# THE ULTRASTRUCTURE OF AMELOBLASTS

# DURING MATRIX FORMATION

# AND THE MATURATION OF ENAMEL

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ABSTRACT

Ameloblasts from different regions of upper incisors of rats were examined with the electron microscope. During matrix formation, the cells resemble secretory cells. They are extremely long, tightly packed, and show considerable polarity. Nuclei are at the basal end of the cell. Mitochondria are proximal and the Golgi apparatus distal to the nucleus. Ergastoplasm is found in all levels but mainly in the distal end. A terminal bar apparatus separates the distal end of the cell from Tomes's process. Next to this is soft enamel. The next incisal region is a transitional zone in which the ameloblasts separate easily from the enamel. Endoplasmic reticulum is dilated and very obviously in communication with the perinuclear space. Mitochondria are present not only proximal, but also distal, to the nucleus. The next incisal zone consists of cells related to the maturation of enamel. They no longer resemble secretory cells, but now have more characteristics of transporting cells. Processes from the distal end of the cell are present with mitochondria closely applied to the base of the processes. A considerable amount of intercellular space exists with microvilli projecting into the space. Iron granules appear in these cells, and the ergastoplasmic cisternae are dilated. In the incisal end of this zone, the iron granules form aggregates. The iron finally leaves the cells to enter the enamel. Free RNP particles and fibrils become more evident after the iron leaves the cells. In the most incisal region, the ameloblasts are further reduced in height. Distal processes are no longer present and fibrils are more conspicuous.

## INTRODUCTION

It is widely accepted that the formation of tooth enamel can be considered as occurring in two major steps: formation of enamel matrix, and maturation of enamel (3, 8, 18). These conclusions are based largely on histological examinations of the enamel and enamel organ such as those of Marsland (15, 16) or on histochemical observations such as those of Symons (31, 32). Although a number of reports on the ultrastructure of ameloblasts and their precursors have appeared (12, 13, 17, 22, 23, 25, 33, 34), these have been confined to studies on cells from the growing end of teeth and have not offered information regarding maturation processes. The secretory nature of these cells has been indicated by Watson (33) and Reith (25). In the present paper, the secretory nature of ameloblasts from the growing end of teeth is again demonstrated, and additional information is offered to illustrate the remarkable degree of cell reorganization that is associated with events which occur subsequent to matrix formation.

## METHODS AND MATERIALS

Adult rats were sacrificed with an overdose of ether. The incisors with adherent enamel organs were removed and fixed in 1 per cent buffered osmium

zone of maturation

zone of
detached ameloblasts

zone of
reduced
ameloblasts

zone of
matrix formation
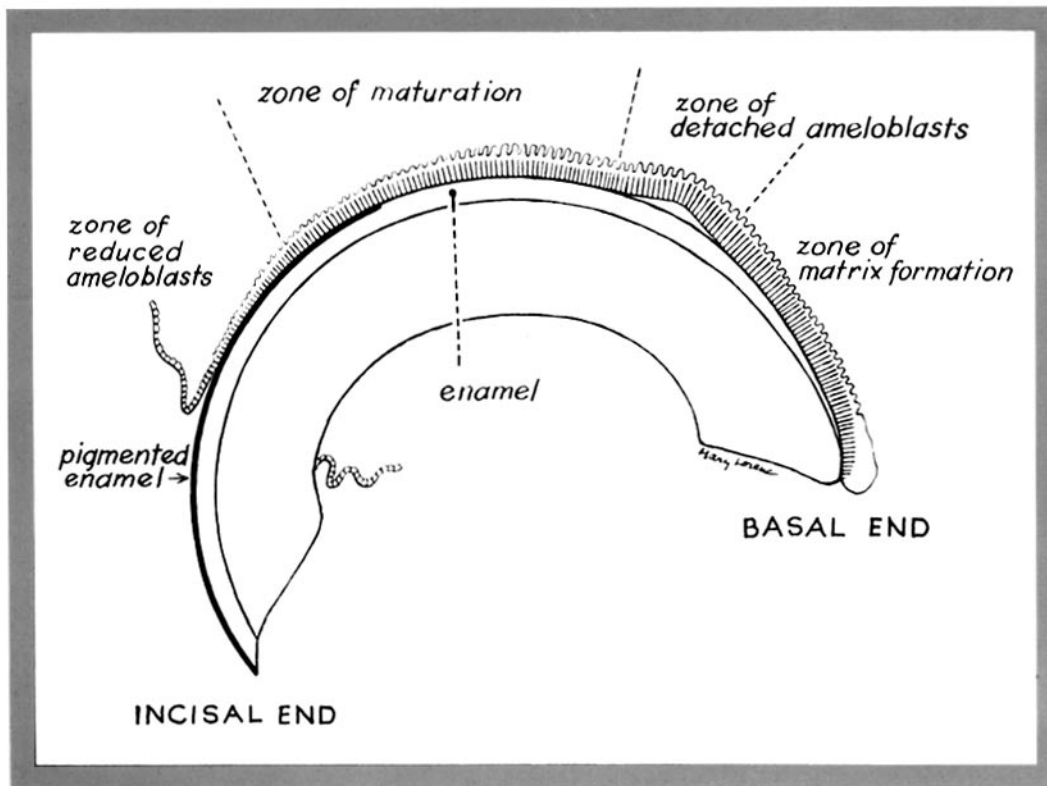
enamel

pigmented
enamel →

BASAL END

INCISAL END

Figure 1

Schematic diagram of upper incisor of rat.

tetroxide (19). After 1 to 2 hours in fixative, the teeth were placed in water at which time they were further trimmed. For sections tangential to the surface of enamel, the enamel organs were left adherent to the tooth; for longitudinal sections they were separated. Tissues were then imbedded in methacrylate. Polymerization was effected with benzoyl peroxide as a catalyst at 60°C. Sections were cut with glass knives on a Porter-Blum microtome. They were mounted on formvar-coated copper grids or slit grids, and examined in an RCA EMU-2e or a Siemens microscope.

Figure 2

Longitudinal section of ameloblasts engaged in matrix formation. Nuclei are at basal end, Golgi apparatus (G) in the center, and ergastoplasm (ER) at all levels but most notably at the distal end. Mitochondria are not shown, but are proximal to nuclei. × 4,210.

