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A TECHNIQUE OF PREPARING THIN GROUND SECTIONS OF HARD TISSUES: TOOTH AND BONE

by

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INTRODUCTION

In the last years several new methods for preparing thin planoparallel sections of undecalcified hard tissues have been devised. *Hallén* and *Röckert* (1960), *Molenaar* (1960) and *Fremlin et al.* (1961) presented techniques well designed for this purpose. There are, however, some small disadvantages associated with their use. The apparatus of *Hallén* and *Röckert* is relatively complicated and expensive and can only be used in special, well equipped laboratories. By contrast, the method by *Fremlin et al.* is extremely simple, but the specimens made by the use of this method may sometimes be markedly thinned out at the outer part. This may be due to the fact that the specimen tilts during grinding.

A new simple grinding device, which can be used in any laboratory and which is intended for the preparation of planoparallel specimens, has recently been designed by *G. Gustafson* (*Gustafson* and *Gustafson*, 1966) and is shown here in Figure 1. It has been the aim of the investigation presented here to work out a simple and inexpensive grinding technique well adapted to this device and to examine the properties of the specimen produced, particularly the degree to which its sides are flat and parallel with each other.

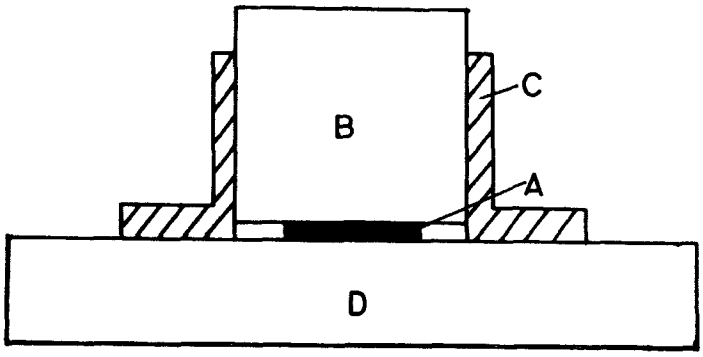

Fig. 1. The grinding apparatus
A = specimen
B = carrier cylinder
C = outer cylinder
D = grinding subsurface (glass)

METHODS AND MATERIAL

Grinding technique

Most of the specimens were prepared from the crowns of vital teeth, mostly premolars, extracted from young adults during orthodontic treatment. Cracks caused by forceps extraction were almost always present in the teeth, and care was taken to exclude them in the final section. Any caries or amalgam was excluded. The teeth were stored in 10 % neutral formalin until used.

Each tooth crown was bisected in a bucco-lingual direction with a dental diamond wheel under running water. The mesial and distal parts of the crown were cut off parallel to the line of bisection to give two slabs of the crown some 4 mm thick. Then the root portion of the tooth was cut off and two sections from each tooth were thus available for further preparation. — The middle part of the crown, which most often is the region chosen for microscopic examination, was thus spoiled. It was, however, considered valuable to obtain two sections from each tooth of very nearly the same material.

The grinding was carried out by hand on thick glass slabs.

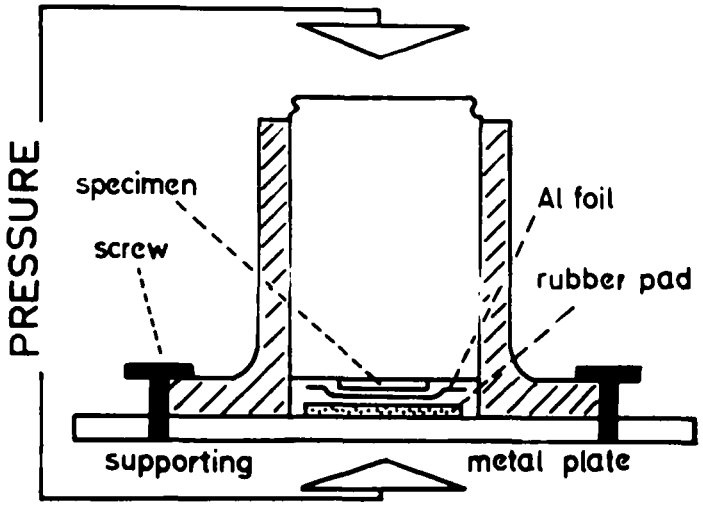

Fig. 2. The specimen is glued to the cooled carrier cylinder. During setting, pressure is applied to this cylinder placed inside the outer cylinder, which in turn is fastened to a metal plate with screws. To prevent cracking of the specimen a hard rubber pad is used and as protection against outsqueezing glue a thin aluminium foil is placed between specimen and rubber pad. This foil can most often be readily stripped off the specimen.

First one surface was prepared without any extra support of the section. This way of preparing the first surface was accepted as it was shown with the measuring techniques described below that the use of the apparatus in this step had no influence on the properties of the resulting specimen.

