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A METHOD FOR COMBINED MICRORADIOGRAPHIC AND HISTOLOGICAL ANALYSIS OF NON-DECALCIFIED HARD TISSUES

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Sectioning of non-decalcified specimens of hard tissue from adult subjects is described. Frozen sectioning using the Jung K microtome was employed. Sections 10 to 50 μm, in special cases 5 μm in thickness were obtained. The entire specimen as well as sections of it could be roentgenographed by different methods including microradiography. The sections could then be histologically stained in the usual way. Thus the same section can be made to yield more than one type of information, and interesting correlation studies become feasible.

Sectioning of hard tissue is usually preceded by a decalcification procedure, which has several disadvantages. One is that it can interfere with the substances to be studied. Furthermore, it becomes impossible to carry out analytical procedures based on the presence of salts, studies involving macro- and microradiography and autoradiography, for example.

In certain types of studies it would thus be advantageous to be able to make serial sections of a specimen without first removing its inorganic components. Such a technique ought to meet the following requirements: It should

preserve all of the macro- and microradiographic characteristics of the specimen

permit uniform sectioning so that the sections can be used for quantitative analyses

permit the use of the usual histological stains

be applicable to the sectioning of large specimens, in which the morphological features, regardless of size, must be strictly preserved.

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Of different techniques tried, frozen sectioning according to *Ullberg* (1958), came closest to satisfying the above requirements. The specimens are embedded in sodium carboxymethylcellulose, in which they are frozen. This method has been in use in a number of laboratories for many years. However, no method for sectioning hard tissues such as dentine or adult bone specimens has come into routine use. The lack of success with these types of specimens is mainly due to the lack of suitable apparatus.

This report points out the possibility of sectioning non-decalcified hard tissue when heavy microtome equipment is used.

METHODS AND MATERIALS

Microtome. The Jung microtome K for cryostat installation, which means that it was cast in corrosion-resistant metal. The distance between the knife and the specimen table was increased 30 mm to permit sectioning of thick specimens.

Power source. An electric motor (380 V delivered by Jung, Heidelberg). The speed of rotation could be regulated continuously without stepwise changes.

Knife. Jung K2 knife for medium-hard material.

Cryostat. A 500 liter standard deep freeze in which the microtome was mounted. The motor and the controls were placed externally.

The base plates were constructed of double standard profiles in brass (50×10 mm in transverse section). Retention screws were of the same material.

Sectioning. Sectioning and collection of the sections was done with the microtome on motor drive in order to guarantee an even rate of feeding. The sections were transferred to tape (Scotch tape no. 810) according to the technique described by *Ullberg* (1954). For routine work the sections were 20 μm thick, but sections up to 50 μm thick or as thin as 10 μm have been used for special purposes. However, specimens from very young individuals, or from other species such as mouse were cut in 5 μm sections.

Freeze-drying. The sections were allowed to remain in the cryostat. The time required for drying was about 10 days. The freeze-dried sections were stained with histological staining procedures and microradiographed. The stains routinely used were Hansen's trihematoxylin, alizarin combined with methyl blue, Bock-hematoxylineosin and alcian blue-PAS. Staining was carried out with the section still on the tape. The tape was removed when the section was mounted. Euparal was the mounting medium used.

Microradiography was performed with the section plus tape in direct contact with the X-ray plates. Kodak Maximum Resolution Plates. The same section was then histologically stained. Philips DS X-ray diffraction generator (1009/30) was used at 30 kV 22 mA. Cu anode, Ni filter.

Material. Hard tissue specimens from man and cat. The structures sectioned were the temporal bone including the pars petrosa, and the mandible and maxilla, with and without teeth.

RESULTS

Hard tissue from both young and adult subjects of both species was sectioned. The possibilities of this method of sectioning are demonstrated by the illustrations, which are of specimens from a human temporomandibular joint (Fig. 1) and from the jaw of a cat (Fig. 2). It was possible to section all hard tissues with the exception of tooth enamel. The artifacts that can occur in the form of crackles or occasionally as breaks in the bone surface seldom create problems in interpretation (Fig. 2). The sections obtained differ from sections prepared with conventional techniques, which is also apparent from the illustrations. The quality of the sections depends on the hardness of the specimen and also on the size and geometry. It is, for exampel, more difficult to cut sections of teeth than sections of bone (Figs. 3, 4).

