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Cellular Renewal in the Enamel Organ and the Odontoblast Layer of the Rat Incisor as Followed by Radioautography Using ^{3}H-Thymidine

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ABSTRACT Renewal of the cell populations of the incisor was studied in 100 gm male rats injected with a single dose of ^{3}H-thymidine and sacrificed at various times from one hour to 32 days after injection. Radioautographs showed that a cohort of labeled cells within the enamel organ, odontoblast layer, and pulp was carried passively with the erupting incisor from the apical end towards the gingival margin where the life cycle of these cells was terminated. Labeled cells in the upper and lower incisor, although traversing different absolute lengths, were found in approximately the same functional stage of their life cycle at similar times after the injection. Thus, by one and one-half days labeled ameloblasts began inner enamel secretion and, by eight days (upper) or nine days (lower), completed outer enamel secretion. By 32 days labeled ameloblasts had traversed the entire maturation zone and were located at the gingival margin. Labeled odontoblasts followed closely the movement of labeled ameloblasts. The mean rate of ameloblast migration was 567 μm/day on the upper incisor and 651 μm/day on the lower. For the odontoblasts this rate was 500 μm/day (upper) and 631 μm/day (lower). Finally, it was found that as the rat aged, the duration of the life cycle for epithelial and pulp cell populations of the incisor increased because of growth within the longitudinal axis of the tooth. It was concluded that the apical end of the incisor literally "grows backward" in the bony socket, and hence, the duration of the life cycle becomes greater simply because it takes cells longer to physically reach the gingival margin.

Cellular renewal is clearly a process of fundamental importance in the rodent incisor since, once the tooth erupts, there must be a perpetual supply of new cells to assure the capacity for renewal of the dental tissues throughout the life of the animal. As with all other renewing systems of the body, renewal of the rodent incisor encompasses the orderly progression of cells through individual "life cycles" comprising separate stages of proliferation, differentiation, function and death (Cleaver, '67). Similar to many other renewing systems, the cells move away from their points of origin as they pass through their life cycles. Thus far most studies of cellular renewal in the rodent incisor have focused, directly or indirectly, on cell proliferation.[3] Consequently, cell migration remains a component of the renewal process which has been only poorly characterized (Hwang and Tonna, '65; Zajicek, '74).

The major problem in characterizing cell migration in the rodent incisor has been to establish a reproducible and precise method for measuring the enormous distances which migrating cells travel in the course of their life cycle. Previous investigators have expressed the location of the migrating front of ^{3}H-thymidine labeled cells by either the number of cell

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[3] Messier and Leblond, '60; Blackwood and Robins, '64; Cotton, '64; Hwang and Tonna, '65; Sasaki, '65; Hwang et al., '66; Chiba et al., '67; Robins, '67; Chiba, '68; Grewe and Felts, '68; Lavelle, '68a,b; Shibata and Stern, '68; Lavelle and Moore, '69; Scott, '69; Zajicek and Bar-Lev, '71a,b; Michaeli and Greulich, '72; Kiely and Domm, '73; Zajicek, '74.

positions advanced from a starting point at one hour after injection (Zajicek and Bar-Lev, '71a; first used by Starkey, '63, in rabbit molars), or, by the distance (μm) moved from some reference point situated near the apical end of the tooth (Hwang and Tonna, '65; Chiba, '68; Zajicek, '74). Although the method for gauging cell movement using measured distance from a single apically located reference point is the most practical and reliable, it does possess two disadvantages. First, to follow cell migration at longer times after injection, yet keep the point of reference, sections of practically the whole length of the incisor are necessary. Paraffin has been the only embedding medium in which this could be achieved, but the thickness of paraffin sections, ultimately limits structural and radioautographic resolution (Nadler, '51; Salpeter et al., '74). Second, well oriented, longitudinal sections of the whole tooth are almost impossible to achieve. Consequently, as the cells move away from the single fixed point of reference, the chance for measurement error due to obliqueness of the section is increased.

The purpose of this report is to follow the migration of ameloblasts and odontoblasts during renewal of the upper and lower incisor in the rat. It was made possible by a technique which allowed Epon to be used as an embedding medium and yielded well oriented 1 μm thick longitudinal sections of sufficient length to conveniently follow the movement of cells (Smith, '74). The morphological classification of Warshawsky and Smith ('74) was used to precisely localize the migrating front of labeled cells in relation to the various zones and regions of amelogenesis. This provided better standardization because of the use of multiple reference points and improved the calculation of the rates of cell migration since both length and time parameters were accurately measured. Finally, the influence of growth in changing the overall length of the incisor was considered. These factors combined to produce a more complete understanding of the dynamics of incisor renewal.

MATERIALS AND METHODS

Thirty male Sprague-Dawley rats weighing 100 ± 5 grams at the time of injection were used. In one experiment animals received a single dose of 1 μC/g body weight of ^{3}H-thymidine intraperitoneally at the same time of day (9:00 AM EST). They were then sacrificed in pairs at intervals ranging from one hour to 32 days after injection. In a second experiment four rats which were expected to weigh about 300 grams at the time of sacrifice received three doses of 1 μC / g body weight of ^{3}H-thymidine intraperitoneally to establish three separate cohorts of labeled cells along the length of the same incisor. For this experiment the injections were always given at 9:00 AM EST and were timed to give labeled cohorts at intervals of one hour, two and nine days after injection.

The jaws were prepared according to the procedure of Warshawsky and Moore ('67) which employs perfusion fixation with 2.5% glutaraldehyde, decalcification in 4.13% isotonic, neutral disodium EDTA, postosmication, dehydration in graded acetone and Epon embedding. Prior to osmication the teeth were prepared for sectioning in their longitudinal axis (Smith, '74). One μm thick longitudinal sections were cut on a Porter-Blum MT-1 ultramicrotome using glass knives. At least 50 serial sections were cut from each block and transfered with a hair to glass slides (Merzel, '71). All sections were then prestained for radioautography with Regaud's hematoxylin (Schantz and Schecter, '65; but, modified to 15 minutes for both the mordant and "ripened" hematoxylin). The slides were dipped in Kodak NTB2 liquid emulsion and exposed for varying periods of time (Kopriwa and Leblond, '62).

Lengths were measured from histological sections using a Leitz sliding ocular eyepiece mounted on a microscope (tables 1, 2; figs. 3, 12–15, 23). The alignment bar was kept perpendicular to the junction between the cell population being measured (ameloblasts or odontoblasts) and the extracellular matrix (enamel or dentin, respectively). The ocular was standardized with a stage micrometer.

Nuclear counts for the cell layers of the enamel organ in each zone of amelogenesis (table 3) were complicated because of the oblong and tightly packed characteristics of ameloblast nuclei. Hence, nu-

TABLE 1

Position of the most advanced mitotic figures in the enamel organ as measured from longitudinal serial sections [1]

Section sampled	Distance in μm from the start of mantle predentin secretion incisally to the most advanced mitotic figure for the:			
	Ameloblasts	Stratum intermedium	Stellate reticulum	Outer dental epithelium
1	74	30	233	NP
11	234	NP	NP	NP
22	149	189	NP	NP
33	128	NP	NP	NP
44	155	NP	NP	NP
55	NP	NP	NP	NP

NP, "None Present" for that section.
[1] From the same lower incisor.

clear counts were markedly influenced by oblique or angled planes of sectioning. Consequently, only sections which were in "good" longitudinal orientation were used. Then, the length of the zone of amelogenesis to be counted was measured. The number of nuclei occupying this length was counted in that section and in each of four immediately adjajcent serial sections. These counts were averaged to obtain a *mean nuclear count.* This value was "normalized"[4] (Cairnie and Bentley, '67) by multiplying it by the ratio of the "mean length" for that zone as given in table 2 divided by the actual measured length. A minimum of 20 different teeth were used to obtain these *normalized nuclear counts* for each zone of amelogenesis and these normalized values were averaged to give the results shown in table 3.

RESULTS

The distinct linear organization of the rat incisor in longitudinal section has been described in detail elsewhere (Warshawsky, '68; Warshawsky and Smith, '74; Smith and Warshawsky, '75). All the immature, proliferative cells giving rise to the epithelium, the odontoblasts and the pulp are located at the apical end of the tooth (figs. 1,2). Anterior to these proliferative sites cellular differentiation results in the formation of dentin and enamel.

Sites of proliferation

Epithelium. In the epithelial tissue mitotic activity extended from the apical loop up to approximately one-fifth of the way into the region of ameloblasts facing dentin (fig. 2, bracket). Mitotic figures were most numerous in the ameloblast and (provisional) stratum intermedium layers. Occasional mitotic figures were found within the stellate reticulum, but were rare within the single layer of outer dental epithelium.

Pulp. In the pulp mitotic figures were often seen in the undifferentiated and partially differentiated cells lying adjajcent to the ameloblasts posterior to the start of mantle predentin secretion (fig. 2, solid line). Mitotic figures were not seen in secretory odontoblasts but were present in those pulp cells which formed a layer beneath the odontoblasts (Warshawsky and Smith '74) and in the endothelium of blood vessels.

Incisal limit of mitotic figures in the epithelium

Within any particular longitudinal section each cell layer of the epithelium contained a mitotic figure which was in front of, or incisal to, all other mitotic figures in that cell layer. This mitotic figure was easily recognized since it was separated from preceding mitoses by fairly long groups of interphase nuclei (fig. 5). Such a mitotis was called the "most advanced mitotic figure" and it was always situated

[4] Normalization as applied here *assumes* a linear relationship between the packing of nuclei and the length for each of the zones of amelogenesis. That is, for a particular zone under examination, a measurement which is "long" relative to the "average" should have a higher than average nuclear count whereas a measurement which is "short" should have a lower than average nuclear count.

TABLE 2

Comparison between the ameloblast and odontoblast of parameters defined by the zones of amelogenesis in the upper and lower incisor

Incisor	Cell type	Parameter	Weight (g ± SD)	Zone of amelogenesis		
				Presecretion [1]	Secretion	Maturation
Upper	Ameloblasts (Enamel organ)	Length of zone (μm ± SD)	110 ± 5	1,210 ± 81	3,784 ± 201	7,335 [2]
			300 ± 16	1,112 ± 92	3,794 ± 412	13,338 ± 457
		Rate of migration (μm/day)	—	664	598	551
	Odontoblasts	Length of zone (μm ± SD)	110 ± 5	1,070 ± 64	3,211 ± 856	6,288 [2]
			300 ± 16	1,083 ± 56	3,413 ± 349	11,434 [2]
		Rate of migration (μm/day)	—	613	524	514
Lower	Ameloblasts (Enamel organ)	Length of zone (μm ± SD)	110 ± 5	1,381 ± 90	5,142 ± 232	10,615 [2]
			300 ± 16	1,217 ± 139	5,159 ± 278	14,792 ± 883
		Rate of migration (μm/day)	—	774	686	643
		Length of zone (μm ± SD)	110 ± 5	1,227 ± 100	4,774 ± 216	9,766 [2]
			300 ± 16	1,144 ± 134	5,025 ± 246	13,608 [2]
		Rate of migration (μm/day)	—	682	641	640

SD = Standard deviation.

[1] Region facing dentin only.

[2] These values are deduced from histological and radiographic measurements (Smith and Warshawsky, '73), hence no standard deviation is given. Note, however, that a deduced value for the length of the maturation zone relative to ameloblasts in the lower incisor of a 300 g rat was 14,665 μm.

TABLE 3

Total number of nuclei (± standard deviation) occupying the length of each zone of amelogenesis

Incisor	Cell population	Zone of amelogenesis: Presecretion [1]	Secretion	110 ± 5 g	300 ± 16 g
Upper	Ameloblasts	529 ± 100	1,194 ± 142	1,620 ± 131	2,946 ± 239
	Stratum intermedium	235 ± 21	312 ± 27	—	—
	Papillary layer	284 ± 40 [3]	652 ± 95	1,321 ± 154	2,402 ± 100
Lower	Ameloblasts	582 ± 81	1,698 ± 232	2,179 ± 256	3,035 ± 356
	Stratum intermedium	294 ± 27	520 ± 43	—	—
	Papillary layer	250 ± 29 [3]	986 ± 99	1,875 ± 197	2,612 ± 275

[1] Region facing dentin only.
[2] Counts are shown at two different weights.
[3] In this zone counts for the outer dental epithelium and stellate reticulum are combined.

incisal to the start of mantle predentin secretion (fig. 2, bracket). Measurements from the start of mantle predentin secretion to the most advanced mitotic figure in the cell layers of the enamel organ, made it apparent that the spatial position of this figure varied considerably from section-to-section (table 1, compare values vertically and horizontally in each column). Plotted measurements from a large number of sections in different incisors (fig. 3) revealed that in *relative terms* the most advanced mitotic figure in the ameloblast, stratum intermedium and stellate reticulum layers was most frequently situated between 50 and 200 μm incisal to the start of mantle predentin secretion (figs. 3, 4). From 200 to 400 μm the frequency gradually decreased. Of these three cell layers, the stratum intermedium showed the greatest frequency of advanced mitotic figures positioned between the 200 and 400 μm interval (figs. 3, 4). In *absolute terms* the most incisally positioned mitotic figure ever measured was located within the single layer of outer dental epithelium, followed apically by mitotic figures in the stratum intermedium, ameloblasts and stellate reticulum (fig. 3, circled points a–d).

Leading edge of labeled cells one hour after ^{3}H-thymidine injection

Epithelium. One hour after the injection of ^{3}H-thymidine, labeled nuclei were distributed throughout the epithelium in essentially the same fashion as mitotic figures (fig. 5). Thus, in the region of ameloblasts facing dentin the "most advanced labeled cells" in the ameloblast, stratum intermedium and stellate reticulum layers showed variable positions similar to the pattern found for the most advanced mitotic figures (fig. 3). The most incisally located labeled cell ever measured, however, was apical to the most incisally positioned mitotic figure in each of these cell layers (fig. 3).