Figure 3

Distal end of ameloblasts engaged in matrix formation. Some Golgi material (G) and granules (gr) can be seen at this level, but the most conspicuous feature is the ergastoplasm (ER). Cell membranes (CM) are close with a minimum of intercellular space. Terminal bars (TB) mark the junction between cells and amorphous material (AM). At the bottom of this figure is soft enamel (S En). × 12,670.
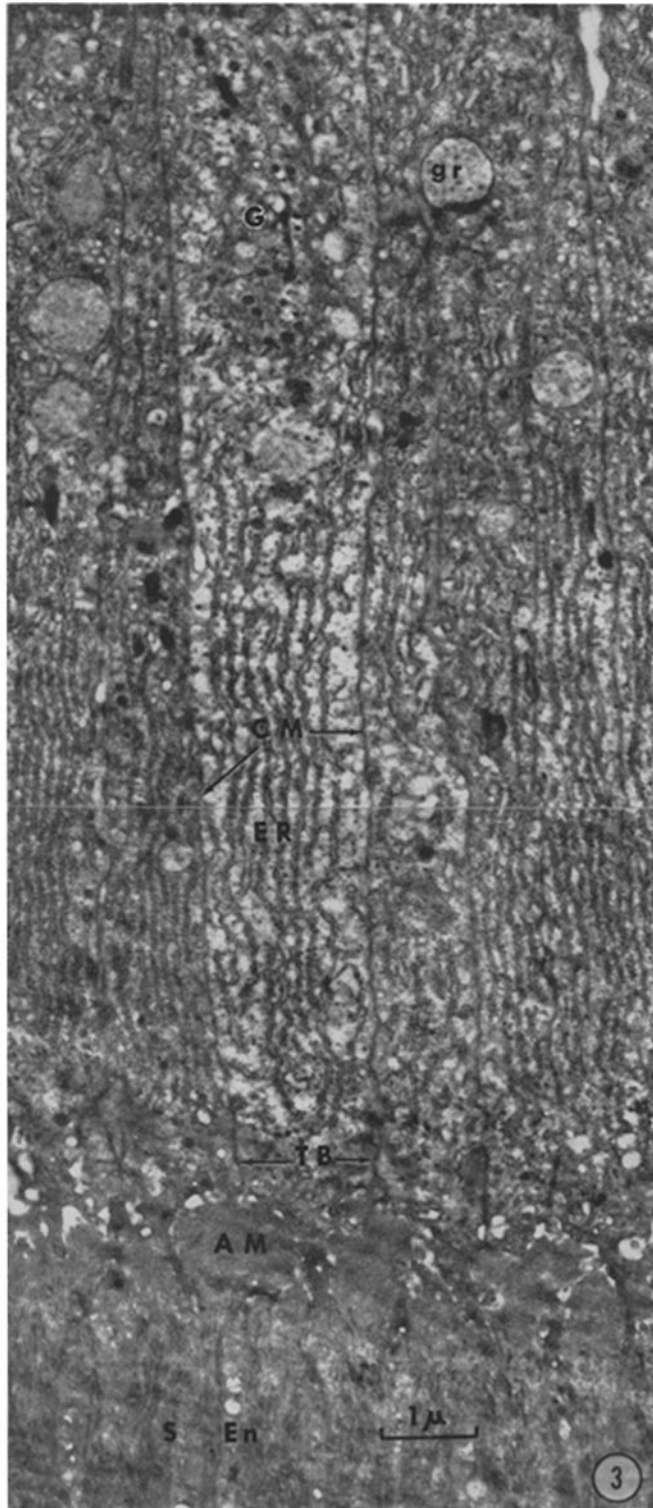
## RESULTS

### General Description

A schematic diagram of an adult rat's upper incisor is shown in Fig. 1. In paraffin sections a short length of enamel organ is consistently seen to be separated from the enamel. The zone basal to this detached zone is described as being engaged in the production of enamel matrix while the zone incisal to the detached zone is described as being related to maturation processes (16, 31, 32). The cells engaged in matrix formation are tall and slender. They are reduced in height when associated with maturation and undergo an additional reduction in height near the gingival border (21).

Note that enamel is present only on the labial surface of the tooth. Basal to the zone of detached enamel organ the enamel is soft and can be cut without the use of decalcifying agents. Incisal to the detached zone the enamel becomes progressively harder so that, with an adult tooth, decalcification is required for routine paraffin sections which include this region. Prior to eruption, pigment enters the enamel from the enamel organ (2) giving the tooth its familiar color.

Ameloblasts have been examined from the entire length of enamel organ referred to in the general description. They will be described under the following headings: ameloblasts related to matrix formation, detached ameloblasts, ameloblasts related to maturation, and reduced ameloblasts.

The growing end of the tooth or toward the growing end will be referred to as basal. The erupted end, or toward the erupted end, will be referred to as incisal. In describing an ameloblast, the term "proximal" will be employed to refer to the end of the cell adjacent to stratum intermedium. Occasionally, the term "basal" will be employed in the same manner. Distal will refer to the end of the cell adjacent to the enamel.

### Ameloblasts Related to Matrix Formation

These cells were the subject of an earlier report (25) which was based upon the examination of sections which were cut in a plane tangential to the enamel surface. Their structure will be briefly reviewed to serve as a basis for comparison with ameloblasts which are located more incisally. In addition, certain features of their structure are not conspicuous in tangential sections, and these will be indicated here.

The marked polarity of these cells is evident in longitudinal sections (Fig. 2). They are extremely tall, measuring as much as 60 or more microns in height but only about 2 to 3 microns in width. They are closely packed with their cell membranes in intimate apposition for almost their entire length. The nuclei are at the basal end of the cell but not all at the same level, so that there appear to be more than one row. Mitochondria are proximal to the nucleus. The Golgi apparatus is organized as a discrete organelle, distal to the nucleus, dominating the center of the cell. Like the nucleus, it is found at somewhat different levels. Large granules are present, surrounded by a membrane.

