Scanning Microscopy

Volume 2 | Number 4 Article 18

7-15-1988

Comparison of the Calcium Distribution Pattern Among Several Kinds of Hard Tissue Forming Cells of Some Living Vertebrates

Yasutoku Kogaya
Asahi University

Kuhei Furuhashi
Asahi University

Scanning Microscopy, Vol. 2, No. 4, 1988 (Pages 2029-2043)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/88$3.00+.00

COMPARISON OF THE CALCIUM DISTRIBUTION PATTERN AMONG SEVERAL KINDS OF HARD TISSUE FORMING CELLS OF SOME LIVING VERTEBRATES

Yasutoku Kogaya* and Kuhei Furuhashi

Department of Oral Anatomy, Asahi University, School of Dentistry,1851 Hozumi, Gifu 501-02, Japan

(Received for publication February 08, 1988, and in revised form July 15, 1988)

Abstract

We investigated the ultrastructural distribution of calcium in several kinds of hard tissue forming cells (secretory and maturation ameloblasts, odontoblasts osteoblasts, chondrocytes, and osteodentine forming cells) of mammals, amphibians, and fish by use of the potassium pyroantimonate technique. The calcium distribution pattern is compared among these cells, and its biological significance is discussed. Except for mammalian odontoblasts, all types of the hard tissue forming cells exhibited fundamentally the same distribution pattern of calcium; the antimonate reaction product was mainly localized on the inner face of the plasmalemma and inside mitochondria. On the other hand, in mammalian odontoblasts, the reaction product was found within secretory granules and in the intercellular spaces. Thus, the calcium distribution pattern in odontoblasts of lower vertebrates differed from that of mammalian odontoblasts and was similar to that of the osteoblasts or chondrocytes of the vertebrates examined. The differences in calcium distribution pattern among these hard tissue forming cells were not related to their origin, ectodermal or mesodermal (ectomesenchymal). We suggest on the basis of previous studies cited in this paper and of the present data that they are closely associated with the phylogeny and physiological system of Ca-ATPase.

Key Words:Calcium, Potassium pyroantimonate, Hard tissue forming cells, Vertebrates, Phylogeny, Electron energy-loss spectroscopy.

*Address for correspondence:
Yasutoku Kogaya
Department of Oral Anatomy
Asahi University, School of Dentistry
1851 Hozumi, Hozumi-Cho, Gifu 501-02
Japan

Phone No. 05832(6)6131

Introduction

It is well known that calcium plays a major role in nearly every aspect of cell function. Intracellular levels of calcium are thought to be maintained by various physiological systems; for instance, sequestration by calcium-binding proteins such as calmodulin, pumping out of calcium by Ca-ATPase, Na-Ca exchange, and/or incorporation into mitochondria (Barritt, 1982; Carafoli and Longoni, 1986; Vincenzi, 1978). The phenomenon of biological mineralization is strictly controlled by hard tissue forming cells and closely connected with the physiological systems described above (Höhling and Fromme, 1984). Therefore, the investigation of the intracellular and extracellular localization of calcium in mineralizing tissue is one of the most important issues in understanding mineralization.

On the other hand, the hard tissues of living vertebrates originate from dermal armour thought to be the earliest hard tissue that primitive vertebrates gained in Ordovician about five hundred million years ago (Halstead, 1964; Moss, 1968; Ørvig, 1968; Poole, 1971). Unfortunately, at present the phylogenetic relationship among the hard tissues (bone, dentin, enamel, and enameloid) remains uncertain. However, it seems that the phylogeny and evolution of the hard tissues involve some of the most important information for the understanding of the mineralization mechanism. In the present paper we will compare the ultrastructural distribution of calcium among several kinds of hard tissue forming cells of some living vertebrates. We suggest that except for mammalian odontoblasts the cells have some features in common with regard to the processing of calcium. The biological significance of these findings is discussed.

Potassium Pyroantimonate (PPA) Technique for the Localization of Cellular Calcium

Since the potassium pyroantimonate (PPA) technique was originally introduced by Komnick (1962) with the primary aim of localizing sodium, this method has been employed with many modifications (Kashiwa and Thiersch, 1984; Klein et al., 1972; Simson and Spicer, 1975; Slocum and Roux, 1982; Wick and Hepler, 1982) and most recent studies using the technique are not related to sodium localization but to calcium localization. The PPA technique has been plagued by uncertainty: lack of specificity for calcium, loss and/or redistribution of calcium in tissues during incubation, and non-specific precipitation of potassium antimonate occurring below pH 7.2 (Landis and Glimcher, 1982). However, some workers (Simson and Spicer, 1975; Wick and Hepler, 1982; Mentre and Halpern, 1988) have pointed out the usefulness of PPA technique for localizing cellular calcium, although some care must be taken in the interpretation of the results obtained. Simson and Spicer (1975) concluded that the technique can contribute to the understanding of cellular calcium distribution resulting from physiologic and pathologic stimuli. Furthermore, Wick and Hepler (1982) showed that it is possible to employ antimonate as a selective electron microscopic histochemical stain for the localization of exchangeable cellular calcium and that in spite of its inevitable limitations, it is a useful tool to explore calcium regulation. The procedure of the PPA technique utilized in this work was carried out according to the guidelines of the technique in conjunction with electron microscopy proposed by Klein et al. (1972). Teeth, bone, and cartilage of some living vertebrates (rat; frog, *Rana nigromacurata*; fish, *Hoplognathus fasciatus* and *Polypterus senegalus*) were investigated.

Potassium Pyroantimonate Technique

Potassium pyroantimonate reagent was made by adjusting 100 ml 0.01 N acetic acid to pH 7.4 with 0.1 N KOH. Four grams of potassium pyroantimonate (Koso Chem. Co. Ltd., Tokyo) were dissolved completely in this solution by shaking for 1 hour in a water bath (about 90 C). The solution was then cooled to room temperature. The fixative was prepared by adding 5 ml of 4% osmium tetroxide to 5 ml of the above solution (4% potassium pyroantimonate), and the final solution was adjusted to pH 7.6 - 7.8 with 0.01 N acetic acid or 0.1 N KOH. Tissue slices were fixed with the potassium pyroantimonate-osmium tetroxide for 2-3 hours at 4 C. After fixation, the specimens were thoroughly washed in potassium acetate buffer and distilled water to remove any unreacted pyroantimonate, dehydrated through a graded series of ethanols, infiltrated with n-butylglycidyl ether and embedded in Taab 812 Resin. Ultrathin sections were cut with a diamond knife using an LKB Ultrotome. Unstained sections were examined with a JEM 1200 EX electron microscopy.

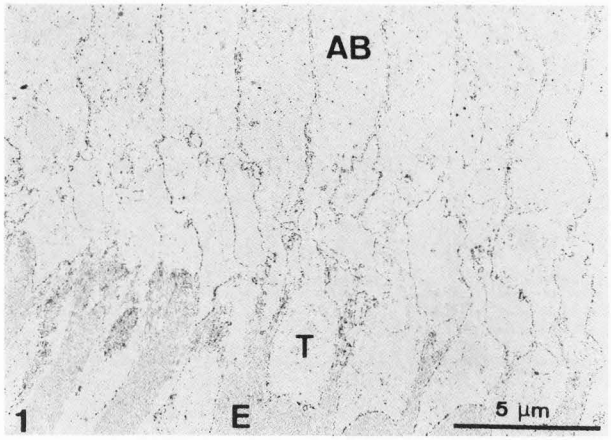
Electron Energy-Loss Spectroscopy (EELS)

Since energy dispersive X-ray microanalysis produces considerable peak overlaps of Ca (K_α 3.69, K_β 4.01 keV) with Sb (L_α 3.60, $L_{\beta 1}$ 3.84, $L_{\beta 2}$ 4.10 keV), it is difficult to identify both simultaneously. In this report, therefore, EELS analysis was utilized to confirm the presence of calcium (Ca-$L_{2,3}$, 350, 346 eV) and antimony (Sb-$M_{4,5}$, 537, 538 eV) in the antimonate reaction product (Eisenmann et al., 1982; Kogaya and Furuhashi, 1986; Makita and Hakoi, 1986; Ashrafi et al., 1987). Unstained ultra-thin sections (about 30-50 nm) on copper grids were analyzed with a JEM 2000 EX electron microscope fitted with Tracor Northern TN-5500, with a microcomputer system and JEM electron energy-loss spectrometer.

