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A SCANNING ELECTRON MICROSCOPIC STUDY ON THE DIFFERENCES BETWEEN NEWLY ERUPTED TEETH AND OLD TEETH WITH REFERENCE TO FISSURE ENAMEL SURFACES AND CONTENTS

BY

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ABSTRACT

Scanning electron microscopy of the fissure enamel surfaces of newly erupted permanent teeth and old sound teeth long-standing against caries demonstrated two distinct features; prismatic enamel and prismless enamel. Prismatic enamel was commonly found on the walls of fissures of newly erupted teeth and rarely found on the bases of fissures of old teeth. Prismless enamel was usually found on both sides of fissures of old teeth.

Various types of calcified contents were almost confined on the prismless enamel surfaces of the occlusal orifice of fissures of old teeth, while organic materials and bacterial contents were found on the prismatic enamel surfaces of newly erupted teeth.

INTRODUCTION

In fissure morphology, fissure carious lesion is generally considered to start early after eruption. Studies of fissure, therefore, have been concerned mainly with the relationship between fissure outline and its caries susceptibility[1–4].

It is known that the prismless enamel layer is most commonly observed on the smooth surfaces[5] and is rarely found on the occlusal surfaces[6]. However, there is no information about the surface layer on the walls of fissures.

Recently, reports have been published concerning the contents gathered both in artificial fissures implanted in human teeth[7] and in natural fissures[8–10]. These authors have not shown satisfactorily the interrelation between fissure enamel surfaces and fissure contents in relation to caries susceptibility.

The purpose of this investigation is to ascertain by scanning electron microscopy what variations exist on the fissure enamel surfaces and contents between newly erupted teeth and old teeth, and to consider what relation these variations may have to caries resistance.

MATERIALS AND METHODS

Ten clinically sound human permanent premolar and molar teeth, known to have been unerupted 6 months previously, were used. Most of them had been removed because of orthodontic reasons and partial impaction. Thirty caries-free, erupted, permanent human molars and premolars from teeth of 45–70 years old humans, removed for periodontal diseases and other reasons,

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were used.

The teeth were fixed in 5 % phosphate-buffered glutaraldehyde immediately after extraction. Shallow slots were made with a diamond disk in line with the fissures on the mesial, distal, buccal, lingual, or platal surfaces of the tooth crowns. The crowns of the teeth had been previously separated from the roots. A wedge-shaped instrument was pushed into the shallow slots to achieve this desired objective. The crowns were split to allow examination of the deepest areas of the fissures. Some teeth were treated with 5 % sodium hypochlorite to digest surface organic materials[11]. After this procedure, fissure surfaces were thoroughly rinsed in a stream of water. The specimens were dehydrated in graded ethanol series at room temperature and placed in absolute ethanol. This was followed by infiltration with isoamyl acetate. Specimens were dried from liquid CO_2 by the critical point methods.

Observations were made with JSM-50A scanning electron microscope operating at 15–25 kV. Microradiographs were prepared from several specimens using copper radiation exposed at 7 kV and 3 mA, and were produced on a Kodak spectroscopic film, 649–0.

Results

Newly erupted tooth group:

Scanning electron microscopy showed adherent organic materials in the contents of fissures of newly erupted teeth to be directly in contact with the walls of fissure surface. These organic materials showed that the areas of cellular features were degenerated and they measured 5 to 7 μm in diameter and often appeared crowded (Fig. 1). No differences were observed between the contents of fissures of premolars and those of fissures of molars.

The organic materials were removed by 5 % sodium hypochlorite and the fissure enamel surfaces were revealed clearly. The enamel surfaces on the fissure walls showed various patterns and the characteristic features present were prismatic enamel. Figure 2a shows rough areas and numerous markedly deep rod-end depressions on the surface of the developing enamel with overhanging walls. Figure 2b shows poorly marked rod-end depressions and some regions of deepest rod-end pits. Porous enamel surfaces were occasionally seen on the walls of third molar teeth (Fig. 2c).

On the base of natural fissure surfaces, all of the specimens showed deepest rod-end pits (Fig. 3a). These longitudinal fractured enamel surfaces revealed subsurface hollowed-out channels indicative of hypomineralized feature (Fig. 3b).

In some teeth in which microradiogram showed fissure enamel carious lesion (Fig. 4, inset), rod-end pits were often seen to form niches for bacterial accumulation (Fig. 4).