Pulp. Secretory odontoblasts were not labeled at one hour after injection (fig. 5). Labeling was confined to cells of the subodontoblastic layer, the endothelium of blood vessels, the differentiating cells adjacent to the ameloblasts and to the undifferentiated pulp cells (figs. 2, 5).

The position of the leading edge of labeled cells in the enamel organ and in the odontoblast layer at one hour was used as the frame of reference for cell migra-

tion. However, as shown in figure 3, this edge is quite variable and has a potential apical-incisal spread of 400 μm. Consequently, standardization (see Appendix) was required if meaningful measurements were to be made within the same tooth, and especially, if valid comparisons were to be made between different teeth.

Movement of ^{3}H-thymidine labeled cells

The radioautographs showed that a cohort of labelled cells from the enamel organ, the odontoblast layer and pulp was carried incisally with the erupting incisor, maintaining almost the same relationship as was seen at the starting position one hour after injection (figs. 5, 16–20, 24, 27).

Ameloblast migration (representative of the cohort in the enamel organ)

The most advanced labeled ameloblast was used as the marker for movement by all cells of the enamel organ (see APPENDIX). From one hour to one and one-half days after the injection, the most advanced labeled ameloblast moved from a position near the start of mantle predentin secretion (figs. 3, 5) to the end of the presecretory zone (fig. 6). By two days it had begun the secretion of the inner enamel matrix figs. 7, 12, 13, 18, 25) and was still secreting inner enamel at four days (figs. 8, 12, 13). On the upper incisor the most advanced labeled ameloblast had completed the secretion of the outer enamel matrix by eight days (fig. 12), while on the lower incisor, the most advanced labeled ameloblast was still secreting outer enamel at eight days (figs. 9, 13, 19, 26) but was finished at nine days (fig. 23). After 16 days the most advanced labeled ameloblast had moved halfway through the maturation zone (figs. 10, 12, 13, 27) and, at 32 days from the initial injection, was found as a reduced cell near the gingival margin of the tooth (figs. 11, 12, 13).

By plotting the distance moved from the starting position against time, the mean rate of ameloblast migration was obtained from the slope of the regression line (fig. 14). This was 657 μm/day for the ameloblasts of the lower incisor and 567 μm/day for those of the upper incisor. The rate of ameloblast migration through each zone of amelogenesis is shown in table 2. These results indicated a "deceleration" as the ameloblasts approached the gingival margin.

Odontoblast migration (representative of the migration of hard tissues). Labeled secretory odontoblasts were not seen until 6 or 8 hours after the injection of ^{3}H-thymidine (figs. 5, 16). At this time a cohort of labeled odontoblasts which originated from the differentiating pulp tissue adjacent to the ameloblasts was at the point marking the start of mantle predentin secretion (fig. 16). Between 8 and 20 hours after injection this cohort advanced incisally, thereby establishing a front of labeled secretory odontoblasts (fig. 17). It was often difficult, however, to determine which cell was the "most advanced labeled odontoblast" because one or more labeled odontoblasts were usually found considerably ahead of the main labeled front (fig. 17, small arrows). From 20 hours to 32 days the front of labeled odontoblasts moved progressively towards the incisal end of the tooth, and kept in pace with the advancing front of labeled cells in the enamel organ (figs. 18–22). In the time intervals between eight hours and eight days after injection, the most advanced labeled odontoblast was apical to the corresponding advanced labeled ameloblast (figs. 18, 19), and the distance between these two cells (relative to perpendiculars at the dentino-enamel junction) remained approximately the same as it had been at eight hours (compare the position of the "squares" in figs. 16, 18, 19). After eight days, the most advanced labeled odontoblast and ameloblast were only slightly out of line from one another (figs. 20–22). The labeled odontoblast, however, was not seen ahead of the labeled ameloblast.

From the slope of the regression line obtained by plotting distance moved versus time, the mean rate of odontoblast migration in the lower incisor was 631 μm/day and 500 μm/day in the upper (fig. 15). The rate of odontoblast migration expressed relative to the zones of amelogenesis is shown in table 2. These values indicated a slightly higher rate of migration

through the presecretory zone, but an almost uniform rate along the rest of the tooth. Note also that the values for the rate of odontoblast migration (either the means, fig. 15 or, relative to zones of amelogenesis, table 2) were consistently less than the corresponding rates for ameloblast migration.

Changes which occurred as ^{3}H-thymidine labeled cells moved with the erupting incisor

Increase in length. Over the 32 days taken by the front of labeled cells to move from one end of the tooth to the other, the absolute length of the upper and lower incisor increased since in that time the animal changed in body weight from 100 to 300 grams (figs. 12, 13; table 2). From measurements of histological sections it was found that of the three zones of amelogenesis, only the zone of maturation had increased in length (table 2, figs. 12, 13). Consequently, it was important to establish experimentally whether there had been any change in the rate of cell migration, and also, to determine where this "new length" appeared relative to the advancing cells in the maturation zone. In this experiment, rats which were expected to weigh about 300 grams at the time of sacrifice were serially injected with ^{3}H-thymidine to provide different labeled fronts along the length of the same incisor. These were then compared to the position of labeled fronts already obtained in the 100 gram rat (figs. 12, 13). It is readily apparent from the results shown in figure 23 that at one hour, two days and nine days after injection there was no difference in the spatial position of the most advanced labeled ameloblast in the 100 and 300 gram rat, and thus, the rate of cell migration remained constant between these two body weights. In addition, from the data in table 2 and in figure 14 it was possible to determine that the front of labeled ameloblasts *should* theoretically take 22 days in the upper incisor and 25 days in the lower to traverse a length equal to the distance between the starting position at one hour after injection and the gingival margin in the 100 gram rat, (i.e. 12,487 μm in the upper incisor and 16,952 μm in the lower, fig. 23). In rats which had been sacrificed at 24 days after the injection of ^{3}H-thymidine, the most advanced labeled ameloblasts in the upper and lower incisors were found at positions which matched fairly closely with the distance theoretically expected to be travelled in 24 days (using the mean rate of ameloblast migration (fig. 14) this was calculated to be 13,924 μm, theoretical, versus 14,542 μm, actual, in the upper incisor; 16,301 μm versus 16,331 μm in the lower incisor). However, in these rats at 24 days after injection the most advanced labeled ameloblasts were located at a considerable distance from the gingival margin. This distance was found to be 4,026 μm in the upper incisor and 3,564 in the lower, as compared to a theoretically expected growth in 24 days of 4,008 μm in the upper incisor and 3,564 in the lower, (unpublished results). In summary, then, between 100 and 300 grams of body weight the rate of cell migration did not change. However, because of growth there was an increase in the overall length of the embedded portion of the incisor. With regard to the zone of amelogenesis, an increase in length was detected only within the maturation zone (table 2), and relative to an advancing front of labeled cells, this "new length" appeared to arise on the side of the maturation zone towards the gingival margin (fig. 23).

Redistribution of labeled cells within the enamel organ as shown by nuclear counts. As the front of labeled cells comprising the enamel organ moved from the apical end of the tooth to the gingival margin, they passed through a series of morphological changes which characterize the life cycle of each type (figs. 5–11; 24–27). During this life cycle certain qualitative changes in the distribution of labeled cells were observed and an attempt was made to quantitate these changes by means of nuclear counts within each of the zones of amelogenesis (table 3, 4).

In the *ameloblast* layer at one hour after the injection of ^{3}H-thymidine the most advanced labeled ameloblast was characteristically isolated from adjacent labeled cells by a group of unlabeled cells (fig. 5). This group of labeled cells was in turn separated from the main body of labeling by a smaller group of unlabeled cells. At

TABLE 4

Number of nuclei per 100 μm length of zone (± standard deviation) in longitudinal section for each zone of amelogenesis

Incisor	Cell population	Presecretion [1]	Secretion	Maturation [2]
Upper	Ameloblasts	43.72 ± 8.26	31.55 ± 5.31	22.09 ± 1.79
	Stratum intermedium	19.42 ± 1.73	8.25 ± 0.71	—
	Papillary layer	23.47 ± 3.31	17.23 ± 2.51	18.01 ± 0.74
Lower	Ameloblasts	42.14 ± 5.86	33.02 ± 4.51	20.52 ± 2.41
	Stratum intermedium	21.29 ± 1.96	10.12 ± 0.84	—
	Papillary layer	18.10 ± 2.10	19.18 ± 1.93	17.66 ± 1.86

[1] Region facing dentin only.
[2] This value is the same in the 100 g and 300 g rat.

20 hours after the injejction (fig. 24), as the ameloblasts were increasing in height, the pattern of labeling was essentially the same as at one hour although the labeled nuclei appeared a little more separated from one another (compare figs. 5, 24). By two days (fig. 25) the ameloblasts at the labeled front were fully differentiated into tall secretory cells and their nuclei were positioned at two staggered levels. The pattern of labeling was comparable to that at 20 hours, but the labeled nuclei again seemed more dispersed by groups of unlabeled nuclei (compare figs. 5, 24, 25). In passing through the secretory zone the labeled front maintained approximately the same pattern as that seen at two days (compare figs. 25 26). In the maturation zone the ameloblasts were shortened cells and their nuclei were positioned mostly at the same level (fig. 27). The pattern of labeling remained comparable to that seen in the secretory zone (compare figs. 25, 26, 27), but there seemed to be a reduction in the number of labeled and unlabeled nuclei comprising the front. Counts of the total number of ameloblast nuclei occupying the length of each zone of amelogenesis are shown in table 3. When these absolute counts were expressed as the number of nuclei per 100 μm length of zone (table 4), it was apparent that between the presecretory and maturation zones a real quantitative change had occurred. Thus, the arrangement of nuclei at "two levels" in presecretion containing 42 nuclei/100 μm length of zone, had changed to "staggered levels" during enamel secretion containing 33 nuclei/100 /μm length of zone, and eventually changed to "one level" of nuclei throughout maturation with 20 nuclei/100 μm length of zone.

In the *stratum intermedium* one hour after the injection, label was distributed among the flattened and irregularly spherical nuclei (fig. 5). Similar to the pattern seen in the ameloblast layer, labeled nuclei in the stratum intermedium were separated by unlabeled groups. This pattern did not change appreciably by two days after injection although pairs of labeled nuclei became apparent (figs. 24, 25). Shortly after the ameloblasts began secreting the inner enamel matrix, the stratum intermedium changed to a single layer of cuboidal cells (figs. 8, 25) with a corresponding separation in the labeled nuclei. After completion of enamel secretion by the ameloblasts and passage through the postsecretory transition region (Warshawsky and Smith, '74), the stratum intermedium was lost as a definitive layer. While traversing the rest of the maturation zone, labeled cells within the papillary layer were seen in a position similar to the site of the former stratum intermedium (figs. 27, 22). Nuclear counts for the stratum intermedium (tables 3, 4) showed that between the presecretory and secretory zones the number of nuclei per

100 μm length of zone dropped almost exactly in half. In the maturation zone all the epithelial nuclei beneath the ameloblasts were counted as part of the papillary layer, consequently no counts are given for the stratum intermedium in tables 3 and 4.

Finally, within the region of ameloblasts facing dentin few cells in either the *stellate reticulum* or *single layer of outer dental epithelium* were labeled at one hour after injection (fig. 5). When present, label was usually seen in cells of the stellate reticulum located near the stratum intermedium (fig. 5). When the front of labeled cells entered the secretory zone at two days after injection, the stellate reticulum and single layer of outer dental epithelium could no longer be distinguished, and, together these layers formed the developing *papillary layer* (fig. 25). Moreover, when the stratum intermedium became a single layer of cuboidal cells, labeling within the developing papillary layer appeared to increase or at least became more noticeable (fig. 26). As the front of labeled cells entered the maturation zone, the papillary layer became fully developed and many of the nuclei, especially those occupying the site of the former stratum intermedium, were labeled (fig. 27). Nuclear counts in relation to the papillary layer (tables 3, 4) showed that between the presecretory and maturation zones the number of nuclei per 100 μm length of zone remained constant. However, it should be noted that within the maturation zone all epithelial nuclei at the base of the ameloblasts were counted as part of the papillary layer, while in the presecretory and secretory zones the stratum intermedium was counted separately from the developing papillary layer. Consequently, the data in table 4 is more comparable if the nuclear counts for the stratum intermedium are added to the counts for the "papillary layer." When this is done a progressive drop in the nuclear count, similar to that seen for the ameloblasts, is apparent from about 40 nuclei/100 μm length of zone (presecretion) to 29 nuclei/100 μm length of zone (enamel secretion) to 18 nuclei/100 μm length of zone (maturation).

Decrease in the apical-incisal distance between the most advanced labeled ameloblast and odontoblast. The interval which separated the most advanced labeled amelobast from the most advanced labeled odontoblast appeared to decrease between the secretory and maturation zones (figs. 19, 20). This decrease was apparent after the labeled front traversed the region of postsecretory transition. However, throughout the rest of the maturation zone the most advanced labeled odontoblast was not seen incisal to the most advanced labeled ameloblast.