The most conspicuous feature of the distal end of the cell is the rough-surfaced endoplasmic reticulum or ergastoplasm. The profiles are arranged in long, parallel, longitudinal rows which come to an end at the level of the broad terminal bars. Many are as long as 15 microns (Fig. 3). In addition, rough-surfaced endoplasmic reticulum is found at other levels of the cell along with free ribonucleoprotein (RNP) particles.

Beyond the terminal bars is the Tomes's process which is made up of an amorphous material.

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Figure 4

Detached ameloblasts. Ergastoplasmic cisternae (*ER*) are dilated and continuous with perinuclear space (3 unlettered arrows). Golgi material (*G*) is less organized than in Fig. 2. Cell membranes are broken. Nucleus (*N*). × 15,000.

Figure 5

Longitudinal view of detached ameloblasts showing area which was enlarged in Fig. 4. × 2,125.
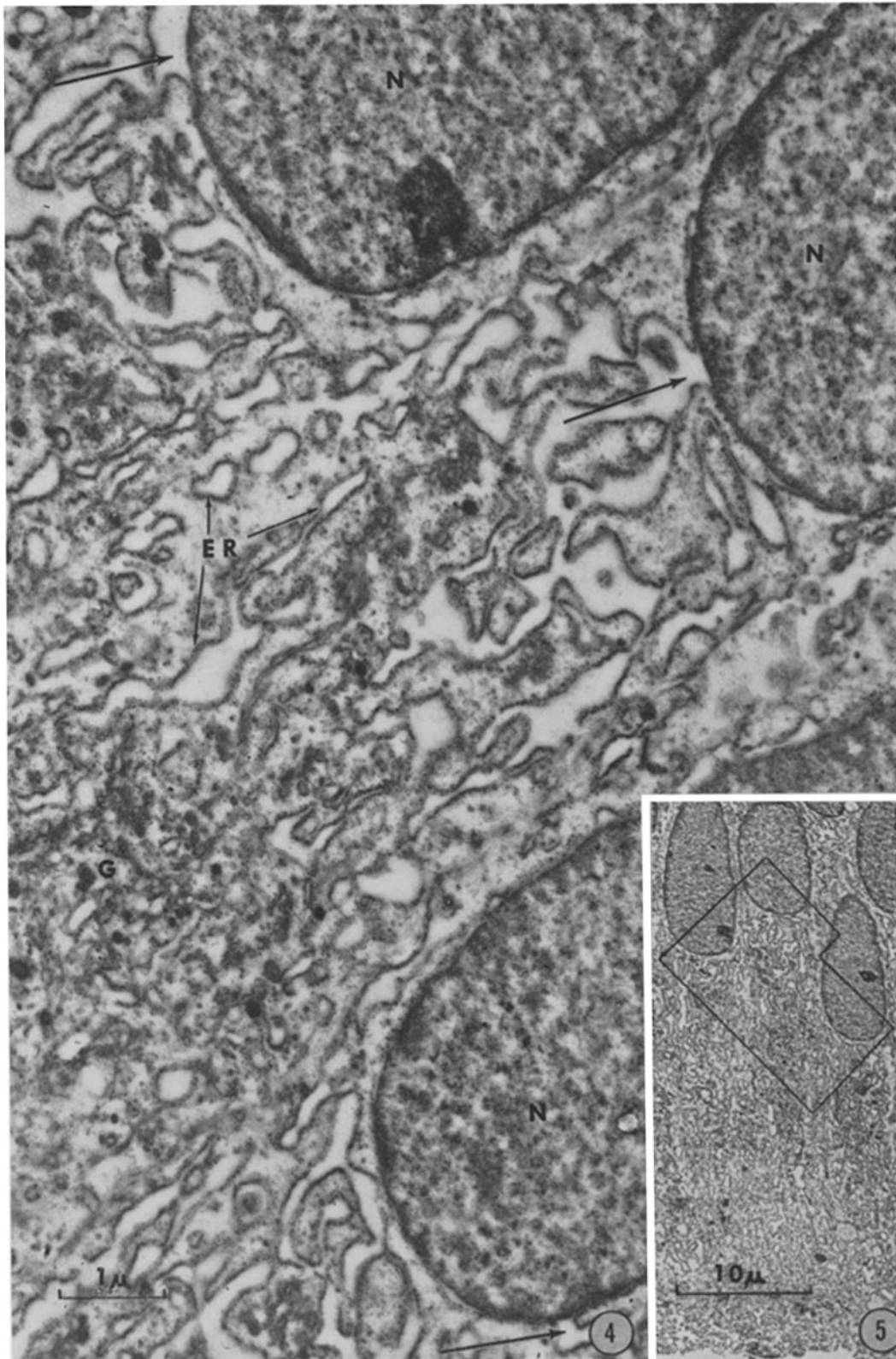
At the bottom of Fig. 3, adjacent to the Tomes's process, is soft enamel.

## Detached Ameloblasts

When the enamel organ is removed from the tooth, it immediately curves upon itself forming a u. The bend consistently occurs at the zone of detached ameloblasts. These cells undergo a reduction in height from about 60 microns at the basal end to about 35 microns at the incisal end of this zone. When examined with the electron microscope, they present a picture of disruption and disorganization (Figs. 4, 5). Cell membranes are broken and cannot be distinguished, and cytoplasmic material escapes into the intercellular spaces. The rough-surfaced endoplasmic reticulum becomes dilated and very evidently continuous with the perinuclear space. Three such communications are shown in Fig. 4. Although not as pronounced, the perinuclear space also shows signs of dilation. The Golgi apparatus is less discrete and no longer dominates a particular region of the cell as in the previous region (Fig. 5). Mitochondria are no longer confined to their proximal position but now are seen distal to the nucleus. In the basal end of this zone, they are still mainly confined to the proximal half of the cell; in the incisal end, many are seen in the distal end of the cell. Large numbers of small granules, about 0.2 micron in diameter, are in the distal extremity of these cells (Fig. 6).

## Ameloblasts Related to Maturation

These cells are adjacent to enamel which becomes increasingly harder and then pigmented toward the incisal end. It is a rather long zone in which noteworthy differences are observed when ameloblasts from one end are compared to those of the other. For this reason, cells from the basal end will be described first, then cells from the incisal end.

