Calcif Tissue Int (1982) 34:211–213

Calcified Tissue International

Correlation of ^{45}Ca Incorporation with Maturation Ameloblast Morphology in the Rat Incisor

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Summary. Rats were injected with ^{45}Ca and horseradish peroxidase to determine the patterns of ^{45}Ca incorporation into incisor enamel and the morphological types of the overlying maturation ameloblasts. ^{45}Ca autoradiography showed no differences in the patterns of incorporation into enamel between routinely embedded and freeze-dried specimens. Enamel overlaid by ruffle-ended ameloblasts was much more heavily labeled while that overlaid by smooth-ended ameloblasts showed only moderate labeling. The observations lend further support to the hypothesis that the ruffle-ended cells are very active in mineralizing enamel and that the smooth-ended cells are in a passive, restorative phase.

Key words: Rat-incisor-ameloblasts-enamel-^{45}Ca autoradiography.

The route and control of calcium movement into developing enamel has been the subject of considerable controversy. Most autoradiographic studies have shown a limited penetration of ^{45}Ca to the surface of secretory enamel and a heavy deposition of the tracer throughout the entire thickness of maturation (rapidly mineralizing) enamel (1-6). The *in vitro* culture study of 8-day-old rat molar tooth buds showed the penetration of ^{45}Ca to the entire thickness of enamel at all developmental stages present when the enamel organ was removed (5). These results were interpreted to indicate cellular control of calcium ingress into secretory enamel and the lack of such control in maturation enamel (5). However, Suga *et al.* (4) found that ^{45}Ca incorporation into guinea pig maturation enamel was not uniform and suggested that the peaks of radioactivity correlated with the cyclic morphological changes in maturation ameloblasts which were first described by Suga (7) and confirmed by others (8-11). These cyclic changes appear as alternating bands of two distinctly different types of ameloblasts, smooth-ended and ruffle-ended. The smooth-ended ameloblasts are characterized by a smooth distal border adjacent to the enamel surface and proximal intercellular junctions. The ruffle-ended ameloblasts have a ruffled distal border with many invaginations and have distal intercellular junctions.

Takano and Crenshaw (12) found that the distal intercellular junctions of the ruffle-ended ameloblasts prevented i.v. perfused lanthanum from reaching the enamel. Takano and Ozawa (10) and Kallenbach (13) also found these junctions were tight to i.v. injected horseradish peroxidase, but this tracer could penetrate to the enamel surface between the smooth-ended maturation ameloblasts (10).

The present study was undertaken to determine the correlation between ^{45}Ca incorporation into rat incisor enamel and the morphology of the overlaying maturation ameloblasts. In a concurrent and independent study, Reith and Boyde (14) found that ^{45}Ca entered maturing enamel in a cyclical manner. They did not make any direct comparisons of the pattern of ^{45}Ca incorporation with the cyclic changes in maturation ameloblasts. Nevertheless, they postulated that ruffle-ended maturation ameloblasts were associated with calcium entry into enamel.

Materials and Methods

$^{45}CaCl_2$ (1 μCi/g body wt.) was i.p. injected into 80 g Sprague-Dawley rats. Horseradish peroxidase (Sigma Type II) (HRP) (8 mg/.16 ml saline) was injected i.v. 25 min later in some rats to delineate the bands of smooth-ended ameloblasts (10). All rats were killed 30 min after the ^{45}Ca injection. The lower incisors with their enamel organs attached were dissected out and freed of alveolar bone. One was frozen in liquid nitrogen and freeze-dried. The other was fixed in 1% glutaraldehyde-formaldehyde, rinsed, incubated with diaminobenzidine (15) if injected with HRP, dehydrated and embedded in Polybed 812.

The freeze-dried incisors were ground, dry, to the midsagittal plane, and the embedded incisors were ground to 50 μm midsagittal sections. They were then placed against X-ray film (Kodak Industrex AA) for 2-5 days. The X-ray images were superimposed upon the respective ground sections in which the bands of smooth-ended ameloblasts were distinguished by the penetration of peroxidase.

