Hyaline bodies and odontogenic epithelium in the follicles of unerupted permanent human teeth

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In 58 embedded teeth and 126 follicles it was found that the enamel-forming epithelium could be heavily disturbed especially in narrow fissures and at the cemento-enamel junction by premature reduction preventing the enamel from being fully mineralized. The congestion of capillaries in the connective tissue of the follicle caused hemorrhage which imbibed the epithelium and the enamel stroma. In the follicles two types of hyaline bodies could be discerned. One type consisted of hyalinized islands of odontogenic epithelium. They could calcify and constituted a fluent transition to odontogenic tumors, e.g. odontoma and dentinoma. They were PAS positive and elastin negative. The other type (the Rushton type) consisted of white thrombi in congested capillaries in inflamed areas. They were PAS negative and elastin positive. The described mechanism might explain the caries susceptibility in molars and premolars with narrow fissural pits and grooves. The same is valid for the cervical areas when the enamel epithelium is prematurely reduced and the enamel stroma imbibed with hematic material.

Key-words: Tooth, impacted; tooth germ; odontogenesis; hyaline substance

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It has been shown that the mineralization of cervical and fissural enamel in embedded teeth can be very deficient (*Hodson*, 1949, 1950; *Arwill*, 1974). It seemed to be of some interest to study the role of the enamel epithelium in the development of these deficiencies. At the same time the material could possibly provide information on such details in the histology of the tooth follicles as the innervation of the follicles, the provenience of the so called »hyaline bodies» and the composition of the follicular tissue structures as judged from their staining properties.

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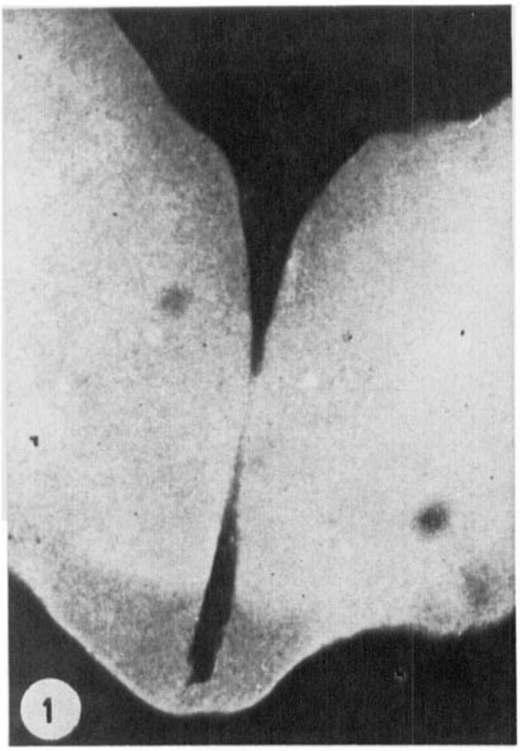
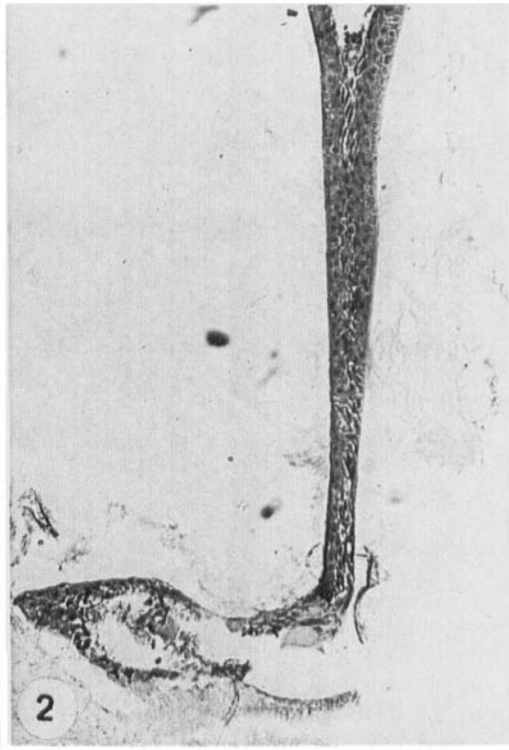
MATERIAL AND METHODS

1) Fiftyeight non-erupted human permanent teeth were decalcified in formic acid after formalin fixation so gently that the enamel stroma still persisted after treatment. The demineralized teeth were embedded in paraffin wax and sectioned serially. The mounted sections were stained in Bock's calcium stain, Hansen's trioxyhematein, Ladewig, Papanicolau, PAS, toluidin blue, Weigert's elastin and Palmgren's silver stain for nerves and reticulin. 2) In 112 patients 126 follicles from non-erupted permanent teeth, surgically removed, were used for histologic study.





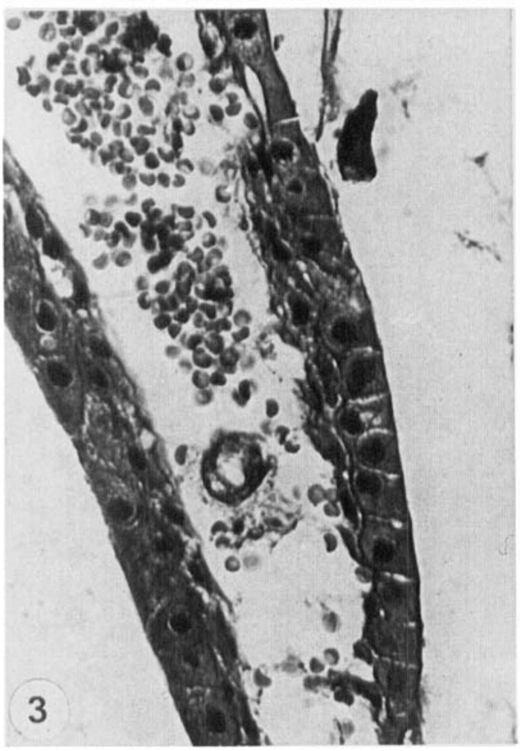


Fig. 1. Tooth 48, partially embedded, ♀ 29 yrs. Deep, invaginated fissure with a narrow opening and an ampulla-like widening at the base. Microradiograph × 125.