*Basal End:* The cells of this zone measure about 35 microns in height. Their nuclei are all at the same level and the cells do not appear tightly packed as they do when related to matrix formation. A large amount of intercellular space exists between the cells. Numerous microvilli project into the space (Fig. 7).

Among the striking differences between cells of this zone and those of more basal zones is the presence of iron granules throughout the cytoplasm. In the basal end of this zone the iron granules are scattered singly throughout the cell (Fig. 10). A little more incisally, these granules also become grouped into aggregates (Fig. 7). Still more incisally, large aggregates are to be seen. These will be described in the next section. When serial thick sections are examined with the light microscope, these aggregations of iron granules appear yellow.

Long ergastoplasmic profiles are no longer a conspicuous feature of these cells. Instead, the endoplasmic reticulum is present as dilated vesicles

FIGURE 6

Distal end of detached ameloblast. Numerous small granules (gr) are seen. × 22,800.

FIGURE 7

Longitudinal section of ameloblasts related to maturation. A considerable amount of intercellular space exists as compared to the arrangement of cells engaged in matrix formation. Microvilli (MV) project into the space. Mitochondria (M) are now also in the distal end of the cell surface. A few fibrils (f) can be seen in these cells. The dark circular bodies are aggregates of iron granules. × 3,145.

FIGURE 8

Distal end of cells shown in Fig. 7. Mitochondria (M) are seen in intimate association with distal processes (dp). Some enamel crystals (ec) are at the bottom of this figure. × 12,250.

FIGURE 9

Golgi (G) zone of ameloblast from same region as shown in Fig. 7. Fibrils (f) are indicated along with aggregates of iron granules (ia). × 13,500.
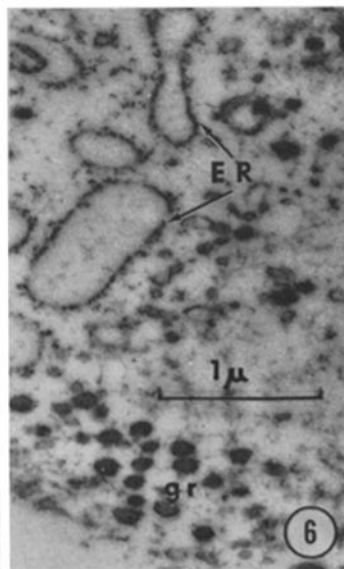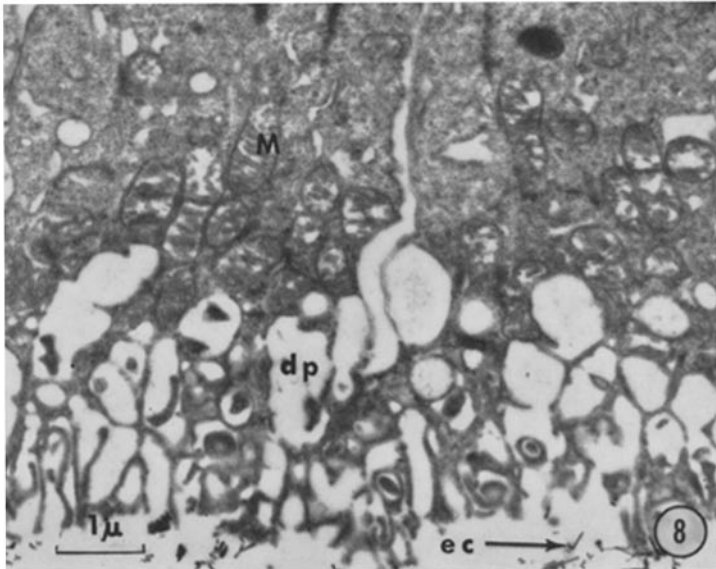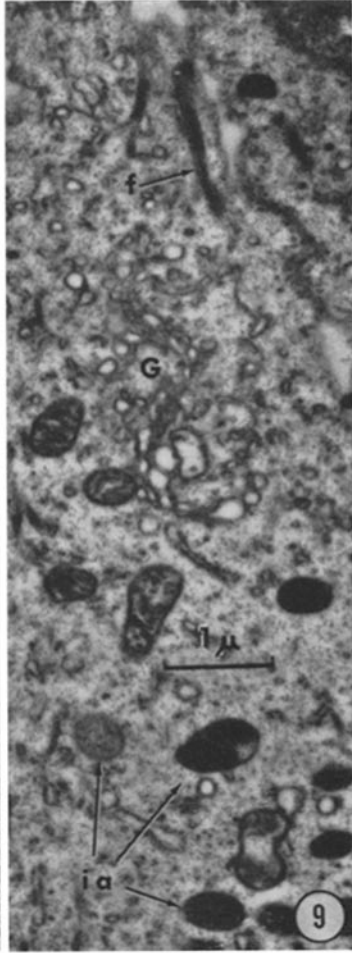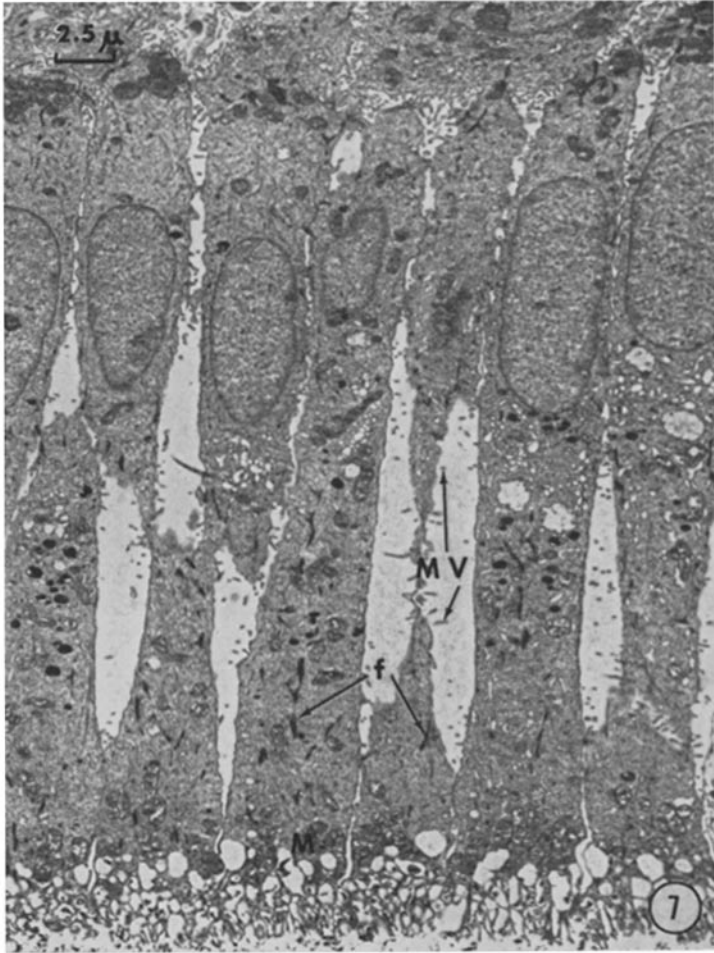
which have few adherent RNP particles. Free RNP particles are present however in conspicuous numbers (Fig. 10). The Golgi apparatus is no longer as discrete and outstanding as in cells related to matrix formation (Fig. 9). A marked difference between these ameloblasts and those involved in matrix formation is in the position of mitochondria. In these cells, they are situated in three main locations. One group is at the proximal end of the cell, another is at the distal end, and a third group is scattered randomly at all levels (Figs. 7, 8, 11).

