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A METHOD OF PREPARING GROUND SECTIONS FOR MICRORADIOGRAPHY AND AUTORADIOGRAPHY

with a description of a grinding apparatus

by

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The preparation of ground sections for microradiography and autoradiography from specimens consisting of a combination of soft and hard tissues — whole jaws containing teeth, and tooth germs, for example — presents a number of technical difficulties beyond those encountered when the section is intended for ordinary optical study under the microscope. Thus, before x-ray exposure the section must be detached from the carrier to which it was secured during the grinding operation. It must then be manipulable independently of the carrier. The same applies in autoradiographic examination when performed by placing the labelled section between two photographic films.

The specimen must be embedded prior to grinding so that the soft tissues are not impaired or displaced in relation to the hard tissues. The introduction of methyl methacrylate as an embedding medium has improved the possibility of grinding thin sections (*Sognnaes,* 1947).

After embedding, the specimen is cut into thin slabs. As these generally cannot be made sufficiently thin for immediate study in the microscope, and are scored by the saw, they must be ground thinner and polished. During the grinding procedure the slabs are usually affixed with Canada balsam (*Patten & Chase,* 1925; *Bödecker,* 1926) or Kollolith (*Meyer,* 1925), on specimen carriers of various kinds.

There are various procedures for obtaining thin sections. *Meyer* (1925), *Gustafson* (1953) and *Ørvig* (1952) recommend manual grinding on glass plates with abrasive powder in liquid; hand grinding may also be done on rotating discs (*Sognnaes,* 1947; *Gustafson & Kling,* 1948). A number of wholly mechanical devices for grinding have been designed (*Lefkowitz,* 1941; *Smreker,* 1943; *Welander,* 1946; *Laude et al.,* 1949; *Jansen,* 1950; *Leblond et al.,* 1950; *Fosse,* 1954). None of these procedures is directly applicable to the purposes mentioned above and a more suitable one is presented in this article with a description of the apparatus used for grinding the sections.

METHOD

Treatment of specimen prior to grinding

Before embedding the specimen is fixed. Ten per cent neutral buffered formalin (*Lillie,* 1948) is used to prevent dissolution of the calcium salts. The specimen is then dehydrated in ascending grades of alcohol and embedded in methyl methacrylate (*Sognnaes,* 1947; *Sognnaes et al.,* 1949). The specimen so embedded is cut into serial sections, for which purpose a rotating diamond saw of 75 millimetres radius is employed.

Mounting and grinding

The slabs, 400 to 600 μ in thickness, are ground thinner after affixing to the specimen carrier of plane glass. (Cleaned 9×12 centimetre photographic plates have been found suitable for this purpose). The surface of the carrier is covered with double-coated Scotch tape.[1] The protective paper having been removed from the tape, as many slabs as possible are pressed on the carrier and grinding may be commenced immediately (Fig. 1). On the other surface of the carrier a rubber suction cup is attached to serve as a holder.

The grinding is performed by means of fine resin bonded silicon carbide abrasive paper[2] which is attached with double-

[1] *Scotch Pressure-Sensitive Tape, No. 400; double-coated tissue;* Minnesota Mining & Mfg. Co.

[2] Wetordry, Tri-M-Ite; Minnesota Mining & Mfg. Co.

coated tape to a plane surface. Abrasive paper is used, with grit Nos. 120, 240 and 600, in this order. The grinding is carried out dry and with gentle pressure on the specimen-holder to avoid the generation of frictional heat. At the same time the likelihood of fracturing the specimen is reduced. The finishing is effected with Emery polishing paper, grit No. 4/0[1].

When one side of the section has been polished the carrier and section are placed in a benzine bath until they separate. They are then dried and new tape is placed on the carrier. The section is pressed on, finished side downwards, and the grinding procedure repeated. The thickness of the section may be checked with a micrometer during the course of the work while it is affixed to the specimen carrier and again after the second surface has been polished and the section removed from the carrier. After microradiographic and radioautographic examination the section may be mounted on a microscope slide in the usual manner.

