From the Department of Operative Dentistry,
Royal Dental School, Malmö, Sweden.

THE INCIPIENT CARIOUS LESION AS OBSERVED IN SHADOWED REPLICAS ('EN FACE PICTURES') AND GROUND SECTIONS ('PROFILE PICTURES') OF THE SAME TEETH

by

FRITZ MANNERBERG

There are still many problems to be solved before the pathology of the earliest stages of the carious process is understood. One of the most discussed questions is the mode of entry of the lesion through the surface zone. As is well known, the earliest changes of the enamel are clinically observed as white spots with lost transparency within the enamel. Clinical and histological observations of the initial lesion make it clear that this phenomenon occurs *within* the enamel while the surface zone apparently remains intact.

Ground sections observed with transmitted light show that in these early carious lesions of the enamel both the striae of Retzius and the cross-striation of the enamel rods become more pronounced. The striae of Retzius and the cross striation of the enamel rods are due to the mineralization pattern of the enamel and changes in their appearance in the early carious lesion emphasize a change in the degree of mineralization.

A similar change of the microscopical pattern can be caused by exposing intact teeth *in vitro* to mineral withdrawing agents (*Pigman & Sognnaes* 1955, *Hals et al.* 1956, *Soni & Brudevold* 1960).

Soni & Brudevold (1960) showed that a "softened" zone produced *in vitro* may penetrate as far as one thousand microns into the enamel and despite this demineralization, a thin zone along the tooth surface remained surprisingly intact. This reduced solubility of a thin superficial layer of the tooth might have several possible explanations. One is that a much higher fluoride concentration is found within the external enamel layer. (*Brudevold*, 1960). Another explanation of the resistance of the surface layer is said to be the concentration of organic material. According to *Schüle* (1960, 1961, 1962) the enamel surface is at all ages largely covered by a thin organic membrane. From the developmental aspect this enamel cuticle is subdivided into (a) the ameloblast cuticle, (b) the epithelial cuticle, and (c) the exogenous aquired pellicle. *Schüle* (1963) further demonstrated that the resistance of the enamel cuticle to cariogenic agents could be explained by its composition. The enamel cuticle of cellular origin probably consists of keratin which is highly resistant to acid.

The stage at which changes in the superficial layer of the tooth surface becomes histologically identifiable has not yet been precisely defined. *Keil* (1949) and *Darling* (1956) investigating ground sections found that the enamel cuticle over demineralized zones was unchanged. *Bhussry & Bibby* (1957) showed that the brown discoloration in teeth of elderly persons is partly due to changes in the organic matter of the surface enamel. According to *Gustafson* (1957) changes in the enamel cuticle do not occur before dissolution of the underlying enamel causes a breakdown in the organic matrix.

Almost all workers in the abundant literature on the pathological changes occurring during the earliest carious attack have utilised longitudinal and transverse sections of the enamel with different microscopical methods. These comprise incident, transmitted and polarised light, dark-field illumination, microradiography and microhardness tests.

It is important to answer the question of when an early lesion may be histologically observed as a change of the normal appearance of the tooth *surface*. This cannot be done satisfactorily with any of the previously described methods.

Using the replica technique, first described by *Wolf* (1940) and later improved by *Scott & Wyckoff* (1946) it should be possible to demonstrate the finest changes in the surface area in an early carious attack. *Scott & Wyckoff* (1949) described the appearance of the tooth surface in cases of carious lesions, when there was already a breakdown of the surface zone, and similar studies have been performed by *Takuma* (1955) using electron microscopy.

Sognnaes (1962) suggested that the surface replica technique should be combined with ground sections to study incipient carious lesions. Such comparative studies do not seem to have been published, but are surely of great importance with a view to obtaining a clearer understanding as to changes occurring in the surface area.

In the present investigation an attempt was made to estimate the depth of the incipient carious lesion as observed in ground sections in transmitted light ("profile pictures") and to compare this with the surface appearance of the same incipient carious zone as observed in shadowed replicas ("en face pictures").

MATERIAL AND TECHNIQUES

Twenty teeth were collected from the Orthodontic Department, Royal Dental School of Malmö, Sweden. The teeth were premolars which had been extracted for orthodontic reasons, the areas of interest being the proximal surfaces showing white spots. All the surfaces exhibiting an actual opaque area within the enamel, were hard to the probe and glossy and did not show any clinically observable surface breakdown. These teeth were assigned to Group I. Ten teeth from patients above 50 years of age were recieved from the Oral Surgery Department, Royal Dental School of Malmö. These teeth, mainly premolars, showed proximal surfaces with wide dark brown areas but no clinically observable loss of tooth substance due to caries. Some, however, had easily observable wide contact facets. Those teeth were assigned to Group II. Prior to any investigation each individual tooth surface was photographed using black and white film.