Fig. 2. Tooth 48, totally embedded, ♀ 23 yrs. The two opposite outer enamel epithelia make contact in the isthmus of the fissure. Hemorrhage in the invaginated fissure. Decalcified section, Hansen × 320.

Fig. 3. Tooth 35, totally embedded, ♀ 15 yrs. Capillary »trapped» between the two outer enamel epithelia with extravasates. Decalcified section, Hansen × 500.

Fig. 4. Tooth 35, ♀ 15 yrs. Two invaginations at the base of the fissure with imbibition of the enamel stroma. Decalcified section, Bock × 320.

91 of the specimens were fixed in 10 % neutral formalin (group A) and 35 in Bouin's solution (group B). After paraffin embedding and serial sectioning the following stains were used: Bock, Hansen, Ladewig, Papanicolaou, and in group A toluidin blue, PAS and amyloid. In group B Weigert's elastin and Palmgren's silver stains were used.

OBSERVATIONS

In narrow, ampulla-formed fissures (Fig. 1.) the enamel epithelia at the mouth of the fissures were pushed against each other by the mineralizing enamel (Fig. 2.). As the ameloblasts were still functioning in the depth of the fissure, their blood supply could become heavily disturbed by congestion of the capillaries in the connective tissue between the enamel epithelia (Fig. 3.). This meant a hemorrhage in this part of the enamel organ which caused an imbibition of the enamel stroma with hemorrhagic products as plasma proteins and hemolysed erythrocytes (Fig. 4.). The imbibed portions of the enamel stroma displayed a pronounced PAS stainability (Fig. 5.) and metachromasia in toluidin blue stain. The same imbibition phenomenon was frequently encountered with at the enamel-cementum junction (Fig. 6.).

In many cases globular structures were found in the stroma similar to droplets which seemed not to be secretion globules (Fig. 7.).

The mesenchymal follicle contained odontogenic epithelium in 100 %, mostly in the form of separate epithelial cells or in a more organized form as solid islands, rings, rosettes or strands (Fig. 8.).

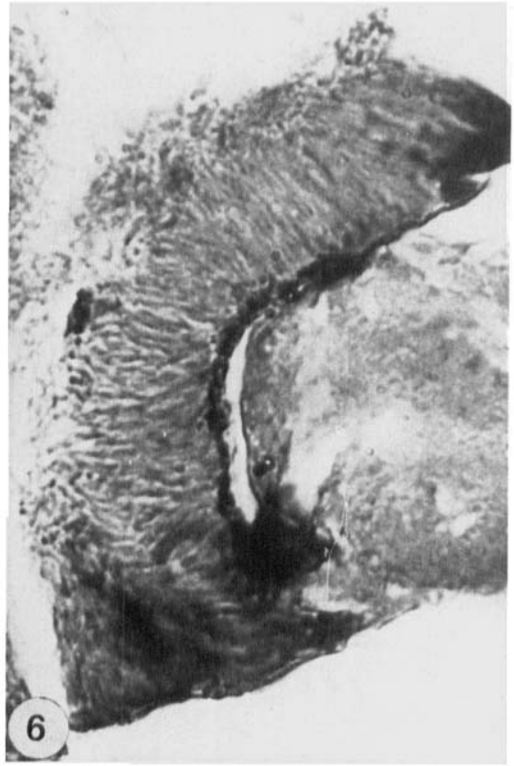
In 34 cases (28 %) ranging from 9 to 35 years of age hyaline and/or calcifying bodies were found (Fig. 9.). They were PAS-positive, at least in their margins, and apparently consisted of degenerating odontogenic epithelial islands or sometimes isolated cells (Figs. 10, 11, 12). The degenerating cells were provided with a pycnotic nucleus, a PAS positive rim and often associated with a small capillary (Figs. 12, 13). The cell clusters could show a chondroid appearance (Fig. 14) and eventually calcify, or the calcification could be similar to coarse woven bone (Fig. 15) or even dentine (Fig. 16). In one case the hyaline bodies were identical with the so called »Rushton bodies», frequently found in the lining of odontogenic cysts. They stained but weakly in PAS, very good, however, in Weigert's elastin (Fig. 17).

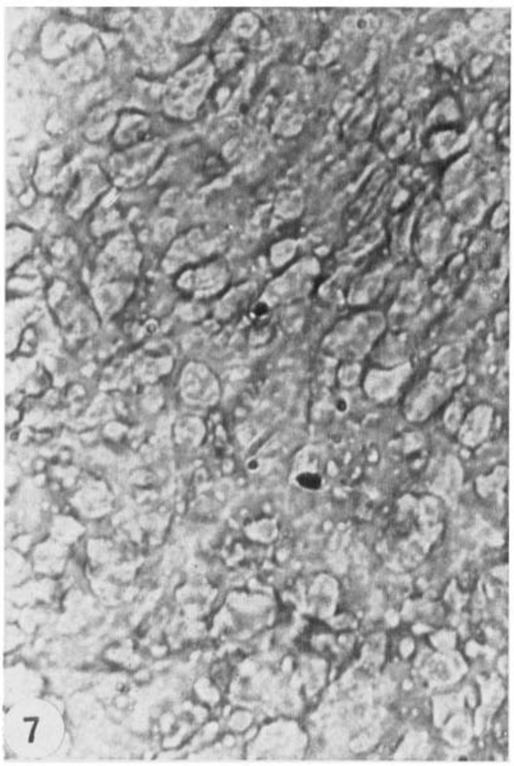
Other observations of interest in the morphology of the tooth follicle were:

The connective tissue generally had a loose, fibrillar texture, but partly displayed a myxomatous character, with stellate or spindleshaped fibroblasts with a random distribution in the follicles. They generally showed metachromasia in toluidin blue stain.

Rather often there was a strong hyalinization of the collagen fibrils. This feature did not seem to bear direct relationship to the patient's age, as it could be found as early as by 11 years of age. On the other hand the loose, myxomatous connective tissue was found in patients up to 26 years.

