The Extracellular Nature of Enamel in the Rat

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ABSTRACT

Developing incisal enamel of the rat has been examined in sections with the electron microscope. Staining the sections with heavy metal and sandwiching them has revealed details hitherto unvisualized because of low contrast and destruction by the electron beam. In particular, it is seen that the cell membrane always lies between the ameloblast and the enamel and therefore that enamel is extracellular and not intracellular. Implications of this with regard to the possible keratinous nature of enamel matrix are discussed.

Although a number of electron microscope studies describing the fine structure of forming dental enamel have been published (1-3), none of these has shown clearly the precise relation of enamel matrix to the ameloblast. In the present communication, we will present evidence which shows that incisal enamel in the rat, like dentine, is extracellular.

Methods

Lower incisors were dissected from the mandibles of 200 gm.—Sprague-Dawley stock rats under ether anesthesia and placed in fixative. The blood supply was interrupted for about 5 minutes before fixation was started. Fixation was for 1 hour at 0-5°C. in 1 per cent OsO4 buffered to pH 7.3 with veronal-acetate containing sucrose (4). The buffer strength was 1/50 that recommended by Caulfield (4). Tissues were dehydrated in ethyl alcohol and embedded in butyl methacrylate. Sections were stained 30 minutes in lead hydroxide (5) and mounted on carbon-filmed grids and sandwiched (6) with evaporated carbon. Microscopy was done with the Siemens Elmiskop I operating at 80 kv. with a 50 μ objective aperture.

RESULTS

The essential part of these observations lies in determining the position of the forming enamel with respect to the cell membrane. It was found that the cell membrane was difficult to see clearly unless the section was sandwiched, and it is for this reason, presumably, that earlier work has failed to reveal these details.

The rat incisor in cross-section is approximately triangular, one side of the triangle representing the labial aspect of the curved tooth. The enamel coats only this surface and is in the form of a ribbon which becomes much attenuated and finally disappears at its edges. In sections cut transversely to the axis of the tooth, it is possible to follow stages of enamel formation by proceeding medially from the edge of the enamel ribbon where no enamel has been deposited to the center of the ribbon where the enamel is thickest. A series of micrographs demonstrating this near the base of the tooth is shown in Figs. 1 to 4. At the extreme edge of the enamel (Fig. 1) essentially only dentine is present, with a well marked space between dentine and cell membrane. Within the ameloblasts, cytoplasmic constituents such as mitochondria and endoplasmic reticulum lie close to

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to the end of the cells toward the dentine. Adhesion
plates or desmosomes are frequently seen between
adjacent ameloblasts. The cells are not columnar
and the nuclei are close to the dentine.

A short distance inwards from the edge of the
enamel ribbon (Fig. 2) three changes are apparent.
Nuclei are no longer close to the dentine, a zone
containing little or no endoplasmic reticulum or
mitochondria is present within the ameloblasts at
the dentinal end, and the beginnings of enamel
formation are discernible. Between the cell mem-
brane and the dentine can be seen globular masses
of finely stippled material as well as occasional
areas of calcification which are recognizable as
enamel by virtue of the length of the dense profiles
of inorganic material. Both the globular masses
and the early enamel are outside the cell mem-
brane.

At a still more advanced stage in development
(Fig. 3) forming enamel entirely covers the den-
tine. Dense, ribbon-shaped profiles can be seen
embedded in finely stippled material like that
described above. Extracellular, globular masses of
this material are also seen between the ameloblasts
at some distance from the enamel surface. The
dense profiles are oriented approximately at right
angles to the dentino-enamel junction which
represents one point of their termination. The
other point of termination of the profiles is at or
close to the cell membrane. Various small, spherical
globules are present in the clear zone of cytoplasm
of the ameloblasts which are suggestive of the
finely granular material outside. These may be
precursors of enamel matrix present in the form
of secretion granules.