Grinding was started with various grades of grinding paper*, the section being held in water and the final "paper step" usually carried out on grade no. 600.—. Grinding was continued with an aloxite grinding powder**, grain size 3—22 μ, in water. The final "powder step" was carried out with a finer powder***, grain size 1 μ, dispersed in glycerin. Between each change of grinding material the section was thoroughly cleaned with soap and water.

The prepared surface of the section was then mounted against

* Water proof. silicon carbide paper. The Carborundum Comp., Niagara Falls, N.Y., U.S.A.

** Aluminium Oxide, Optical finishing Powder, no. 50. The Carborundum Comp.

*** 1549 AB Micropolish. Beuhler Ltd. Evanston, Ill., U.S.A.

the inner cylinder of the apparatus with a special glue°, stored in the refrigerator. Cylinder and section were first put in the refrigerator for some 10 minutes, then glued together and left undisturbed under pressure for at least 5 hours (Fig. 2). To secure an even pressure over the section, this had been prepared roughly planoparallel. After setting of the glue the section on the cylinder was ground down to the desired thickness using the same steps as described above. The section thickness was continuously estimated with an ordinary screw micrometer (accuracy ± 5 μ), the cylinder thickness being known and the thickness of glue disregarded. Finally the glue was dissolved by immersion of the specimen and cylinder in a special chemical°°.

Technical remarks

The "paper steps" — which rapidly reduce the section thickness to around 40 μ — were easily carried out. However, as discussed below, the papers caused a gradual grinding away of the periphery of the specimen and therefore when the thickness had fallen to around 40 μ, grinding powders had to be used. It should be pointed out that the grinding papers were not glued to the grinding subsurface and that the specimens were not embedded. If such precautions are taken, paper grinding will probably yield thinner specimens than 40 μ without loss of the periphery. These details were not made use of here, because very thin specimens ($\sim$ 10 μ), which were presently aimed at, must anyhow be finally prepared with powder grinding.

Grinding with the powders was, on account of the hard surface of the glass slab and the thinness of the specimens, carried out more cautiously. Hard pressure resulted in pronounced scratches and with the coarser powder a single large grain could pierce the specimen and split it. Though in fact the 3—22 μ powder together with very gentle pressure could be used below a specimen thickness of 10 μ, the finer powder was usually applied when the specimen thickness reached some 15 μ.

If the final powder was dispersed in water it was nearly impossible to move the grinding device and the specimen against

° Eastman 910 Adhesive. Eastman Chemical Products, Kingsport, Tenn., U.S.A.

°° Dimethylformamide.

the grinding surface without damaging the specimen. Therefore glycerin was used instead of water, but of course any lubricant not offensive to the specimen or the glue could be used.

The way in which the glueing was performed proved to be of great importance. If the cylinder was not cooled down part of the specimen was very often ground away when the specimen thickness, as determined with the screw micrometer, approached 10 μ. It may be that this difficulty in glueing, leaving a wedgeshaped glue layer and a wedgeshaped specimen, is particularly due to the special glue which was used. Moreover, the nature and structure of the surface against which the section is glued may be of importance. Finely made grooves on this surface, intended for squeezing out superfluous glue, were of no advantage. In fact they allowed the introduction of air bubbles between section and cylinder. When a section was glued on an object glass, circularly shaped to fit a cavity made in the cylinder, the time required to dissolve this section from the glass in the chemical was prolonged. Maybe glass is superior as a base surface to metal, and results in a thinner layer of glue and, as a consequence, makes the glue step easier. An inner cylinder of glass is presently being prepared. An ideal glue for the present purpose should be easy to handle, give an extremely thin layer and be easy to dissolve out. The last requirement should involve a solvent which does not affect either the organic or inorganic parts of the specimen.

Evaluating procedures

The final thickness measurements were based on the endprofile technique and carried out at various points of the specimen, scattered in a similar way as the crossmarkings shown in figure 3 (specimen no. 4, Fig. 4). Five independent measurements were made at each point, the thickness being estimated as the mean. A special technique made these measurements permissible: the thin specimen was dried and glued between two transparent plastic quadrants[z]. It could thus be studied from the side with unaided eye or with magnifying glass during the procedure and by grinding the block down stepwise, measurements could be carried out at recognizable levels. A thin layer of immersion

z Bioplastic. Ward's Natural Science Est., Rochester, N.Y., U.S.A.

