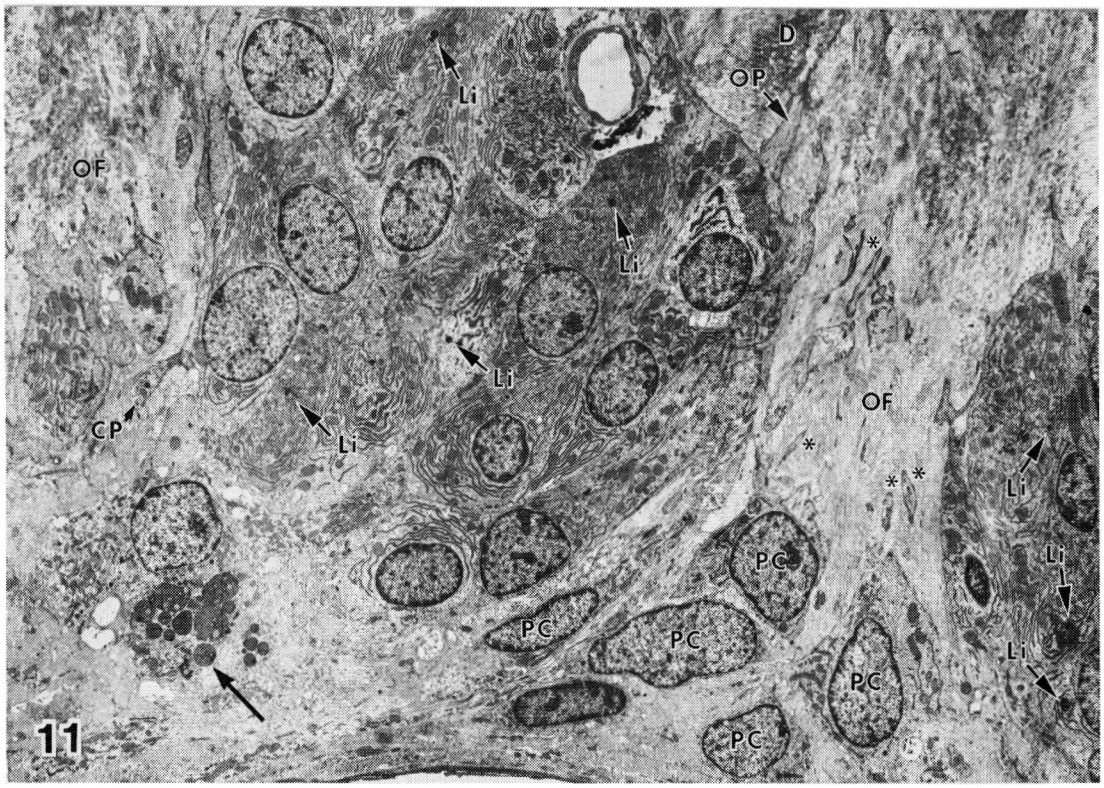


Fig. 11. The odontoblasts constructing cell clusters observed in the apical-thirds of the incisor of 1 αOHD$_3$-treated animal. Odontoblast-free zones (OF) are noted between the cell masses. There can be seen substantial cytoplasmic fragments and cytoplasmic extentions (*) in the collagenous ground substance in this zone. Note the pulp cells (PC) gathering at the bottom of this free zone. A degenerative pulp cell with many lipids (arrow) is also seen in the subodontoblastic area. Most of the odontoblasts in the cluster contain small lipid droplets (Li). A cytoplasmic projection (CP) extended from the proximal aspect of odontoblast can be seen. D, dentin; OP, odontoblast process. ×1,840

packed in the dentinal defects were atrophic with decreasing cell size and organelles and incorporated into the dentin matrix, separating from each other (Fig. 10). Small blood vessels were found penetrated into the defects. These findings are consistent with those of the light microscopy (Fig. 3). Some of the vessels were enclosed within the dentin matrix, but circulation must have still continued for a while after the embedding, since erythrocytes were frequently observed in them. Both necrotic odontoblasts and vessels were also seen in the irregular dentin above the dentinal defects.

In the region of the apical-third of the pulp, the odontoblasts, which were deprived of the characteristics of "old odontoblasts"[16] and containing lipid droplets, constructed cell clusters, and between them there was a "odontoblast-free zone" (Fig. 11). This zone consisted of an extracellular compartment of ground substance, including dispersed substantial cytoplasmic fragments, broken cytoplasmic extentions, and collagen fibers. Several pulp cells were found gathered together at the bottom of the free zone. The cytoplasmic fragments must be of these cells, because no other cells could be seen just above this zone. In addition, a lot of degenerative pulp cells with many lipids were also observed in the subodontoblastic area in this region.