A specialization of the cell surface is present at the distal end of the cell (Fig. 7). Examination of this structure by both longitudinal (Fig. 8) and transverse sections (Figs. 11, 12) indicates that it consists of irregular, thin, ridge-like projections which branch and communicate with each other. They extend as far as 3 microns from the distal end of the cell. At the base of these projections, the adjacent cell membranes are intimately apposed (Fig. 7) in contrast to the wide separation seen at higher levels.

*Incisal End:* These cells are also 30 to 35 microns in height with their nuclei at the same level. There is less intercellular space between these cells, but microvilli are still very numerous. Mitochondria are arranged in the same general manner as in the basal end (Fig. 13).

The most conspicuous change in these cells is the increase in number and size of the aggregates of iron granules. Before the iron leaves these cells, the centers of many aggregates become thin, giving them the appearance of oval or circular rings (Fig. 14). Higher magnification of one of these rings is shown in Fig. 16 which illustrates certain aspects of their structure. The granules

have a diameter of about 60 A and give indications of being composed of subunits (Fig. 17) as observed by other workers (10, 11, 29). The cytoplasmic background associated with clusters of these granules is more opaque than that where no granules are present. This is noticeable both within the center of the ring and in the ring itself (Fig. 16). Even in cells where large and numerous aggregates are seen, individual free granules are still found in the cytoplasm. They have not, however, been found in nuclei or mitochondria. Whereas the large aggregates seen in these cells are not surrounded by a membrane, occasionally small aggregates in other regions, both basally and incisally located (Figs. 9, 18), appear to be enclosed by a membrane.

In the most incisal end of this zone, the cells yield most of their iron to the enamel and certain features of their cytoplasm are more readily observed. Large numbers of free RNP particles are present (Fig. 18) although there are few signs of endoplasmic reticulum, except possibly in the form of vesicles of various size. An additional component of the cytoplasm is now more evident, namely intracellular fibrils. They are present in more basally located cells (Figs. 7, 9), but are studied more advantageously in these cells since they are more numerous, and there is less iron to cloud the picture. These fibrils consist of parallel filaments which measure about 60 A in width (Fig. 18). Their length is difficult to ascertain in these thin sections.

*Reduced Ameloblasts*

These cells are adjacent to hard pigmented enamel. They measure about 15 microns in height (Fig.

FIGURE 10

Cross-section of two cells related to maturation of enamel, but a little more basal than cells in Fig. 7. Dilated ergastoplasmic vesicles (*ER*), free RNP particles (*p*), and iron granules (*ig*) are indicated. Intercellular space (*IS*) and microvilli (*MV*) are also shown. × 43,000.

FIGURE 11

Cross-section of same cells as in Fig. 10 through the level of the base of the distal projections (*B of dp*). Mitochondria (*M*) are seen close to these projections. Arrows indicate limits of adjacent cells. × 13,000.

FIGURE 12

Cross-section of distal projections of cells in Fig. 10. Note complex arrangement of the irregular branching ridges. × 13,000.