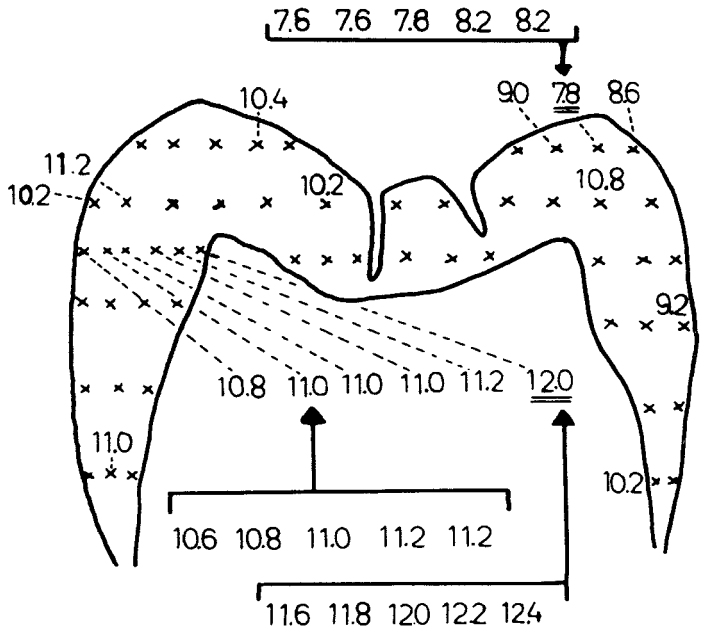

Fig. 3. Schematic representation of the scattered thickness measurements (i. e. the crossmarkings). Specimen no. 4, Fig. 4.

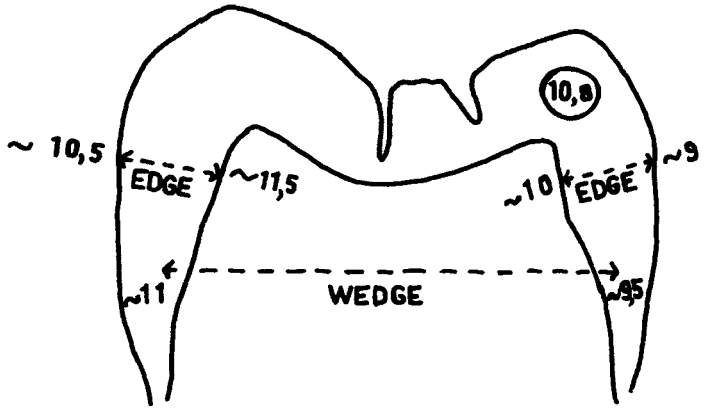

Fig. 4. Schematic representation of the thickness variations in specimen no. 4 (see also figure 3). The encircled value is considered the result of prism orientation perpendicular to the ground surfaces.

oil on the block surface during the measurements eliminated disturbing light glare. Enclosed in this way the thin specimen was held flat and could be oriented in an upright position — any lat-

eral deviation was easily detected in the microscope through the transparent material.

The microscope used was Leitz Ortholux equipped with Leitz Ultropak (obj. × 11 and × 22) and a measuring ocular (× 10). Calibration of the latter was made with an ordinary object micrometer, the unit on the ocular measuring drum corresponding to approximately 0,2 μ using the × 11 objective.

The error in the thickness determinations was estimated as follows. The position of the interface between the specimen and its enclosing material, the glue layer, was determined 5 times. The total deviation of these ocular readings was ± 0,3 μ. Further measurements, up to 20, did not alter the magnitude of deviation. The error in a thickness determination would consequently be ± 0,4 μ (root mean square), and when the mean value of the total deviations from all thickness measurements in the respective specimens was calculated this was always of the same order. Of course, the resolving power of the × 11 objective under the present conditions is around 2 μ. The problem now, however, was to locate the midline within an area and not to resolve two different structures.

The surface structure of the specimens was studied by means of formwar replicas seen under phase contrast illumination using the same microscope as above, together with Leitz phase-contrast equipment (obj. × 40, ocular × 10).

Table I. Results of thickness (in μ) determinations. d = thickness of specimen as determined with a screw micrometer prior to loosening the specimen from the cylinder, n = number of final measurements (endprofile technique), $\bar{x}$ = mean thickness, S.D. = standard deviation, r = difference between max. and min. thickness values.

Tooth	Specimen	d	n	$\bar{x}$	r	S.D.
A	1	10	43	7.3	3.2	± 0 8
	2	12	45	7.3	2.4	± 0.6
B	3	11	49	9.0	3.9	± 0.9
	4	10	53	10.4	4 2	± 0.8
C	5	12	48	8.7	. .	. .
	6	9	45	8.0	3.2	± 0.8

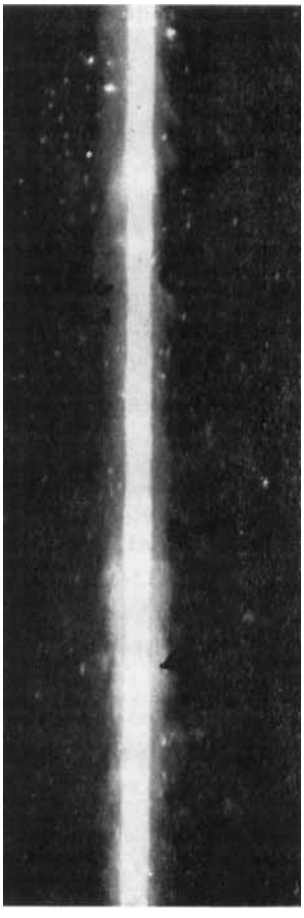

Fig. 5. Thickness deviations in relation to Hunter-Schreger bands. Orig. magn. × 110.