Calcium Distribution in Hard Tissue Forming Cells of Some Living Vertebrates

Mammals

Ameloblasts The ameloblast during its cytodifferentiation goes through several well documented stages: 1) early secretory stage (secretory ameloblasts without Tomes' process), 2) secretory stage (tall secretory ameloblasts with a Tomes' process), 3) maturation stage (ruffle-ended and smooth-ended maturation ameloblasts which undergo several cyclic morphologic changes). Autoradiographic studies (Bawden and Wennberg, 1977; Hall and Höhling, 1969; Munhoz and Leblond, 1974; Nagai and Frank, 1975; Oka and Shimizu, 1972; Reith and Cotty, 1962) with ^{45}Ca have demonstrated that calcium is transported to the calcifying enamel matrix through the enamel organ including the ameloblasts layer, although there exist some inconsistencies with regard to calcium localization in the time course for the passage of calcium into the enamel. Crenshaw and Takano (1982) and Takano et al. (1983) showed that most of the radiocalcium is lost from soft tissue when the specimens are processed aqueously, and suggested that this might account for the inconsistent distributions of radiocalcium in the ameloblasts layer reported in these autoradiographic studies. El-Zainy et al. (1987) found a consistent pattern of ^{45}Ca labelling over dental tissues processed with PPA which



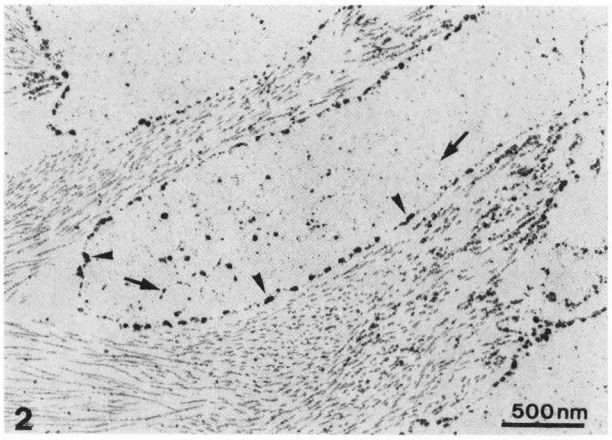
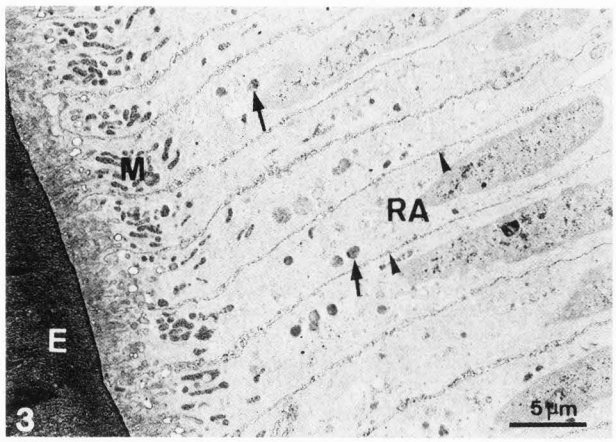


Fig. 1. Secretory ameloblasts (AB) of rat incisor tooth. T=Tomes' process, E=enamel

Fig. 2. A higher magnification image of Tomes' process. The antimonate reaction product is detected on the inner face of the plasma membrane (arrowheads) of Tomes' process and on the outer surface of secretory granules (arrows).



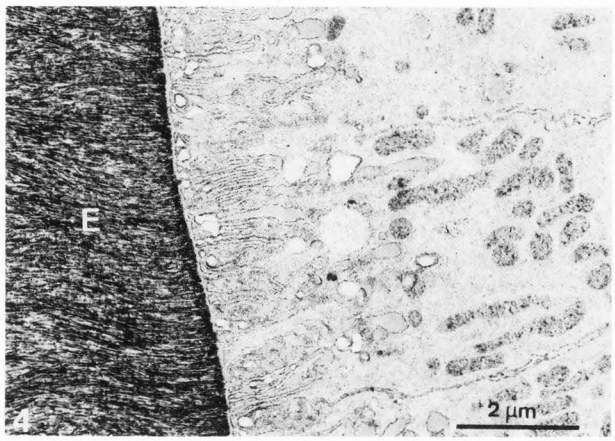


Fig. 3. Ruffled-ended maturation ameloblasts (RA) of a rat incisor. The antimonate reaction product is observed associated with the plasmalemma (arrowhead), mitochondria (M), and multi-vesicular like bodies (arrows). E=enamel

Fig. 4. A high magnification picture of the distal portion of ruffle-ended maturation ameloblasts. The reaction product is located on the plasma membrane including ruffled border and mitochondria. E=enamel

---

was quantitatively more pronounced, but qualitatively similar to that in the specimens processed without PPA. This would suggest that the PPA treatment enhances calcium retention during processing for autoradiography. The cyclic pattern of Ca incorporation into maturation enamel (Suga et al., 1970) was related to the pattern of ameloblast modulation; the heaviest calcium uptake was observed associated with ruffle-ended maturation ameloblasts (Reith and Boyde, 1981; Takano et al., 1982). However, the precise route for calcium through the cell is unresolved. It has been proposed (Nagai and Frank, 1975) that there are two pathways for the translocation of calcium into the enamel, one involving the direct movement of calcium from capillaries to the mineralizing enamel matrix through the extracellular spaces between ameloblasts, and the other involving a transcellular route via secretory granules originating from the Golgi apparatus.

Several studies (Appleton and Morris, 1979b; Eisenmann et al., 1979; Ozawa et al., 1979) have provided some

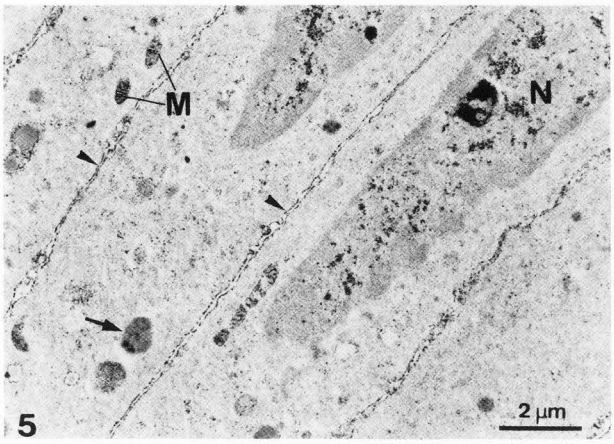
support for the hypothesis that antimonate reaction product is localized within the Golgi apparatus and secretory granules and in the intercellular spaces. On the other hand, some of those studies (Appleton and Morris, 1979b; Deporter, 1977; Eisenmann et al., 1979; Ashrafi et al., 1987) reported that the reaction product is also localized associated with the plasmalemma of secretory ameloblasts. Eisenmann et al. (1984), Chen et al. (1986), and Ashrafi et al.(1987) suggested that secretory ameloblasts may be actively controlling the availability of calcium to enamel by a mechanism involving the cell membrane. Crenshaw and Takano (1982) and Takano et al. (1983) stated that calcium must enter the enamel mainly through an intracellular route through the secretory ameloblasts because the distal tight junction of these cells would prevent intercellular passage. Reith (1983) emphasized that a reexamination of data shows that occasionally the reaction product can be found on the inner face of the plasmalemma. He proposed that the plasma membrane of ameloblasts, but not the secretory granules, might have a direct role in the transcellular transport of calcium, because it has been shown that time frame for the passage of calcium from the blood to the developing enamel is under 30 seconds (Munhoz and Leblond, 1974), while the enamel matrix proteins pass through the organella complex of the secretory granules in time frame from 20-60 min (Weinstock and Leblond, 1971; Frank, 1970). Subsequently, Reith and Boyde (1985), Lyaruu et al. (1985), and Kogaya and Furuhashi (1986) reported that the antimonate reaction product is mainly detected on the inner face of the plasma membrane of secretory ameloblasts (Figs. 1, 2). Frank (1979) observed using Ca-autoradiography at 5 min after injection, the most intense labeling in the basal peripheral cytoplasm adjacent to the endoplasmic reticulum as far as the basal terminal web and no labeling within the secretory granules.

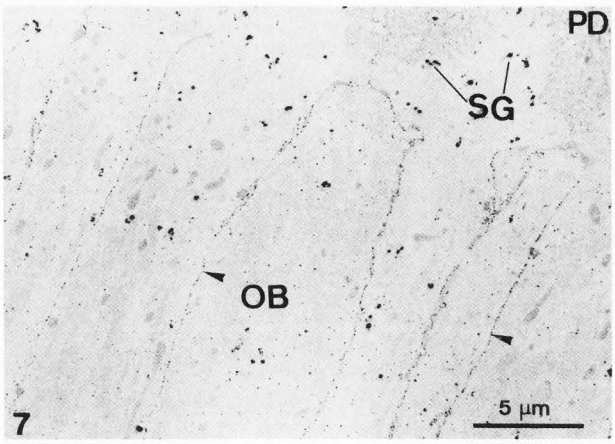
Little information exists as to the ultrastructural localization of calcium in maturation ameloblasts, ruffle-ended and smooth-ended ameloblasts. As demonstrated in Figs. 3-5, the antimonate reaction product appears mainly associated with the plasma membrane, nuclei and mitochondria of ruffle-ended ameloblasts but there is no specific calcium localization pattern in smooth-ended ameloblasts (Fig. 6).