DISCUSSION

Incisal limits of proliferative compartments in the epithelial tissue

The significance of the most advanced mitotic figure as defining the incisal limit of the proliferating compartment has been controversial. One source of confusion originated with the report of Starkey ('63) who postulated that ameloblasts pass directly into differentiation without a terminal cell division (i.e., they differentiate from the G_2-phase in the cell cycle). Although subsequent studies by Sasaki ('65), Hwang et al. ('66) and Chiba et al. ('67) established conclusively that ameloblasts which incorporate ^{3}H-thymidine eventually divide, these authors elected to use the most advanced labeled cell at one hour for defining the incisal limit of the proliferative compartment. Chiba et al. ('67) also made measurements of the length of the proliferative compartment in relation to the ameloblast and stratum intermedium layers, but they were unable to demonstrate any major difference in the *mean spatial position* for the most advanced mitotic figure and most advanced labeled cell at one hour. Zajicek and Bar-Lev ('71a) simply rejected Starkey's paper ('63) and redefined the incisal limit of the proliferative compartment in the ameloblast layer by means of the most advanced mitotic figure (a definition carried over to later reports: Zajicek et al., '72 and Michaeli et al., '72). These authors further stated that the most advanced mitotic figure was positioned "about sixty cells" in front of the most advanced labeled ameloblast at one hour. The data in figure 3 has provided an explanation for

many of these previous inconsistencies. That is, the last cell division prior to differentiation occurs over a wide range of positions relative to where the odontoblasts begin secreting the mantle predentin. This would account for why there is such a wide variability from section-to-section in the measured position of the most advanced mitotic figure (table 1), and why in any given section the most advanced mitotic figure is not always seen in front of the most advanced labeled cell at one hour. Moreover, this also explains why Chiba et al. ('67) were unable to demonstrated a difference in the mean position for the most advanced mitotic figure and most advanced labeled cell (the same data used in fig. 3 indicates that the mean position for the most advanced mitotic figure in the ameloblast layer is 125 ± 66 μm incisal to the start of mantle predentin secretion and 186 ± 82 μm for the most advanced labeled ameloblast). Finally, it is clear from figure 3 that, in absolute terms, mitotic figures are positioned incisal to labeled cells at one hour. This implies that a labeled cell undergoes mitosis in a more incisal position. Such a result offers no support for Starkey's contention ('63), and agrees with the definition of the proliferative compartment given by Zajicek and Bar-Lev ('71a). However, figure 3 further shows that in the ameloblast layer the interval which separates the most incisally positioned mitotic figure from the most incisally positioned labeled ameloblast at one hour is considerably less than 60 cells (it is only about 30 cells).

True starting position for cell migration in the epithelial tissue

While it was possible with ^{3}H-thymidine labeling to *directly* demonstrate the progressive movement of cells *away from* their respective proliferative compartments, it was not possible to establish with certainty the exact point at which these cells begin their incisal migration. Most previous investigators were not concerned with, or have been vague about, the question of the movement by the proliferating cells themselves (Ness and Smale '59; Hunt and Paynter, '63; Starkey, '63; Chiba, '65; Sasaki, '65; Chiba et al., '66, '67; Robins, '67; Shibata and Stern, '68). In their first report Hwang and Tonna ('65) more or less implied that the position of the most advanced labeled cell at one hour represented the starting point at zero velocity from which the differentiating cells accelerated. In a later study (Hwang et al., '66) this view was somewhat expanded to allow for migration of ameloblasts through the proliferative compartment. Zajicek and Bar-Lev ('71a,b) felt that cells of the ameloblast layer (inner enamel epithelium; "metablasts") began their incisal movement from a point corresponding to the anterior limit of the region of ameloblasts facing pulp, posterior portion (classification of Warshawsky and Smith, '74). Previously (Smith and Warshawsky, '75; and Smith, '75), it was concluded that *all cells of the epithelial tissue* originate from the bulbous part of the odontogenic organ. According to this interpretation, the cells giving rise to the enamel organ begin their incisal movement from a point near the apical loop (in essecnce the posterior limit of the region of ameloblasts facing pulp, posterior portion). Consequently, even though the most advanced mitotic figure and most advanced labeled cell at one hour are used as static reference points for measurement, these points do not represent the true starting position for migration, and it is likely that the proliferative cells are already moving at the mean rate of migration (e.g., 651 μm/day, lower incisor) by the time they enter the region of ameloblasts facing dentin. Therefore, the data in figure 3 can also define the spatial limits of the last division cycle prior to differentiation. For example, the most incisally positioned mitotic figure ever found in the ameloblast layer was located only 350 μm incisal to the start of mantle predentin secretion. However, the *majority* of most advanced mitotic figures were located between 50 and 200 μm from the start of mantle predentin secretion. If the ameloblasts are moving from the region facing pulp and entering the region facing dentin at 651 μm/day (lower incisor), then the distance travelled by any cycling cell should be about 600 μm (the distance travelled by an ameloblast in the cell cycle = rate of migration x generation time, which is about 22 hours for the ame-

loblasts; Zajicek and Bar-Lev, '71a). Consequently, if a cycling cell entered the G_1 phase of the last division cycle right at the start of the region facing dentin, then its mitotic division would be expected to occur about 600 μm from that point. But in fact no mitotic figures are seen further than 350 μm from the start of predentin secretion. It is therefore concluded that cells in the ameloblast layer (and likely all the other cells of the epithelial tissue as well) begin the G_1 phase of the last division cycle *before they leave the region of ameloblasts facing pulp.*

Life cycle of the ameloblast in the upper and lower incisor

In general the most advanced labeled ameloblast in the upper and lower incisor is found at an equivalent functional stage of its life cycle at equivalent times after ^{3}H-thymidine injection. Thus, it takes about 1.5 days for the ameloblast to differentiate into a secretory cell from the S phase of the last division cycle, about 7 days to secrete the enamel matrix and form it into rods and, in this study, about 23 days for the cell to complete the many activities associated with the maturation zone. There are however, some specific differences between the upper and lower incisor in the timing of enamel secretion. For example, the entire enamel layer is secreted in *exactly* 6.6 days on the upper incisor and 7.5 days on the lower. In addition, the ameloblasts in the upper incisor spend about 4.5 days secreting the inner enamel matrix and 2.1 days secreting the outer enamel matrix. In the lower incisor the ameloblasts require about 6.2 days for inner enamel secretion and only 1.3 days for outer enamel secretion. It is not possible with the present level of understanding about the process of enamel secretion to explain these time differentials, especially in regards to a genetic or an environmental control (Helmecke, '63).

Maturation is the most time-variable phase in the life cycle of the ameloblast. Not only are there differences between upper and lower incisors (fig. 23), but also ameloblasts take progressively longer amounts of time to traverse the maturation zone as the weight or age of the rat increases because of growth in the length of this zone. Thus, when a rat weighs 100 grams ameloblasts at the gingival margin on the lower incisor had spent about 16 days traversing the maturation zone, whereas, when the same animal weighs 300 grams ameloblasts at the gingival margin had taken 23 days to complete the same stage. This ultimately implies that the maturation stage of amelogenesis contains some functional activity which is growth dependent, and becomes a step of progressively longer duration as the weight of the animal increases. In the rat incisor changes in the maturation zone occur in two steps: the first step, as in other tooth systems, is the final calcification of the matrix (Deakins, '42; Weinmann et al., '42; Reith and Cotty, '62; Suga et al., '70; Robinson et al., '74); the second step, characteristic of only certain tooth systems, is the addition of an iron pigment to the fully calcified enamel (Butcher, '53; Stein and Boyle, '59; Reith, '59; Kallenbach, '70; Lindemann, '70; Halse, '72a,b). The final calcification of the enamel matrix is presumably a *time-constant step* (Chase, '40; Halse, '72b) which should, therefore, occupy a fixed length within the maturation zone (Suga et al., '70; Halse, '72b; Robinson et al., '74). However, pigmentation of the enamel is clearly an "extra" event in relation to the calcification process and one which is known to undergo certain changes. For example, under conditions of normal eruption (impeded) the length of the region of pigmentation, and correspondingly the amount of pigment in the enamel increases progressively with the age of the rat (Addison and Appleton, '15; Pindborg et al., '42; Schour and Massler, '49; Lindemann, '70; Halse, '72b). Moreover, under conditions of accelerated eruption (unimpeded) there is a decrease or total loss of pigment from the fully calcified enamel (Taylor and Butcher, '51; Bryer, '57; Matena and Kindlova, '66). Thus, pigmentation is a functional activity which meets all the requirements for a *time-variable step,* and is presumably the event ultimately affected by the growth which occurs within the longitudinal axis of the incisor.

Life cycle of the cells which form the papillary layer

It is clear from this report that a cohort of cells from each layer of the epithelial tissue is carried incisally with the erupting incisor and all of the cells in this cohort reach the gingival margin at the same time relative to their starting position at one hour. Moreover, all of the epithelial cells associated with the ameloblasts eventually become a part of the papillary layer. Not all of these cells, however, contribute to the papillary layer at the same time, but it occurs in three distinct steps. The first contribution is made by the stellate reticulum and single layer of outer dental epithelium which become indistinct as separate cell layers by the time enamel secretion begins (1.5 days relative to the starting position at 1 hour). A second contribution is made by the stratum intermedium when it changes from a double layer of mostly flattened cells into a single layer of cuboidal cells. This change occurs approximately one day after the ameloblasts begin secreting the inner enamel matrix (or 3 days relative to the starting position at 1 hour). The final contribution in the development of the papillary layer is also made by the stratum intermedium when it is no longer seen as a distinct cell layer of cuboidal cells after the region of postsecretory transition (about 9 days on the upper incisor and 10 days on the lower relative to the starting position at 1 hour). From this point until the gingival margin is reached, all the hypertrophied epithelial cells at the base of the ameloblasts constitute cells of the papillary layer. Finally, similar to the ameloblasts, the duration of the life cycle for the cells of the papillary layer progressively increases with the weight of the rat because of the growth which occurs in the length of the maturation zone. This lengthening of the life cycle presumably affects the cells of the papillary layer in a fashion similar to that described for the ameloblasts.

Changes in the distribution of cells within the enamel organ during amelogenesis

The results in table 4 offer conclusive proof that the cellular organization as measured by nuclear number per unit length is similar in the upper and lower incisors. These results also demonstrate that the cellular organization changes in the course of amelogenesis. Since a cohort of cells is carried with the erupting incisor through each zone of amelogenesis during renewal the steady decrease in the number of nuclei per unit length between the presecretory and maturation zones must mean that either cells are lost when passing between these zones or the cells spread out to occupy different unit areas (or both). Morphologically, the only evidence of a cell loss in the enamel organ is seen at the start of the maturation zone (region of postsecretory transition; Symons, '62; Reith, 70; Moe and Jessen, '72; Kallenbach, '74). The question of cell loss and cell spreading is best resolved by determining the population kinetics (Cleaver, '67) within each of the zones of amelogenesis and on that basis it was concluded that the decrease in the number of nuclei per 100 μm length of zone in the ameloblast layer between the secretory and maturation zones is due to a loss (death) of ameloblasts (Smith and Warshawsky, '74). Consequently, the other decreases in this value for both the ameloblast and papillary layers (stratum intermedium included) shown in table 4 must be caused by changes in the unit area over which cells are distributed. Finally, the full significance of the "high" and "low" level positioning of the ameloblast nucleus remains to be established.

Life cycle of the odontoblast

The secretory odontoblast arises very quickly, appearing within six or eight hours from the S phase of the last division cycle of its precursor cell. Most or all of the odontoblasts on the labial surface of the incisor originate from the partially differentiated pulp tissue lying adjacent to the ameloblasts just apical to the start of mantle predentin secretion (Chiba, '65; Hwang and Tonna, '65; Robins, '67; Takuma and Nagai, '71). Odontoblasts are carried progressively forward with the erupting incisor and reach a point opposite the gingival margin about the same time as a similar cohort of cells from the enamel organ and what remains of the pulp

proper. However, as measured in the longitudinal axis of the incisor, the most advanced labeled odontoblast appears closer to the most advanced labeled ameloblast at the gingival margin than it did at the apical end of the tooth. Such a finding is in direct contrast to the results of Hwang and Tonna ('65) in the mouse incisor who reported that the ameloblast gains distance relative to the odontoblast.

The few labeled odontoblasts which were found well in front of the main body of labeled odontoblasts at early times after injection cannot be fully explained. It is possible these labeled cells represent an addition from the subodontoblastic layer, since this layer shows considerable labeling at one hour and some cells are often so close to the odontoblasts that it is difficult to distinguish between them (Cotton, '64). It is also likely that such labeling is the result of incorporation of ^{3}H-thymidine by, and subsequent division of, secretory odontoblasts themselves. Although in this work no mitoses or labeling at one hour of secretory odontoblasts was observed, the mitotic figure shown by Chiba ('65: fig. 6) would have been classified as a mitotic secretory odontoblast in this investigation.

Finally, growth within the longitudinal axis of the incisor effects the odontoblasts as it does cells in the enamel organ. Thus, the duration of the odontoblast life cycle must increase with the weight of the rat. The longer life cycle for the odontoblast must also mean that an increasingly thicker layer of dentin is produced as measured at the gingival margin. In this regard, growth of the incisor includes an increase in the circumference as well as length of the tooth (Schour and Massler, '49; unpublished results) and this larger size would provide the space necessary for such an increase in the absolute thickness of the dentin layer.

Rate of migration of the ameloblast (representative of cells in the enamel organ) versus rate of migration of the odontoblast (representative of the migration of hard tissues)

Similar to the findings of Hwang and Tonna ('65), this report does not offer conclusive evidence that ameloblasts move at a rate which is any different than that of odontoblasts. Neither cell, however, traverses the same absolute length as measured in the longitudinal axis of the incisor; that is, the odontoblast is carried across the inside of an arc whose radius progressively shortens towards the incisal end because of the continued appositional growth of dentin (Hwang and Tonna, '65). Consequently, with a uniform rate of cell migration it is not surprising that the most advanced labeled odontoblast appears to "catch up" with the most advanced labeled ameloblast since this would be expected by the geometry of the system. Nevertheless, it is surprising that at the gingival margin the most advanced labeled odontoblast does not actually appear ahead of the most advanced labeled ameloblast. The consistently higher rate of migration noted in table 2 for the ameloblast, though small and within the limits of error, may therefore mean that cells of the enamel organ actually do move slightly faster than the odontoblasts.