RESULTS

Thickness of specimens

As an example, the results from the thickness determinations of 6 specimens, i.e. 2 specimens out of 3 consecutive teeth, will be given. The presentation of consecutive specimens is made to stress the reproducibility of the grinding technique when deliberately preparing thin specimens. Thickness values expressed in μ are given in Table I and the notations represent: d = thickness of specimen as determined with the screw micrometer prior to loosening specimen from cylinder, n = number of final measuring points (endprofile technique) spread uniformly in the

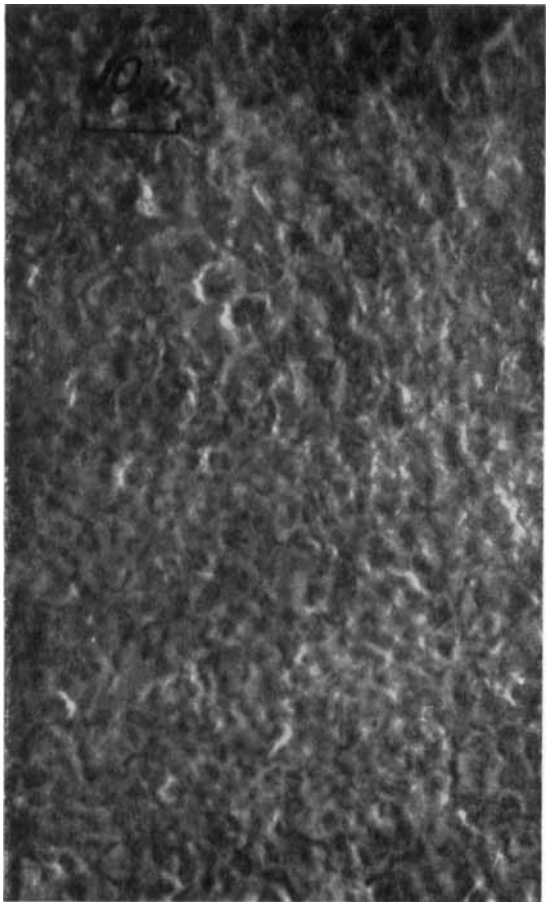

Fig. 6. Surface replica of finished specimen seen under phasecontrast illumination. The prism structure is shown as is a gradual loss of finer detail. Orig. magn. × 400.

enamel of the specimen, $\bar{x}$ = mean thickness, S.D. = standard deviation, and r = difference between highest and lowest thickness values in the section.

Planoparallelity of specimens

In all cases the range (r) of the specimen thickness was greater than the estimated accuracy of the determinations. This indicates what was easily recognized in the microscope, that true thickness deviations always occurred. As, however, the main of the devia-

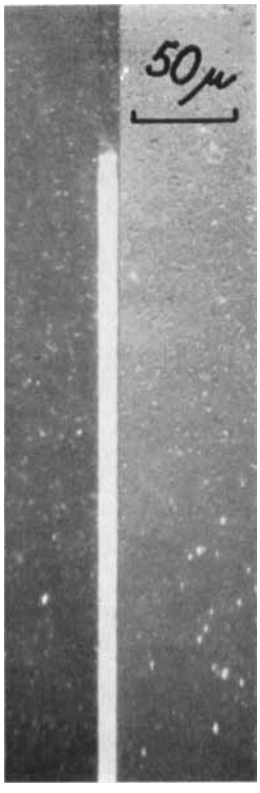

Fig. 7. Area of enamel towards the outer surface of a specimen showing good planoparallelity. Note, however, the extreme outer end. Orig. magn. × 110.

tions were preferentially distributed within the specimens, they will be further discussed.

1. The most obvious form of deviation was a continuous one, the specimen being more or less wedge-shaped. This effect was particularly often marked if the glueing procedure was carried out with non-refrigerated specimen and cylinder and it is thus ascribed to an uneven adhesive layer under the specimen. As stated above, the use of another glue and perhaps a specially selected and prepared cylinder surface might overcome this difficulty and so give less wedgeshaped specimens. *Hallén* and *Röckert* (1960) tested the thickness of their glue film and found it to be less than 0,3 μ, and these conditions might be adapted to the present procedures. With the glueing technique now used, the wedgeshape was not completely eliminated (Fig. 4; compare to Fig. 3). The thickness difference of the wedge could still sometimes

