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HUMAN DENTAL ENAMEL IN POLARIZED LIGHT AND CONTACT MICRO-RADIOGRAPHY

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INTRODUCTION

Some of the greatest advances in modern dentistry have been made in the field of caries prophylaxis. Many of these were developed as a result of new knowledge about the structure of enamel. This knowledge was gained by research with many different experimental methods. There has, however, been a lack of correlation between the results obtained with these varying techniques. Any new research method is often used first by technicians in a wide variety of unrelated fields. This has always been so in the field of histology and seems likely to continue. Eventually, however, each new method becomes an accepted technique and it is then that its results are compared with those obtained in other ways. Electron microscopy is a typical example of a technique which has developed in this manner.

This paper describes an attempt to evaluate results obtained with two methods of investigation, micro-radiography and examination by polarized light. Both were used primarily to study morphology but variations in mineralization were also considered. Since the theory underlying the use of polarized light has been dealt with by many authors including ourselves (*G. Gustafson,* 1945; *G. Gustafson,* 1957; *A.-G. Gustafson,* 1959), it will not be considered here. The theoretical background to microradiography has been adequately covered by previous workers (*Engström*, 1949; *Bergman & Engfeldt*, 1954; *Engfeldt, Bergman, & Hammarlund-Essler*, 1954; *Engström*, 1955; *Engström & Omnell*, 1958).

MATERIAL

The experimental material was composed of ground sections from extracted teeth. These included clinically "normal" teeth from Malmö and fluorosed teeth from Isparta in Turkey.

The carious teeth examined were all from Malmö. Etching experiments were also carried out on normal and fluorosed teeth (*Gustafson & Ata*, 1959). A separate investigation of the relationship between variations in the teeth and their age, origin etc., is being carried out at present. These factors will not, however, be considered here as they are of no importance in the present context.

Only enamel was studied. In order to keep the treatment to which it was subjected to an absolute minimum, the teeth were not embedded and sections were ground by hand. This makes the production of sections of even thickness very difficult but it was found that satisfactory sections could be obtained by grinding by hand on glass plates. Considerable care and experience with this method are, however, necessary if reliable results are to be obtained, and of course, all sections which are not of even thickness must be discarded. The ground sections varied in thickness between 30 and 50 microns. The thickness and evenness of the sections can readily be measured with a micrometer screw.

The thickness of some of the sections was also estimated by the "edge" method. After the investigation of the section was completed, it was embedded in a plastic material which was allowed to set hard. Then grinding was carried on through the section at right angles to the original plane of section. It was found that the estimated thickness determined with this method was always less than that found with the micrometer. Unfortunately, the "edge" method is destructive so it was only used as a check on the method of grinding and not for direct measurement of the strength of birefringence.

In the "edge" method, the thickness was measured with the Leitz Ultropak with an ocular micrometer calibrated from a micrometer slide. The measurements in one particular case (Fig. 1) were started 35 microns from the outer enamel surface and measurements were taken every 35 microns to the dentine-enamel junction. Five measurements were taken at each level and the figures given below (in microns) are the averages of these. The 16 points of measurement extend from the surface through 0.8 mm of enamel. The results starting from the outer surface, were: — 33.1, 32.5, 33.2, 33.4, 33.4, 33.4, 33.7, 34.2, 34.0, 34.2, 34.3, 33.4, 33.7, 32.6, 33.9, 33.4.

Even if there is a slight degree of unreliability in these measurements, they do show that this particular ground section was of sufficiently even thickness for the morphologic studies which were carried out. Different measurements may vary from the average thickness of the section by about 5 %. There are, of course, proportionately greater variations with extremely thin sections. *Thewlis* (1940) calculated that the greatest permissible variation was 10 microns. *Hammarlund-Essler* (1958) estimated the thickness of sections prepared by her method (embedding in a plastic) to lie between 73 and 83 microns.

These estimations of thickness refer to measurements extending inwards from points on the outer enamel surface. There are, however, greater variations in section thickness between enamel at the neck of the tooth and other parts of the crown. This is of no significance for this investigation but it must be remembered that valid comparisons between the birefringence and x-ray absorption of two areas at a distance from one another cannot be made on hand-ground sections. Comparisons between different teeth are, of course, equally unreliable.

INVESTIGATIONS WITH POLARIZED LIGHT

In a paper published in 1945, one of the present writers (G. G.) pointed out that all the methods of investigation which had been used to study tooth enamel were not completely reliable. This difficulty, particularly with the use of polarized light, was also dealt with by the other writer (A.-G. G.) in 1959. The following quotation is from this latter paper: — "A number of objections can be raised against investigations of ground sections of enamel in polarized light. The method must be used with great care and the possible errors borne in mind ... Even if the difficulties can be overcome, the results must be confirmed by other methods."

In the latter paper, the great variations existing in the structure of all enamel, even if it appears clinically sound, were also described. Most of the publications dealing with caries histopathology (including that of *G. Gustafson,* 1957) have failed to take proper account of these variations. It is possible, and indeed probable, that variations in morphology and mineralization can so affect the histopathology of caries that descriptions of the caries process failing to take account of these are inaccurate. It was for these reasons that the present investigations were planned. Changes in our ideas of the development, structure, and histopathology of enamel must be expected as new methods of investigation become available and particularly as their results can be compared with those obtained by the older methods.

Polarized light can be used in three different ways: — 1) for chemical analysis of certain substances, 2) for investigation of sub-microscopic structures, and 3) for morphologic studies where birefringent substances are incorporated into larger morphological units.

The present investigations belong entirely to the third of these groups and are dependent upon prior assessment by other methods of the way in which the sub-microscopic birefringent units are oriented in relation to certain structures which are visible in the light microscope.

The prime prerequisite for studies of tooth enamel in polarized light is that the individual sub-microscopic crystals lie in the long axis of the prism. Virtually all those who have studied this problem are agreed that this is the case, at least within the limits which are of importance in morphological studies of enamel (see *A.-G. Gustafson,* 1959).

There is, to date, no experimental or other evidence to suggest that, in disturbances of mineralization, the sub-microscopic crystals become orientated differently from the normal with their long axes mainly parallel to the long axis of the prism. Since, however, there is a theoretical possibility it has to be considered.

