A HISTOLOGICAL STUDY OF THE EPITHELIAL ATTACHMENT IN THE WISTAR RAT

A Thesis

Presented in Partial Fulfillment of the Requirements
for the Degree Master of Science

by

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The Ohio State University
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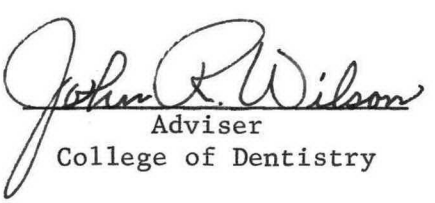
Approved by



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INTRODUCTION

The question of whether an organic union exists between the surface of enamel and the epithelium of the gingiva has been the subject of many investigations, yet an unequivocal answer is lacking. The morphology and histochemistry of the tissues of the developing rat molar were studied in an attempt to obtain additional knowledge pertaining to the relationship between epithelium and enamel after eruption.

From the results of this study it was concluded that an organic attachment between epithelium and the surface of enamel does exist in the white rat.

HISTORICAL REVIEW

Black, in 1915, stated that after eruption the epithelium was attached at the cemento-enamel junction and the whole crown was exposed.[1] This view was held by many others.[2,3,4,5,6,7,8,9,10,11,12,13] In 1921, Gottlieb[14] presented the theory of an epithelial attachment to enamel. He believed that an organic connection remained between the epithelium of the enamel organ and the surface of the enamel even after enamel maturation. Detachment of the epithelium from the enamel occurred when the cusp tip emerged and continued as eruption proceeded. After complete eruption, however, the apical portion of the anatomic crown was still in connection with the surrounding epithelium.

Orban and Mueller[15] were the first investigators to completely review and confirm the results of Gottlieb's study. In the following years his concept was supported both by clinical studies[16,17] and observations by the light microscope of ground and decalcified sections.[18,19,20,21,22,23] In addition, theories concerning the mode of attachment were proposed. Manley[24] felt that the oral epithelium, enamel cuticle, and prism sheaths were all in organic connection, while Baume[22] was of the opinion that tonofibrils of the attached epithelium were continuous with the primary enamel cuticle. Ussing et al.[25] thought that the attachment was due to a keratinization process which connected the cuticle to the other epithelial layers.

Not all investigators have agreed with Gottlieb's theory. Weski[26]

believed that when the anatomic crown was fully developed and had reached the line of occlusion, the bottom of the crevice was at the cemento-enamel junction.

In 1952, Waerhaug[27] repudiated the existence of an epithelial attachment. After many detailed experiments he concluded that the pocket was formed immediately after eruption, at which time its bottom was found at the cemento-enamel junction. Clinical and histological studies by others[28,29] have supported his findings.

Waerhaug's work stimulated renewed interest in the controversy. Orban and co-workers[30] in 1956 and Weinreb[31] in 1960 repeated some of Waerhaug's key experiments and reported complete disagreement with his findings.

Recent studies using electron microscopy have shown what appears to be a definite organic union between the enamel and the epithelium. Reith[32] reported the formation of "tonofibrils" by the ameloblasts and Ussing[33] demonstrated submicroscopic fibrils passing into the enamel cuticle from both the ameloblasts and the enamel.

METHODS AND MATERIALS

The Wistar strain of rat was used, and the maxillary first molar selected for observation.*

The rats were killed daily from the first day through the twenty-fifth day post partum. The tooth and contiguous tissue to be studied were excised and fixed in 80% alcohol. Sodium citrate - citric acid buffer, pH 4.5, was used to decalcify the specimens. After decalcification the specimens were washed, dehydrated, and embedded in rubber paraffin. Each specimen was sectioned serially at 8 microns in a labio-lingual direction.

Four stains were used in this study, with every fourth section receiving the same stain. These stains were:

1. Hematoxylin and eosin.

2. Masson's Trichrome Stain. This stain was used to contrast staining of nuclei, cytoplasm, and collagen tissue with the reactions of hematoxylin and eosin.

3. Periodic acid-Schiff. This histochemical stain demonstrates the presence of carbohydrates through aldehyde reactions.[34] The carbohydrates stain red, but the technic is not specific for any particular compound.[35]

4. Toluidine Blue. This histochemical stain indicates the

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*These experiments were conducted according to the "Rules Regarding Animal Care" as established by the American Medical Association.

presence of acid polysaccharides in tissues.[36] It also shows changes occurring in tissue components through a reaction known as "metachromasia."[37] In this reaction certain components of the tissue stain a different color from that of the dye solution itself.[34]

OBSERVATIONS

The Outer Enamel Epithelium

At birth the outer enamel epithelium consisted of a sheet of cuboidal cells which was one layer in thickness. In a few places capillaries and fibroblasts were seen penetrating through the outer enamel epithelium into the stellate reticulum (Fig. 1). The invasion of blood vessels and connective tissue elements continued and increased until after nine days the outer enamel epithelium consisted of isolated cells rather than a layer of cells (Figs. 2,3).