DISCUSSION

Comparison with other techniques

Serial sectioning of hard tissue without previous decalcification can, for certain purposes, provide information which could otherwise require two histological techniques, namely serial sectioning of a decalcified specimen plus the preparation of a ground section of a non-decalcified specimen. Serial sections of non-decalcified tissue are suitable for evaluation at the macroscopic level, but can also be used for microscopic analysis. However, one cannot expect histological details to be preserved to the same degree as in serial sections of a decalcified specimen. This difference is partly due to the fact that it is nearly impossible to obtain serial sections of non-decalcified tissue of adult specimens thinner than about 10 μm. This is, however, much thinner than the ordinary ground section. Unlike ground sections, the non-decalcified sections contain minor crackles. These can be kept below a tolerable level if the apparatus is in proper condition, and the speci-

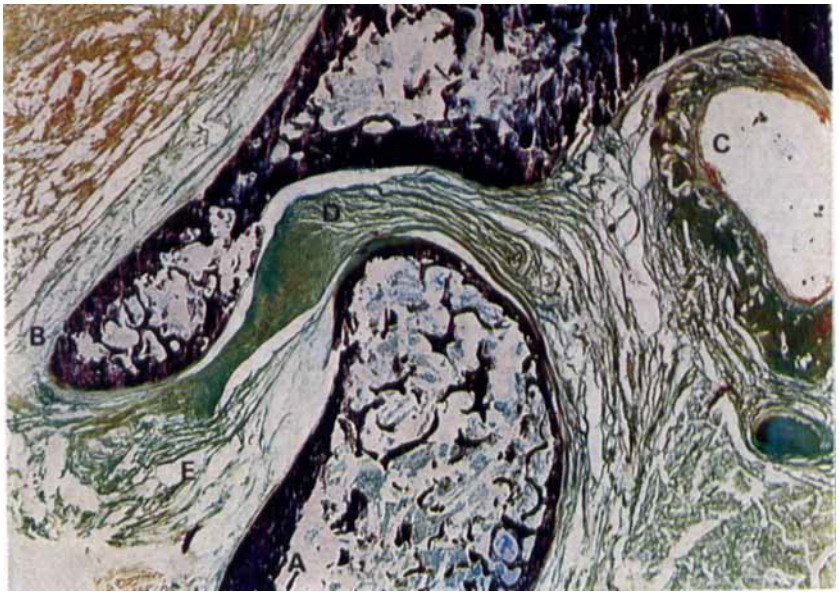

Fig. 1. Human jaw. Male 53 years old. Stain: alizarin methyl blue. Section 20 *µm*. Anatomy, Figs. 1 and 3: A. head of the mandible, B. articular tubercle, C. external auditory canal. D. articular disc, E. fibres from the lateral pterygoid muscle, F. auditory ossicles.