Replicas of the actual area were taken and placed on slides in the correct position. The cleaning of the tooth surface before the replica taking will be described later.

The following is a short description of the interpretation of the replica:

On inspection of the replica under the microscope alternating dark and light portions are seen. All parts facing the wire filament during shadowing are dark, all the others are light. The replica therefore gives a clear and detailed *image* of the relief of the tooth surface. The collodion film is always placed with the incisal or occlusal part towards the short edge of the slide and this end is placed towards the wire filament during shadowing.

If a replica is photographed and a copy made of the negative film, the copy will show the same picture as one sees on inspection of the replica in the microscope. *To facilitate interpretation, the photograph may be regarded as the tooth surface.* The photographic copy is placed so that *light* appears to fall into the picture antero-posteriorly from above the observer. In this way a furrow, for example, or a scratch in the tooth surface, will appear as a furrow to the examiner (see Fig. 17). In the present investigation all photographs and figures were placed in this way. The incisal or occlusal parts of the replica are thus always uppermost in the illustrations. For a complete description of the preparation, orientation and interpretation of replicas the reader is referred to *Mannerberg* (1960).

In some cases the outer border of the white spot was marked with an instrument (described by *Mannerberg,* 1960) in order to make it possible to orientate and observe microscopically on the replica the very area with the white spot and the adjacent area for any differences in appearance.

After the replica of the actual area had been taken, a ground section was made through the white spot area. Of half of the teeth in Group I and of all teeth in Group II transverse sections were made. Of the remaining teeth in Group I longitudinal sections were prepared.

Care was taken to prepare the section through the contact area if one existed.

All ground sections were embedded in Canada-balsam.

The replicas and ground sections were photographed at the same magnification. A scale was put on the photographs according to a method described by *Mannerberg* (1960). The "en face" and "profile" pictures of the same teeth were placed side by side and compared.

RESULTS

On the basis of the chart filled in for each tooth, the replica findings and ground section findings could be tabulated and compared from different points of view. The results of the comparisons are given in Table I and Figs. 5—26. Table I is based on thirteen teeth from Group I (Nos. 1, 2, 3, 4, 10, 11, 13, 14, 15, 22, 26, 27, 28) and three teeth from Group II (Nos. 5, 6, 9).

In the actual opaque area five types of surface appearance could be differentiated in the replica study.

Type I. The original tooth surface appearance with marked perikymata pattern and well observed prism ends. Fig. 1.

Type II. A tooth surface with marked perikymata pattern but with the prism end openings barely discernible or diffuse in small areas. Fig. 2.

Type III. A tooth surface with loss of the perikymata pattern and prism end openings in small distinct areas. In other parts no changes of the pattern were observed. Fig. 3.

Type IV. In some parts the replica showed no tooth structures but the surface had a granulated appearance. Fig. 4.

Type V. In the contact area the typical tooth surface structures were lost due to the mechanical wear, and the replica showed a flat surface with little differentiation. Fig. 5.

The different types of tooth surfaces will be discussed later.

It may be seen from Table I that six cases in Group I showed no change in the typical tooth surface appearance as observed on the replicas, namely Tooth No. 1, No. 10 (outside the contact area), No. 13, No. 22 (outside the contact area), No. 26 (outside the contact area), and No. 27 (outside the contact area).

The deepest penetration of changes in the enamel in these teeth as noted on the ground sections is 200, 200, 150, 250, 300, and 250 microns, respectively.

In five teeth from Group I (Nos. 2, 3, 4, 11, and 14) the prism end openings are diffuse in some parts, which may indicate that an alteration of the tooth surface structure has occurred. In these cases the deepest structural alterations in the histological sections reached 275, 400, 400, 500, and 500 microns, respectively.

Table I

Data on teeth examined by means of shadowed surface replicas and ground sections.

Group I Tooth No.	Clinical appearance of opaque area	Appearance of opaque area as observed on the replica	Appearance of opaque area as observed on ground section	Deepest penetration observed on ground section
1 (Figs. 6, 7)	Circular distinct opaque area, 1.5 mm in diameter	Perikymata pattern without changes, prism end openings clearly visible. *Type I surface*	Transverse section Zone I	200 microns
2	Circular distinct opaque area, 2 mm in diameter	Perikymata pattern without changes, prism end openings in some parts barely discernible. *Type II surface*	Transverse section Zones I, II, III	275 microns
3	Oval distinct opaque area, 3 mm in length, 2 mm in height. Within opaque area contact area with transparent appearance	Outside contact area: Perikymata pattern without changes, prism end openings in some parts barely discernible. *Type II surface*. Within contact area: no perikymata pattern, no prism end openings. *Type V surface*	Transverse section Outside contact area: Zones I, II, III Within contact area: Zone I	Outside contact area: 400 micr. Within contact area: 200 micr.
4	Circular distinct opaque area, 2 mm in diameter	Perikymata pattern without changes, prism end openings in some parts only just discernible. *Type II surface*	Transverse section Zones I, II, III	400 microns
10	Oval opaque area with diffuse borders, 5 mm in length, 1.5 mm in height. Within opaque area contact area with transparent appearance	Outside contact area: Perikymata pattern without changes, prism end openings clearly visible. *Type I surface* Within contact area: No perikymata pattern, no prism end openings. *Type V surface*	Transverse section Outside contact area: Zone I Within contact area: Zone I	Outside contact area: 200 micr. Within contact area: 100 micr.