The reduction of the stellate reticulum brought the cells of the outer enamel epithelium in closer approximation to the stratum intermedium (Figs. 2,3). By the fourteenth day the stratum intermedium cells had proliferated so as to encompass the cells of the outer enamel epithelium (Fig. 4). It was not possible to distinguish between the cells of the outer enamel epithelium and the stratum intermedium.

The Stellate Reticulum

The invasion of the stellate reticulum by capillaries and fibroblasts from the dental sac was apparent from the day of birth. The cells of the stellate reticulum nearest the stratum intermedium were more numerous than those adjacent to the outer enamel epithelium (Fig. 1).

This layer was reduced in size until by fifteen days it was indistinguishable as a definite entity.

The Stratum Intermedium

At birth, the stratum intermedium varied in cell morphology and thickness in different areas of the tooth. The cells were cuboidal and three layers thick in the occlusal area. In the cervical region they appeared squamous and one layer thick (Fig. 1).

Occlusally, an acidophilic intercellular space was seen between the stratum intermedium and the ameloblasts. The cytoplasm of the cells of these two layers appeared to merge in this zone.

Through the ninth day the stratum intermedium remained distinguishable as a separate layer. The cell layers had been gradually reduced to the thickness of one cell and the cells appeared more cuboidal throughout all regions. The intercellular space between the stratum intermedium and the ameloblasts extended from the occlusal to the cervical areas (Fig. 2).

On the eleventh day the stratum intermedium in the occlusal area began to proliferate (Fig. 4). The intercellular space was no longer visible. These changes advanced in a cervical direction until on the fourteenth day the stratum intermedium cells in all areas had proliferated.

The following day these cells had merged with those of the remaining outer enamel epithelium and those of the oral epithelium. This process advanced cervically in following days (Fig. 5). It was

not possible morphologically to distinguish between the cells of the proliferated stratum intermedium and those of the oral epithelium.

A correlation was seen between the proliferation of the stratum intermedium, the start of ameloblastic degeneration, and the end of enamel matrix production, all of which began simultaneously.

The Ameloblasts

At birth the ameloblasts were thickly packed and columnar in the occlusal area. Cervically, the ameloblasts thinned out and were cuboidal (Fig. 1). The nuclei were elongated and located at all levels of the cells.

On the second day enamel matrix was seen. The following day the nuclei of ameloblasts adjacent to the matrix migrated to the proximal side of the cells. At the distal end of the ameloblasts were light-stained Tomes' processes in which filament-like connections could be seen between the cells and the matrix (Fig. 6). With the appearance of matrix in the more cervical regions, the nuclei of adjacent ameloblasts migrated to the proximal end of the cells (Fig. 6).

On the eleventh day there was a reduction in height of the ameloblasts in the occlusal portion of the tooth. In later sections, the ameloblasts in the cervical areas showed a similar shortening. This change occurred in conjunction with the end of matrix production and proliferation of the stratum intermedium cells.

At the time of eruption and for three days following, ameloblasts

could be seen in close apposition to the matrix (Figs. 5,7). During this period the ameloblasts progressively became more squamous in the occlusal areas where the combined oral epithelium and stratum intermedium cells had merged (Fig. 5). The ameloblasts appeared to be converting to a keratin-like substance. Cervical to these transformed cells, cuboidal ameloblasts remained (Fig. 8). The keratin of the oral epithelium and the keratinous material contiguous with the ameloblastic layer were continuous and stained a bright red with Masson's Trichrome stain (Fig. 8).

On the twenty-third day no enamel matrix was retained in the sections. The occlusal ameloblasts had degenerated and showed pyknotic nuclei and keratinous changes (Figs. 9A,9B). However, they were still in continuity with the cuboidal cells in the more cervical region (Fig. 10). The ameloblasts in the most occlusal area had split off from the underlying epithelium.

Histochemical Staining Observations

Periodic acid-Schiff

On the first day a slightly positive reaction was evident at the distal ends of the ameloblasts and in the odontoblasts (Fig. 11).

The eleventh day specimen revealed positive reactions at the junction of the ameloblasts and the enamel matrix and where the enamel matrix was in apposition with the dentin.

On the thirteenth day, the enamel matrix in the occlusal area

exhibited a more positive reaction than the rest of the matrix. This appeared to progress cervically on successive days. The oral epithelium and the stratum intermedium cell layers stained similarly. Their basement membranes showed a pa-S positive reaction.

In specimens examined later in the study the degenerating ameloblasts in the occlusal area stained strongly positive. This reaction was not evident in the ameloblasts of the cervical region.