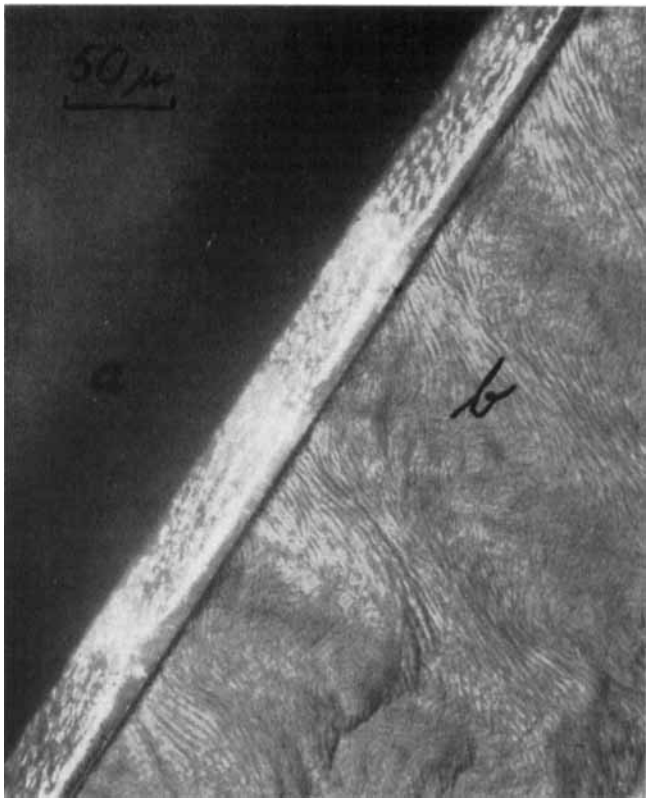

Fig. 8. Profile (a) and transmitted light view (b) of a specimen with one surface prepared by polishing, the other by paper grinding (profile view photographed before etching and staining the specimen). Prisms lying parallel to the plane of the specimen show less resistance to removal.

amount to around 2 μ, this value being the distance between the extreme outer parts of the wedge, adjacent points showing the same thickness. That the glueing of the specimen has to be carried out with the greatest care was illustrated by one of the specimens presented (Table I, nr. 5), which after slipping was forced back into a position in the middle of the cylinder surface. The glueing was thus not perfect and it is clear that an attempt to save a specimen that has slipped in the first attempt by fixing it again to the cylinder is not worth while.

2. Another form of deviation is also continuous as there is a gradual decrease in thickness from the inner of the enamel to-

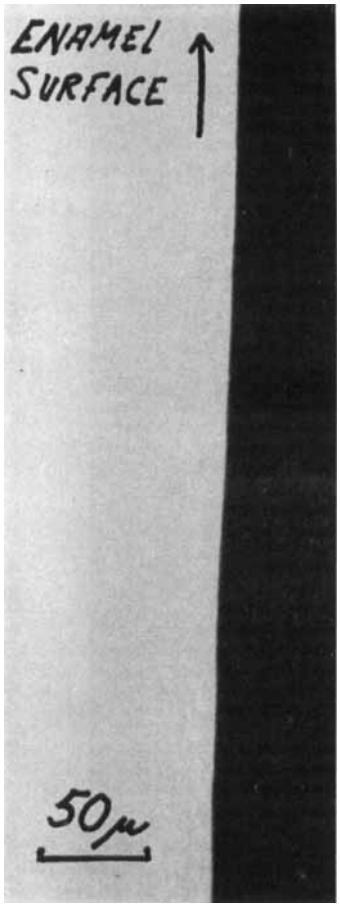

Fig. 9. Surface topography of area perpendicular to the paper prepared by polishing on cloth surface. Outer part of the enamel, showing plane appearance.

wards the outer surface all over the specimen (Fig. 4). This edge-effect was particularly pronounced when specimens during preliminary studies were prepared by paper grinding alone. When successively finer powders were used the edge-effect was considerably reduced but not quite eliminated. Its magnitude was generally in the order of 1 μ.

As to the cause of the edge-effect, two possibilities exist. It could be due to the grinding itself operating preferentially at the advancing edge of the specimen. In this case the edge-effect could be reduced by embedding the specimens. The other possibility is that the edge-effect is the result of the properties of the specimen. The outer enamel presents regularly arranged prisms, ori-

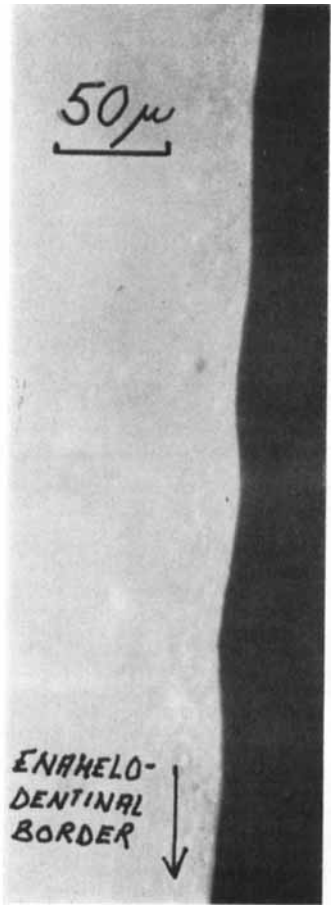

Fig. 10. Same specimen and treatment as in figure 9. Area perpendicular to paper plane chosen towards the inner part of enamel (area of Hunter-Schreger lines) and showing scalloped contour.

ented perpendicular to the tooth surface and thus nearly parallel to the plane of the sections used here. The inner enamel presents intermingled prism groups, of which approximately a half will be more or less perpendicular to the plane of the section. If prisms lying parallel to the plane of the section are more easily ground down than prisms perpendicular to it, the resulting specimen will probably show an edge-effect.

