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Phagocytosis and Elimination of Amelocyte Debris by Stratum Intermedium Cells in the Transitional Zone of the Enamel Organ of the Rat Incisor*

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Summary. In the transitional zone of the enamel organ (rat) some of the amelocytes perish. Their debris is phagocytized and digested by stratum intermedium cells and macrophages. These two cell types also seem to remove cytosegresomes expelled from those amelocytes which survive and redifferentiate into transporting amelocytes. Digestion of the amelocyte debris in the stratum intermedium cells is effected rapidly and completely. Degeneration of stratum intermedium cells was not observed in the transitional zone.

Key words: Tooth — Enamel organ — Cell differentiation — Phagocytosis — Electron microscopy.

Introduction

It is generally accepted that the process of enamel formation involves two phases. Amelocytes engaged in these two phases are called secretory and transporting amelocytes respectively. They represent stages in the same cell cycle and are located within a zone of enamel matrix formation and a zone of enamel maturation. Between these two zones is a narrow transitional zone in which a redifferentiation of secretory amelocytes into transporting amelocytes takes place. This functional transition is manifested in drastic changes in the morphology of the amelocytes and of the stratum intermedium cells.

Under the light microscope, numerous globular structures are apparent in the amelocyte layer and in the adjacent stratum intermedium cells of the transitional zone (Wassermann, 1944; Marsland, 1951). These globules contain pyroninophilic, eosinophilic and Feulgen-positive material and were assumed by Symons (1962) to represent degenerating cells or cell constituents. They were thought to correspond to the "cytosegresomes" or "autophagosomes" in amelocytes and stratum intermedium cells, recently described in electron microscope studies (Elwood and Bernstein, 1968; Kurahashi and Moe, 1969; Reith, 1970). None of these reports give detailed information on the origin and fate of the "globules" of the light microscopists. Furthermore, it has not been established whether cells degenerate during the reorganization of the enamel organ, and if so, which.

The present paper presents evidence that amelocytes perish in the transitional zone. Their debris is phagocytized and eliminated by stratum intermedium cells. These cells may also remove cytosegresomes expelled from redifferentiating amelocytes. The stratum intermedium cells do not degenerate. They cooperate with macrophages as scavengers in the enamel organ (Jessen and Moe, 1972).

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Materials and Methods

Albino rats, weighing approximately 60 g, were perfused via the ascending aorta with a fixative containing 3% glutaraldehyde and 2% Dextran T40 in 0.15 M cacodylate buffer at pH 7.4. The mandible was dissected free and bisected. Demineralization of the mandible halves was effected at 4° C in a 2.5% solution of EDTA in 0.2 M glucose adjusted to a pH of 7.4, for three weeks. The demineralized incisors were sliced in sections of 1 mm thickness. The specimens were washed in 0.1 M cacocylate buffer overnight and postfixed by immersion in 2% osmium tetroxide in 0.15 M cacodylate buffer at pH 7.4 for 2 hours, prior to dehydration and embedding in Epon. Series of sections were cut through the transitional zone at intervals less than 10 microns. They were stained with both uranyl acetate and lead citrate and examined in a Philips 300 electron microscope.

Observations

In the transitional zone, tall and crowded secretory amelocytes are transformed into shorter and less crowded transporting amelocytes. Based on differences in morphology of the amelocyte layer, two regions can be distinguished in this zone: a region of regression and a region of redifferentiation. The region of regression arises when the enamel matrix has attained its ultimate thickness. Two of the main features of this region are the occurrence of a large number of cytosegresomes within the amelocytes and the presence of conspicuous amounts of cellular debris between and below these cells.

The Amelocyte Debris

An essential part of the cellular debris originates by degeneration of entire amelocytes. In this process, the amelocytes disintegrate into fragments of varying size. Some of these may be very large and contain the major part of a dying amelocyte. In other cases, the amelocyte will disintegrate into smaller fragments situated in a row between the neighbouring vital amelocytes (Fig. 1). The fragments are surrounded by a plasma membrane and contain either pyknotic nuclei or cytoplasmic components such as granular endoplasmic reticulum, mitochondria, ribosomes, Golgi components, filaments, and various granules, or both (Figs. 1, 2). The organelles often appear intact. However, fragments of disintegrating amelocytes are easily recognized because of the greater density of their cytoplasmic matrix compared to that of intact amelocytes.

