From: The Department of Physical Cell Research (Head: Professor ARNE ENGSTRÖM), Karolinska Institutet, Stockholm, and The Department of Dental Histopathology at The Royal School of Dentistry, Stockholm

STUDIES ON MINERALIZED DENTAL TISSUES[1]

II. Microradiography as a method for studying dental tissues and its application to the study of caries

by

GUNNAR BERGMAN

BENGT ENGFELDT

INTRODUCTION

Microscopic details in a tissue may be recorded on photographic emulsions by means of x-rays. Hitherto, the usual way of obtaining such a radiograph has been to place the object in close contact with a photographic emulsion and to expose with x-rays as nearly parallel as possible. The contrast in the radiograph is dependent on the variations in intensity of the emergent x-ray beam which in turn varies with the chemical composition of the object and on the properties of the x-rays, while the definition is largely decided by the geometric conditions.

The procedure for rendering microscopic details visible by means of x-rays was given the name *microradiography* by *Pierre Goby* (1913). He applied the method to the study of biological material. The preparation was radiographed in close contact with a finegrained photographic emulsion using as source of radiation an x-ray tube with a small anticathode to ensure good definition. In these early experiments magnifications of up to 25× of good quality were obtained.

This paper provides a survey of earlier work on the application of microradiographic methods to the dental tissues; the principles underlying the technique are stated and an account is given of recent advances in the field. To demonstrate the scope of modern methods and to illustrate the results that may be obtained, some problems on mineralization in normal and carious tissue of the teeth have been studied.

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SURVEY OF EARLIER WORK ON THE APPLICATION OF X-RAY ABSORPTION METHODS TO THE DENTAL TISSUES

Bödecker & Applebaum (1931) were probably the first to study the pathology of caries by x-ray absorption methods. X-rays were passed through ground sections on to a photographic film. The radiograph showed a light zone, the position of which corresponded to that part of the dentine lying beneath an occlusal lesion. This zone was interpreted as indicating precipitated calcium salts or fatty substance in the affected dentine. No minute description of the technique is given but it is evident that it did not permit study of the detail.

Heiwinkel, who published similar pictures in 1932, held that the light zone, that sometimes occurs beneath a carious lesion corresponds to the transparent zone in the dentine. She gives no details of the thickness of the sections or of the x-rays used. The radiographs, however, give the impression that the sections were not planoparallel.

In a paper from 1932 *Applebaum* publishes a radiograph of a ground section from a tooth with incipient caries. This enamel has a lower x-ray absorption than the surrounding healthy enamel.

These three investigations were of the nature of pioneer work. It is probable that the authors used thick ground sections, hard x-rays and ordinary coarse-grained dental x-ray film. As a result it was impossible to study the detail.

Improvements in the technique introduced by *Applebaum, Hollander & Bödecker* (1933) rendered possible the examination of finer structures. These workers used planoparallel ground sections 150 to 200 μ in thickness, soft x-rays at a potential difference down to 5 kilovolts (grenz rays), and finegrained emulsion. In some of the radiographs the striae of Retzius, Owen's lines and Schreger's bands stand out, suggesting that these structures may be due to variations in the degree of mineralization in the hard tissues of the teeth. They found a dentine zone of increased x-ray absorption beneath the carious lesion, and confirmed the earlier discovery that incipient caries has a lower absorption than normal enamel. *Hollander et al.* (1935) verified the suggestion that Schreger's bands may be due

to variation in mineralization. Ground sections of 100—130 μ were used.

Up till then no attempt had been made at a quantitative determination of x-ray absorption in ground sections of the teeth. In papers by *Van Huysen et al.* (1933) and *Warren et al.* (1934), however, a method is described for carrying out quantitative measurements of the differences in absorption between different parts of the dentine of one tooth or between the dentines of different teeth. The teeth were ground planoparallel to a thickness of about 1 mm. The reference system used was a stepped penetrometer of aluminium that has roughly the same x-ray absorption as dentine. The sections and the reference system were placed side by side on the film and exposures made with a Coolidge tube at 30 kV and 30 mA. The distance between the tube and the film was 38 cm. After development, the density of the film was measured with a densitometer. A density-thickness curve was drawn for each film, with the meter values as ordinates and the thickness of the aluminium as abscissae. The meter values for the dentine could then be converted into the equivalent thickness of aluminium by means of the curve. The error of the method was given as approximately 5 per cent, with double determinations on one tooth and using various films. With this technique it was possible to show that the dentine beneath the carious lesion had in one case an absorption about 25 per cent. higher than the surrounding normal dentine. On account of the area of the surface measured — 0.5 mm^2 — it was difficult to confine the measurements to sufficiently well-defined parts of the preparation. This was also evident from the authors' cautious interpretation of the values for pathologically changed dentine.

Van Huysen, Bale & Hodge (1935) compared the accuracy of the above-mentioned densitometric procedure with an ionization method. In this the x-rays were passed through a masked part of the preparation to a gas ionization chamber. The current resulting from the ionization of the gas was measured and compared with the values obtained when the reference system replaced the ground section. It proved that the two methods had roughly the same accuracy. It was, however, difficult to determine the position of the area of the preparation the absorption

of which was measured by the ionization method. This disadvantage, together with the large area of measurement (about 0.2 mm^2) would seem to indicate that the method is less suited for studying caries.

The quantitative method described by *Warren et al.* (1934) was employed in other investigations. *Van Huysen* (1935) was able to show that transparent and opaque dentine beneath carious lesions had a 2—3 per cent. higher absorption than "unmodified" dentine. On the other hand he was unable to find any clear connection between the staining properties of the sections in basic fuchsin and their absorption. He used 5 ground sections of 0.80—0.85 mm thickness.

In a later paper *Van Huysen* (1936), with the same method, tried to find a normal absorption value for unchanged dentine. He used 160 planoparallel ground sections of 1 mm thickness and found on interpolation that the mean absorption was equivalent to 0.94 ± 0.03 mm aluminium. The absorption was greater in the crown than in the root, and the dentine in intact teeth had the same value as unchanged dentine from carious teeth. Dentine from older persons proved to have a somewhat higher absorption than from younger.

The same method was used by *Van Huysen, Hodge & Warren* (1937) in an investigation of dentine changes under abraded surfaces. They found areas with high absorption similar to those beneath carious lesions.

As early as 1934 *Thewlis* had shown on his microradiograms that a line of width about 100 μ — corresponding to the superficial enamel layer — presented a lower density than the rest of the enamel. He interpreted this line as an indication that the enamel was more strongly mineralized at the surface than below. This paper gave rise to a discussion. *Hollander & Saper* (1935) asserted that the light marginal line was a photographic artefact arising during development of the film. They held that such a line always appeares on a film at the boundary between two strongly contrasting areas (Mackie line) and found in microradiograms of enamel fragments and thin aluminium plates similar lines along the edges. They could make the line completely or partly disappear by laying an x-ray contrasting sub-

stance — phosphate cement — in contact with the enamel margin before exposing.

This criticism lead *Thewlis* (1937) to take up the question for renewed study by microphotometry. He used 200 μ ground sections of deciduous teeth and compared the photometric curves for intact enamel with corresponding curves for interrupted sections. The Mackie line could certainly be discerned along the interrupted surfaces, but the curves showed that it was weaker than the corresponding line along the surface of the enamel. The line in the latter case was therefore only partly an artefact. Thewlis therefore maintained that the external layer of the enamel is in many cases markedly hypercalcified.

When *Applebaum* (1935) showed by means of x-rays the existence of a strongly absorbing superficial layer in incipient caries the question arose whether the light line in this case was the Mackie line or an indication of greater mineralization in the superficial enamel layer. *Applebaum* (1940) considered that there was strong evidence that the superficial layer of incipient caries was mineralized to a greater degree than the underlying carious enamel. He pointed out that the light line was uneven and thicker than the Mackie line, and that the contrast necessary for the appearance of the Mackie line was frequently absent in his material. The problem cannot be considered as completely resolved, however, and further study is indicated.