The possibility that both these factors may operate together must be entertained.

3. A third form of deviation in thickness was always found in the specimens and it is considered due to specimen properties. It

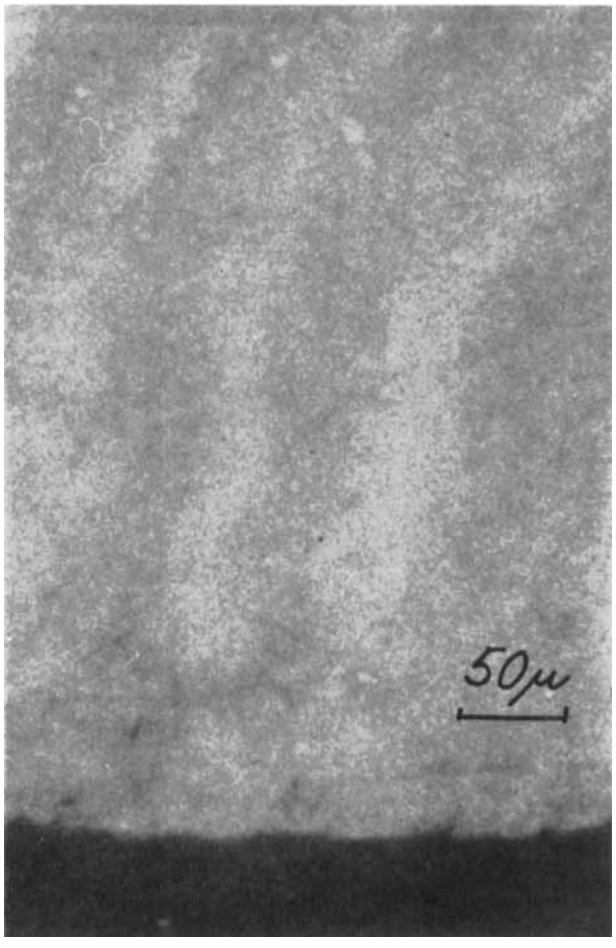

Fig. 11. Microradiogram of thin enamel specimen, showing emulsion density differences in relation to Hunter-Schreger bands.

was found in the inner enamel, i.e. the area showing the Hunter-Schreger bands (Fig. 5). A positive relation between the Hunter-Schreger bands and true thickness deviations was obtained when the thickness of a specimen was determined at right angles to the bands. Then recurring differences in thickness appeared at approximately the same distance from each other as the bands. The magnitude was variable within each specimen, being most marked nearer to the amelo-dentinal junction where enamel tufts

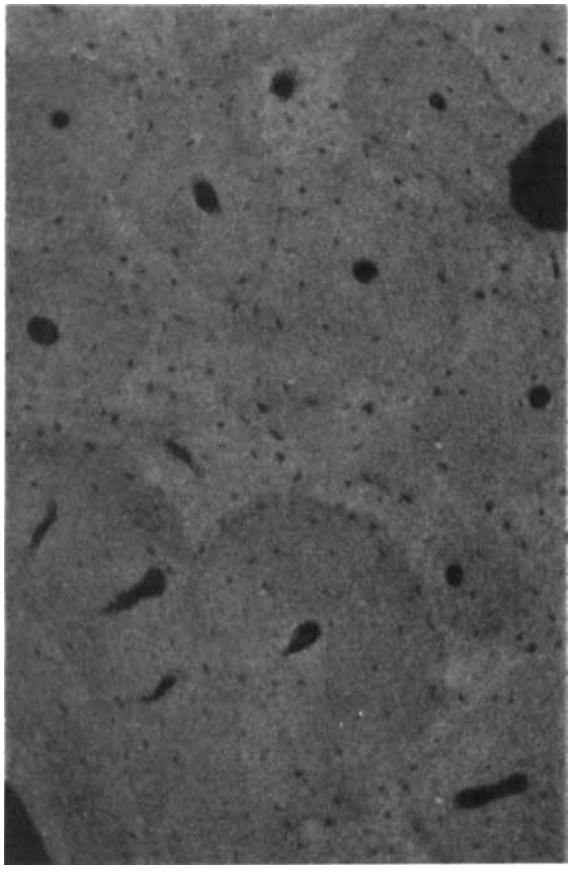

Fig. 12. Microradiogram of thin bone specimen, showing emulsion density differences in relation to haversian and interstitial systems.

may also contribute to the variation in thickness. In the specimens presented it generally did not exceed the order of 1.5 μ.

