THE ULTRASTRUCTURE OF AMELOBLASTS DURING EARLY STAGES OF MATURATION OF ENAMEL

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Investigations with the electron microscope have added significantly to our understanding of the early stages of amelogenesis (Sicher, 1962). Much less information has been obtained regarding the cytology of later stages because the enamel becomes increasingly more mineralized as maturation proceeds, and the relation between cell and enamel is more difficult to ascertain. Recently a number of rat incisors have been prepared in such a way that maturing enamel was obtained along with ameloblasts for examination with the electron microscope. The results reveal certain structural relations between enamel and ameloblasts which shed light on the mechanism of maturation.

In an earlier paper (Reith, 1961) it was pointed out that ameloblasts related to maturation of enamel possess certain characteristics of transporting cells. These cells form a relatively long zone of the enamel organ, and as one proceeds incisally, *i.e.*, away from the growing end and toward the mature end, iron appears in progressively increasing amounts within the cytoplasm. The cells of this zone are about 35 microns in height and have irregular cytoplasmic processes from their distal end. Mitochondria are present in large numbers at the distal end of the cells, adjacent to the distal processes. The cells of this zone which are closest to the growing end of the tooth do not yet contain iron. These cells are presumably engaged in the earliest activities related to maturation of enamel and are the basis of the present report.

METHODS AND MATERIALS

Upper incisor teeth of adult rats were removed with the enamel organ still adherent to the tooth. These were immediately fixed in buffered OsO_4, dehydrated, embedded in Epon, and sectioned on an LKB ultratome. Serial thick sections were prepared for examination with the phase contrast microscope. Thin sections were mounted on Formvar-coated copper grids, stained with uranyl acetate, and examined with a Siemens electron microscope.

RESULTS

A thick plastic section, photographed with phase optics, illustrating the region under investigation is shown in Fig. 1. The cells are adjacent to the enamel, which presents a relatively smooth surface at this magnification. Upon closer examination of the enamel surface with the electron microscope, the free ends of apatite crystals can be seen projecting from the main mass of mineralized enamel (Fig. 4). In addition to the apatite crystals, there is present a less dense, amorphous, finely granular material which can be seen in Figs. 2, 3, and 4 (*om*). This material can be seen to advantage at the free edge of the main mass of mineralized enamel, where it is located between the surface apatite crystals. It is also situated in the interval between the main mass of mineralized tissue and the cell membrane of the ameloblasts. This interval measures from 400 to 800 A. It is difficult to determine from these electron micrographs whether the subsurface apatite crystals are also separated by a similar less dense material. Much of the lighter material between the dense apatite crystals is probably due to apatite crystals which have the flat side facing the electron beam.

Ameloblasts are not in direct contact with the mineralized component of enamel in this region of the enamel organ, but rather are related to the

contents of the interval in such a way as to suggest the presence of an attachment device. This consists of a footlike expansion of the distal process, a thickening of the cell membrane along the free edge of the foot, and a membrane or patch which is parallel to the thickened cell membrane. This attachment membrane or patch appears as a thin line in sections and is a constituent of the 400- to 800-A interval along with the amorphous, finely granular material.

On the basis of its presence and appearance in electron micrographs, the amorphous, finely granular material is regarded as a non-crystalline, polymeric, organic material rather than a crystalline material. Moreover, it is not fibrous, although in a number of places there are indications of network organization. Of particular interest is the relation of ameloblasts to this material. In a number of places this material can be seen to extend between the elements of the distal processes of the ameloblasts (Fig. 4). In addition, near the distal processes there are membrane-bounded granules that contain an amorphous or finely granular material which appears to be similar to the material found between the distal processes, and beyond the cells, on the enamel surface (Figs. 2 and 3). The size of the granules differs in Figs. 2 and 3. This may represent a difference in degree of activity, since the cells shown in Fig. 3 are taken from a region slightly closer to the growing end of the tooth. It should be indicated, however, that the cells illustrated in Figs. 2 and 3 are from different animals.

In the center of the cell another type of granule of variable size is present in large numbers. These granules are also bounded by a single membrane (Figs. 5 to 8). However, their contents vary markedly. In some instances, the contents are dense; in other instances, they are less dense. Moreover, small circular profiles or vesicles are evident within many granules. Small vesicles of about the same or slightly larger size are usually found in the immediate vicinity of the granules which have intragranular vesicles.