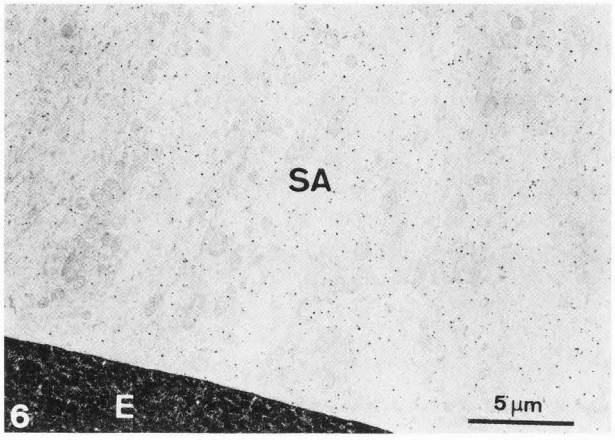
Odontoblasts Höhling and Fromme (1984) using ^{45}Ca autoradiography, found that 10 min after injection ^{45}Ca had already accumulated inside odontoblasts, especially in the rough endoplasmic reticulum but some started to appear in the central Golgi region; after 60 min ^{45}Ca was observed within the odontoblast process and after 120 min within the mineralizing dentin. Nagai and Frank (1974) demonstrated that calcium from dental papilla was transported through odontoblasts and/or through intercellular spaces between odontoblasts. Frank (1979) stated that the calcium transfer via elongated dense secretory vesicles was not observed in the odontoblasts, although as a relative low dose of ^{45}Ca was used in the study, it might be that calcium transfer via secretory vesicles requires a certain mineral level of radioactivity for detection. This observation contrasts with the ultrastructural cytochemical findings utilizing the histochemical techniques as described below. Fromme et al. (1971) and Höhling and Fromme (1984) showed calcium (as oxalate precipitates) to be present intracellularly in the region of organelles as well as in the odontoblasts process. On the other hand, previous studies (Appleton and Morris, 1979a; Kogaya and Furuhashi, 1986; Ozawa, 1972; Reith, 1976) using the PPA technique demonstrated that the reaction product was mainly localized within secretory granules in the Golgi region and odontoblast process, inside pinocytotic vesicles, and in the intercellular spaces, but little or no precipitate was observed within mitochondria (Figs. 7, 8). It was also suggested that the mitochondria of odontoblasts show considerable variation in the amounts of the precipitates (Appleton and Morris, 1979a). Kogaya and Furuhashi (1988b) reported that the calcium distribution pattern in mitochondria changed with the dentin formation stages. Thus, it is clear that there are distinct differences in the calcium distribution pattern between odontoblasts and ameloblasts (Kogaya and Furuhashi, 1986).

It is known that in the prefunctional stage, the anterior apex of the rat incisor is composed of a mass of osteodentin outlined and confined by a thin layer of dentin (Takuma et al., 1977). We investigated the osteodentin forming cells in the rat incisor using the PPA technique. Interestingly, the antimonate reaction product, unlike odontoblasts in the rat incisor, was mainly localized on the plasma membrane, within nuclei, and inside mitochondria but little or none in the intercellular spaces (Fig. 9).

Osteoblasts Landis et al. (1977, 1980) observed dense mineral granules in mitochondria of osteoblasts prepared anhydrously in organic solvents. Burger and DeBruijn (1979), and Burger and Matthews (1978) demonstrated the presence of an antimonate reaction product on the plasmalemma and within mitochondria of young and mature osteoblasts in the periosteum. With the matrix mineralization, the anti-







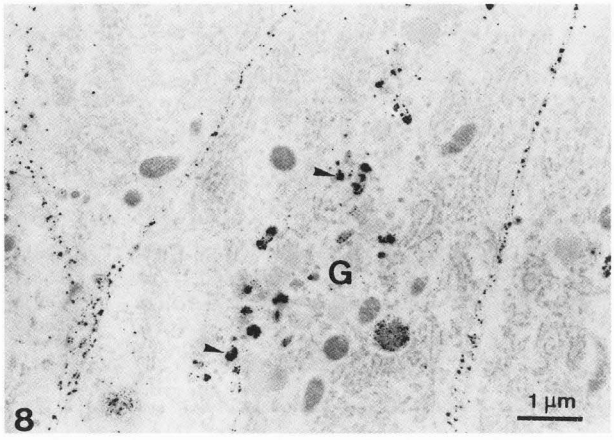


Fig. 5. A higher magnification image of proximal portion of ruffle-ended ameloblasts. The reaction product is localized on the inner face of the plasmalemma (arrowheads) and within mitochondria (M), nuclei (N), and multi-vesicular-like body (arrow).

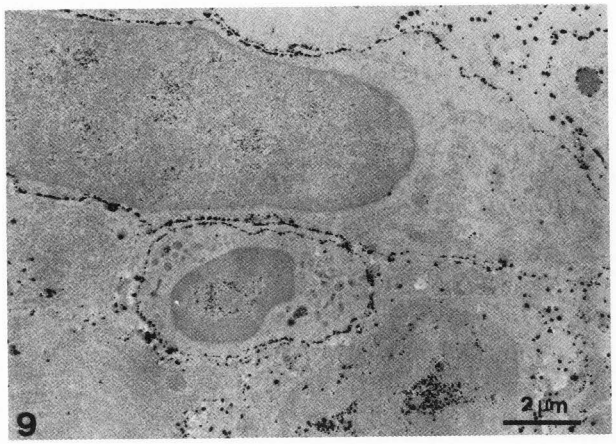
Fig. 6. In smooth-ended maturation ameloblasts (SA), there is no specific distribution pattern of antimonate reaction product. E=enamel

Fig. 7. Odontoblasts (OB) of rat incisor tooth. The antimonate reaction product is localized in the lateral intercellular spaces (arrowheads) between odontoblasts, and within secretory granules (SG). PD=predentine

Fig. 8. Golgi area (G) of the odontoblasts. The antimonate reaction product is seen associated with various Golgi vesicles (arrowheads).

monate reaction product on the plasmalemma rapidly disappeared (Burger and Matthews, 1978). Fig. 10 also shows that the precipitates are localized on the inner face of the plasma membrane and inside mitochondria of osteoblasts in the developing rat calvaria and that the cells are sharply outlined by the reaction product. Frank (1979) observed using ^{45}Ca-autoradiography that there is no label within the secretory granules of osteoblasts, suggesting that calcium transport is not synchronized with the secretion of bone organic matrix.

Chondrocytes Mitochondrial granules, which vary in size and are closely associated with mitochondrial membranes, are observed principally within the chondrocytes located between the middle proliferative and the lower hypertrophic cartilage zone (Landis and Glimcher, 1982). It has also been reported that granule-containing mitochondria are present most frequently in the zone of hypertrophic cells (Carson et al., 1978; Martin and Matthews, 1969) but are absent in the rachitic growth plate (Matthews et al.,



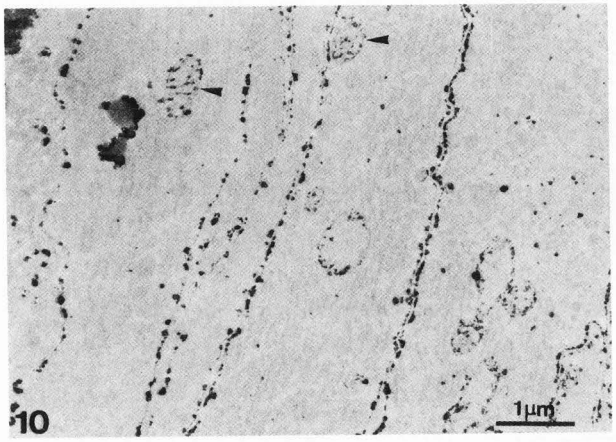


Fig. 9. Osteodentine forming cells in the anterior apex of rat incisor. The reaction product, unlike in odontoblasts, is detected on the inner face of the plasma membrane.

Fig. 10. Osteoblasts in developing rat calvaria. The cells are sharply outlined by antimonate reaction product, which is also seen within mitochondria (arrowheads).

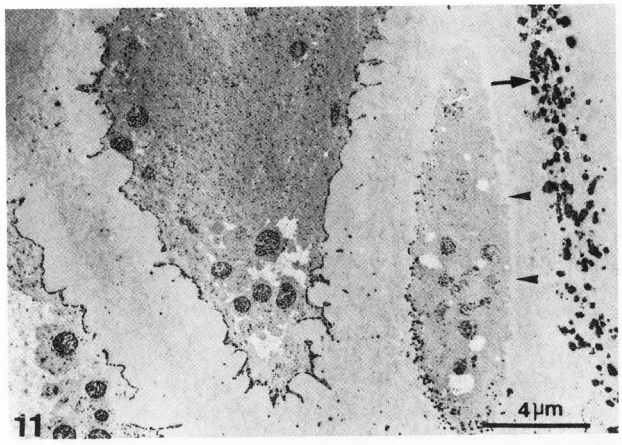


Fig. 11. Chondrocytes of mouse Meckel's cartilage. The antimonate reaction product is localized on the plasma membrane and within mitochondria. Note absence (arrowheads) of reaction product associated with the cells adjacent to the region where bone collar matrix calcification has started (arrow).