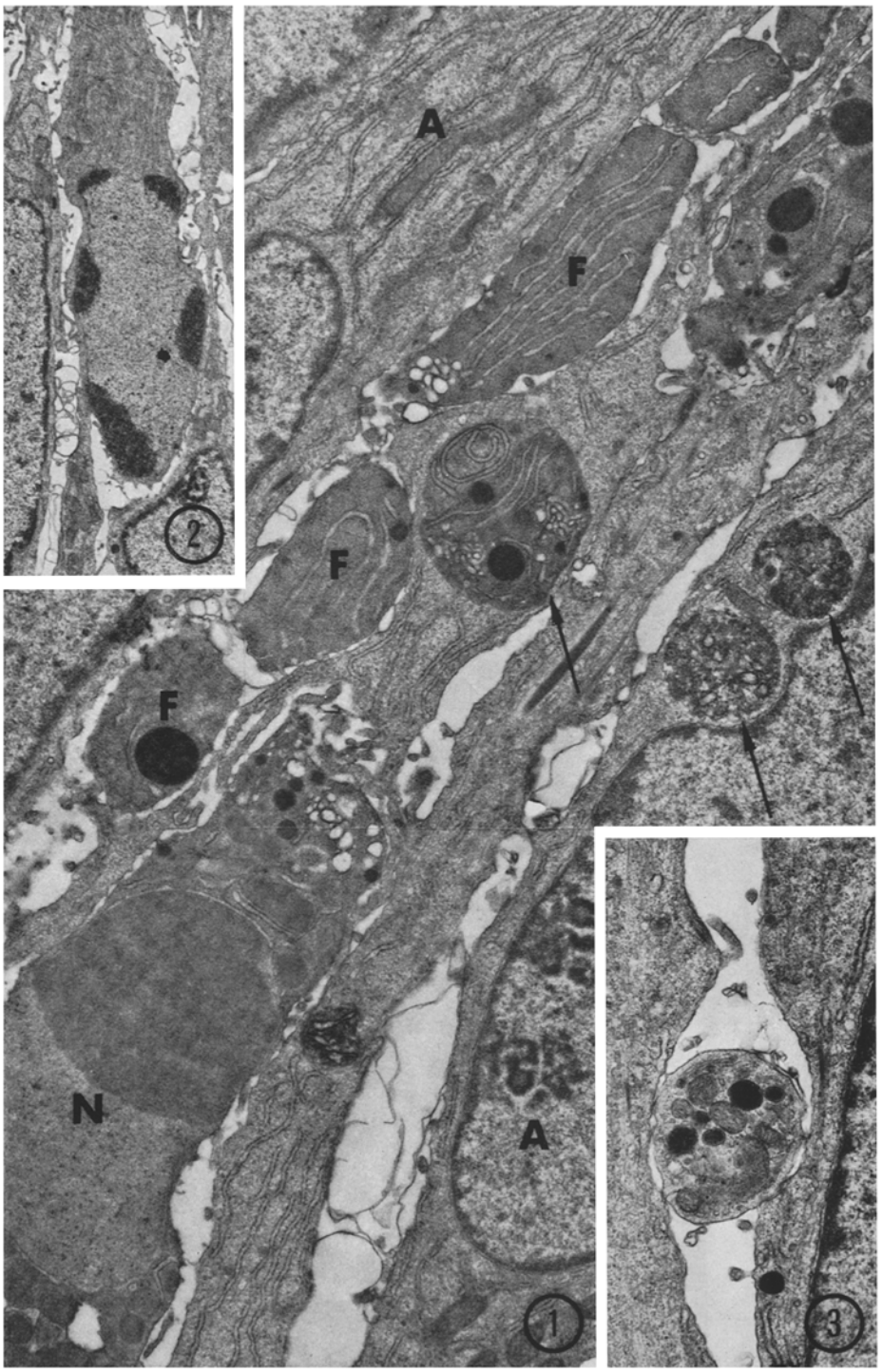
In the area of disintegration, wide intercellular spaces appear between the vital amelocytes.

Amelocytes in the transitional zone contain a large number of cytosegresomes (autophagic vacuoles). A part of the cellular debris between the amelocytes

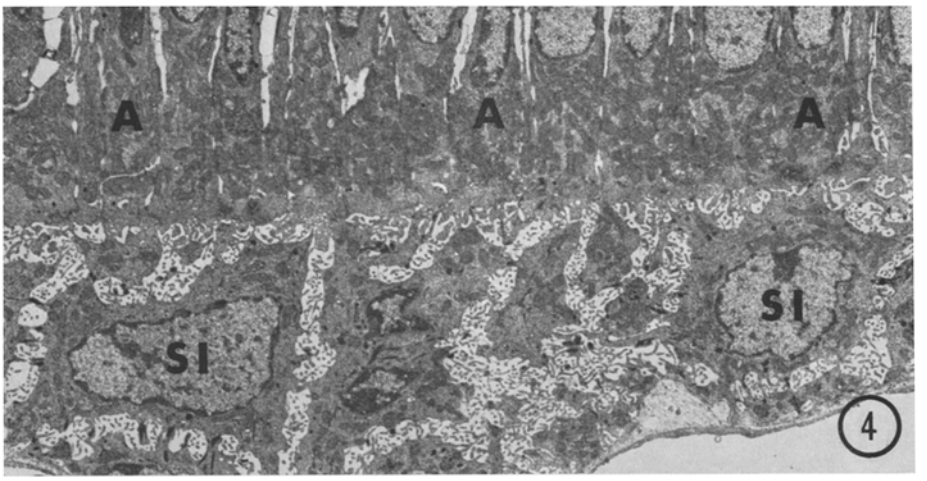
Fig. 1. Micrograph showing amelocyte debris in the basal part of the amelocyte layer. A large fragment contains a pyknotic nucleus (*N*). Several smaller fragments (*F*), containing cytoplasmic components, are situated in a row between vital amelocytes (*A*). Autophagic vacuoles or cytosegresomes (arrows) are seen within vital amelocytes. × 10000