Rate of cell migration versus rate of tooth eruption

It is generally accepted that all hard tissues of the continuously erupting incisor, whether contained within the socket or exposed in the oral cavity, must physically move through space at a uniform velocity. This movement is usually measured by means of the hard tissues exposed in the oral cavity and is termed the rate of eruption. Previous investigators have reasoned that since cells make the hard tissues, these cells likely also move at a velocity which is similar to the rate of eruption (Ness and Smale, '59; Starkey, '63; Chiba, '65). Hwang and Tonna ('65) measured the parameters of tooth eruption (impeded) and rate of cell (ameloblast) migration in the mouse incisor and reported equal values for both. Consequently, subsequent workers have accepted this view and have thereafter equated these two rates (Robins, '67; Shibata and Stern, '68; Michaeli et al., '72; Zajicek et al., '72). The findings of this investigation, however, indicate a marked difference between the rate of cell migration and rate of tooth

eruption (impeded). For example, the mean rate of odontoblast migration was 631 μm/day in the lower incisor and 500 μm/day in the upper, whereas the rate of eruption (impeded) has been found by other workers to average about 400 μm/day on the lower incisor and 300 μm/day on the upper (Addison and Appleton, '15; Massler, '49; Sturman, '57; Sessle, '66; Marshall, '21; Donaldson, '24; Schour and Weinreb et al., '67; Chiba et al., '68; Lavelle, '69). Since the rate of odontoblast migration is considered synonymous with the rate of hard tissue migration, this difference represents a discrepancy of major proportions because it would imply that over the 32 days of this study the hard tissues moved almost 200 μm/day faster inside the socket than outside, a situation which is clearly not possible. Consequently, either the values determined in this report for rate of cell migration are in error, the values for rate of eruption were underestimated by previous investigators, or what is termed "rate of cell migration" in this report, in fact, represents a *compound rate* which includes a component of *true physical movement* (equal to the rate of eruption) and a component of *apparent movement* resulting from the growth within the longitudinal axis of the incisor. Of these three possibilities the last one is considered the most likely for the following reasons. First, if we ignore for the moment the growth which occurs between 100 and 300 grams of body weight and simply calculate at what rate odontoblasts would move in order to traverse in 32 days a length equal to the distance between the starting position at one hour and the gingival margin in the 100 gram animal (i.e., 10,662 μm in the upper incisor and 15,842 μm in the lower), then mean rates of odontoblast migration of 333 μm/day in the upper incisor and 495 μm/day in the lower are obtained. Though still somewhat high, these values are clearly a closer match with the generally accepted rate of eruption (impeded). Second, if there were no growth in the length of the incisor, then one would theoretically expect a complete steady state balance between the rates of cell production, cell migration, tooth eruption, and attrition (i.e., rate of cell production = rate of cell migration = rate of eruption = rate of attrition; see Michaeli et al., '72; Zajicek et al., '72). Carrying this reasoning one step further, since it has been concluded that the true rate at which the hard tissues move (eruption) and the true rate at which cells move (migration) are equal, growth in the length of the incisor could, then, only arise if the rate of cell production is greater than the true rate at which cells leave the proliferative compartments and/or if the rate of attrition is less than the rate of eruption (i.e., rate of cell production > true rate of cell migration = rate of eruption > rate of attrition). An imbalance between the rate of attrition and rate of eruption is expected to result in a gradual increase in the length of the erupted portion of the incisor.[5] Similarly, an imbalance between the rate of cell production and true rate of cell migration should result in a gradual increase in the length of the embedded portion of the incisor.[6] But consider how this would be achieved. If cells are being produced faster than they are being re-

[5] It is possible using the data shown in figures 12 and 13 to calculate the *actual growth rate* of the erupted portion of the incisor between 100 and 300 grams of body weight (an average value, assuming that growth is linear with time). For example, the length of the erupted portion of the upper incisor at 300 grams of body weight is 5,140 μm and 4,343 μm at 100 grams; for the lower incisor these lengths are 7,030 μm. and 5,062 μm, respectively. Since it took about 32 days for the rat to change in body weight from 100 to 300 grams, the growth rate for the erupted portion of the upper incisor is 25 μm/day and 62 μm/day for the lower. According to the rationale given, a similar growth rate for the erupted portion of the incisor should result by subtracting the rate of attrition from the rate of eruption (impeded). Using the values for rates of eruption and attrition reported by Sessle ('66) and Weinreb et al. ('67), *theoretical growth rates* of 28 μm/day in the upper incisor and 25–42 μm/day in the lower are obtained. These values clearly match the observed rates quite closely.

[6] The *actual growth rate* for the embedded portion of the incisor can easily be determined from the data in table 2 by subtracting the length of the maturation zone at 100 grams of body weight from the length of the same zone at 300 grams of body weight and dividing the difference by 32 days. When this is done, the *actual growth rate* for the embedded portion of the upper incisor is 161 μm/day and 120 μm/day for the lower (assuming growth is linear). Since it is concluded that the mean rate of cell migration determined in figures 14 and 15 represents a compound rate consisting of a portion due to true physical movement and another portion due to growth, a theoretical value for the growth rate of the embedded portion of the incisor can be obtained by subtracting the values which were determined for the theoretical rate of cell migration without growth (i.e., 333 μm/day in the upper incisor and 495 μm/day in the lower) from the mean rate of cell migration (i.e., 500 μm/day in the upper incisor and 631 μm/day in the lower). When this calculation is made, the *theoretical growth rate* for the embedded portion of the upper incisor is 167 μm/day and 136 μm/day in the lower. Once again, these values match the actual observed rates fairly closely.

moved, then the proliferative tissues at the apical end of the incisor should literally "grow backwards" in the bony socket. Since it is clear from table 2 that the distances between certain reference points on the tooth itself remain constant, this backwards spatial repositioning of the proliferative (apical) end of the incisor must also be accompanied by an apical shift of these reference points. Thus, the points marking the maximum convexity of the apical loop, the start of predentin secretion, start of enamel secretion and start of maturation would have to shift their position in three dimensional space posteriorly, and in unison. The point marking the gingival margin would however, remain fixed. Consider next what observations would be made if it were assumed that the tooth is growing backward in the bony socket and reference points are shifting posteriorly. First, there would be growth, or increase in the length of the embedded portion of the incisor (as is shown in figs. 12 13). Second, this growth would appear to occur *only* within the maturation zone, and significantly, there would be the illusion that the growth occurs on the side of the maturation zone towards the gingival margin (as is shown in fig. 23). Third, labeled cells moving incisally at a speed equal to the rate of eruption would in fact appear to move faster than they actually do because the points of reference used to gauge their speed are shifting posteriorly; that is, in a direction opposite to their movement. Finally since the gingival margin remains as a more or less fixed point relative to advancing labeled cells, these cells would appear to "decelerate" as they move closer to the gingival margin (as is shown in table 2). Although many authors have argued that the apical end of the rat incisor remains fixed in three dimensional space (Sicher, '42; Baume et al., '54; Robins, '67; Weinreb et al., '69), it was noted in embryological studies that during development of the incisor the tooth elongates away from the oral epithelium (Addison and Appleton, '15; Schour and Massler, '49), a process which could conceivably be carried over into adulthood. Moreover, restraining experiments have demonstrated that the incisor is capable of "growing backwards" in the socket (Ness, '56; Bryer, '57; Lavelle, '68a; Berkovitz '72). Restraining experiments also showed that ameloblasts in functional stages characteristic of maturation are present in spatial positions normally characteristic of enamel secretion of differentiation (Metena and Kindlova, '66; Weinreb et al., '69; Berkovitz, '72). This indicates that points of reference are capable of shifting posteriorly. In summary, then, it is concluded that the true rate at which cells move and the rate at which hard tissues move are equal. However, because there is growth in the length of the embedded portion of the incisor, the rate of cell migration, as measured relative to the zones of amelogenesis, appears to be faster than the rate of eruption (impeded), as measured in the oral cavity, and this difference is equal to the growth rate. Finally, it is suggested that the increase in the length of the embedded portion of the incisor arises by backwards spatal repositioning of the apical end of the incisor in the bony socket.

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APPENDIX

Method for standardizing measurements at the labeled front

Enamel organ. The ameloblast layer was se-

lected as the cell population upon which measurements would be made to represent movement by all cells comprising the enamel organ. The ameloblasts were chosen for several reasons: first, the radioautographs clearly showed that the cells forming each layer of the enamel organ advance incisally as a cohort, and thus, what is measured for one cell layer will apply to all; second, ameloblast nuclei were the most well defined of all the epithelial nuclei comprising the enamel organ; and lastly, the ameloblast layer had the highest packing of nuclei per unit length (table 4) in the enamel organ, and thus, this was the one cell layer most likely to express proper relationships at the labeled front in the fewest number of sections (fig. 3).

For each sample from the various time intervals, the position of the most advanced labeled ameloblast was measured in fifty serial sections. This gave an *average* value for the position of the most advanced labeled ameloblast in that sample. The averages from several different teeth representing the same time interval were then used to obtain a *mean* value for the position of the most advanced labeled ameloblast. This procedure eliminated the variability which would arise in trying to deal in absolute positions when the sampling number was limited, and gave the best balance between sampling number and the variability inherent to the system (fig. 3).

The distance over which the most advanced labeled ameloblast moved relative to the starting position at 1 hour after injection was calculated indirectly by using *average histological lengths* previously determined for the three zones of amelogenesis (Warshawsky and Smith, '74; table 2). For example, once the position of the most advanced labeled ameloblast at one hour had been accurately determined (for the lower incisor this was 186 ± 82 μm incisal to the start of mantle predentin secretion), the only other value required throughout the experiment was the position of the most advanced labeled ameloblast relative to the start or finish of the zone of amelogenesis being traversed at the time of Sacrifice. Consider the most advanced labeled ameloblast at four days after injection in the lower incisor. It was located 2,116 ± 45 μm incisal to the point marking the start of enamel secretion. Since the starting position at one hour had been 186 ± 82 μm incisal to the start of mantle predentin secretion, the distance traveled in four days was equal to the average length of the region of ameloblasts facing dentin (measured at the apex of the ameloblast; table 2) minus the position at one hour (1,381 — 186 = 1,195 μm) added to the distance advanced into the secretory zone, or 3,311 μm. This procedure eliminated the inaccuracies which would have resulted through direct measurement since, again, the sampling number in the case of the radioautographs was extremely low in comparison to the number of teeth which had been used to calculate the average length for each zone of amelogenesis. In addition, the farther the most advanced labeled ameloblast moved from the point marking the start of mantle predentin secretion, the greater was the chance for error due to obliqueness in the plane of section.

Odontoblasts. For odontoblast migration a similar procedure using serial sections and average histological lengths was employed. As shown in table 2, lengths which are analogous to the zones of amelogenesis are different when measured at the apex of the odontoblast. Also, the starting position of the most advanced labeled odontoblast was different than that of the ameloblast (fig. 2) which was, for example on the lower incisor, 75 ± 45 μm *apical* to the start of mantle predentin secretion. To calculate the distance moved by the most advanced labeled odontoblast a simple addition procedure was used. For example, at four days the most advanced labeled odontoblast was positioned 1,326 ± 125 μm incisal to the start of enamel secretion. The distance traveled in four days was then equal to this length added to the distance between the start of mantle predentin secretion and enamel secretion (measured at the apex of the odontoblast; table 2; 1,326 + 1,227 = 2,553 μm) plus the distance between the starting position at one hour and the start of mantle predentin secretion (75 μm) or a total of 2,629 μm.

PLATES

Abbreviations

Aa, Ameloblasts of the region facing pulp, anterior portion
Ad, Ameloblasts of the region facing dentin
af, Apical foramen
Aies, Ameloblasts of the region of inner enamel secretion
al, Apical loop
Amat, Ameloblasts of the region of maturation proper
Aoes, Ameloblasts of the region of outer enamel secretion
Ap, Ameloblasts of the region facing pulp, posterior portion
Apg, Ameloblasts of the region of pigmentation
Ar, Ameloblasts of the region of reduced ameloblasts
D, Dentin
E, Enamel Matrix
ER, Erupted portion of the incisor
ES, Enamel Space
H▽, "High" level nucleus in the ameloblast layer
L△, "Low" level nucleus in the ameloblast layer
Lab, Labial
M, Multiple layer of outer dental epithelium
MAT, Maturation Zone
O, Odontoblast
Pd, Predentin
Pp, Pulp, partially differentiated
Pu, Pulp, undifferentiated
PL, Papillary Layer
PSEC, Presecretory Zone
PSI, Provisional Stratum Intermedium
S, Single layer of outer dental epithelium
SEC, Secretory Zone
SI, Stratum Intermedium
SL, Subodontoblastic Layer
SR, Stellate Reticulum
tp, Tomes' process

PLATE 1

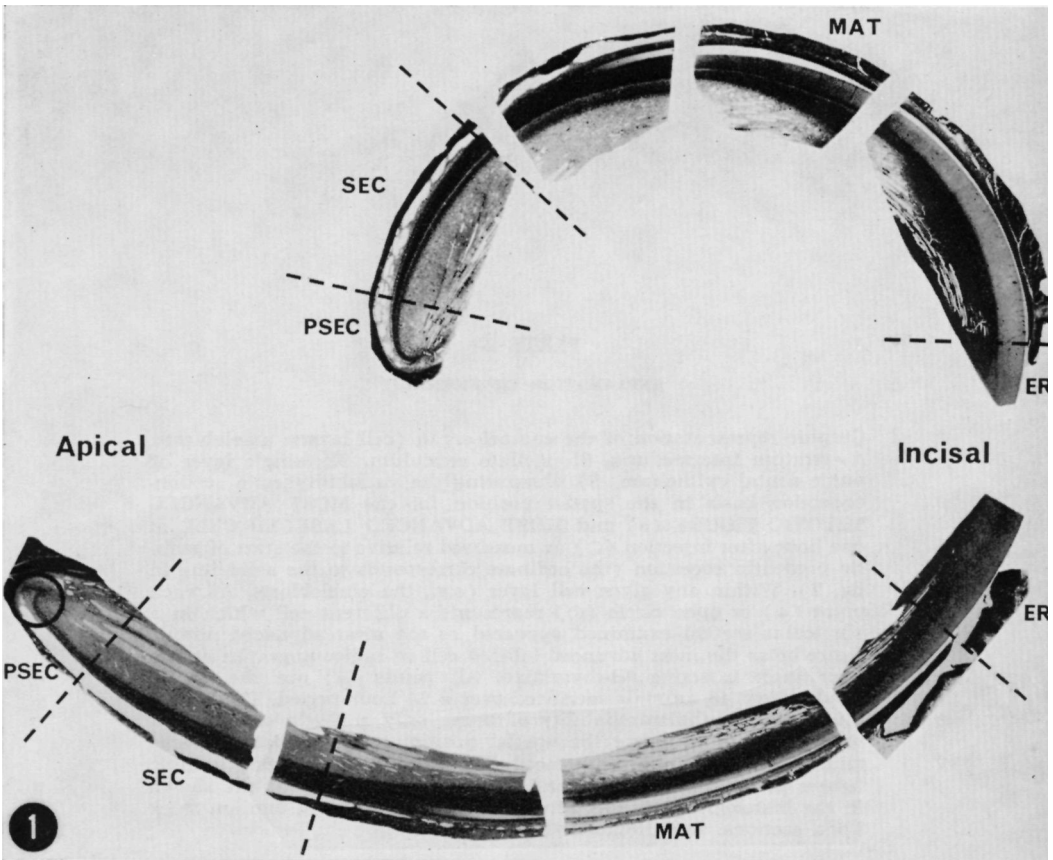
EXPLANATION OF FIGURES

1 Montage using 1 μm. thick longitudinal sections illustrating the upper (top) and lower (bottom) incisor. Toluidine blue. x 7.