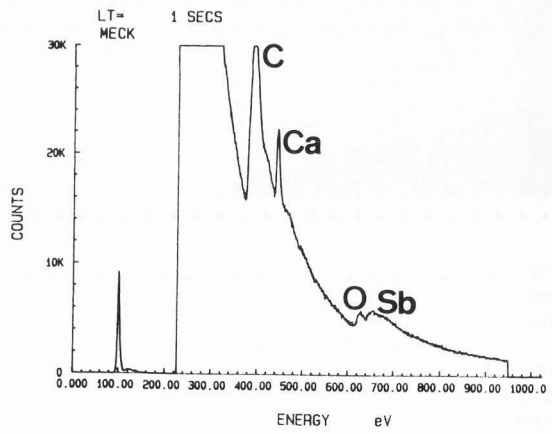
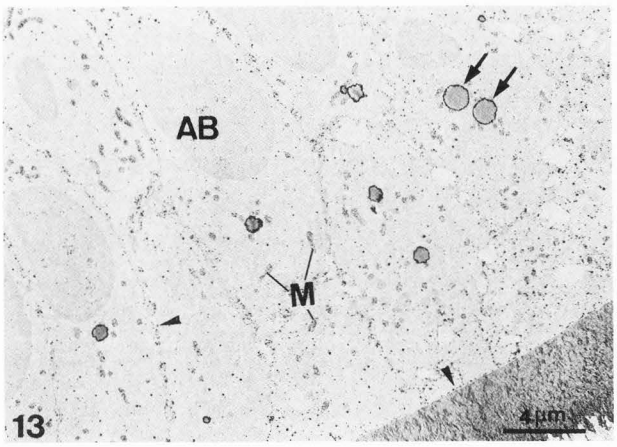
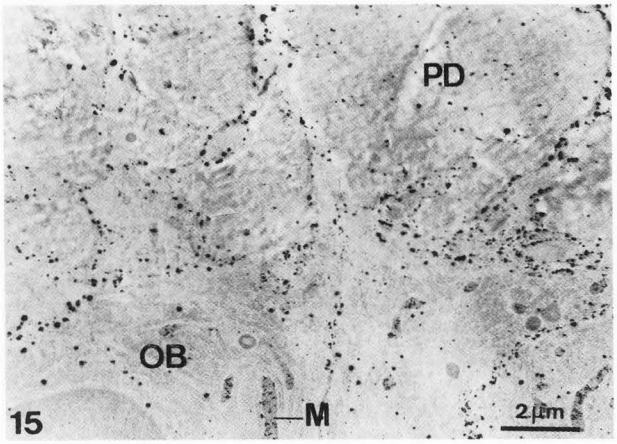


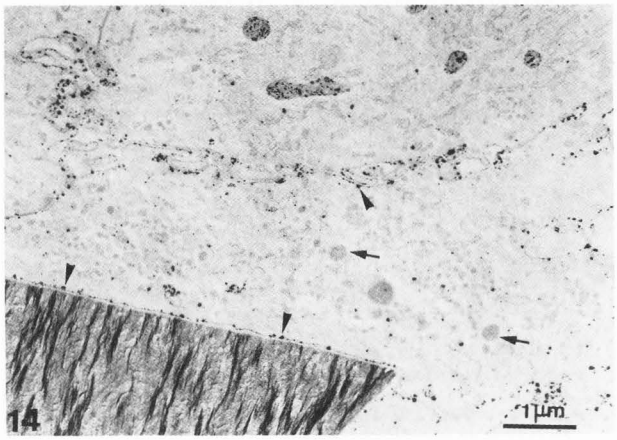
Fig. 12. EELS spectrum from the antimonate reaction product on the plasmalemma of a chondrocyte. The edges of calcium (Ca) and antimony (Sb) are closely detected. C=carbon O=oxygen

1970). On the other hand, Appleton et al. (1985) stated that mitochondrial granules consisting of calcium and phosphorus precipitates were not observed except where chondrocytes were damaged as a result of the freezing process, and suggested that mitochondrial granules only appear when tissue is damaged because of inadequate preservation. Brighton and Hunt (1976) showed that the antimonate reaction product is located predominantly in mitochondria and cell membranes throughout most of the growth plate. In the degenerating zone the reaction product is gradually lost from mitochondria and cell membranes and concomitantly accumulated by extracellular matrix vesicles which are thought to be one of the possible sites of initial mineralization. This would suggest that intracellular calcium plays a significant role in matrix calcification. Similar results were observed from Meckel's cartilage (Fig. 11).

Most recently, Barckhaus et al. (1985) demonstrated the presence of large







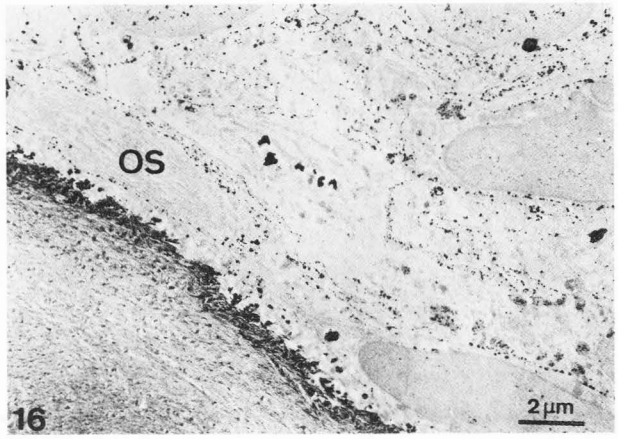


Fig. 13. Ameloblasts (AB) of the frog, Rana nigromaculata. Antimonate reaction product is observed along the plasma membrane (arrowheads), within mitochondria (M), and on the periphery of lipid droplet (arrows).

Fig. 14. A higher magnification image of the distal portion of the ameloblasts. The antimonate reaction product is observed on the inner face of the plasmalemma (arrowheads) but not within secretory granule-like structures (arrows).

Fig. 15. Odontoblasts (OB) of the frog, Rana nigromaculata. Unlike in mammalian odontoblasts, the antimonate reaction product is localized on the plasma membrane including odontoblast process and within mitochondria (M). PD=predentine

Fig. 16. Osteoblasts (OS) in maxilla of the frog, Rana nigromaculata. The antimonate reaction product is mainly detected on the plasmalemma.

quantities of sodium and potassium together with calcium associated with the plasma membrane of chondrocytes in tibia growth plate. As described previously, since the PPA technique is also capable of precipitating in situ sodium or potassium in additon to calcium, one cannot rule out a possibility that the reaction product on the plasmalemma of chondrocytes may be a complex Na/Sb or K/Sb. However, previous studies (Burger and Matthews, 1978; Burger and DeBruijn, 1979; Morris and Appleton, 1980) using X-ray microanalysis have shown that the X-ray emission of sodium was too small to allow analysis of its distribution. Furthermore, our data using electron energy-loss spectroscopy (Fig. 12) indicated that the antimonate reaction product localized at the plasma membrane of chondrocytes of Meckel's cartilage does contain Ca and Sb. Therefore, it seems that the PPA reacts preferentially with calcium rather than with other cations.

Reptilia

To the best of our knowledge, no literature concerning calcium distribution in hard tissue forming cells of Reptilia has been published. We have not yet examined the calcium distribution pattern using the PPA technique.

Amphibia

El-Zainy et al. (1987) demonstrated with ^{45}Ca-autoradiography that 60 min after injection, silver grains are detected in dentin and enamel as well as in the layer of odontoblasts and ameloblasts of the frog, Rana pipiens. According to our data with regard to ultrastructural distribution of calcium in hard tissue forming cells of the frog, Rana nigromaculata, determined by the PPA technique, antimonate reaction product is observed on the inner face of the plasma membrane, on the outer surface of lipid droplets, inside nuclei, and within mitochondria of ameloblasts but not within secretory granule-like structures and in the enamel matrix (Figs. 13, 14). In odontoblasts, the precipitates are localized on the inner face of the plasmalemma and inside mitochondria, although the localization pattern is not so distinct as that in ameloblasts (Fig.15). In osteoblasts, similar results were obtained (Fig. 16).