Discussion

The present study clearly demonstrated that the daily overdosage of 1αOHD$_3$ affects the dentinogenesis and results in the formation of osteodentin in the incisors of adult rats, though this effect is dose-dependent. The narrowing or lack of predentin suggests that the odontoblasts were interrupted their dentinogenetic activities. This can also be speculated from the results that the most severely affected odontoblasts in the dentinal defects showed atrophy with decreasing cell size and amount of organelles and losing their polarity. On the basis of cytological characteristics of odontoblasts classified by Takuma and Nagai[16], these cells were apparently different from old odontoblasts and considered to be in regressive conditions. Such odontoblasts must become unable to recede toward the pulpal side from their original positions because of functional lesions and be consequently embedded in the dentin matrix formed by neighboring active odontoblasts. This inference is strongly supported by the findings that most of the embedded cells were observed in the irregular dentin matrix just above the atrophic odontoblasts in the dentinal defects and fewer or no dentinal tubule was seen in such dentin matrix. Lack of dentinal tubules suggests that the odontoblasts could make no recession, since their configuration indicates the course taken by the odontoblasts during dentinogenesis. It is known that the incisal tip of the fetal or young rat incisors is composed of a mass of osteodentin in the prefunctional stage[27]. Takuma et al.[16,28] indicated that this osteodentin is formed as a result of lowered dentinogenetic activities of the "short odontoblasts" that have changed from "old odontoblasts" in the apical region. Although no short odontoblasts can be seen after the eruption because of the attrition of incisal tip[17], the procedure of osteo-

dentin formation in the present study is similar to that described by Takuma et al. in a certain aspect. The vascular inclusion in the dentin matrix is interpreted as follows : the blood vessels penetrated deep into the odontoblastic layer were pinched off by the irregularities of this cell layer and eventually embedded in the dentin matrix.

On the other hand, it can also be suggested that the formation of predentinoid tissue participates in the production of osteodentin as described by Kubota[8]. It is reasonable to assume that only the "functional fibroblasts"[29] and odontoblasts are able to produce collagenous matrix in the tooth pulp. In the present investigation, no functional fibroblast-like cell was recognized in the small mass of predentinoid tissue, but on the proximal side of some odontoblasts we often found very similar circumstances to those of the early dentinogenesis. Although no reversal of the cell polarity was observed in such odontoblasts, it seems likely that these cells initiate the secretion of predentinoid matrix from the proximal side toward the subodontoblastic area. In addition, the accumulation of pulp cells at the pulpal aspect of the large mass of predentinoid tissue suggests that these cells might have differentiated into new odontoblasts and taken part in forming this tissue after the initial production by odontoblasts described above. This suggestion is substantiated by the finding that the mass of dentinoid tissue often included tubular structures akin to dentinal tubules. Since the length of cytoplasmic projections extended from the proximal aspect of the odontoblasts was limited and such cells could not recede toward the dentinal side, the tubular structures might have been formed by the cytoplasmic processes of newly differentiated odontoblasts. The appearance of "odontoblast-free zone" might be a result of the embedding of odontoblast population. The pulp cells at the bottom of this zone might have gathered in order to compensate the gap with producing collagenous matrix.

The effect of the overdosage of 1αOH-D_3 on the odontoblasts is considered to be preferential, because (1) little change was observed in the region of the basal-third of the pulp-dentin complex, (2) the groups of regressive odontoblasts appeared at some intervals, and (3) the odontoblasts between these groups were not so affected in the basal part of the middle-third of the pulp. As the initial atrophic odontoblasts emerged at some distance incisally from the transitional region where "young odontoblasts" differentiated into "old odontoblasts"[16], and at the same time the regressive odontoblasts increased in number toward the apical direction, it can be suggested that odontoblasts are affected during the stage of old odontoblasts. It is known that the old odontoblasts frequently demonstrate partial cytological reorganizations as a certain metabolic changes[30]. The old odontoblasts may possibly be inclined to be affected by the overdosage of 1αOHD_3 under a particular cytological condition in their cell cycle, though we have no evidence of the preferential effect at present. As immature or young odontoblasts are better able to resist various disturbances than the mature or older cells[31],

the odontoblasts in the basal-third of the incisor might not be affected. Becks et al.[4] also reported that Hertwig's epithelial sheath of the developing molars of young dogs was very resistent to the toxic effects of hypervitaminosis D_3. Their result coincide with ours.