Old sound tooth group, long-standing against caries:

Scanning electron microscopy of longitudinal fractured surfaces of the fissures of old teeth, long-standing against caries, usually showed an absence of prism pattern in the outermost layer of the enamel. This prismless enamel layer was approximately 3 μm thick and formed an acute angle with periphery and a crystallite orientation was arranged almost perpendicular to the enamel (Fig. 5a). The wall of fissure was often barred by amorphous materials and slight carious lesions were found to occur bilaterally on the walls of fissure. This prismless surface layer on slight carious lesions (arrows) was wider than that of the adjacent sound prismless layer (Fig. 5b). In carious lesion of prismless layer, cuboidal crystals in size of 1 μm were found (Fig. 5c).

Observations on the fissure surface revealed no rod patterns by treatment with sodium hypochlorite and often the presence of calcified contents. These contents were of several types. Firstly, calcified spheroidal bodies were commonly located in the region of the orifice of the fissure (Fig. 6a, inset). In some cases, these contents showed large, hexagonal angulated crystals in association with irregular spherical bodies measuring up to 0.7 μm which were manifested by treatment with sodium hypochlorite (Fig. 6a). One side of these hexagonal crystals was about 20 μm in length. Irregular spherical bodies, in the present study, with regard to their size and morphology, may be said to be calcified bodies of microorganisms. Calcified contents of another type were often seen in the part of damaged enamel (Fig. 6b). These were many cuboidal crystals measuring approximately 1 μm in size similar to that of Figure 5c. Figure 6c shows a large mass of cuboidal crystals on the wall of the fissure. Also, in some specimens, fissures were totally filled with calcified contents which showed radioopacity microradiographically (Fig. 6d, inset). Scanning electron microscopy showed these contents to be needle-shaped crystals closely packed together and arranged in tufts (Fig. 6d).

The base of fissures often showed shallower depressions than those in the base of newly erupted teeth (Fig. 7a). Occasionally slight carious lesions were seen confined with amorphous materials. These longitudinal fractured enamel surfaces showed a lesion whose spread in the deeper enamel was limited. Mineral loss within sheaths of the prisms and the subsurface area was observed (Fig. 7b).

Discussion

Galil and Gwinnett[12] reported using a light and scanning electron microscope that the contents of the fissure of unerupted human teeth consisted mainly of ameloblasts and the cells constituting the enamel organ, and also that these cellular elements existed in a presumably viable state until or shortly after eruption, although the ultimate result would be uncertain. On the smooth surface of human teeth, Newman[13] showed degeneration of both ameloblasts and cells of the reduced enamel epithelium from the findings through a transmission electron microscope. This present scanning electron microscopic observations of the organic materials on fissure enamel surfaces of newly erupted teeth showed the areas of the cell features indicative of degeneration. These areas seem to be a pathway for decalcifying agents. It is of interest that Apostolopoulos and Buonocore[14] demonstrated a much greater solubility of dentin and bone when the organic constituents were removed by treatment with ethylenediamine.

A classical description of enamel fissure caries lesion was made by Bödecker[15] that it occurs at the base of the fissure. Mortimer[3,16], on the other hand, found that carious attack lesions were more often initiated from the sides of the fissure and also many centers of carious attack lesions were distributed along the fissures. He stated that Bödecker's case seemed to be a case of advanced lesion in which bilateral areas of attack had fused. It seems, therefore, that there are some risky factors to fissure enamel development for caries susceptibility. Crabb, in a series of papers[17–19], observed the areas of lower mineralized enamel and porous outer enamel in unerupted teeth. He also observed similar features in fissure areas and considered these features involved the risk of caries susceptibility and potentially easy carious attack. Awazawa[20], using an electron microscope, showed a hypomineralized enamel area at the base of the fissures in human adult teeth. By means of a scanning electron microscope, Boyde[11)] considered that the highest caries incidence on approximal surfaces just below the contact area might be correlated with the deepest rod-end pits which would form the best microstagnation area. The present study showed the features of prismatic enamel on both sides and bases of the fissure surfaces on newly erupted teeth and these features fitted in with caries susceptible areas shown by Boyde[11)] in approximal surface. Indeed, carious lesions in this study of newly erupted teeth showed that marked pits of the fissure enamel surface were more susceptible to bacteria than poor rod-end depressions.

Recent reports have shown a distinct prismless layer in the outermost enamel on smooth and occlusal surfaces, but not on fissure surface. This layer varies in thickness, the average of which is about 30 μm and is distributed unevenly over the enamel surface[6,21,22)]. It is lamellated parallel to the outer surface and crystallites are orientated approximately perpendicular to the surface, similar to that found in the present study on the fissure surface of old teeth[5,23)]. It is generally accepted that the prismless layer may possess certain characteristics related to caries. The present study indicated that usually there were prismless surface layers approximately 3 μm in thickness overlying the intact enamel surface, and also on slight subsurface carious lesions in fissure enamel surfaces of old teeth, long-standing against caries. Wider prismless surface on subsurface carious lesions than that of the adjacent sound layer may suggest the fact that remineralization occurs on the prismless layer during carious attack. Furthermore, the observation that prismless enamel predominated in old teeth suggested that this might be the result of the influence of posteruptive maturation changes and these changes might be related to caries resistance. Therefore, the prismless layer of the fissure enamel surface may possess caries resistance characteristics but these have to be investigated more in detail.