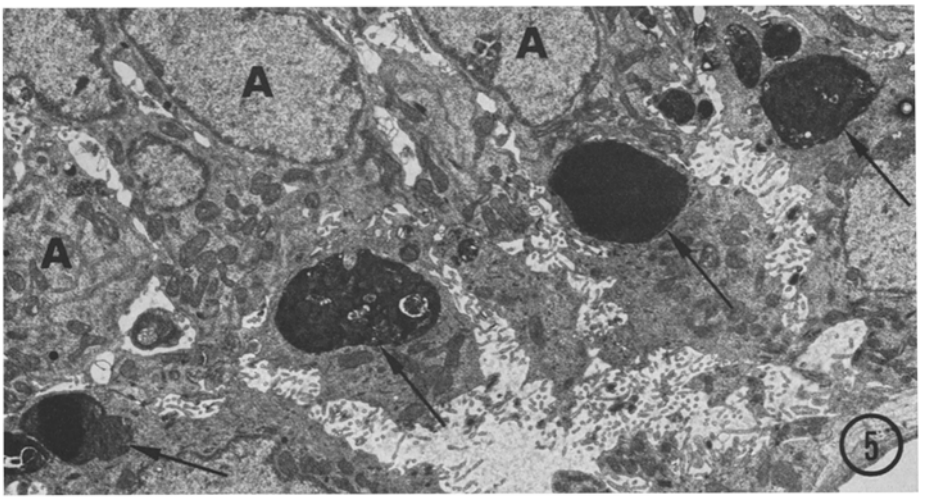
Fig. 2. Micrograph showing a part of an amelocyte whose nucleus contains chromatin condensations, indicating early pyknotic changes. × 4000

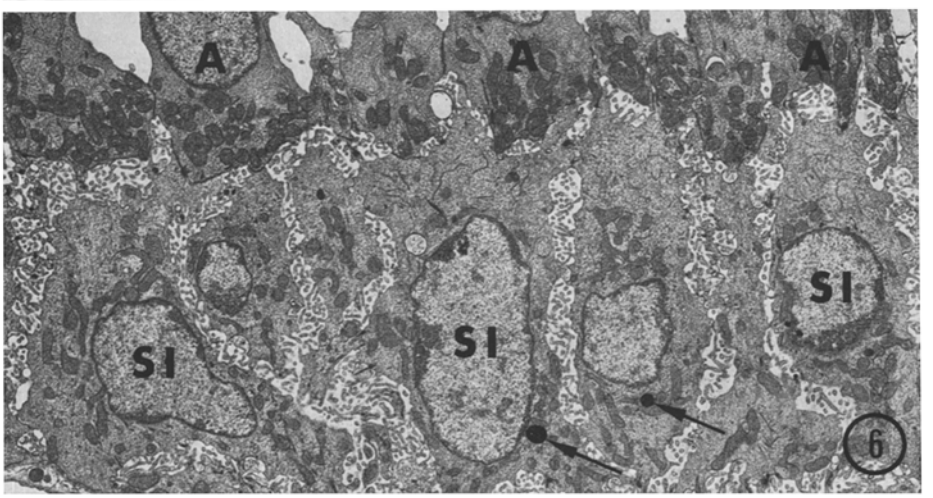
Fig. 3. Cytosegresome expelled into the intercellular space between two amelocytes. The organelle content of the cytosegresome appears intact. × 15000



Figs. 1—3







Figs. 4—6

probably represents cytosegresomes extruded into the intercellular space (Fig. 3). The expelled cytosegresomes are spherical bodies surrounded by a membrane. They vary in size and content. Their main content is granular endoplasmic reticulum, ribosomes, Golgi components, and mitochondria. The organelles are often more closely packed than they are in the fragments of degenerating amelocytes and may appear intact. Some extruded cytosegresomes contain lysosome-like bodies, and degenerative changes of the organelle content can be seen.

The majority of the extruded cytosegresomes originates in the supranuclear portion of the transitional amelocytes. After their extrusion they apparently pass through the intercellular spaces towards the stratum intermedium cells.

The Stratum Intermedium Cells

In the zone of enamel matrix formation, the stratum intermedium consists of a row of cuboidal cells with many microvilli and short spinous processes (Fig. 4). The cells are attached primarily to one another, but also to the amelocytes and the stellate reticulum cells by desmosomes and occasional tight (gap) junctions. They contain bundles of tonofilaments attached to the desmosomes interconnecting the cells. The cells may be somewhat polarized, i.e. several small Golgi complexes and small dense bodies, with the morphology of primary lysosomes, may occur mainly on the dental side of the nucleus. The cells contain many mitochondria and a moderate number of free ribosomes. Granular endoplasmic reticulum is scarce.

In the incisal part of the matrix formation zone, where the amelocytes contain reformed basal webs (Moe, 1971) and their Tomes processes disappear, the stratum intermedium cells send long, slender cytoplasmic processes between the bases of the amelocytes. These processes extent beyond the attachment zones of adjacent amelocytes and thus reduce the number of junctions between the basal ends of the amelocytes (Fig. 7). This particular type of process from the stratum intermedium cells is seldom observed in the transitional zone.