A variety of imbibing media were used for the investigations in polarized light. These give varying appearances which depend on their effect on the form-birefringence of the enamel. The most detailed picture of the morphology of non-carious enamel is obtained after imbibition with Canada-balsam since the refractive index of the latter is as close as possible to that of enamel so that refractive phenomena at the junction between the ground section and the imbibing medium are avoided.

Canada-balsam is, however, soluble in xylol so that if the enamel is subsequently immersed in xylol, reduction in the form birefringence and a marked increase in the intrinsic birefringence from the crystals take place. This gives an appearance of increased mineralization in some areas.

In order to retain the advantages of Canada-balsam for morphological study and yet avoid the disadvantages of xylol, *A.-G. Gustafson* (1959) dried the sections in air and then embedded them in very thick Canada-balsam. A comparison between the results with polarized light and those with micro-radiography was described in that paper and it was found that, as far as Retzius lines and areas of hypo-mineralization were concerned, there was no change in the amount or type of birefringence. The same type of comparison was made in greater detail in the present investigation and was also extended to include carious enamel.

The polarized light studies were carried out with a Leitz Ortholux microscope with polarizing attachments and with a Leitz Polarlux. The black and white photographs were taken on Adox 14 and 17 Din films and the colour photographs on Kodak Ektachrome.

MICRO-RADIOGRAPHIC INVESTIGATIONS

The micro-radiographs were made with a Philips X-Ray Diffraction Apparatus Type PW 1009 (copper target) using a target-film distance of about 25 cm. Kilovoltages varying from 10 kV. to 35 kV. were used with the corresponding milliamperage. The best results were obtained with 12 kV. and with long exposures of from 20 minutes to 3 hours. The resultant micro-radiographs were then photographed with an Ortholux microscope using periplan objectives and a Hasselblad camera with Adox 14 and

17 Din films. Direct comparison between the micro-radiographs and the appearances in polarized light was possible with the Leitz comparison attachment and two Ortholux microscopes.

The way in which the ground section is pressed against the photographic plate affects to a considerable extent the quality of the micro-radiograph. Most workers recommend that the section be pressed against the plate with cellophane which is wound round both section and slide. This method did not prove entirely satisfactory since, as the cellophane band only presses on the sides of the section, its central part is not pressed against the plate. A new technique was therefore developed in which the area being examined was pressed firmly against the plate by a rubber cushion on the end of two metal springs. The details can be seen in Fig. 3. The rubber cushion (ordinary pencil eraser) has a hole in its middle through which the rays can pass to the part of the section which is being photographed. A number of cushions with holes of different diameters can be used to produce photographs of areas of different size and these all gave an adequate degree of pressure on the sections without damaging them in any way. The parts of the plate surrounding the exposed area must be painted dense black so that, when it is examined in the microscope, no light is refracted into the objective.

Because of the great differences in radiodensity is it very difficult to obtain a radiograph showing details both of a carious lesion and of the surrounding normal enamel. In most published micro-radiographs of carious enamel, the adjacent non-carious enamel appears quite white. Yet many authors have formed definite opinions on the structure and mineralization of these inadequately exposed regions.

To obtain some degree of blackening of every part of a micro-radiograph, the proper amount of voltage, current, and exposure time must be calculated carefully. Variations in these factors can result in entirely different appearances of one particular area in different films.

If the total effect of voltage, current, and exposure time is just enough to produce x-rays which will penetrate the most highly mineralized part of the section, this can be taken as 100 units of x-rays. If less than 100 units of x-rays are emitted, they will not penetrate all parts of the section and the most highly mineralized areas will appear white and devoid of detail. The variations in the appearance of the resultant radiographs can be illustrated as in Fig. 5.

If the total quantity of x-rays is only enough to penetrate to line A, the film will only be dark in two small areas with the lowest radiodensity. If the quantity is slightly greater as in B, the dark areas will increase in size and, if it is as in C, a new dark area will appear between the two which first appeared. If the penetrating effect is still greater (as in D) most of the plate will be darkened by the x-rays and only one part will still remain white.

As was pointed out earlier, all the variations in mineral content of an area of enamel only become visible if the total quantity of x-rays is sufficient to penetrate every part of the section. Then, when the total quantity of x-rays equals or exceeds 100 units, all the variations in mineralization in the section should theoretically be visible. In this case, however, the less-mineralized areas may appear very dark and lack detail.

PRISM SHEATHS

The classical view of the structure of enamel is that there are accumulations of organic material at the periphery of the prisms, and these have been termed prism sheaths. The precise nature of these sheaths has been wrongly interpreted by a number of authors as something quite different in character from the surrounding enamel.

On the evidence of replicas of undecalcified sections of enamel *Helmcke* (1959), among others, doubted the existence of prism sheaths. He completely overlooked the results of earlier studies with the light microscope which demonstrated great variations in the appearance of prism sheaths and showed that the sheath often fails to extend round the whole of the periphery of the prism. Electron microscopic studies of decalcified material have shown structures corresponding to the classical picture of the prism sheaths (*Scott*, 1955; *Frank*, 1957).

More recent work has demonstrated that the only difference between the prism sheaths and the inner part of the prism is in the relative proportions of the component parts.

It is generally accepted now that the prism sheaths are composed of condensations of organic fibrils around the prisms. The name given to this structure is of no real importance but as the term prism sheath has been used for many years and the part of the enamel to which it refers is widely known, there seems little reason for adopting a new term.

Like the prism sheath, the interprismatic substance varies in both character and quantity. In some areas of enamel it may be completely absent while in others it is present in relatively great quantity (*G. Gustafson*, 1945).

Unfortunately, it is extremely difficult to distinguish between the prism sheath and the interprismatic substance and this has led to some confusion.

The presence of a band of low mineralization (a prism sheath and/or interprismatic substance) is a prerequisite if the prism itself is to be distinguishable on a micro-radiograph. Since many investigations have shown that individual prisms can be distinguished in micro-radiographs, it follows that these variations in mineralization must exist.

COMPARISON OF FINE DETAILS

Before any valid comparison of major structural features of enamel, such as Hunter-Schreger lines, Retzius lines and areas of hypo-mineralization, can be made, preliminary studies of the finer structural details is required to permit an evaluation of the degree of agreement between the results of the various methods of investigation; in this case polarized light and micro-radiography.

Figs. 2 and 4 are of the same area of enamel; one (Fig. 2) is taken in polarized light, the other (Fig. 4) is a micro-radiograph. Both were taken with exactly the same microscope arrangement.