The grinding apparatus

The grinding is performed by means of an apparatus designed for use with different abrasive materials (Fig. 1). The motor and stand are taken from an electric household machine (An Electrolux Assistent). A turnable and a collecting tray for the grindings are made from the two bowls which are standard parts of the Assistent (Fig. 2). On the under side of these there are an axle and a supporting ring, which are removed from the bowl that has been adapted as the collecting tray (Fig. 2 e). In place of the axle a hole is bored in which a tube, 2.8 cm in length, is welded so as to project through the bottom to one half of its length. This tube fits exactly the driving socket on the machine. In another hole drilled near the periphery of the tray is welded an outlet tube 5 cm in length and 1.2 cm in diameter; through this the grindings are expelled (Fig. 2 g). The bowl is cut off to a height of 4 cm and the edge covered by a moulded rubber rim, by way of protection (Fig. 2 a). The collecting bowl is pressed down on the bearing of the machine; to ensure that it goes down far enough a small recess is made in the stand.

[1] Emery Polishing Paper; Norton Berh-Manning.

Fig. 1. The grinding apparatus and accessories. On the turntable is the glass plate carrying the abrasive paper. In the foreground are the grinding discs, micrometer gauge and two specimen carriers with sections of jaws of dogs.

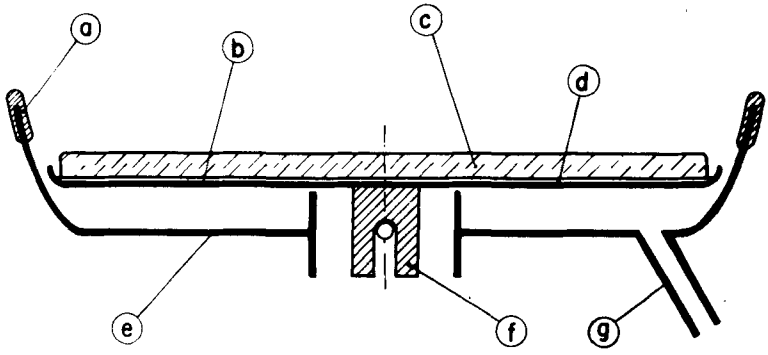

Fig. 2. Diagram of the grinding apparatus. Rubber rim (a), turntable (b), disc of plate glass (c), rubber mat (d), collecting tray (e), axle (f), outlet tube (g).

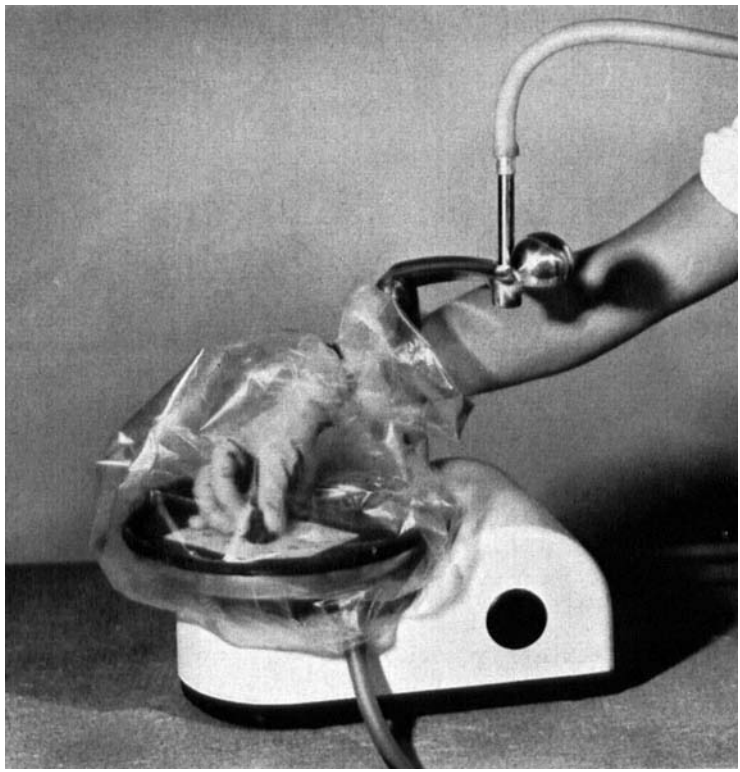

Fig. 3. Grinding under the dust shield. A rubber glove protects the hand against radioactive dust.