In addition to the investigations referred to above mention may be made of *Applebaum* (1938 a) who showed that the striae of Retzius in certain cases appeared more definitely in the part of the microradiogram corresponding to the carious lesion. *Applebaum & Adam* (1938) published a microradiogram showing that fissure caries in the white rat bears some resemblance to fissure caries in man. *Applebaum* (1938 b, 1943) used soft x-rays to study the mineralization of teeth during their development, and *Lefkowitz* (1940) employed the same method to examine whether phosphate cement had any visible effect on enamel. He was able to show that the cement dissolved the enamel at the surface. *Gottlieb* (1947) has published a large number of microradiograms of caries, these being prepared by Applebaum with soft x-rays. They largely confirm the findings of earlier authors.

PRINCIPLES UNDERLYING MICRORADIOGRAPHY AND THEIR APPLICATION

1. *General principles*

In microradiography of heterogeneous objects the transmission of the x-rays varies in different structures, and these variations are registered on photographic emulsions. The absorption of a not too wide or divergent beam of monochromatic x-rays follows the formula:

$$E = \ln \frac{I_o}{I} = \frac{\mu}{\varrho} \cdot q \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 1$$

E is the x-ray extinction and I_o and I represent the incident and transmitted x-ray intensities, $\frac{\mu}{\varrho}$ the mass-absorption coefficient $(cm^2 \cdot g^{-1})$ and q the mass of absorbing screen $(g \cdot cm^{-2})$.

If several elements 1, 2 ...... n are present in the absorbing screen the attenuation of x-rays can be written

$$E = \ln \frac{I_o}{I} = \frac{\mu_1}{\varrho_1} \cdot q_1 + \frac{\mu_2}{\varrho_2} \cdot q_2 + \quad . \quad . \quad . \quad . \quad \frac{\mu_n}{\varrho_n} \cdot q_n \quad . \quad . \quad . \quad 2$$

Within certain limits the mass-absorption coefficient $\left(\frac{\mu}{\varrho}\right)$ increases with increasing atomic number of the absorbing material, and with increasing wavelength of the x-rays according to the formula $\frac{\mu}{\varrho} = k \cdot \lambda^3 \cdot Z^4$. However, the x-ray absorption spectra of elements have discontinuities, absorption edges which appear at certain critical wavelengths. The position of these absorption edges is a property of the atoms and from the practical aspect independent of their chemical combination. These absorption edges are usually known as K, L, M etc. and of these the K-edge has the shortest wavelength. The position of the absorption edges within the x-ray spectrum is displaced towards the higher wavelength with decreasing atomic number of the absorbing element. For general references see *Siegbahn* (1931), *Compton & Allison* (1947) and *Engström* (1946).

By employing these characteristic absorption discontinuities it is possible to determine the quantity of an element by absorption measurements of monochromatic x-ray radiation on both sides of the absorption edge. The procedure thus involves measurement of the transmission of x-rays of two different wavelengths; these should lie as close to the absorption edge as possible so as to minimize the correction for the presence of elements other than the one intended.

For an elementary analysis by this method it is necessary to have a certain mass of the material in question per unit area. If 5 per cent. is taken as the least difference in transmission which can be measured on both sides of the absorption edge, it is possible to calculate roughly the minimum amount of a given material that is detectable (*Engström* 1946). The difference in the numerical value of the mass-absorption coefficient on both sides of the absorption edge is a factor of importance. This difference increases with decreasing atomic number, a relationship that is important in analysis of biological material that consists mainly of elements of low atomic number. It is thus possible to determine the amount of carbon, nitrogen and oxygen in ordinary biological material in sections of 1—10 μ thickness. If the sections are up to 20 μ in thickness sulphur and phosphorus can also be determined, and in still thicker sections a large number of other elements. An exception to these conditions is to be found in mineralized tissue where calcium salts and phosphorus can be determined in very thin sections on account of the high local concentration of the element.

By using very soft x-rays (8—12 Å) it is possible to determine the density of biological soft tissues. Such tissue is composed mainly of carbon, nitrogen and oxygen and has an average atomic number of about 7. Other elements occur only in small concentrations and their mass-absorption coefficients do not differ greatly from that of nitrogen in the wavelength range used. On this condition are based the methods for weighing cells or cell constituents (*Engström & Lindström* 1950). This method of weighing the structures in biological soft tissue may, however, be modified for application to tissue of high x-ray absorption — mineralized tissue, for example. For instance an absorption picture of bone tissue taken with x-rays of the wavelength

2—3 Å (K-absorption edge for calcium is 3.06 Å) shows distributions of mineral salts in the section. As will be shown below the x-ray absorption of the organic component in this tissue is negligible in relation to the inorganic parts at the wavelengths used here.

The composite mass-absorption coefficient for mineralized tissue, for instance bone, can be expressed as

$$\left(\frac{\mu}{\varrho}\right)_{\text{bone}} = a \cdot \left(\frac{\mu}{\varrho}\right)_{\text{inorganic}} + b \cdot \left(\frac{\mu}{\varrho}\right)_{\text{organic}} \quad \ldots\ldots\ 3$$

where a and b are weight proportions of the inorganic and organic fraction of bone.

The mass-absorption coefficients for apatite and for the organic fraction, which is represented by nitrogen, are given in the following table for various wavelengths:

Table 1

Mass-absorption coefficient for apatite (inorganic phase) and nitrogen (representing the organic phase) at various wavelengths.

λ Å	Mass absorption coefficient $cm^2 \cdot g^{-1}$	
	$\frac{\mu}{\varrho}$ apatite	$\frac{\mu}{\varrho}$ org.(N)
0.5	~ 3.5	~ 0.4
1.0	26	2.1
1.5	81	7.7
2.0	178	19
2.5	330	29
3.0	545	57

From the table it is evident that the mass-absorption coefficient for the organic fraction of mineralized tissue at the wavelengths used in our microradiographic procedure are only about 1/10 of that for apatite. Another point which will further minimize the contribution to the absorption of the organic fraction is the fact that it constitutes only about 1/4 of the total mass. Thus if we have a section of dry bone tissue 100 μ in thickness, the weight will be 0.02 g/cm^2 and the weight of the inorganic fraction will be 0.015 g/cm^2 and of the organic 0.005 g/cm^2. For

a wavelength of 1 Å the mass-absorption coefficients for the inorganic fraction is 26 and for the organic 2.1. From this follows that

$$\frac{E_{inorganic}}{E_{organic}} = \frac{40}{1}$$

which means that 97.5 % of the extinction comes from the inorganic part. Thus a microradiogram registered under the condition mentioned above shows the distribution of mineral salts.

One of the most important factors for the definition of the radiograph is constituted by the geometric conditions obtaining during exposure. Since the focus of an x-ray tube consists of a surface, at least a part of the shadow produced by the object consists of penumbra (cf. Fig. 1). The part of the shadow consisting of umbra may generally be expressed as

$$u = \frac{ad - bf}{a - b}$$

where a is the distance from focus to image plane, b the distance from object to image plane, f the diameter of the focus, and d the diameter of the object.

Differentiation with respect to b gives

$$\frac{du}{db} = a\,\frac{(d - f)}{(a - b)^2}$$

$$\frac{du}{db} = (d - f)$$

if $d > f$; $\frac{du}{db} > 0$ then u increases with b;

if $d = f$; $\frac{du}{db} = 0$ then u is constant;

if $d < f$; $\frac{du}{db} < 0$ then u decreases as b increases.

The size of the penumbra (P) is given by the expression

$$P = \frac{b}{a - b} \cdot f$$

Fig. 1. The geometry of the image formation in microradiography. f = focal spot, a = focus to image plane distance, b = object to image plane distance, d = diameter of the object, u = the umbra, p = the penumbra, t = the total shadow.

and the total shadow (t) is then

$$t = \frac{ad + bf}{a - b}$$

A condition for obtaining an "image" is that the value for the umbra is greater than zero. The dimension of the smallest object that gives an image proportional to the size of the object is represented by $\frac{b}{a} \cdot f$.

