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THE ENAMEL AND DENTINE OF SOUND HUMAN TEETH UNDER THE ELECTRON MICROSCOPE

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The discovery and application of the electron microscope has opened a new chapter in histology. Knowing the technique of preparation, it must seem obvious that the dental tissue was the first of the animal tissue to be examined by means of that new tool. Since dental tissue contains a considerable amount of organic substances, it was comparatively easy to develop a technique by which replicas of surface impressions can be used in biological examinations.*

It seems from the literature that SOUNDER and SCHOONOVER [13] (1944), GEROULD [2] (1944), RICHARDS [9] (1944) were the first to apply the replica technique in the electron microscopic examination of the dental tissue. Since then a great number of investigators have availed themselves of the electron microscope to study the enamel and dentine of both sound and pathological teeth. Their investigations yielded much new information about the dental tissue and gave a new foundation to dental histology. Since the morphology of dental tissue has not yet been studied in Hungary by means of the electron microscope, it was deemed necessary to subject the sound dental tissue to micromorphological examinations before attacking problems of pathological histology and aetiology.

Method

Using the gold-aluminium (positive)-replica technique, the enamel and dentine of sound human teeth taken form 50 adults were examined in the present experiments. Various methods of replica are described in the works of WYCKOFF [17] (1949), WOLF [16] (1953), GUBA and SUGÁR (1953), and GUBA (to be published). The surfaces of both untreated and demineralized ground sections were replicated. To demineralize the surfaces they were exposed to a 5% dilution of nitric or hydrochloric acid for 30 seconds.

The ground sections were covered with concentrated collodion and, after drying, separated from the collodion by mechanical stripping. A gold-aluminium film, from 80 to 100 Å thick, was then evaporated on that side of the collodion which bore the impression. This done, the collodion was dissolved from under the metal film. Placing the films thus obtained on the screen of a TTC electron microscope, they were ready for examination (40 kV, 0,5 mA).

*Microreliefs for examination under the optical microscope were first used by J. Wolf (1937—39) who invented the term "Reliefhistologie" for the new method. It is identical with what British authors later called "replica technique", and as the latter term has found general acceptance it has been adopted in this paper.

We had to work with pseudoreplicas in some cases ; these are, however, not less characteristic of the structure than genuine ones and, duly evaluated, do not mar the morphological picture.

Submicroscopic structure of the enamel

Optical microscope and polarized light show the enamel to be composed of prisms and interprismatic substance. The electron microscope reveals also the fine structural details of the enamel tissue. On the strength of electron

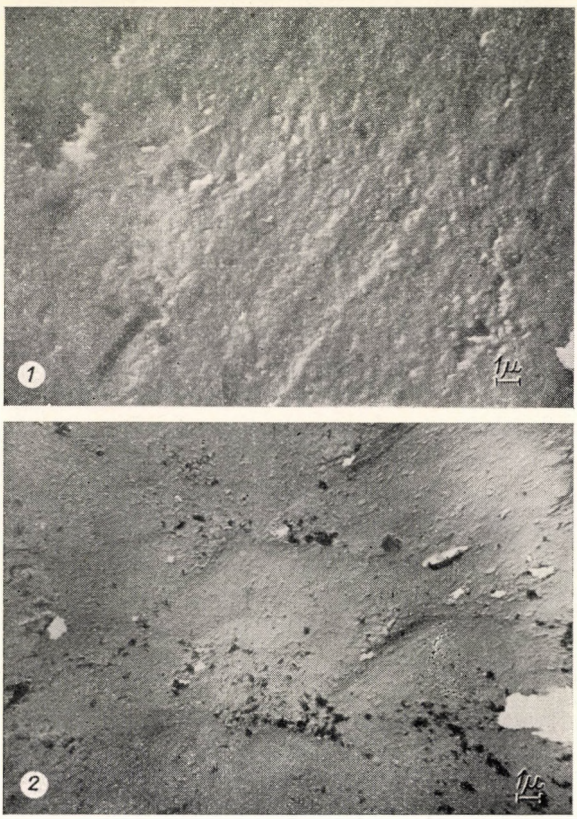


Fig. 1. Longitudinal ground section of untreated enamel. Enamel boundary dimly visible Au-Al replica

Fig. 2. Untreated enamel cut transversely. Outlines of hexagonal enamel prisms. Au-Al replica

microscopic observations, some authors doubt the existence of an interprismatic substance, a problem to which we shall revert later in this paper.

