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MICRORADIOGRAPHY OF DENTINE USING CHARACTERISTIC X-RAYS

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SUMMARY

Typical microradiographs made with titanium, calcium, silicon and aluminum K-radiations are given of sections of the healthy and carious dentine of human teeth. They are offered to indicate the kind of new information about composition which can be obtained by using monochromatic instead of heterochromatic X-rays for microradiography.

INTRODUCTION

Radiography, whether on a gross or a micro scale, is important as the way to visualize the interior of a solid opaque to light. What it shows depends on the amount of X-rays absorbed at different points in the sample, and this absorption is determined by the structure and composition of the object as well as by the quality of the X-rays. In conventional radiography the X-rays have a wide range of wave lengths and contrast is largely the result of differences in density throughout the sample. These X-rays, as generated in most commercial tubes, are so penetrating that the specimen must be thick and its details relatively large to yield the requisite absorption. The more strongly absorbed X-rays that can reveal minute detail in thin specimens must be produced in an especially made tube equipped with the thinnest possible window and operated at a low voltage.

Two methods[1] have been devised for microradiography. In contact microradiography, as in the radiography of gross objects, the sample is photographed in contact with the film or plate. To reveal minute detail the contact must be excellent, the specimen thin and the photographic emulsion unusually fine-grained. In projection microradiography the X-ray source must be as small as the detail we hope to see and the specimen and film are separated to furnish an enlarged shadowgraph. Both methods have their advantages, and disadvantages. The equipment for projection microradiography is relatively costly since it involves a stable electron optical system to supply the minute X-ray source. It is limited in the number of different wave lengths it can use but has the outstanding advantage that the specimen can be very thick without a loss of sharpness in the microradiographic image. The techniques of contact microradiography are simple and inexpensive and it offers the important possibility of using

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the characteristic X-rays of all the elements (except the rare gases). Its chief limitation is to be found in the fact that the specimens must be very thin if small details are to be portrayed. We are using both types of microradiography in the study of biological structures but the present paper deals only with contact microradiography.

In principle X-ray absorption can serve to identify chemical elements present in a substance as well as to outline its morphology as indicated by differences in density. An element absorbs very strongly wave lengths shorter than those corresponding to the energy required to excite its characteristic radiation and is particularly transparent to its own radiation and to wave lengths somewhat longer. Radiographs made with these different X-rays therefore outline regions where a particular element is in high concentration. In contrast to the many applications already made with heterochromatic X-rays, few attempts[1] have yet been made to work with these characteristic radiations. Some years ago one of the present authors employed them to identify minerals[2] in petrographic sections; their use is currently being further investigated, especially for the lighter elements. Teeth, employed here to illustrate their possibilities, have frequently been investigated with polychromatic X-rays[3].

METHODS

Characteristic X-ray microradiography requires the use of the radiations from a wide range of elements. Such X-rays can be produced in one of two ways: either by fluorescence or in a tube equipped with a target of the desired element.

Fluorescent X-rays are excited when a secondary target is irradiated with an intense beam of wave lengths shorter than those characteristic of the target element. This method of producing characteristic X-rays for microradiography is especially simple because a high-powered commercial tube can supply the primary X-rays and the secondary target can be placed more or less at one's convenience with respect to it and the camera. Fluorescent excitation has the advantage for microradiography that only the characteristic spectral lines are produced, unaccompanied by the background of continuous radiation that results from electron bombardment. It has the disadvantage that sufficiently intense fluorescent sources are too large to yield micrographs of the highest resolution. Furthermore, the intensity of fluorescent radiations falls rapidly for the lighter elements: thus the efficiency of excitation diminishes from about 30% for iron radiation to only 2% for magnesium. This results in prolonged exposure times and in a loss of spectral purity through an increasingly important scattering of primary radiation by the fluorescing target.

A fluorescent source was employed in our earlier work[2] with mineral specimens. In view of the success there obtained, a preliminary series of microradiographs was made of sectioned teeth using the fluorescent K-radiations of titanium and calcium excited under near-optimal conditions by the output of a tungsten target Philips FA-60 X-ray tube operated at 1200 W. With the relatively coarse Eastman Fine Grain Positive emulsion used for minerals, the exposure time was of the order of a few minutes but for the far less sensitive ultra fine-grained emulsion needed for work at magnifications above approx. 30-fold, exposure times were impractically long.

All subsequent work has consequently been carried out with directly excited characteristic X-rays. The demountable tube employed for this purpose was constructed in this laboratory; it will be described elsewhere. For the study of teeth the most

useful radiations have been those of titanium, calcium, silicon and aluminum. The tube window was of 6 μ mylar film and because of the high absorption of all these X-rays in air the path from the tube window to the specimen and film was filled with helium. Light from the tube filament was excluded by interposing an opaque foil which was of titanium for titanium X-rays. It was of evaporated beryllium when a silica target was used and of aluminum for the other radiations. All micrographs were made on Eastman Spectroscopic Film Type 649-0.