11 (Figs. 8, 9)	Kidney-shaped opaque area, 3 mm in length, 1.5 mm in height	Perikymata pattern without changes, prism end openings in some parts barely discernible. *Type II surface*	Transverse section Zones I, II, III	500 microns
13 (Figs. 10, 11)	Oval opaque area with diffuse borders, 5 mm in length, 1—2 mm in height	Perikymata pattern without changes, prism end openings clearly visible. *Type I surface*	Transverse section Zone I	150 microns
14 (Figs. 12, 13)	Oval opaque area with distinct borders, 5 mm in length, 1—2 mm in height. Within the opaque area a contact area with transparent appearance, 1 mm in diameter	Outside contact area: Perikymata pattern without changes, prism end openings in some parts barely discernible. *Type II surface* Within contact area: No perikymata pattern, no prism end openings. *Type V surface*	Transverse section Outside contact area: Zones I, II, III Within contact area: Zone I	Outside contact area: 500 micr. Within contact area: 150 micr.
15	Circular opaque area, 4 mm in diameter. Within the opaque area a contact area with transparent appearance, 1 mm in diameter	Outside contact area: Occlusally to the contact area surface with no perikymata pattern and no prism end openings, but a granulated appearance. *Type IV surface* Cervically to the contact area, surface with no change in perikymata pattern, prism end openings in some parts barely discernible. *Type II surface* Buccally and lingually to the contact area perikymata pattern and prism end openings partly lost. *Type III surface* Within contact area: No perikymata pattern, no prism end openings. *Type V surface*	Transverse section Outside contact area: Zones I, II, III Within contact area: Zone I	Outside contact area: 600 micr. Within contact area: 250 micr.

Table I cont.

Group I Tooth No.	Clinical appearance of opaque area	Appearance of opaque area as observed on the replica	Appearance of opaque area as observed on ground section	Deepest penetration observed on ground section
22 (Figs. 16, 17)	Triangle-shaped opaque area, 5 mm in length, 1—3 mm in height. Within the opaque area a contact area with transparent appearance, 2 mm in diameter	Outside contact area: Disturbance in the development of the tooth, observed in the appearance of the perikymata pattern. No other change of the perikymata pattern, prism end openings clearly visible. *Type I surface*	Longitudinal section Outside contact area: Zones I, II, III	Outside contact area: 250 micr.
		Within contact area: No perikymata pattern, no prism end openings. *Type V surface*	Within contact area: Zone I The contact area is not seen in Figs. 16 and 17	Within contact area: 100 micr.
26 (Figs. 18, 19)	Triangle-shaped opaque area, 7 mm in length, 1—3 mm in height. Within opaque area a contact area with transparent appearance, 1.5 mm in diameter	Outside contact area: Perikymata pattern without any changes; prism end openings clearly visible. *Type I surface*	Longitudinal section Outside contact area: Zone I	Outside contact area: 100 micr.
		Within contact area: *Loss of tooth substance*	Within contact area: Zones I, II, III	Within contact area: 300 micr.
27 (Figs. 20, 21)	Oval opaque area, 4 mm in length, 2 mm in height. Within opaque area a contact area with transparent appearance, 1.5 mm in diameter	Outside contact area: Cervically no change in perikymata pattern; prism end openings clearly visible. *Type I surface* Occlusally to the contact area surface with no perikymata pattern and no prism end openings, but a granulated appearance. *Type IV surface*	Longitudinal section Outside contact area: Zones I, II, III	Outside contact area: 300 micr.
		Within contact area: No perikymata pattern, no prism end openings. *Type V surface*	Within contact area: Zones I, II, III	Within contact area: 300 micr.