Since the structural details under discussion are very small, the usual technics of labelling with arrows and letters are not suitable. Instead, the polarized light photograph was placed on top of the micro-radiograph and a number of holes were made with a pin through both. These holes were made to mark special features such as single prisms and small areas of low birefringence. When prints were made from the negatives, the holes appeared as light spots.

Although the photographs with the two methods were entirely different from each other in appearance, it is possible to distinguish particular areas in each. For instance the four parallel prisms marked by the pinholes can be seen to have a higher birefringence than their surroundings and also to have a higher degree of x-ray absorption. Note also the long band up to the right of the most highly birefringent prism. This band has both low birefringence and low x-ray absorption.

The other points also mark areas where corresponding appearances can be clearly seen.

With the technique described above it is thus possible to prepare ground sections which, when they are examined in polarized light, will give a variety of appearances corresponding to variations in mineralization. Therefore we can now pass on to consider larger structural features in which prisms are component parts.

RETZIUS LINES

Retzius lines are probably the most striking structural features of "normal" enamel, and they have been observed in micro-radiographs by a number of workers.

In an earlier morphological study (*A.-G. Gustafson*, 1959) it was pointed out that comparable results could be obtained by studying sections with polarized light and with micro-radiography, even if they were embedded in Canada-balsam. Retzius lines could either be more or less mineralized than the surrounding enamel. The lines which absorbed many x-rays were negatively birefringent while those which absorbed few x-rays were isotropic or occasionally positively birefringent. In 1959 these studies were carried out using a technique in which the section was not pressed against the x-ray plate as firmly as is desirable so that there was some lack of detail in the micro-radiographs. For this reason, the same investigations were repeated with our new and more accurate technique and the earlier conclusions were confirmed.

Well-mineralized areas appear in ordinary transmitted light to be more transparent than their surroundings. For this reason, comparative studies in transmitted light were also carried out.

Figures 6, 7, and 8 and Colour Plate *a* show an area of enamel with two prominent Retzius lines. These appear transparent in ordinary transmitted light (Fig. 6), negatively *birefringent in* polarized light (Fig. 7 and Colour Plate *a*) and they are less radiolucent than the surrounding enamel in the micro-radiograph (Fig. 8).

The photographs also show lines which are less transparent, isotropic, and more radiolucent than their surroundings. They thus confirm the earlier assumption that ground sections embedded in relatively thick Canada-balsam reveal variations in mineralization when they are examined in polarized light. The validity of these results has been confirmed by micro-radiography. This is demonstrated in Fig. 9 which shows a composite photograph of some Retzius lines. The upper half of the photograph shows a micro-radiograph and the lower the adjacent area in polarized light.

Retzius lines display a wide range of appearances in different teeth and even in different parts of the same tooth. Fig. 10 shows Retzius lines composed of narrow bands of very low mineralization. The lines can be traced across from the micro-radiograph on the left to the picture in polarized light on the right.

It is not always possible to demonstrate the correspondence between the two methods with a single photograph taken in polarized light. This is because the strength of the birefringence varies with the position of the prism in relation to the polarizing planes of the filters. The results can only be evaluated correctly if the area under investigation is placed at an angle of about 45° in the negative quadrants (see *A.-G. Gustafson*, 1959).

HUNTER-SCHREGER LINES

In 1935 *Hollender et al.* concluded that "the bands of Schreger are not merely some sort of optical manifestation of the angle of section of the individual enamel rods. It is far more probable that the difference in appearance by reflected light between different areas of the enamel is related to a difference in density of calcification which, in turn, may be the result of structural differences in the organic matrix itself". "The fact that the bands of Schreger can be observed by means of the grenz-ray suggested to us that the phenomenon may be of more fundamental importance than has hitherto been assumed."

Krüger & Rakuttis (1952) also found differences in the degree of darkening of micro-radiographs corresponding to the Hunter-Schreger lines. They attributed this phenomenon to variations in the course of the prisms such as are found in this part of the enamel. Thus the Hunter-Schreger lines show only relatively minor differences in mineralization from the adjacent enamel. These differences were considered to be due to variations in the quantity of prism sheaths depending on whether the groups of prisms lie in the same plane or lie perpendicular to each other.

Baud & Held (1956) also found Hunter-Schreger lines on micro-radiographs of enamel and believed that they were due to bands of enamel with different degrees of mineralization. They found support for this opinion in the increased staining with silver in the band showing the least absorption of x-rays.

Darling (1956) found on micro-radiographs that "differences were seen which appeared to correspond to the band of Schreger".

Own Observations

Where the Hunter-Schreger lines were very prominent in polarized light, they were also evident in micro-radiographs of the same area. The dark lines seen in polarized light (Fig. 12) correspond to dark lines in the micro-radiograph (lower part). The dark bands in polarized light consist of transversely cut prisms which show no birefringence in this position, apart from very slight birefringence from the prism sheaths. This means that the dark bands are those in which the prisms run perpendicular to the plane of the section and parallel to the x-rays.

Because of the way in which enamel is built up of prisms whose outher layer (prism sheath) is less mineralized than their centres, the degree of darkening of the micro-radiograph depends on whether the rays pass through the sides of the prism or via its long axes.

With the proportions shown in the diagram in Fig. 13 (left), in which the prism sheaths and interprismatic substance are combined, the relation between organic and inorganic materials is 0.25 if the rays pass through at right angles to the long axes of the prisms. The underlying photographic plate will then be uniformly darkened.

If, however, the prisms run at right angles to the plane of the section (to the right) and are thus more or less parallel to the x-rays, we get a varying degree of darkening depending on the relationship of organic to inorganic material. As can be seen from the diagram this relationship may vary considerably. Some of the rays may pass through inorganic material only (f) so that more of the rays are absorbed than if they had passed perpendicularly to the prisms. If, on the other hand, the rays pass through organic material only (e) there is little absorption and correspondingly great darkening of the film. A strength of current and an exposure time producing very slight darkening of the film where the rays meet the prisms transversely, would produce obvious darkening of the film where the rays pass down the long axes of the prisms.

It is thus evident that differences in the degree of darkening of parts of micro-radiographs corresponding to Hunter-Schreger bands may be due entirely to differences in the direction of the prisms in relation to the x-rays.