Figure 10 labels: P, ER, IS, MV, ig, 0.5 µ, 10

Figure 11 labels: M, B of dp, 1 µ, 11

Figure 12 labels: 1µ, 12
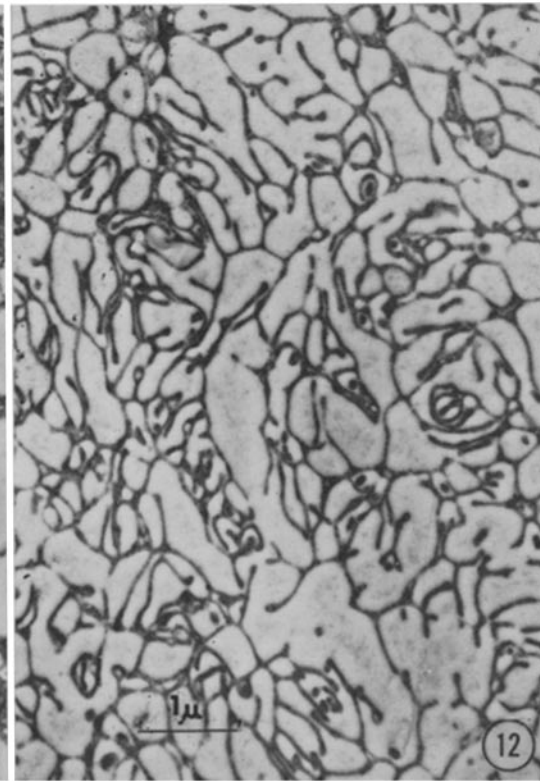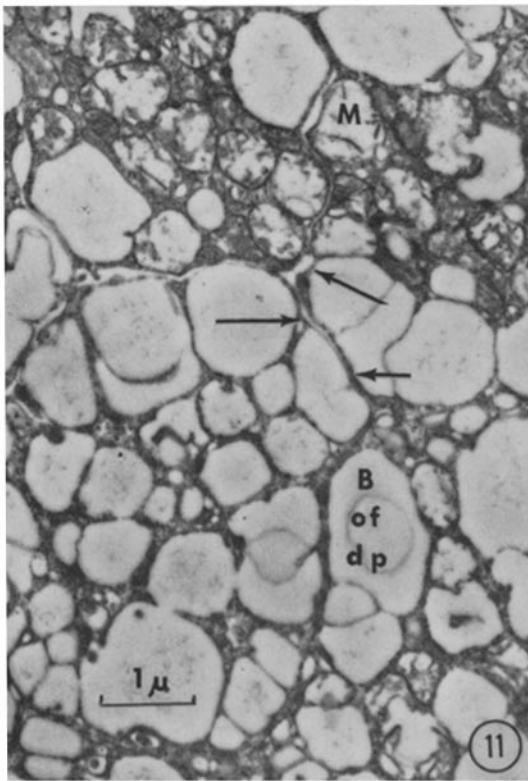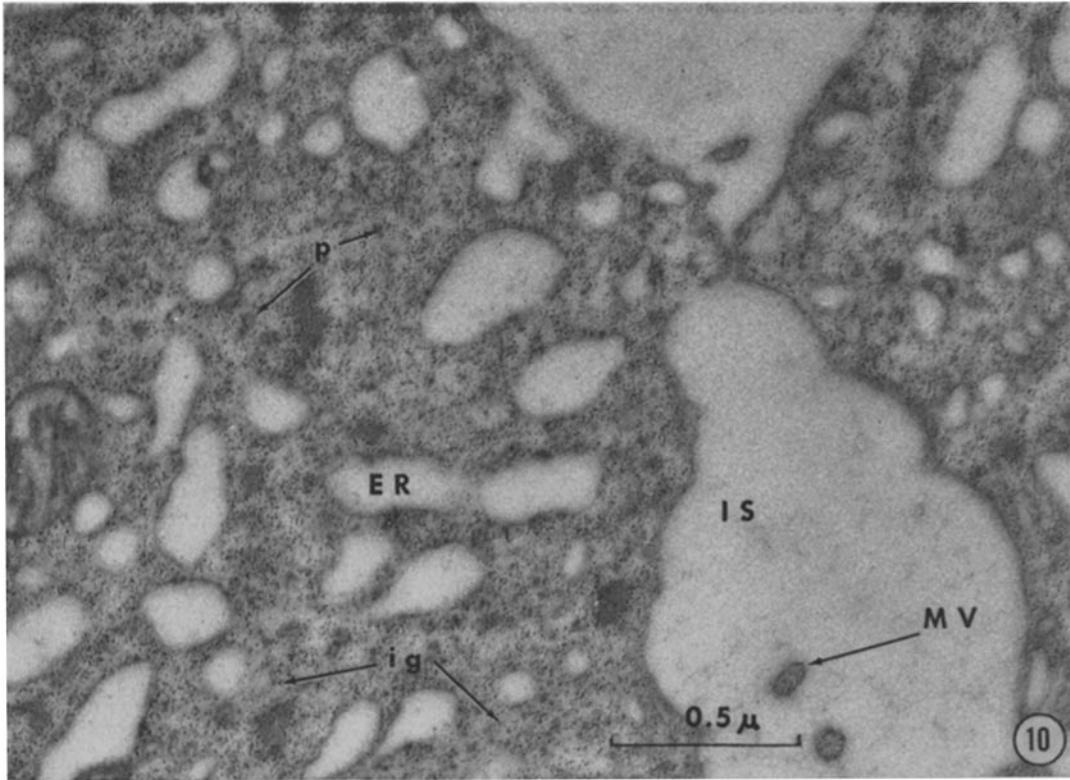
15). Nuclei are at the proximal half of the cell, with most of the mitochondria in the central region of the cell. The surface specialization of the previous region has completely disappeared, having been replaced by a relatively even cell membrane. Microvilli are still present between the sides of these cells. The fibrils which are more conspicuous in many of these cells suggest a network arrangement.

## DISCUSSION

The secretory nature of ameloblasts engaged in matrix formation has been pointed out in an earlier investigation (25) and again in this paper. Additional support for this view was presented by Watson (33) who was able to demonstrate a cell membrane between ameloblasts and their product. The stippled material of Watson resembles the amorphous material described in an earlier paper (25) and referred to again here. In its location just distal to the terminal bars it occupies the same position as the structure which is referred to as Tomes's process when examined with the light microscope. In an earlier report (25) ameloblast cytoplasm was shown distal to the terminal bars in the same position as Tomes's process. Thus it is seen that Tomes's process consists of a different fine structure in different locations very likely related to the production of inner and outer enamel (34), the predominantly "cytoplasmic" Tomes's process being related to inner enamel and the predominantly "amorphous" Tomes's process being related to outer enamel. Aside from these differences in Tomes's processes, no other differ-

ences were noted in ameloblast structure which would suggest that the production of material for inner enamel is different from the production of material for outer enamel.

The detached cells are considered to be transitional for a number of reasons. The full thickness of enamel is present at this point, and one can reasonably conclude, on the basis of this, the altered morphology, and the loose attachment to enamel, that matrix formation has ceased or is coming to an end in this zone. Moreover, the position of mitochondria in these cells is intermediate between the positions they occupy in the two adjacent zones. This, along with the reduction in height, relates them to the next incisal zone.

The absence of intact cell membranes in the detached cells must be interpreted as being due to the gross distortion which occurs upon removal of this region from the tooth. It is not possible to tell with the available evidence whether the dilated endoplasmic reticulum is related to a cessation of large-scale protein synthesis (30) or is a reflection of cell damage. In this connection, Goldberg and Green (7) have demonstrated similar dilation of endoplasmic reticulum and communication with the perinuclear space resulting from cell damage. There is also the possibility that these communications between endoplasmic reticulum and perinuclear space, which are not seen in other zones, are related to the altered function which these cells are about to undergo.

