

AN APPROACH TO DETERMINE IF AMELOBLASTS MOVE TRANSVERSELY DURING RAT INCISOR AMELOGENESIS.

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INTRODUCTION

The prism pattern of rat incisor enamel implies that adjacent, transverse rows of ameloblasts slide sideways past each other in opposite directions, i.e. mesially and distally, during secretion of inner enamel (1-3). An alternative hypothesis suggests that the ameloblasts move along the path of their curved cell bodies (4). In either case, ameloblasts of adjacent rows will be displaced transversely relative to one another.

As the cells of the inner dental epithelium move with the erupting incisor in an incisal direction, they go through different phases: mitosis, differentiation, inner enamel secretion, outer enamel secretion, maturation, and regression (5-6). Using a single injection of ³H-thymidine, all the cells of the inner dental epithelium, which were in the S-phase of the mitotic cycle during the period when the radioactive thymidine was available, will be labeled and can be identified in the microscope by radioautography (6). The front of labeled ameloblasts will mark the position of the cells which were chronologically of the same age relative to the last S-phase of their cell cycle. In the rat incisor the front of labeled ameloblasts is characteristically C-shaped, with a short mesial and a long lateral arm directed incisally (7). The lines demarcating the start of inner enamel secretion, outer enamel secretion, and maturation are correspondingly C-shaped, which means that the ameloblasts along the C-shaped front are in the same functional stage (7).

The C-shaped front of labeled ameloblasts offers an opportunity to test the hypothesis of the transverse movement of ameloblasts during secretion of inner enamel in rat incisors. As the labeled ameloblasts enter into the secretory phase, they should start to move with an outward, incisal, and transverse component relative to the enamel-dentin junction (3). Depending on the direction of movement of the transverse rows to which they belong, the individual ameloblasts constituting the front of labeled cells will either move mesially or distally. The overall result will be a transverse narrowing of the C-shaped front (Fig. 24:1). The aim of the present study was to try to detect the predicted change in outline of the C-shaped front.

MATERIAL AND METHODS

The material is described in detail elsewhere (7). In short, two male Sprague-Dawley rats, weighing 107±5 g, were injected with a single dose of ³H-thymidine. The animals were sacrificed after two and eight days by perfusion with glutaraldehyde. After decalcification in EDTA and embedding in Epon, 1 µm-thick serial cross sections of the lower right incisors were cut with glass knives, transferred to glass slides, and processed for radioautography. Since at two days the ameloblasts had just started secreting inner enamel, and at eight days had just finished inner enamel secretion, this six-day interval was expected to give maximum narrowing of the C-shaped front. Graphic reconstruction of the C-shaped front was based on enlarged (X 200) drawings of the serial sections. The drawings, which were produced using a drawing tube, included the outline of the enamel-dentin junction, the outline of the enamel surface, and the outline of the ameloblast layer with the position of the most centrally placed labeled ameloblasts on the mesial and distal aspects (Fig. 24:2). Since the surface area of the enamel increases as the enamel grows in thickness, the C-shaped front at the onset and at the end of inner enamel secretion is not directly comparable. Therefore, the position of the labeled ameloblasts was projected onto the