In the transitional zone, the stratum intermedium cells change their shape and no longer constitute a uniform layer. They persist, but become more irregular, and their mutual desmosomal connections are reduced. Concurrently, more junctions (desmosomes and tight (gap) junctions) are formed with the basal ends of the amelocytes. From the opposite aspect, long processes extend to form junctions with the epithelial cells in the papillary ridges. This reorganization of the stratum intermedium results in more elongated and irregular cells (Fig. 6). The dental part of the stratum intermedium cells exhibits a particular plasticity during

Fig. 4. Stratum intermedium cells (*SI*) and part of adjacent amelocytes (*A*) in the incisal part of the enamel matrix formation zone. In this area the stratum intermedium cells do not contain phagosomes. × 3000

Fig. 5. Micrograph showing stratum intermedium cells in the transitional zone. Large phagolysosomes are present in the cells (arrows). *A* amelocyte. × 4500

Fig. 6. Stratum intermedium cells (*SI*) in the incisal part of the transitional zone. In this area no phagolysosomes or residual bodies are seen. A few lipid droplets are present (arrows). *A* amelocytes. × 3500

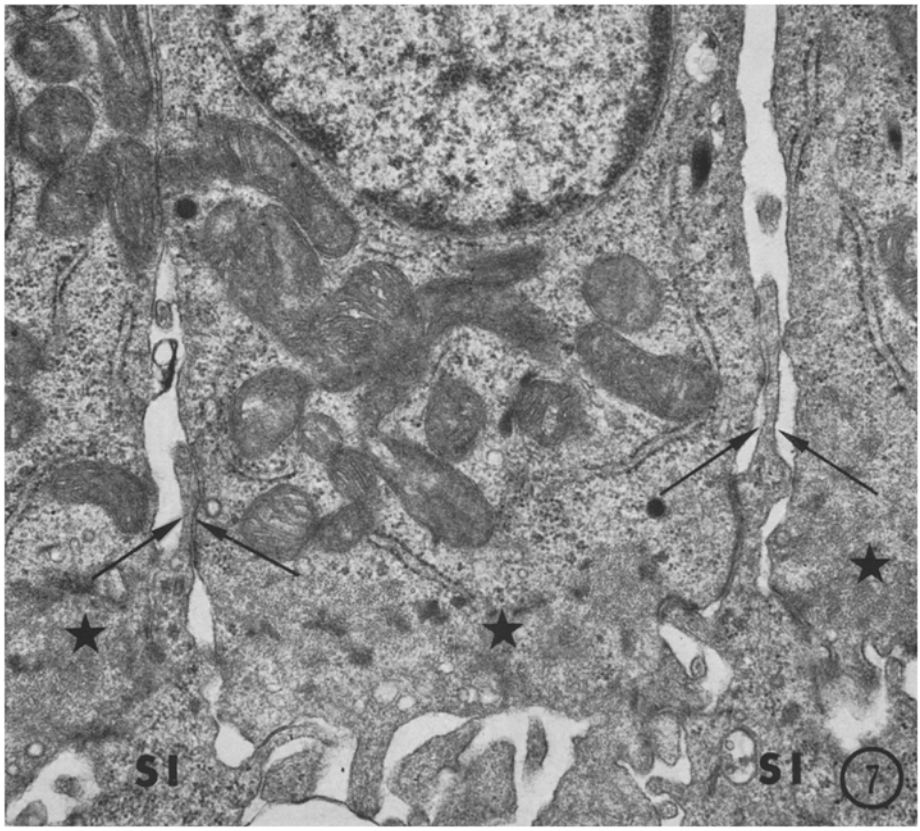


Fig. 7. Micrograph showing the basal part of amelocytes late in the matrix formation stage Slender cytoplasmic processes (arrows) from a stratum intermedium cell (*SI*) extend beyond the attachment zones between the amelocytes. Basal webs are present in the amelocytes (stars). × 23500

the regressive events in the amelocyte layer. This feature reflects the phagocytic activity of the cells, since it is most evident in areas with large amounts of amelocyte debris. In such areas, the stratum intermedium cells remove debris by extending cytoplasmic processes into the intercellular spaces between the basal parts of the amelocytes (Figs. 8, 9). In other cases, however, the debris apparently passes between the basal parts of the amelocytes into the intercellular spaces between the amelocytes and the stratum intermedium cells before it is phagocytized. The stratum intermedium cells engulf the amelocyte debris by surrounding it with slender cytoplasmic processes. During this process the debris is enveloped by a plasma membrane from the stratum intermedium cell and ingested as a

Fig. 8. Micrograph showing a stratum intermedium cell (*SI*) in the process of engulfing large fragments of amelocyte debris. Some fragments contain pyknotic nuclei (*N*). *A* vital amelocytes. × 12000