2. *Methods for obtaining magnified radiographs*

Radiographs with microscopic details are obtainable by various procedures. The hitherto most common techniques are illustrated in Fig. 2 A and B. In Fig. 2 B a thin preparation is placed in

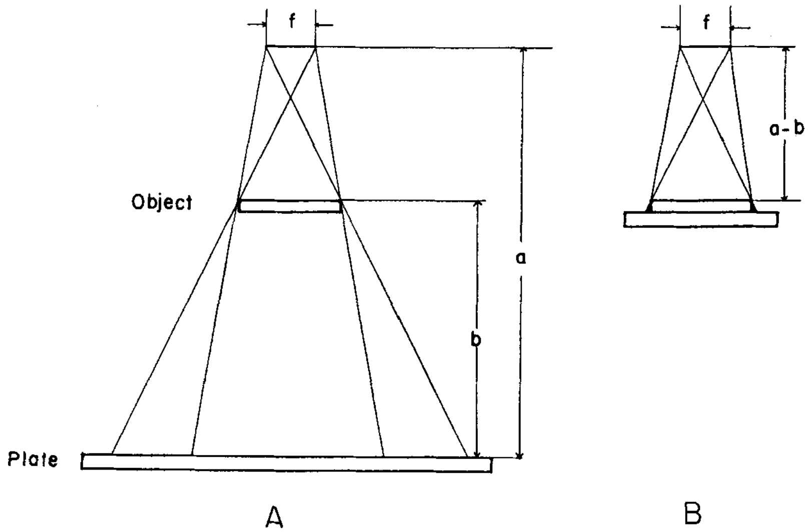

Fig. 2. Different principles for microradiography. A. Primary magnification. B. Secondary magnification.

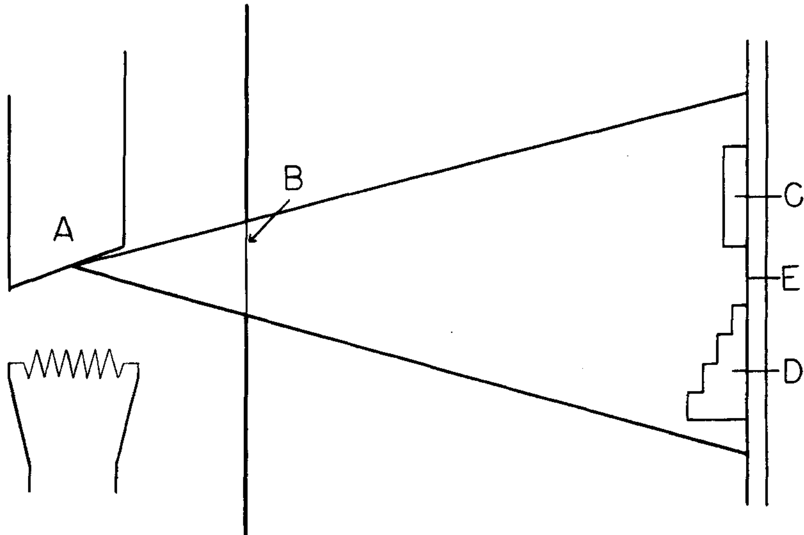

Fig. 3. General arrangement for microradiography. A = the target of the x-ray tube. B = the window filter, C = the sample, D = reference system, E = the photographic emulsion.

direct contact with a photographic emulsion of extremely fine grain and exposed to x-rays of suitable wavelength. In this way a radiograph (microradiogram) is obtained to a scale of 1:1. This x-ray absorption image may then be enlarged with the aid of the microscope (secondary magnification).

Another method for obtaining magnified radiographs is based on the use of divergent ray bundles originating at a very small focus (primary magnification) (Fig. 2 A). Such x-ray microscopes have been constructed by *von Ardenne* (1940) among others. In this method the object recordable is of the same order of magnitude as the focus, since a and b in this case have rather similar values, and the smallest object that gives an image proportional to its size is represented by $\frac{b}{a} \cdot f$. (p. 108). The production of a focus of very small area involves great technical difficulties. The resolving power with this method is given as about 1 μ at present (*Cosslett & Nixon* 1951, 1952).

The x-rays are refracted at the boundary of the media of different refractive indices. The effect is very small, however, and the principle of refraction cannot be applied in the design of x-ray magnifying systems. Radiographs with considerably greater definition than provided by the methods described above (down to about 70 Å) will probably be possible in the near future with an x-ray microscope designed by *Kirkpatrick et al.* (1948). The principle of the x-ray microscope is based on the fact that a divergent beam of x-rays having grazing incidence on a concave surface is converted to a convergent beam and thus a focusing effect is obtained. By combining several such surfaces in succession a picture of good quality is obtained.

It seems that for the present direct (contact) microradiography is the simplest and most convenient method of performing x-ray studies of biological material. In this procedure there are several factors that influence the definition of the microradiogram. The geometric factor may easily be brought down to 0,5 μ with a suitable arrangement. The point is then reached when the definition is practically dependent on the photographic emulsion provided soft x-rays are used. Photographic emulsions such as are used on Lippman film, Eastman Kodak Spectroscopic Plates No 548 and No 649 and Kodak Maximum Resolu-

tion Plates have all a resolving power of at least 1000 lines per millimetre. With this procedure it is thus easy to obtain a resolution of about 1 μ. Photographic emulsions with still better resolution are being developed and thus a resolving power of the same order of magnitude as applies to the ordinary light microscope is obtained.

It is clear, then, that microradiography is no new technique, but its application in biological research has been restricted by several factors. One has been the difficulty of obtaining a sufficiently high intensity of x-rays in the soft range of wavelength (2—50 Å). Another factor is the high absorption of soft x-rays in air. A third, the difficulty of getting photographic emulsions of sufficiently high resolving power. This last factor has been solved fairly satisfactorily by the introduction of the above-mentioned fine grain emulsions, while considerable improvements in respect of the other factors have recently been made, as will be seen below.

3. *X-ray equipment used in the experiments*

In the experiments carried out by the present authors on the process of mineralization in healthy and diseased dental and osseous tissues microradiography has been used mainly for studying the amount and distribution of mineral salts in the ground sections. With the aid of ordinary histological staining procedures the presence of calcium salts in tissue can be revealed but quantitative data cannot be so obtained. The mineral content in two adjacent structures may be compared by microradiography, and it has been shown that as small differences in mineral content as 2 per cent. may easily be detected (*Amprino & Engström,* 1952). The principle of this procedure is illustrated in Fig. 3. The reference system necessary in quantitative measurements here consists of the step wedge.

Various types of x-ray equipment have been employed in these experiments. The usual instrument was a standard Machlett-tube AEG 50 with beryllium window 1 mm in thickness. The softest x-rays transmitted by this filter were about 4 Å. The tube may be run at a P.D. of up to 50 kV and a very wide range of wavelengths may thus be obtained. For thin ground sections of osseous tissue 6—8 kV was used (*Engström & Wegstedt,* 1951).

For preparations that are large or have a high mineral content — completely mineralized dental enamel for instance — a Philip's diffraction unit with a copper target served as radiation source. The tube was run at 20—30 kV, with a focus-film distance of 25 cm. For microradiography of histological sections of soft tissue or of very thin ground sections (less than 5 μ) it is necessary to employ considerably softer radiation. The apparatus for such experiments was an open specially-built x-ray tube with Al-anod and the exposure had to be made in a high vacuum.

The abovementioned Machlett tube has recently been provided with a beryllium window only some tenths of a millimetre thick, which transmits wavelengths above 4 Å. An x-ray tube made by Philips having a beryllium window of 50 μ has quite recently been tested in this laboratory; very soft rays are obtained that permit mass determinations without the timeconsuming high-vacuum procedure.