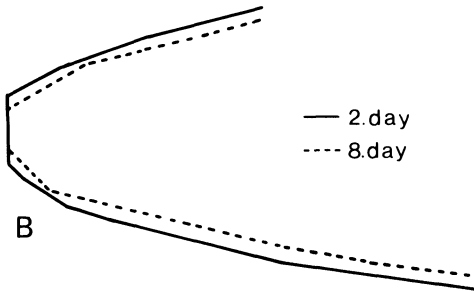
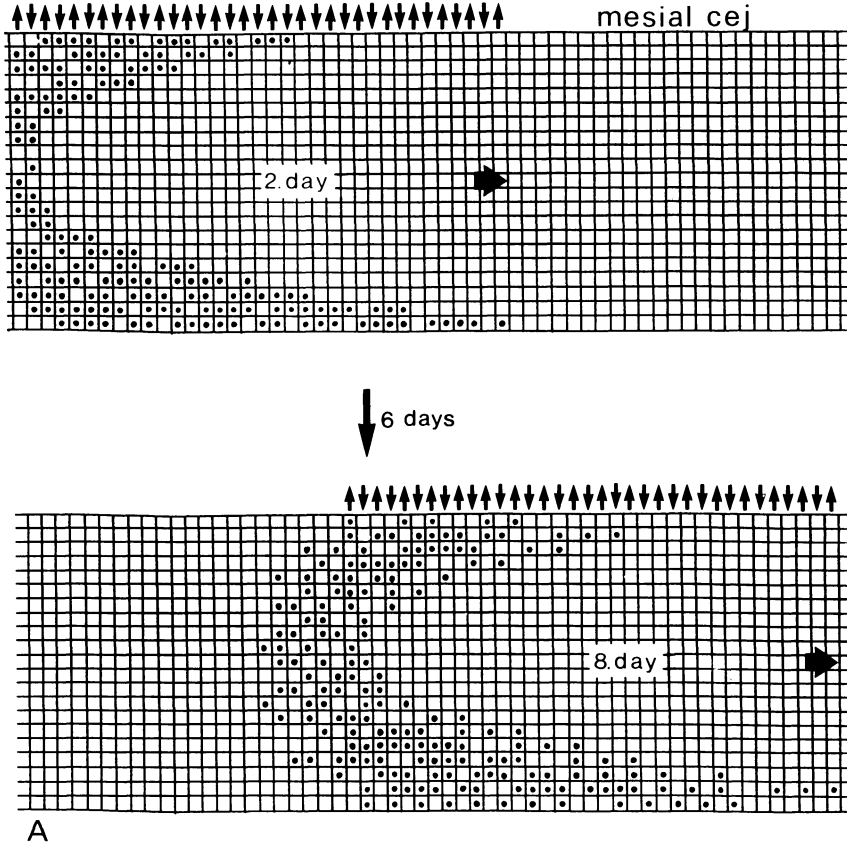


Fig. 24:1. Schematic representation of theoretical basis for the present study. (A) Labial aspect of rat incisor erupting in the direction of the large arrow. A cohort of labeled (dots) ameloblasts (squares) move with the incisor in incisal direction. As the ameloblasts begin secreting inner enamel (at about the second day), the transverse rows of ameloblasts, including the labeled ones, start moving transversely, with alternate rows moving in opposite directions (small arrows). After 6 days the cohort of labeled ameloblasts have moved through the zone of inner enamel secretion and the C-shaped front of labeled ameloblasts has narrowed. The transverse movement depicted is of about the same magnitude as that expected relative to the size of the tooth, cej = cemento-enamel junction. (B) The C-shaped fronts from the two stages are superimposed and compared. The front at eight days (broken line) is somewhat narrower than the front at two days (unbroken line).