It was, however, noted that (A) accessory teeth invariably were associated with loose connective tissue in their follicles irrespective of the patient's age, and (B) hyalinization of the follicle was always accompanied by rather heavy fibrin clots and hemorrhage in the tissue (Fig. 18). The hyalinization did not appear to be dependent of age in other way than that the





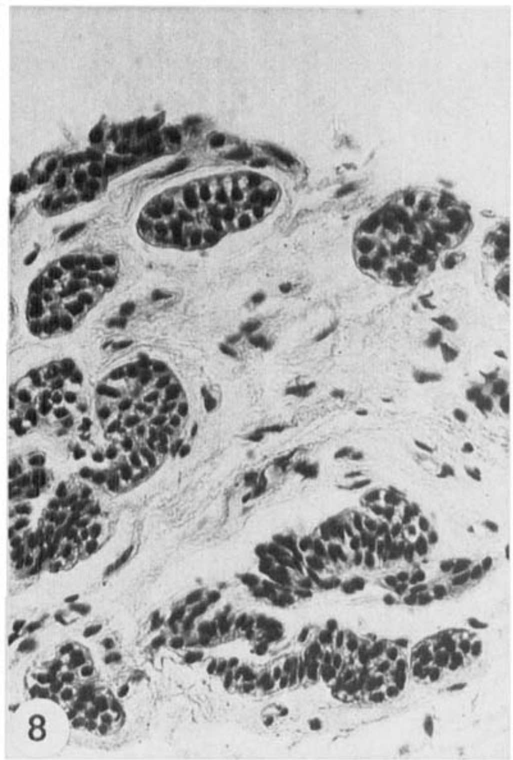


Fig. 5. Tooth 35, totally embedded, ♂ 28 yrs. Imbibed zones of the enamel stroma are strongly PAS positive, the rest negative. Decalcified section, PAS × 125.

Fig. 6. Tooth 35, totally embedded, ♂ 28 yrs. Imbibition and hyalinization at the enamel-cementum junction. Decalcified section, Hansen × 1250.

Fig. 7. Accessory tooth regio 11, ♀ 12 yrs. A hemilateral complex odontoma in connection with the enamel stroma which contains numerous irregular globules. Hansen × 1250.

Fig. 8. Mesiodens, ♂ 9 yrs., totally embedded. Odontogenic epithelium proliferating from the basal membrane of the lining epithelium. Non-decalcified section. Hansen × 320.

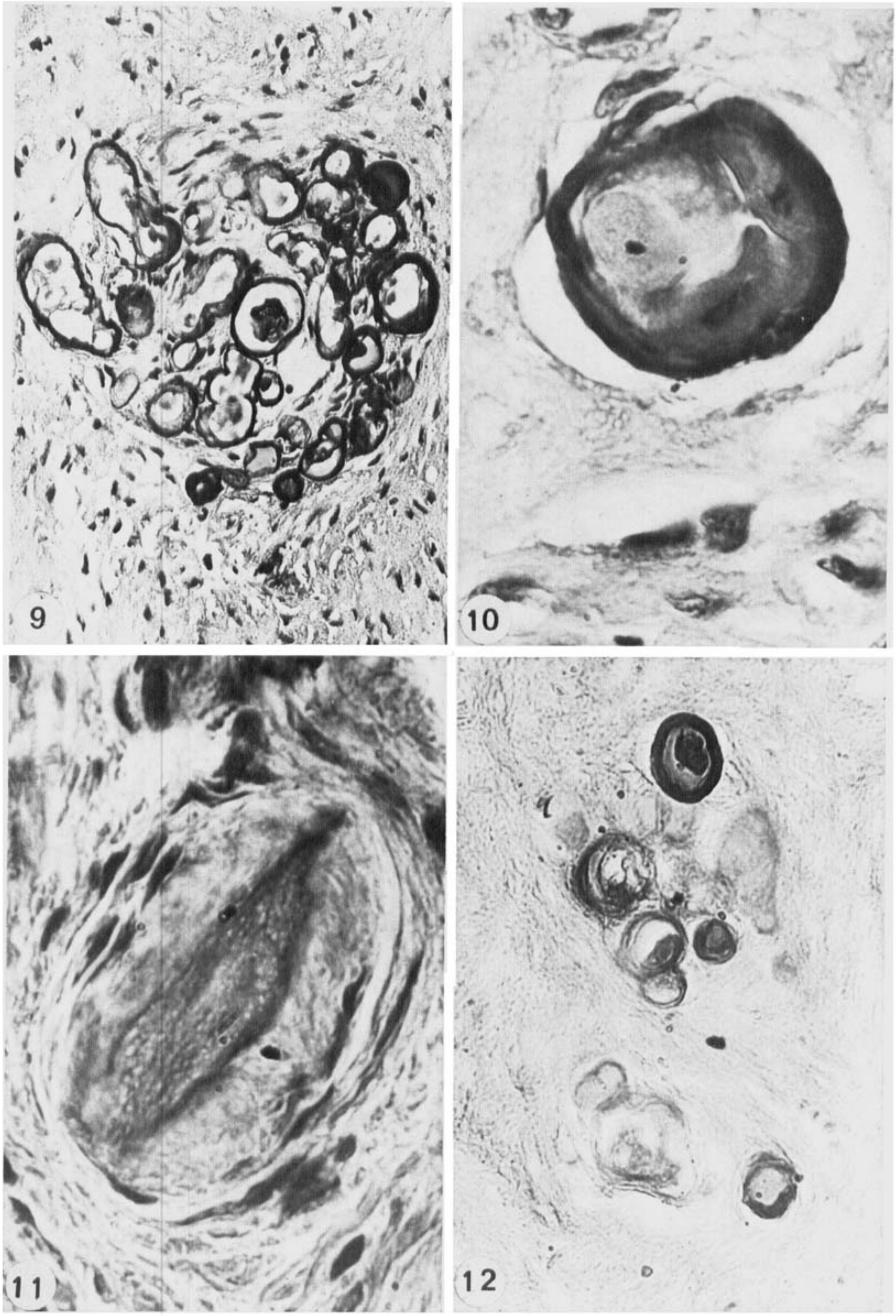


Fig. 9. Tooth 13, totally embedded, ♀ 19 yrs. Calcifying bodies in the follicle, annular and globular. Bock × 320.