As has been demonstrated by many workers (e.g. *G. Gustafson*, 1945) the enamel tufts are interwoven with the Hunter-Schreger lines, since they run a wavy course in the long axis of the prism. Thus, the Hunter-Schreger lines may be affected by the tufts. Since the latter are less mineralized than the surrounding enamel they may cause some of the darkening of the micro-radiographs which has been attributed to the Hunter-Schreger lines.

In examining micro-radiographs of the inner half of the enamel, it is important that the morphology of the enamel in this region be borne in mind when the degree of mineralization is being assessed.

Baud & Held's (1956) observation that the dark bands in the micro-radiographs correspond to bands which stain more heavily with silver might be due to the silver nitrate penetrating the sheaths of the transversely cut prisms more extensively than those of the longitudinally cut prisms (*G. Gustafson,* 1945). Thus, the difference in staining need not be due to a greater content of organic material.

IRREGULAR HYPO-MINERALIZED AREAS

Irregular areas of enamel exhibiting positive birefringence were described by *Gustafson, Nyström & Stelling* (1947) in teeth from several members of a family, all of whom displayed hereditary amelogenesis imperfecta.

Gustafson & Kling (1949) studied the micro-hardness of enamel and observed that, "Areas of more or less pronounced hypo-calcification were also found in the thick enamel layers buccally and lingually and even incisally (occlusally). Especially in buccal or lingual direction there were areas within which mineralization was so low that, besides areas of pseudo-isotropy, areas with positive birefringence too could be found. In places with positive birefringence we know that the hardness values will be very low". These variations in mineralization in the enamel have been found to coincide with the Retzius lines and the tufts. There are, however, other less well-defined areas in enamel which exhibit variations in mineralization. *Darling* (1956) described how, "... a linear radiolucent zone was seen, situated in an area which appeared dark brown when the section was examined in transmitted light and which showed positive birefringence in polarized light". *Darling* (1956) also found "... irregular zones of hypo-calcification close to the amelodentinal junction" and "... hypo-calcified zones related to morphologic defects".

Allan (1959) prepared micro-radiographs and photo-micrographs taken in polarized light of the same hypo-mineralized areas and studied the effect of different fluids used for imbibition.

Hodson (1955) found "... almost complete agreement between the dark areas in the water-mounted sections and those observed on the x-ray negative after exposure to x-rays". The same sections, "... when cleared and mounted in the routine histological manner, became translucent".

Most authors claim that if sections of enamel are immersed in a clearing agent (such as Canada-balsam), the zones seen when the sections are examined in water, will disappear. *Keil* (*Schmidt & Keil*, 1958 and *Keil*, 1959) showed, however, fairly large areas of positive birefringence surrounded by areas of negative birefringence in sections embedded in Canada-balsam.

Canada-balsam embedded ground sections were previously used by the present authors to study variations in morphology and in mineralization of enamel (*A.-G. Gustafson*, 1959). Certain precautions were taken, however. The sections were dried after the final polishing and were embedded in very thick Canada-balsam. Studies on carious enamel (*G. Gustafson*, 1957) showed that, after a certain degree of decalcification has taken place, even thick Canada-balsam will penetrate the opened-up spaces in the enamel. It thus seems likely that thick balsam can also enter the spaces in hypo-mineralized enamel, and doubt has been cast on the validity of using Canada-balsam embedded sections for studies of the morphology and mineralization of enamel (*Carlström*, 1960, *Torell*, 1960). Comparative studies of balsam embedded sections using micro-radiography and polarized light have been carried out and will now be presented in greater detail.

An area of hypo-mineralized enamel photographed by polarized light with crossed Nicols and a first degree red compensator is shown in Colour Plate *b*. There is an area of positive birefringence (yellow) surrounded by an isotropic band (red) between the positive and the negatively birefringent enamel. A micro-radiograph of the same area (Fig. 11) shows that the area of positive birefringence is less radio-opaque than the "normal" enamel. There is thus correspondence between the results obtained with polarized light and with micro-radiography.

If the Canada-balsam, or the xylol which is its solvent, had penetrated into the enamel in this area of positive birefringence, it would have become negatively birefringent as the refractive index of the balsam is very near that of the enamel. The positive "form" birefringence would thus have been extinguished. In this specimen, however, the positively birefringent area has remained unchanged even after months of immersion in thick Canada-balsam.

In some ground sections there are also areas of high negative birefringence. If the section is relatively thick, these areas show up as a yellow colour of the second order as in Colour Plate *c* (if a first order red compensator is used). As they are negatively birefringent they must be highly mineralized. They can also be distinguished from the hypo-mineralized yellow (first order) areas as the latter are separated from the blue (second order) by an isotropic band (red of first order). There can thus be no confusion between hypo-mineralized and hyper-mineralized areas. Although both appear yellow, the hypo-mineralized areas exhibit positive birefringence while the hyper-mineralized areas are negatively birefringent.

More detailed examination reveals that, in addition to defective mineralization, the prisms in hypo-mineralized enamel follow an abnormal course. This gives rise to difficulty in interpretation when photographs taken in polarized light are compared with micro-radiographs of the same area. The strength of the birefringence is dependent upon the relation of the prisms to the axes of the polarizing filters, whereas the exposure of micro-radiographic films is unaffected by prism direction provided they run in a plane perpendicular to the x-rays. When a well-mineralized prism following an irregular course is examined in polarized light, different parts of the prism may appear blue, red, and yellow although all are equally well mineralized. The only method of comparing the results of this method with those of micro-radiography is to use a comparison microscope in which the section can be rotated while being examined in polarized light and simultaneously compared with the micro-radiograph.

Thus, a photo-micrograph such as that in Fig. 14 cannot be directly compared with its corresponding micro-radiograph (Fig. 15). The left part of Fig. 14 is a micro-radiograph while the right part was photographed in polarized light. The variations in the intensity of birefringence are due partly to variations in direction of the prisms and partly to variations in mineralization. Fig. 15 is a micro-radiograph which reveals the great variations in mineralization in this same region.

Carlström (1960) has cast doubt on the existence of hypomineralization. It must, of course, be realized that where variations do exist they can only be demonstrated if the correct voltage, current, and exposure time are used in the production of the micro-radiographs. With these, micro-radiographs like that in Fig. 16 can be produced, and this reveals great variations in radio-density both along the Retzius lines and in irregular areas. When the same section is examined in polarized light and rotated to the appropriate positions, the results correspond exactly to those obtained by micro-radiography.