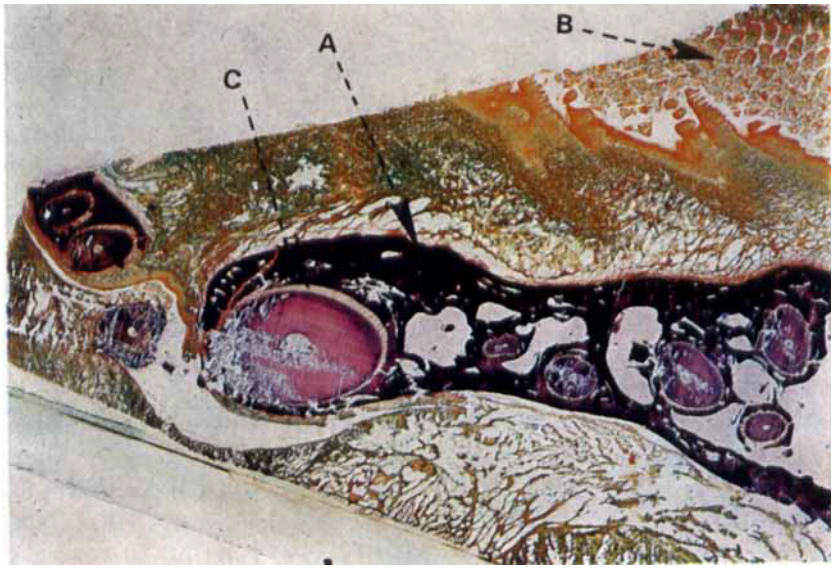

Fig. 2. Cat jaw. Stain: alizarin methyl blue. Section 20 *µm*. A. jaw bone, B. tongue, C. Sharpey's fibres.

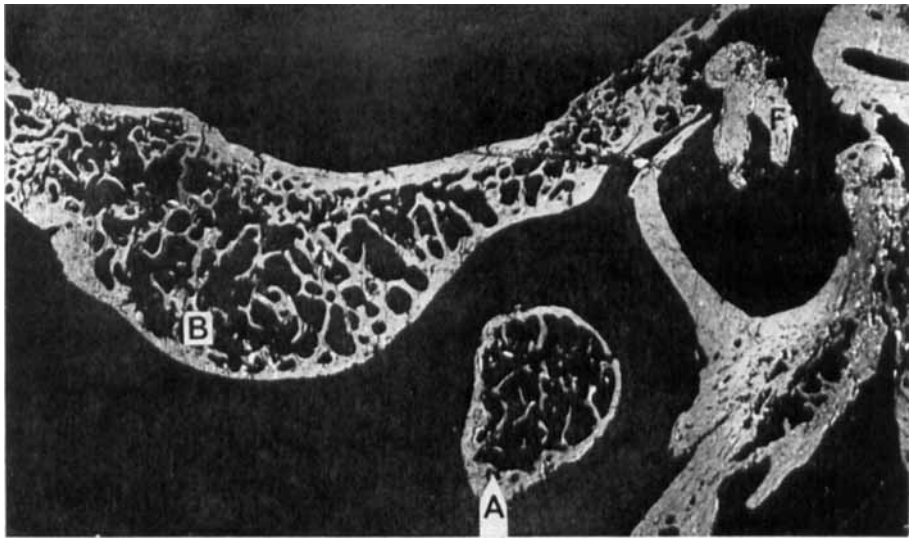

Fig. 3. Microradiograph of a human temperomandibular joint. Male 53 years old. Section 20 μm.

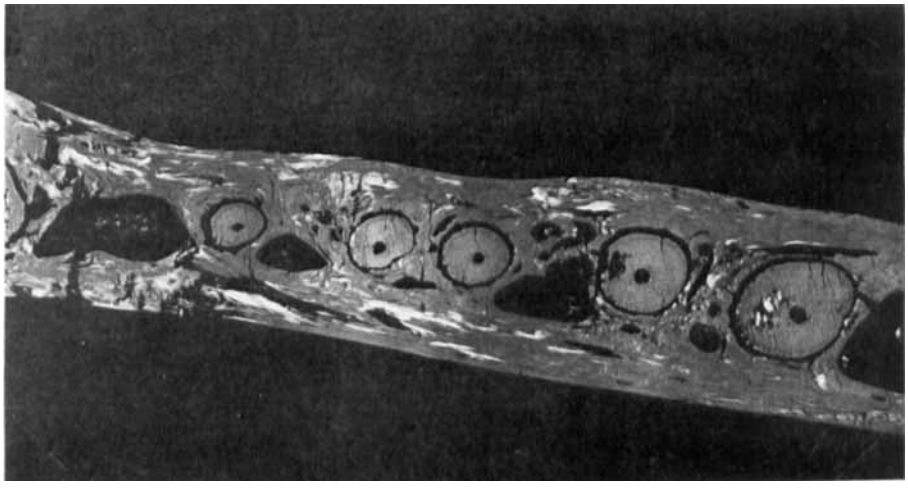

Fig. 4. Microradiograph of a jaw from an adult cat. The teeth are cut transversely. Note that the teeth are fairly well centered in the alveoli even after sectioning. The pulp cavities are intact. There are minor cracks in the bony surface and in the dentine caused by the knife edge.