4. The ultimate deviations from planoparallelity will naturally lie in subtle surface irregularities caused either by scratching or by preferential removal of material due to different resistance between the enamel subunits, i.e. prisms, prism sheaths and interprismatic substance. The final result in this respect with the present technique is illustrated in Fig. 6. It is evident that the surface structure is dependent on the histological structure of enamel. The magnitude of the final irregularities was not further

investigated. It was certainly too small to be estimated by the measuring technique applied.

A definite area, comprising the outer third of the enamel, and thus corresponding to the area where all prisms are most nearly parallel to one another, always showed the best planoparallelity (Fig. 7). Although thickness differences are sometimes found at seemingly impredictable points (see for instance the lowest value in Fig. 3), deviations within this area can generally be related to the preferential removal due to the structure of the enamel subunits.

CONCLUSIONS

Whenever ground specimens of hard tissues are prepared, the final properties of the specimen will be influenced by both technical treatment and structure of the tissue. In particular, polishing the specimens might give marked thickness variations. The dependence of surface structure related to polishing was commented on by *Gustafson* (1945), who furthermore pointed out that the resultant relief, consciously produced, could also be used as an advantage for further investigations of the specimen structure. The influence of treatment and structure is illustrated here by figures 8, 9 and 10. The specimen seen in figure 8 has been treated on one side by paper grinding, on the other side by polishing on a soft subsurface with a grinding powder with extremely small grain size (0,1 μ). Paper grinding has given a flatter surface than the polishing procedure, the result of which is markedly influenced by the structure of the specimen. Prisms lying parallel with the plane of the section have been preferentially removed. Figures 9 and 10 are taken from the same surface of another specimen prepared by polishing. Figure 9 is the aspect towards the enamel outer surface where the prisms are all parallel to each other, whereas figure 10 is taken from the inner part of the enamel where the prisms are arranged in alternating bundles, perpendicular and parallel to the polished surface.

The results obtained with the present grinding technique, also draw attention to the problem of the last preparatory step. The hard surface of the glass slab was chosen as it could be supposed to minimize the deviations from planoparallelity. In spite of this

deviations were found probably due to the properties of the specimen. As pointed out before, glycerin was used in combination with 1 μ powder and with only light pressure in the final preparatory step. It may be possible that the glycerin in reality acts as a soft polishing layer between the surface of the specimen and the surface of the glass slab. A specimen surface appearance similar to that seen in figure 6 has been produced — among others by *Placková* and *Pribyl* (1958) — by polishing enamel specimens on a soft (velvet) subsurface.

To what extent the deviations from planoparallelity discussed in relation to the Hunter-Schreger lines in reality are inevitable in ground sections can not for the present be determined. From a practical point of view, however, the dilemma is evident: thin specimens can not usually be prepared by paper grinding. Powder grinding produces or enhances thickness variations in the specimens. It thus seems as if the desirable specimen properties of thinness and planoparallelity are mutually opposed.

The deviations in thickness in thin specimens might endanger for instance the interpretation of microradiographic results. Figure 11 is a microradiogram of a thin enamel specimen and with an uncertainty in specimen topography the informations from radiolucency and radiotransparency might easily be misinterpreted. Figure 12 is a microradiogram of a thin bone specimen, which shows slight emulsion density differences between the Haversian and the interstitial systems. Detailed thickness measurements of bone specimens have not yet been carried out, but it follows from the study of enamel that, here also, thickness deviations must be accurately known if quantitative measurements are carried out.

An additional point intimately connected with the discussion of last preparatory steps will be mentioned. Lately *Boyde, Switsur* and *Stewart* (1963), while studying enamel surface erosion under ionic bombardment, noticed that differential erosion, — i.e. erosion of material in relation to histological structure — did not commence until a few microns of the surface material had been eroded. They attributed this finding to the polishing applied to the enamel surfaces. Several other workers have studied the result of mechanical damage to hard tissues surfaces. It is evident that a specimen surface may be mechanically damaged in principally two ways, both leaving a view where — in enamel — de-

tails of prism, prism sheath and interprismatic substance are erased. Either the smooth appearance is the result of the plastic deformation of the inorganic material (involving melting in the crystalline lattice — *Berlin,* 1959, *Schmidt,* 1961) or it is the result of a gradual filling up of porosities with grinding dust (*Adolph,* 1958; see also *Mathiesen* and *Fremlin,* 1963). Though the surface appearance of the enamel subunits is preserved with the present technique, the finer details of structure apparently become filled up. The appearance also differs markedly from replica pictures of fracture surfaces of enamel.

SUMMARY

A simple grinding technique to obtain microscopic objects of hard tissues is presented. Use is made of the grinding device designed by *G. Gustafson* (*Gustafson & Gustafson,* 1965), and the procedures result in a reproducible preparation of specimens, 10 μ or less thick.

The planoparallelity of the produced specimens is analysed and discussed and it is shown that deviations in thickness are easily caused if a careful attention is not paid to specimen mounting technique and to the choice and use of abrasive materials and grinding subsurfaces. The presented specimens show thickness deviations in relation to their histological structure, particularly the Hunter-Schreger bands. In thin specimens all forms of thickness deviations must be exactly known to permit quantitative measurements. The specimen surface appearance shows differential grinding in relation to the individual enamel prisms as well as a gradual disappearance of finer detail.