Near the center of the enamel sheath (Fig. 4)
the structure has taken on the characteristic
appearance of enamel rods when viewed in sections
(1). In this area only small amounts of the finely
stippled material are present and are continuous
with the areas of the enamel containing ribbon-
shaped elements. Both the stippled material and
the enamel proper are separated from the
cytoplasm of the ameloblasts by the cell mem-
brane. Numerous small, spherical globules of
the type described above are present in parts of the
clear zone of ameloblast cytoplasm close to the
enamel.

Masses of the stippled material described above,
although not always present, are a frequent
finding in the rat incisor at later stages of inner
enamel formation (Fig. 5). Whether their presence
bears any systematic relation to enamel develop-
ment we cannot say. This material does not rep-
resent enamel matrix, once calcified, but subse-
sequently decalcified by the staining procedure, be-
cause it can also be found in unstained sections (7).

One further point of interest which we should
like to emphasize is the large amount and high
degree of development in the ameloblast of the
rough surfaced endoplasmic reticulum, that is,
endoplasmic reticulum bearing ribonucleoprotein
particles. Greatly extended elements are oriented
predominantly parallel to the long axis of the
columnar cells (Fig. 6). The rough surfaced endo-
plasmic reticulum occupies a major portion of the
cytoplasmic volume of the ameloblast. It is inter-
esting to note that most of the ribonucleoprotein
particles are arranged in circles or spirals similar
to formations described by Palade (8) in other cells.

DISCUSSION

The stippled material which is present in var-
iable amounts at the surface of forming inner
enamel in the rat incisor appears to be continuous
with the organic matrix in which the enamel
crystallites are embedded. Sometimes large,
globular masses of this material can be found;
however, what relation it may bear to various
granules and vacuoles (9) which have been de-
scribed in light microscope studies is not clear.
One may presume either that this represents the
enamel matrix precursor in the process of deposi-
tion as suggested by Fearnhead (3) or that it is
organic material lost from the enamel as calcifica-
tion proceeds. Radioautographic studies by
LeBlond, Bélanger, and Greulich (10) show that
injected S\(^{35}\)-labeled sulfate is earliest deposited in
greatest amounts at the surface of the enamel.
This suggests, therefore, that this stippled material
which is sometimes present in such large amounts
has only recently been synthesized and that it is
not organic matrix leaving the enamel. Whatever
its role, it is continuous with and shares with the
enamel a location which is extracellular.

The presence of large amounts of highly
organized, rough surfaced endoplasmic reticulum
gives the ameloblasts an outstanding morp-
ho logical feature of secretory cells and in this respect
puts them in a class with pancreatic acinar cells,
and active fibroblasts. Cells in which the major
synthetic product remains intracellular, such as
normoblasts, the cortical cells of hair (11), or the
cells which finally make up the cornified epithelium
of the tongue, although containing many ribo-
nucleoprotein particles possess relatively little
endoplasmic reticulum. Ameloblasts, though of 
estodermal origin, thus differ from some other 
estodermal cells in that morphologically they re-
semble strongly secretory cells. The product which 
is of a "structural" nature does not resemble hair 
and cornified epithelium in that the enamel is not 
made up of cornified cells but is wholly extra-
cellular.

These observations disagree with earlier work 
of ours and of others (1-3) which suggested that 
 enamel might be intracellular. The present 
finding that enamel is extracellular was reached 
because it was possible by sandwiching sections 
between high melting materials to prevent certain 
local distortions under the electron beam and 
thus clearly to observe the cell membrane near 
the enamel. It seems reasonable to presume that 
the secretory features of the ameloblast cytoplasm 
are connected with the elaboration of enamel 
matrix.

If, indeed, enamel is to be regarded as a eukera-
tin as has been reported in several studies (12-
14), we have in this case the first example to the 
author's knowledge of a protein that may be 
found in either extra- or intracellular locations. In 
this connection, we should stress that by intrac-
cellular we mean present within the cytoplasm 
and not surrounded by a membrane. Thus, muscle 
protein and hemoglobin would properly be called 
intracellular, whereas pancreatic digestive enzymes 
while existing within the cell boundary as secretion 
granules, are surrounded by a membrane (15) and 
would, here, be considered as extracellular.