SYRRIST [14] (1949) and MENKE [6] (1950) demonstrated that the enamel prisms consist of submicroscopic crystals. Since these formations did not always appear to have the shape of true crystals, they termed them "crystallites".

Different prismatic forms have been described. We encountered all these forms, which have been mentioned also by SCOTT [12], viz. hexagonal, oval, round, scale-like and other prisms.

Admitting that the form of a prism may show misleading variations according to the plane of section, we do not doubt that there are real differences in both shape and size between the various prisms. So far, we are in the dark about the laws that govern the development of the various forms, nor do we know their biological significance. Form and diameter of the prisms may vary

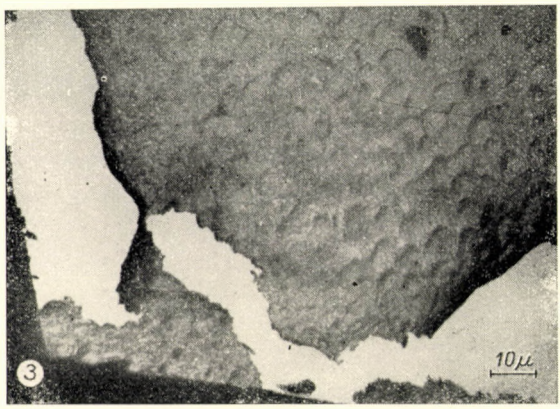


Fig. 3. Cross section of slightly decalcinated enamel. Characteristic scale-like structure with arcuate prism sheath. Au-Al replica, low electron-microscopic magnification

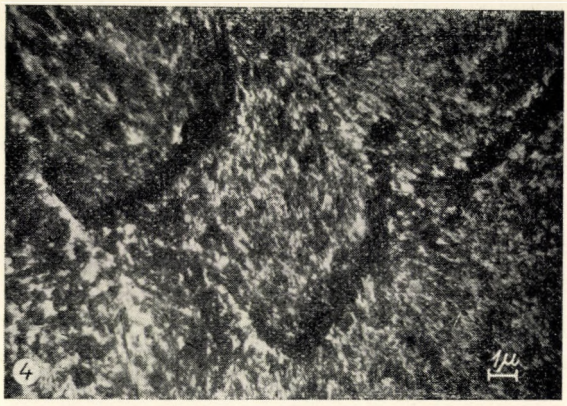
within one and the same preparation, and it would seem that conditions of local pressure play a certain part in their formation.

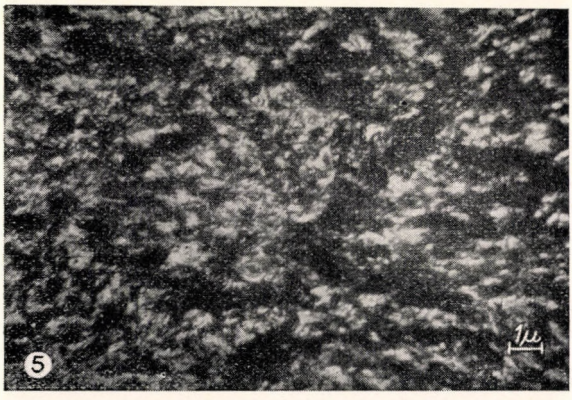
Fig. 1 shows the electron micrograph of a replica taken from the longitudinally, and Fig. 2 that taken from the transversely, cut ground section of an untreated enamel prism.

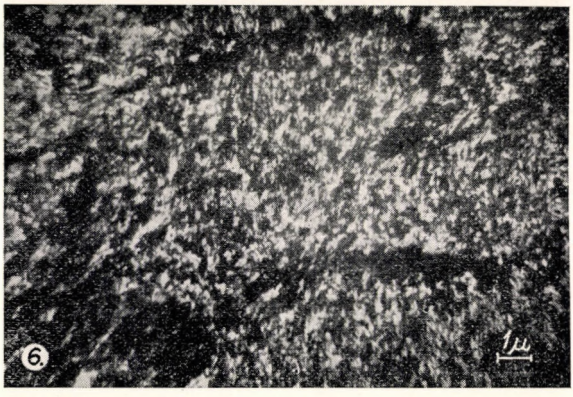
The electron micrographs of the decalcified ground sections reveal the fine details of the inner structure.

A low magnification of the electron microscopic image shows the same characteristic structure which is observable under the ordinary light microscope. The replica of a scale-like demineralized enamel prism is presented in Fig. 3. A higher magnification reveals the fine structure of similar prisms (Figs. 4, 5, 6). No circular prism sheath can be seen to surround the scale-like structure ; it has the shape of an arc and extends to a width of 0,5 to 2 micra. With the exception of the still organic arc-like portion, the prism sheaths are calcified.