In the present investigation, the dentinoid tissue seemed to be calcified and must have been formed by the mineral deposition in the predentinoid tissue. It has been known that the overdosage of vitamin D results in hypercalcemia and occasionally causes ectopic calcification in the soft tissue[32,33]. The classic studies [2,4-6] reported that large dosage of vitamin D induced amorphous pathologic calcification or denticle formation in the tooth pulp. In our study, serum calcium concentration of the animals in the highest dose group was significantly raised (11.74 ± 0.46mg/dl, mean$\pm$SD, $P<0.01$) compared with that of the untreated animals (9.32 ± 0.47)[34]. Therefore, it is very probable that mineral deposition occurred in the predentinoid tissue.

In addition, the small round membrane-bound vesicles found near the proximal cytoplasmic projections might possibly initiate the mineralization in the predentinoid tissue, because they were about the same as so-called extracellular matrix vesicles[22-26]. We speculate that the source of these vesicles might be odontoblasts which protruded the projections, for no odontoblast-like cell was observed at the pulpal aspect of the initially formed predentinoid matrix.

On the other hand, we also found reticular atrophy of the pulp accompanied with the decrease in pulp cells and increase in vascular supply with dilatation. The decrease in pulp cells was reported by Kubota[8] and Ratcliff & Itokazu[35] in rat incisors and molars respectively, and the increase in vascularity by Ratcliff & Itokazu[35] in rat molars, after a daily administration of overdose of DHT. However, they described few explanations of these findings. The dilatation of blood vessels must have induced effusion. It is evident that the pulp tissue is damaged when effusion occurrs, since this tissue is enclosed within the calcified walls of dentin and consequently interfered with swelling. Therefore, it is probable that the reticular atrophy in the pulp was a result of the vascular lesion. As the cell density of a cell-rich zone became more scarce toward the incisal direction and at the same time many vacuoles were observed especially in the subodontoblastic area, it seems likely that these changes relate to the regression of odontoblasts. However, we have no firm explanation for the increase in vascularity with dilatation induced by the overdosage of 1α OHD_3 at present.

Recently, Pitaru et al.[9] also demonstrated that besides the osteodentin formation there was a decrease in pulp cells in rat incisors after daily injection with large dose of $1,25\ (OH)_2\ D_3$, suggesting that these changes were a result of direct hormonal activity on odontoblasts and fibroblasts. On the contrary, Sjögren et al.[36] showed by the use of autoradiographic technique that no uptake of ^{14}C-vitamin D_3 was noted in the developing teeth of rats. If hypercalcemia was the only factor responsible for the present findings, it would be expected that the entire

pulp-dentin complex should have been influenced. Our opinion is consistent with that of Kubota[8] and Pitaru et al.[9] in this respect. However, whether 1αOH D_3 has a direct effect on odontoblasts and pulp cells can not be decided just by the morphological results alone.

Finally, we conclude that the daily overdosage of 1αOHD_3 affects the dentinogenesis in the incisors of adult rat and causes the embedding of odontoblasts in the dentin matrix, which consequently results in the formation of osteodentin in the apical-third of the dentin. Osteodentin may be formed at least through two different procedures, i.e., the partial regression of odontoblast population and predentinoid tissue formation in the subodontoblastic area initially by odontoblasts and consequently by pulp cells.

和文抄録：雄ラット（体重320—350g）20匹を4群に分け，1群は対照群とし，実験群に 0.1，0.5，2.5 μg/kg の 1α-OH-D_3 を30日間連続経口投与を行った。上顎切歯を材料として，ビタミンD_3の象牙質形成に及ぼす影響，特にOsteodentin形成を光顕ならびに電顕により観察し，次に示す結果を得た。