The examinations of contents in fissures by a scanning electron microscope have revealed interesting findings. Gwinnett and Buonocore[24)] found spheroidal bodies in aggregate clumps held together by a matrix on the fissure surface. Galil and Gwinnett[25)] reported two zones of light and heavy bacterial aggregation in fissure surface. The heavy plaques were usually located at the occlusal orifice of fissures which were often the site of initiation and spread of occlusal caries. These authors further described the phenomenon of the mineralization of fissure contents and showed the fissure contents to be totally mineralized from top to base with no sign of any microorganisms in about 2 % of the specimens[10)]. They, also, considered that it would seem that if fissure became totally mineralized they would be less susceptible to caries. The present observations on old teeth, long-standing against caries, revealed that the orifice in most fissures was almost barred by various types of calcified contents and in about 10 % of the specimens the fissure contents were totally calcified from top to base.

The study of Backer Dirks[26)] showed that natural caries process in an examination of of children did not progress in more than 50 % of the proximal surfaces that showed enamel lesions radiographically but a study of fissures could not furnish much information because of the too rapid progress of fissure caries process. The present scanning electron microscopic observation that some specimens showed resistant carious lesions both on prismless enamel of the fissure wall and on prismatic enamel of the fissure base in old teeth suggests following conjectures.

That is, prismless layer may block the diffusion channels leading deeper into the subsurface carious lesion and also the deposition of calcified materials in caries-attacked area, and calcified contents may have a favorable influence on caries resistance. This is also supported by the fact that old teeth often showed slighter carious lesions than those found by Mortimer and Tranter[27], which showed to be hollowed-out channel structure on fractured enamel surfaces in fissure caries examined by a scanning electron microscope.

In view of the above considerations, it would seem from the preventive standpoint that the fissure surface of newly erupted teeth involves potentially grave risk to caries and, therefore, it is popularly treated with fluoride gels, fissure sealants, and food restriction in immediate post-eruptive period. The addition of calcium and phosphate to oral environment[28] in relation to fissure contents and the investigative efforts of the laser on enamel surface[29,30], have been employed to increase resistance to caries. Further work of the laser is in progress on fissures in hope of increasing resistance in relation to enamel structure and contents of newly erupted teeth.

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Explanation of Figures

Fig. 1. Natural fissure surface of the wall of newly erupted tooth, showing organic material covering the enamel surface with cellular regions. ×1000
Inset shows high magnification of the areas of cellular features indicative of degeneration. ×1500

Fig. 2. The fissure enamel surface treated with sodium hypochlorite on the wall of newly erupted teeth.
- a) Rough areas on the surface of the developing enamel with overlapping walls. ×1000
- b) Poorly marked rod end depressions and some deepest rod end pits. ×1000
- c) Porous enamel surface. ×1000

Fig. 3. Natural fissure surface on the base of newly erupted tooth.
- a) Deepest rod end pits. ×3000
- b) Longitudinal fractured surface of figure 3a. Hollowed-out channels in subsurface enamel. ×3000

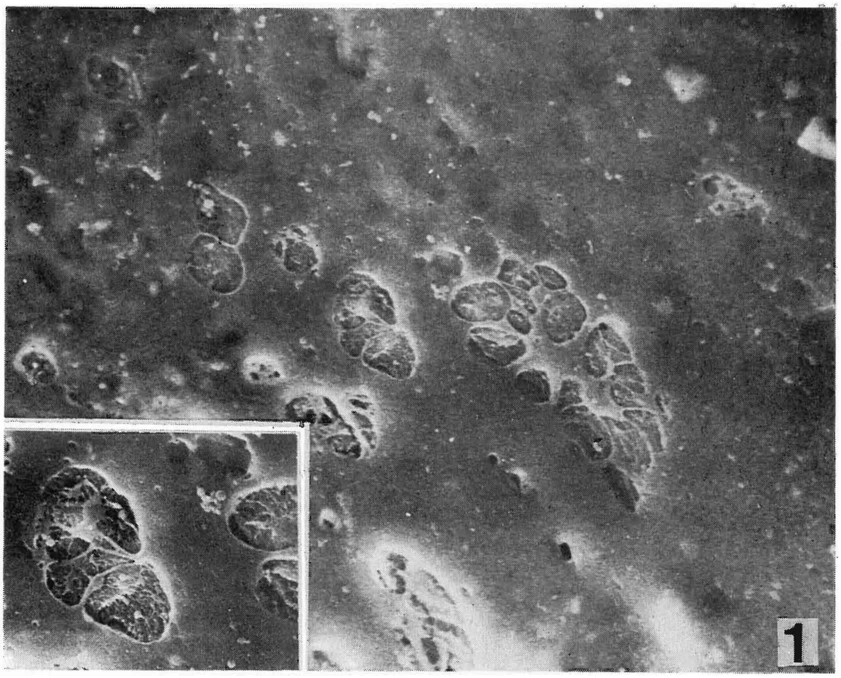
Fig. 4. The wall of fissure on carious lesion of newly erupted tooth. Rod end pits showing bacterial accumulation. ×3000
Inset shows enamel carious lesion microradiographically.

