J. Anat. (1981), **133**, 3, pp. 381–388
With 9 figures
Printed in Great Britain

The arrangement of ameloblasts on the surface of maturing enamel of the rat incisor tooth

EDWARD J. REITH* AND ALAN BOYDE

* *Department of Anatomic Sciences, Temple University School of Dentistry, 3223 North Broad Street, Philadelphia, Pa. 19140, U.S.A. and Department of Anatomy and Embryology, University College London, London WC1E 6BT, U.K.*

(*Accepted 22 October* 1980)

INTRODUCTION

The formation of enamel involves two major stages of cytological activity, designated matrix production and maturation, which are readily visualized in paraffin sections (Marsland, 1951, 1952; Reith & Butcher, 1967; Suga, 1959; Symons, 1955; Wassermann, 1944). It has been demonstrated in several recently published papers that in the rat incisor (Boyde & Reith, 1976, 1977; Josephsen & Fejerskov, 1977) and in the rat molar (Reith & Boyde, 1979) the maturation ameloblasts undergo changing structural patterns which in the incisor, at least, are cyclical.

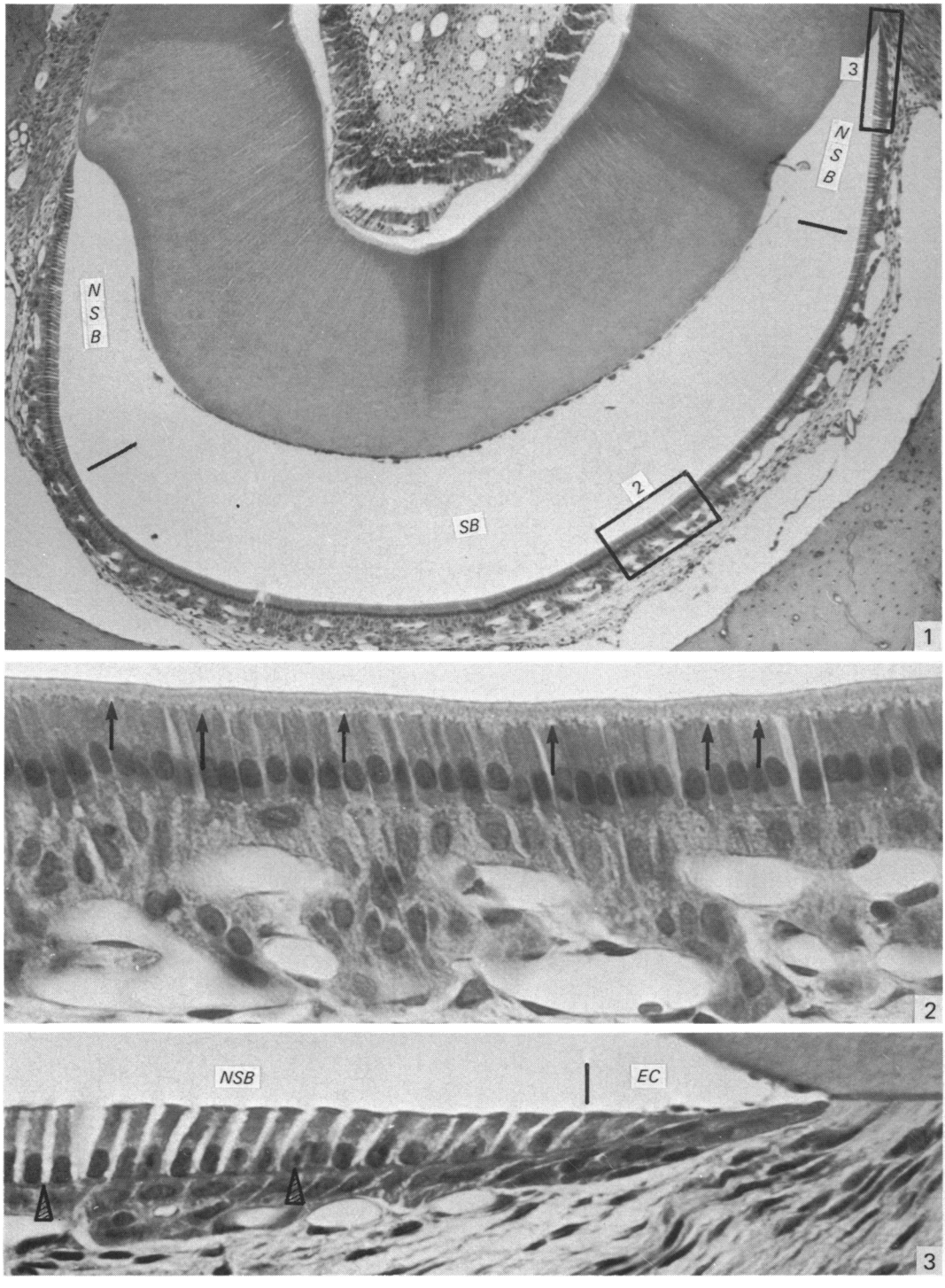
At the ultrastructural level, both with the transmission as well as the scanning electron microscope, it has been shown (Boyde & Reith, 1976, 1977; Reith & Boyde, 1979; Josephsen & Fejerskov, 1977) that during one period of the cycle the maturation ameloblasts possess a 'striated border' or a distinctive apical specialization, whereas during another period they lack a discernible striated border. Although they did not allude to cyclical alteration in maturation ameloblasts, the data of Kallenbach (1968, 1970), Kurahashi & Moe (1969), Reith (1961, 1970) and Warshawsky & Smith (1974) also indicate the presence of maturation ameloblasts with and without a striated border. What has not been determined in any of the above studies is how the different cell types are related to each other in two dimensional terms on the surface of the maturing enamel. Accordingly, an analysis of serial sections of a segment of the incisor tooth of the rat has been carried out in order to answer the following question: Does one of the patterns constitute islands of cellular activity which are surrounded by the other, or do each of the patterns alternate successively on the tooth surface? The following results provide our findings on this question.