Enamel matrix calcifies partially just as soon as it is formed. This occurs at the growing end of the incisor while more matrix is still being produced. It is only after the full thickness of matrix has been

FIGURE 13

Longitudinal section of ameloblasts related to enamel maturation. These cells are taken from the incisal end of this zone. The amount of intercellular space has decreased. The conspicuous dark oval and round bodies are aggregates of iron granules. × 3,800.

FIGURE 14

Cross-section of ameloblast from same region as in Fig. 13. Parts of four cells are included; the nuclei (N) are labeled. One of the iron aggregates (ia) has lost iron from its center and appears as a ring (R). × 12,600.

FIGURE 15

Longitudinal section of reduced ameloblasts. Note the even cell membrane at the distal end. Mitochondria (M) are mostly in the center of the cells. The fibrils (f) suggest a network arrangement. × 5,600.
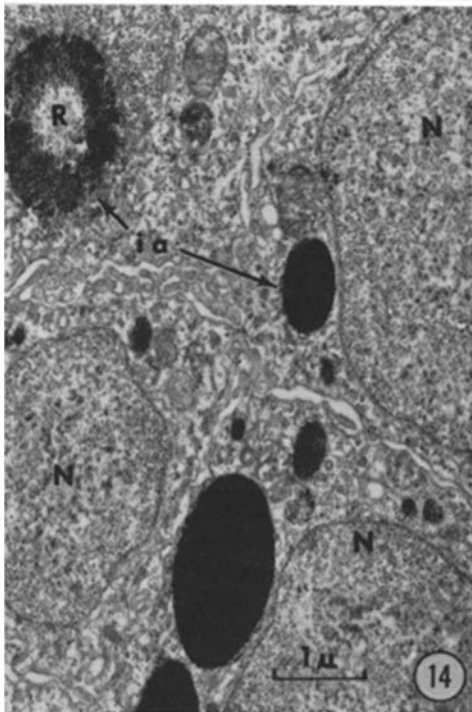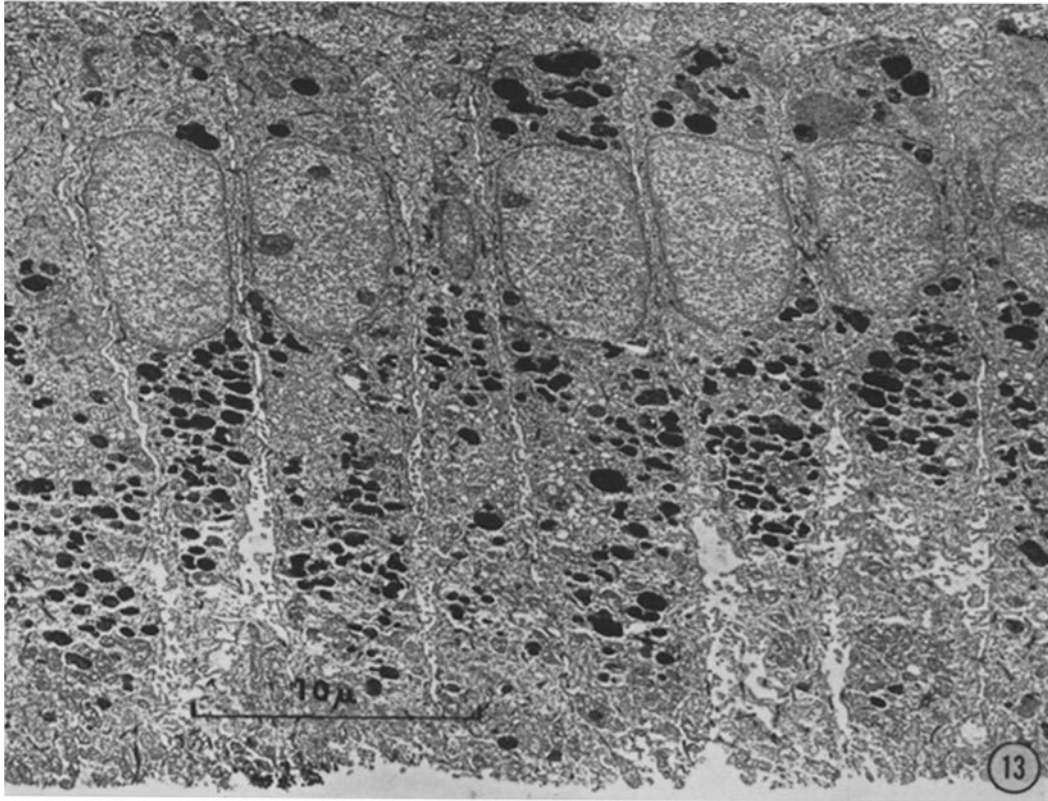
formed that the enamel becomes fully calcified. This latter process, during which final hardening of the enamel occurs without concomitant matrix formation, is referred to as maturation (8). In view of the sharp contrast in ultrastructure between the cells producing matrix and the cells incisal to the detached zone which are adjacent to maturing enamel, it is reasonable to conclude that their function has altered and that they are now involved in the maturation of enamel. A most striking feature of these cells is the appearance of projections at their distal ends. The special nature of the distal end of the cell was noted earlier (24) when it was observed that pigment does not appear there. Pindborg and Weinmann (21) also noted this specialization, referring to it as a striated border. The intimate relationship of mitochondria to these projections suggests that mitochondrial activity is associated with the work of the projections. It is interesting to note that these ameloblasts adjacent to maturing enamel are alkaline phosphatase positive (31). Moreover, the distal projections are periodic acid–Schiff positive (26). Upon considering the changes which occur during the maturation of enamel, which include an influx of calcium salts and a decrease in water (4), along with the ultrastructure and histochemical picture of these cells, one is tempted to regard them as "transporting" cells. This is especially so when one recalls that proximal tubule cells (27) and intestinal epithelium (5) also possess projections from their free border, and are alkaline phosphatase and PAS positive (3, 14).

The presence of iron in the enamel organ has been demonstrated previously by chemical and histochemical analysis (9, 24) and the disposition of iron granules in ameloblasts as seen with the electron microscope is described in this paper. It should be noted that they are found only in the region of the enamel organ associated with maturation and that even though they "fill" the cytoplasm of the cell, it appears to continue its function. High magnification of these granules

indicates that they consist of subunits similar to those described for ferritin (10, 11, 29). There is evidence that the iron granules are associated with some opaque material in these cells. Unfortunately, we have as yet no information on the nature of this opaque material or any other material with which iron is associated in the ameloblast. The function of the iron in the enamel organ is not known.