Fig. 10. Tooth 13, totally embedded, ♀ 19 yrs. Degenerating odontogenic epithelial nidus with cell remnants. Non-decalcified section. Bock × 1250.

Fig. 11. Tooth 12 with invaginated crown, totally embedded, ♂ 9 yrs. Degenerating cell of odontogenic epithelium with granular content (kerato-hyaline granules?). Non-decalcified section, Hansen × 1250.

Fig. 12. Tooth 13, totally embedded, ♀ 19 yrs. Calcifying body with PAS positive centre and outer rims. PAS × 500.

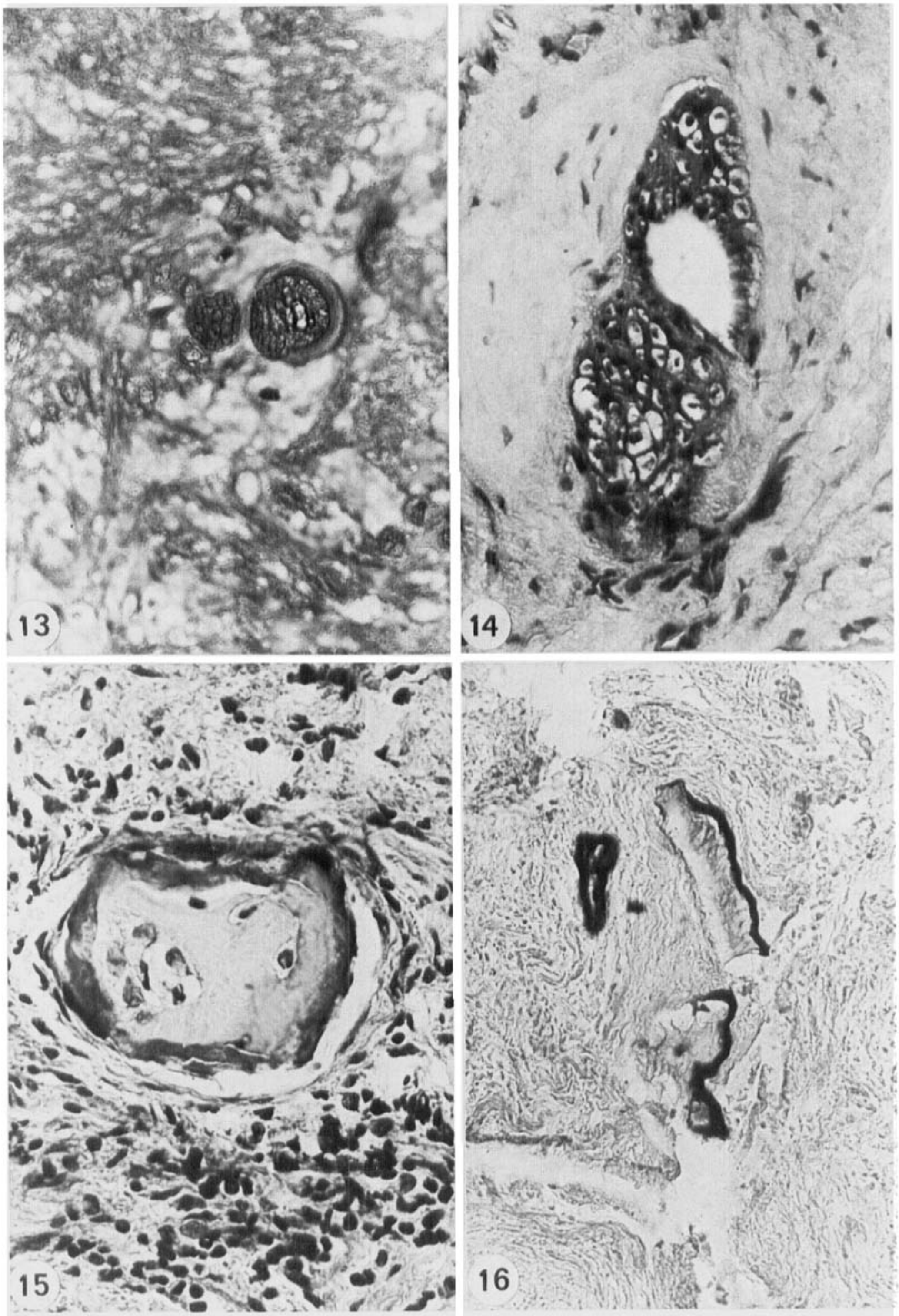
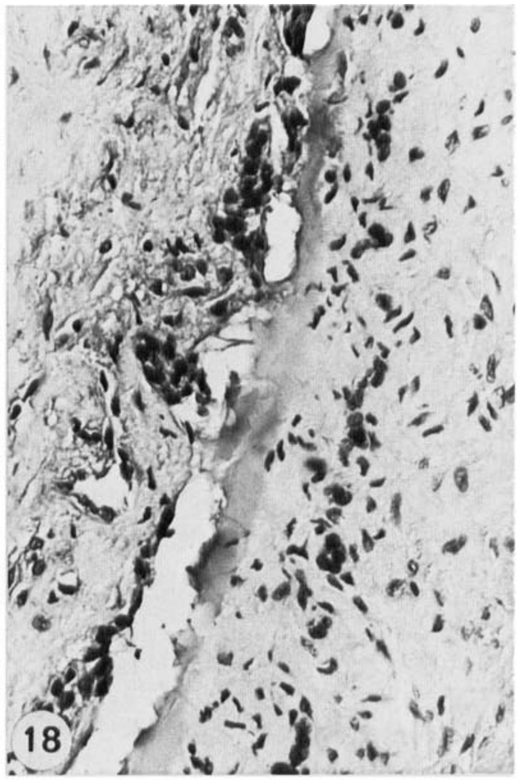


Fig. 13. Tooth 13, totally embedded, ♂ 13 yrs. PAS positive zone surrounding epithelial cell nest. In the centre a penetrating capillary with PAS positive border. PAS. × 320.