Structural examinations revealed the presence of a reticularly arranged organic substance within the prisms. We think that this cementing material surrounds the bundles of crystallites in the manner of a space grid, and that it is connected with the organic prism sheat (Fig. 5). The bundles of crystallites



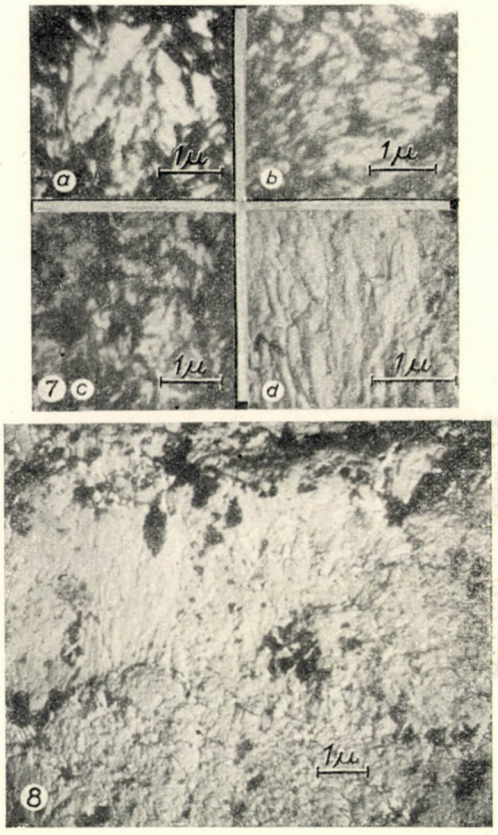




Figs. 4, 5, 6. Same as Fig. 3 with higher magnification. The arc-like prism sheath and the grid-like organic binding material in pseudoreplica

are still evident after mild treatment (Figs. 7a, b, c). An untreated bundle is shown in Fig. 7d.

We are in agreement with BERNICK [1] that calcification may lead to a fusion of adjacent prisms (Figs. 4 and 6). To this we wish to add the following.



Figs. 7a and *7b*. Agglomerations of crystallites in slightly treated enamel prisms with higher magnification

Fig. 7c. Agglomerations of crystallites in untreated enamel prisms. Magnification as in Figs. *7a* and *7b*

Fig. 8. Untreated preparation of hypercalcified enamel prism. Separate prisms no longer visible but inferable from arrangement of crystallites. Au-Al replica

In some preparations mineralization is so complete that the prisms enwrapped can hardly be considered to be in organic sheats. However, the original prisms can still be regarded as separate units, their structural independence being characterized by the orientation of the intraprismatic crystallite bundles. There remain „prism territories". Closely as these prisms are united, it is safe to assume that originally they were separated by organic sheats. The cross

section of a hypercalcified enamel surface before treatment is shown in Fig. 8., and after treatment in Fig. 9.

There are two theories concerning the interprismatic substance. TAKUMA et al [15] (1949), further BERNICK et al [1] (1953), do not admit the existence of an interprismatic substance in the dental tissue of adults, while D. B. SCOTT

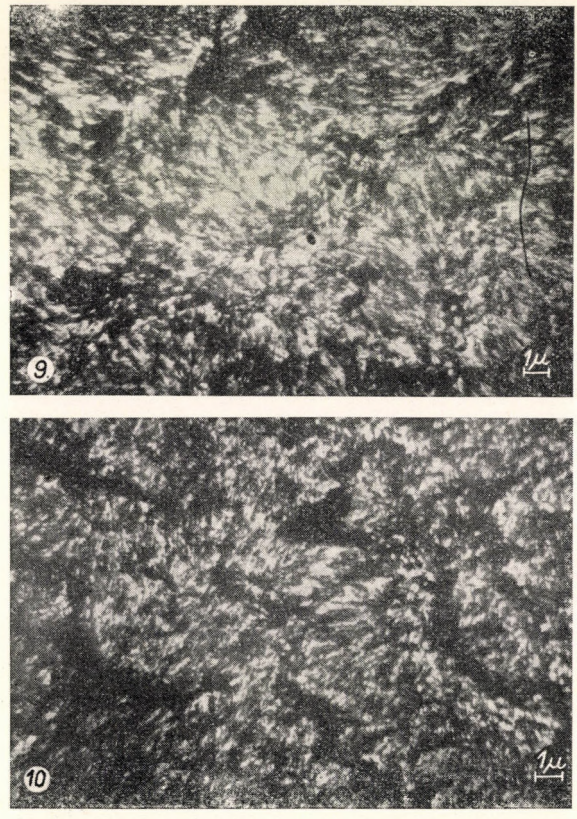


Fig. 9. Mildly treated preparation of hypercalcified enamel prism. Separate prisms no longer visible, but inferable from arrangement of crystallites. Au-Al replica