Toluidine Blue

Until the ninth day, metachromasia was seen in the cytoplasm of the stratum intermedium and ameloblasts, and in the enamel matrix.

On the thirteenth day, the matrix in the occlusal area became more metachromatic (Fig. 12). This reaction progressed toward the cervical area in succeeding days. There was no apparent change in the ameloblasts and stratum intermedium cells.

Metachromasia persisted in the enamel matrix although the reaction was not as strong in the last specimens studied. The cytoplasm of the cells of the reduced enamel epithelium continued to indicate some metachromasia, but it was not possible to observe this in the degenerated ameloblasts.

DISCUSSION

The Outer Enamel Epithelium

The invasion of the outer enamel epithelium by capillaries and fibroblasts has been reported.[38,39,40,41] Johnson and Bevelander, and Uohara reported that the outer enamel epithelium was completely destroyed and, therefore, did not contribute to the reduced enamel epithelium. In this work, isolated groups of cells from the outer enamel epithelium could be identified and appeared to be encompassed by the proliferating cells of the stratum intermedium. It was not possible to distinguish between the cells of these two layers after this proliferation, but it was thought that the combined cells formed part of the reduced enamel epithelium. Others[38,39,42] also believed that the outer enamel epithelium became a part of the reduced enamel epithelium.

The Stellate Reticulum

In this study, a reduction of the stellate reticulum and its replacement with connective tissue and proliferating stratum intermedium were observed. However, it was not possible to determine if all the cells of this layer were destroyed. As a result, it was felt that the stellate reticulum may contribute to the reduced enamel epithelium.

The Stratum Intermedium

The development of the cells of the stratum intermedium confirm the observations of others. The intimate relationship of the stratum intermedium and the ameloblasts as described by Noyes,[39] and Johnson and Bevelander[42] was seen. Mitotic activity and proliferation of the stratum intermedium which have been described previously[38,39,43,44,45] were also observed. The intercellular space separating the cytoplasm of the ameloblasts and the cells of the stratum intermedium was decreased. At the same time, the completion of matrix formation, an altered morphology of the ameloblasts, and proliferation of the stratum intermedium were observed. These were consistent with the findings described by Wasserman[46] and Marsland.[44]

The findings in this study left little doubt that the stratum intermedium became a part of the reduced enamel epithelium.

The Ameloblasts

This study confirmed the morphologic changes which occurred in the ameloblastic layer as described by others.[14,22,33,45,46,47,48] From the time matrix was first observed the fibrillar connections between the ameloblasts and the matrix were seen, as reported by Baume[22] and Reith[32] (Fig. 6). This relationship continued as long as matrix remained.

Since the enamel matrix was retained for three days after tooth eruption, an organic union with the ameloblasts could be observed

(Figs. 5,7). This also has been reported[19,22,31,33,38,47] in other experimental animals. Due to the union of the ameloblasts and enamel it seems apparent that the gingival crevice does not reach to the cemento-enamel junction at the beginning of tooth eruption.

Ussing[33] described amelogenic degeneration which occurred either by a quick positional change or by a gradual decrease in height. In this study, the latter process was observed. It was concluded that degeneration was due to a combination of two factors: 1) the end of the life cycle of ameloblasts, and 2) a reduction in the nutritional supply to the ameloblasts caused by the downward migration of the oral epithelium behind them. The latter has been cited by Skillen[21] and by Becks.[20]

Although it has been stated that the degenerating ameloblasts secrete or produce keratin as their final product,[38,39,49] these cells seemed to form keratin through a process of conversion rather than secretion[42] (Figs. 8, 9B).

Controversies regarding the origin, structure and existence of the primary and secondary cuticle abound in the literature.[20,22,33,38,49,50,51,52] No primary cuticle was observed in this study.

The secondary cuticle has been reported to form from the reduced enamel epithelium.[38] In these tissue sections, the secondary cuticle appeared to form from the ameloblastic layer only. The degeneration and conversion of the ameloblasts resulted in the formation of a keratinous cuticle (Fig. 9B).

The epithelial attachment consisted of the remaining layers of the reduced enamel epithelium. This attachment was observed to be in organic union with enamel matrix after tooth eruption had occurred (Fig. 7). The exact mode of attachment could not be determined. However, the ameloblasts which were apical to the degenerated ameloblasts remained vital and in connection with the surface of the enamel.

Histochemical stains were used in this study to determine whether they would reveal the composition or the mechanism of the epithelial attachment. Periodic acid-Schiff and toluidine blue stains did not prove of value in this study.

Lack of a control slide[35] with the use of periodic acid-Schiff makes it difficult to decide what intensity of staining can be considered a positive reaction. Authors differ on their interpretations[43,53,54,55] and relative values need to be established for accurate observations.