To determine what the patterns of ^{45}Ca incorporation were in the absence of the enamel organ,

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0171-967X/82/0034–0211 $01.00

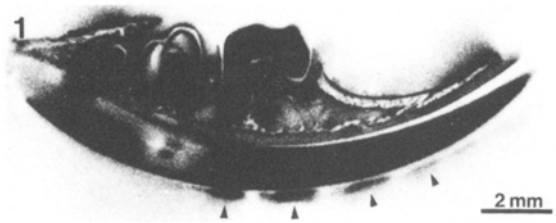

Fig. 1. Autoradiograph of a mandible ground to the midsagittal plane. 30 min after ^{45}Ca injection. Freeze-dried. Arrowheads show peaks of label in maturation enamel.

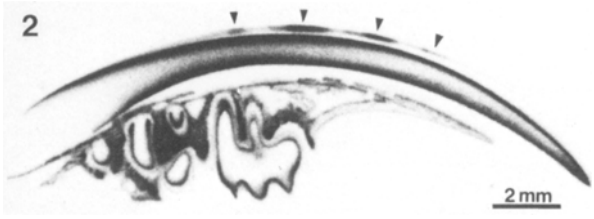

Fig. 2. Autoradiograph of mandible 30 min after injection of ^{45}Ca. Plastic-embedded ground section. Arrowheads show peaks of label in maturation enamel.

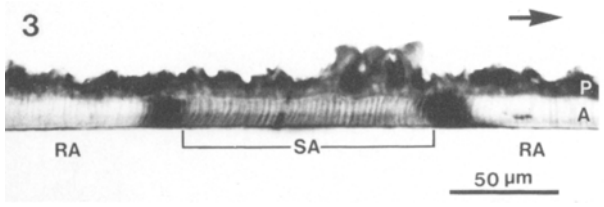

Fig. 3. Light micrograph of a longitudinal ground section of a lower incisor from a rat injected with ^{45}Ca and peroxidase. Smooth-ended ameloblasts (SA) are delineated by the penetration of the peroxidase. The papillary layer (P) is also labeled. Note the most intense labeling at the transitions of ruffle-ended ameloblasts (RA) to SA and of SA to RA. A, ameloblast layer. The arrow points in the incisal direction.

the lower incisors from rats were taken out, wiped free of cells and incubated in saline containing 13 µCi/ml ^{45}Ca for 30 min at 37°C. Other lower incisors were treated with 2.5% sodium hypochlorite for 30 min before they were incubated with ^{45}Ca in order to examine the effect of the matrix removal on the calcium deposition. These teeth were dehydrated, embedded in plastic, ground to the midsagittal plane and placed against X-ray film for 20 min.

Results

The patterns of ^{45}Ca distribution were essentially the same whether the specimens were freeze-dried or embedded in plastic, except that the diffuse labelings in the soft tissues and alveolar bone were lost in the embedding process (Fig. 1,2). Several peaks of radioactivity were evident in the maturation enamel of the incisors

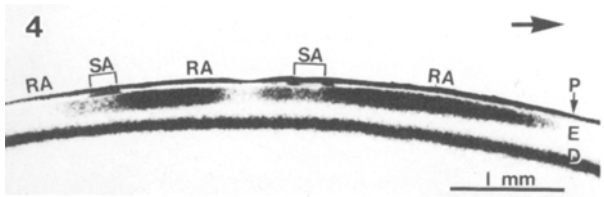

Fig. 4. Autoradiograph superimposed on the light micrograph of a lower incisor from a rat injected with ^{45}Ca and horseradish peroxidase. Two cycles of ^{45}Ca uptake in enamel (E) are shown. The bands of smooth-ended ameloblasts (SA) are delineated by the penetration of the peroxidase. RA, ruffled-ended ameloblasts. D, dentine. P, papillary layer. The arrow points in the incisal direction.

prepared by either method. Horseradish peroxidase injection also did not affect the patterns of ^{45}Ca distribution.