The second bowl, modified to fit the turntable, is cut down so that only a flange 0.5 cm high remains (Fig. 2 b). The axle on the bottom of the bowl has been retained and is fitted into the hollowed driving axle of the machine (Fig. 2 f). On the turntable is laid a rubber mat and then a disc of plate glass 0.8 cm in thickness and 20.3 cm in diameter (Fig. 2 d, e). This plate serves as a base for the abrasive paper which is affixed to it with double-coated tape. A separate plate is provided for each type of paper.

During the grinding operation the disc rotates in a horizontal plane at 150 r.p.m. The specimen carrier is moved to and fro over the paper, and the thinner the section becomes the less should be the pressure on the carrier. Two minutes' grinding

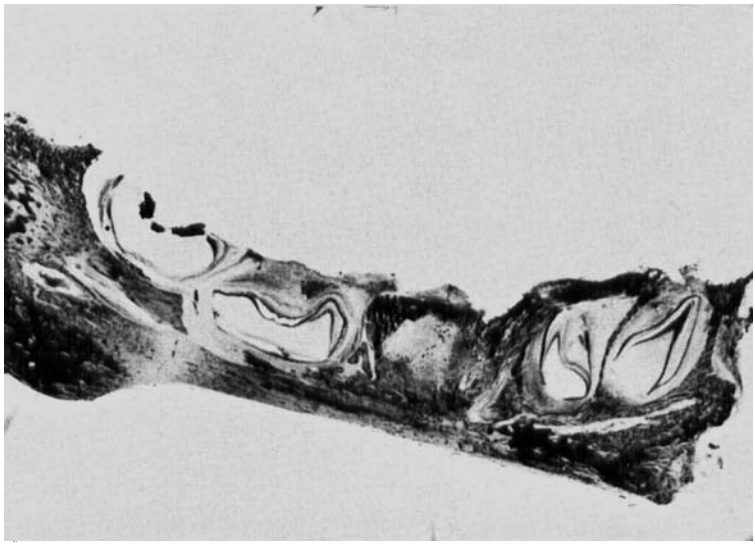

Fig. 4. Photomicrograph of a 50 μ ground section through the mandible of a newborn infant. The tooth germs of 6—, 05—, 02— and 01— are visible. × 2.

with gentle pressure on each of the papers of grit Nos. 120, 240 and 600 followed by polishing on grit No. 4/0 reduces the thickness of the section by roughly 200 μ.

The grindings fall into the collecting tray from which they are sucked away through the tube which is connected by rubber tubing to an air pump. A dust shield (Fig. 3) of transparent polyethylene is tied round the wrist. When grinding radioactive specimens the hand is protected by a rubber glove. On changing from coarser to finer abrasive paper measures should be taken against contamination with grains of the coarser material as the section may otherwise be scored. It is advisable to change the dust shield at the same time as the abrasive paper.

The type of abrasive paper used with this grinding procedure is employed also for wet grinding. With certain histochemical staining methods the specimen cannot be embedded before grinding and must then be kept from drying during the operation. This may be effected by a stream of water directed on the paper by a tube attached to the spring arm of the motor stand. Emery polishing paper cannot be used in wet grinding, the section then being polished on a soft bed with a soap solution of aluminium