MATERIALS AND METHODS

Adult rats were fixed by perfusion with a mixture of glutaraldehyde and formaldehyde. The lower jaws were removed, trimmed and demineralized in 15% formic acid. They were then embedded in paraffin, serially cross sectioned through the incisor and stained with haematoxylin and eosin. Three sections were placed on each slide and one of the three cross section sections was selected for analysis.

For the analysis, the cross sectioned profile of the enamel organ was divided into 10 segments, and the presence or absence of a striated border in each segment was plotted on a sheet of paper marked off to show the face of the maturing enamel as a

* Reprint requests to Edward J. Reith.


3
NSB
NSB
2
SB
1
2
NSB
EC
3

two dimensional uncurved surface. It was possible to cut a single block of tissue without changing the orientation of the block and plot the distribution of the ameloblast apical configurations. The results of such a plot are given in this report. As a reference point, the serial sections were taken from a segment of the jaw which also included the first molar tooth.

RESULTS

In every section of this series, cells with a striated border and cells without a striated border could be readily identified. Those with a striated border occupied about 71 % of the enamel surface, whereas those without a striated border occupied about 23 % of the surface. Unclassifiable cells located towards the edges of the enamel organ occupied the remaining 6 % of the surface.

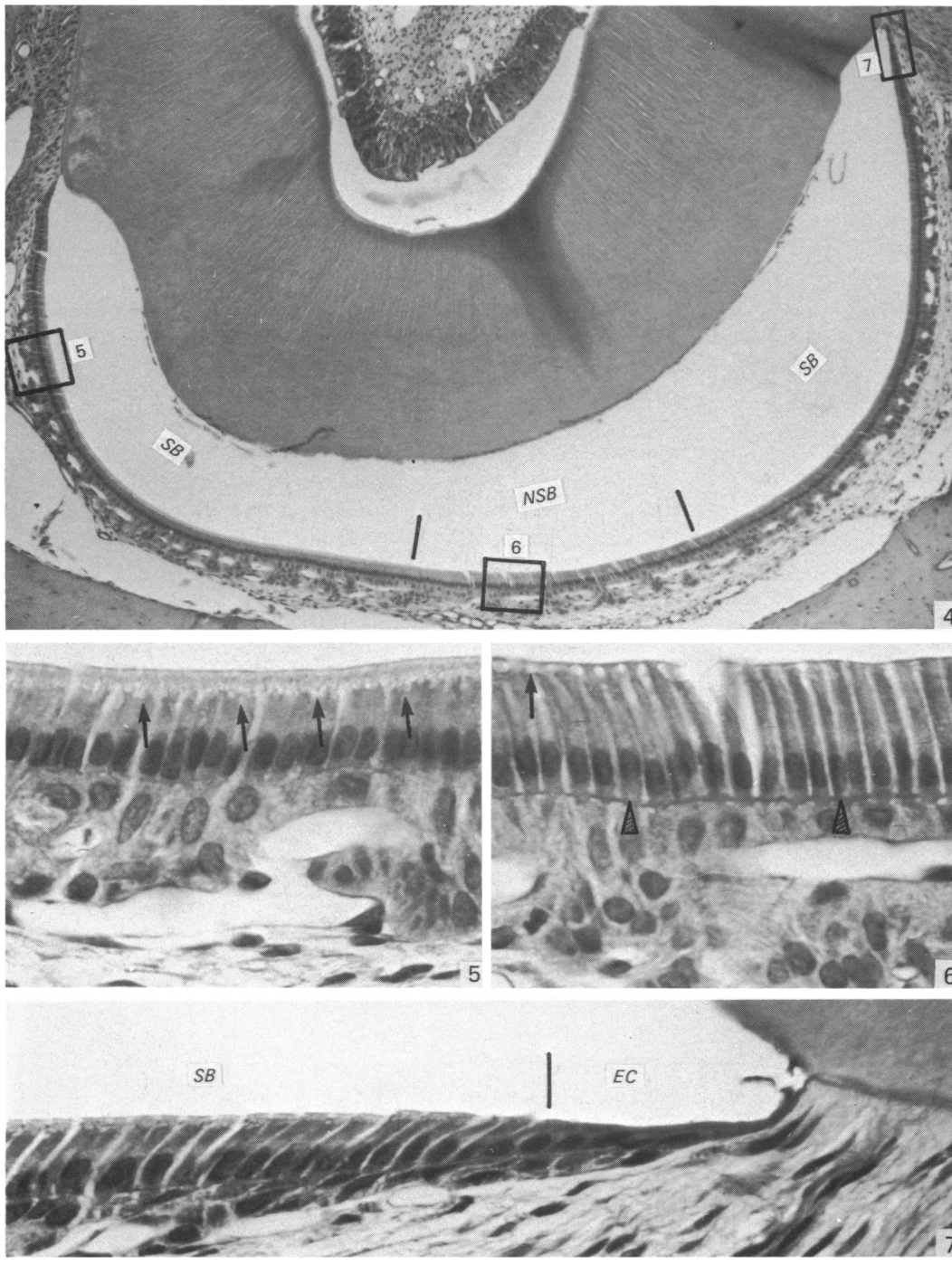
A representative cross section through the incisor tooth is shown in Figure 1. The specimen was on slide 40 of the collection, and this is shown by a horizontal line drawn through the plot in Figure 9. The maturing enamel was lost during the demineralization process and the maturation ameloblasts are seen on one edge of the enamel space. The central region was made up of