28 (Figs. 22, 23)	Oval opaque area, 3 mm in length, 2 mm in height. Within opaque area a contact area, 1 mm in diameter with transparent appearance. Within opaque area also small area with clinically observable loss of tooth substance, 1 mm in diameter, cervicolingually to the contact area	Outside contact area: Loss of perikymata pattern and prism end openings in several places. *Type III surface* Within contact area: No perikymata pattern, no prism end openings. *Type V surface* Not possible to take replica of the area with loss of tooth substance	Longitudinal section Outside contact area: Zones I, II, III Within contact area: Zone I	Outside contact area: 300 micr. Within contact area: 100 micr.
Group II 5 (Figs. 24, 25)	Circular dark brown area, 6 mm in diameter	In some parts of the area a discernible perikymata pattern, but no prism end openings visible. In some parts a granulated appearance. *Type IV surface*	Transverse section No clear division into zones	1000 microns
6	Oval brown area, 4 mm in length, 2 mm in height	No perikymata pattern and no prism end openings visible, but a granulated appearance. *Type IV surface*	Transverse section No clear division into zones	1000 microns
9 (Fig. 26)	Triangle-shaped dark brown area, the base cervically 6 mm, the height 3 mm. Within and partly outside this area, circular contact area, 3 mm in diameter	No perikymata pattern and no prism end openings visible in the area, but a granulated appearance. *Type IV surface*	Transverse section No clear division into zones	1000 microns

Finally, in two teeth from Group I, viz. No. 15 (facially and lingually to the contact area) and No. 28 (outside the contact area) the perikymata pattern and prism end openings had wholly or partly disappeared. The deepest alterations in the histological sections were 600 and 500 microns, respectively.

In the three teeth in Group II no prism end openings were visible according to the replicas. The perikymata pattern was seen in some parts and in some parts a type of tooth surface referred to as Type IV. In all these cases the alterations in the histological sections had penetrated down to 1000 microns. The Type IV surface was also observed in two teeth in Group I, viz. No. 15 and No. 27.

The results show that in teeth from young people with an opaque area, the first change in the appearance of the tooth surface was observed on the replica when the demineralized zone was 275—500 microns deep, as measured from the ground section of the same part of the tooth. In three teeth (from elderly people) with brown discoloured areas (but with no clinically observed loss of tooth substance due to caries) the typical surface structures were usually lost and the alterations were 1000 microns deep.

DISCUSSION

Due to technical difficulties in replica taking and the production of ground sections only 16 teeth out of 30 were finally accepted for tabulation.

Replicas will often show folds due to the curvatures of the tooth surfaces. There is, however, no difficulty in identifying this type of artifact. Typical folds are seen in Figs. 6 c and 10 c.

Before the replica is taken the tooth surface must be carefully cleaned without causing mechanical damage. As soon as possible after extraction, the tooth is sprayed with distilled water and cleaned with a soft dental prophylaxis brush and 3 per cent hydrogen peroxide. The tooth is then sprayed again with distilled water and dried with compressed air.

Bergman has recently (1963) published an interesting report showing that enamel fluid may spontaneously appear on the surface of extracted teeth.

It might be presumed that the enamel fluid could penetrate the surface zone of the tooth and alter the surface appearance during the replica taking. The replica taking lasts about five minutes before which the surface, as mentioned, is completely dried with compressed air. No small droplets are observed on the replica after such a short time. The author has, however, observed similar droplets if the replica taking is prolonged.

Clinically, the simplest classification of caries is (1) incipient caries with an intact surface and (2) with a break in surface continuity. While the cavity is the most obvious manifestation of a carious lesion, deviations from the normal translucent tooth colour, viz. opaque areas, are an even earlier diagnostic guide. In teeth from elderly people such opaque areas are seldom seen but the enamel may aquire a dark brown colour in contrast to the yellowish colour of the sound enamel. It may be suggested that these dark brown areas are arrested carious lesions.

The replicas all showed proximal tooth surfaces without scratches of any kind, which means that the teeth had not been damaged by mechanical impact. Exceptions of course were the *contact areas* appearing on some of the teeth.

Different types of tooth surface

The term "perikymata" has been used in a way in the literature that may give rise to misinterpretation. It has been used by some authors (*Wolf,* 1940; *Orban,* 1954) to designate the ridges and by others (*Gustafson,* 1945; *Scott,* 1952; *A.-G. Gustafson,* 1959) to designate the grooves observed on the tooth surface between the ridges. When the term perikymata is used in this paper it refers to the ridges.

The perikymata pattern, i.e. the ridges, is especially well observed in Fig. 1 b. No definite structural details can be observed on the ridges. It seems possible that the original organic cuticle is especially thick in this part. In the grooves fine pits are seen. These pits vary in size and distribution from tooth to tooth and from surface to surface and may probably be regarded as breaks in the organic cuticle disclosing the underlying enamel prisms.

Type I

In this investigation, tooth surfaces without observable damage of prism end openings or perikymata pattern were called Type I surfaces (Fig. 1). Demineralisation of the enamel is a sequela of acid formation in the plaque material. The acid diffuses through the minute portals of the surface — probably the prism end openings — without damaging the original organic cuticle. In this way the enamel may be demineralised 200—300 microns deep, as is observed e.g. in Fig. 7 b, without damaging the normal tooth surface appearance as observed on the replica (Fig. 7 a).