Fish

Except for the teeth of the coelacarhid fish, Latimeria chalumnae, the surface layer of which is true enamel (Sasagawa et al., 1984), the teeth of most fishes are covered by enameloid. This is thought to be a product of ectodermal and mesodermal (or ectomesenchymal) cells and is composed of collageneous matrix (Herold et al., 1980; Meinke, 1982; Poole, 1967; Prostak and Skobe, 1985; Shellis, 1975, 1978; Shellis and Miles, 1974; Slavkin et al., 1983), unlike true enamel in which enamel proteins (amelogenins and enamelins) and certain sulfated glycosaminoglycans and glycoproteins are involved (Eastoe, 1979; Fincham and Belcourt, 1984; Fukae, 1972; Goldberg and Septier, 1986; Kogaya and Furuhashi, 1988a; Nanci et al., 1985; Nagai and Nagai, 1977; Sasaki et al., 1982; Suga et al., 1970). As shown in Figs. 17-20, the antimonate reaction product in the hard tissue forming cells, odontoblasts, inner enamel epithelium (ameloblasts equivalents), osteoblasts, and chondrocytes of the fishes, Polypterus senegalus and Hoplognathus fasciatus, is localized associated with the plasma membrane and mitochondria. Kogaya and Furuhashi (1987) have reported similar calcium distribution pattern in true odontoblasts of Hoplognathus fasciatus.

Biological Significance of the Localization of Calcium on the Inner Face of the Plasmalemma of Hard Tissue Forming Cells

The plasma membrane and its calcium transporters are responsible for the maintenance of an intracellular free calcium concentration of about 10^{-8} to 10^{-7} M in resting or unstimulated cells, since intracellular membranes and enzymes, which have evolved to function optimally at 10^{-8} to 10^{-6} M Ca, are damaged at higher concentrations (Barritt, 1982; Carafoli and Longoni, 1986). Mitochondria, endoplasmic reticulum, calmodulin, and/or other calcium binding proteins play an important part in regulating the concentration of free calcium in the cytoplasm. Wick and Hepler (1982) reported that fixation at a slightly basic pH, directly in osmium-antimonate appears to retain bound or sequestered calcium according to its in vivo distribution while other soluble physiological cations seem to be lost. Therefore, it seems that the calcium on the inner face of the plasmalemma of hard tissue forming cells is in a loosely bound, exchangeable state, presumably in relation to certain calcium-binding protein such as calmodulin. Ca-ATPase is stimulated by calmodulin, which is known to increase the affinity of the ATPase for Ca down to a Km below 0.5 µM (Gopinath and Vincenzi, 1977; Jarrett and Penniston, 1977). It has been reported that Ca-ATPase is localized in the Golgi cisternae, cytoplasmic vesicles and along the outer surface of the presecretory and secretory ameloblasts (Inage and Weinstock, 1979; Takano et al., 1986; Sasaki and Garant, 1986, 1987a; Salama et al., 1987), whereas it is totally absent from odontoblasts (Takano et al., 1986). Granstrom et al. (1978) showed Ca-ATPase activity in the Golgi region of odontoblasts but not in associated plasmalemma. Highsmith et al. (1987) indicated that Ca-ATPase activity of odontoblast microsomes was not associated with a calcium pump. Sasaki and Garant (1986) described that when the calmodulin blocker trifluoperazine is administered to the rats, Ca-ATPase activity was almost completely abolished from the plasma membranes of secretory ameloblasts, suggesting that Ca-ATPase of secretory ameloblasts has a high affinity for calcium, which is modulated by an endogenous calmodulin. Their subsequent work with the protein A-gold immunocytochemical technique (Sasaki and Garant, 1987b) demonstrated that specific immunolabelling is detected in association with nuclei, mitochondria, cytosol, and plasmalemma of secretory ameloblasts. On the other hand, concerning Ca-ATPase activity of maturation ameloblasts, Salama et al. (1987) revealed that ruf-

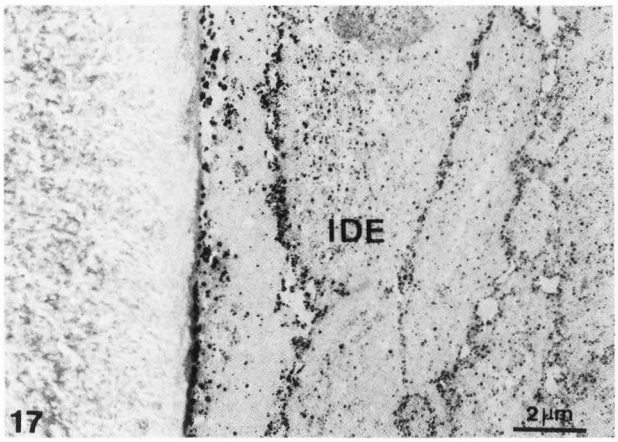


Fig. 17. Inner dental epithelium (IDE) of the fish, Polypterus senegalus at the collar enameloid formation site. The antimonate reaction product is mainly observed associated with the plasmalemma.

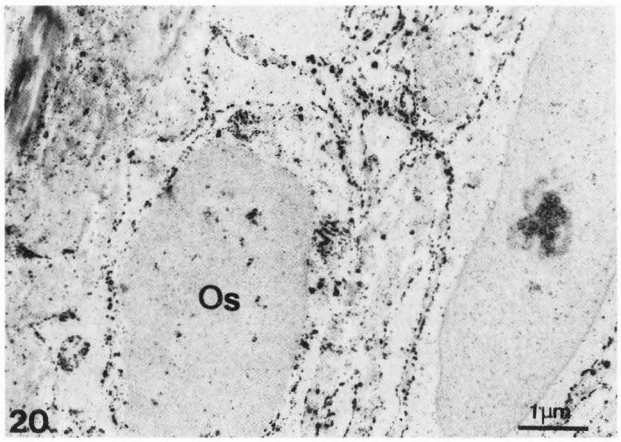
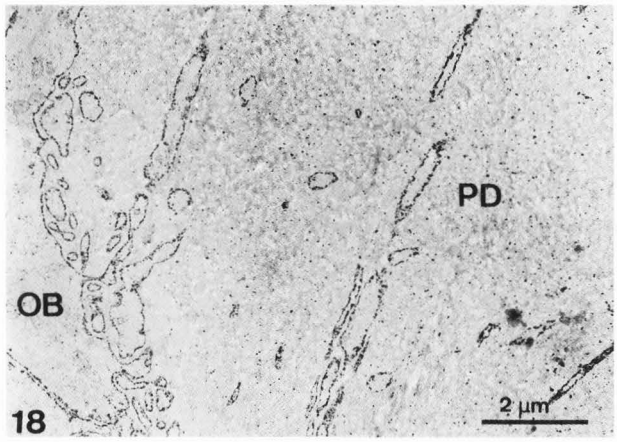
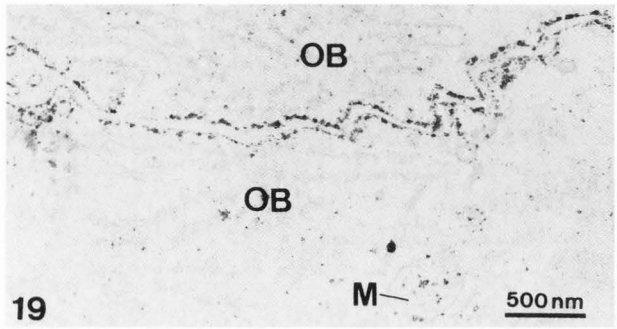


Fig. 20. Osteoblasts (Os) in dentary of the fish, Polypterus senegalus. The antimonate reaction product is observed associated with the inner face of the plasma membrane and within mitochondria.





Figs. 18, 19. Odontoblasts (OB) of the fish, Hoplognathus fasciatus. The antimonate reaction product is localized on the plasmalemma including odontoblast process and within mitochondria (M). PD=predentine

fle-ended maturation ameloblasts exhibit intense reaction product along their lateral and distal plasma membrane. In contrast, in smooth-ended maturation ameloblasts no reaction product is present at the distal plasmalemma, although substantial reaction product is found along the lateral and proximal surface. Furthermore, Salama et al. (1987) demonstrated a higher overall intensity of Ca-ATPase reaction product in ruffle-ended compared to smooth-ended maturation ameloblasts. Takano and Akai (1987) showed intense reactions of Ca-ATPase along the outer surface of ruffled border membrane and in the adjacent tubulo-vesicular structures of the ruffle-ended maturation ameloblasts but not on the lateral plasma membrane. As shown in this paper (Figs. 1-5), the antimonate reaction product is mainly localized on the plasma membrane of secretory ameloblasts and ruffle-ended maturation ameloblasts. In contrast, no specific distribution pattern of calcium is observed in smooth-ended maturation ameloblasts. This calcium distribution pattern appears to be mostly in agreement with that of Ca-ATPase activity, not only of mammalian ameloblasts but also of mammalian chondrocytes (Akisaka and Gay, 1985) and osteoblasts (Bab et al., 1979; Sandhu and Jande, 1982), and amphibian ameloblasts (Zaki and Hand, 1983). Although there is no literature on the Ca-ATPase of fish odontoblasts, from the calcium distribution pattern in these cells, it can be inferred that fish odontoblasts may have a similar mechanism to process calcium. Calcium was detected in the intercellular spaces and within secretory granules of

mammalian odontoblasts (Ozawa, 1972; Reith, 1976; Appleton and Morris, 1979a, b; Kogaya and Furuhashi, 1986) but calcium and Ca-ATPase activity were absent on the plasma membrane (Granstrom, 1984; Granstrom et al., 1978; Takano et al., 1986). This suggests that in odontoblasts of mammals calcium is mainly transported through a transcellular route via secretory granules in which calcium is presumably bound to certain organic materials such as phosphophoryn, glycosaminoglycans or glycoproteins.