Sometimes, a type of hypo-mineralization which is localized to the cemento-enamel junction can be demonstrated by micro-radiography (Fig. 17), in polarized light, and in transmitted light. This type is particularly common in fluorosed teeth.

Since Canada-balsam can penetrate some parts of enamel decalcified by caries, it is theoretically possible that even *thick* balsam can enter some of the "empty spaces" of hypo-mineralized enamel. More than 200 ground sections embedded in thick balsam were examined and in only 2 of these had the balsam penetrated the enamel causing the birefringence of the first zone to change from positive to negative. Both teeth from which the sections were prepared were from the same individual; they were contralateral upper first premolars. The area which had been penetrated by the balsam was of low radiodensity (Fig. 18) and the balsam could readily be dissolved out and then allowed to re-enter the same area.

Areas of positive birefringence in Canada-balsam embedded sections were thus usually found to have low radio-density. Control studies using other imbibing fluids with different powers of penetration and different refractive indices were carried out. These are, however, important only when the degree of mineralization is being investigated and are unnecessary in morphologic studies.

As was pointed out earlier, there is no single experimental method which can be used to investigate all the components of tooth enamel. Evidence has been put forward to show that ground sections embedded in Canada-balsam are suitable for studies of the morphology of enamel and of certain of the variations in mineralization. The great advantage of embedding sections in Canada-balsam is that the structural details are clearly visible. They are visible to a far greater extent than in sections embedded in water or watery media of low refractive index. This does not *mean* that Canada-balsam is the best medium for every type of investigation, since other media have their advantages. No one method or technique of investigation is universally superior to all others. The choice depends on the aim of the investigation.

THE CARIOUS LESION

Many investigators have tried to define distinct zones within carious lesions (see *G. Gustafson*, 1957). These zones can readily be seen in polarized light but their existence has been questioned by some workers because they could not find them in micro-radiographs. This criticism would be valid if demineralization was the only process involved in caries but there are, of course, others.

The advantage of dividing a carious lesion into zones is that particular changes can be localized to more or less well-defined areas. This is true even if there is merely continuous demineralization. Different parts of the enamel are not, however, dissolved at the same rate and the decalcifying substances must also have some effect on the organic matrix. The best method for defining the different zones within a carious lesion is the one which will give the most detailed information. Methods showing only one type of change provide useful confirmatory evidence and also give detailed information about certain areas. It is wrong to assert that one or other method is the best; each one is superior in certain respects and inferior in others. All possible sources of information should be fully utilized.

Zone 1

Round the edge of the carious lesion, a zone with increased negative birefringence has often (but not always) been observed (*G. Gustafson*, 1957). It was thought that this was a zone of hyper-mineralization in which mineral salts which had been dissolved out from the second zone had been precipitated. There may, however, be other explanations for this phenomenon. Some workers have denied the existence of this first zone as they could not find it in their micro-radiographs. They have, however, ignored the observation made by *G. Gustafson* (1957) that this zone is not always present since all lesions do not develop in the same way and at the same speed.

As was stressed earlier, certain conditions must be fulfilled before micro-radiographs can reveal all the variations in mineralization in a ground section. Many published micro-radiographs show large white areas devoid of detail. Since the first zone, if it exists, is situated in these under-exposed areas it is often overlooked. An attempt was therefore made in this investigation to ensure that all parts of the enamel including the non-carious areas were exposed sufficiently for some blackening of the film to show up.

Fig. 20 shows a micro-radiograph of a caries lesion on the buccal aspect of an upper first incisor. All parts of the film show some degree of blackening and, round the edge of the lesion there is a zone of varying width which appears lighter than other areas of the enamel. This zone corresponds to the zone of high negative birefringence around the lesion (Fig. 21 and Colour Plate *c*). It appears transparent in ordinary transmitted light. The white points in the photo-micrograph and the micro-radiograph are for ease of comparison. In the highly negative birefringent area there are also areas showing yellow birefringence of the second order.

Relatively radiopaque areas such as this are present around many, but not all, lesions. Fig. 19 shows an early lesion in the fissure of a premolar. A narrow radiopaque band can be seen at the junction of the lesion with the normal enamel.

This zone thus contains a greater amount of minerals than did the enamel before the caries attack. The most probable explanation of this phenomenon is that the minerals are first dissolved out in the lesion and then precipitated in the surrounding enamel; probably in prism sheaths and the interprismatic substance.

As was stressed earlier, a zone of increased negative birefringence is not present around all caries lesions (Fig. 22). Where it is absent there is, of course, no increased radiopacity in the micro-radiograph (Fig. 23).

Zone 2

The second zone is where the demineralization first starts. It is very difficult to define exactly, particularly in micro-radiographs where its appearance depends entirely on the amount of radiation. The size of the lesion appears to vary considerably with variations in exposure etc.; within certain limits, the greater the amount of radiation and consequent blackening of the film, the nearer does the size and form of the lesion correspond to what is seen with polarized light.

From the point of greatest radiopacity to the point of lowest radiopacity visible in the micro-radiograph (Fig. 20) there is a gradual and continual fall in mineral content. Also, the same areas show a gradual change from negative birefringence to isotropy (Fig. 21 and Colour Plate *c*). This change can be seen between the two points at the top left of Figs. 20 and 21. The micro-radiograph shows that the end of the isotropic area seen in polarized light corresponds to the end of the visible minerals. With reduced exposure, however, minerals can be demonstrated in the centre of the lesion.

The same can be seen in Figs. 24 and 25. The row of points runs along the edge of the isotropic area and corresponds to a sudden fall in radiopacity. Closer examination of these figures reveals that here too there is a continuous decrease in mineralization within the isotropic zone.

It seems logical to assume that all the isotropic enamel is due to demineralization. Normal enamel is impermeable to Canada-balsam so some demineralization must take place before changes become visible in polarized light.

The sections shown in these photo-micrographs were immersed in Canada-balsam and the interior of the lesions thus show negative birefringence although they contained less minerals than the parts of the enamel which appeared isotropic. This was demonstrated by using other imbibition media. The triangular area in Figs. 24 and 25 (marked by the six square points in line) appears light in the micro-radiograph although it is dark in the photo-micrograph taken in polarized light. This is because the dark appearance in polarized light is produced by an isotropic zone identical with that seen near the normal enamel.

The present authors have assumed that sections imbibed with Canada-balsam are suitable for morphologic studies of enamel. It is therefore interesting to study the effect of the balsam on the birefringence in carious enamel and to compare the results with those obtained with micro-radiography.