The spaces between consecutive sections are intentionally included. A line drawn perpendicular to the labial surface at the gingival margin grossly divides the incisors into erupted (ER) and embedded (MAT, SEC, PSEC) portions. The embedded portion is further subdivided, by using the points which mark the start and finish of enamel secretion, into the presecretory (PSEC), secretory (SEC) and Maturation (MAT) zones. Cells are born at the apical end of the incisor and move toward the gingival margin. The part of the presecretory zone circled in the lower incisor is shown at higher magnification in figure 2.

2 Longitudinal section through the apical end of the lower incisor. One μm Epon section. Toluidine blue. x 80.

Mitotic activity in the epithelial tissue and in the partially differentiated pulp (Pp) lying adjacent to the ameloblasts (Aa) at the labial side (Lab) stops near the point which marks the start of mantle predentin secretion (solid line) by the odontoblasts (O). The brackets indicate where the most advanced mitotic figures are usually seen. The cessation of mitotic activity by these cells parallels the progressively more differentiated appearance when passing from the posterior aspect of the apical foramen (af) anteriorly (al, Ap, Aa, Ad; Pu, Pp, O). Pulp cells often appear condensed as a layer (SL) beneath the odontoblasts when predentin secretion first begins.


MAT
SEC
PSEC
ER
Apical
Incisal
PSEC
SEC
MAT
ER
1

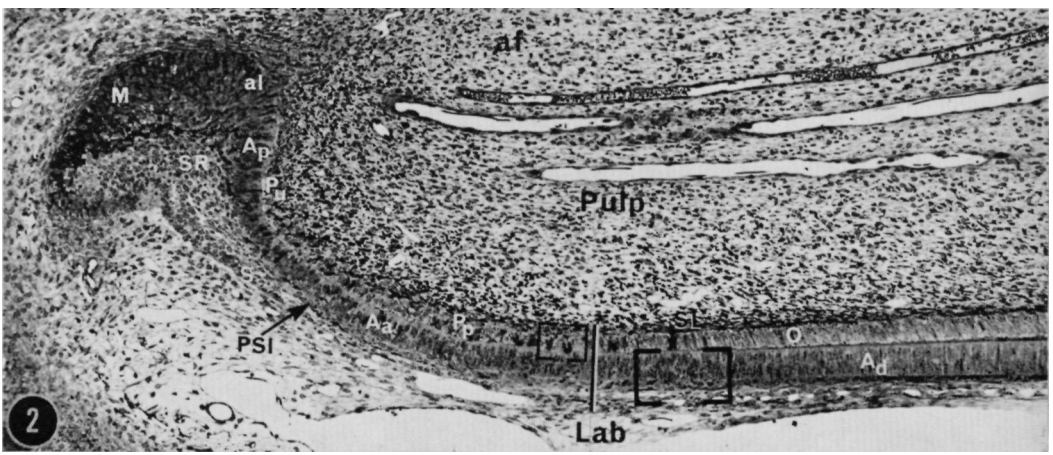
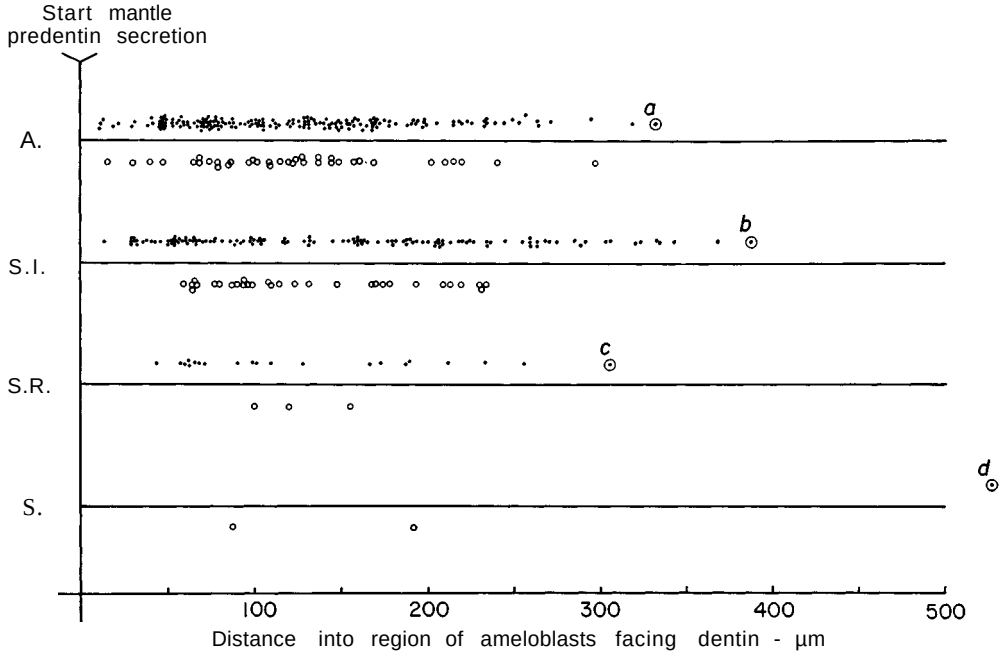

af
M
al
SR
Pulp
PSI
Lab
2

PLATE 2

EXPLANATION OF FIGURES

3 Graphic representation of the enamel organ (cell layers; ameloblasts, A; stratum intermedium, SI; stellate reticulum, SR; single layer of outer dental epithelium, S) illustrating the variability on a section-to-section basis in the spatial position for the MOST ADVANCED MITOTIC FIGURE (•) and MOST ADVANCED LABELED CELL at one hour after injection (O) as measured relative to the start of mantle predentin secretion (the ordinate corresponds to the solid line in fig. 2). Within any given cell layer (e.g., the ameloblasts, A) each point (•) or open circle (O) represents a different cell which in a particular section examined appeared as the most advanced mitotic figure or as the most advanced labeled cell in radioautographs at one hour after injecting ^{3}H-thymidine. All points (•) are the pooled results from 18 animals sacrificed over a 24 hour period. This graph clearly shows the unreliability of using only a single or very few measurements to define the spatial position of the most advanced mitotic figure or most advanced labeled cell. The actual mitotic figures giving points a, b, c and d in the graph (circled) are shown in the histological sections beneath the graph (a,b,c,d, one μm thick Epon sections. Iron Hematoxylin. x 720.


Start mantle predentin secretion
A.
S.I.
S.R.
S.
a
b
c
d
100
200
300
400
500
Distance into region of ameloblasts facing dentin - µm

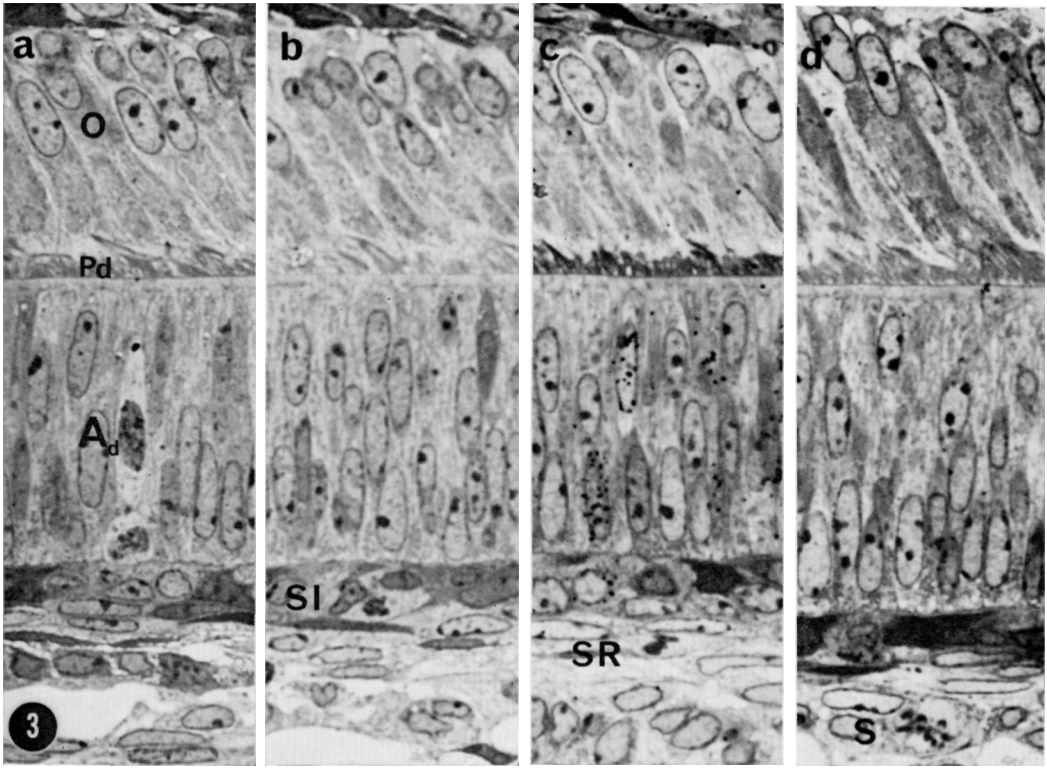

a
O
Pd
Ad
b
SI
c
SR
d
S
3

PLATE 3

EXPLANATION OF FIGURE

4 Histograms showing the percent frequency at which the most advanced mitotic figures in the ameloblast, stratum intermedium and stellate reticulum layers are found positioned at various distances incisal to the start of mantle predentin secretion (ordinate) as determined from the results plotted in figure 3.

The width of each bar represents 20 μm and the height indicates the percent relative frequency of points that fall within this width. Each cell layer in the enamel organ is calculated separately. These histograms illustrate that the most advanced mitotic figure will usually be found between the 50 to 200 μm interval and, of the three cell layers, the most advanced mitotic figure in the stratum intermedium is often situated between the 200 to 400 μm interval.

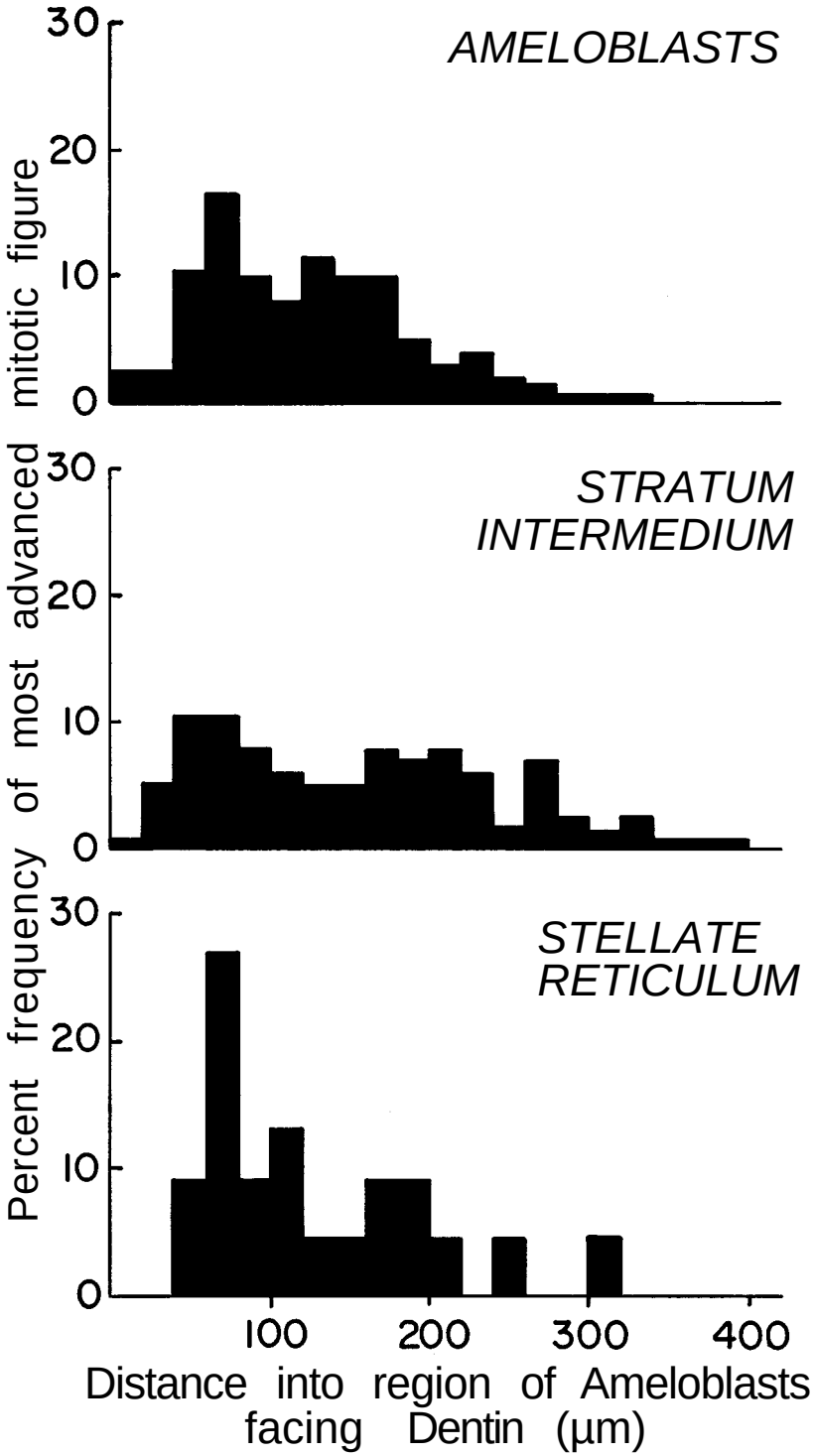

AMELOBLASTS
STRATUM INTERMEDIUM
STELLATE RETICULUM
Percent frequency of most advanced mitotic figure
30
20
10
0
100
200
300
400
Distance into region of Ameloblasts facing Dentin (µm)

PLATE 4

EXPLANATION OF FIGURES

5 Radioautograph showing the characteristics of labeling in the tissues around the most advanced labeled ameloblast one hour after injecting ^{3}H-thymidine. One μm thick Epon section. Iron hematoxylin. x 400.