It is probable that in cases showing Type I surfaces re-mineralisation or rather re-calcification of the inner parts of the enamel may occur through diffusion from the saliva which is supersaturated with calcium salts.

This may occur under "favourable circumstances", favourable circumstances meaning that the surface is kept clean instead of being covered with plaque. In this way diffusion between the supersaturated saliva and the enamel fluid could result in a re-calcification instead of demineralisation. The composition of the enamel fluid will thus depend upon the conditions *on* the tooth surface, because the enamel fluid is in dynamic equilibrium with the saliva. This is the *Andresen* (1921) re-mineralisation theory. He found that under certain circumstances incipient caries may "heal".

Type II

The first sign of a change in the appearance of the tooth surface is manifest when the prism end openings are only just discernible. During the preparation for replica taking it was sometimes hard to remove the plaque from the fine details in the grooves. In limited areas the plaque material may be retained and interfere with the shadowing. It is also possible that *in vivo* the plaque material is more easily retained in the grooves and that the earliest surface alterations are to be found there.

A tooth surface which in some places showed areas with barely discernible prism end openings was called a Type II surface (Fig. 2). The fact that in all cases where Type II surfaces were observed the alterations in the enamel according to the ground sections had penetrated *deeper* than in case of Type I surfaces, might mean that discernible prism end openings is the first sign of a damaged organic cuticle possibly due to proteolytic processes.

Type III

When, according to the replica, the prism end openings and the perikymata pattern were lost in the actual area, the case was referred to as Type III. This is characterized by a breakdown of the surface zone and a loss of tooth substance including a real alteration of the tooth surface appearance as distinct from Type I and Type II surfaces. In the Type III cases the ground section showed histological enamel alterations to a depth of between 600 and 300 microns (Teeth Nos. 15 and 28, Figs. 15 and 23, respectively). It should be emphasized, however, that in these cases no changes could be observed in the outer profiles of the enamel in the ground sections even though the surface changes according to the replicas were well documented.

In the case of Type III surfaces it seems likely that a recalcification of the inner parts of the enamel might occur under similar circumstances as mentioned above under Type I surfaces, i.e., that the surfaces are kept clean making it possible for the super-saturated saliva to diffuse into the enamel and deposit calcium salts there.

One thing is clear, however. *The original tooth surface appearance will never be "repaired"* (restored) in Type III surfaces. The prism end openings and the perikymata pattern cannot be restored.

Type IV

The changes in tooth surfaces in cases of intensive *mechanical* wear and tear through a long sequence of years were demonstrated by *Mannerberg* (1960) and it was suggested that after mechanical injuries the tooth surface will be covered by a precipitation from the saliva.

Wolf & Neuwirt (1941) and *Mannerberg* (1962) also showed that after a serious *chemical* attack by acid, a precipitation occurs on the surface. This can be observed on replicas taken at intervals.

If a chemical attack resulting from bacterial activity in the dental plaque stops as a consequence of the surface being kept clean, it is possible that on a Type III surface a precipitation from the saliva may occur both *on* the tooth surface and *inside* the enamel.

Under such circumstances the author suggests that the term "tooth surface" is a more adequate name for the surface of the crown than "enamel surface".

The Type IV tooth surface (Fig. 4) has no prism end openings or perikymata pattern but a "granulated" appearance. The latter is probably typical of arrested caries and caused by a precipitation from the saliva as described above.

In this investigation such a surface appearance was found in all three cases in Group II (i.e. teeth from elderly people, Nos. 5 and 9 — Figs. 25 and 26 respectively — as well as in Tooth No. 6) and in two cases in Group I (Teeth Nos. 15 and 27 — Figs. 15 b and 21 a, respectively).

Type V

Finally, the surface observed in the contact area was classed as Type V (Fig. 5). The surface is rather flat without any structural detail except small mechanical scratches.

In the investigation of the histological sections, zones with different appearance can be observed. It is generally accepted that this differentiation reflects an uneven distribution of the mineral pattern. To what extent this uneven distribution reflects *de*-mineralisation or *re*-mineralisation in the zones is a matter for speculation. The three zones referred to in this study are the first three zones described by *Gustafson* (1957).

The first zone is transparent in transmitted light. The second zone is dark, and the third zone is transparent. It was suggested by *Nishimura* (1926) and by *Gustafson* (1957) that the increased solution of minerals, which have been initially dissolved within the enamel, later precipitate when the enamel fluid becomes supersaturated. This theory has been disagreed to by others (*Darling*, 1958, 1961) and should be the object for further investigation.

Where the ground sections passed through the contact area, zones I, II, and III were observed *outside* the contact area but *inside* the contact area only zone I was observed (especially well demonstrated in Figs. 12 and 14). The explanation of this state might be that due to mechanical wear, cleansing of the contact area was sufficient and the carious attack was thus prevented here while it proceeded in the surrounding area.