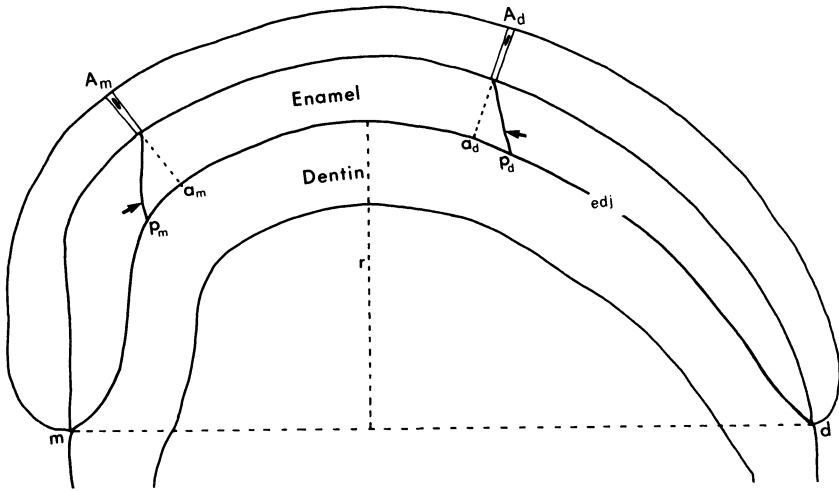


Fig. 24:2. Drawing of transverse section of lower rat incisor. Position of most centrally placed labeled ameloblasts (A_m and A_d) on mesial and distal aspects respectively, is projected onto the enamel-dentin junction (edj) at a_m and a_d . The starting position of labeled ameloblasts at enamel-dentin junction (p_m , p_d) is found by back-tracing their path of movement along the prisms (arrows). The following distances were measured: m-d, m-d along enamel-dentin junction, m- p_m , m- a_m , m- a_d , m- p_d , and r.

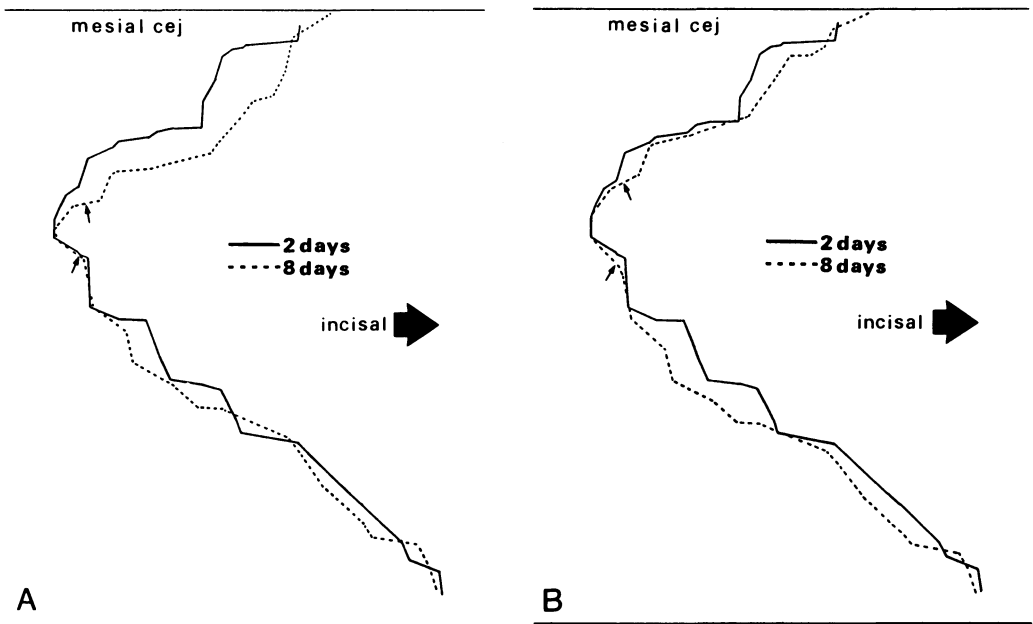


Fig. 24:3. Reconstructions of C-shaped fronts of labeled ameloblasts at two (unbroken line) and eight (broken line) days. The apical summit at eight days (from small arrows) had to be established by extrapolation. cej = cemento-enamel junction. (A) Fronts projected onto enamel-dentin junction. The curve at eight days seems slightly narrowed compared with the curve at two days. (B) Fronts traced back along the prisms to the starting position of the labeled ameloblasts at enamel-dentin junction. The curves are nearly congruent.

enamel-dentin junction, which is supposed to be stable throughout amelogenesis. In addition, the starting position of the labeled ameloblasts at the enamel-dentin junction was determined by tracing its path of incisal and transverse movement back along the prisms. The prism path was obtained from SEM micrographs of transverse and longitudinal sections cut through mature enamel of slightly older animals. Various distances were measured on the drawings (Fig. 24:2) using the MOP/AM01 manual optical image analyzing system (Kontron). The curved surface of the enamel-dentin junction was straightened out to a flat plane when the C-shaped fronts were reconstructed and compared. The reconstructed fronts were corrected for incisal ameloblast movement. Due to some missing sections, the apical summit of the front at eight days had to be established by extrapolation.