striated border cells. A higher magnification of part of this region (rectangle 2 in Fig. 1) is shown in Figure 2. It reveals the presence of maturation ameloblasts with a distinctive apical specialization. The apical specialization consisted of both a striated border and also of relatively large vacuoles at the base of the striated border. Generally, the vacuoles appeared to be empty. However, two areas were observed where the vacuoles of the striated border cells did not display an empty appearance (Fig. 8). These areas correspond approximately to the location of the letters A and B in Figure 9. Serial sections showed that these areas were surrounded by cells where the vacuoles were clearly apparent. There was relatively little intercellular space between the ameloblasts with a striated border when compared to the ameloblasts without a striated border. However, this was a relative feature, and some ameloblasts with a striated border displayed larger amounts of intercellular space.

The lateral regions in this section contained non-striated border cells. A higher magnification of one of these regions (rectangle 3 in Fig. 1) is shown in Figure 3. The non-striated border cells were typically surrounded by a considerable amount of extracellular space. The presence of this large extracellular space made it possible to identify cells of this arrangement at both extremities of the enamel organ strip, even at the relatively low magnifications such as those shown in Figure 1. A feature of the non-striated border cells was that they were joined at their basal or nuclear poles, thereby separating a lateral inter-ameloblast space from the space between the papillary-ridge cells (Figs. 3, 6). It was not possible in these preparations to be certain that the striated border cells also formed contacts which would separate the lateral inter-ameloblast space from the space between the papillary-ridge cells.