At one hour the most advanced labeled ameloblast (arrowhead) is located at the apical limit of the region of ameloblasts facing dentin (Pd). In this section the most advanced mitotic figure in the ameloblast layer (arrow) is seen well in front of the most advanced labeled ameloblast, as is a labeled cell in the stratum intermedium (SI). Other labeled nuclei are present in the stellate reticulum (SR) and in the pulp, but labeling is not seen in the secretory odontoblasts (O) nor (in this example) within the outer dental epithelium (S).

Figures 6 to 11 are radioautographs of the most advanced labeled ameloblast in the lower incisor from 20 hours to 32 days after injection of ^{3}H-thymidine. One μm thick Epon sections. Iron hematoxylin. x 720.

6 Twenty hours after injection, the most advanced labeled ameloblast (Ad) has begun to differentiate. It is still within the region of ameloblasts facing dentin, but more incisally located than at one hour as indicated by the thicker layer of predentin (Pd) at its apex. Note the labeled nuclei in the outer dental epithelium (S).

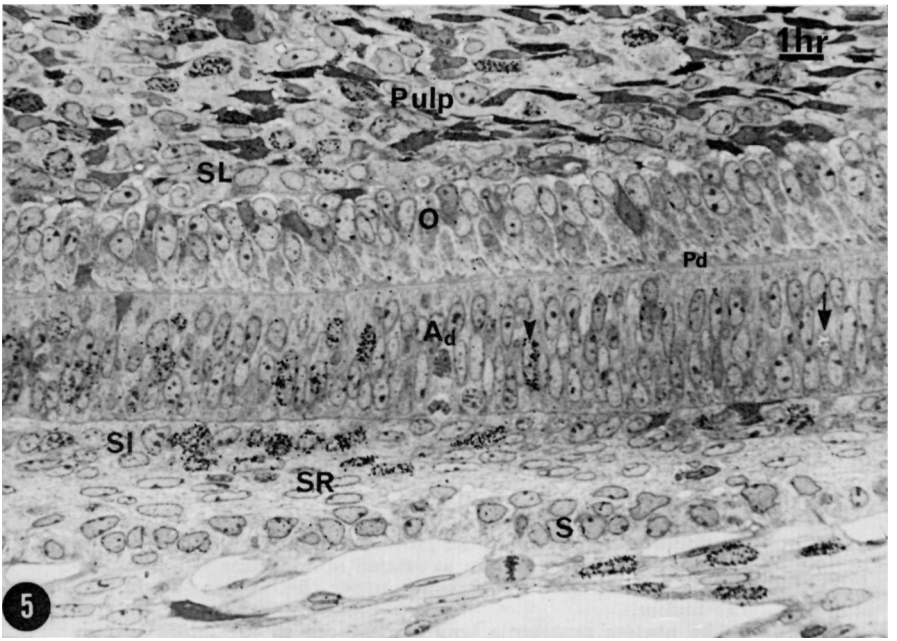
7 Two days after ^{3}H-thymidine injection the most advanced labeled ameloblast (Aies) has differentiated into a tall secretory cell with a Tomes' process (tp) and has begun the secretion of the inner enamel matrix (E).

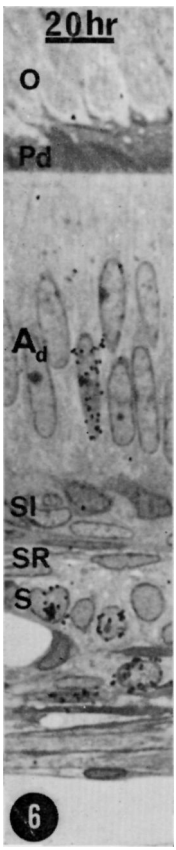
8 Four days after ^{3}H-thymidine injection the most advanced labeled ameloblast (Aies) is still secreting the inner enamel matrix.

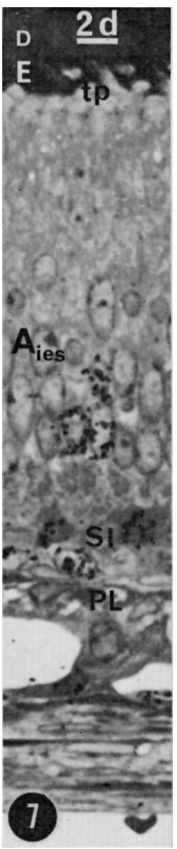
9 Eight days after ^{3}H-thymidine injection the most advanced labeled ameloblast (Aoes) is secreting the outer enamel matrix as is evident by the appearance of Tomes' process.

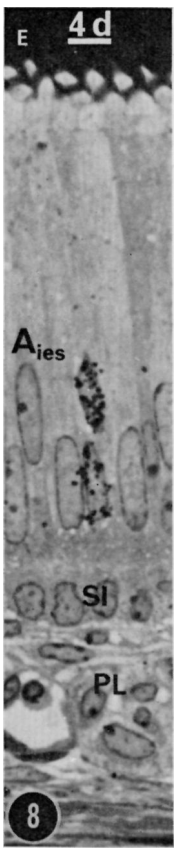
10 At 16 days after ^{3}H-thymidine injection the most advanced labeled ameloblast (Amat) is about halfway along the maturation zone and is related to the enamel space (ES) since all of the matrix at its apex has been lost during decalcification. In this example the ameloblasts have an unmodified apex.

11 By 32 days after injecting ^{3}H-thymidine the most advanced labeled ameloblast (Ar) is found as a reduced cell near the gingival margin of the incisor.


1hr
Pulp
SL
O
Pd
Ad
Sl
SR
S
5


20hr
O
Pd
Ad
Sl
SR
S
6


D
E
2d
tp
Aies
Sl
PL
7


E
4d
Aies
Sl
PL
8


8d
Aoes
9


16d
ES
Amat
PL
10

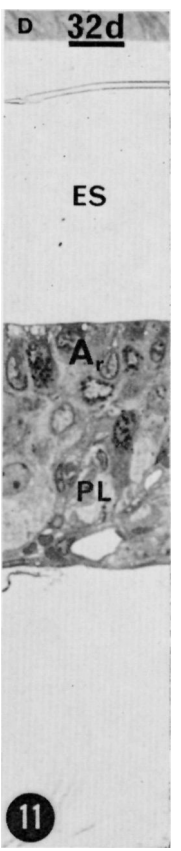
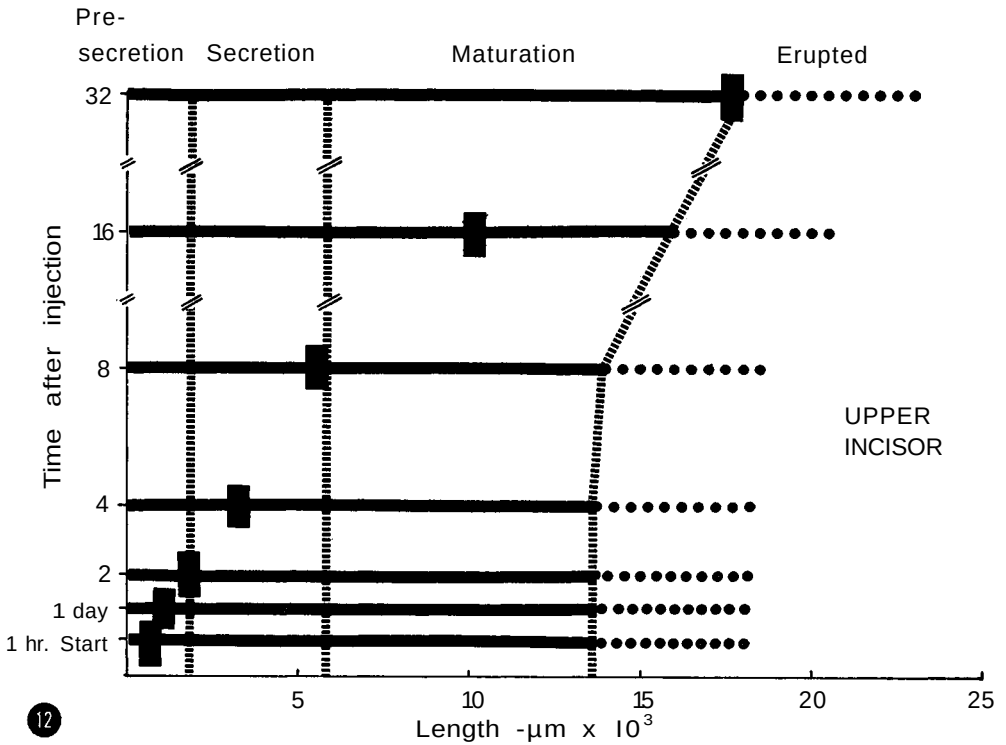

D
32d
ES
Ar
PL
11

PLATE 5

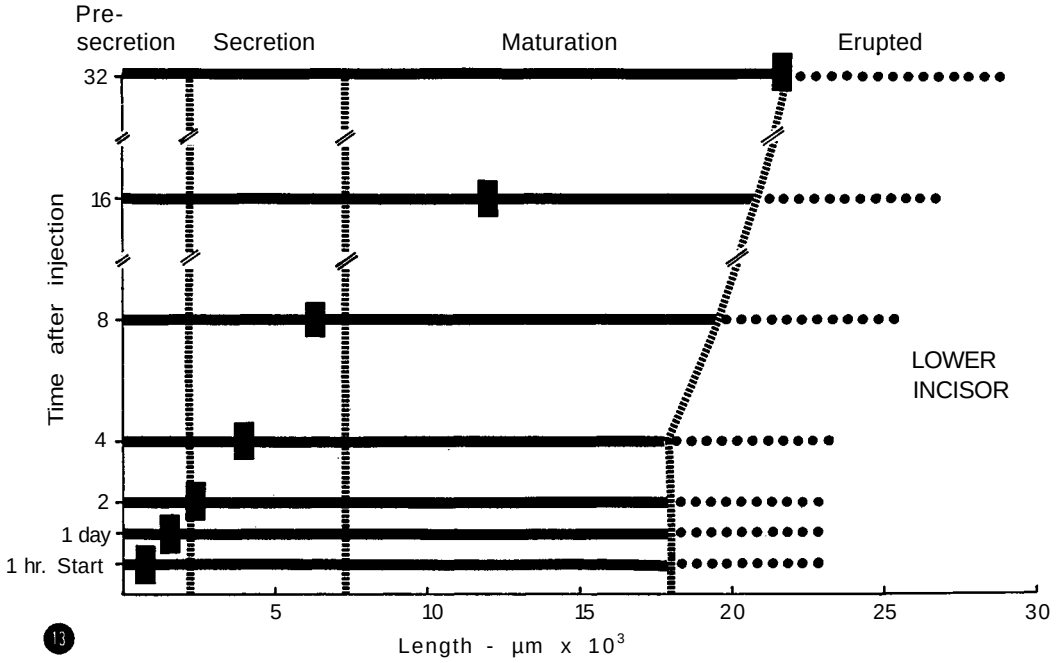
EXPLANATION OF FIGURES

12, 13 Figures 12 and 13 are graphic representations illustrating the position of the most advanced labeled ameloblast at various times after ^{3}H-thymidine injection as related to the zones of amelogenesis and to total tooth length in the upper (fig. 12) and lower (fig. 13) incisor.

The abcissa represents length measured at the labial surface from the apical loop to the incisal tip of the incisor. The solid lines indicate the embedded portion of the tooth while the dotted lines are the erupted portion. The embedded portion is subdivided by the dashed vertical lines into the zones of presecretion, secretion and maturation. The solid rectangles represent the position of the most advanced labeled ameloblast at the times indicated on the ordinate. Note that between one hour and 32 days the length of the incisor changes, since the rat grows in body weight from 100 to 300 grams in this period of time. The measurements in table 2 indicate that the increase in embedded tooth length occurs only within the maturation zone.