1．ビタミンD_3低投与群では変化はみられず，高投与群で著明な形態的変化が認められた。

2．象牙質と象牙芽細胞の配列の乱れは最初に基底側⅓にみられ，切端に向って周期的に生じる。

3．象牙質と象牙芽細胞の配列が乱れる部位では，象牙前質が消失し，退行性の象牙芽細胞が多数出現してその一部は不規則な象牙質中に埋入されていく。

4．象牙芽細胞の配列が乱れる部位では象牙芽細胞直下の歯髄側に前象牙質様の構造物が出現し，この構造物はコラーゲン基質から成っている。

5．上記の前象牙質様構造物に接する象牙芽細胞の近位端には象牙芽細胞の突起が出現し，基質小胞様の構造物が出現してくる。

6．上記の結果からビタミンD_3の過剰投与により，周期的に象牙芽細胞に退行性変化が生じ象牙芽細胞は自ら分泌した基質中に埋入して Osteodentin を形成していく可能性が示唆される。

References

1) Shour I., Ham, A. W. : Action of vitamin D and of the parathyroid hormone on the calcium metabolism as interpreted by studying the effect of single dose on the calcification of dentin. *Arch. Path.* 17 : 22-39, 1934.

2) Becks, H. : Dangerous effects of vitamin D overdosage on dental and paradental structure. *JADA* 29 : 1947-1968, 1942.

3) Ziskin, D. E., Gibson, J. A., Skara, A. and Bellows, J. W. : Effects of large daily doses of vitamin D on teeth and jaws of rats and on humans. *J. dent. Res.* 22 : 457-468, 1943.

4) Becks, H., Collins, D. A. and Axelrod, H. E. : The effects of a single massive dose of vitamin D_2 (D-stoss therapy) on oral and other tissue of young dogs. *Am. J. Ortho. oral. Surge.* 32 : 452-462, 1946.

5) Becks, H., Collins, D. A. and Freytag, R.M. : Changes in oral structures of the dog persisting after chronic overdoses of vitamin D. *Am. J. Ortho. oral. Surge.* 32 : 463-471, 1946.

6) Hendricks, J. B., Morgan, A. F. and Freytag, R. M. : Chronic moderate hypervitaminosis D in young dogs. *Am. J. Physiol.* 149 : 319-332, 1947.

7) Irving, J. T., Weinmann, J. P., Schour, I. and Tweedy, W. R : Experimental studies in calcification VIII. The effects of large doses of calciferol on the dentin of the incisor in mormal and nephrectomized rats. *J. dent. Res.* 28 : 362-368, 1949.

8) Kubota, Y. : Disturbed formation of dental tissue by the overdose of dihydrotachysterol. *Odontology* (Shigaku, Japan). 60 : 401-434, 1972.

9) Pitaru, S., Blaushild, N., Noff, D., and Edelstein, S. : The effect of toxic doses of 1,25-dihydroxycholecalciferol on dental tissue in the rat. *Arch. oral. Biol.* 27 : 915-923, 1982.

10) Holick, M. F., Semmler. E. J., Schnoes,

H. K. and DeLuca, H. F. : 1α-hydroxy derivative of vitamin D_3 : A high potent analog of 1α,25-dihydroxyvitamin D_3. *Science*. 180 : 190-191, 1973.

11) Haussler, M. R., Zerwekh, J. E., Hesse, R. H., Rizzardo, E. and Pechet, M. M. : Biological activity of 1α-hydroxycholecalciferol. A synthetic analog of the hormonal form of vitaminD_3. *Proc. Nat. Acad. Sci.* USA. 70 : 2248-2252, 1973.

12) Kaneko, C., Yamada, S., Sugimoto, A., Eguchi, Y., Ishikawa, M., Suda, T., Suzuki, M., Kakuta, S., and Sasaki, S. : Synthesis and biological activity of 1α-hydroxyvitamin D_3. *Steroids*. 23 : 75-92, 1974.

13) Zerwekh, J. E., Brumbaugh, P. F., Haussler, D. H., Cork, D. J. and Haussler, M. R. : 1α-hydroxyvitamin D_3. An analog of vitamin D which apparantly acts by metabolism to 1α,25-dihydroxyvitamin D_3. *Biochem*. 13 : 4097-4102, 1974.

14) Kawashima, H., Hoshina, K., Okamiya, Y., Makita, T., Hashimoto, Y., Noguchi, T. and Orimo, H. : Biological activity of 1α-hydroxycholecalciferol (II) . Effect on the metabolism of calcium and phosphorous in rats. *Folia pharmacol. japon*. 74 : 361-382, 1978.

15) Kurahashi, Y. : Perfusion fixation with glutaraldehyde and its application for cytochemistry of developing teeth. *J. electron. Microsc*. 14 : 337-338, 1965.