men properly embedded so that all parts are surrounded by the embedding medium. In spite of the drawbacks mentioned, serial sectioning of non-decalcified tissue has the advantage of permitting one to follow processes of interest, section for section, without the loss of valuable material, as in ground sections. There is no interference by chemical agents, as in decalcified sections. The main advantage of the method, however, lies in the fact that one and the same specimen can be roentgenographed before and after sectioning, using macro- and microradiography respectively. Autoradiography and fluorescence studies on tetracycline-marked preparations, as well as the common histological staining methods, can also be carried out on the same or adjoining sections. This makes possible interesting combinations and correlations in research on the physiology and pathology of bones and teeth.

Knives

A prerequisite for the succesful sectioning of hard tissue from adult subjects is access to a heavy, stable microtome, which keeps the knife and the embedded specimen in a fixed position to each other. Various combinations of knives have been tried. A robust knife with a wide edge angle (44°), Jung K3, crackled the preparations more than could be accepted. The Jung K1 knife, with a narrow edge angle (15°), was advantageous for sectioning, but the blade developed fractures almost at once. The Jung K2 knife was chosen as a compromise (35° edge). This knife gave sections with a moderate degree of crackling. The knives usually held for 1000 sections when working with the cortex of adult bone, but in certain situations, for example if the cortex was too hard, if the specimen was unfavourably oriented with respect to the cutting edge, or if the section contained enamel or dentine, the edge might develop fractures after a couple of sections.

Thickness of the sections and sectioning capacity

In order to obtain sections of uniform thickness, such as are required for quantitative analyses, the resilience of the embedding material must be taken into account. When the knife encounters a harder part during sectioning, an uneven section may result, due mainly to the elasticity of the embedded specimen as a whole. The unfavourable effects of this resilience decrease as the height of the embedded specimen is decreased. There is a

greater risk of variations in thickness if the specimen contains components that differ markedly in hardness, and which, in addition, may be unevenly distributed. A smooth cutting movement, such as can be achieved with a motor-driven microtome, obviously counteract tendencies to variation in thickness and gives sections more uniform in quality and with fewer crackles. The angles of the cutting edge and of the knife as a whole are also important factors in obtaining sections of uniform thickness. The optimal angle is related to the properties of the single specimen.

The size of the embedded specimen that can be sectioned is limited by the breadth of the knife, but also of the hardness of the specimen. With the K microtome it is theoretically possible to section specimens 80×200 mm in area. The specimens shown in this report were embedded in a block measuring $50 \times 50 \times 120$ mm, where 120 mm is the length.

Histological staining

At first, the sections were stained and mounted while still on the tape. This gave rise to difficulty in microscopic examination due to diffusion of light in the tape. However, the greatest disadvantage was in mounting. Certain stains caused the tape to shrink; this was very marked when staining with alizarin-methyl blue. It was necessary to apply pressure to the coverglass when mounting. Such a mount, if acceptable to begin with, was soon ruined by further shrinkage of the tape which, among other things, allowed air to enter under the coverglass so that the preparation dried out. Later, to avoid these problems, the tape was removed before the coverglass was applied. The ease with which this could be done depended on the degree of adhesion between the stained section and the tape. This was most easily separated after staining with alizarin-methyl blue. There was more adhesion after the use of other stains. The tissue structures were locally disorganized when the tape was removed after the use of Hansen's stain. This was most marked with the joint specimens, in which a discontinuity in the tissue slice, due to the presence of the joint space, made the sections especially susceptible to distortion.

Comparison between stained frozen sections, and stained sections of decalcified specimens reveals that the former sometimes contain impurities, probably originating in the glue layer of the tape used. Thus one have to change staining as well as rinsing baths more often than in conventional histological technique.

Finally it may be added that it has been possible to omit the freeze drying and stain the sections directly after sectioning. Even remove of the tape has been successful. This direct technique is interesting because of the elimination of changes in the sections during the freeze drying period.

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