In certain cases it has proved of great value to be able to localize a structure in the third dimension. In stereomicroradiography exposures of the object are taken in two directions. This is managed by tilting the object and film in relation to the normal, the two radiographs so obtained being examined directly in a microstereoscope, or enlarged and viewed in a mirror stereoscope (*Engström,* 1951; *Bellman,* 1953). With this technique the above-mentioned Philip's diffraction unit was employed with a Siegbahn spectrograph especially adapted for this procedure (cf. *Bellman,* 1953).

APPLICATION OF MICRORADIOGRAPHY TO DENTAL TISSUES

That the published papers report no noteworthy application of microradiography to the study of details in the dental tissues is probably because suitable x-ray equipment has not been available, the film used has not been of sufficiently fine grain, or the ground sections were generally too thick.

A renewed study of the dental tissues therefore seemed to be indicated with application of the advantages inherent in the improved methods. Special attention was devoted to enamel and dentine caries.

The mechanism of the caries process is not fully understood. The literature contains extremely divergent opinions on the etiology and pathogenesis. Whatever view may be held on these problems, it is indisputable that loss of calcium salts is an important factor in the occurrence of carious cavities in the teeth. Under certain conditions it seems that there may actually be an increase in the mineral content of the tissues of the teeth in caries. It follows that dental caries is a suitable field for studies employing microradiographic procedures.

MATERIAL AND METHOD

The material consisted of carious human teeth. Immediately after extraction they were fixed in alcohol and then embedded in methyl metacrylate. Slabs through the centre of the carious lesions in the longitudinal direction of the teeth were cut with a rotating diamond saw. These were ground planoparallel and polished by a "dry" technique that will be described by *Hammarlund-Essler* (1954). The thickness of the sections varied between 20 and 120 μ. They were mounted on thin mica sheets.

The microradiographic technique is described above.

RESULTS

The most important findings are illustrated below in the figures showing the ground sections in transmitted light and the corresponding microradiograms and detail pictures. The dark areas on all the microradiograms represent lower x-ray absorption and thus a lower mineral salt content.

1. *Enamel caries*

Examples of caries in the enamel are provided by one case of incipient caries on a proximal surface (Figs. 4—6), 2 cases with superficial proximal cavities (Figs. 4, 7—10) and 2 cases with fissure caries (Figs. 11—15).

The microradiograms of *incipient caries* presented here show that the enamel has a lower x-ray absorption in the carious area, indicating that the mineral salts are partly dissolved away. A narrow zone on the surface of the lesion has a higher absorption, however (Fig. 5). Higher magnification brings out the prism structure of the inner region of the lesion (Fig. 6). The

low absorption suggests that this is the main area of demineralization, the striae of Retzius being revealed.

The microradiograms of cases with *shallow enamel cavities* show two different situations. In one case (Fig. 7) there is no counterpart to the strongly absorbing superficial layer in incipient caries, and the striae of Retzius are not visible. Instead, the "palissade" structure has appeared through demineralization along the enamel prisms.

In the second case the carious lesion has begun in a hypoplastic pit in the enamel (Fig. 8). The striae of Retzius appear clearly on the microradiogram, as does the prism structure and the cross striation (Fig. 9). With high magnification the microradiogram gives the impression that the prisms are thinner at those places where they cross the striae of Retzius (Fig. 10). Whether this picture is due to caries or is connected with a disturbance of development it is impossible to decide at present. The strongly absorbing superficial layer that was earlier revealed in incipient caries is present also on this tooth.

Fissure caries in the enamel is illustrated by three microradiograms (Figs. 12, 14 & 15) showing two stages of caries. In the earlier stage (Figs. 12 & 14) the carious enamel appears as two long areas with low x-ray absorption in the inner part of the enamel near the fissure. It is possible that the demineralization occurs mainly in the deeper enamel, but the possibility cannot be excluded that the enamel is undermined by the action of acid from the adjacent parts of the fissure that are not visible in the section. In a later stage (Fig. 15) the region with low absorption advances to the dentine, the striae of Retzius and the prism structure being revealed.

2. *Dentine caries*

As examples of the changes in dentine caries, choice has been made of one case of proximal caries (Fig. 8), 2 cases of fissure caries (Figs. 11, 12, 16, 17, 20) and one case of cervical caries (Figs. 11, 12, 18, 19).

Proximal caries. The microradiogram (Fig. 8) shows that the dentine beneath the earlier-described shallow enamel cavity has an area with low absorption which suggests progressive demineralization of the dentine.

Fissure caries. In spite of the superficial character of the fissure lesions (Fig. 12), the reaction in the dentine may clearly be traced as strongly absorbing bands extending to the dentine-predentine junction. A microradiogram of the same region at higher magnification (Fig. 20) shows that the canal structure has disappeared within the region with high x-ray absorption.

Fig. 16 is the classical picture of a conical transparent zone in the dentine associated with a fissure lesion. The corresponding microradiogram (Fig. 17) shows in this case close, although not complete, correspondance between the transparent zone on the ground section and a region with high x-ray absorption due to increased mineral salt content. This increase may have occurred through precipitation of calcium salts in the dentine.

Cervical caries (Fig. 12). This lesion has a strongly absorbing superficial layer, which may possibly be interpreted as stationary state in the development of the cavity. The reaction in the dentine follows the S-form of the dentine tubules. There is no evident agreement between, on the one hand, the dark and transparent zones on the ground section and, on the other hand, the x-ray absorption on the microradiogram. It is evidently impossible with unstained ground sections studied in transmitted light to estimate the degree of mineralization in the dentine.

An investigation of the same lesion under greater magnification shows that the region of increased absorption coincides only partly with the transparent zones on the pictures of the ground section (Figs. 18 & 19).

3. *Other observations*

Fig. 21 shows how the dentine tubules appear on a microradiogram. The illustration is of unimpaired dentine near the pulp. On account of the low absorption of the predentine zone compared with the mineralized dentine it was overexposed and is not visible. The dark area corresponds therefore to the pulp cavity and predentine. Roughly 30 μ from the junction between the dentine and predentine there is in the dentine a low absorption band about 20 μ wide, parallel to the pulp wall and thus less mineralized than the surrounding tissue.

The incremental lines of the dentine are seen in Fig. 22, which is a microradiogram. The varying dark and light lines at intervals of about 25 μ show clearly that the lamellar structure of the dentine is to a certain extent at least an indication of variations in the calcium salt content.

Fig. 23 is of an unimpaired region of the dentine. The figure shows how the interglobular dentine and the calcium salt globuli appear in a microradiogram.

The microradiograms in Figs. 24 (a) and (b) form a stereopair illustrating the possibility of studying the structures in incipient caries in three dimensions.

SUMMARY AND CONCLUSIONS

A survey of previous work on the application of x-ray absorption techniques to the study of dental tissues is followed by an account of the principles underlying microradiography and of recent advances in the field. The opportunities offered by new techniques are illustrated in an examination of a number of carious teeth. It has been shown that the method constitutes an extremely valuable aid in the study of caries, providing accurate information on the degree of mineralization in the tissue under examination. On account of the high definition of the microradiograms with the technique employed it has been possible to reveal some details concerning the mineral content in normal and carious tissues of the teeth, which, so far as the authors are aware, have not been demonstrated hitherto by the microradiographic method; wiz,

(1) dentine tubules in healthy dentine,

(2) incremental lines in the dentine, 25 μ in width,

(3) the prism structure in enamel caries,

(4) cross striation in enamel caries,

(5) increased x-ray absorption in the superficial parts of a dentine lesion, and

(6) interglobular spaces and calcium globuli.

Where indicated the scope of the microradiographic method may be widened by applying the stereoscopic technique that enables a structure to be localized in three dimensions (Figs. 24 (a) and (b).)