Fig. 14. Tooth 13, totally embedded, ♀ 24 yrs. Epithelial strand with degenerative changes in form of vacuolization and increased stainability. Bock × 500.

Fig. 15. Tooth 45, partially embedded, ♀ 16 yrs., inflammation in the follicle. Bone-like calcifying body. Hansen × 500.

Fig. 16. Tooth 12, totally embedded, ♂ 9 yrs., invagination. Dentine-like calcifying body. PAS positive border zone. PAS × 320.



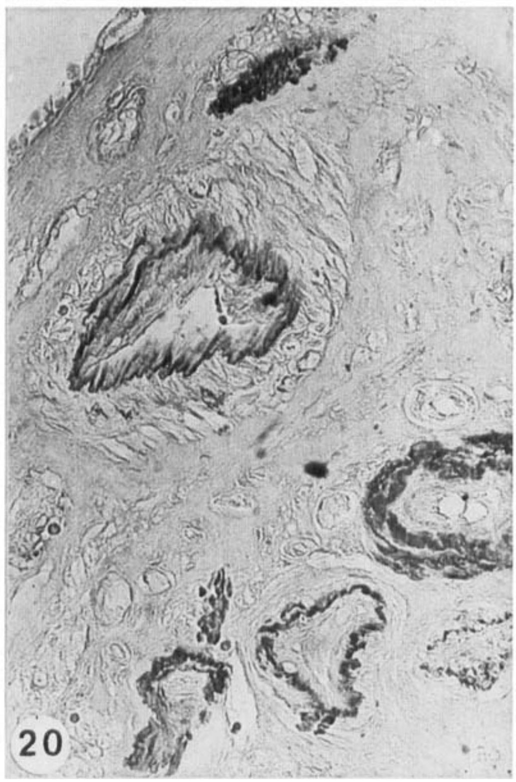


Fig. 17. Tooth 13, totally embedded, ♀ 33 yrs. Hyaline bodies of the Rushton type in the epithelial lining of a follicular cyst cavity. While some bodies (»white thrombi») are elastin positive, erythrocyte aggregates and other bodies (»red thrombi») show reduced stainability with Weigert's elastin stain. × 320.

Fig. 18. Tooth 15, totally embedded, ♀ 11 yrs. Fibrin clot lining a connective tissue cleft with hyalinization of the adjacent parts. Bock × 320.

Fig. 19. Tooth 15, totally embedded, ♀ 11 yrs. Organizing fibrin clot with red blood corpuscles of a ghosty appearance and prospective »calcifying bodies» with increased stainability. Bock × 320.

Fig. 20. Tooth 48, totally embedded, ♂ 16 yrs. Elastic fibres in the capillary walls. Elastin × 320.

risk of the occurrence of hemorrhage seemed to be greater the longer time the tooth was impacted. Consequently the older patients showed more frequent hyalinization than the younger. The hyalinized areas occasionally stained positive in Congo red for amyloid.

The PAS-activity was very variable and strongest in the endothelial cells. The proliferating odontogenic epithelium was bordered by a moderately PAS-positive zone, corresponding to the basement membrane (Fig. 19).

The capillarization was pronounced The vessels often seemed to be related to the odontogenic epithelium (Figs. 18, 19).

Elastic fibres in the capillary walls were found in 11 cases, an incidence of 8 % (Fig. 20). In two of these signs of elastin degeneration were noted.

Nerve fibre bundles were not always found in the follicles, but when they could be observed they numbered from 1 to 16 at the most with a mean of about 6 bundles per section (Fig. 21).

Reticulin fibres were invariably found, especially in the loose connective tissue and the organizing fibrin clots. The main bulk of fibres, however, was collagenous.

DISCUSSION

Kraus and *Jordan* (1965) in deciduous molars showed the fissural regions to be the last to be mineralized. The same is obviously true in permanent molars and premolars, as judged from the present material. In a chemical analysis of the enamel stroma *Pincus* (1948) stated that the enamel stroma of the fissural regions in molars was of a different composition than the rest of the stroma, and that the tissue captured in the fissures probably in part was of mesenchymal origin.

This investigation confirms these observations and further stresses the role of hemorrhage in the connective tissue which is trapped in between the mineralizing fronts of the mouth of the fissure.

It is generally accepted that the post-secretory involuting ameloblasts resorb water and organic material from the formed but unsufficiently mineralized enamel. Ameloblasts in this stage use e.g. the proteolytic enzyme naphtylamidase (*Hammarström, Toverud & Hanker*, 1971). The change in the metabolic activity of the ameloblasts due to their altered function demands a rich supply of blood-mediated products. Consequently the capillaries of the connective tissue proliferate and establish an intimate contact with the enamel organ (Fig. 22). Red blood corpuscles thereby can leak out from the vessels and pass in between the epithelial cells (Fig. 22). The outer epithelial cells form sprouts which proliferate into the connective tissue of the follicle by which the intersurface between epithelium and the blood vessels is considerably increased. The sprouts form starting points for the immigration of odontogenic epithelium into the follicle. The combination of odontogenic epithelium with blood plasma proteins and/or erythrocytic lysates seem to produce a hyalinized structure which is PAS positive (Fig. 11). It is possible to follow the disintegration of the epithelial cell rests and their nuclei (Figs. 10, 11). These hyalinized nests of odontogenic epithelium constitute the so called »hyaline bodies» of the follicle. They are heavily stained in hematoxylin and readily calcified (*Villa*, 1951). They are surrounded by a PAS positive zone which can be so intense that the easiest way to localize them was to scrutinize the PAS-stained sections.