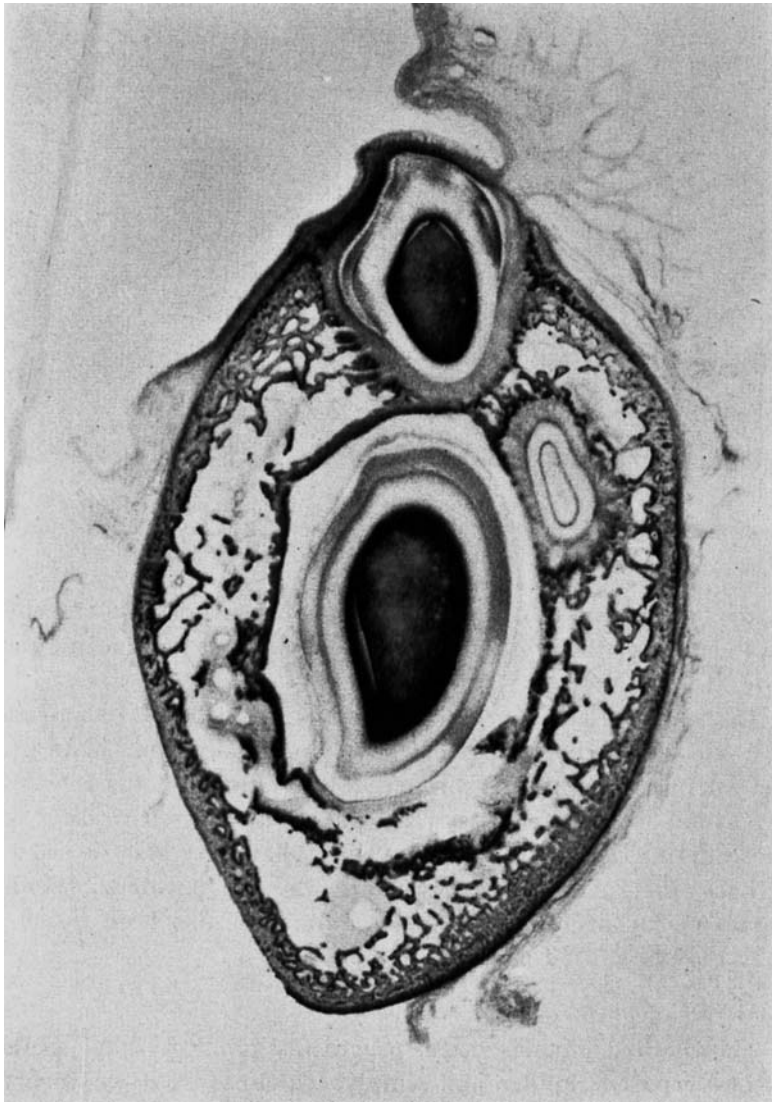

Fig. 5. Autoradiogram of a 50 μ ground cross-section through the mandible of a young dog, S^{35}-labelled *in vitro*, showing first premolar and permanent and deciduous canine. × 6.

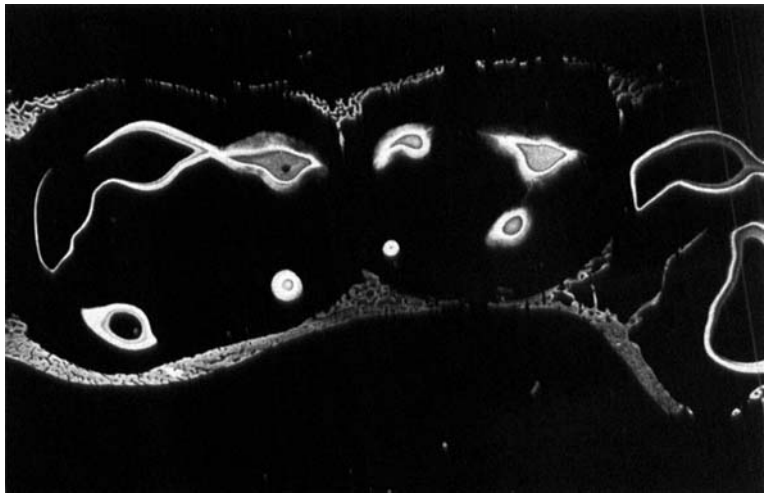

Fig. 6. Microradiogram of a 50 μ ground section from the mandible of a newborn infant — horizontally through the cusps of —05 and —04 and through —03 and —02. × 5.

oxide (grain size 1 μ)[1]. For this purpose a piece of flannel is stretched over the glass plate and turnable and tied on the under side.

The apparatus may be used with other abrasive materials. A series of grinding discs has been made from plate glass the surface of which is prepared by etching or sand blasting to various grades, from very fine to very coarse. Grinding with these discs requires a flow of water. They may also be used as a base for grinding with various abrasive powders. Grinding stones of carborundum and corundum have also been tested, as they may be placed on the turntable.