At the present time there appear to be two 
qualitatively different ways in which ribonucleo-
protein (RNP) particles are associated morpho-
logically with proteins undergoing synthesis. On 
the basis of present evidence, it is not unreasonable 
to assume that some proteins such as pancreatic 
digestive enzymes are formed within membranous 
vesicles which have on the outer surface of the 
membrane an investment of attached RNP 
particles. Other proteins such as hemoglobin or 
hair and epidermal keratins appear to form 
"naked" within the cytoplasm in regions close to 
RNP particles, but in the absence of any inter-
posed membrane. Since the roles of RNA and of 
RNP particles in protein synthesis are far from 
clear, it would be fruitless to speculate on possible 
effects of a membrane enclosing the synthesizing 
product; however, these observations and con-
siderations do suggest that enamel protein may 
well differ in important respects from other 
eukeratins. The presence in enamel of proline and 
hydroxyproline, much glycine, and little cystine is 
pointed out by Battistone and Burnett (14) as not 
typical of the keratins.

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EXPLANATION OF PLATES

Figs. 1 to 4. A series of sections cut transversely to the axis of the lower incisor of the rat near the base of the tooth. Fig. 1 is taken at the edge of the ribbon of enamel which coats only the labial aspect of the incisor while Figs. 2, 3, and 4 are taken at points progressively more medial to this. All sections were stained with lead hydroxide for 30 minutes and were sandwiched with carbon. The calcified portion of the tooth appears at the right of each micrograph and the cytoplasm of the ameloblasts is on the left.

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Fig. 1. The only calcified material appearing at the edge of the enamel ribbon is represented here by the dense image of dentine. No enamel has been deposited. Portions of two ameloblasts are shown. The ameloblast cell membrane follows a complicated path on the dentinal side of the cells and is spaced from the dentine by a region containing fine filaments and some uncalcified collagen. Nuclei are close to the dentine, and all parts of the cytoplasm contain the usual cell components such as mitochondria and endoplasmic reticulum. X 24,000.

Fig. 2. Very early stages in enamel formation are detectable. Masses of stippled material (s) are present between calcified dentine and the cell membranes of the ameloblasts. At one or two points (e) the beginnings of enamel calcification can be seen. Ameloblast nuclei are no longer close to the dentinal end of the cells, and the cytoplasm at that end now contains relatively few mitochondria and little endoplasmic reticulum. X 24,000.
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FIG. 3. Dentine is now completely covered by a rudimentary layer of enamel (e). Large amounts of stippled material (s) are present with the lathe-shaped elements of calcifying enamel embedded within it. Other masses of stippled material are noticeable at some distance from the enamel. The enamel and stippled material are separated from the ameloblast cytoplasm by the cell membrane. Within the ameloblast are numerous, rather dense, membrane-enclosed globules which might represent granules of enamel matrix before secretion. X 24,000.

FIG. 4. Enamel is assuming the characteristic, highly ordered appearance of enamel rods in the inner enamel of the rat. Indentations in the serrated surface of the forming enamel are occupied by extensions of ameloblasts. The ameloblast cytoplasm is at all points covered by cell membrane and is not directly in contact with enamel. At some points stippled material (s) can be seen in contact with the enamel and apparently continuous with the matrix surrounding the dense, lathe-shaped elements. X 24,000.
Fig. 5. The surface of forming inner enamel at a much more advanced stage than that shown in the previous micrographs. At this stage, very large masses of stippled material (s) are present in the rat. This material is continuous with enamel matrix (arrow), but, like the enamel, is extracellular. Attenuated arms of ameloblast cytoplasm extend between the masses of stippled material and terminate within pockets in the surface of the forming enamel. × 24,000.

Fig. 6. Cytoplasm of the ameloblast at some distance from the forming enamel. Large amounts of endoplasmic reticulum are present having a dense investment of ribonucleoprotein particles. The particles are characteristically arranged in the ameloblast in short spirals. The high development of "rough-surfaced" endoplasmic reticulum suggests that the ameloblast is strongly secretory. × 24,000.
(Watson: Extracellular nature of enamel)