The area of the curves representing the reconstructed C-shaped fronts was calculated. The baseline chosen was the transverse line passing through the most incisally situated point on the curve.

RESULTS

Figure 24:3 shows the reconstructed C-shaped fronts of labeled ameloblasts at two and eight days after injection with ³H-thymidine. When the fronts were projected onto the enamel-dentin junction, the front at eight days seemed slightly narrowed compared with the front at two days (Fig. 24:3A). The area at eight days was 3 % smaller than at two days. When the fronts were traced back along the prisms to the starting position of the labeled ameloblasts at the enamel-dentin junction, the two fronts were closer to congruence (Fig. 24:3B). The difference in area was only 0.6%.

DISCUSSION

The expected narrowing of the C-shaped front of labeled ameloblasts due to their transverse movement during inner enamel secretion, is relatively modest (Fig. 24:1). Therefore, it was not surprising that the front at eight days seemed only slightly narrower than at two days (Fig. 24:3A). Traced back along the prisms to the starting position of the labeled ameloblasts at the enamel-dentin junction, the two curves were expected to be identical. Except for a slight overall lateral displacement of the back-traced front at eight days compared with the back-traced front at two days, which may be due to individual variations or to slightly oblique sectioning, the two curves were congruent (Fig. 24:3B).

The expected theoretical decrease in curve area from the second to the eight day is about 7.7 %, provided that all ameloblasts at the C-shaped front move centrally. Since it is likely that some of the ameloblasts constituting the C-shaped front move away from the center, the actual narrowing would be smaller. In the present study the detected decrease in curve area was 3 %.

Aside from the prism pattern itself, there are several morphological features suggestive of a transverse movement of rat incisor ameloblasts: the arrangement of ameloblasts into transverse rows just prior to and during amelogenesis (2,4,8-15), the orientation of the trailing Tomes' processes in the direction of the prisms (4,11-13), and the polarity of the distal cell web (2,10-12,15).

There is an apparent contradiction between the notion of transverse ameloblast movement and the existence of fixed mesial and lateral cemento-enamel junctions. A reconciliation may be sought in the following facts: the prism rows (and presumably the ameloblast rows) do not extend across the whole mesio-distal width of the enamel and deviate apically toward the lateral cemento-enamel junction (16), prisms (and presumably ameloblasts) may change direction in the inner enamel (16), and some of the ameloblast loss observed during amelogenesis (14) appears to be due to ameloblast degeneration in the secretory zone along the lateral and especially the mesial cemento-enamel junctions (17).

Although the present study tends to support the transverse sliding of ameloblast rows during rat incisor amelogenesis as premised by the prism pattern, the uncertainty of the method (few animals, missing

apical summit of front at eight days, irregular outline of fronts) precludes an unequivocal conclusion to be drawn.

ACKNOWLEDGEMENTS

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Poster 24

Goldberg: Dr. Risnes I think that it is a very interesting result and the shape which is seen as an arch shaped structure is fascinating. What could be expected from a functional point of view are lateral banding, as it is seen in the maturation part. What is their concept? Is this arch shape related to the eruption?

Risnes: I think maybe the C-shape is related to the organization of the enamel organ at the far apical part of the incisor where the cells are generated.

Warshawsky: I would like to amplify that comment. The shape of the C is something which Charlie Smith demonstrated in 1976 (*Am. J. Anat.* 145: 225-260, 1976), and it reflects the shape observed during the sequence of differentiation from a presecretory cell related to pulp, then related to dentine, then related to inner enamel secretion and outer enamel secretion. All of these transitions occur on that C-shape curve. The distribution of the division of cells, which is what the curves in the poster represent, as indicated by nuclei labelled with ^3H -thymidine, follows that differentiation pattern. What happens in maturation, in terms of giving these C shaped bands, is probably related to the modulation pattern, but not very directly.