In contrast to these hyaline bodies the

Fig. 21. Tooth 48, totally embedded, ♂ 16 yrs. Nerve fibre bundle peripherally in the follicle. Palmgren's silver stain × 500.

Fig. 22. Tooth 23, follicular cyst with inflammation. ♀ 10 yrs. Between the ameloblasts a cleft can be seen where red blood cells penetrate the cell layer. Bock × 1250.

Fig. 23. Tooth 23, totally embedded, ♀ 21 yrs. Hyalinization and clot formation in the enamel epithelium. Note the clear nuclei and the heavy PAS positivity in the hyaline membrane. PAS × 320.

Fig. 24. Mesiodens, ♀ 43 yrs., totally embedded. PAS positive hyaline membrane between enamel and cementum. PAS × 320.

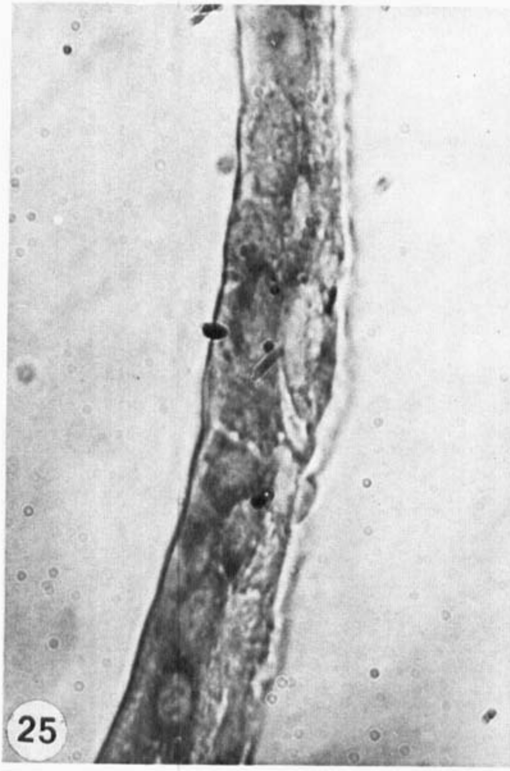
hyaline bodies of Rushton (*Rushton*, 1955) which usually are found in the lining of odontogenic cysts are very weakly stained with PAS but excellently in Weigert's elastin. This means that they have a different etiology and pathogenesis than the hyaline bodies derived from the odontogenic epithelium. In fact they consist of white thrombi in congested capillaries preferably in inflammatory areas, which long ago was proposed by *Dewey* (1918) and corroborated by *Boyssou* and *Guilhem* (1965). The difference in staining properties between the two types of hyaline bodies might be referred to their provenience, in one case from enamel-forming epithelium, in the other case from endothelium and white blood cells. In both cases, however, the hyalinization seems to be mediated by hematic products.

In a comprehensive investigation *Schüle* (1962) found that the »primary cuticle» (ameloblastic cuticle) did not stain consistently with PAS. In the present investigation the outer hyaline membrane of the enamel not infrequently was PAS positive (Figs. 23, 24). In high magnification it was possible to discern the enamel epithelial cells being incorporated in the »hyaline membrane» of the enamel. the nuclei surrounded by a PAS positive zone (Fig. 25). The similarities between the behaviour of the enamel epithelium in the hyaline bodies of the follicle and the outer hyaline membrane of the enamel seems to be too great to be pure incidence (Figs. 26, 27) (*Wertheimer, Fullmer & Hansen*, 1962). In fact the rate of PAS positivity seemed to be related to the imbibition by blood plasma products, and so was the toluidin blue metachromasia. The locations of these two staining reactions were often but not consistently identical (Figs. 28, 19). That such invasion of hematic products in the outer enamel hyaline membrane can happen is shown in Fig. 30. In one case there was a developing hemilateral complex odontoma associated with the embedded tooth. The same epithelial changes as just described also were observed in the tumour (Figs. 31, 32). In all degenerating epithelial cells the nuclei displayed lamellation (Figs. 27, 32) or scattered light globular structures (Fig. 11). Thereafter a disintegration and incorporation of nuclear material in the epithelial cell took place. Occasionally there was an incorporation even in the enamel stroma of the nuclei of the odontogenic epithelial cells (Fig. 33). If this phenomenon contributed to a change in the stainability is difficult to say.

In another case the follicle contained a »dentinoma» as judged from the hard tissue as a separate entity. But the fact that the dentinomatous structure was found in connection with an embedded tooth launches the diagnoses »odontoma».

The two odontogenic tumours in this material thus stresses the possibilities of transformation of odontogenic epithelial nests to tumour producing cells. It might be inferred, therefore, that impacted human permanent teeth must be regarded as potential odontogenic tumour producers.

The enamel globules in the fissures and at the cemento-enamel junction earlier described (*Arwill*, 1974) very closely correspond to the degenerated and partly imbibed enamel epithelial cells. This is consistent with an identical picture in teeth in amelogenesis imperfecta (*Bergman et al.*, 1964) and in epidermolysis bullosa (*Arwill, Bergenholtz & Olsson*, 1965). It can be discussed, however, if the described pathologic changes have any bearing to the carious predilection sites. The disturbances in the formation