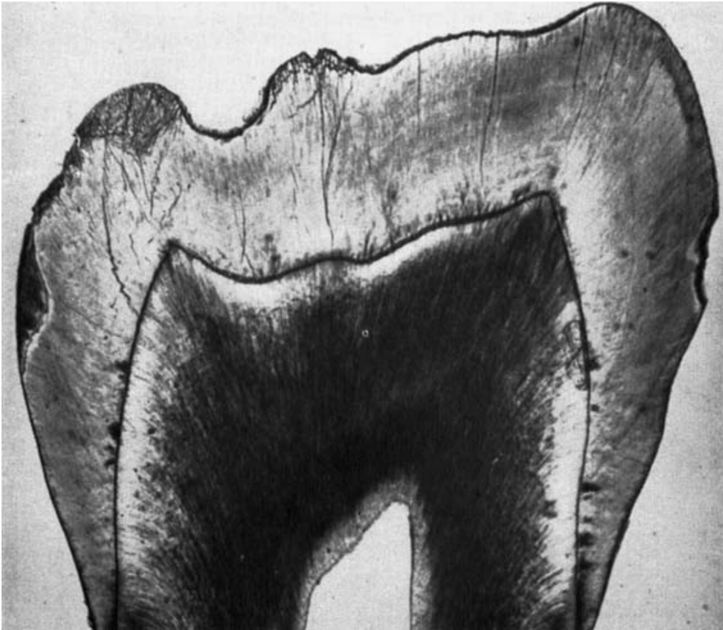

Fig. 4. 90 μ unstained ground section in transmitted light. Two proximal lesions in the enamel; on the right shallow cavity, and on the left incipient caries (× 10).

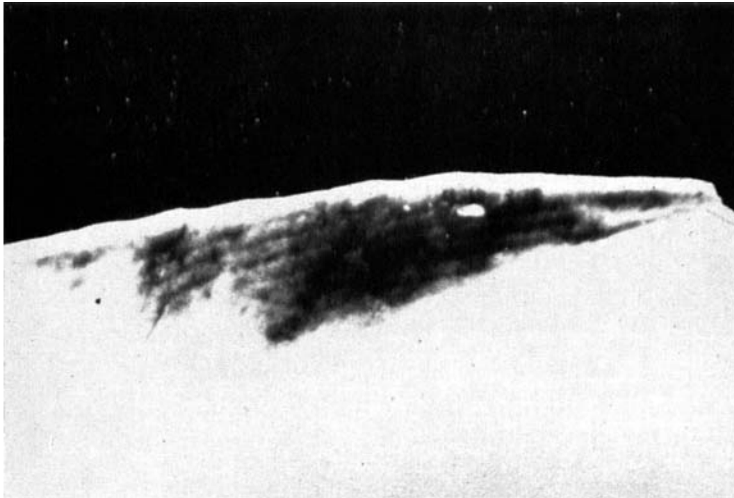

Fig. 5. Microradiogram of incipient caries in Fig. 4. Low x-ray absorption in the inner part of the lesion. Unmasked striae of Retzius (× 50).

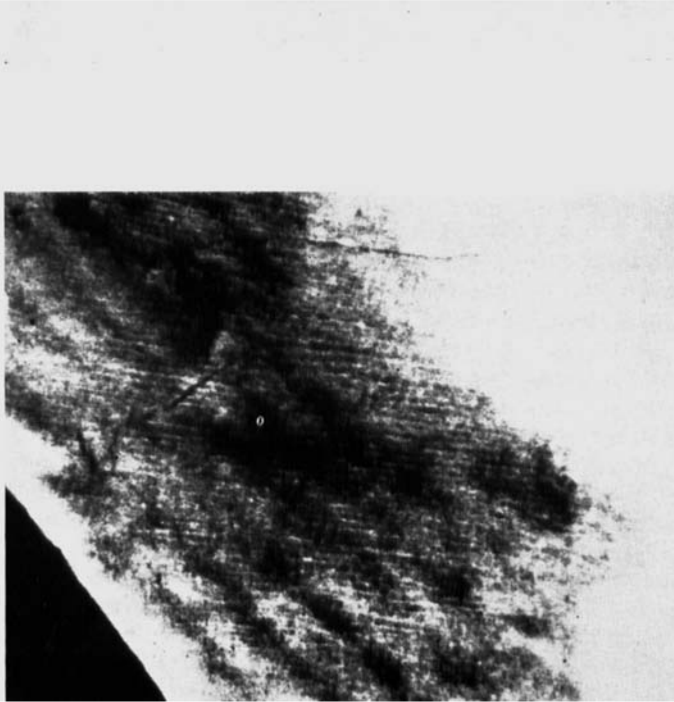

Fig. 6. Microradiogram of incipient caries in Fig. 4 under high magnification. The prisms that run vertically on the figure are crossed by unmasked striae of Retzius. The surface of the enamel is seen in the upper left (× 250).

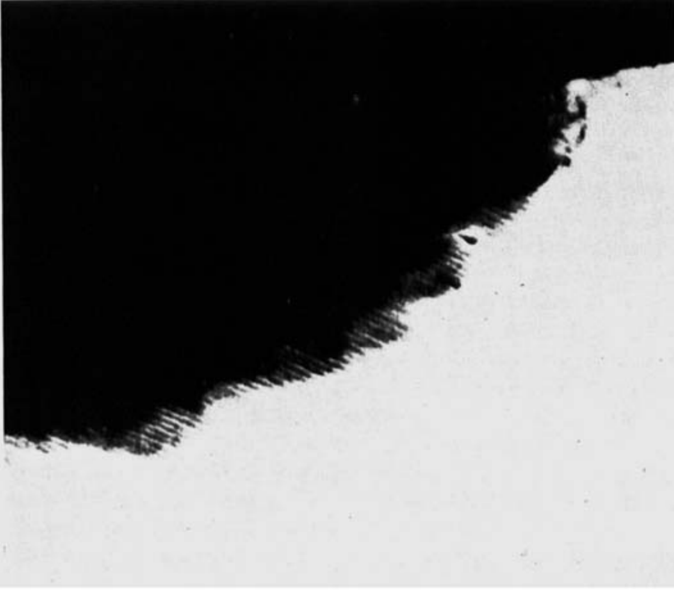

Fig. 7. Microradiogram of the shallow cavity in Fig. 4. The "palissade structure" has appeared through dissolution of the calcium salts along the enamel prisms (× 165).

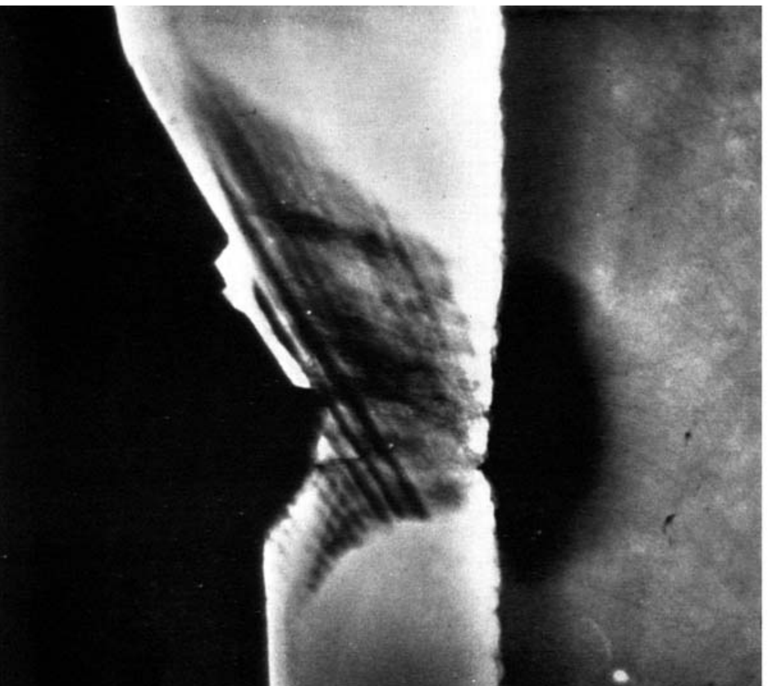

Fig. 8. Microradiogram. Superficial cavity in the enamel. Reduced x-ray absorption in the deeper carious enamel, particulary along the striae of Retzius. The dark area in the dentine indicates partial dissolution of the calcium salts (× 45).