Fig. 1. Cross section through lower incisor of rat (slide 40 of series). Most of the ameloblasts are striated border cells (*SB*). Those within the rectangle marked 2 are shown at higher magnification in Fig. 2. Non-striated border cells (*NSB*) are at each end of the ameloblast strip. Those within rectangle 3 are shown at higher magnification in Fig. 3. × 100.

Fig. 2. Striated border cells from rectangle 2 in Fig. 1. The arrows point to vacuoles at the base of the striated border. × 650.

Fig. 3. Edge of the enamel organ strip showing non-striated border cells (NSB) and edge cells (EC). Basal cell-to-cell contacts of non-striated border cells are evident (arrowheads). The basal cell-to-cell contacts are not seen at basal pole of edge cells. × 650.



Figures 3 and 7 also show that neither the non-striated border nor the striated border cells went entirely to the edge of the enamel organ. The ameloblasts here were short, possessed no apical specialization discernible with the light microscope, were not surrounded by a large amount of extracellular space, and were not clearly joined at their apical poles. Hence no attempt was made to classify them as striated border

or non-striated border cells. These cells are represented by the intermediate shading in Figure 9. It should be noted that they were found along the entire edge of the enamel organ on both the mesial and lateral sides.

Another representative cross section through the incisor tooth is shown in Figure 4. This specimen was on slide 85 of the collection, and this is also shown by a horizontal line through the plot in Figure 9. In this cross section the non-striated border cells occupied a centre position in the enamel organ strip and striated border cells extended to each end as far as the 'edge cells' (Fig. 7). Again, in those maturation ameloblasts which showed a well developed apical specialization (Fig. 5), large vacuoles were present at the base of the striated border.

When the distribution of maturation ameloblasts based on the presence or absence of striated border was plotted for each slide in the cross section series, a pattern was obtained as shown in Figure 9. The darkly shaded areas in the Figure represent the location of cells without a striated border. One band (I) began at the mesial side of the enamel and extended across the enamel surface to the lateral side. In doing so, it separated a territory of striated border cells (A) from another territory (B) of cells with similar apical configurations. In the upper right of the Figure, another band (II) extended partly across the enamel surface and again it was disposed between two territories (B and C) of striated border cells. In the lower left there was a small length of another band of non-striated border cells. As already mentioned, the edge of the enamel organ consisted of short obliquely positioned ameloblasts.

DISCUSSION

It should be emphasized that excluding the 'edge' cells, the plotting of cell positions on the enamel surface was limited to cells with discernible apical specializations (striated border cells) and those without such a specialization at their apical pole. No attempt was made to characterize cells at the junction between these two cell groups, although transitional configurations do occur (see Fig. 6). The transitional forms have been clearly identified and characterized by Josephsen & Fejerskov (1977). These workers showed that ameloblasts in transition from one configuration to another (R-SA and S-RA, Fig. 43, Josephsen & Fejerskov, 1977) display fewer apical vacuoles adjacent to the striated border. Moreover, the membrane infoldings were less pronounced in these transitional forms. By the use of the two dimensional plotting of cells on the surface of maturing enamel it was possible to show in the present investigation that additional groups of striated border cells without numerous 'empty' vacuoles, not located in a transitional position, also occur. The presence of these less vacuolated cells (Fig. 8) suggests that, even within the territory of the striated border cells, there is a wave of activity. This lesser degree of vacuolation may reflect the beginning or the end of a period of endocytosis (or exocytosis) by the

---

Fig. 4. Cross section through lower incisor of rat (slide 85 of series). Most of the cells are striated border cells (SB) but at this level non-striated border cells (NSB) are at the centre of the strip. The rectangular areas are shown at higher magnification in Figs. 5, 6 and 7. × 100.

Fig. 5. Typical striated border cells with large vacuoles (arrows) at base of striated border. × 650.

Fig. 6. Typical non-striated border cells on right side of Figure. On the left an apical terminal bar apparatus is forming (arrow). Arrowhead indicates basal cell-to-cell contacts of non-striated border cells. × 650.

Fig. 7. Edge of enamel organ strip showing striated border cells (SB) and edge cells (EC). × 650.