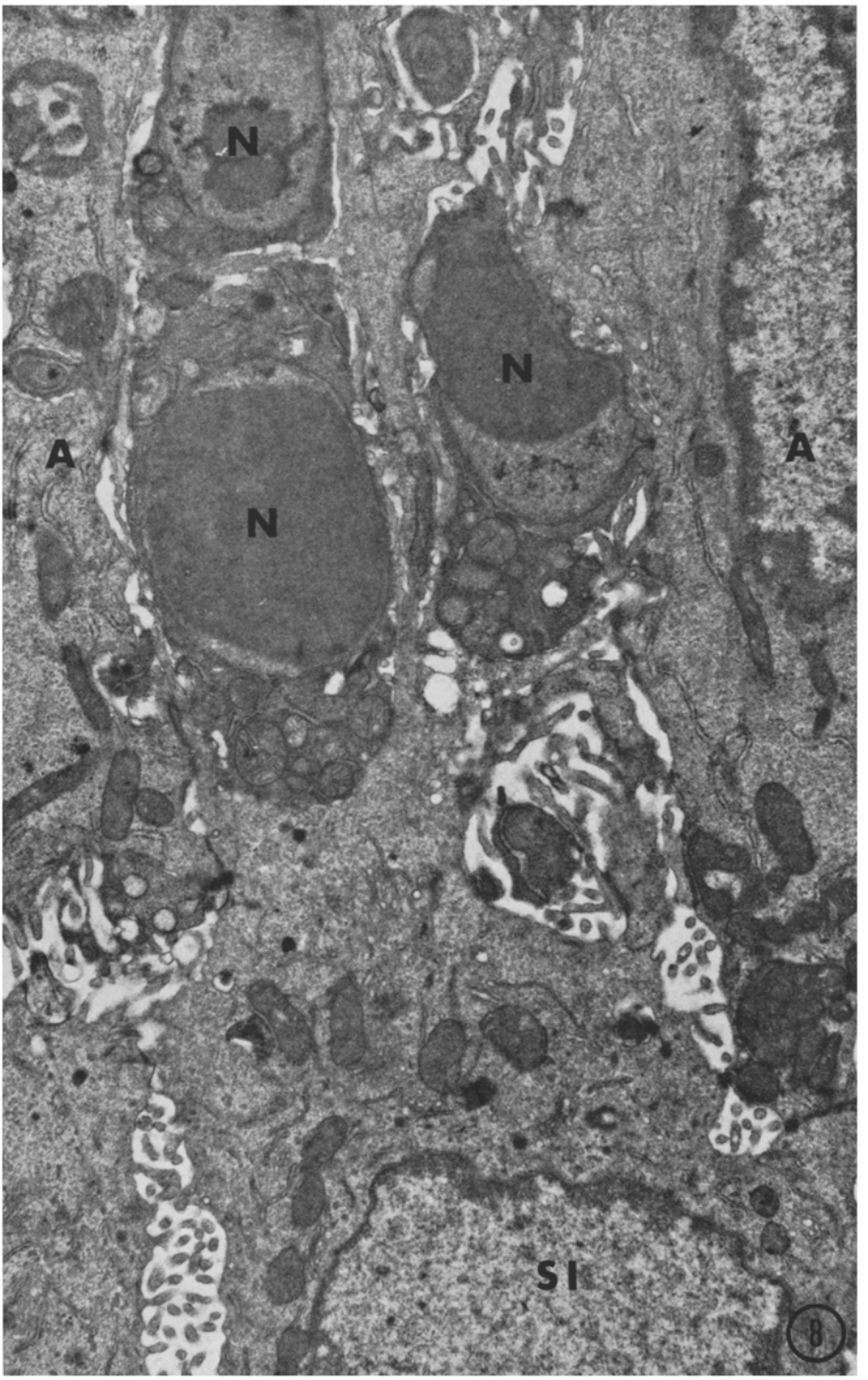


Fig. 8

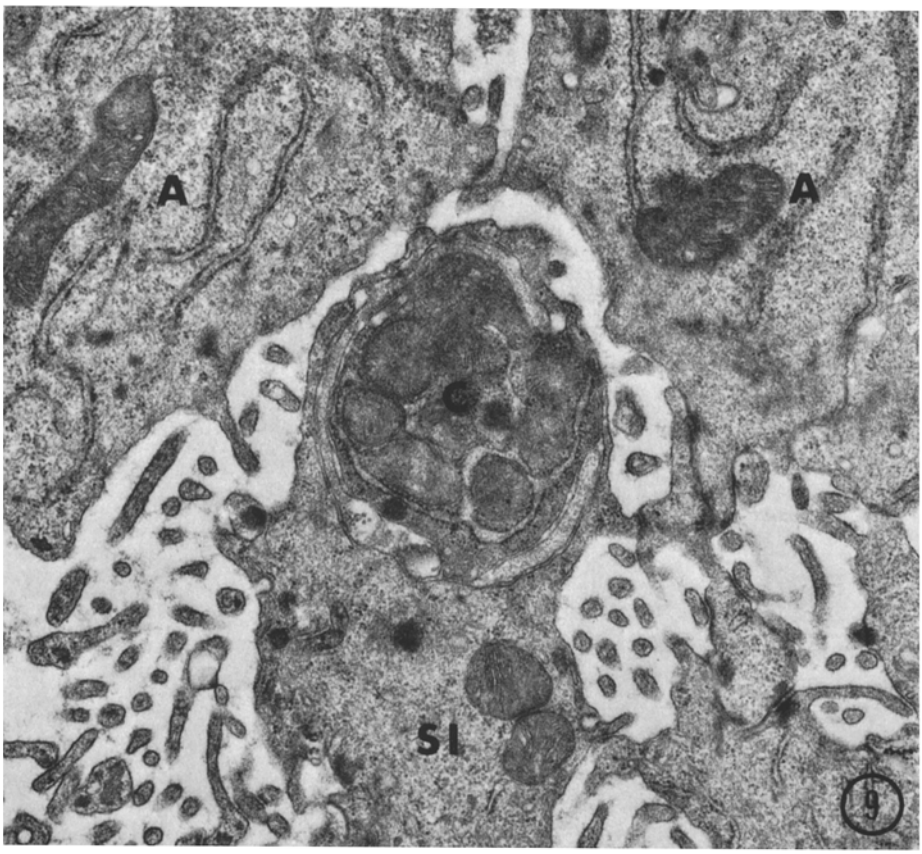


Fig. 9. Micrograph showing a body with features of a cytosegresome (*C*) surrounded by slender processes from a stratum intermedium cell (*SI*). The body contains apparently intact mitochondria and granular endoplasmic reticulum. *A* bases of amelocytes. × 19500

phagosome. The phagosomes are usually retained in the dental parts of the cells (Fig. 10).

Proximally in the regressive part of the transitional zone, many of the phagosomes in stratum intermedium cells are very large and may contain a major part of a degenerating amelocyte. An individual stratum intermedium cell may contain several large phagosomes (Fig. 10). Occasionally, one phagosome may contain two or three amelocyte fragments each surrounded by a membrane. The contents of the phagosomes may be more or less tightly packed and show varying