Horseradish peroxidase penetrated the extracellular space between the smooth-ended ameloblasts to the enamel surface so that the bands of smooth-ended ameloblasts were well delineated (Fig. 3). In many cases heavier peroxidase staining was evident at both ends of these bands where the transitions from ruffle-ended to smooth-ended and from smooth-ended to ruffle-ended ameloblasts take place. The extracellular space in the papillary layer was so heavily labeled that, at low magnification, the entire papillary layer seemed to be stained by the peroxidase (Fig. 3,4).

When the autoradiographs were superimposed over the respective peroxidase-stained sections, a correlation of the cyclic ^{45}Ca uptake by enamel with the bands of overlaying ameloblasts was discerned (Fig. 4). One cycle can be described as follows. There was a moderate incorporation of ^{45}Ca into enamel overlaid by ameloblasts undergoing the transition from ruffle-ended to smooth-ended cells. This region of moderate incorporation extended through the incisally adjacent band of smooth-ended ameloblasts. An immediate increase in ^{45}Ca uptake corresponded with the beginning of the incisally adjacent band of ruffle-ended ameloblasts. This uptake decreased in the incisal one-third to one-quarter of the ruffle-ended band where it was barely above background (Fig. 4).

The exposed enamel of the incisors incubated *in vitro* with ^{45}Ca were labeled uniformily in the secretory and maturation stages. The label was gradually confined to the surface in the late maturation stage until it was barely detectable at the pigmentation stage (Fig. 5). Sodium hypochlorite treatment did not affect this pattern.

Discussion

Our results show that routine histological processing is appropriate for the autoradiographic study of ^{45}Ca incorporation into enamel, at least at the level of low power light microscopy. We obtained the same patterns of uptake with the routine methods as those we obtained

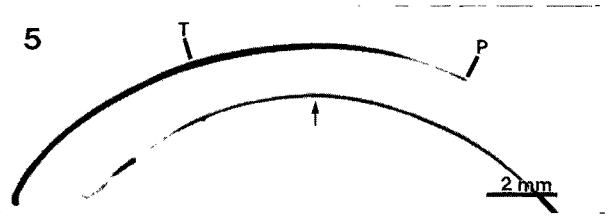

Fig. 5. In vitro ^{45}Ca uptake. Enamel organ wiped off. T, transitional stage. P, start of pigmentation. Arrow, cementum.

when the tissues were quick-frozen and processed dry.

We confirmed the results of Suga et al. (4) by showing that ^{45}Ca incorporation into rat incisor enamel was not uniform over the maturation stage. The autoradiographic patterns that we obtained were also similar to those obtained by Reith and Boyde (14) in their concurrent study.

Our in vitro experiments indicate that calcium can be incorporated into enamel without the presence of any active transporting mechanisms and that matrix removal was not a significant factor for calcium deposition. Therefore, the moderate incorporation of ^{45}Ca into enamel overlaid by smooth-ended ameloblasts and apically adjacent band of transition from ruffle-ended to smooth-ended ameloblasts can be ascribed to diffusion through the intercellular channels between these cells which have been shown to be open to the passage of horseradish peroxidase (10). Evidence for the diffusion of ^{45}Ca into enamel overlaid by the bands of transition from smooth-ended to ruffle-ended ameloblasts was not obtained. This failure may have been due to the shorter length of this transition (9), the limited resolution of ^{45}Ca autoradiographs and the high uptake into the adjacent enamel overlaid by ruffle-ended ameloblasts.

The more intense ^{45}Ca incorporation into enamel overlaid by ruffle-ended ameloblasts appears to be an active process because diffusion between these cells was prevented by tight intercellular junctions (10,12,13). It should be emphasized that this active process is restricted to the apical portion of the ruffle-ended cycle, and that it is not a characteristic of all ruffle-ended ameloblasts.

Takano and Ozawa (10) hypothesized that the ruffle-ended maturation ameloblasts are active transporting cells until they become smooth-ended which is the restorative phase of the cycle. Our observations of the ^{45}Ca incorporation patterns, especially that the most intense incorporation occurred at the apical portion of the ruffle-ended band, are consistent with this hypothesis.

Acknowledgments. This research was supported, in part, by NIH grants DE02668 and RR05333.

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