RÉSUMÉ

TECHNIQUE POUR LA PRÉPARATION DE COUPES MINCES DE TISSUS DURS PAR USURE: DENT ET OS

Une technique d'usure simple pour la réalisation de coupes de tissus calcifiés est décrite. Un dispositif d'usure mise au point par *G. Gustafson* (*Gustafson & Gustafson,* 1965) est utilisé et permet d'obtenir des coupes de 10 microns ou moins d'épaisseur de façon répétée.

Le plano-parallélisme des coupes obtenues est étudié et discuté. Il apparait que des différences d'épaisseur sont facilement produites si les specimens ne sont pas montés avec précaution. Le choix et l'utilisation de produits abrasifs ainsi que les supports pour l'usure sont également importants. Les specimens étudiés montrent des différences d'épaisseur en rapport avec leur structure histologique et plus particulièrement les bandes de Hunter-Schreger. Dans les coupes minces, toutes les différences d'épaisseur doivent être connues très exactement pour permettre des mesures quantitatives. L'aspect des surfaces dentaires ainsi préparées montrent une usure différentielle en rapport avec des prismes d'émail individuels et la disparition graduelle des détails les plus précis.

ZUSAMMENFASSUNG

DIE TECHNIK FÜR DIE PRÄPARATION VON DÜNNEN SCHLIFFEN DER HARTEN GEWEBE: ZAHN UND KNOCHEN

Eine einfache Schleiftechnik für das Erhalten mikroskopischer Präparate der harten Gewebe wird beschrieben. Man verwandte dabei den Schleifapparat nach *G. Gustafson* (*Gustafson & Gustafson*, 1965) und die Technik resultiert in einer reproduzierbaren Präparation von Schliffen, die 10 μ oder dünner sind.

Ob die produzierten Schliffe planparallel waren, wurde untersucht und diskutiert, und es ist gezeigt, dass Abweichungen der Dicke leicht zustande kommen, wenn man nicht genügend aufmerksam bei der Befestigung der Schliffe ist, und wenn die Wahl und Verwendung des Schleifmaterials und der Schleifunterlage nicht sorgfältig geschieht. Die präsentierten Schliffe zeigen Dikkenabweichungen in bezug auf ihre histologische Struktur, insbesondere die Schregerschen Streifen. In dünnen Schliffen müssen alle Arten der Dickenabweichungen bekannt sein, um quantitative Messungen des Mineralisationsgrades machen zu können. Die Schleifflächen zeigen differentialen Abrieb in bezug auf einzelne Prismen und wiederum eine gradweise Verwischung der feineren Strukturen.

REFERENCES

Adolph, W., 1958: Elektronenmikroskopische Untersuchungen an maschinell bearbeiteten Dentinoberflächen von Zähnen mit lebender Pulpa. Dtsch. zahnärztl. Z. *13:* 635—644.

Berlin, V., 1959: Elektronenmikroskopische Untersuchungen mechanischer und chemischer Verletzungen des Schmelzes. In *"Proceedings of the 6th ORCA Congress"*. University of Pavia, p. 207—214.

Boyde, A. V. R, Switsur & A. D. G. Stewart, 1963: An assessment of two new physical methods applied to the study of dental tissues. In *"Proceedings of the 9th ORCA Congress"*. Pergamon Press, p. 185—193.

Fremlin, J. H., J. Mathiesen and J. L. Hardwick, 1961: The preparation of thin sections of dental enamel. Arch. oral Biol. *5:* 55—60.

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

Gustafson, G. & A.-G. Gustafson, 1966: Micro-Anatomy and histochemistry of enamel. In *"Structural and Chemical Organisation of Teeth"*. Academic Press. In press.

Hallén, O. & H. Röckert, 1960: The preparation of plane parallel sections of desired thickness of mineralised tissues. In *"X-ray microscopy and X-ray microanalysis"*. Elsevier Publishing Comp. Amsterdam, p. 169—176.

Mathiesen, J. & J. H. Fremlin, 1963: Phase-contrast illumination of surface structure of thin tooth sections. Brit. dent. J. *114:* 354—369.

Molenaar, I., 1960: A new apparatus for the preparation of very thin ground sections for high resolution microradiography. In *"X-ray microscopy and X-ray microanalysis"*. Elsevier Publishing Comp. Amsterdam, p. 177—183.

Plačková, A. & J. Pribyl, 1958: Das Mikrorelief und das Mikroröntgenogram der initialen und der stationären Karies des Schmelzes. Dtsch. Zahn-, Mund- u. Kieferheilk. *28:* 455—463.

Schmidt, A. G. E., 1961: Elektronenmikroskopische Untersuchungen mechanischer und chemischer Verletzungen des Schmelzes. Arch. oral Biol. Special Suppl., *4:* 151—155.

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