Toluidine blue did not delineate cell morphology (Fig. 12) and the changes seen with this stain were not significant in the area of the epithelial attachment.

CONCLUSIONS

1. The reduced enamel epithelium was composed of the remaining cells of the enamel organ.

2. An organic attachment of epithelium to enamel matrix was apparent after tooth eruption in the Wistar rat.

3. There was no evidence of a primary cuticle.

4. The secondary cuticle formed from the ameloblastic layer only and was a result of the degeneration and conversion of the ameloblasts into a keratinous substance.

5. The histochemical stains used were of little value in determining the development of the epithelial attachment.

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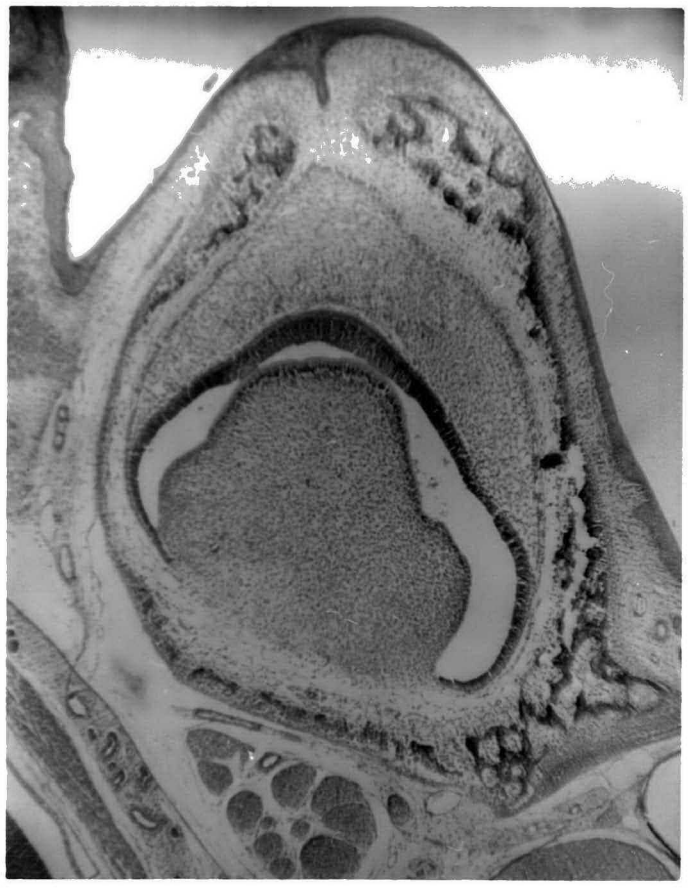

Fig. 1. Developing rat molar one day after birth. Orig. mag. 21x.

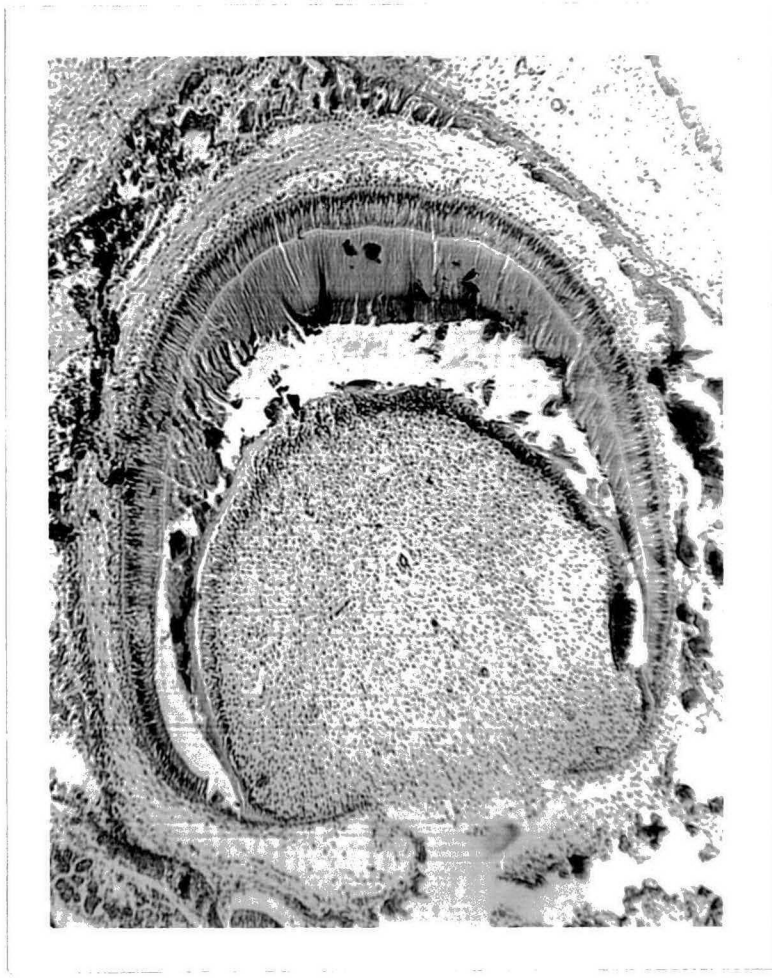

Fig. 2. Developing rat molar nine days after birth. Note reduction of stellate reticulum layer and break-up of outer enamel epithelial layer. Orig. mag. 21x.