Phylogenetic Consideration of Hard Tissue Forming Cells and Their Calcium Distribution Pattern

The exoskeletal elements such as teeth, dermal bone, and scales as well as endoskeletal bone have the same basic origin (see references, Halstead, 1964, 1969, 1974; Moss, 1968; Ørvig, 1967, 1968; Lumsden, 1981; Poole, 1971). The earliest hard tissue in vertebrates originates from dermal armour, which is believed to have developed first as a phosphate store (which was laid down in the skin in the form of calcium phosphate), and then, secondly, as a protection. Although the preservation of the dermal armour of the primitive jawless vertebrates is such that it is possible to examine their light microscopic structure in considerable detail, soft tissues including hard tissue forming cells can not naturally be observed in fossil vertebrates. Therefore, the data from hard tissue forming cells of lower and higher living vertebrates is very important. What is the nature of the earliest type of mineralized tissue? According to Halstead (1964, 1969, 1974), heterostracan dermal armour, which is composed of three main layers (a basal layer of lamellar aspidin in which thin sheets of hard tissue lie on top of each other, a very thick middle layer of spongy aspidin, in which the calcified tissue forms a meshwork around interconnecting vascular spaces, and a superficial layer of dermal denticles), is considered as the most primitive type of calcified tissue, from which true bone could have been derived. On the other hand, aspidin is considered by Ørvig (1967) to be irrelevant for the early development or possible origin of bone; he claims that dentin is derived from primitive bone and that mesodentine is the most primitive type of dental tissue. It would be expected that cells exist that are a basic type of connective tissue cell and that have risen, phylogenetically, to be both osteoblasts and odontoblasts, although there is no evidence from the fossil record to indicate their precise origin. As shown in this work, the calcium distribution pattern in bone forming cells of fish, amphibian, and mammals is fundamentally similar to that in dentin forming cells except for the mammalian odontoblasts. Even in the osteodentin forming cells of rat incisor, a similar distribution pattern can be observed. Furthermore, interestingly, the calcium distribution pattern seems to coincide with that of Ca-ATPase activity. It should be noted that only mammalian odontoblasts have specific features concerning the calcium distribution pattern and that there is no Ca-ATPase activity on the plasmalemma. Therefore, it may be that at least with regard to the processing of calcium that is presumably in conjunction with Ca-ATPase, the osteoblasts of mammals, reptiles and fish and the odontoblasts of lower living vertebrates have preserved the primitive characters which the hard tissue forming cells of the earliest vertebrates might already have gained. In addition, mammalian odontoblasts appear to have gained another mechanism by which calcium is transported mainly via secretory granules into the mineralizing front.

Acknowledgments

This study was supported in part by Miyata Research Grant (1985) and Grant-in-Aid No. 61771432 from the Ministry of Education Science and Culture, Japan. The authors wish to thank Tomoyoshi Watabe (JEOL, Tokyo) for his excellent technical assistance.

References

Akisaka T, Gay CV (1985) Ultrastructural localization of calcium-activated adenosine triphosphatase (Ca-ATPase) in growth-plate cartilage. J. Histochem. Cytochem. 33: 925-932.

Appleton J, Morris DC (1979a) An ultrastructural investigation of the role of the odontoblasts in matrix calcification using the potassium pyroantimonate oamium method for calcium localization. Archs oral Biol. 24: 467-475.

Appleton J, Morris DC (1979b) The use of potassium pyroantiminate-osmium method as a means of identifying and localizing calcium at the ultrastructural level in the cells of calcifying system. J. Histochem. Cytochem. 27: 676-680.

Appleton J, Lyon R, Awindin KJ, Chesters J (1985) Ultrastructure and energy-dispersive X-ray microanalysis of cartilage after rapid freezing, low temperature freeze drying, and embedding in Spurr's Resin. J. Histochem. Cytochem. 33: 1073-1079.

Ashrafi SH, Eisenmann DR, Zaki AE (1987) Secretory ameloblasts and calcium distribution during normal and experimentally altered mineralization. Scanning Microsc. 1: 1949-1962.

Bab IA, Muhlrad A, Sela J (1979) Ultrastructural and biochemical study of extracellular matrix vesicles in normal bone of rats. Cell Tissue Res. 202: 1-7.

Barckhaus RH, Schmidt PF, Quint P, Höhling HJ (1985) Potassium concentration in membrane-associated particles in the epiphyseal growth plate. Cell Tissue Res. 242: 217-219.

Barritt GJ (1982) Calcium movement across the cell membrane. In: The role of calcium in biological systems. Anghileri LJ, ed. CRC Press, Boca Raton, Florida. pp. 17-30.

Bawden JW, Wennberg A (1977) In vitro study of cellular influence on ^{45}Ca uptake in developing rat enamel. J. Dent. Res. 56: 313-319.

Brighton CT, Hunt RM (1976) Histochemical localization of calcium in growth plate mitochondria and matrix vesicles. Fed. Proc. 35: 143-147.

Burger EH, DeBruijn WC (1979) Mitochondrial calcium of intact and mechanically damaged bone and cartilage cells studied with K-pyroantimonate. Histochemistry. 62: 325-336.

Burger EH, Matthews JL (1978) Cellular calcium distribution in fetal bones studied with K-pyroantimonate. Calcif. Tissue Res. 26: 181-190.

Carafoli E, Longoni S (1986) The plasma membrane in the control of the signaling function of calcium. In: Cell calcium and membrane transport synaptosomal calcium regulation. Mandel LJ, Eaton DC, ed. The Rockefeller University Press, New York. pp. 22-29.

Carson FL, David WL, Matthews JL, Martin JH (1978) Calcium localization in normal rachitic, and D treated chicken epiphyseal chondrocytes utilizing potassium pyroantimonate-osmium tetroxide. Anat. Rec. 190: 23-40.

Chen S, Eisenmann DR, Zaki AE, Ashrafi SH (1986) Cytochemical calcium distribution in secretory ameloblasts of the rat in relation to enamel mineralization. Acta Anat. 126: 34-40.

Crenshaw MA, Takano Y (1982) Mechanisms by which the enamel organ controls calcium entry into developing enamel. J. Dent. Res. 61(Sp Iss): 1574-1579.

Deporter DA (1977) The early mineralization of enamel. Fine structural observations on the cellular localization of calcium with the potassium pyroantimonate technique. Calcif. Tissue Int. 24: 271-274.

Eastoe JE (1979) Enamel protein chemistry-past, present and future. J. Dent Res. 58: 753-763.

Eisenmann DR, Ashrafi SH, Neiman A (1979) Calcium transport and the secretory ameloblasts. Anat. Rec. 193: 403-422.

Eisenmann DR, Ashrafi SH, Zaki AE (1982) Multi-method analysis of calcium localization in the secretory ameloblasts. J. Dent. Res. 61: 1555-1561.

Eisenmann DR, Ashrafi SH, Zaki AE (1984) Calcium distribution in freeze-dried enamel organ tissue during normal and altered enamel mineralization. Calcif. Tissue Int. 36: 596-603.

El-Zainy MA, Zaki AE, Eisenmann DR (1987) Comparisons of processing with and without potassium pyroantimonate in quantitative autoradiography of calcium in developing teeth of the frog Rana pipiens. Archs oral Biol. 32: 143-149.

Fincham AG, Belcourt AB (1984) Amelogenin biochemistry, current concepts. In: The chemistry and biology of mineralized tissue. Butler WT, ed. Ebsco Media, Birmingham. pp. 240-247.

Frank RM (1970) Autoradiographique de la dentinogenese en microscopie electronique a laide de la proline tritee chez le chat. Archs oral Biol. 15: 583-596.

Frank RM (1979) Electron microscope autoradiography of calcified tissues. In: International review of cytology. Bourne GH, Danielli JF, ed. Academic Press, New York. pp. 183-253.

Fromme HG, Höhling HJ, Riedel H (1971) Elektronenmikroskopische Studien uber die Dentinbildung. 1. Mitteilung; Lokalisation von Calcium und alkalischer Phosphatase. Dtsch. Zahn- arztl. z. 26: 359-364.

Fukae M (1972) The studies on mucopolysaccharides in developing bovine enamel. Jap. J. Oral Biol. 14: 100-102.

Goldberg M, Septier D (1986) Ultrastructural location of complex carbohydrates in developing rat incisor enamel. Anat. Rec. 216: 181-190.

Gopinath RM, Vincenzi FF (1977) Phosphodiesterase protein activator mimics red blood cell cytoplasmic activator of (Ca+Mg) ATPase. Bioch. Biophys. Res. Comm. 77: 1203-1209.