Fig. 5. Longitudinal fractured fissure enamel surface of old tooth, long-standing against caries, by treatment with sodium hypochlorite.
- a) Lack of prism patterns in the outermost 3 μm thick. ×3000
- b) The wall of fissure barred by amorphous materials and wider prismless enamel surface layer than adjacent sound layer on bilateral slight carious lesion. ×300
- c) High magnification of figure 5b. A small cuboidal crystal is seen in damaged area. ×3000

Fig. 6. Fissure enamel surface of long-standing erupted teeth against caries.
- a) Calcified contents treated with sodium hypochlorite. Large hexagonal angulated crystals of roundish bodies are seen. ×1000

Inset shows calcified contents located in the region of the orifice of the fissure.
- b) Distinct cuboidal crystals similar to that of Fig. 5c can be seen in damaged part. ×3000
- c) Large mass of cuboidal crystals. ×3000
- d) Needle-shaped crystals filled in the fissure. ×1000

Fig. 7. Natural fissure surface on the base of old teeth, long-standing against caries.
- a) Shallower depressions than those of newly erupted teeth. ×3000
- b) Slight carious lesion confined with amorphous materials. ×3000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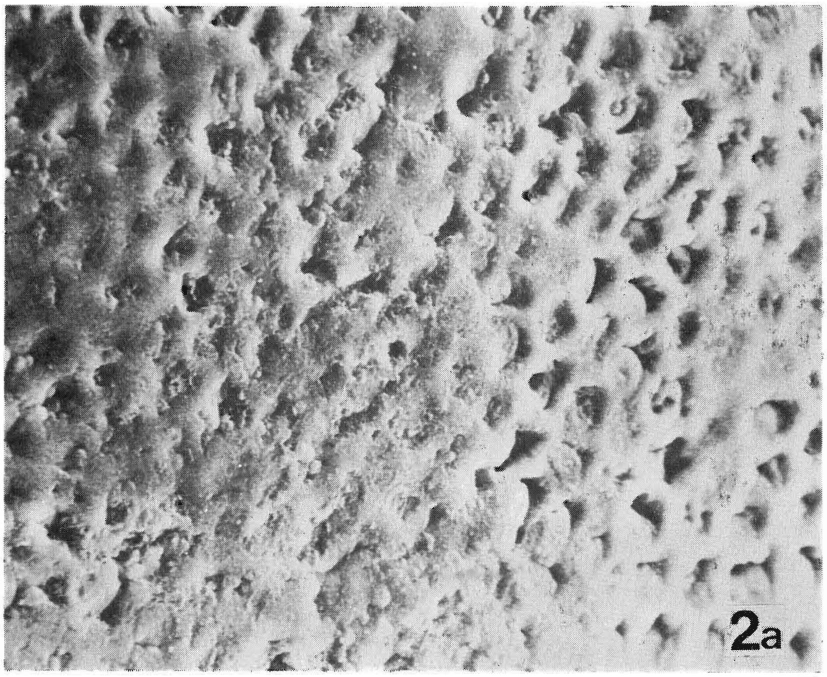
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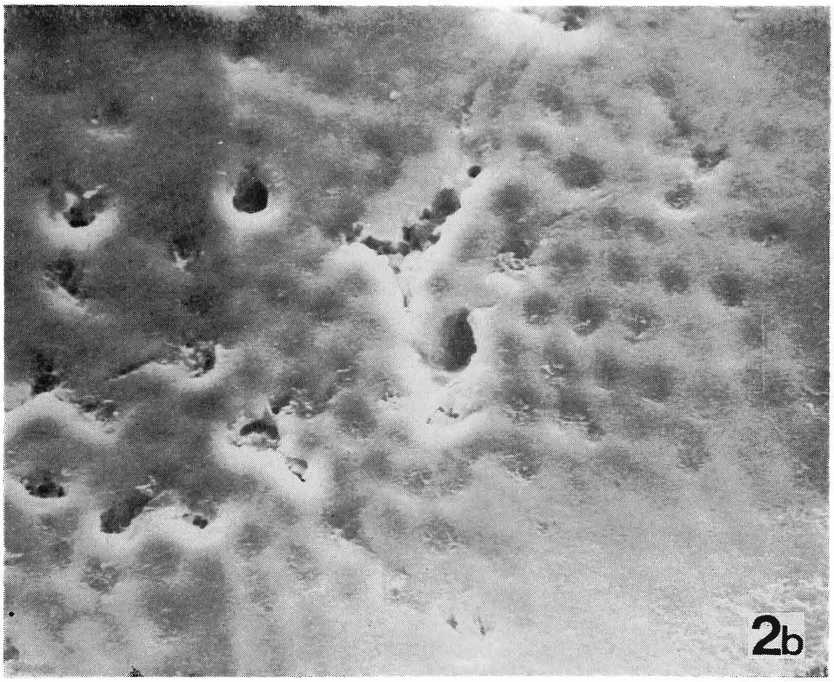
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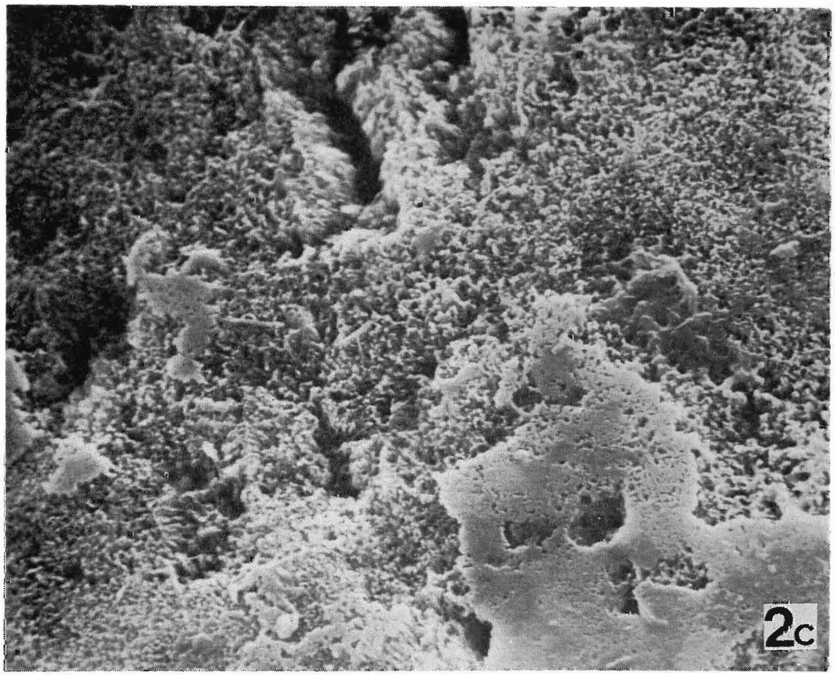
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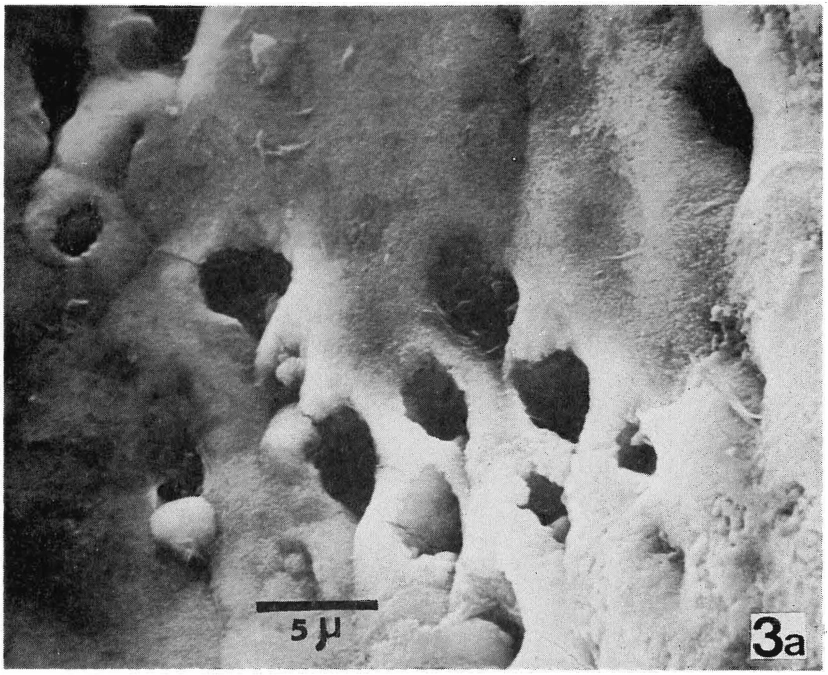
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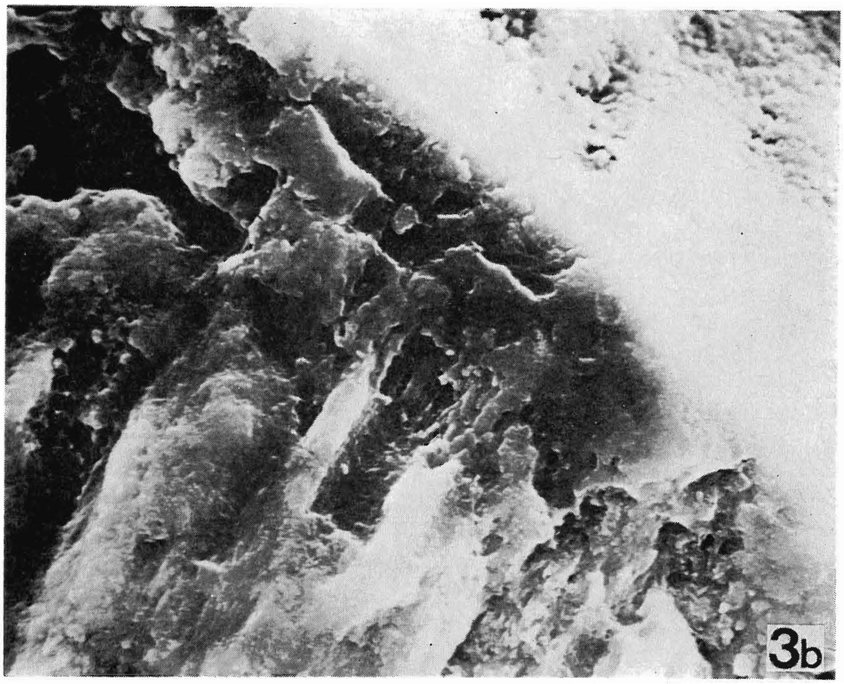
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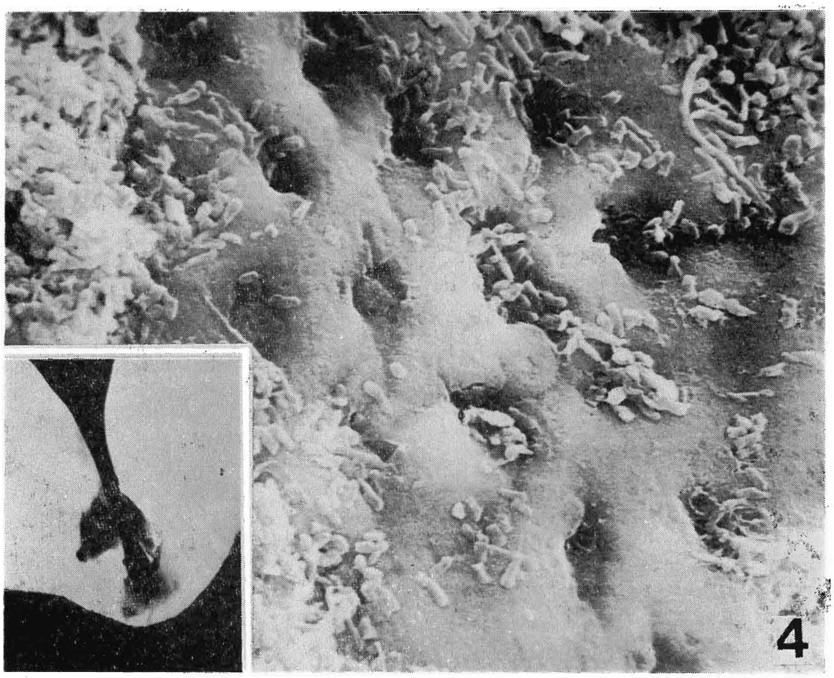