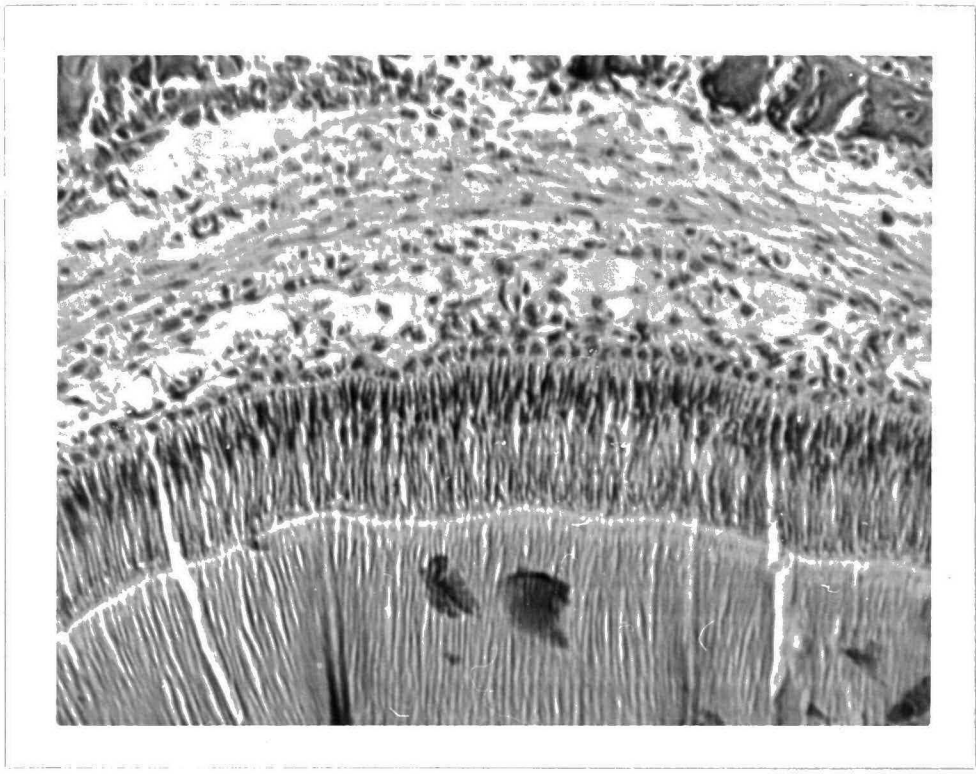

Fig. 3. Magnification of Fig. 2. Note reduction of stellate reticulum layer, break-up of outer enamel epithelium layer, single layer of stratum intermedium cells, and intercellular space between ameloblasts and stratum intermedium layer. Orig. mag. 60x.

Fig. 4. Developing rat molar twelve days after birth showing proliferation of the stratum intermedium cells. Orig. mag. 60x.