Titanium K-radiation is especially strongly absorbed by the calcium and somewhat less strongly by the phosphorus in a sample. Calcium-rich details will appear with much reduced contrast in microradiographs made with calcium K-radiation, though since this element occurs in teeth as phosphate, the strong absorption of phosphorus will partially neutralize the high transmissibility of the calcium. Nevertheless the diminished contrast with calcium X-rays is apparent, as in Fig. 4b. Minimal contrast should be obtained with phosphorus K-radiation but because a target has not yet been prepared to supply such X-rays, silicon, as the next lighter element, has been used instead. The absorption of the organic material in a section is negligible for radiations as penetrating as that of titanium; but with silicon and even more with targets of the lighter aluminum and magnesium, its absorption is comparable with that of apatite and sufficient to bring out details of the matrix in sections a few μ thick (Fig. 1b and 2b). By photographing a section of a tooth with titanium and calcium radiations it is thus feasible to see directly the mineral of a tooth; if one wishes to learn more about the organic matrix, this can be visualized with aluminum or magnesium radiation after demineralization with EDTA. Similar studies are being made of bone and other calcified tissues. An even sharper delineation of the calcium distribution is possible in shells of invertebrates because their calcite and aragonite do not offer the complication arising through the superimposed absorptions of phosphorus and calcium in apatite.

RESULTS

In order to illustrate the possibilities of this microradiography using characteristic X-rays, series of contact microradiographs were made through sections of extracted human teeth, choosing for photography regions in both the healthy and the carious dentine. The sections were sawed from teeth preserved in formalin and reduced to the desired thinness by manual grinding. A first group of experiments was carried out to ascertain the best thickness for the radiations in question. This has proved not to be highly critical and to depend somewhat on whether major interest lies in healthy or in carious structures. For general purposes thicknesses between 25 and 40 μ are satisfactory. Sections as thin as 15 μ yield somewhat less than optimal contrast when photographed with the relatively hard titanium radiation while those much thicker than 50 μ require prolonged exposures to the softer X-rays and frequently show some superposition of detail.

Most sections through normal dentine photographed with titanium radiation have shown a high absorption which is greater within the often ill-defined tubules (Fig. 1a). In some areas the tubules have been much more clearly outlined as a consequence of being only partially filled with calcium phosphate (Fig. 2a). The dense mineral lining these tubules is evidently the optically refractile zone that has been