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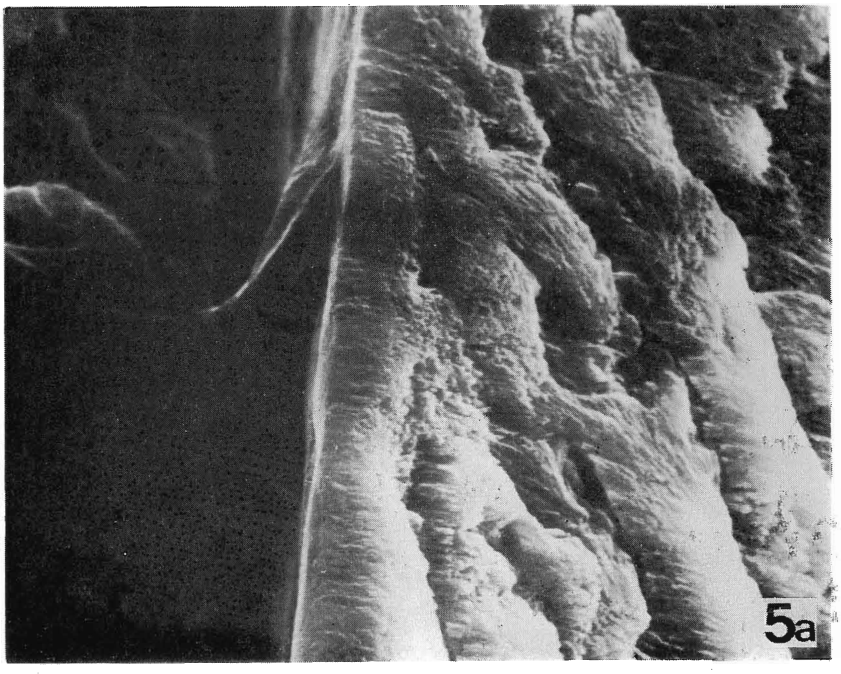