As may be seen from Table I and the illustrations, alterations in the structure of the tooth surface start earlier in cases of incipient caries than has been suggested. When the alterations in the enamel (according to the ground sections) were 275—300 microns deep, alterations were observed in the appearance of the prism end openings and perikymata patterns.

Sognnaes (1962) raises the interesting question whether or not caries susceptibility is related to some pre-existing inadequacies in microstructure stemming from incomplete development. As far as the present author has observed from the microscopical study of shadowed replicas, no special irregularities in the appearance of the tooth surface could be related to the incipient caries lesion. It is, however, possible that an electron microscopic investigation might reveal new information. It is also possible that enamel quality, i.e. the homogeneity of mineralisation, is of greater importance in caries susceptibility than variations in structure of the tooth surface.

SUMMARY

In this investigation the depth of the change in the mineralisation pattern was determined on ground sections from teeth with incipient carious lesions. Before the ground sections were produced replicas of the actual tooth surfaces were made.

Comparison of the ground section (profile picture) with the surface appearance (en face picture) of the same incipient carious lesion was then made. A series of thirteen teeth (from young people), called Group I, were compared in this way.

In six teeth with a change in mineralisation pattern extending

to a depth of 150—300 microns into the enamel, as measured on the ground sections, no changes of the normal tooth surface appearance were observed on the replicas of the actual regions.

The first sign indicating that an alteration of the surface appearance occurred was that the prism end openings were diffuse or only discernible in some areas. In these cases no change of the perikymata pattern could, however, be observed.

In five teeth such changes in surface appearance were observed. The change in mineralisation pattern in these cases extended 275—500 microns into the enamel as measured on the ground sections.

In two teeth the prism end openings and perikymata patterns had partly disappeared in the actual region, indicating that the normal tooth surface appearance was eliminated. The deepest alteration in the mineralisation pattern was 300 and 600 microns in these cases.

In another series of three teeth (from elderly people), called Group II, longitudinal sections were compared with the observations on replicas of wide, dark-brown areas, (apparently arrested caries, but without clinically observable loss of tooth substance). The ground sections revealed that the changes in mineralisation pattern were 1000 microns deep. The "granulated appearance" of the tooth surface in these cases was presumed to indicate that a precipitation of calcium salts from the supersaturated saliva had occurred giving the impression of "repair" of the surface.

The investigation shows that the incipient caries attack does not change the appearance of the tooth surface before the change in mineralisation pattern is more than 300 microns deep.

The explanation may be that there exists an organic cuticle more resistant to the acid and/or inorganic surface layer with the same property.

RÉSUMÉ

LÉSION DE LA CARIE INITIALE TELLE QU'ELLE APPARAÎT SUR LES RÉPLIQUES OMBRÉES (IMAGES DE FACE) ET SUR LES COUPES PAR USURE (IMAGES DE PROFIL) DES MÊMES DENTS

Dans cette étude, la profondeur des modifications de la minéralisation a été évaluée sur des coupes par usure de dents présentant des lésions de carie initiale. Avant les coupes par usure,

des répliques de la surface des dents elle-même avaient été préparées.

Des comparaisons entre les coupes par usure (images de profil) et l'aspect de la surface (images de face) des mêmes lésions de carie initiale ont alors été faites. Une série de treize dents (provenant de jeunes sujets), appelée groupe I, a été comparée de cette manière.

Dans six dents présentant une modification de la minéralisation atteignant, suivant les mesures faites sur les coupes par usure, 150—300 microns à l'intérieur de l'émail, aucune modification de l'aspect normal de la surface de la dent ne pouvait être observée sur la réplique de la région en question.

Le premier signe indiquant qu'une altération de l'apparence de la surface pouvait s'être produite était un aspect diffus de l'ouverture des extrémités des prismes, ou le fait que l'ouverture ne pouvait être discernée que dans certaines parties. Dans ces cas, il n'était cependant pas possible d'observer de modifications du dessin des périkymaties.

Dans cinq dents il fut possible d'observer de telles modifications de l'aspect de la surface. Les modifications de la minéralisation, dans ces cas, atteignaient 275—500 microns à l'intérieur de l'émail, suivant les mesures faites sur les coupes par usure.

Dans deux dents l'ouverture des extrémités des prismes et le dessin des périkymaties avaient en partie disparu dans la région considérée, l'aspect normal de la surface étant ainsi altéré. La modification de la minéralisation la plus profonde dans ces cas était à 300 et à 600 microns.

Dans une autre série de trois dents (provenant de sujets âgés), appelée groupe II, des coupes longitudinales ont été comparées avec les observations faites sur des répliques de larges zones brun foncé (carie arrêtée, apparemment, mais sans perte de substance dentaire observable à l'examen clinique). Les coupes par usure montraient que la modification de la minéralisation atteignait une profondeur de 1000 microns. L' "aspect grenu" de la surface de la dent dans ces cas semblait indiquer qu'une précipitation de sels de calcium s'était produite à partir de la salive sursaturée, donnant une sorte de "réparation" de la surface.