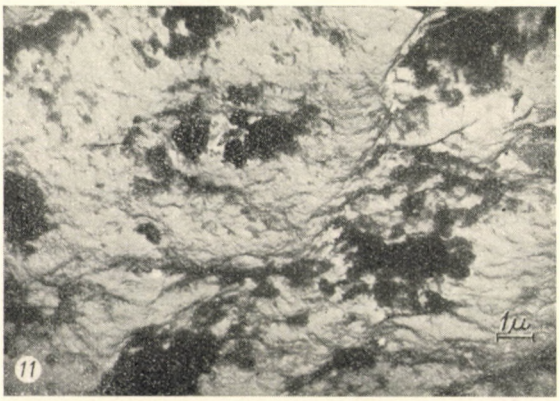
Fig. 10. Replicas of cross section of variously shaped enamel prisms

claims that it is demonstrable both with the replica technique and also in ultra-thin sections. The term „interprismatic substance" is applied by some authors to one, by others to another, structure, although there exists a morphological difference between the two structures so termed. One of them, described by SCOTT and others as an interprismatic substance of fibrillar architecture, is, in our opinion, no enamel structure at all, but rather the dentinal matrix which projects like a finger into the enamel and shows the fibrillation so characteristic of the matrix. The other structure described as interprismatic substance has

undoubtedly a morphological appearance that corresponds to this term. We are in agreement with BERNICK who regards these elements as prisms checked in their development by physical forces (Figs. 9, 10). (They are, according to BERNICK, prismatic processes.)

We are of the opinion that while the adult human tooth may include hypo- or hypercalcified areas it is devoid of interprismatic substance.

HELMCKE's [5] studies, made by means of X-ray diffraction and electron microscope, and published during our investigations, seem to confirm our claim.



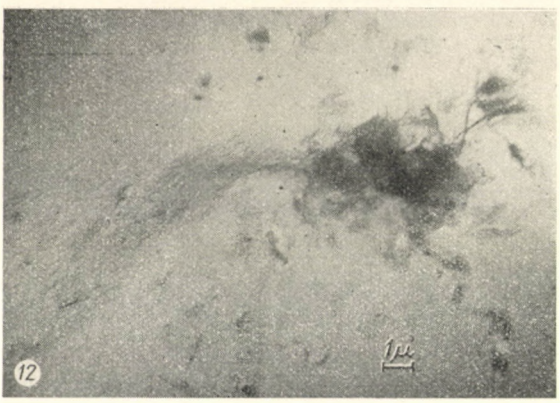


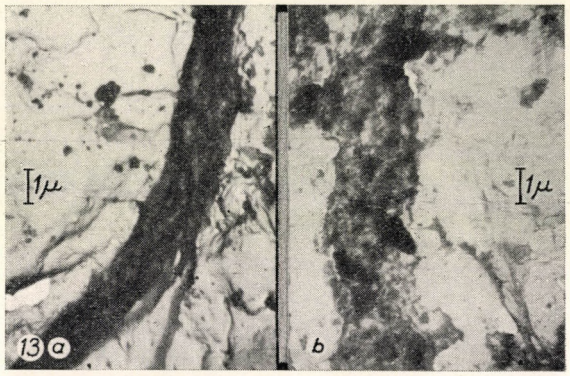
Fig. 11. Cross section of coronal dentine. Tomes' fibres visible in dentinal tubules. Note fibrils 100 Å wide in dentinal matrix

Fig. 12. Cross section of radicular dentine. Size of area shown in picture same as in Fig. 11. Of the tubular system, one tubule and several side channels, as seen in cross section

Submicroscopic structure of dentine

This is similar to that of the osseous tissue, with the difference that dentine does not include cells ; only the projections of the odontoblasts are encountered in the dentinal tubules. The ground substance of dentine, a calcified connective tissue, consists of fibrils which branch out among the calcified areas. It is still

a matter of controversy whether the dentinal tubules should be regarded as channels with special walls (Neumann's sheath) or as simple cavities bounded by the dentinal matrix. ROUILLER and co-workers [7] (1952) compared the