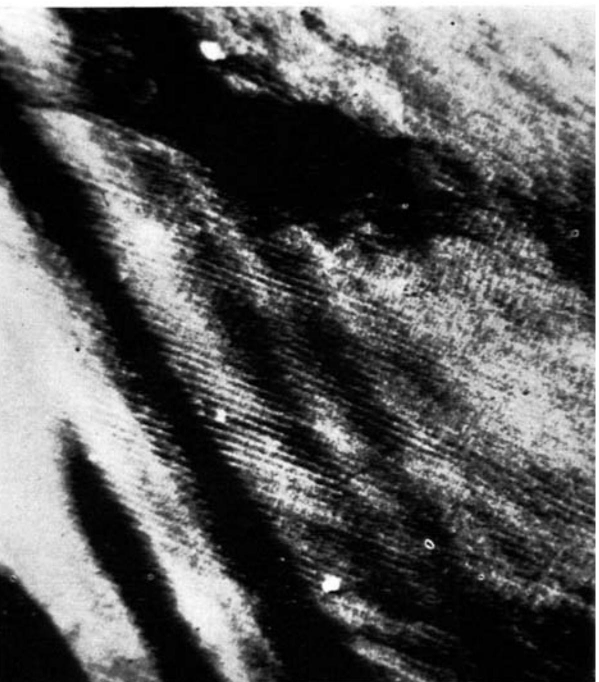

Fig. 9. Microradiogram, magnification of detail from the carious enamel in Fig. 8. Unmasked striae of Retzius crossing the prisms. Transverse striation is visible (× 260).

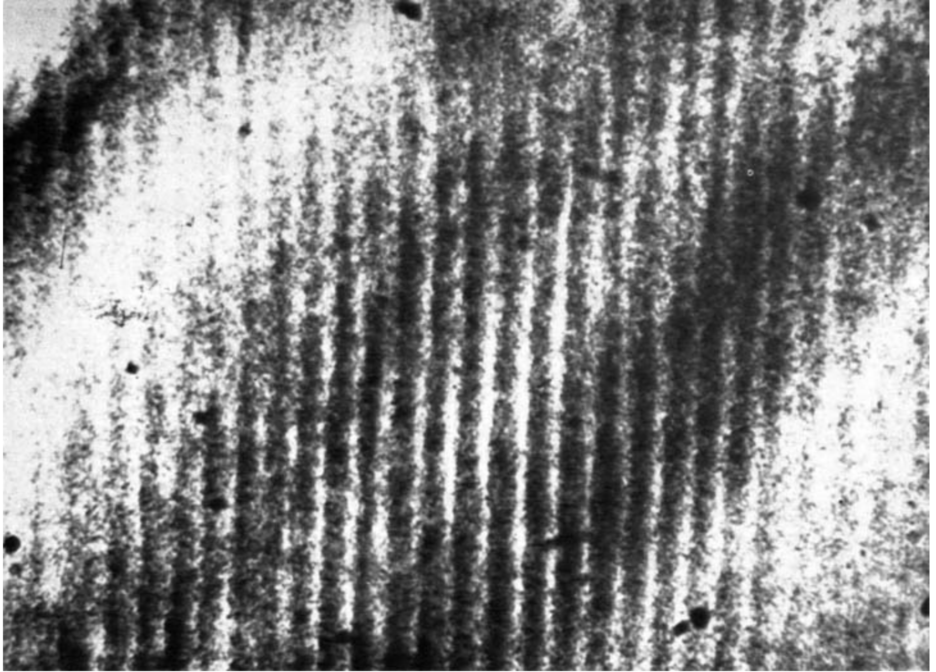

Fig. 10. Microradiogram from the same lesion as in Fig. 9. Parallel prisms crossed by striae of Retzius. The latter run diagonally in the figure (× 900).

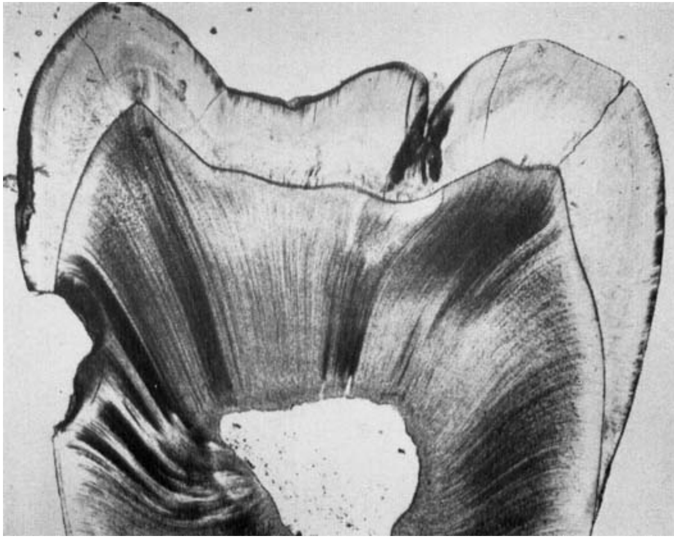

Fig. 11. 120 μ unstained ground section in transmitted light. Superficial fissure caries and cervical caries in the dentine. Light and dark zones alternate in the dentine (× 9).

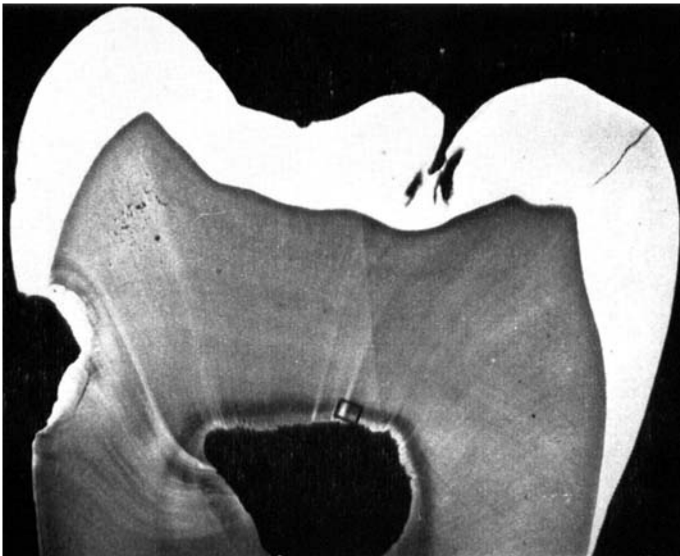

Fig. 12. Microradiogram from the same section as in Fig. 11. The fissure caries lesion appears as regions in the enamel of low x-ray absorption. The reaction in the dentine may be traced as strongly absorbing bands down to the pulp boundary. The cervical lesion has a strongly absorbing superficial layer. The reaction in the dentine follows the S-form of the dentine tubules. The squared area is seen in higher magnification in Fig. 20. (The sharp boundary line that passes through the dentine and the upper part of the pulp is due to the fact that the mica foil is thinner to the right of the boundary). (× 9).

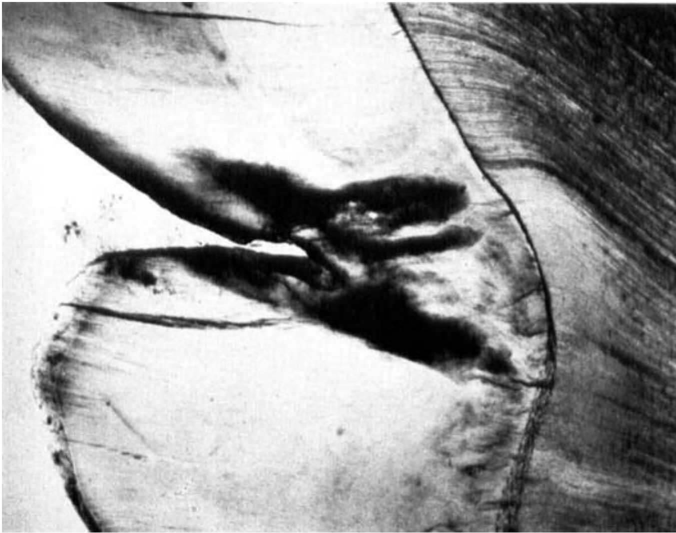

Fig. 13. Detail enlargment of fissure lesion in Fig. 11 (× 40).