density. However, pyknotic nuclei, granular endoplasmic reticulum, mitochondria, and other cytoplasmic constituents of dead amelocytes can often be recognized within the large phagosomes. Some of the phagosomes have a very compact and heterogeneous content, whose components cannot be identified. These bodies probably represent phagolysosomes at an advanced stage of digestion (Figs. 5, 11).

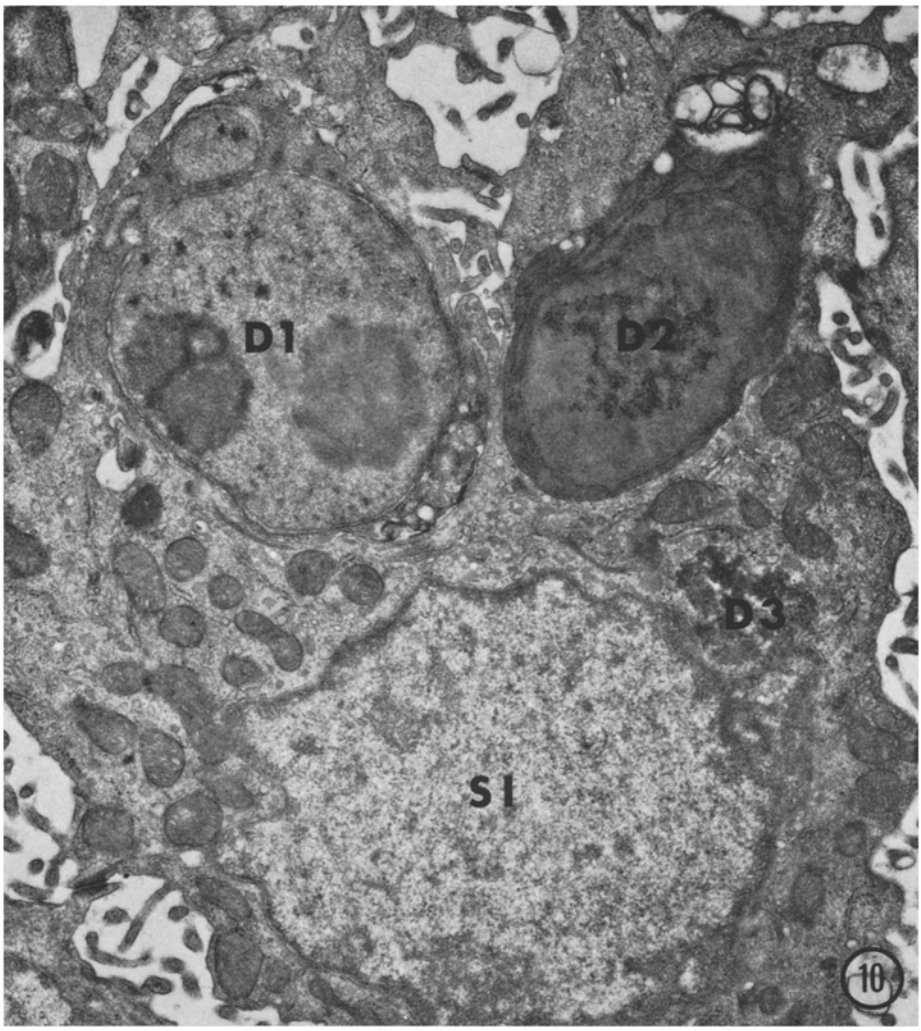


Fig. 10. Stratum intermedium cell (*SI*) containing ingested amelocyte debris in various stages of degradation (*D1, D2, D3*). × 15000

Incisally in the regressive region the phagolysosomes are smaller. At this stage, organelle remnants cannot be recognized in the bodies. In the transitional zone, in the region of redifferentiating amelocytes, the stratum intermedium cells contain very few or no phagolysosomes (Fig. 6). Lipid droplets and an occasional residual body may be present.