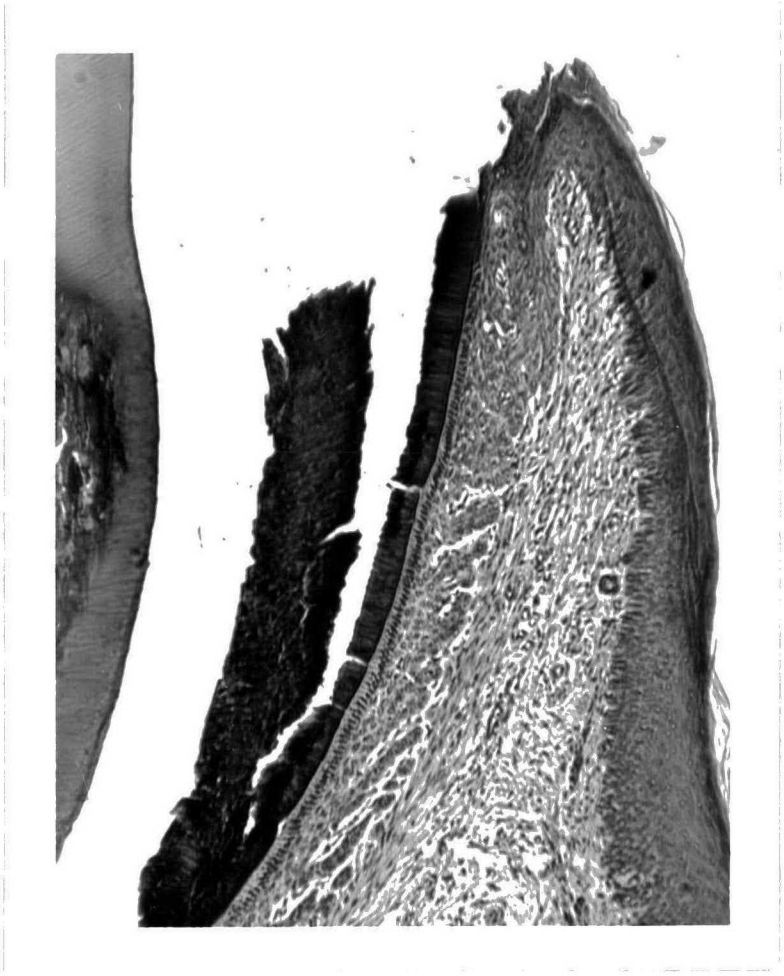

Fig. 5. Developing rat molar twenty-one days after birth. Note proliferation of the stratum intermedium cells in all areas and its merging with the remaining outer enamel epithelium and the oral epithelium. Ameloblasts remain in apposition to enamel matrix. Orig. mag. 60x.

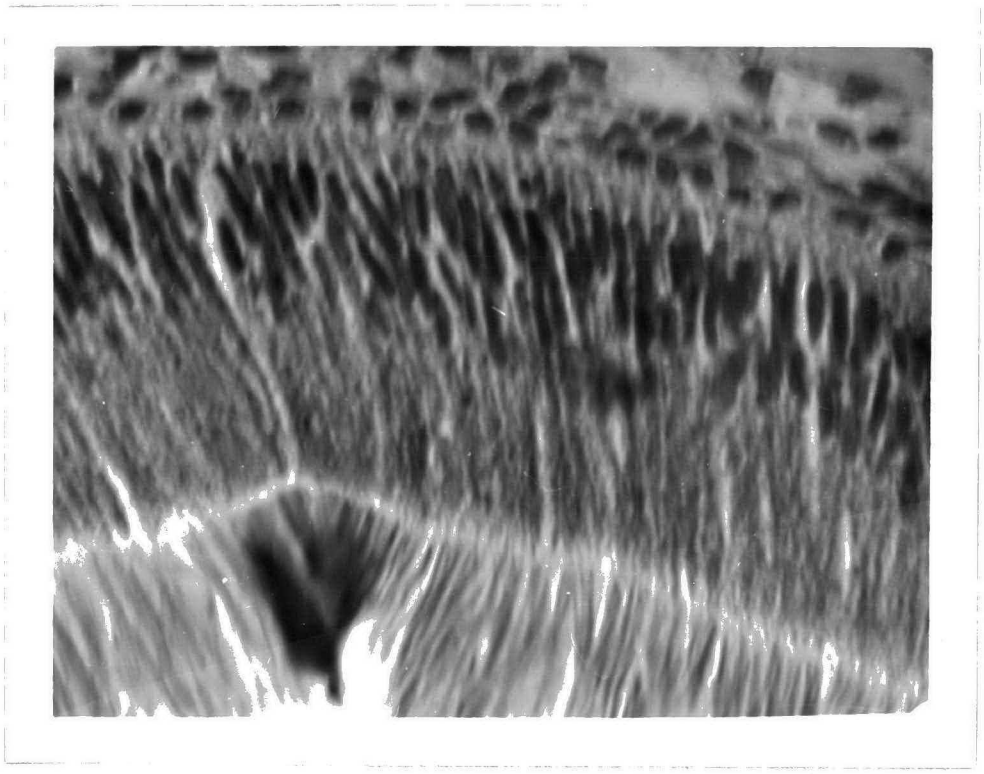

Fig. 6. Developing rat molar six days after birth. Note filament-like connections between the ameloblasts and the enamel matrix. Orig. mag. 270x.

Fig. 7. Developing rat molar twenty-two days after birth. Note close apposition of ameloblasts and enamel matrix three days after tooth eruption. Orig. mag. 60x.