Pre-
secretion
Secretion
Maturation
Erupted
32
16
8
4
2
1 day
1 hr. Start
Time after injection
UPPER
INCISOR
5
10
15
20
25
Length -μm x 10³

12


Pre-
secretion
Secretion
Maturation
Erupted
32
16
8
4
2
1 day
1 hr. Start
Time after injection
LOWER
INCISOR
5
10
15
20
25
30
Length - μm x 10³

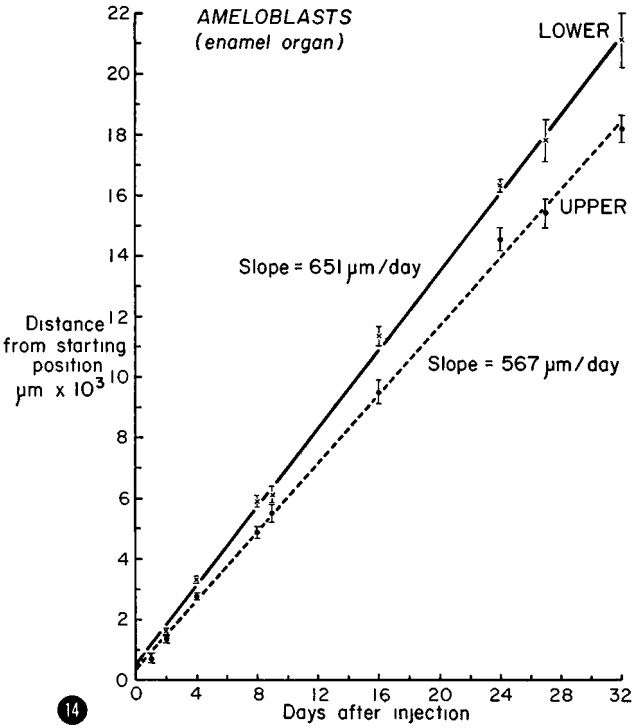
13

PLATE 6

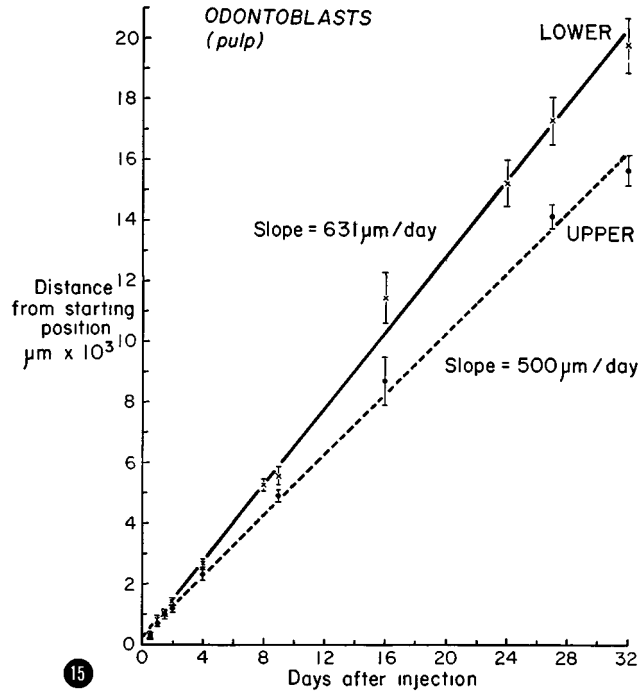
EXPLANATION OF FIGURES

14, 15 In figures 14 and 15 the distance moved by the most advanced labeled ameloblast (fig. 14) and the most advanced labeled odontoblast (fig. 15) from their starting position one hour after injecting ^{3}H-thymidine is plotted against time.

The slopes of the regression lines indicate the rate of migration of these cells in μm/day. Note that for both the ameloblasts and odontoblasts, the cells move 100 μm/day faster on the lower incisor (—) than on the upper (——). Moreover, the rate of migration for the ameloblasts (fig. 14) appears slightly faster than the odontoblasts (fig. 15) in both the upper and lower incisor. The regression lines do not cross the origin of these graphs indicating that either the cells at first accelerate from the starting position, or, are already moving at one hour and, therefore, have some other "true" starting position at zero velocity.


AMELOBLASTS
(enamel organ)
LOWER
UPPER
Slope = 651 μm/day
Slope = 567 μm/day
Distance from starting position μm x 10³
Days after injection

14


ODONTOBLASTS
(pulp)
LOWER
UPPER
Slope = 631 μm/day
Slope = 500 μm/day
Distance from starting position μm x 10³
Days after injection

15

PLATE 7

EXPLANATION OF FIGURES

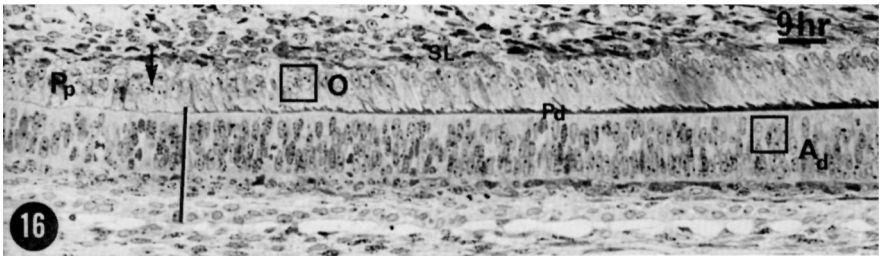
Figures 16 to 19 are radioautographs showing the relationship between the most advanced labeled odontoblast and ameloblast at various times after the injection of ^{3}H-thymidine. One μm thick Epon sections. Iron hematoxylin. x 180, except figure 17, which is x 250.

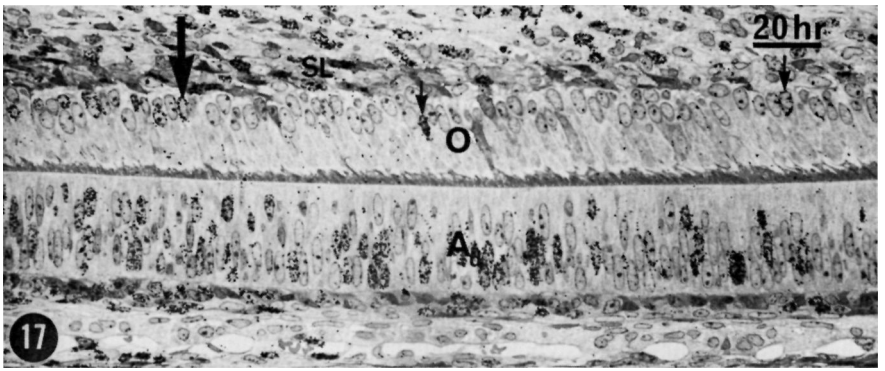
16 Labeled secretory odontoblasts begin to appear between six and eight hours after ^{3}H-thymidine injection. In this radioautograph at nine hours, the most advanced labeled odontoblast (0, in square) is slightly in front of the point marking the start of mantle predentin secretion (solid line). Cells in the main body of labeling (arrow) from the partially differentiated pulp (Pp) will soon become secretory odontoblasts. (Most advanced labeled ameloblast, Ad, in square.)

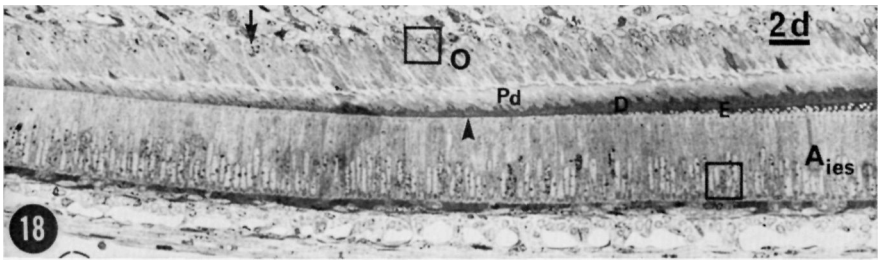
17 Radioautograph at 20 hours after ^{3}H-thymidine injection showing the main body of labeled odontoblasts (large arrow) and other labeled odontoblasts well in advance of the main front (small arrows). Labeling in the subodontoblastic layer (SL) is seen.

18 Two days after ^{3}H-thymidine injection the most advanced labeled odontoblast (0, in square) is opposite ameloblasts which are about to begin the secretion of the inner enamel matrix (indicated by arrowhead). The arrow marks the main body of labeling in the rest of the odontoblast layer. (Most advanced labeled ameloblast, Aies, in square is shown at higher magnification in fig. 25).

19 Eight days after injecting ^{3}H-thymidine the most advanced labeled odontoblast (0, in square) is opposite ameloblasts which are about to begin the secretion of the outer enamel matrix (indicated by arrowhead). (Most advanced labeled ameloblast, Aoes, in square is shown at higher magnification in fig. 26).


9hr
SL
R_p
O
Pd
A_d
16


20hr
SL
O
A_d
17


2d
O
Pd
D
E
A_{ies}
18

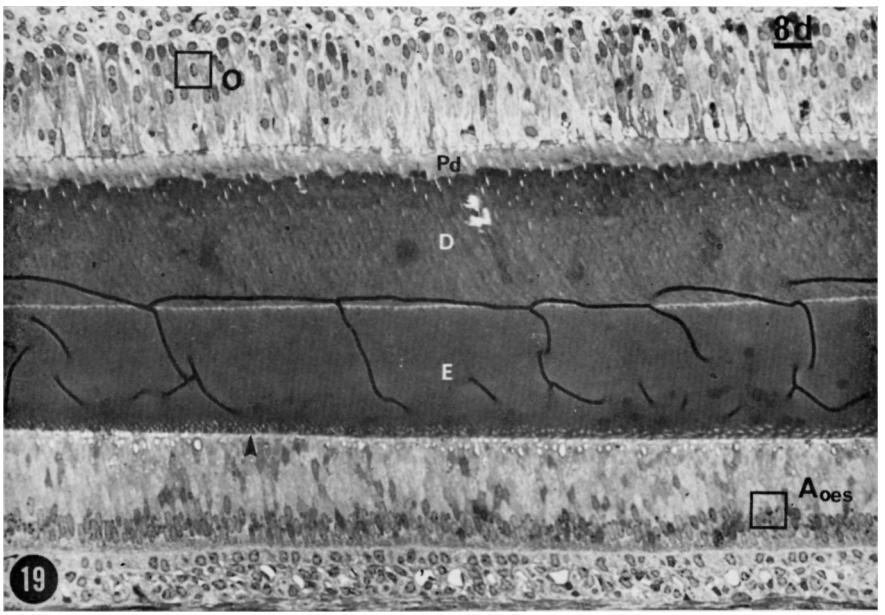

8d
O
Pd
D
E
A_{oes}
19

PLATE 8

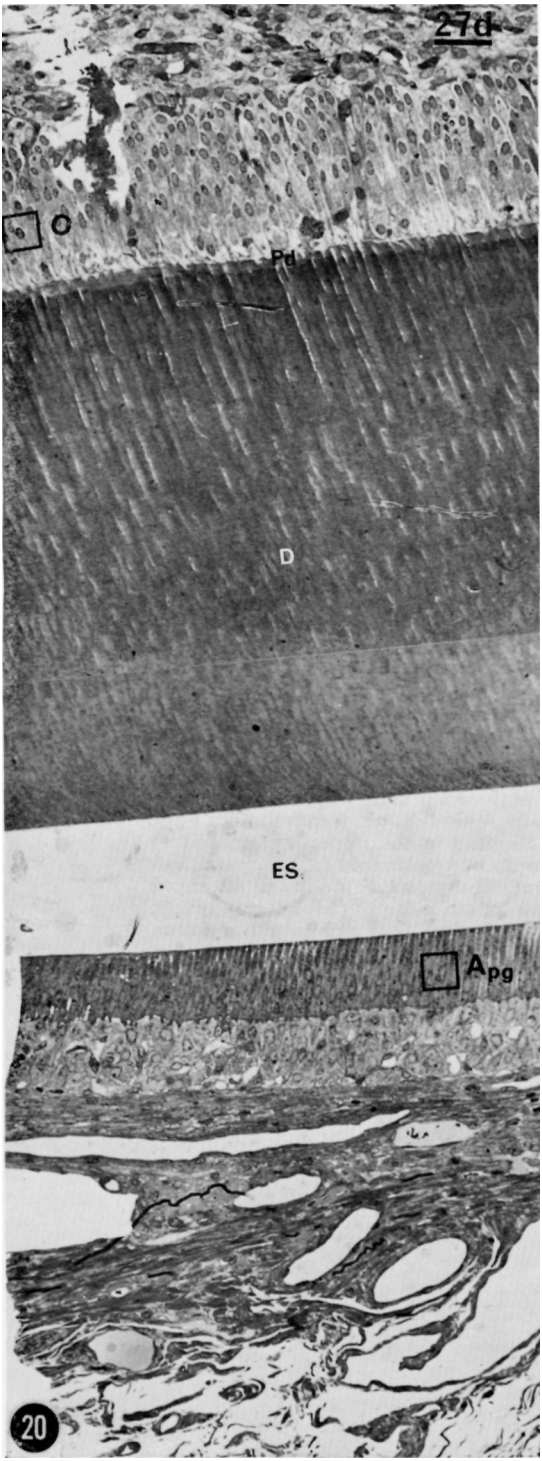
EXPLANATION OF FIGURES

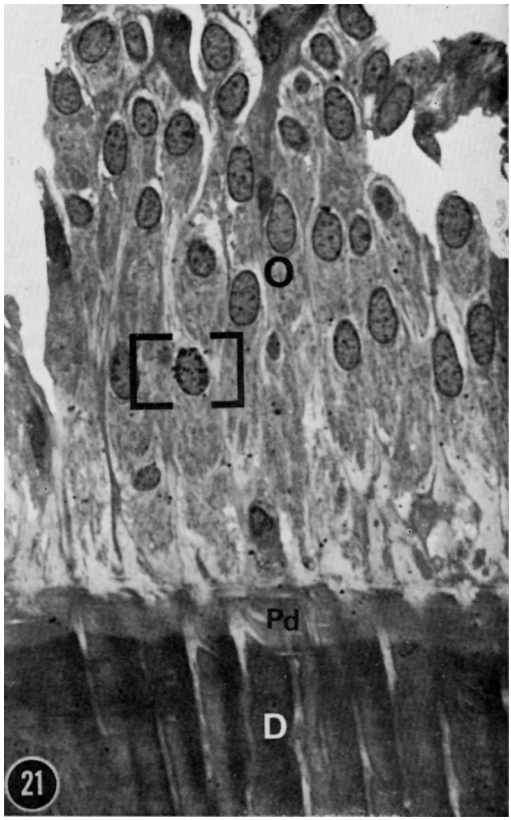
20 Radioautograph showing the most advanced labeled odontoblast and ameloblast 27 days after injecting ^{3}H-thymidine. (One μm thick Epon section. Iron Hematoxylin. x 180.