Degeneration of stratum intermedium cells was not observed in the transitional zone.

The Macrophages

A considerable number of macrophages assist the stratum intermedium cells in the removal of amelocyte debris. In addition to cellular debris, the macrophages

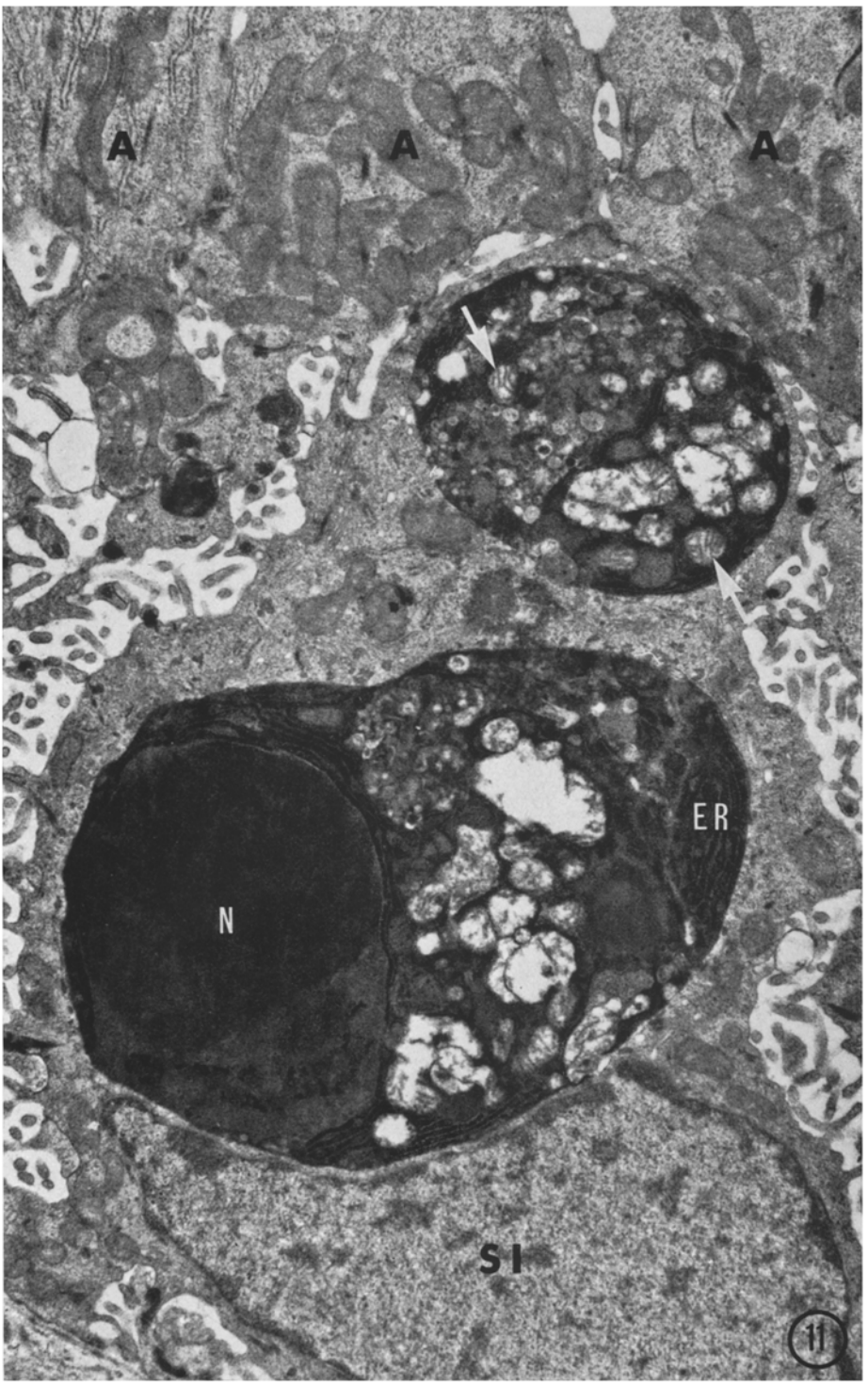


Fig. 11

appear to phagocytize extracellular, granular material (Jessen and Moe, 1972). Phagocytosis of this material by stratum intermedium cells was not observed.