Granstrom G (1984) Further evidence of an intravesicular Ca-pump in odontoblasts from rat incisor. Archs oral Biol. 29: 599-606.

Granstrom G, Linde A, Nygren H (1978) Ultrastructural localization of alkaline phosphatase in rat incisor odontoblasts. J. Histochem. Cytochem. 26: 359-368.

Hall TA, Hohling HJ (1969) The application of microprobe analysis to biology. In: Int. Congr. on X-ray optics and microanalysis. Mollenstedt G, Gaukler KH, ed. Springer-Verlag, Berlin. pp. 582.

Halstead LB (1964) The origin of bone. In: Bone and tooth symposium, Blackwood HJJ, ed. Pergamon Press, New York. pp. 3-15.

Halstead LB (1969) Calcified tissues in the earliest vertebrates. Calcif. Tissue Res. 3: 107-124.

Halstead LB (1974) Vertebrate Hard Tissue. Wykeham, London. pp. 58-63.

Herold R, Graver H, Christner P

(1980) Immunohistochemical localization of amelogenins in enameloid of lower vertebrate teeth. Science. 207: 1357-1358.

Highsmith S, Bloebaum D, Smith D, Claydon N (1987) The Ca-ATPase activity of rat-incisor odontoblast vesicles. Archs oral Biol. 32: 745-749.

Höhling HJ, Fromme HG (1984) Cellular transport and accumulation of calcium and phosphate during dentinogenesis. In: Dentin and dentinogenesis, Linde A, ed. Vol. 2. CRC Press, Boca Raton. pp. 1-15.

Inage T, Weinstock A (1979) Localization of the enzyme ATPase in the rat secretory ameloblasts by means of electron microscopy. J. Dent. Res. 58(Sp Iss): 1010-1011.

Jarrett HW, Penniston JT (1977) Partial purification of the Ca-Mg ATPase activator from human erythrocytes. Its similarity of the 3':5'-cyclic nucleotide phosphodiesterase. Biochem. Biophys. Res. Comm. 77: 1210-1216.

Kashiwa HK, Thiersch NJ (1984) Evaluation of potassium pyroantimonate/sucrose/glutaraldehyde concentration and incubation time as essential variables for localizing calcium bound to organic compounds in epiphyseal chondrocytes. J. Histochem. Cytochem. 32: 1055-1065.

Klein RL, Yen SS, Thureson-Klein A (1972) Critique of the K-pyroantimonate method for semiquantitative estimation of cations in conjunction with electron microscopy. J. Histochem. Cytochem. 20: 65-78.

Kogaya Y, Furuhashi K (1986) The differences in calcium distribution pattern between preodontoblasts and preameloblasts in developing rat molar tooth germs. Calcif. Tissue Int. 39: 78-85.

Kogaya Y, Furuhashi K (1987) Calcium distribution in true odontoblasts of the fish Hoplognathus fasciatus at dentine mineralization stage. Archs oral Biol. 32: 665-669.

Kogaya Y, Furuhashi K (1988a) Sulfated glycoconjugates in rat incisor secretory ameloblasts and developing enamel matrix. Calcif. Tissue Int. (in press)

Kogaya Y, Furuhashi K (1988b) Ultrastructural localization of calcium in matrix vesicles and preodontoblasts of developing rat molar tooth germs during initial dentinogenesis. Acta Anat. 132: 100-108.

Komnick H (1962) Elektronmikroscopische Lokalization von Na und Cl in Zell und Geweben. Protoplasm. 55: 414-418.

Landis WJ, Glimcher MJ (1982) Electron optical analytical observations of growth plate cartilage prepared by ultracryomicrotomy: The failure to detect a mineral phase in matrix vesicles and the identification of heterodispersed particles as the initial solid phase of calcium phosphate deposited in the extracellular matrix. J. Ultrast. Res. 78: 227-268.

Landis WJ, Paine MC, Glimcher MJ (1977) Electron microscopic observations of bone tissue prepared anhydrously in organic solvent. J. Ultrast. Res. 59: 1-30.

Landis WL, Paine MC, Glimcher MJ (1980) Use of acrolein vapors for the anhydrous preparation of bone tissue for electron microscopy. J. Ultrast. Res. 70: 171-180.

Lumsden AGS (1981) Evolution and adaptation of the vertebrates mouth. In: A comparison to dental studies. Dental anatomy and embryology. Osborn JW, ed. Blackwell Scientific Publications, Carlton. pp. 88-117.

Lyaruu DM, Bronckers ALJJ, Burger EH, Woltgens JHM (1985) Localization of calcium in differentiating early dentinogenesis and amelogenesis in hamster tooth germs. J. Histochem. Cytochem. 33: 595-603.

Makita T, Hakoi K (1986) Advantageous usage of EELS to detect Ca in pyroantimonate staining. Acta Histochem. Cytochem. 19: 289-295.

Martin JH, Matthews JL (1969) Mitochondrial granules in chondrocytes, osteoblasts, and osteocytes. Clin. Orthop. 68: 273-278.

Matthews JL, Martin JH, Sampson HW, Kunin AS (1970) Mitochondrial granules in normal and rachitic rat epiphysis. Calcif. Tissue Res. 5: 91-99.

Meinke DK (1982) A histological and histochemical study of developing teeth in Polypterus (Pisces, Actinopterygii). Archs oral Biol. 27: 197-206.

Mentre P, Halpern S (1988) Localization of cations by pyroantimonate. II. Electron probe microanalysis of calcium and sodium in skeletal muscle of mouse. J. Histochem. Cytochem. 36: 55-64.

Morris DC, Appleton J (1980) Ultrastructural localization of calcium in the mandibular condylar growth cartilage of the rat. Calcif. Tissue Int. 30: 27-34.

Moss ML (1968) The origin of vertebrate calcified tissues. In: Current problems of lower vertebrate phylogeny. Orvig T, ed. Almqvist & Wiksell, Stockholm. pp. 359-371.

Munhoz COG, Leblond CP (1974) Deposition of calcium phosphate into dentine and enamel as shown by radioautography of sections of incisor teeth following injection of Ca into rats. Calcif. Tissue Res. 15: 221-235.

Nagai N, Frank RM (1974) Autoradiographie du Ca en microscopie electronique au cours de la dentinogenese. Cell Tissue Res. 155: 513-523.

Nagai N, Frank RM (1975) Transfert du Ca par autoradiographie en microscopic electronique au cours de l amelogenese.

19: 211-221.

Nagai N, Nagai Y (1977) Sulfate metabolism of amelogenesis in teeth-germs of young cats. Bull. Tokyo Dent. Coll. 18: 1-12.

Nanci A, Bendayan M, Slavkin HC (1985) Enamel protein biosynthesis and secretion in mouse incisor secretory ameloblasts as revealed by high-resolution immunocytochemistry. J. Histochem. Cytochem. 33: 1153-1160.

Oka N, Shimizu T (1972) Electron microscopic studies on mineral movement of calcifying process on the hard tissues. I. Uptake of Ca on the stage of enamel matrix. Jap. J. Oral Biol. 14: 560-570.

Ørvig T (1967) Phylogeny of tooth tissues: evolution of some calcified tissues in early vertebrates. In: Structural and chemical organization of teeth. Miles AEW, ed. Academic Press, New York. pp. 45-110.

Ørvig T (1968) The dermal skeleton: general consideration. In: Current problems of lower vertebrate phylogeny. Orvig T, ed. Almqvist & Wiksell, Stockholm. pp. 373-397.

Ozawa H (1972) Calcium localization and movement in the calcified tissues by means of light and electron microscopic calcium detecting methods. Jap. J. oral Biol. 14: 125-130.

Ozawa H, Yamamoto T, Yamada M, Uchida T (1979) Frozen ultrathin-section for X-ray microanalysis of rat tooth germs. J. Dent. Res. 58(B): 1016-1018.

Poole DFG (1967) Enameloid and enamel in recent vertebrates. In: Structural and chemical organization of teeth. Miles AEW, ed. Academic Press, New York. pp. 111-147.

Poole DFG (1971) An introduction to the phylogeny of calcified tissues. In: Dental morphology and evolution. Dahlberg AA, ed. The University of Chicago Press, Chicago. pp. 65-79.

Prostak K, Skobe Z (1985) The effect of colchicine on the ultrastructure of the dental epithelium and odontoblasts of teleost tooth buds. J. craniofac. Genet. devl. Biol. 5: 75-88.

Reith EJ (1976) The binding of calcium with the Golgi saccules of the rat odontoblasts. Am. J. Anat. 147: 267-272.

Reith EJ (1983) A model for transcelluar transport of calcium based on membrane fluidity and movement of calcium carriers within the more fluid microdomains of the plasma membrane. Calcif. Tissue Int. 35: 129-134.

Reith EJ, Boyde A (1981) Autoradiographic evidence of cyclical entry of calcium into maturating enamel of the rat incisor tooth. Archs oral Biol. 26: 983-987.