16) Takuma, S. and Nagai, N. : Ultrastructure of rat odontoblasts in various stages of their development and maturation. *Arch. oral.* Biol. 16 : 993-1011, 1971.

17) Tanaka, S. : Microstructure of anterior extremity of the rat incisor. *Shikwa Gakuho* (The Journal of the Tokyo Dental College Society). 74 : 1651-1676, 1974.

18) Weinstock, M. : Collagen formation-observations on its intracellular packaging and transport. *Z. Zellforsch*. 129 : 455-470, 1972.

19) Frank, R. M : Etude autoradiographique de la dentinogenèse en microscopie électronique à l' aide de la proline tritiée chez le chat. *Arch. oral. Biol*. 15 : 583-596, 1970.

20) Reith, E. J. : Collagen formation in developing molar teeth of rats. J. Ultrastruct. Res. 21 : 383-414, 1968.

21) Torneck, C. D. : Dentin-pulp complex. In : Ten Cate A. R. (ed) Oral histology. The C. V. Mosby Company, Saint Louis Toronto London, 161-166, 1980.

22) Bernard, G. W. : Ultrastructural observations of initial calcification in dentin and enamel. *J. Ultrastruct. Res*. 41 : 1-17, 1972.

23) Eisenmann, D. R. and Glick. P. L. : Ultrastructure of initial crystal formation in dentin. *J. Ultrastruct. Res*. 41 : 18-28, 1972.

24) Larsson, Å. : Studies on dentinogenesis in rat. Ultrastructural observations on early dentin formation with special reference to "dentinal globules" and alkaline phosphatase activity. *Z. Anat. Entwickl-Gesch*. 142 : 103-115, 1973.

25) Yanagisawa, T. : Electron-microscopic study of matrix vesicles and their alkaline-phosphatase activity. *Bull. Tokyo Dent. Coll*. 16 : 109-118, 1975.

26) Ozawa, H., Yamada, M. and Yajima, T. : The ultrastructure and cytochemical aspects of matrix vesicles and calcification processes. In : Talmage R. V. Ozawa H (ed) Formation and calcification of hard tissue. Shakai Hoken Publishing CO., LTD., Tokyo, Japan, *Coll*. 9-57, 1978.

27) Addison, W. H. F. and Appleton, J. L. : The structure and growth of the incisor teeth of the albino rat. *J. Morph*. 26 : 43-96, 1915.

28) Takuma, S., Kurahashi, Y. and Tsuboi, Y : Electron microscopy of the osteodentin in rat incisors. In : Symons N. B. B (ed) Dentin and pulp : Their structure and reactions. Univ. of Dundee, Dundee, 169-195, 1968.

29) Harris, R. and Griffin, C. J. : Histogenesis of fibroblasts in the human dental pulp. Arch oral Biol 12 : 459-468, 1967.

30) Nagai, N. : Life cycle and function of odontoblast. *The Cell* (New Science Co., Tokyo, Japan) 10 : 2-14, 1978.

31) Jenkins, G. N. : The effects of hormones on the oral structures. The calciotraumatic response. In : The physiology and biochemistry of the mouth. 4th ed. Blackwell Scientific Publications, Oxford London Edinburgh Melbourne, 223, 1978.

32) Harris, L. J. and Innes, J. R. M. : XLV. The mode of action of vitamin D. Studies on hypervitaminosis D. The influence of the calcium-phosphate intake. *Biochem. J*. 25 : 367-390, 1931.

33) Eisenstein, R. and Grott, W. : Experimental hypervitaminosis D : hypercalcemia, hypermucoproteinemia, and metastatic calcification. *Proc. Soc. exp. Biol. Med*. 94 : 441-444, 1957.

34) Makita, T., Uotani, Y., Izawa, Y., Kawashima, H., Hashimoto, Y. and Tsubura, Y. : Safety evaluation studies on the hormonal form of vitamin D_3 (I) Acute and subacute

toxicity of 1 α-hydroxycholecalciferol in rodents. *IYAKUHIN KENKYU* 8 : 560-588, 1977.
35) Ratcliff, P. A. and Itokazu, H. : The effect of dihydrotachysterol on the teeth and periodontium. *J. Periodont.* 35 : 320-325, 1964.
36) Sjögren, S., Bawden, J., Hammaström, L. E. and Larsson, Å. : No demonstrable accumulation of ^{14}C-vitamin D_3 in developing rat teeth and bones. *Acta. Odont. Scand.* 36 : 51-55, 1977.