DISCUSSION

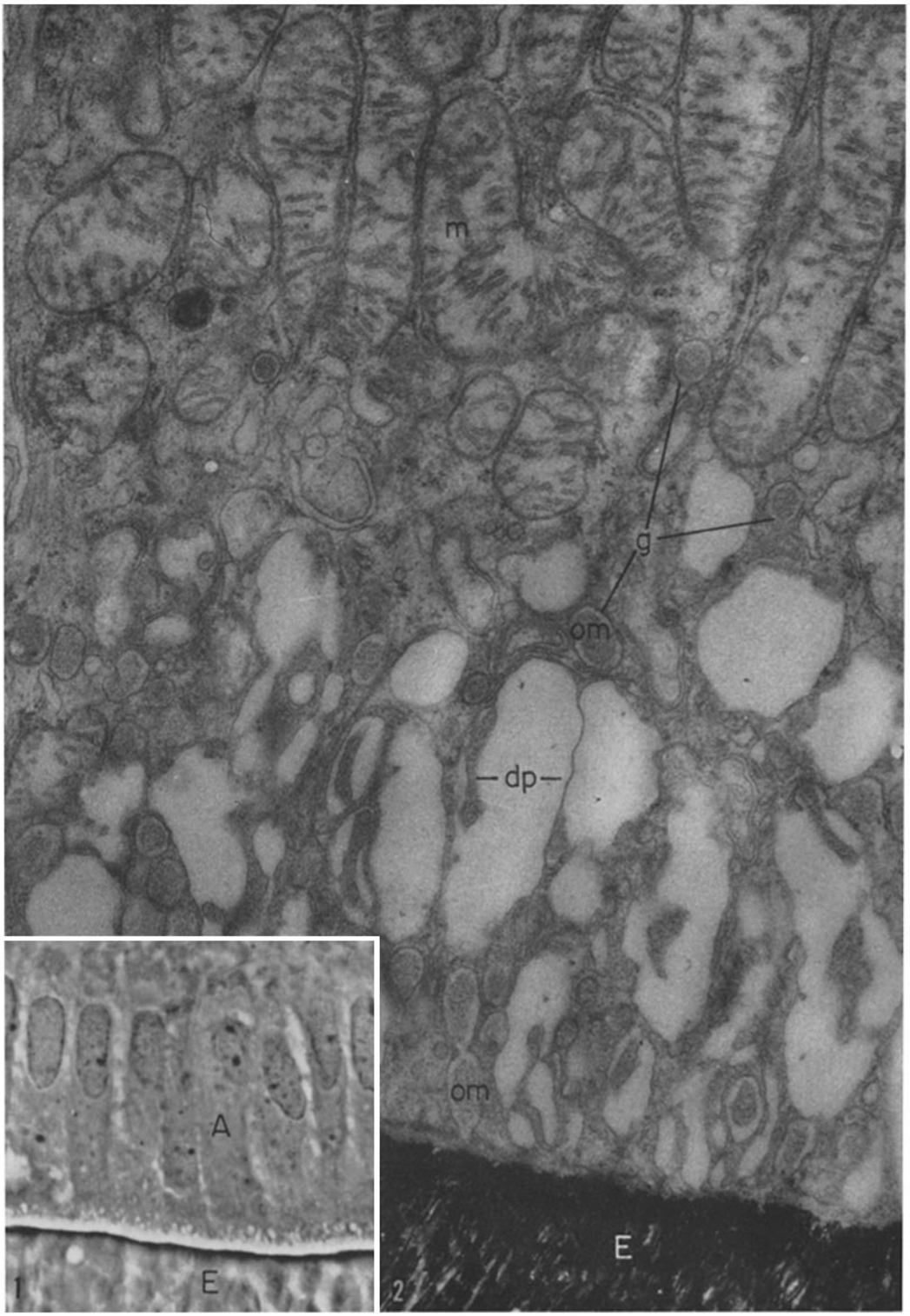
It is thought that the results reported above demonstrate that some organic substance is being moved either into or out of the enamel. The direction of movement, however, is not demonstrated. Several reports in the literature have a direct bearing on this question.

In a study of unerupted pig molar teeth, Weinmann *et al.* (1942) were able to show that shortly after matrix formation ceases there is a marked reduction in the organic content of enamel. By correlating their chemical analysis with histological examination of related teeth in the opposite side of the jaw, they were able to show that the reduction in organic content occurs where the enamel becomes soluble to demineralizing acids. That is, the enamel matrix at the growing end of the tooth that remains in decalcified histologic sections represents enamel with a higher organic content than the enamel which is lost in the demineralizing process. In the same year, Deakins (1942) also reported a decrease in the organic content of maturing pig molar enamel. This was found in enamel which could still be cut easily with a scalpel, but which would soon be hard so that cutting with a scalpel would be difficult or impossible. This decrease probably corresponds to the one that was recorded by Weinmann *et al.* (1942).