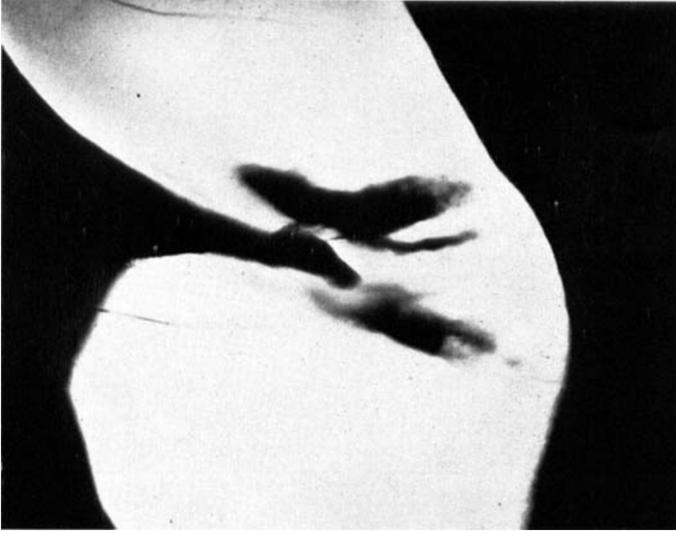

Fig. 14. Microradiogram of the same region as in Fig. 13. Reduced x-ray absorption in the inner enamel adjacent to the fissure due to the partial dissolution of the calcium salts as a result of the caries (× 40).

Fig. 15. Microradiogram showing fissure caries in the enamel. The prism structure and the striae of Retzius are visible. Penetration to the dentine (× 150).

Fig. 16. 100 μ unstained ground section in transmitted light. Conical transparent zone in the dentine in fissure caries (× 40).

Fig. 17. Microradiogram of the same regions as in Fig. 16. The transparent zone in Fig. 16 corresponds to dentine of high x-ray absorption (× 40).

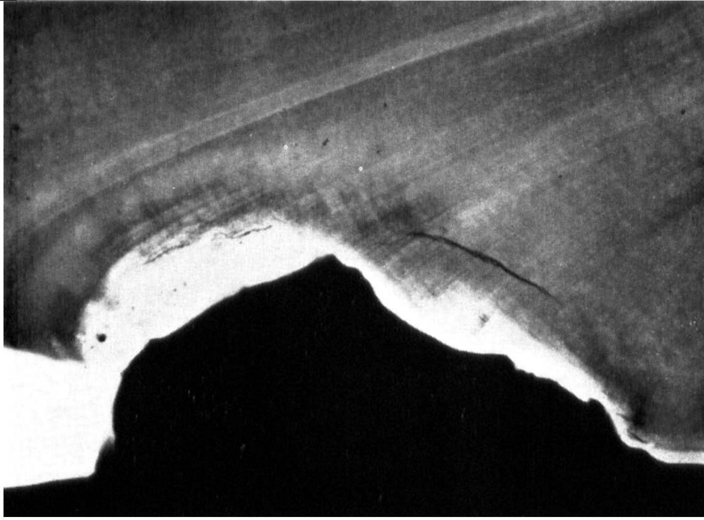

Fig. 19. Microradiogram of the same region as in Fig. 18. The floor of the cavity is strongly absorbing (× 35).

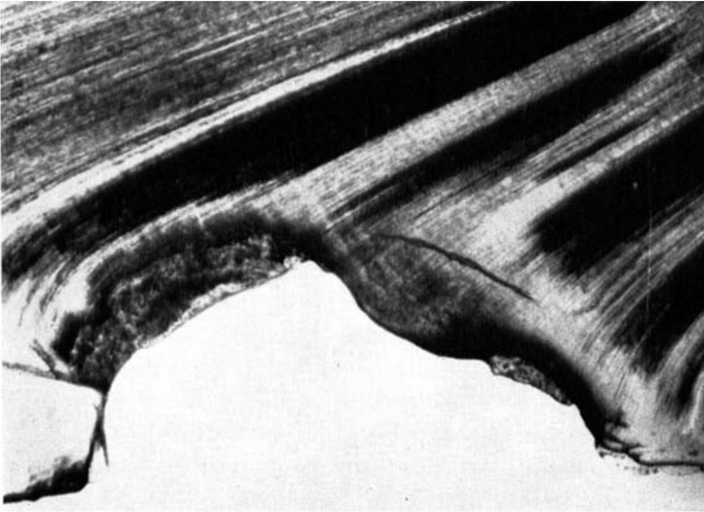

Fig. 18. Detail enlargment of cervical caries in Fig. 11. Opaque and transparent zones alternate in the dentine (× 35).

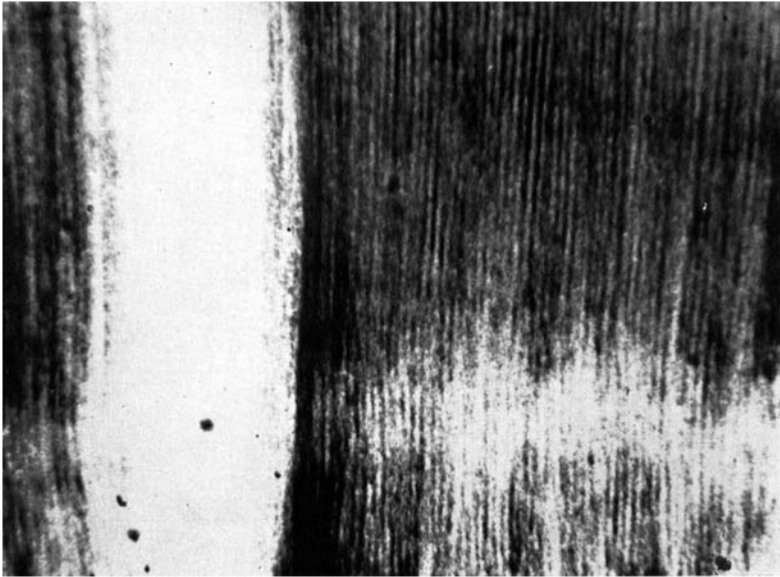

Fig. 20. Microradiogram. On the left is a band of dentine of high x-ray absorption. The figure corresponds to the squared area in Fig. 12. The dentine tubules are probably filled with calcium salts (× 250).

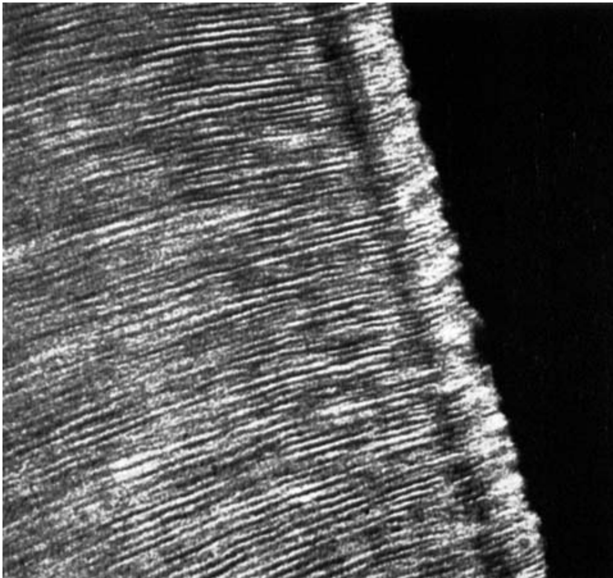

Fig. 21. Microradiogram of the dentine near the pulp in a sound part of the tooth. The dentine tubules are dark. Near the junction between the dentine and the predentine is a band about 20 μ in width with lov x-ray absorbtion (× 165).