Figs. 26 and 27 show a carious lesion by polarized light and by micro-radiography. The right hand side of the lesion can be seen clearly in the micro-radiograph (Fig. 27), and appears partly isotropic and partly negatively birefringent when viewed by polarized light. There is no positive birefringence. Little can be seen in the micro-radiograph of the left hand side of this same lesion whereas, in polarized light, an area of positive birefringence is visible (Colour Plate *c*). If the Canada-balsam had penetrated this part there would have been no positive birefringence, only negative birefringence like that seen to the right.

This positive birefringence to the left is unaltered by prolonged immersion in Canada-balsam. This is probably because the "empty" spaces (i.e. devoid of crystallites) are not filled up by the balsam because the caries process has not progressed far enough to permit its access. If, however, the section was imbibed by a penetrating fluid the positive area turned over to negative (Colour Plate *f*).

In a previous investigation it was found that solution of the minerals started at the periphery of the prisms and proceeded towards their centres. Fluorosed enamel was also found to be demineralized from the periphery of the prisms when it was etched with acids (*Gustafson and Ata,* 1961). *Darling* (1958), however, observed that the centres of the prisms were devoid of minerals in the later stages of caries and concluded that demineralization started in the prism core.

In the present investigation, areas of carious enamel were found with some prisms having their centres unaffected and demineralization at their peripheries while others showed greater mineralization at the periphery than in the centre. This difference could be seen both with polarized light and with micro-radiography.

In Fig. 28, part of the lesion in Figs. 20 and 21 is shown in a comparison microscope. The left side of the figure is taken in polarized light and the right side by micro-radiography. Crossing the junction between the two parts is a well-marked Retzius line and inside it there is a white band of greater negative birefringence and greater radiopacity. Part of this area is shown in Fig. 29 in which the dark Retzius line dominates the picture. There are square dark areas corresponding to prism sections

(*A.-G. Gustafson*, 1959). On both sides of these there are prism sections surrounded by radiopaque lines; the centres of these sections being highly radiolucent. The corresponding picture is seen in polarized light with the fine radiopaque lines showing negative birefringence while the dark centres are isotropic.

Fig. 30 shows another part of the same lesion in which the prisms are being attacked from the sides.

If the prisms in Fig. 29 had been cut transversely, it would have appeared that demineralization was most advanced in the centres of the prisms. This is because the cross-striations of the prisms would not be visible. As, however, the cross-striations are as well-defined as are the sides of the prisms, demineralization cannot have started in the centre of the prisms. In that case there would have been some destruction of the cross-striations. We have no satisfactory explanation for this phenomenon.

Non-carious enamel does, however, exhibit so many structural variations (*A.-G. Gustafson*, 1959) that it seems likely that many of the differing pictures seen in carious enamel are due to pre-existant structural variations. There is need for much more study of "normal" enamel.

The various zones in the carious lesion show differing birefringence when viewed in polarized light. There is negative intrinsic birefringence from undissolved minerals, positive form-birefringence from "empty" spaces and positive intrinsic birefringence from the organic fibrils. In assessing the implications of these appearances the possible effect of the imbibing fluid must also be considered (*G. Gustafson*, 1957).

Changes are visible in polarized light before they can be seen in micro-radiographs. However, the greater the intensity of x-rays and consequent blackening of the film, the more do micro-radiographs resemble the picture seen in polarized light. Thus it can be accepted that examination by polarized light is a more sensitive method than is micro-radiography.

Zone 3

Outside the isotropic area (zone 2) in the lesion there is a gradual transition to negative birefringence. (The second zone may sometimes show positive birefringence). As has been pointed out previously (*G. Gustafson,* 1957) the centre of the lesion displays negative birefringence if the section has been imbibed with Canada-balsam. If the section is imbibed with alcohol, however, most of this central area can be seen to be extensively demineralized. In some lesions there are small areas which are not influenced by imbibition but which display negative birefringence whichever fluid is used. One of us has previously suggested (*G. Gustafson,* 1957) that these are areas of "pathologic calcification". This view is supported by the increase in micro-hardness which was found in some parts of these areas.

The existence of this third zone and, in particular, the view that it is more highly calcified, have been questioned by some workers. *Applebaum* (1938) believed that "... certain microradiographic studies cast doubt on the possibility of salivary remineralization of areas of incipient caries". *Bergman et al.* (1958) observed that: "In a few cases of more advanced caries, distinct zones were visible in the enamel. They were reminiscent of those observed by *G. Gustafson* (1957) at polarization microscopic examination".

The investigation which was referred to (*G. Gustafson,* 1957) was carried out without micro-radiography since the necessary equipment was not available. It therefore seemed advisable to re-examine this problem.

This third zone does not of course exist in all lesions and is indeed, something of an exception. As was pointed out in 1957: "The carious lesion does not, of course, develop at the same rate in all teeth. Where a carious lesion develops very rapidly, the different zones are not formed in the way just described. Sudden cavitation can take place without visible re-mineralization in zones one and three and without mineralization of the surface".

The third zone is also characterized by increased transparency to transmitted light. Theoretically, this can only be due to greater homogeneity. The latter can, however, arise in three different ways. There can be an increase in the amount of the original substance; in this case the minerals in the form of crystallites fill the "empty" spaces. Alternatively, these "spaces" can be filled with another material with the same refractive index as the enamel. The third possibility is that the organic fibrils swell and are pressed together so that the "spaces" are eliminated. This would take place if watery solutions were used as imbibing media.

We have found no evidence of any increased calcification in the third zone in our micro-radiographs. This probably means that there is no re-calcification although it is also possible that micro-radiography is too coarse a method to demonstrate small but real differences.

The increase in micro-hardness and in negative birefringence in this zone may be due to pre-existent variations in enamel structure. It is clear that certain of the variations in the lesion, particularly those along the Retzius lines, are not caused by caries but are developmental variations in enamel mineralization. Micro-hardness measurements across the prominent Retzius lines in Figs. 28 and 29 may show increased hardness.

Since there is no evidence of pathological calcification in this third zone in micro-radiographs, our previous theory is still "not proven". Because of these conflicting results a further study of this problem has been started using polarized light, micro-hardness tests, fluorescence microscopy and micro-radiography.