Some results obtained by the method

The method enables large, practically plane parallel sections to be prepared rapidly and simply. Thicknesses down to about 50 μ are readily obtainable, and in many cases it is possible to grind still thinner without fracturing the brittle dental enamel. The method is economical of time as several sections may be ground simultaneously. The sections are of uniform thickness,

[1] Linde B Air Products, Company.

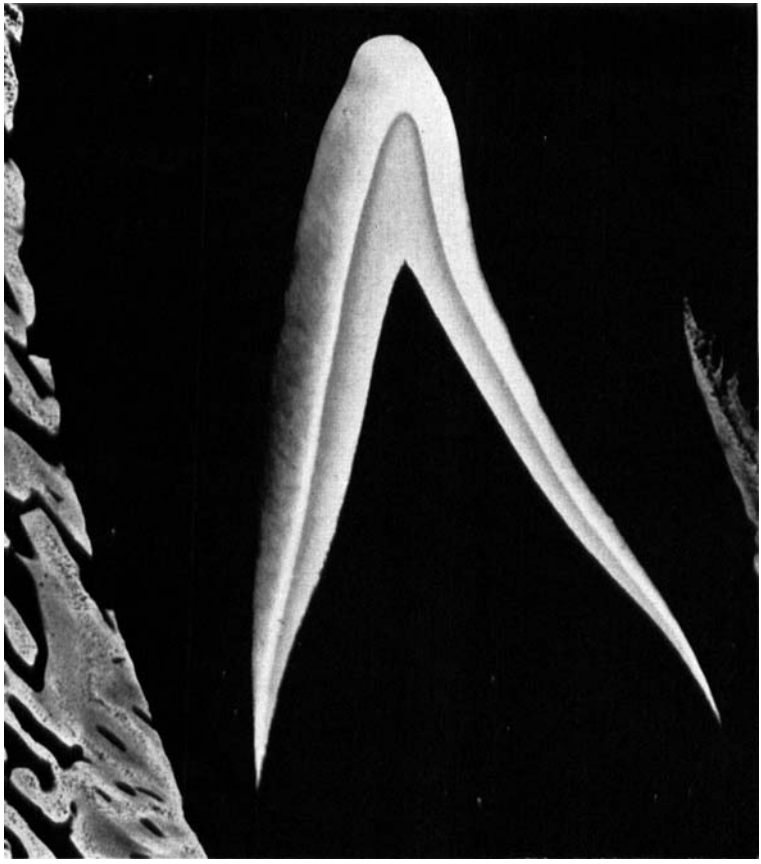

Fig. 7. Microradiogram of a 50 μ ground section through a lower lateral incisor of a newborn infant. The dark parts are regions with low x-ray absorption, indicating a low mineral content. $\times$ 20.

a valuable advantage in the subsequent examination. As an illustration of what is attained Figures 4 to 7 are reproduced.

DISCUSSION

Methyl methacrylate has proved superior to other embedding materials for specimens consisting of both hard and soft tissues that are to be prepared as ground sections. The section embedded in plastic cannot however be fixed in the usual manner or cemented with plastic on plastic plates (*Sognnaes,* 1947), as the material is dissolved by such solvents as xylol and chloroform

that must be used for removing the section from the carrier. The plastic that has infiltrated into the section must be left if thin sections of soft and hard tissues are to be handled apart from the carrier. It is inadvisable to detach the section by means of heat as the plastic has a tendency to buckle. A satisfactory solution to the problem of mounting the section has been found in the use of Scotch tape. The section can easily be removed from the carrier by immersion in benzine that dissolves the rubber film on the tape but does not affect the plastic. The double-coated tape adheres well to the glass and the section, and the mounting has the additional advantage that the grinding may be commenced immediately after the section is pressed on the holder. As many sections may be affixed on the carrier it lies stable on the abrasive paper, thus facilitating the preparation of plane parallel sections.