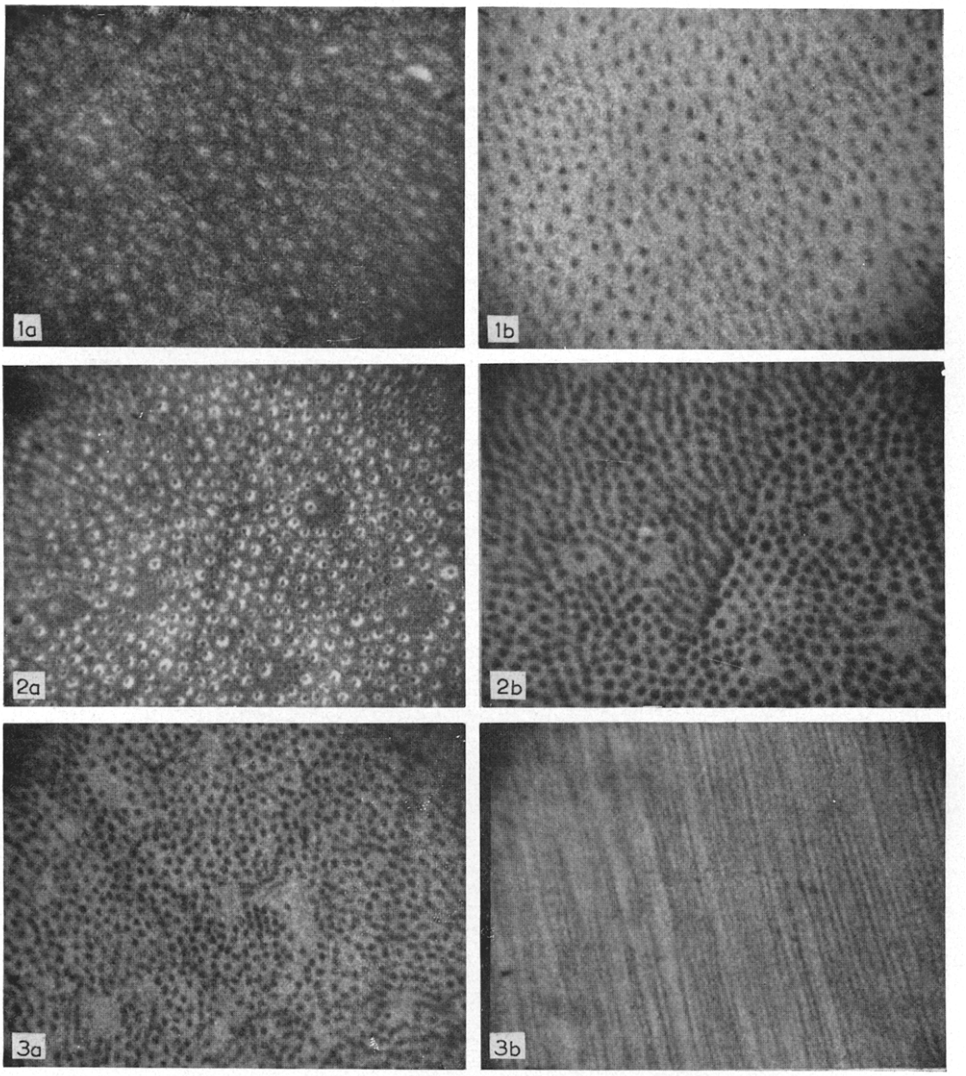

Fig. 1. (a) A microradiograph made with titanium radiation of a ground section of healthy human dentine in a region not far from the enamel-dentinal junction. The magnification in this and all other photographs of this paper is 500 ×. (b) A micrograph of a field adjacent to that of Fig. 1a after decalcification of the section with EDTA. Aluminum radiation.

Fig. 2. (a) A region of healthy dentine where most of the tubules are incompletely filled with calcium phosphate. Titanium radiation. (b) The same region as that of Fig. 2a after decalcification of the section with EDTA. Aluminum radiation. The opacity of the intertubular spaces is due to the residual organic matrix.

Fig. 3. (a) A transverse section through undiseased dentine close to the pulp. There is little calcium within any of the tubules. Titanium radiation. (b) A longitudinal section through a region close to that providing Fig. 3a. Titanium radiation.