Unfortunately, no comparable report on the enamel of continuously growing rat incisors is available, and one might rightly question the validity of taking data from molar teeth and applying them to continuously growing incisor teeth. This difficulty has not been resolved. However, it should be pointed out that maturing incisor enamel also becomes acid soluble (Marsland, 1952) as it gets harder, and this acid solubility may well re-

FIGURE 1 Ameloblasts (*A*) in contact with enamel (*E*). The enamel surface is relatively even, matrix formation is no longer in progress, no pigment is present within the cells. Phase contrast photomicrograph. × 1200.

FIGURE 2 Distal end of ameloblasts containing numerous mitochondria (*m*), in contact with enamel (*E*). Beyond the mitochondria are the distal processes (*dp*). Finely granular material (*om*) is present between the cells and the mineralized component of enamel. It also appears to be the constituent of granules (*g*); it is also found between the distal processes. × 36,000.



flect a decrease in organic content similar to that which was observed for pig molar teeth. Ameloblasts for the present study were adjacent to maturing enamel which could still be cut without the use of decalcifying acids, but which would soon become acid soluble. In view of the location from which these cells were taken, and in consideration of the enamel solubility that will shortly follow, coupled with the observations of Weinmann *et al.* (1942) and Deakins (1942) on developing pig molar teeth, it is thought that the present report represents a visualization of organic material as it is being removed from enamel by ameloblasts.

It is difficult to say what connection, if any, the membrane-bounded granules in the center of the cell have with the processes occurring at the distal end of the cell. These granules bear a striking morphological resemblance to lysosomes (Novikoff, 1961). However, granules with a similar appearance are also present in secretory ameloblasts, which are regarded as producing matrix at the growing end of the tooth (Reith, 1960). In this connection it should be recalled that, following the production of enamel matrix, ameloblasts undergo a striking alteration in ultrastructural morphology (Reith, 1961). It is tempting to speculate on the relationship of the granules in secretory ameloblasts to the reorganization of cell structure following the production of enamel matrix, or the relationship of the granules (Figs. 5 to 8) to the processing of material that is found on the surface of the enamel. However, this can be done more fruitfully after a survey of their distribution and histochemical characteristics.

The granules that are found at the distal end of the cell (Figs. 2 and 3), with contents that resemble the material on the surface of the enamel, are similar morphologically, in certain respects, to granules that are found at the distal end of secretory ameloblasts (Fig. 3, Watson, 1960). The most that can be said for either observation, on morphological grounds, is that in each case the granular contents may be related to the extracellular material near by. However, by considering the events that are taking place in the process of tooth development, one can obtain circumstantial evidence on their nature. Therefore, Watson (1960) considered that the granular contents might represent enamel matrix before secretion. On the other hand, for the reasons discussed above, the granular contents observed in this report are considered more likely to be absorbed material.

The author wishes to express his appreciation to Miss Anne-Marie Lindgren for technical assistance.

This work was supported by United States Public Health Service Grant D-797 (C3).

Received for publication, March 20, 1963.

REFERENCES

DEAKINS, M., *J. Dental Research*, 1942, **21**, 429.