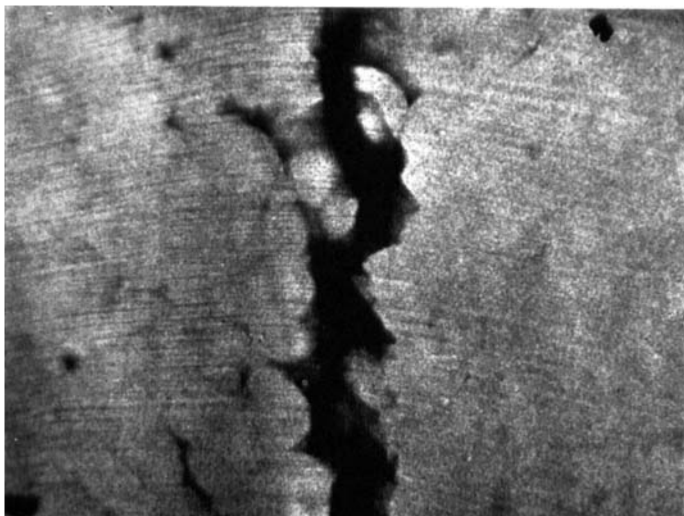

Fig. 23. Microradiogram. Interglobular spaces with low x-ray absorption and calcium globuli with high absorption (× 200).

Fig. 22. Microradiogram. The horisontal lines are incremental lines at intervals of about 25 μ. The vertical parallel lines indicate the direction of the dentine tubules (× 120).

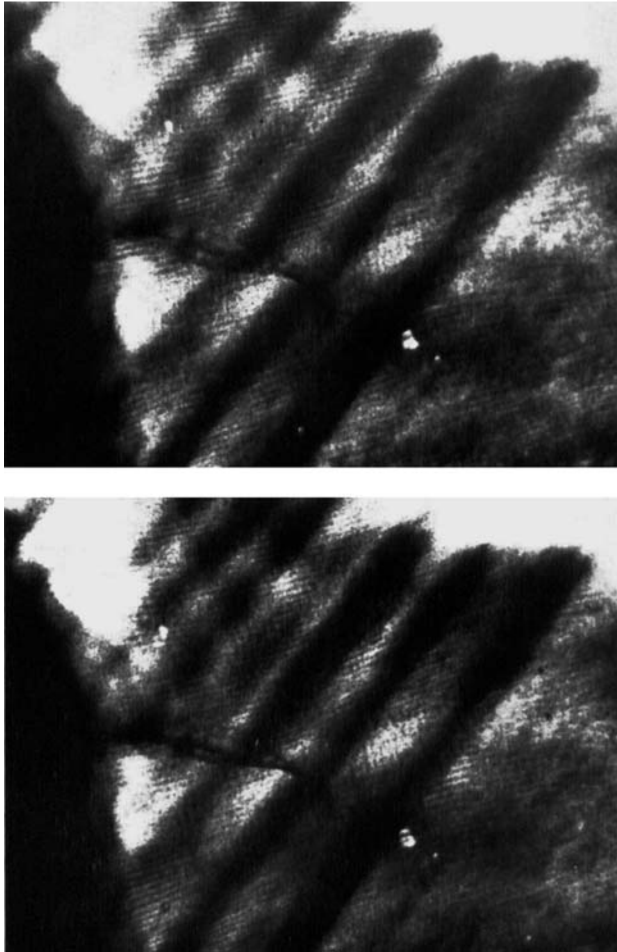

Fig. 24 (a) & (b). Microradiogram (stereo-pair) showing carious enamel (× 250).

ZUSAMMENFASSUNG

STUDIEN AN MINERALISIERTEN ZAHNGEWEBEN

II. Die Mikroradiographie als Methode zur Untersuchung von Zahngeweben und ihre Anwendung beim Studium der Karies

Es wird ein Überblick über frühere Arbeiten gegeben, die sich mit der Anwendung von Röntgenstrahlen zum Studium der Zahngewebe befassen. Es folgt eine Darstellung der Prinzipien der Mikroradiographie und der Fortschritte, die in neuerer Zeit auf diesem Gebiet erzielt wurden. Die Vorteile der neuen Methoden werden an einer Reihe kariöser Zähne gezeigt. Das angewandte Verfahren stellt eine aussergewöhnlich wertvolle Hilfe beim Studium der Karies dar, da es sichere Schlüsse auf den Grad der Mineralisierung des untersuchten Gewebes zulässt. Die mit dieser Methode zu erzielende Deutlichkeit der Mikroradiogramme ermöglichte ein Erkennen von Einzelheiten im Hinblick auf den Mineralgehalt gesunder und kariöser Zähne, die nach Wissen der Autoren durch mikroradiographische Methoden bisher nicht gezeigt werden konnten, z.B.

1) Dentinkanälchen in gesundem Dentin
2) Streifenförmige Verkalkung des Dentins in Abständen von 25 μ
3) Prismenstruktur bei Schmelzkaries
4) Querstreifung bei Schmelzkaries
5) Erhöhte Absorption von Röntgenstrahlen in den oberflächlichen Bezirken einer Dentinläsion und
6) Interglobularräume und Kalkglobuli.

Falls indiziert, kann der Anwendungsbereich der Mikroradiographie durch stereoskopische Verfahren erweitert werden, die eine dreidimensionale Darstellung der Strukturen ermöglichen (Abb. 24 (a) und (b)).

RÉSUMÉ

ETUDES SUR LES TISSUS DENTAIRES MINÉRALISÉS

II. La microradiographie, une méthode pour l'étude des tissus dentaires et son application à l'étude de la carie dentaire

Après avoir donné un résumé des travaux antérieurs où avaient été employées des méthodes d'absorption des rayons X pour l'étude du tissu dentaire, les auteurs ont rendu compte du principe de la microradiographie et des conquêtes nouvelles effectuées en ce domaine. Les possibilités offertes par ces méthodes modernes ont été démontrées dans une recherche sur les dents cariées. Il est apparu que ces méthodes sont d'une très grande valeur pour l'étude de la carie dentaire et qu'elles fournissent des indications exactes sur le degré de minéralisation du tissu examiné. Par suite de la haute résolution des microradiographies dans la technique employée, il a été possible de constater quelques détails au sujet de la teneur minérale du tissu normal ou carié des dents, détails qui, à notre connaissance, n'ont pas été relevés auparavant par les méthodes microradiographiques:

1. Des tubes dans la dentine saine.
2. Des zones de croissance larges de 25 μ dans la dentine.
3. La structure prismatique dans la carie de l'émail.
4. Des stries transversales dans la carie de l'émail.
5. Des tubes dentaires remplis de sels de calcium dans la zone translucide.

   D'autres faits ont encore été démontrés, qui prouvent la fécondité de cette méthode:

6. Une absorption accrue des rayons X dans les parties superficielles d'une cavité carieuse dentinaire.
7. Des espaces interglobulaires et des globules de chaux.

Le domaine de la méthode microradiographique peut encore être étendu dans certains cas par l'application d'une technique stéréoscopique qui permet une localisation des structures dans la troisième dimension (Comparez fig. 24 a et b).

Il faut enfin remarquer que la microradiographie n'est pas seulement applicable à la carie dentaire; elle est aussi un auxiliaire précieux dans l'étude de la pathologie des dents en général, par exemple du rachitisme, du scorbut, de l'osteogenesis imperfecta etc.

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Addresses:

Bergman
Royal School of Dentistry
Stockholm 3
Sweden

Engfeldt
Karolinska Institutet
Stockholm 60
Sweden