At this time the labeled front has traversed most of the length of the maturation zone. The most advanced labeled odontoblast (0, in square) is still posterior to the most advanced labeled ameloblast (Apg, in square), but the longitudinal distance between them appears shorter. The decreasing radius of curvature for the odontoblasts due to the continued deposition of dentin (D) is evident.

21 The most advanced labeled odontoblast from figure 20 is shown at higher magnification (0, in bracket). x 720.

22 The most advanced labeled ameloblast from figure 20 is seen at higher magnification (Apg, in bracket). x 350.


27d
O
Pd
D
ES
Apg
20


O
Pd
D
21

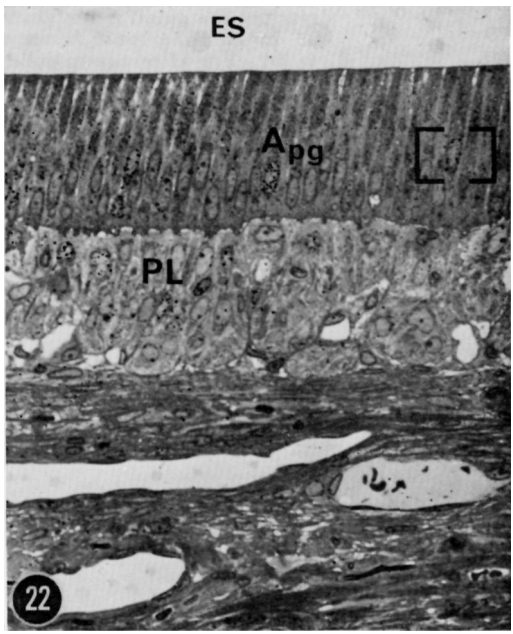

ES
Apg
PL
22

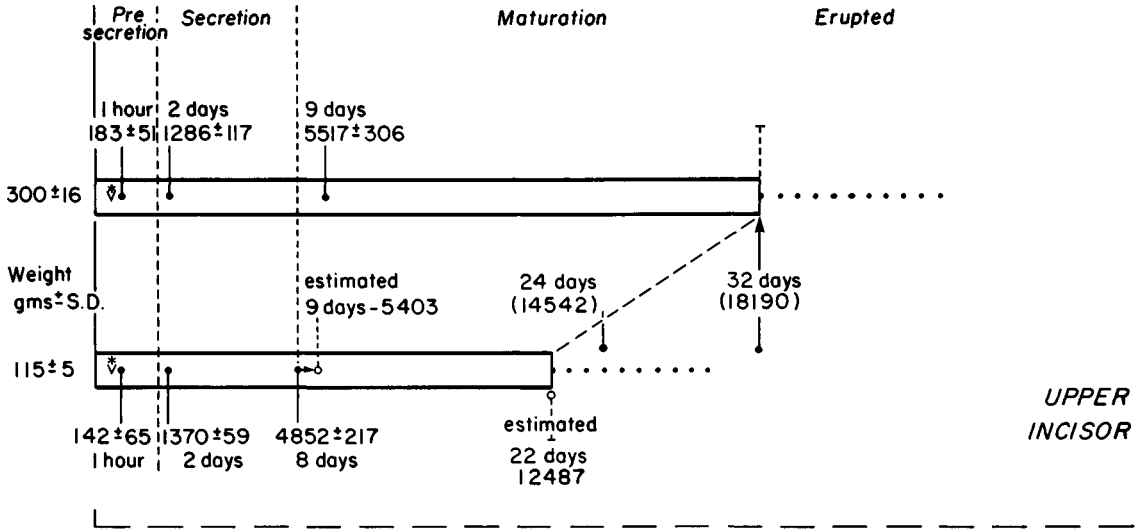
PLATE 9

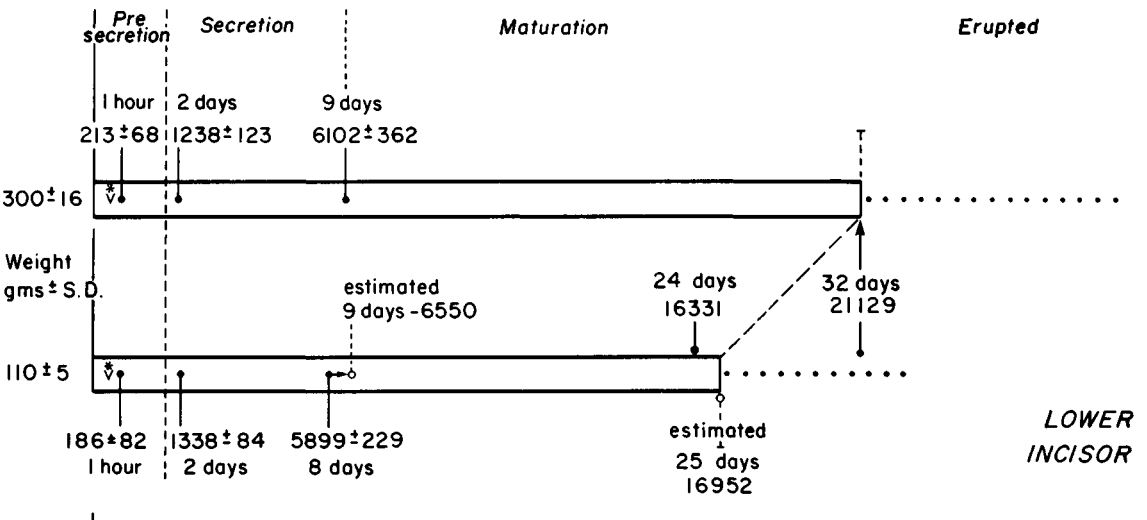
EXPLANATION OF FIGURE

23 Graphic representation of the position of the most advanced labeled ameloblast in the upper (top) and lower (bottom) incisor at various times after ^{3}H-thymidine injection in rats weighing 100 and 300 grams.

The elongated bars represent the length of the embedded portion of the incisor measured at the labial side from the apical loop to the gingival margin, and the dotted lines indicate the length of the erupted portion. The presecretory, secretory and maturation zones are indicated. The solid points (•) show the measured position of the most advanced labeled ameloblast (in μm ± standard deviation) at one hour, two days, eight or nine days, 24 days and 32 days after injection. For the points at one hour the distance represents the position measured from the start of mantle predentin secretion. For all other points the distance indicates the number of μm moved from the one hour position. The points at 32 days represent the distance travelled from the one hour position in the 100 gram rat. The open points (o) indicate the estimated position of the most advanced labeled ameloblast as calculated from points actually measured.

This graph shows that the most advanced labeled ameloblast at one hour, two and nine days is found in the same position in the 100 and 300 gram rat. Moreover, the graph indicates that the increase in length which occurs within the maturation zone appears "ahead" of the advancing cells. Theoretically, the labeled front should have reached the gingival margin at 22 days in the upper incisor and at 25 days on the lower if no growth had occurred. In rats sacrificed at 24 days after the injection of ^{3}H-thymidine the most advanced labeled ameloblast had traveled a length close to the original length of the maturation zone (compare estimated (o) and actual positions (•)). The most advanced labeled ameloblast, however, was not at the gingival margin and the distance remaining between it and the gingival margin was 4,026 μm on the upper incisor and 3,564 μm on the lower. Thus additional length appeared to arise ahead of the advancing ameloblast.





23

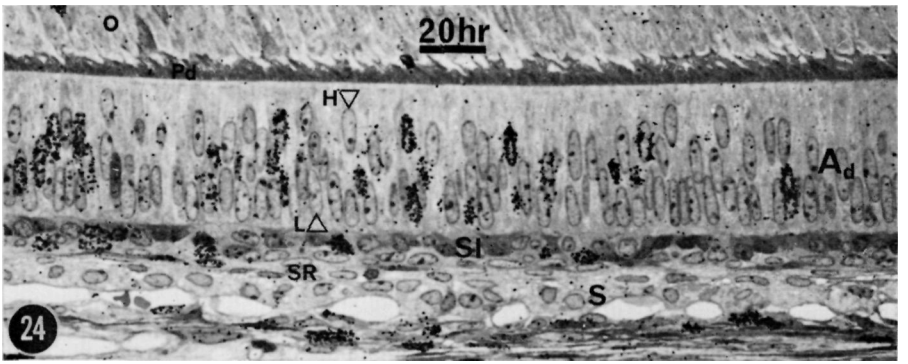
Start mantle predentin secretion

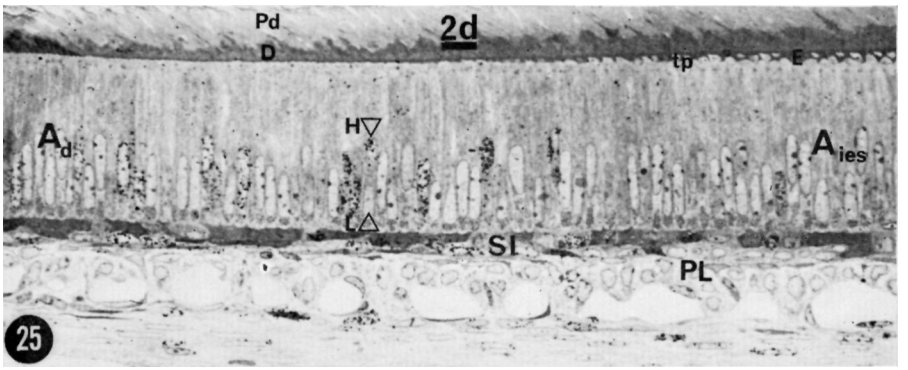
PLATE 10

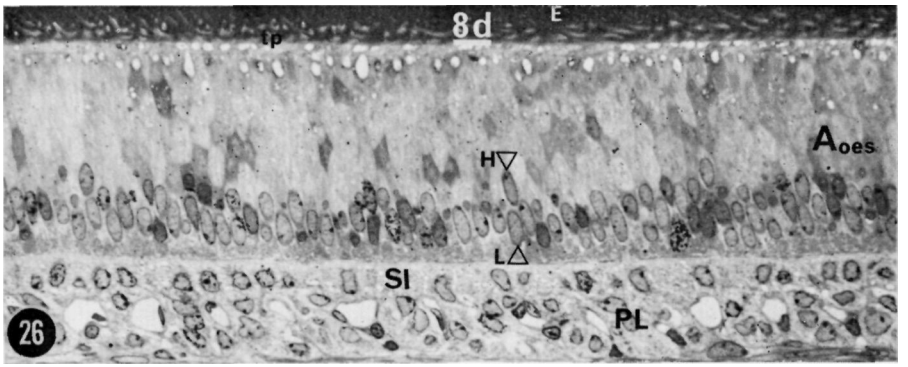
EXPLANATION OF FIGURES

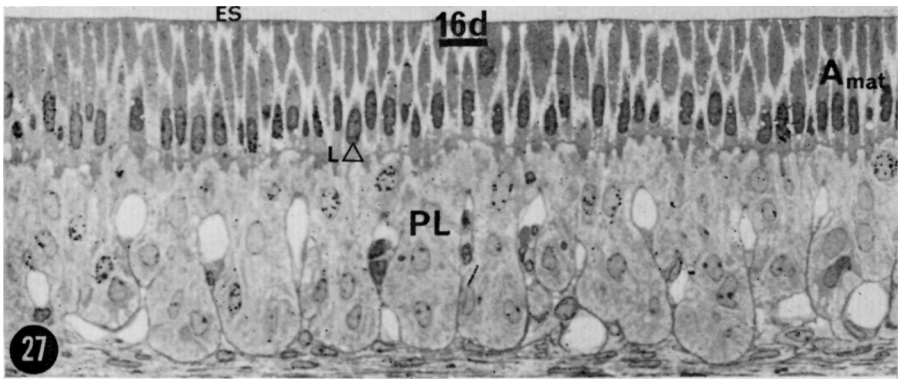
24–27 Figures 24 to 27 are radioautographs of the leading edge of labeled cells in the enamel organ from 20 hours to 16 days after the injection of ^{3}H-thymidine. The most advanced labeled ameloblast is seen near the right-hand side of each figure. One μm thick Epon sections. Iron hematoxylin. x 350.

These figures show the changes which occur in the distribution of cells and nuclei within each layer of the enamel organ during amelogenesis. In the ameloblast layer the nuclei at 20 hours after injection (Ad, fig. 24) appear gathered at two distinct levels which become staggered levels during differentiation and remain as such throughout enamel secretion (Aies, fig. 25, Aoes, fig. 26). Within the maturation zone, shown in figure 27 (Amat) at 16 days after injection, most of the ameloblast nuclei are aligned at one level. The stratum intermedium (SI) is bilayered until the ameloblasts begin secreting the inner enamel matrix (figs. 24, 25) and then becomes a single layer of cuboidal cells throughout the rest of enamel secretion (fig. 26). The stratum intermedium is lost as a definitive layer at the start of maturation. The papillary layer (PL) undergoes progressive development throughout enamel secretion (figs. 24, 25, 26) and is fully developed at the start of maturation (the example in fig. 27 at 16 days after injection is about halfway through the maturation stage).


O
20hr
Pd
H▽
Ad
L△
SI
SR
S
24


Pd
D
2d
tp
E
Ad
H▽
Aies
L△
SI
PL
25


tp
8d
E
Aoes
H▽
L△
SI
PL
26


ES
16d
Amat
L△
PL
27