Cette étude montre que l'attaque de la carie initiale ne modifie pas l'aspect de la surface de la dent avant que la modification de la minéralisation ait atteint une profondeur de plus de 300 microns.

Ce fait peut s'expliquer par l'existence d'une cuticule organique plus résistante aux acides, ou d'une couche superficielle inorganique ayant les mêmes propriétés, ou bien par la présence simultanée des deux.

ZUSAMMENFASSUNG

FRÜHSTADIEN DER SCHMELZKARIES BEOBACHTET AN REPLIKATEN (EN FACE BILDER) UND SCHLIFFEN (PROFILBILDER) DERSELBEN ZÄHNE

Das erste Karieszeichen im Schmelz tritt auf, wenn er seine normale Transluzens verloren und ein kreideartiges Aussehen angenommen hat. Es ist eine klinische Erfahrung, dass keine Veränderung der Oberflächenstruktur des Zahnes in diesem Anfangsstadium zu geschehen scheint. Histologische Schliffe scheinen auch zu bestätigen, dass diese Veränderung im Mineralisationsmuster des Schmelzes *innerhalb* des Schmelzes geschieht, während die Oberfläche intakt verbleibt. Die interessante Frage, wie tief die Veränderung der Mineralisierung im Schmelz eingedrungen ist, bevor Veränderungen an der normalen Oberfläche des Zahnes histologisch beobachtet werden können, scheint nicht klar beantwortet zu sein und kann knapp eine zufriedenstellende Antwort alleine durch das Studium von Schliffen erhalten.

In dieser Untersuchung wurde die Tiefe der Veränderung des Mineralisierungsmusters im Schmelz an Schliffen von Zähnen mit typischen kreidigen Flecken gemessen. Bevor die Schliffe angefertigt wurden, wurde das Aussehen der aktuellen Zahnoberflächen mit der Replikatmethode registriert. Der histologische Schliff (Profilbild) und das Replikat (en face Bild) desselben Initialstadiums der Karies konnten dann zusammengestellt und verglichen werden. Eine Serie von 13 Zähnen (an jungen Individuen entfernt), Gruppe I benannt, konnten auf diese Weise verglichen werden.

An sechs Zähnen, wo die Veränderung im Mineralisierungsmuster des Schmelzes eine Tiefe von 130—300 μ, am Schliff gemessen, erreichte, konnte keine Veränderung der normalen Oberflächenstruktur des Zahnes am Replikatbild des aktuellen Gebietes beobachtet werden.

An den Replikaten von fünf anderen Zähnen konnten Veränderungen in der Oberflächenstruktur beobachtet werden. Das erste Zeichen einer Veränderung der Oberflächenstruktur war, dass die Prismaendöffnungen diffus oder nur erkennbar in gewissen Teilen des aktuellen Gebietes waren. In diesen Fällen konnte doch keine Veränderung im Perikymatamuster beobachtet werden. Veränderungen im Mineralisationsmuster erstreckten sich bei diesen bis 275—500 μ in den Schmelz hinein.

An Replikaten, genommen an zwei Zähnen, waren die Prismaendöffnungen und das Perikymatamuster im aktuellen Gebiet teilweise verschwunden, was darauf deutet, dass die normale Oberflächenstruktur des Zahnes zerstört war. Die Veränderung im Mineralisationsmuster war in diesen Fällen 300 und 600 μ tief.

An einer anderen Serie von drei Zähnen (von einem älteren Klientel), Gruppe II genannt, wurden die histologischen Schliffe und die Replikate an weitgestreckten braunen Gebieten (wahrscheinlich sogenannte "Kariesmarken", ohne klinisch wahrnehmbaren Verlust an Zahnsubstanz) verglichen. Die Schliffe zeigten, dass die Veränderung im Mineralisationsmuster in diesen Fällen 1 mm tief war. Es wird angenommen, dass das granulierte Aussehen der Zahnoberfläche in diesen Fällen auf eine "reparative" Ausfällung von Kalziumsalzen, von dem übersättigten Speichel auf die Zahnoberfläche hin, zurückzuführen ist.

Die Untersuchung zeigt, dass der Kariesangriff während seines Initialstadiums nicht die Oberflächenstruktur des Zahnes verändert, wenn die Veränderung im Mineralisationsmuster eine Tiefe von 300 μ nicht überschreitet.

Die Erklärung kann sein, dass der Schmelz von einer organischen Kuticula bekleidet ist, welche einer Säureeinwirkung besser standhält und/oder von einer anorganischen Schicht mit den gleichen Eigenschaften bedeckt ist.