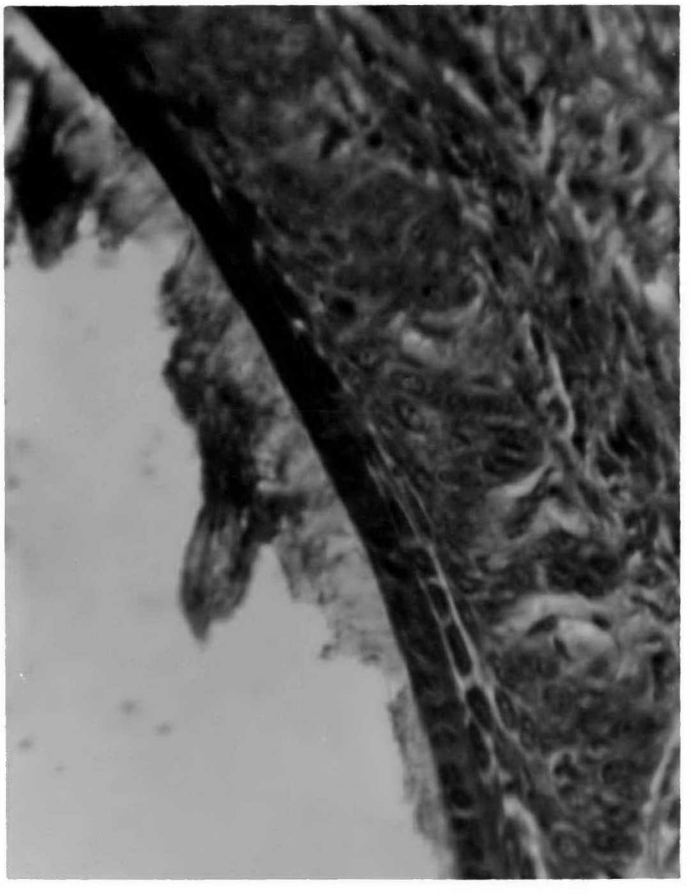

Fig. 8. Developing rat molar twenty-two days after birth. Note conversion of occlusal ameloblasts to a keratinous substance. (Masson's trichrome stain) Orig. mag. 270x.

Fig. 9A. Developing rat molar twenty-three days after birth. Note continuity of oral keratin and degenerated occlusal ameloblasts. (Masson's trichrome stain) Enamel matrix is no longer evident. Orig. mag. 60x.

Fig. 9B. Magnification of Fig. 9A. Note continuity of oral keratin and degenerated ameloblasts (Masson's trichrome stain). Orig. mag. 270x.

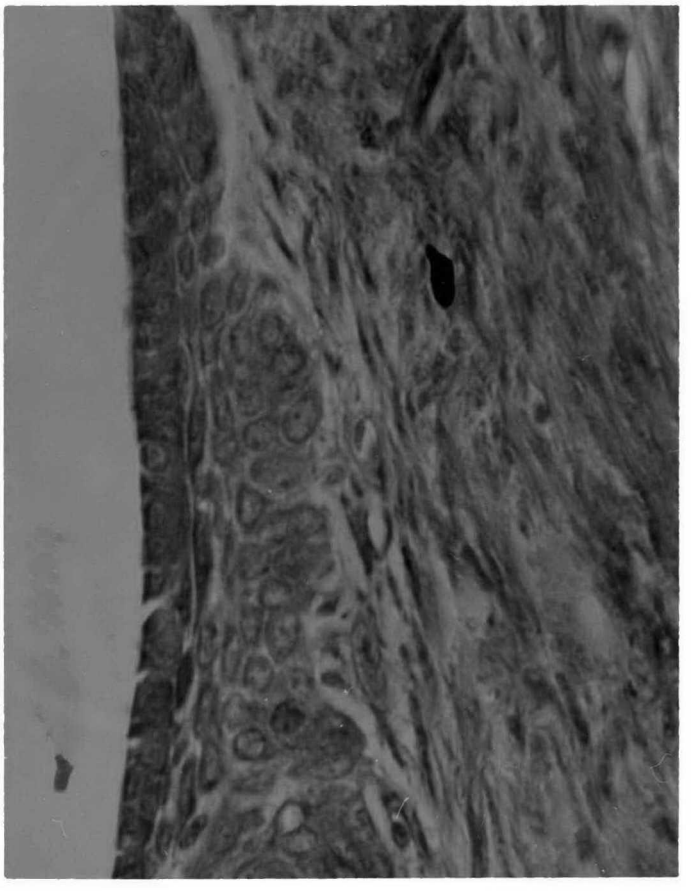

Fig. 10. Developing rat molar twenty-four days after birth. Note the presence of cuboidal ameloblasts in the cervical area of the tooth five days after eruption. Orig. mag. 270x.