Discussion

This study furnishes evidence that some of the amelocytes do perish in the transitional zone of the enamel organ of the rat incisors. Furthermore, it reveals that amelocyte debris is ingested and eliminated by the stratum intermedium cells. As previously reported, amelocyte debris is also removed by macrophages present in the enamel organ (Jessen and Moe, 1972).

All these events take place in the proximal portion of the transitional zone and result in a certain thinning out of the cells in the amelogenetic cell layer. This may contribute to the widening of the intercellular spaces characteristic of this region. Another characteristic feature of the region is the drastic reduction in height of the surviving amelocytes. This is caused by regressive processes in connection with the conclusion of matrix secretion and the ensuing obsolescence of the secretory machinery. Superfluous cell constituents are confined and digested in cytosegresomes or autophagic vacuoles, especially in the supracellular portion of the cells. Since the amelocytes which survive redifferentiate into transporting cells after the regression, the transitional zone may appropriately be divided into a region of regression and a region of redifferentiation. A similar division was suggested by Wassermann (1944), who used the term progressive for the last mentioned region, however.

Amelocytes in the processes of degeneration are recognizable by chromatin condensations typical of pyknosis, increased cytoplasmic density, crowding of the organelles, and fragmentation of the cell body. The adjacent stratum intermedium cells ingest the dying amelocytes by surrounding the debris with cytoplasmic projections which extend from their dental aspects. During this activity, their lateral and abdental surfaces are secured by junctions to adjacent cells. Most of the debris containing nuclei is engulfed by the stratum intermedium cells. The macrophages may also ingest large debris, but these cells seem mainly to remove the smaller cell fragments. The effectiveness of this work is evident, since no cellular debris was observed in the intercellular spaces external to the stratum intermedium.

There is some uncertainty as to the origin of the smaller pieces of debris. Many of these fragments undoubtedly come from totally degenerating amelocytes. Some debris, however, contains organelles without recognizable degradation. These pieces might represent cytosegresomes segregated by surviving amelocytes without incorporation of lysosomal elements, and thereafter discharged from the cells. A few of our electron micrographs may be interpreted as showing cytosegresomes of this type in the process of discharge. It may be mentioned in this connection that Kerr (1970) reports on defaecation of cellular debris into the intercellular

Fig. 11. Stratum intermedium cell (*SI*) containing large fragments of amelocytes at an advanced stage of digestion. Nuclear material (*N*), mitochondria (arrows), and endoplasmic reticulum (*ER*) can still be recognized. *A* amelocytes. × 12500

spaces by hepatic cells extremely active in autophagocytosis. During regression, the amelocytes are subjected to extensive formation of autophagic vacuoles.

In a recent paper, Reith (1970) conceived the large inclusions in the stratum intermedium cells to be autophagic vacuoles and suggested that the great number of inclusions might indicate that some of these cells perish. We do not agree with this interpretation, but consider the inclusions to be phagosomes. They contain degenerating nuclei without degenerative changes occurring in the nucleus of the stratum intermedium cell itself. Furthermore, stratum intermedium cells passing the region preceding the transitional zone did not contain inclusions that could be regarded as initial stages in the formation of large autophagic vacuoles. Occasionally a cytosegresome did occur in a few of the stratum intermedium cells in the matrix secretion zone, but these bodies were always very small. We also disagree with Reith in his statement that the proximal "terminal bar apparatus" persists through the entire transitional stage. On the contrary, the junctions between the bases of the amelocytes in the transitional zone are considerably weakened. This situation probably facilitates the passage of material from the spaces between the amelocytes to the spaces between the adjacent cells of the enamel organ. In the transitional zone, not only cell debris leaves the amelocyte layer but also lumps of intercellular granular or stippled material. It must be added, however, that Reith studied the molar teeth of the rat, in which the processes may be less clear-cut than is the case in the incisors.

The digestion of the phagosomes in the stratum intermedium cells is effected rapidly and apparently completely. The digestive enzymes probably originate from lysosomes present in the cellular debris and from lysosomes contributed by the stratum intermedium cells. Residual bodies were relatively rare. When the stratum intermedium cells enter the enamel maturation zone, they are virtually without inclusions except for a few lipid droplets.

The degeneration of the amelocytes, their ingestion by stratum intermedium cells, and the elimination of the phagosomes in these cells occur in a zone the extent of which in midsagittal sections of the incisors of the rats used in this study was about 200 microns or less. From studies with tritiated thymidine it is known that the amelocytes and the stratum intermedium cells of the rat and mouse incisors move forward in the jaw with the tooth and at the same rate (Hunt and Paynter, 1963; Hwang and Tonna, 1965). The eruption rate of the lower incisors in the rat has been measured by several authors to be about 400 microns per 24 hours (Addison and Appleton, 1915; Downs, 1930–31; Shadle *et al.*, 1936; Sturman, 1957; Chiba, 1965; Robins, 1967). From these figures, it is apparent that it takes about 10 to 12 hours for the amelocytes to pass the momentous regression region and less than 12 hours for moribund amelocytes to be completely eliminated. Quantitative studies are needed to establish the number of amelocytes which become moribund. Roughly, we would estimate the percentage to be in the order of 10 to 15 per cent.

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