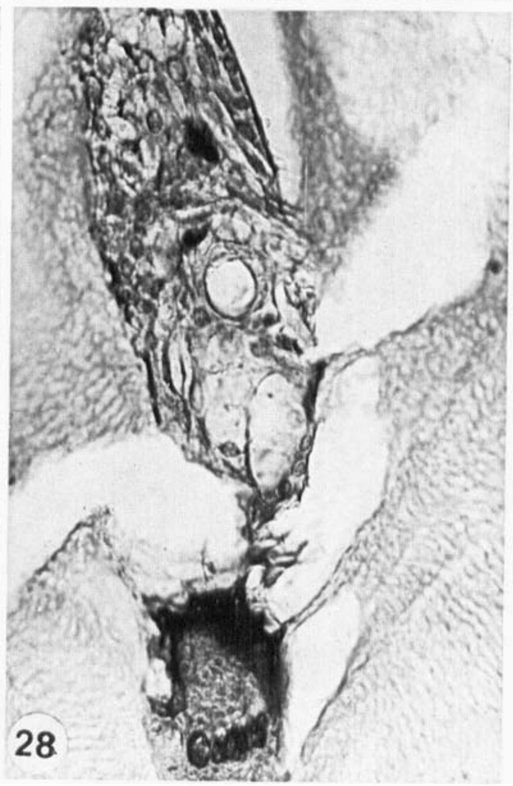
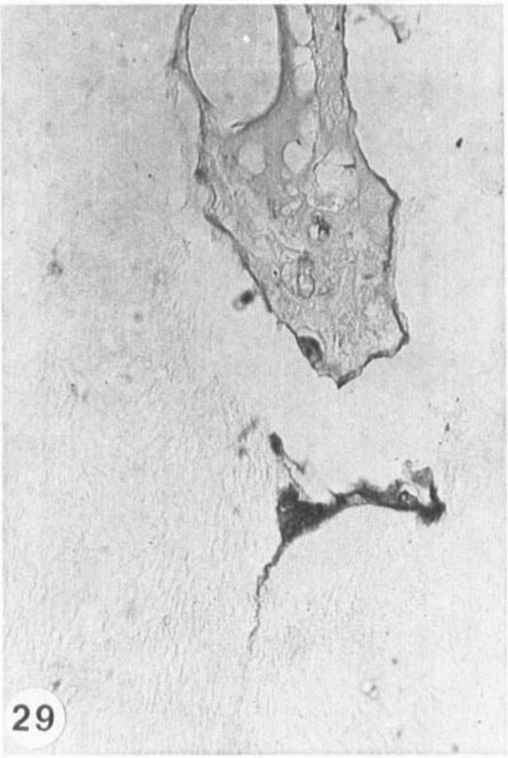


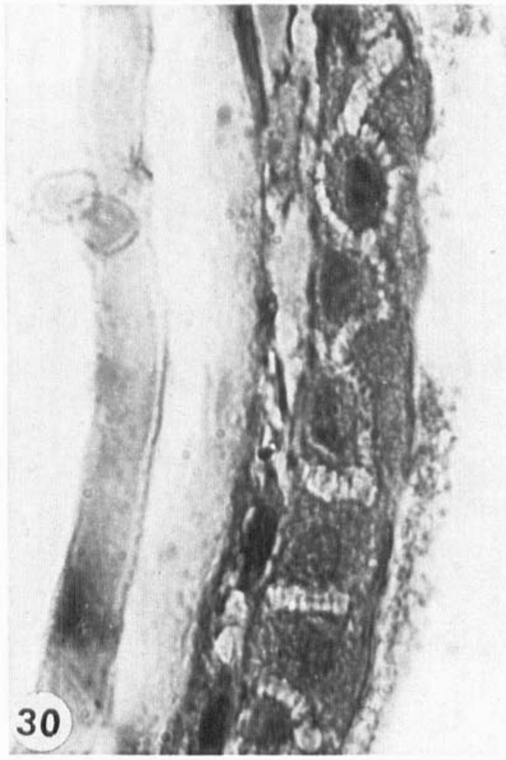
Fig. 25. Tooth 38, totally embedded, ♂ 49 yrs., follicular cyst. The enamel hyaline membrane with incorporated cell nuclei. PAS × 1250.

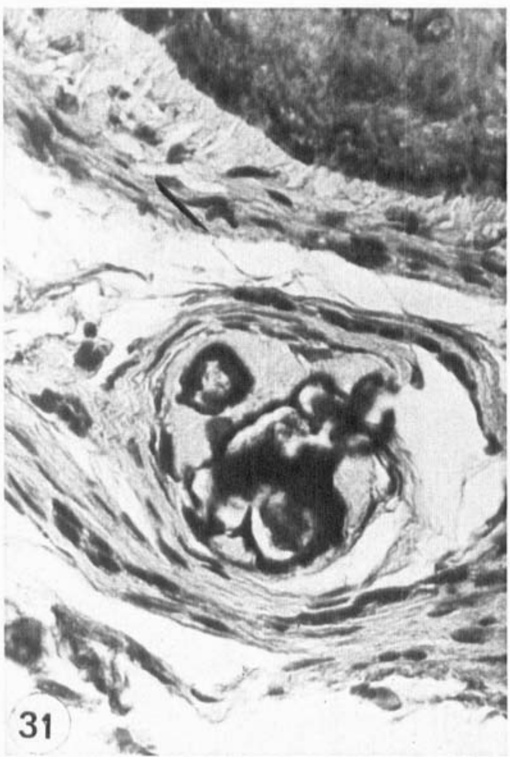
Fig. 26. Tooth 38, totally, embedded, ♀ 16 yrs. Hemorrhage and clotting in the fissural invagination. The enamel stroma contains hyaline globular regions. Hansen × 1250.

Fig. 27. Tooth 38, totally embedded, ♀ 16 yrs. Part of the follicle with hyaline bodies similar to those in the enamel stroma of Fig. 26. Bock × 1250.

Fig. 28. Tooth 48, totally embedded, ♀ 21 yrs. Hemorrhage in the fissure. Imbibition zones and hyalin lamellae metachromatic. Toluidin blue × 500.