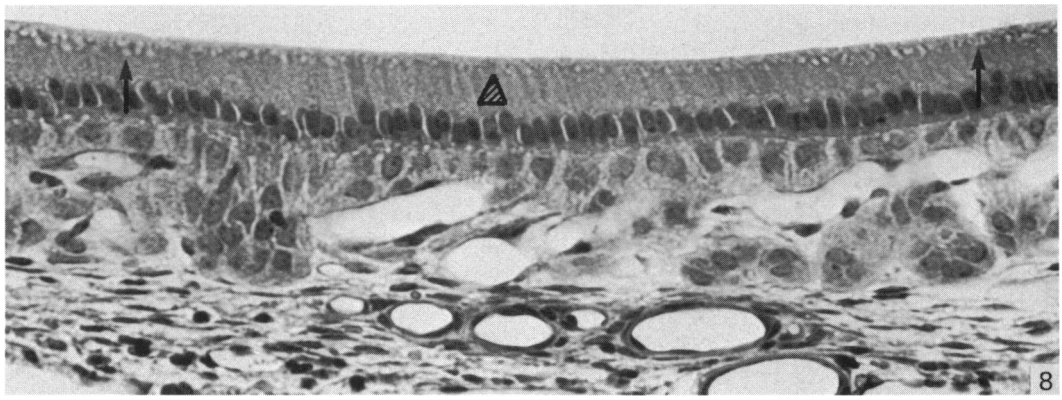


Fig. 8. Segment of striated border cells (slide 142 of series) showing vacuoles (arrows) which are readily apparent and a small area (arrowhead) where vacuoles are not so apparent. × 490.

striated border cells. Secondly, in some areas the striated border cells appeared to be surrounded by more extracellular space than in other areas. These differences with regard to extracellular space were difficult to display convincingly in photomicrographs (*e.g.*, compare Fig. 7 with Figs. 5 and 2) and they need to be quantified with optical systems which provide greater resolution. Again, these differences appeared to be a question of degree, and conceivably they reflect some phases in the functioning of the striated border cells.

Although it was hoped that the paraffin sections would provide clear-cut indications of where maturation ameloblasts form apical contacts which serve to separate the lateral inter-ameloblast space from the space of the papillary-ridge cells, this proved to be not possible. Although the role of papillary cells undoubtedly relates to the function of the maturation ameloblasts (Kallenbach, 1966), this relationship has not yet been clarified (Skobe & Garant, 1974).

The fact that the bands of striated border ameloblasts cross the long axis of the tooth obliquely is not surprising. Smith & Warshawsky (1976) have shown that the ameloblasts enter the maturation zone as an obliquely oriented front with a U-shaped curve to it. Thus, any cyclical changes in ameloblast behaviour would be expected to follow this pattern. Moe's (1979) data also show that the secretory-transitional zone boundary is curved, although not quite the U-shape reported by Smith & Warshawsky.

Perhaps the most significant finding in this study is the fact that the non-striated border cells cross the enamel surface obliquely from the mesial to the lateral edge. It seems reasonable to conclude that successive narrow bands of non-striated border cells are interposed between wider bands of striated border cells which also cross the developing incisor surface obliquely from one side to the other. While the functional significance of this arrangement is not clear at present, several points emerge. One is that a simple cross section through the enamel of a rat incisor does not represent enamel being worked on by one maturation ameloblast cell type. Thus, the biochemical changes reported by Robinson, Briggs, Atkinson & Weatherall (1979) probably reflect the activity of at least two maturation cell types. A second point is that, at present, it is not possible to assess what each cell type is doing. Certainly, the striated border cells display features of absorptive cells. On the other hand, the non-striated border cells do not yield clues as to their function. Nevertheless, the