Limeback: If this C shape represents changes in the organisation of the enamel organ cells and, presumably, the activity of the maturation ameloblasts would follow this pattern as well, then how would you explain the swirling patterns that Marc McKee has shown in porcine unerupted canines?

Risnes: The C-shaped front that we deal with here is related to movement of cells while the swirling figures obtained by Dr. McKee is more like a wave sweeping across the ameloblasts, it is not the cells themselves that move in that pattern.

Warshawsky: There is obviously a difference of opinion between the Norwegian author and the Canadian contingent, that is not very fundamental. Nevertheless, when Dr. Risnes says movement of cells I immediately react as I am sure Dr. Smith does too, because this does not represent a movement of cells. This represents a movement of the tooth. The cells are just sitting passively on this surface. What we are trying to determine in this poster is whether there is a movement of cells in a lateral direction, not in an anterior/posterior direction.

McKee: The swirling patterns result from a transmission of some sort of a message from one cell to another in an apical to incisal direction. What I would predict is that a modulation wave is set up whereby the maturation ameloblasts communicate with one another in a straight and oblique banding pattern in the rat incisor but in a swirling pattern in the pig canine.

Snead: Dr. Warshawsky, since gap junctions transmit information almost instantaneously, how is it then, that the apparent elapse of time can occur? I find the idea of the gap junctions, superimposing a second set of informational molecules, with the biomechanical and biophysical limitations of gap junctions, to be somewhat difficult to imagine.

Berdal: Do you not think that ionic calcium could be the message for the modulation process?

Young: We had the pleasure of having Dr. Warshawsky with us last year as a visiting Professor and he did spend some time trying to explain the object of this particular experiment to us. I must say it was always very enlightening. As I understand it, it is a device to explain how the prisms of uniserial rodent enamel

are formed in marked decussations. This suggests that individual ameloblasts in different cohorts move in different directions. From what Dr. Risnes has presented to us this does not appear to occur. So we are left with a fascinating dilemma as to how to explain this three dimensional organisation. Having posed the problem to us, can you now give us your world shattering solution?

Risnes: I agree that the main purpose of this presentation is the approach itself. The material is small and therefore the result is uncertain. Personally, and I may disagree with my co-authors in this, I think that there is a certain tendency which tends to support the transverse movement of ameloblasts.

Robinson: I think we should congratulate these authors in view of their disagreements, that they ever got together at all to produce an abstract.

Warshawsky: In 1971, when Dr. Smith started these experiments with thymidine, he had prepared, a series of transverse sections cut serially at $1\mu\text{m}$ through the entire length of the incisors at various times after the injection of ^3H -thymidine. We had used this data to put together a number of papers that dealt with the anterior migration of the tooth and how the cells behaved on its surface. These papers of course became known to Steinar Risnes in Norway, and about 10 years ago, Steinar visited us and said "here is an excellent way in which we can test the sliding row hypothesis". I had always been intrigued by the comments contained in (Tooth Enamel I, pg 197-198, 1965. publ. John Wright and Sons), between the late Ed Reith and Alan Boyde about the "dancing ameloblasts" on the surface of the incisors, as they make enamel rods that decussate, and I have always been looking for these "dancing ameloblasts". So I was delighted when Dr. Risnes suggested that this was a possible way in which we could test the movement of cells. Dr. Risnes came to Montreal for 6 months to use the slides that Dr. Smith had prepared. He laboriously transferred the information from sections to a surface plot which is the result shown in poster 24. On the last day of his visit, the two plots which were produced at the beginning and the end of secretion, were superimposed, and we ceremoniously sat there and said "it proves nothing, there is no sliding". Dr. Risnes could not accept this and so he went back to Norway saying that "he would repeat the experiments". Now anybody in their right mind would be crazy to repeat this type of experiment and consequently he did not. So what we have now is the data and these curves. I still believe that the ameloblasts do not slide, Dr. Risnes still believes that they do. The data shows for the first time an experimental approach that was tried to verify the sliding rows, and I think as such it is worth reporting here, but we still have an equivocal result.