Reith EJ, Boyde A (1985) The pyroantimonate reaction and transcellular transport of calcium in rat molar enamel organs. Histochemistry. 83: 539-543.

Reith EJ, Cotty VF (1962) Autoradiographic studies on calcification of enamel. Archs oral Biol. 7: 365-372.

Salama AH. Zaki E, Eisenmann DR (1987) Cytochemical localization of Ca-Mg adenosine triphosphatase in rat incisor ameloblasts during enamel secretion and maturation. J. Histochem. Cytochem. 35: 471-482.

Sandhu HS, Jande SS (1982) Investigations of alkaline phosphatase Ca-ATPase and Na, K-ATPase during beta-APN-induced initial bone mineralization. Acta anat. 112: 242-248.

Sasagawa I, Ishiyama M, Kodera H (1984) Fine structure of the pharyngeal teeth in the coelacanthid fish (Latimeria chalumnae). In Tooth Enamel IV. Fearnhead RW, Suga S, ed. Elsevier Science Publishers, Amsterdam. pp. 462-466.

Sasaki T, Garant PR (1986) Ultracytochemical demonstration of ATP-dependent calcium pump in ameloblasts of rat incisor enamel organ. Calcif. Tissue Int. 39: 86-96.

Sasaki T, Garant PR (1987a) Calmodulin blocker inhibits Ca-ATPase activity in secretory ameloblasts of rat incisor. Cell Tissue Res. 248: 103-110.

Sasaki T, Garant PR (1987b) Calmodulin in rat incisor secretory ameloblasts as revealed by protein A-gold immunocytochemistry. Calcif. Tissue Int. 40: 294-297.

Sasaki S, Shimokawa H, Tanaka K (1982) Biosynthesis of rat enamel matrix components in vivo. J. Dent. Res. 61(Sp Iss): 1479-1482.

Shellis RP (1975) A histological and histochemical study of the matrices of enameloid and dentine in teleost fishes. Archs oral Biol. 20: 183-187.

Shellis RP (1978) The role of the inner dental epithelium in the formation of teeth in Fish. In: Development, Function and Evolution of Teeth. Butler PM, Joysey KA, ed. Academic Press, New York. pp. 31-42.

Shellis RP, Miles AEW (1974) Autoradiographic study of the formation of enameloid and dentine matrices in teleost fishes using tritiated amino acid. Proc. R. Soc. Lond. Ser. B 185: 51-72.

Simson JAV, Spicer SS (1975) Selective subcellular localization of cations with variants of the potassium (pyro)-antimonate technique. J. Histochem. Cytochem. 23: 575-586.

Slavkin HC, Nelson S, Bringas P, Nanci A, Santos V (1983) Selachian tooth development. II. Immunolocalization of amelogenin polypeptides in epithelium during secretory amelogenesis in Squalus acanthias. J. craniofac. Genet. devl. Biol. 3: 29-41.

Slocum RD, Roux SJ (1982) An improved method for the subcellular localization of calcium using a modification of

the antimonate precipitation technique. J. Histochem. Cytochem. 30: 617-629.

Suga S, Murayama Y, Musashi T (1970) A study of the mineralization process in the developing enamel of guinea pig. Archs oral Biol. 15: 597-612.

Takano Y, Akai M (1987) Demonstration of Ca-ATPase activity in the maturation ameloblasts of rat incisor after vascular perfusion. J. Electron Microsc. 36: 196-203.

Takano Y, Crenshaw MA, Reith EJ (1982) Correlation of Ca incorporation with maturation ameloblasts morphology in the rat incisor. Calcif. Tissue Int. 34: 211-213.

Takano Y, Ozawa H, Crenshaw MA (1983) The mechanism of calcium and phosphate transport to the enamel. In: The mechanisms of tooth enamel formation. Suga S, ed. Quintessence Publishing, Tokyo. pp. 49-64.

Takano Y, Ozawa H, Crenshaw MA (1986) Ca-ATPase and ALPase activities at the initial calcification sites of dentin and enamel in the rat incisor. Cell Tissue Res. 243: 91-99.

Takuma S, Yanagisawa Y, Lin WL (1977) Ultrastructural and microanalytical aspects of developing osteodentin in rat incisors. Calcif. Tissue Int. 24: 215-222.

Vincenzi FF (1978) Regulation of a plasma membrane calcium pump; a speculative model. In: Calcium transport and cell function. Scarpa A, Carafoli E, ed. The New York Academy of Sciences, New York. pp. 229-231.

Weinstock A, Leblond CP (1971) Elaboration of the matrix glycoprotein of enamel by the secretory ameloblasts of the rat incisor as revealed by radioautography after galactose- H injection. J. Cell Biol. 51: 26-51.

Wick SM, Hepler PK (1982) Selective localization of intracellular Ca with potassium antimonate. J. Histochem. Cytochem. 30: 1190-1204.

Zaki A, Hand AR (1983) Cytochemical localization of Ca-Mg ATPase in developing teeth of the frog Rana pipiens. J. Dent. Res. 62: 287.

Discussion with Reviewers

S. H. Ashrafi: What were the experimental conditions to localize exchangeable Ca by using PPA technique? Why do we see two types of precipitates 1) discrete lump type and 2) fine granular type?
Authors: Fixation at a slightly basic pH, either directly in osmium-antimonate or in phosphate-buffered glutaraldehyde followed by osmium-antimonate, appears capable of retaining bound or sequestered cellular Ca (Wick and Hepler, 1982). The size of the antimonate reaction product is related to several factors such as the rate of antimonate penetration into the tissue, the concentration of the reactive tissue cations, and the rate of precipitaion formation.

J. Appleton: Mitochondria are involved in calcium metabolism in most cells. Why do you think there is an absence of pyroantimonate precipitation in the mitochondria of mammalian odontoblasts?
Authors: Recent works concerning localization of dentin phosphophoryn, which is strongly anionic with a high capacity for binding calcium ions, have demonstrated that it is mainly present in circumpulpal dentin of human teeth, but deficient in mantle dentin and secondary dentin. Our previous study (Kogaya and Furuhashi: 1988b) indicated that at an early stage of mantle dentin mineralization (matrix vesicle-mediated calcification) the antimonate reaction product is detected in mitochondria of the rat odontoblasts and that at the subsequent collagen calcification stage, definite antimonate reaction product is no longer seen within mitochondria. It may be that most calcium at least associated with circumpulpal dentin mineralization in odontoblasts binds to calcium-binding proteins such as phosphophoryn.

J. Appleton: Hard tissue formation is not continuous but is incremental and can be modified amongst other things by the action of hormones, e.g., parathormone. Could you speculate on a mechanism for the action of hormones on hard tissue forming cells which would result in an interference with matrix mineralisation?
Authors: It is reported that PTH interferes with the cytodifferentiation of preodontoblasts into mature odontoblasts and inhibits predentin collagen synthesis. In contrast, PTH has little influence on the secretory metabolism of ameloblasts (Sakakura, 1987). Furthermore, according to Bawden et al. (1983), the net transport of calcium through the enamel organ at the secretory stage is not changed when rat molars were exposed to PTH. This may be a reflection of the differences in calcium transport mechanism between odontoblasts and ameloblasts.

D. M. Lyaruu: In the secretory ameloblasts studied the pyroantimonate reaction for calcium was consistently found associated with the inner aspect of the lateral plasma membranes. This calcium is presumably destined for enamel mineralization. Therefore, this membrane associated calcium, whether in "true" ionic form or bound to a calcium-binding protein or other macromolecules, has to be first transported through the cell in the direction of the enamel mineralization front before being extruded into the extracellular space via the Tomes' pro-

cess. Is there a specific mechanism which controls the extrusion process of calcium ions from the intracellular compartment of the Tomes' process into the enamel mineralization front?

Authors: According to Lyaruu et al. (1985), deep in Tomes' processes of secretory ameloblasts, the membrane and cytoplasmic antimonate activity was very low or absent, but it increased gradually toward the ameloblasts cell body. Our experience has shown that in some specimens it is hard to detect the calcium associated with the plasma membrane of Tomes' process, compared with that of the lateral plasma membrane. The calcium on the inner face of the plasma membrane of Tomes' process may be in an unstable state. The Ca-ATPase along the plasma membrane of Tomes' process might have an important role in controlling the extrusion process of calcium ions from the intracellular compartment into the enamel mineralization front.

D. M. Lyaruu: The pattern of calcium distribution in the odontoblasts of lower vertebrates was different from that of higher mammals. Is this difference also reflected in the type and/or composition of the mineral deposited in the dentin of these species?

Authors: No evidence is presently available to indicate it.

Additional References

Sakakura Y (1987) Effects of parathyroid hormone on odontogenesis of the mouse embryonic molar tooth in vitro. Calcif. Tissue Int. 40: 49-54.

Bawden JW, Deaton TG, Crenshaw MA (1983) The effects of parathyroid hormone, calcitonin, and vitamin D metabolites on calcium transport in the secretory rat enamel organ. J. Dent. Res. 62: 952-955.