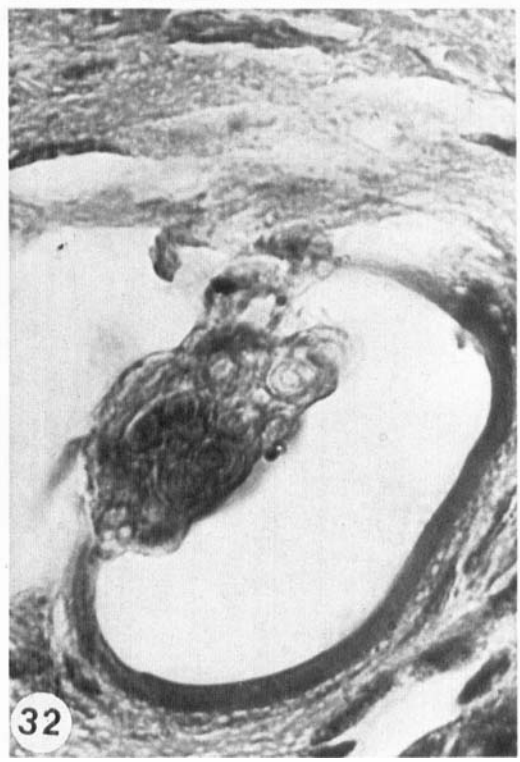


Fig. 29. Tooth 48, totally embedded, ♀ 21 yrs. Imbibed zones of the enamel stroma PAS positive PAS × 320.

Fig. 30. Tooth 48, totally embedded, ♀ 21 yrs. The reduced enamel epithelium. Spinocellular cells to the right, flattened pycnotic cells in the centre and the hyaline outer enamel membrane to the right. Two red blood cells associated with the membrane. Hansen × 1250.

Fig. 31. Accessory tooth regio 11, ♀ 12 yrs. Enamel epithelium with hyaline bodies of the type which usually is found in the odontogenic epithelium of the follicle. Hemilateral complex odontoma in association with the totally embedded tooth. Bock × 500.

Fig. 32. Accessory tooth regio 11, ♀ 12 yrs., totally embedded. Vacuole in the enamel epithelium with disintegrating epithelial cells showing lamellated nuclei which apparently are getting incorporated in the degenerating cell. The border zone is hyaline. Hansen × 1250.

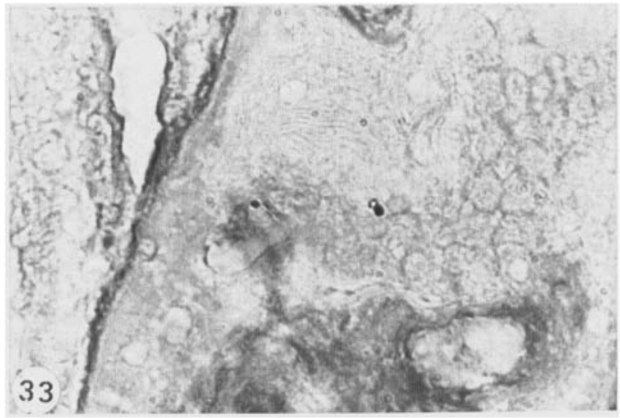


Fig. 33. Tooth 48, totally embedded, ♀ 21 yrs. Enamel stroma with a lamellar-globular pattern. Hansen × 1250.

of the enamel stroma in impacted teeth could of course be a sequel of the impaction. The longer the eruption lasts, the greater is the chance for hemorrhage in the follicle due to traumatic injury or, possibly, the pure growth pressure. The subepithalial hemorrhage might even be useful in sloughing of the enamel epithelium (*Villa et. al.*, 1951). If the tooth erupts in normal time, the reduced enamel epithelium will fulfill its function in an ordinary way and the outer enamel layer will be fully mineralized. If, however, the reduction of the enamel epithelium due to the anatomical form of the crown will be premature, the enamel areas to be last mineralized will be impaired. These areas are the fissural and the cervical regions. Thus it seems to be logical to conclude that these regions not necessarily need to be badly mineralized, but if they are (as very frequently is the case in narrow ampullaformed fissures and at the cemento-enamel junction in 60 % in the present material) they will constitute an excellent starting point for a microbial colonization after eruption. If the mineralization disturbances are moderate it seems possible to prevent a carious attack by sealing with varnish or polymers. In grave cases these methods are likely to fail.

REFERENCES

Arwill, T. 1974. A qualitative microradiographic study on the enamel and the dentine in ground sections of impacted human teeth. Acta Odont. Scand. 32, 1—13

Arwill, T., Bergenholtz, A. & Olsson, O. 1965. Epidermolysis bullosa hereditaria. III. A histologic study of changes in teeth in the polydysplastic dystrophic and lethal forms. Oral Surg. 19, 723—744

Bergman, G., Arwill, T., Welander, E. & Wennström, A. 1964. Observations on enamel and ectodermal lesions in some cases of amelogenesis imperfecta. Odont. Revy, 15, 1—9

Bouyssou, M. & Guilhem, A. 1965. Recherches morphologiques et histochimique sur les corps hyaline intrakystique de Rushton. Bull. G.I.R.S. n:o 2, 81—102

Dewey, Kaethe, 1918. Cysts of the dental system. Dent. Cosm. 60, 555—570

Hammarström, L., Toverud, S. & Hanker, J. S. 1971. Naphtylamidase in ameloblasts during enamel maturation. Calcif. Tiss. Res. 7, 267—276

Hodson, J. J. 1949. Invaginations of the enamel capsule. T-hypoplasia, a new factor in the pathology of human enamel caries. Brit. Dent. J. 87, 167—180

Hodson, J. J. 1950. A study of some of the developmental, structural and pathological aspects of tubular hypoplasia in human enamel. Brit. Dent. J., 88, 34—38

Kraus, B. S. & Jordan, R. E. 1965. *The human dentition before birth.* Lea & Febiger, Philadelphia

Pincus, P. 1948. Further local factors affecting dental caries: The shape and contents of occlusal grooves. Brit. Dent. J. 84, 25—28

Rushton, M. A. 1955. Hyaline bodies in the epithelium of dental cysts. Proc. Roy. Soc. Med. 5, 407—409

Schüle, H. 1962. *Das Schmeltzoberhäutchen.* Georg Thieme Verlag, Stuttgart
Villa, V. G. 1951. Calcification of the epithelial rests and portion of the reduced enamel epithelium in the tooth follicle of an embedded molar. Oral Surg. 4, 877—885
Wertheimer, F. W., Fullmer, H. M. & Hansen, L. S. 1962. A histochemical study of hyaline bodies in odontogenic cysts and a comparison to the human, secondary cuticle. Oral Surg. 15, 1466—1473