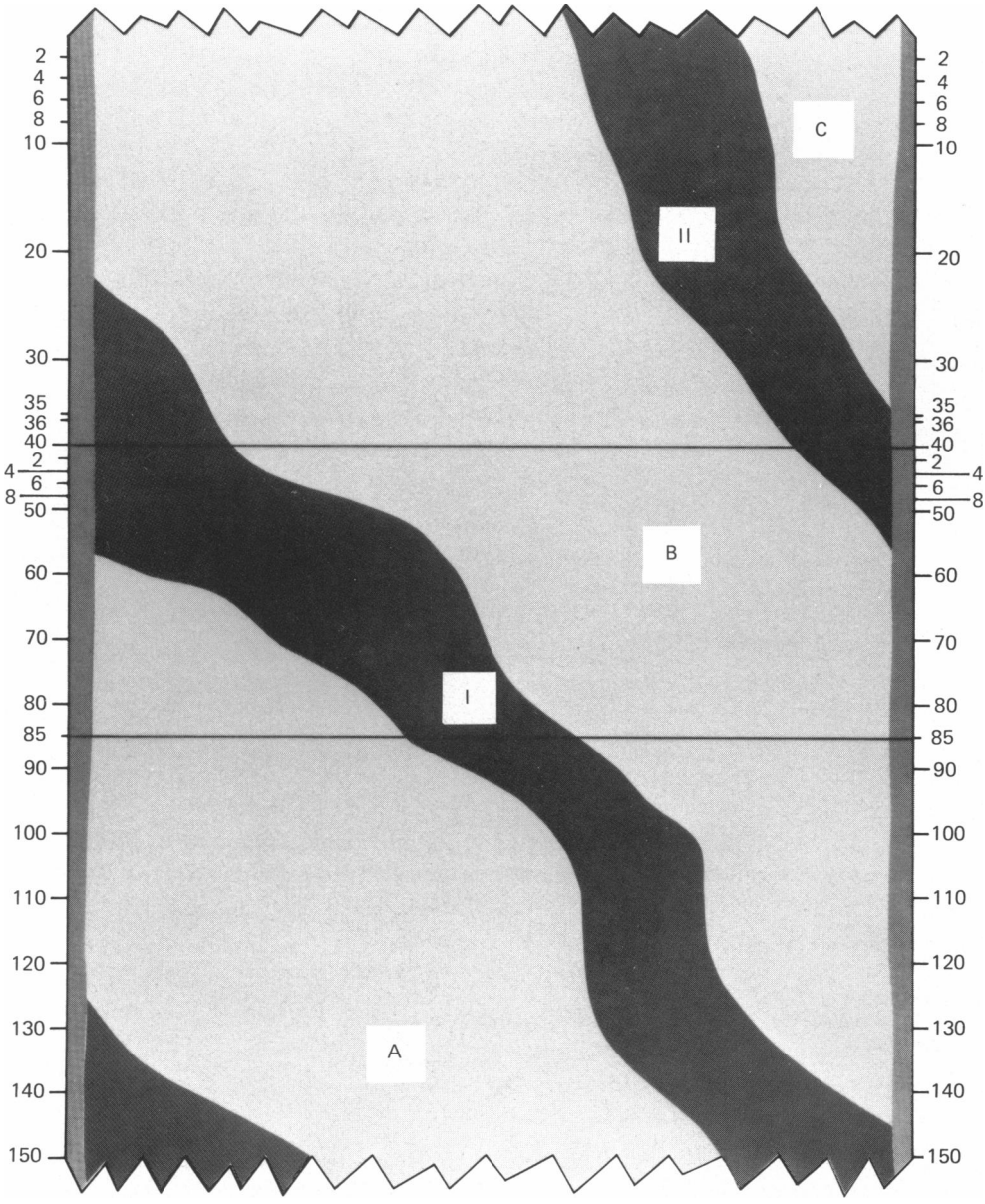


Fig. 9. Plot of striated border cells (light shading), non-striated border cells (dark shading) and edge cells (intermediate shading) on maturing enamel surface of rat incisor tooth. Serial numbers of sections shown along each margin.

pattern displayed in Figure 9 outlines those which need to be considered when looking for correlations between cellular activity and changes in the enamel.

SUMMARY

The location of striated border cells verus non-striated border cells during enamel maturation in the rat incisor was studied by light microscopy. Serial cross sections of the lower incisors were examined from a segment of the incisor in sections which also

included the first molar tooth. In the series recorded here every cross section showed both striated and non-striated border cells. A map showing the distribution of cells on the enamel surface, as plotted from their position in each of the cross sections, reveals that the non-striated border cells traverse the enamel as oblique bands. Between the narrow bands of non-striated border cells were wide bands of striated border cells. The non-striated border cells were joined at their basal nuclear poles by contacts which appeared to separate the lateral inter-ameloblast space from the space between the papillary-ridge cells. Neither the striated border cells nor the non-striated border cells went entirely to the edge of the enamel organs. The above pattern of striated border cells and non-striated border cells is regarded to be a manifestation of the cyclical activity of maturation ameloblasts.