There are, however, certain indications that re-mineralization can take place within a carious lesion. Types of crystallites which are not found in "normal" enamel have been found in the lesion (*Torell*, 1955, *Sognnaes*, 1959). As *Mézl* (1959) has recently shown, these can only be found if re-mineralization has taken place.

Soni & Brudevold (1959) are undoubtedly correct in their observation that: "... the hardness of enamel is not necessarily related to its degree of mineralization". They also found that: "There is no doubt, therefore, that fluoride originating either from external sources or from enamel undergoing dissolution will deposit in residual carious enamel and that zones of increased hardness and increased resistance to decalcification may thus be produced".

If there is re-mineralization, the new crystallites are probably laid down along the axes of the organic fibrils.

There are thus many unsolved problems relating to enamel caries and it is clear that the structure of "normal" enamel, and its variations must be considered to a far greater extent than has hitherto been the case.

SUMMARY

The mineralization of fluorosed teeth and the histopathology of caries were investigated.

A comparison between the results obtained with polarized light and micro-radiography was made on ground sections.

For micro-radiography a new apparatus for pressing the ground section against the photographic plate was employed.

The effect of exposure on one and the same ground section was diagrammatically represented.

Complete agreement between photomicrographs taken in polarized light and micro-radiographs of the same area was demonstrated. In most cases, however, photographs in polarized light manifested greater detail than micro-radiographs. This is possibly due to changes in the enamel other than the amount of mineralization present.

Enamel structures in ground sections must be investigated in more than one way. In this study transmitted light, polarized light, and micro-radiography were employed.

Irregular low-mineralized areas can be demonstrated both by polarized light and with micro-radiography.

Although some Hunter-Schreger lines show up on the micro-radiographs as darker areas, there may be no differences in mineralization between different lines.

This effect is thought to be due to changes in direction of prisms, which allow the x-rays to penetrate more easily along and between the cross-cut prisms.

Micro-radiographs and photomicrographs taken in polarized light showed that there was an increase in mineralization in the first zone at the edge of the carious lesion.

It was not possible to demonstrate a higher radiodensity in the third zone (negative birefringence).

The importance of "normal" enamel structure for the development of caries is pointed out.

RÉSUMÉ

L'ÉMAIL DENTAIRE HUMAIN EN LUMIÈRE POLARISÉE ET EN MICRO-RADIOGRAPHIE DE CONTACT

Les auteurs ont étudié la calcification des dents fluorotiques et l'histopathologie de la carie.

Une comparaison entre les résultats obtenus en lumière polarisée et par microradiographie a été faite sur des coupes par usure.

Pour la microradiographie, un nouvel appareil destiné à maintenir la coupe par usure contre la plaque photographique a été utilisé.

L'effet de l'exposition sur une seule et même coupe par usure a été présenté sur un diagramme.

Des concordances totales entre les microphotographies en lumière polarisée et les microradiographies des mêmes régions ont été mises en évidence. Dans la plupart des cas, cependant, les photographies en lumière polarisée montraient plus de détails que les microradiographies. Il est possible que cela soit dû à des modifications de l'émail autres que le degré de calcification.

Les structures de l'émail dans les coupes par usure doivent être examinées de plusieurs façons différentes. Dans la présente étude, on a employé la lumière transmise, la lumière polarisée et la microradiographie.

Des régions irrégulières peu calcifiées ont peut-être mises en évidence tant par la lumière polarisée que par la microradiographie.

Bien que quelques lignes d'Hunter-Schreger apparaissent comme des régions plus sombres sur les microradiographies, il peut n'y avoir pas de différence de calcification entre différentes lignes.

On considère que cet effet est dû à des changements de direction des prismes, permettant aux rayons X de pénétrer plus facilement le long des prismes sectionnés transversalement et entre eux.

La microradiographie et la microphotographie en lumière polarisée de l'émail carié ont mises en évidence une augmentation de la calcification autour des surfaces cariées dans la première

zone. Le rapport entre l'aspect des microradiographies et l'aspect en lumière polarisé fait l'objet d'une discussion.

Il n'a pas été possible de mettre en évidence une radioopacité plus élevée dans la troisième zone (biréfringence négative).

Les auteurs soulignent l'importance de la structure "normale" de l'émail pour le développement de la carie.

ZUSAMMENFASSUNG

ZAHNSCHMELZ IM POLARISIERTEN LICHT UND IM MIKRORÖNTGEN

Die Mineralisierung fluorotischer Zähne und die Veränderungen des Schmelzes in Kariesbezirken wurden untersucht.

Die Untersuchung besteht hauptsächlich aus einem Vergleich von Resultaten, die durch Untersuchungen im polarisierten Licht und mittels Mikroröntgenaufnahmen erreicht wurden.

Um einen besseren Kontakt zwischen den photographischen Platten und den Dünnschliffen zu erhalten, wurde eine neuartige Apparatur angewandt.

Es wird an Hand eines Diagramms gezeigt, dass verschiedene Resultate je nach der Intensität der Bestrahlung gewonnen werden können.

Eine grosse Übereinstimmung zwischen den Resultaten, die einerseits mit polarisiertem Licht und andererseits mit Mikroröntgenaufnahmen gewonnen wurden, wurde festgestellt. In vielen Fällen zeigten aber die Mikrophotographien in polarisiertem Licht mehr Einzelheiten als die Mikroröntgenaufnahmen. Es wird deshalb angenommen, dass auch andere Veränderungen als Mineralisationsunterschiede im polarisierten Licht sichtbar werden.

Es wird hervorgehoben, dass für die Untersuchung von "normalem" und kariösem Schmelz immer mehr als nur eine Methode angewandt werden sollte.

Obgleich einige der Hunter-Schregerschen Streifen in den Mikroröntgenaufnahmen dunkler hervortreten als die Nachbarstreifen, wird angenommen, dass hier keine Unterschiede im Mineralisationsgrad vorliegen. Diese verschiedene Schwärzung kommt dadurch zustande, dass die Röntgenstrahlen diejenigen Teile des Schmelzes, in denen die Prismen quer getroffen sind, leichter durchdringen.

Bei der Schmelzkaries ist die erste sichtbare Veränderung eine stärkere Röntgenstrahlenabsorption gerade in dem Gebiet, in dem auch eine stärkere negative Doppelbrechung vorhanden ist. Es wird deshalb angenommen, dass die sogenannte erste Karieszone wirklich aus einem Niederschlag von vorher gelösten Mineralsalzen bestehen kann.