After the iron leaves the cell it becomes evident that the cell is engaged in yet another activity; namely, the formation of fibrils. These fibrils have about the same appearance and dimensions as tonofibrils (28) and are associated with large numbers of RNP particles. The cytoplasm of these cells stains intensely with toluidine blue but not after ribonuclease digestion (26). It may be that these fibrils are related to the enamel cuticle of the tooth which is considered to be the last product of the ameloblasts and of a keratinous nature (18).

The reduced ameloblasts exhibit still further changes. In addition to the reduction in height, they show a reorganization of organelles and a loss of the surface specialization.

We cannot agree with the view of Frank and Sognnaes (6) who question the validity of considering amelogenesis as occurring in two stages; namely, matrix formation and maturation. While there is no doubt that calcification itself is a continuous process, the above results indicate that the function of ameloblasts in the early stages of enamel formation is different from their function in later stages.

In conclusion, the ultrastructure of a cell which undergoes a series of activities has been described. At one stage the cell is engaged in production of organic material including protein (1) for export. This is done with a complement of organelles similar to those which one sees in protein-secreting cells such as pancreatic cells (20). A transitional stage is then observed. The cell next engages in an activity which requires a cell organization not

FIGURE 16

An aggregation of iron granules (ig) with fewer granules in the center, thereby appearing as a ring in sections. Some opaque material (OM) is associated with the granules. The aggregate is pressed against the nucleus (N) of the cell. × 109,440.

FIGURE 17

Enlargement of some iron granules indicating subunits in the granules. × 418,000.
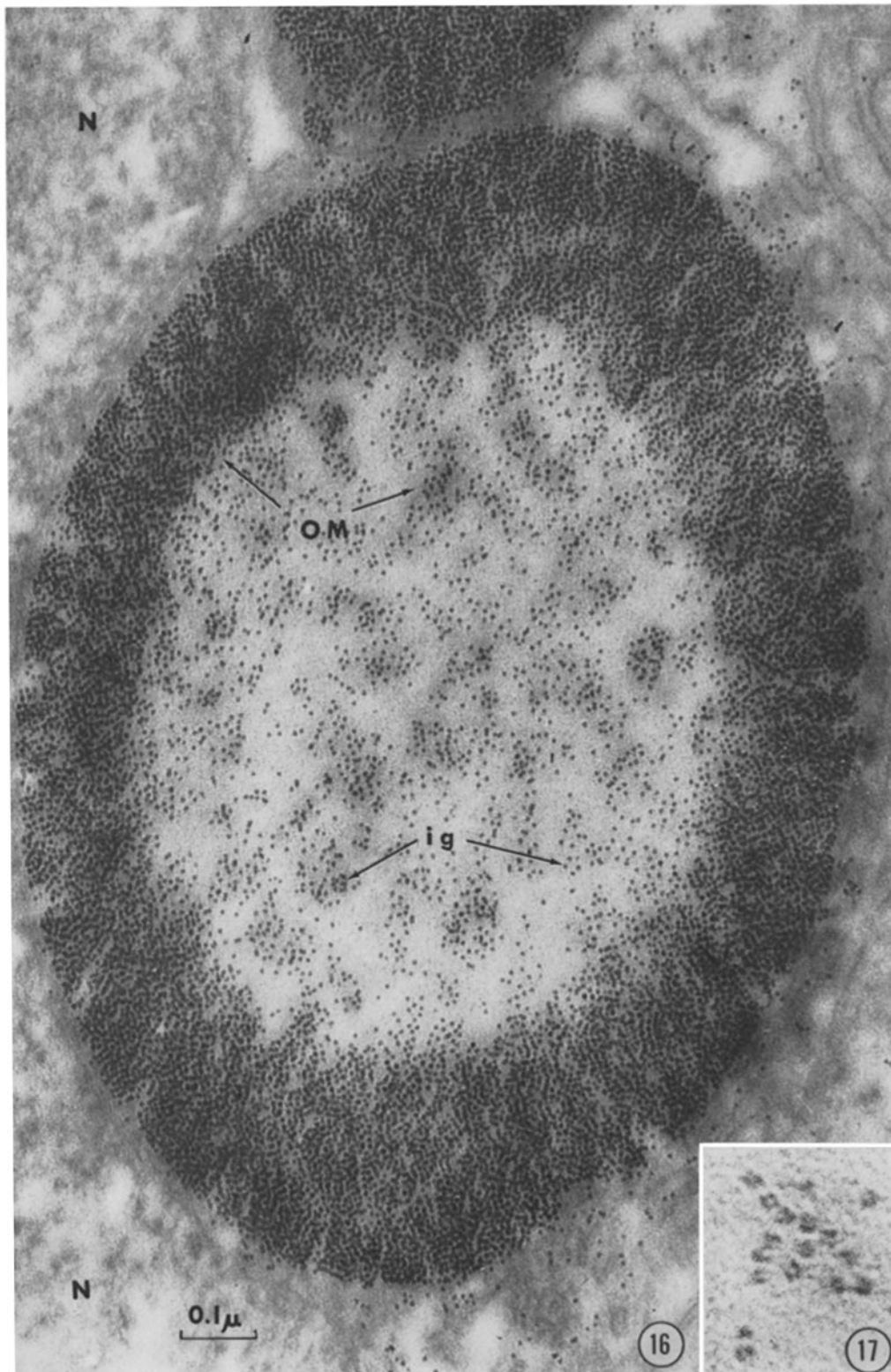
usually associated with protein synthesis. It is concluded that this function is mainly a transporting function, related to the maturation of enamel. Finally, the cell again shows evidence of engaging in protein synthesis, but this time, in a manner similar to that of epidermal cells, that is, with the product not enclosed by a membrane, and the participation of free RNP particles.

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FIGURE 18

Enlargement of two adjacent cells from same region as in Fig. 13, after iron has left the cells. Free RNP particles (*p*) are present along with fibrils (*f*). Iron granules (*ig*) and part of a mitochondrion (*M*) are also shown. × 57,000.