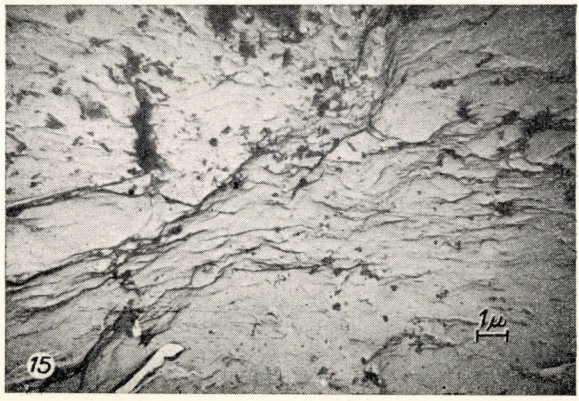
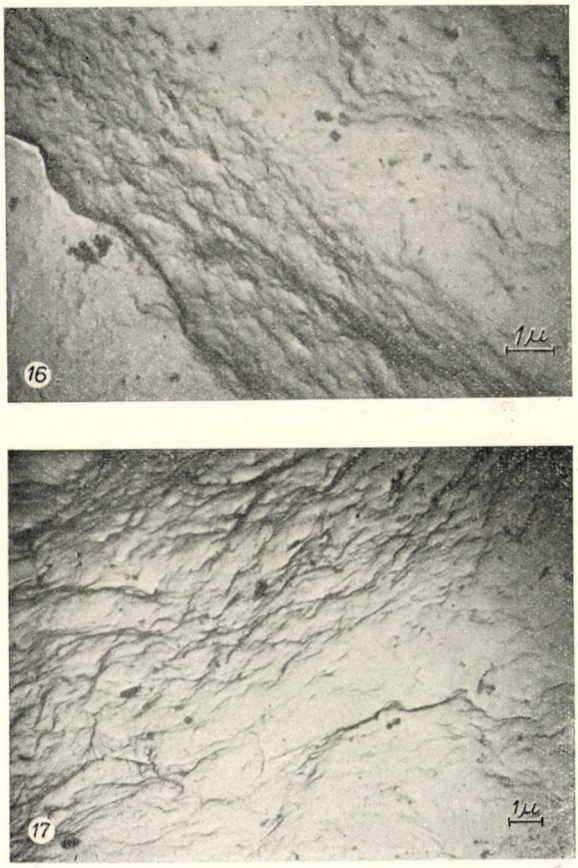


Fig. 13a. Dentinal tubule with fibrillar lining, longitudinal section. Pseudoreplica
Fig. 13b. Replica of the deeper tubular regions. Note granular architecture of protoplasm
Figs. 14, 15. Fine fibrillar texture of dentinal matrix. Au-Al replica

osseous tissue and the dentine. They found the dentinal matrix to consist of a mass of collagen fibrils embedded in the calcified organic substance in the same manner as in osseous tissues. Examinations in polarized light (ORBÁN [8]), proved the longitudinal axis of the collagen fibrils to be parallel with that of the prisms surrounding them. ROUILLER claims that the hypercalcified wall of

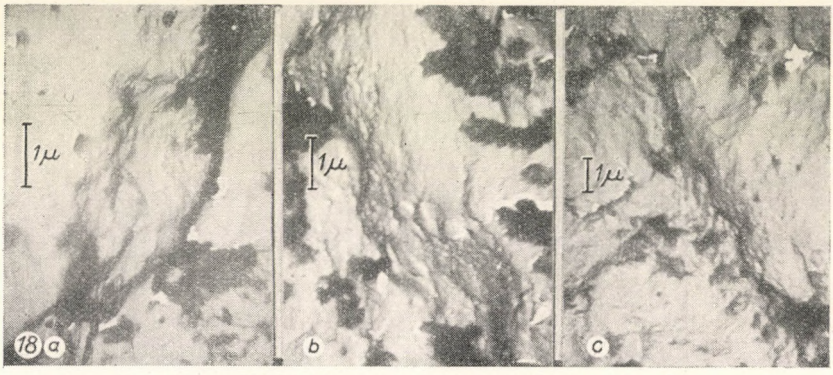


Figs. 16, 17. Alternating fibrillar and calcified zones in dentinal matrix. Au-Al replica

the dentinal tubules is coated by an inner layer of collagen, while the cavity is filled with the projection of the odontoblast, namely Tomes' fibre.