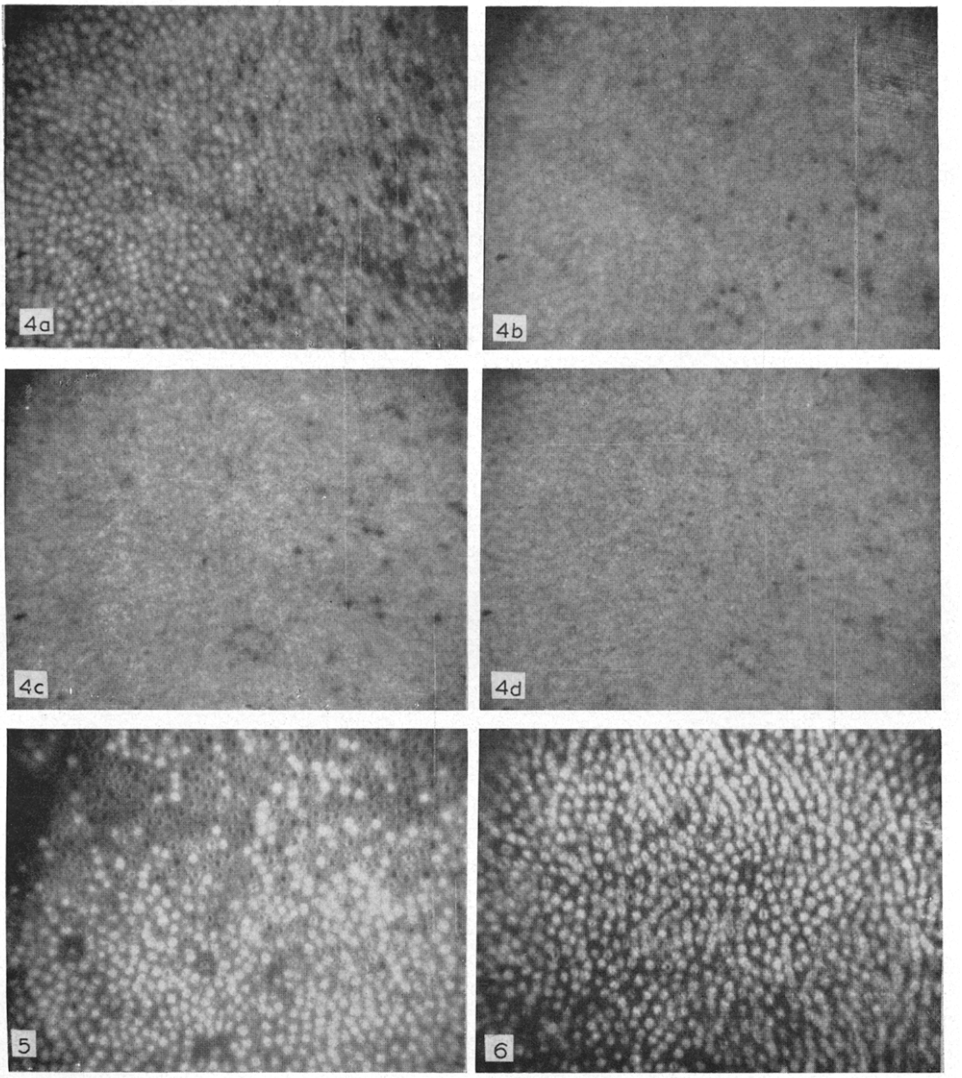

Fig. 4. (a) A section through carious enamel. Most of the tubules are more or less completely filled with calcium phosphate but a certain number, on the right side of the photograph, appear completely transparent. Titanium radiation. (b) The same section photographed with calcium radiation. Most of the contrast has been lost. Most but not all of the "empty" tubules of Fig. 4a remain transparent. (c) The same section photographed with silicon radiation. There is so little contrast that the tubules filled with calcium phosphate are hard to distinguish from the intertubular matrix. (d) The same section photographed with aluminum radiation. The absorption of the organic material is sufficient not only to suppress the contrast between tubules and matrix but to render nearly invisible the "empty" tubules of Fig. 4a.

Fig. 5. A section towards the border of a cavity showing many calcified and partly filled tubules. Titanium radiation.

Fig. 6. A region with a carious area showing many calcified and "ringed" tubules in a matrix that seems deficient in calcium phosphate. Titanium radiation.

noted since early times and has been recently demonstrated by heterochromatic microradiography[4,5]. Close to the pulp, partly or seemingly completely empty tubules have been numerous (Fig. 3).

Within and in the neighbourhood of cavities the tubules have stood out with special clarity. In the titanium pictures fully calcified (opaque) tubules have been interspersed with many that appeared entirely empty (Fig. 4a) in an intertubular matrix rather poor in calcium (Figs. 5 and 6). As one would expect, most of this high contrast has been lost in the calcium photographs (Fig. 4b); when photographed with silicon (Fig. 4c) and aluminum (Fig. 4d) X-rays the tubules, even those that were transparent to titanium radiation, are scarcely discernible. Since the latter manifest a considerable opacity to these very long X-rays, it is obvious that they are not indeed empty but must contain organic matter.

The relative distribution of the inorganic and organic matter in the tubules and intertubular spaces of normal dentine is seen in radiographs of a section before and after demineralization. Thus Fig. 1b is an aluminum photograph of a field adjacent to that of Fig. 1a made after treatment of the section with EDTA; Fig. 2b is the same field as Fig. 2a after decalcification. There is complete reversal of contrast through this treatment, the initially more opaque tubules becoming more transparent than the spaces between.

DISCUSSION

The foregoing photographs are presented, not as a specific contribution to the histology of teeth, but as an illustration of the possibilities that lie in the use of characteristic X-rays for microradiography. Besides the kind of information about details visible at several hundred times magnification shown in these figures, the examination of larger areas at low magnifications can contribute much concerning the processes of calcification and decalcification. Thus around areas of decay we have often seen very sharply delimited zones of reduced calcium phosphate content, and sometimes especially strongly marked zones of an enhanced opacity (in titanium photographs) that could represent bands of secondary calcification. Within carious areas it is easy to distinguish, at both high and low magnifications, the progressive loss of calcium and then of organized structure which is decay. It is proposed in subsequent work to illustrate these applications to developing and diseased teeth and to indicate what this form of microradiography can show about the structure of healthy and diseased bone.

There are a number of ways in which the techniques employed here can profitably be developed and improved. The X-ray tubes were not specifically designed for this type of microradiography and it would not be difficult, in the light of the experience already gained, to construct others far more convenient to use and giving X-ray beams of much greater intensity. Further work is needed to show how these low voltage tubes should be operated to give an output as monochromatic as possible. This involves the selection of suitable materials for targets, a determination of optimal voltages and the production of filters to improve the monochromatic character of the X-ray beam. The microradiographs of this paper yield qualitative information only. In principle this information could be made quantitative though at this time, when the possibilities of the method are so inadequately known, it is improbable that such results would be worth the effort they require. Nevertheless it would not be hard to

render this microradiography semi-quantitative through the use of standard absorbers to be photographed at the same time as the specimens. All these developments are straightforward and of an engineering character and it is intended to introduce them as the work proceeds.

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