This project was supported in part by NIH Grant DE 04059-05. The authors wish to express their appreciation to Helen Ruane and Marguarita Schmid for their excellent technical assistance and to Margaret Pickard for typing the manuscript.

REFERENCES

BOYDE, A. & REITH, E. J. (1976). Scanning electron microscopy of the lateral cell surfaces of rat incisor ameloblasts. *Journal of Anatomy* **122**, 603–610.

BOYDE, A. & REITH, E. J. (1977). Scanning electron microscopy of rat maturation ameloblasts. *Cell and Tissue Research* **178**, 221–228.

JOSEPHSEN, K. & FEJERSKOV, O. (1977). Ameloblast modulation in the maturation zone of the rat incisor enamel organ. A light and electron microscopic study. *Journal of Anatomy* **124**, 45–70.

KALLENBACH, E. (1966). Electron microscopy of the papillary layer of rat incisor enamel organ during enamel maturation. *Journal of Ultrastructure Research* **14**, 518–533.

KALLENBACH, E. (1968). Fine structure of rat incisor ameloblasts during enamel maturation. *Journal of Ultrastructure Research* **22**, 90–119.

KALLENBACH, E. (1970). Fine structure of rat incisor enamel organ during late pigmentation and regression stages. *Journal of Ultrastructure Research* **30**, 38–63.

KURAHASHI, Y. & MOE, H. (1969). Electron microscopy of the ameloblasts in the later stage of the matrix formation stage and in the maturation stage of the enamel in rat. In *Hard Tissue Research* (ed. S. Araya *et al.*), pp. 256–285. Tokyo: Ishiyaku Press.

MARSLAND, E. A. (1951). A histological investigation of amelogenesis in rats. I. Matrix formation. *British Dental Journal* **91**, 251–261.

MARSLAND, E. A. (1952). A histological investigation of amelogenesis in rats. II. Maturation. *British Dental Journal* **92**, 109–119.

MOE, H. (1979). Physiological cell death of secretory ameloblasts in the rat incisor. *Cell and Tissue Research* **197**, 443–451.

REITH, E. J. (1961). The ultrastructure of ameloblasts during matrix formation and the maturation of enamel. *Journal of Biophysical and Biochemical Cytology* **9**, 825–840.

REITH, E. J. (1970). The stages of amelogenesis as observed in molar teeth of young rats. *Journal of Ultrastructure Research* **30**, 111–151.

REITH, E. J. & BOYDE, A. (1979). A correlated scanning and transmission electron microscopic study of maturation ameloblasts in developing molar teeth of rats. *Cell and Tissue Research* **197**, 421–431.

REITH, E. J. & BUTCHER, E. O. (1967). In *Structural and Chemical Organization of Teeth* (ed. A. E. W. Miles), vol. 1, ch. 9. New York: Academic Press.

ROBINSON, C., BRIGGS, H. D., ATKINSON, P. J. & WEATHERALL, J. A. (1979). Matrix and mineral changes in developing enamel. *Journal of Dental Research* **58B**, 871–880.

SKOBE, Z. & GARANT, P. R. (1974). Electron microscopy of horseradish peroxidase uptake by papillary cells of the mouse incisor enamel organ. *Archives of Oral Biology* **19**, 387–395.

SMITH, C. E. & WARSHAWSKY, H. (1976). Movement of entire cell populations during renewal of the rat incisor as shown by radioautography after labeling with ^{3}H-thymidine. *American Journal of Anatomy* **145**, 225–260.

SUGA, S. (1959). Amelogenesis: some histological and histochemical observations. *International Dental Journal* **9**, 394–420.

SYMONS, N. B. B. (1955). Alkaline phosphatase activity in developing teeth of the rat. *Journal of Anatomy* **89**, 238–245.

WARSHAWSKY, H. & SMITH, C. E. (1974). Morphological classification of rat incisor ameloblasts. *Anatomical Record* **179**, 423–446.

WASSERMANN, F. (1944). Analysis of the enamel formation in continuously growing teeth of normal and vitamin C deficient guinea pigs. *Journal of Dental Research* **23**, 463–509.