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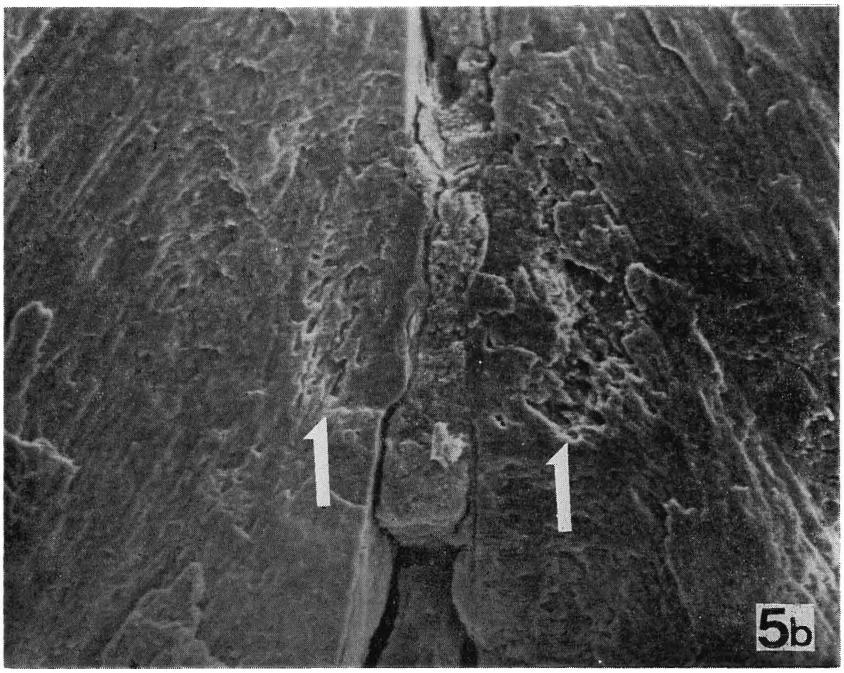
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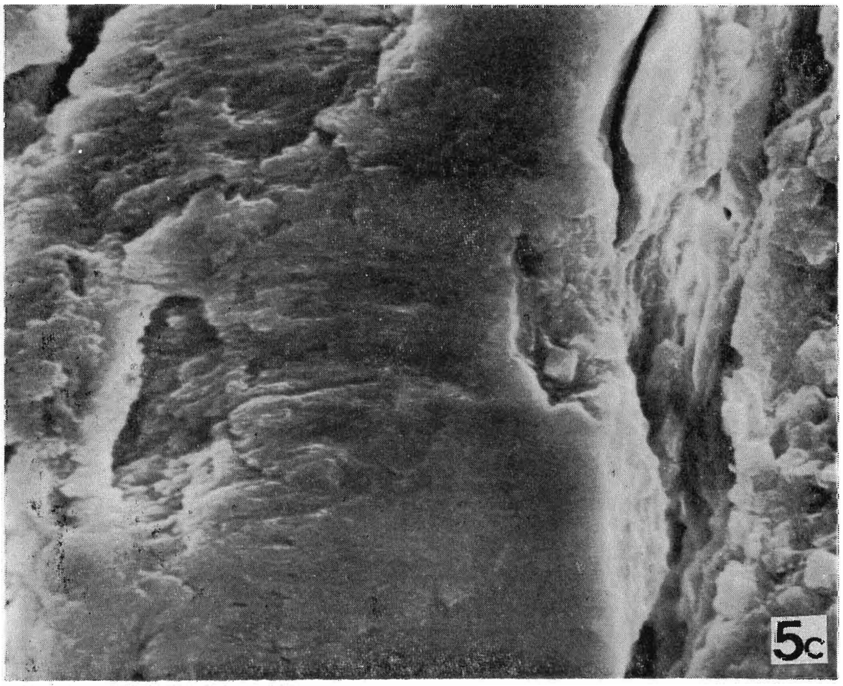
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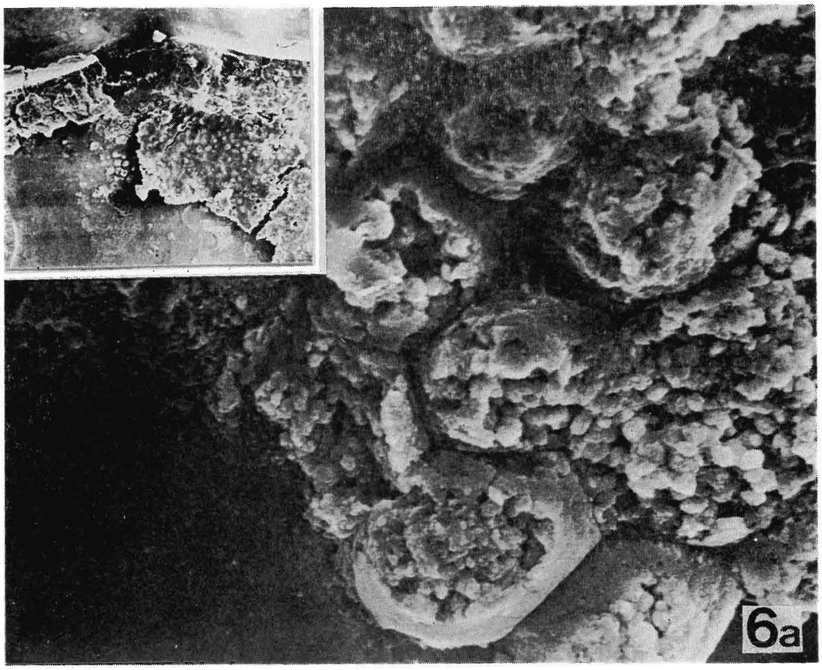
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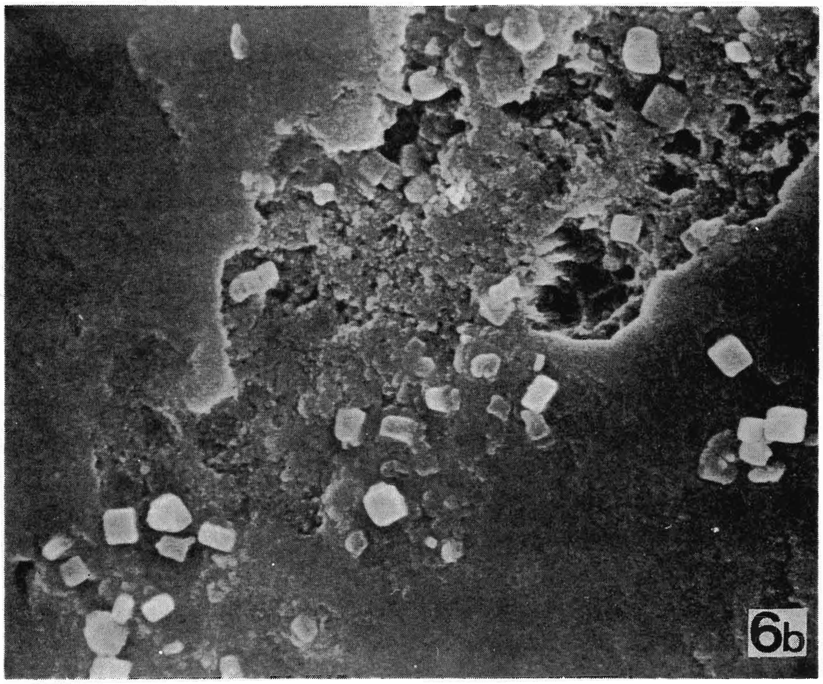
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