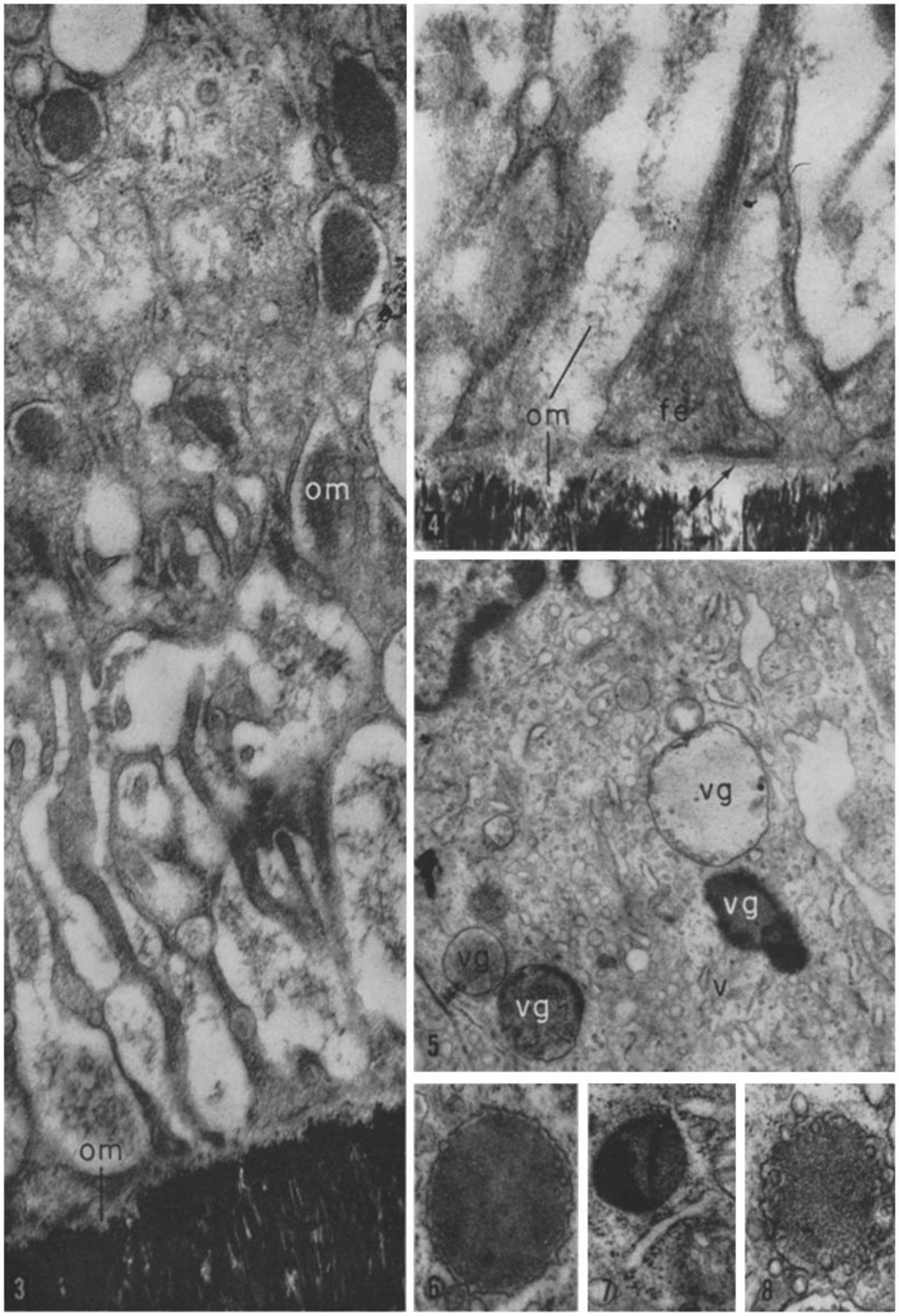
MARSLAND, E. A., *Brit. Dental J.*, 1952, **92**, 109.

FIGURE 3 Distal end of ameloblast in contact with enamel, showing the finely granular material (*om*) on the surface of enamel, between the distal processes, and as the constituent of granules. The material between the distal processes and in the closest granules suggests a network organization. × 37,000.

FIGURE 4 Distal processes of ameloblasts in contact with enamel, showing a footlike expansion (*fe*), the thickened cell membrane, and, parallel to it, the attachment membrane or plaque (arrow). The location of the amorphous, finely granular material (*om*) is shown between apatite crystals, between ameloblast and main mineralized mass, and between the distal processes. × 63,000.

FIGURE 5 A group of granules (*vg*) with contents of variable density is shown near the center of the cell. Part of the nucleus and numerous small vesicles (*v*) are also shown. × 16,000.

FIGURES 6 TO 8 Three granules, two of which show intragranular vesicles. × 9000.



NOVIKOFF, A. B., *in* The Cell, (J. Brachet and A. E. Mirksy, editors), New York, Academic Press, Inc., 1961, **2,** 423.

REITH, E. J., *Arch. Oral Biol.*, 1960, **2,** 253.

REITH, E. J., *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 825.

Sicher, H., Orban's Oral Histology and Embryology, St. Louis, C. V. Mosby Co., 5th edition, 1962.

Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1960 **7,** 489.

WEINMANN, J. P., WESSINGER, G. D., and REED, G., *J. Dental Research*, 1942, **21,** 171.