REFERENCES

Andresen, V., 1921: Über Mineralisation und Remineralisation des Zahnschmelzes. Dtsch. Mschr. Zahnheilk. *39:* 97—122.

Bergman, G., 1963: Techniques for microscopic study of the enamel fluid in vitro. Odont. Revy *17:* 1—7.

Bhussry, B. R. & B. G. Bibby, 1957: Surface changes in enamel. J. dent. Res. *36:* 409—416.

Brudevold, F., 1960: Depth of penetration of fluoride in erupted teeth. J. dent. Res. *39:* 1118—1124.

Darling, A. I., 1956: Studies of the early lesion of enamel caries with transmitted light, polarised light and radiography. Part II. Brit. dent. J. *101:* 329—341.

—»— 1958: Studies of the early lesion of enamel caries with transmitted light and microradiography. Its nature, mode of spread, points of entry and its relation to enamel structure. Brit. dent. J. *105:* 119—135.

—»— 1961: The selective attack of caries on the dental enamel. Ann. roy. Coll. Surg. Engl. *29:* 354—369.

Gustafson, A.-G., 1959: A morphologic investigation of certain variations in the structure and mineralization of human dental enamel. Odont. T. *67:* 361—472.

Gustafson, G., 1945: *The structure of human dental enamel.* Odont. T. *53:* Suppl.

—»— 1957: The histopathology of caries of human dental enamel. Acta odont. scand. *15:* 13—55.

Hals, E. et al., 1956: Laktatbuffers virkning pa emaljen in vitro. Odont. T. *4:* 245—275.

Keil, A., 1949: Über die Initialstadien der Schmelzkaries und ihre Erzeugung in Kaubrei-Versuchen in vitro. Dtsch. zahnärztl. Z. *4:* 947—959.

Mannerberg, F., 1960: *Appearance of tooth surface as observed in shadowed replicas.* Odont. Revy *11:* Suppl. 6.

—»— 1962: Effect of lemon juice on different types of tooth surface. A replica study in vivo. Acta odont. scand. *20:* 153—164.

Nishimura, T., 1926: Histologische Untersuchungen über die Anfänge der Zahnkaries, speziell der Karies des Schmelzes. Schweiz. Mschr. Zahnheilk. *36:* 491—545.

Orban, B. J., 1957: *Oral Histology and Embryology.* C. V. Mosby, St. Louis.

Pigman, W. & R. F. Sognnaes, 1955: Histologic studies of carious-like lesions produced in the artificial mouth. Oral Surg. *8:* 530—538.

Schüle, H., 1960: Spontane Reparationsvorgänge an oberflächlichen Schmelzdefekten. Klin. Wschr. *38:* 733—743.

—»— 1961: Chemische Zusammensetzung und physikalische Eigenschaften des Schmelzoberhäutchen. Arch. oral Biol. Special supp. *4:* 40—49.

—»— 1962: *Das Schmelzoberhäutchen. Untersuchungen über die morphologischen, chemischen und physikalischen Eigenschaften.* Thieme, Stuttgart.

—»— 1963: The enamel cuticle in incipient caries: Morphological and biochemical investigations. Int. dent. J. *13:* 465—470.

Scott, D. B., 1952: Microscopic studies of dental tissues. II Optical microscopy of tooth surfaces. Oral Path. *8:* 638—645.

Scott, D. B. & R. W. G. Wyckoff, 1946 a: Shadowed replicas of tooth surfaces. Publ. Hlth Rep. (Wash.) *61:* 697—700.

—»— 1946 b: Typical structures on replicas of apparently intact tooth surfaces. Publ. Hlth Rep. (Wash.) *61:* 1397—1400.

—»— 1949 a: Metal shadowing for optical microscopy of certain tissues. Amer. J. clin. Path. *19:* 63—66.

—»— 1949 b: Studies of tooth surface structure by optical and electron microscopy. J. Amer. dent. Ass. *39:* 275—282.

Sognnaes, R. F., 1962: *Chemistry and Prevention of Dental Caries.* Charles C. Thomas, Springfield.

Soni, N. N. & F. Brudevold, 1960: Microradiographic and polarized light studies of artificially produced lesions. J. dent. Res. *39:* 233—240.

Takuma, S., 1955: The electron microscopy of the enamel surfaces of teeth under various abnormal conditions. J. dent. Res. *34:* 152—163.

Wolf, J., 1940: Plastische Histologie der Zahngewebe. Dtsch. Zahn-, Mund- u Kieferheilk. *7:* 265—284, 507—538, 678—690.

Wolf, J. & F. Neuwirt, 1941: Reparative Vorgänge an der Oberfläche natürlicher und künstlich hervorgerufener Schmelzdefekte. Dtsch. Zahn-, Mund- u Kieferheilk. *8:* 222—228, 349—382.

Address: *The Royal Dental School*
Malmö S
Sweden

(Plates overleaf)