Wet grinding methods are, however, not suitable for sections attached to the carrier with tape as the paper in the tape disintegrates in water and the sections come away; moreover, a thin embedded section that has become wet sometimes buckles. Grinding on the above-mentioned silicon carbide paper without water has been carried out with satisfactory results. A dry grinding method is also of value in the case of grinding specimens treated with water-soluble stains — for example, basic fuchsin — or water-soluble radioactive labelled isotopes. Silicon carbide paper has proved preferable to abrasive powder in cases where wet grinding is necessary as less manual skill is required and there is less tendency for the abrasive material to penetrate the section. Grinding paper also gives better results than the other abrasive materials tested.

SUMMARY

A method is described for the preparation of ground sections of specimens consisting of a combination of hard and soft tissues. The specimens are embedded in plastic and then cut into a series of slabs. These are ground dry on resin bonded silicon carbide paper and finished on Emery polishing paper.

During the grinding procedure the sections are fastened to the carrier of plate glass by double-coated tape. The sections are removed from the carrier by immersing in benzine. This method

provides sections that may be handled independently of a carrier — a prerequisite for the examination by microradiography and certain autoradiographic procedures.

The grinding is performed with the aid of an apparatus built from parts of an electric kitchen machine, the stand of which is used without modification. The grinding apparatus is so constructed that it may be used also for testing various abrasives employed in the preparation of ground sections.

RÉSUMÉ

METHODE POUR LA PRÉPARATION DE COUPES PAR USURE POUR LA MICRORADIOGRAPHIE ET L'AUTORADIOGRAPHIE

Une méthode est décrite pour la confection des coupes par usures des préparations composées de tissus durs et mous. Les préparations sont imprégnées et incluses dans du méthyl-méthacrylate et ensuite découpées en série en tranches minces. L'épaisseur des tranches est diminuée par usure à sec sur du papier de carbure de silicium imperméable. Le polissage se fait sur du papier-émerisé supérieur. Pendant l'usure et le polissage, les tranches sont fixées avec du papier collant double-face sur des porte-objets en verre à surface plane. Par immersion dans de la benzine les coupes sont séparées de cette plaque.

Les coupes par usures obtenues par cette manière sont possible de manipuler sans base, ce qui est la condition indispensable pour l'examen micro-radiographique ainsi que pour certains procédés auto-radiographiques.

L'usure est effectuée au moyen d'une machine construite de pièces d'un appareil électrique de cuisine, dont le socle est employé sans changement. Cette machine est construite d'une manière permettant également l'essai des différents abrasifs employés pour la préparations de coupes par usures.

ZUSAMMENFASSUNG

EINE METHODE ZUR ANFERTIGUNG VON SCHLIFFEN FÜR MIKRORADIOGRAPHIE UND AUTORADIOGRAPHIE

Es wird eine Methode zur Anfertigung dünner Schliffe von Hartsubstanzen mit dem dazugehörigen Weichgewebe angegeben. Die Präparate werden in Kunstharz (Methyl-Methakrylat) ein-

gebettet und können danach in Serien dünner Scheiben gesägt werden. Die Scheiben werden ohne Wasser auf mit kunstharzgebundenem Siliziumkarbidpapier dünn geschliffen und auf Schmirgelpolierpapier poliert.

Während des Schleifens sind die Scheiben mit doppelseitig klebenden Klebestreifen auf planen Glasträgern befestigt. Durch Eintauchen in Benzin lassen sich die Schliffe wieder vom Träger ablösen. Mit dieser Methode hergestellte Schliffe können ohne Unterlage verarbeitet werden und eignen sich daher besonders für mikroradiographische und gewisse autoradiographische Untersuchungen.

Das Schleifen wird mit einem Apparat durchgeführt, dessen Hauptbestandteile einer elektrischen Haushaltsmaschine entstammen. Der Apparat ist so gebaut, dass er sich für die Benutzung verschiedener Schleifmittel zur Herstellung dünner Schliffe eignet.

Es werden einige Beispiele gezeigt, bei denen diese Methode zur Anfertigung von Schliffen für Mikrophotogramme, Mikroradiogramme und Autoradiogramme angewandt wurde.

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