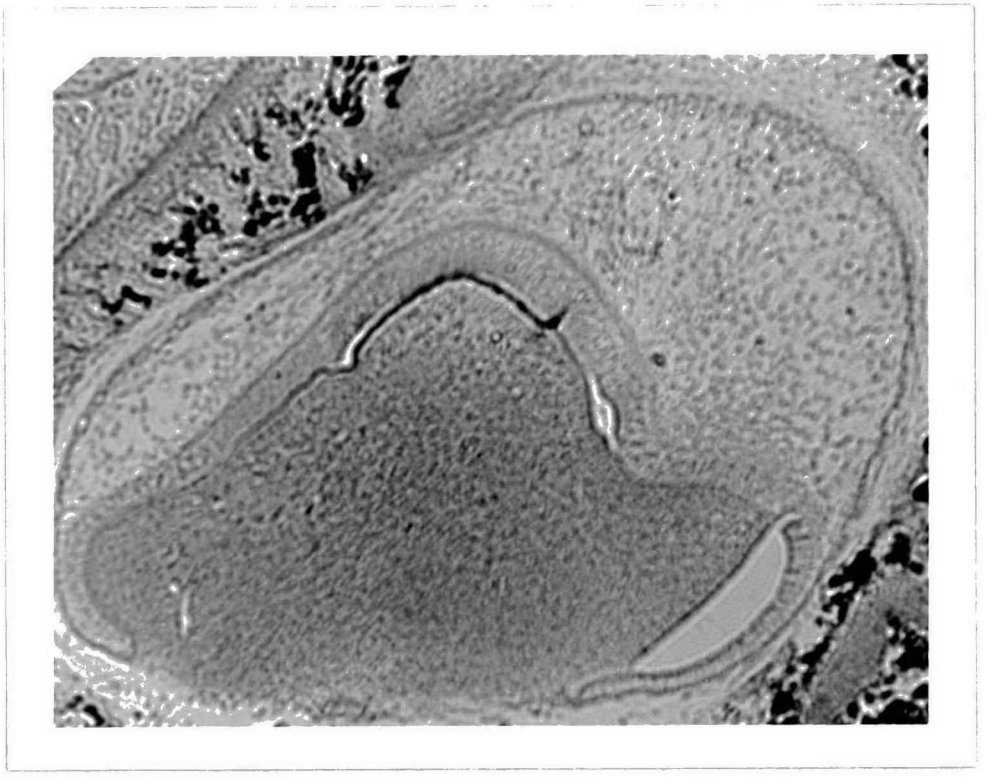

Fig. 11. Developing rat molar one day after birth. Note slightly positive reaction in the area of the ameloblasts and odontoblasts. (Periodic acid-Schiff stain) Orig. mag. 21x.

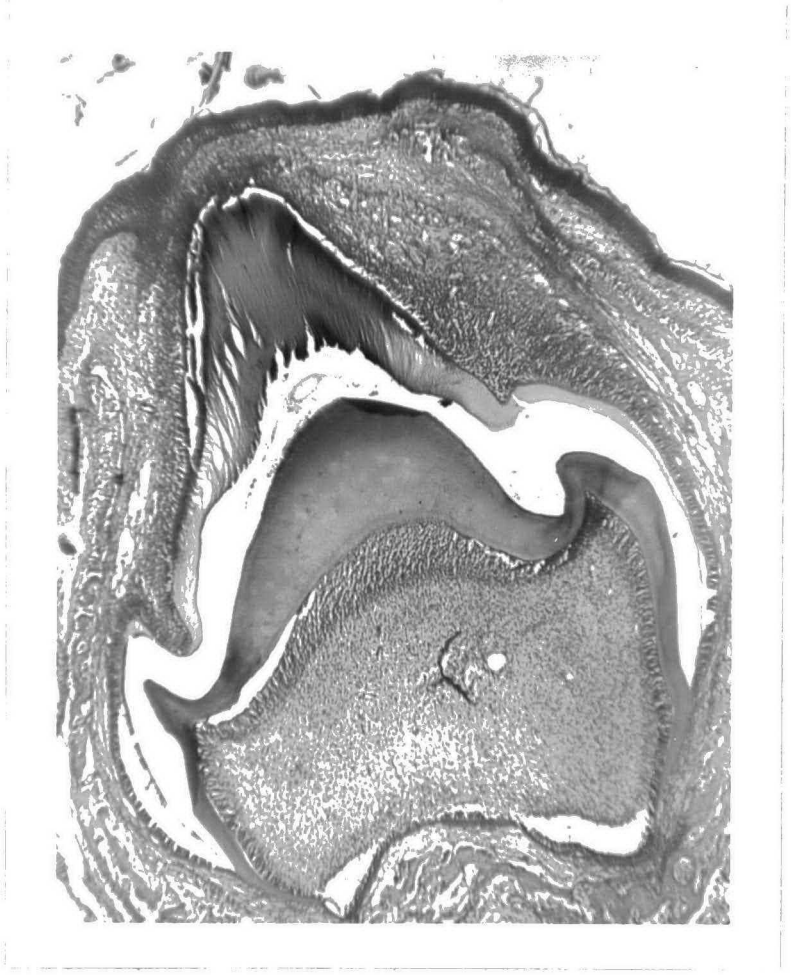

Fig. 12. Developing rat molar thirteen days after birth. Note metachromasia in the enamel matrix in the occlusal area. Orig. mag. 21x.