The cross sections of dentinal tubules are represented in Figs. 11 and 12 (Coronal dentine in Fig. 11 and radicular dentine in Fig. 12). A fine fibrillar system between the tubules is brought to view by demineralization. SCOTT [11] (1952) assumes that additional fibrils form part of the tubular system, and this theory seems to be substantiated by our micrographs.

The pliancy of Tomes' fibre has made it impossible to demonstrate its true structure on the replicated longitudinal sections of dentinal tubules. We are nevertheless inclined to assume that within the fibrillar structure, as represented in Fig. 13*a*, a granular structure — characteristic of the protoplasm — can be visualized (Fig. 13*b*). The fibrillar structure of the dentinal matrix too becomes visible only after demineralization. Two types of fibrils are mentioned in the literature. One of them is the collagen fibril with characteristic cross striations



Figs. 18a, b, c. Ground section of dentino-enamel junction. Note laminiform projections extending from dentine into enamel. Au-Al replica

at intervals of 650 Å, the other a much finer unstriated fibril. While the collagen fibrils of dentine have been demonstrated by numerous authors, the correlation between the thin fibrils devoid of structure and the typical striated collagen has not yet been elucidated. In attempting to solve the problem, it should be remembered that electron-microscopic examinations have revealed three forms of collagen fibril : first, the striated form with intervals of about 650 Å ; second, the LS-form with cross striations at about 2000 Å intervals ; third, the structureless collagen.

Bundles of extremely delicate fibrils with diameters between 100 and 200 Å and devoid of structure were found in our preparations (Figs. 14 and 15). Figs. 16 and 17 show various degrees of calcification ; the calcified zones of the dentine are well distinguishable from the fibrillar zones.

Photographs made of the dentino-enamel junction show enamel tufts projecting from the dentine into the enamel tissue (Figs. 18*a*, *b*, *c*).

Summary

Enamel tissue is composed of prisms. The latter consist of submicroscopic crystals held together by an organic substance which, arranged in the manner of a space grid anastomizes with the prism sheath. No interprismatic substance in the enamel was observed. The organic prism sheath calcifies in some cases, causing a lateral fusion of adjacent prisms.

Tomes' fibres in the dentinal tubules are assumed to be surrounded by a network of fibrils. Extremely fine fibrils with diameters from 100 to 200 Å were encountered in the dentinal matrix.

Many unsolved problems may be cleared by, and great facilities for the practice may be expected from, further electron-microscopic investigations.

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ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ ЭМАЛЕВОГО ВЕЩЕСТВА И ДЕНТИНА ЗДОРОВЫХ ЗУБЬЕВ

ДЬ. ХАЙОШШИ, Ш. КОХАРИ и К. БОНА

Эмалевая ткань состоит из призм. Призмы построены из субмикроскопических микрокристаллов. Между микрокристаллами имеется в качестве спаивающего межуточного вещества органическая сеть, располагающаяся в виде пространственной сети, которая анастомозует с влагалищами призм. В эмали авторам не удалось выявить межпризматического вещества. Органическое влагалище призм обызвествляется в виде чешуеобразных структур и призмы латерально сливаются.

Волокна Томса, находящиеся в дентинных каналцах, окружены предположительно фибриллярными волокнами. В ложе дентина наблюдаются весьма тонкие фибриллы диаметром в 100–200 Å.

Электронномикроскопическое исследование зубного вещества может выяснить еще много вопросов и послужить основой для решения ряда практических задач.

L'ÉMAIL ET LA DENTINE DE DENTS SAINES EXAMINÉS AU MICROSCOPE ÉLECTRONIQUE

GY. HAJÓSSI, S. KOHÁRI et K. BÓNA

Le tissu de l'émail est constitué par des prismes d'émail. Les prismes sont constitués par des microcristaux submicroscopiques. Il existe entre les microcristaux une matière organique servant de substance adhésive qui s'anastomose avec la gaîne prismatique. Cette matière est disposée dans l'espace sous forme de grilles, entre croisées. Nous n'avons pas mis en évidence de substance interprismatique dans l'émail. La gaîne prismatique organique se calcifie dans cette structure en écail de poisson et les prismes s'agglutinent latéralement.

On peut supposer que les fibres de Tomes qui se trouvent dans les canaux de la dentine sont entourées par des fibrilles. On trouve dans les matrices de la dentine des fibrilles très fines de 100—200Å de diamètre.

L'étude au microscope électronique du tissu dentaire est susceptible de résoudre de nombreux problèmes et servir de point de départ à la solution de questions d'ordre pratique.

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