Dagegen ist es den Verfassern nicht gelungen eine stärkere Röntgenabsorption in der Gegend der dritten Karieszone festzustellen. Diese dritte Zone muss deshalb Gegenstand weiterer Untersuchungen werden.

Die Rolle der Variationen im "normalen" Schmelz für das spätere Aussehen des Kariesangriffes wird betont.

REFERENCES

Allan, J. H.: The polarized light appearances of human dental enamel. J. dent. Res. *38*, 60—66, 1959.

Andresen, V.: Physiological and artificial remineralization of enamel. Tidskr. Tandl. *25*, 79, 1923.

Applebaum, E.: Tissue changes in dental caries. J. Amer. dent. Ass. *25*, 1223—1235, 1938.

Applebaum, E.: Grenz ray studies of enamel matrix formation and calcification. J. dent. Res. *22*, 7—11, 1943.

Baud, C. A. & A. J. Held: Silberfärbung, Röntgenmikrographie und Mineralgehalt der Zahnhartgewebe. Dtsch. zahnärztl. Z. *11*, 309—314, 1956.

Bergman, G. & B. Engfeldt: Studies on mineralized dental tissues. II. Microradiography as a method for studying dental tissues and its application to the study of caries. Acta odont. scand. *12*, 99—132, 1954.

Bergman, G., E. Hammarlund-Essler & L. Lysell: Studies on mineralized dental tissues. XII. Microradiographic study of caries in deciduous teeth. Acta odont. scand. *16*, 113—126, 1958.

Carlström, D.: Polarisationsmikroskopi på tandemalj. Odont. Revy. *11*, 1—22, 1960.

Darling, A. I.: Studies of the early lesion of enamel caries with transmitted light, polarized light and radiography. Brit. dent. J. *101*, 289—297, and 329—341, 1956.

—»— Some observations on amelogenesis imperfecta and calcification of the dental enamel. Proc. R. Soc. Med. *49*, 759—765, 1956.

—»— Studies of the early lesion of enamel caries. Its nature, mode of spread, and points of entry. Brit. dent. J. *105*, 119—135, 1958.

Engfeldt, B., G. Bergman & E. Hammarlund-Essler: Studies on mineralized dental tissues. I. A microradiographic and autoradiographic investigation of teeth and tooth germs of normal dogs. Exp. Cell Res. *7*, 381—392, 1954.

Engström, A.: Microradiography. Acta radiol. Stockh. *31*, 503—521, 1949.

—»— Microradiography in biological and medical research. Medica Mundi. *1*, 119—128, 1955.

Engström, A. & K.-Å. Omnell: De hårda tandvävnadernas biofysik. Nordisk klinisk Odontologi. kap. 2, del III. Band 1, 1958.

Engström, A.: X-Ray microscopy. Mellors Analytical Cytology. Second Edition. 343—379, New York, 1959.

Frank, R. M.: Contributions à l'étude au microscope électronique des tissus calcifiés normaux et pathologiques. Thèse Médecine, Strasbourg, 1957. 1—97.

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

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

Gustafson, G., P. Nyström & Em. Stelling: Five cases of pronounced enamel hypocalcification within the same family. Odont. Tidskr. *55*, 183—207, 1947.

Gustafson, G. & Ö. Kling: Micro-hardness measurements in the human dental enamel. Odont. Tidskr. *56*, 24—70, 1949.

Gustafson, G.: The histopathology of caries of human dental enamel. With special reference to the division of the lesion into zones. Acta odont. scand. *15*, 13—55, 1957.

Gustafson, G. & P. Ata: Untersuchungen über die Säureresistenz der Fluorosezähne von Isparta in der Türkei. Dtsch. Zahn-, Mund- u. Kieferheilk. *31*, 109—124, 1959.

Helmcke, J.-G.: Eine Betrachtung über die Grenzen polarisationsmikroskopischer Methoden in der Zahnhistologie. Naturwissenschaften. *46*, 233—234, 1959.

—»— Kariesforschung mit dem Elektronenmikroskop. Fortschr. Med. *77*, 175—176, 1959.

—»— Untersuchung der Schmelzoberfläche menschlicher Zähne mit Interferenz- und Phasenkontrastmikroskopie sowie Licht- und Elektronen-Stereomikroskopie. II. Mikroskopie. *13*, 345—364, 1959.

Hodson, J. J.: Identification of hypocalcified and demineralized areas in sections of human enamel. Nature, Lond. *175*, 261—263, 1955.

Hollander, F., Ch. F. Bödecker, E. Applebaum & E. Saper: A study of the bands of Schreger by histological and grenz-ray methods. Dent. Cosmos. *77*, 12—20, 1935.

Keil, A.: Über mangelhafte Schmelzreifung mit Bezug auf Odontomzähne. Dtsch. zahnärztl. Z. *14*, 1235—1238, 1959.

Krüger, V. & G. Rakuttis: Das Röntgenbild der Hartsubstanzen des normalen und kariösen Zahnes. Dtsch. zahnärztl. Z. 7, 141—155, 1952.

Mézl, Z.: Histopathology of enamel caries. J. Canad. dent. Ass. *26*, 585—597, 1960.

Newborn, E. & F. Brudevold: Studies on the physical properties of fluorosed enamel — I. Microradiographic studies. Arch. oral Biol. 2, 15—20, 1960.

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

Schmidt, W. J. & A. Keil: Die gesunden und die erkrankten Zahn-Gewebe des Menschen und der Wirbeltiere im Polarisationsmikroskop. Carl Hanser Verlag. München, 1958.

Scott, D. B.: The electron microscopy of enamel and dentin. Ann. N. Y. Acad. Sci. *60*, 575—584. Art. 5, 1955.

Sognnaes, R. F.: Microradiographic observations on demineralization gradients in the pathogenesis of hard tissue destruction. Arch. oral Biol. *1*, 106—121, 1959.

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

Thewlis, J.: The structure of teeth as shown by x-ray examination. Special Report Series No. 238, Medical Research Council. K 411, London 1940.

Torell, P.: X-ray diffraction studies of substances from intact carious and acid treated dental enamel. Göteborgs Tandläkare-Sällskaps Årsbok. 65—72, 1955.

—»— Imbiberingsmedel vid polarisationsmikroskopisk bedömning av kariös och syrabehandlad tandemalj. Odont. Revy *11*, 23—31, 1960.

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