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APPLICATIONS OF A DIAMOND KNIFE FOR ULTRATHIN SECTIONING TO THE STUDY OF THE FINE STRUCTURE OF BIOLOGICAL TISSUES AND METALS*

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PLATES 8 TO 15

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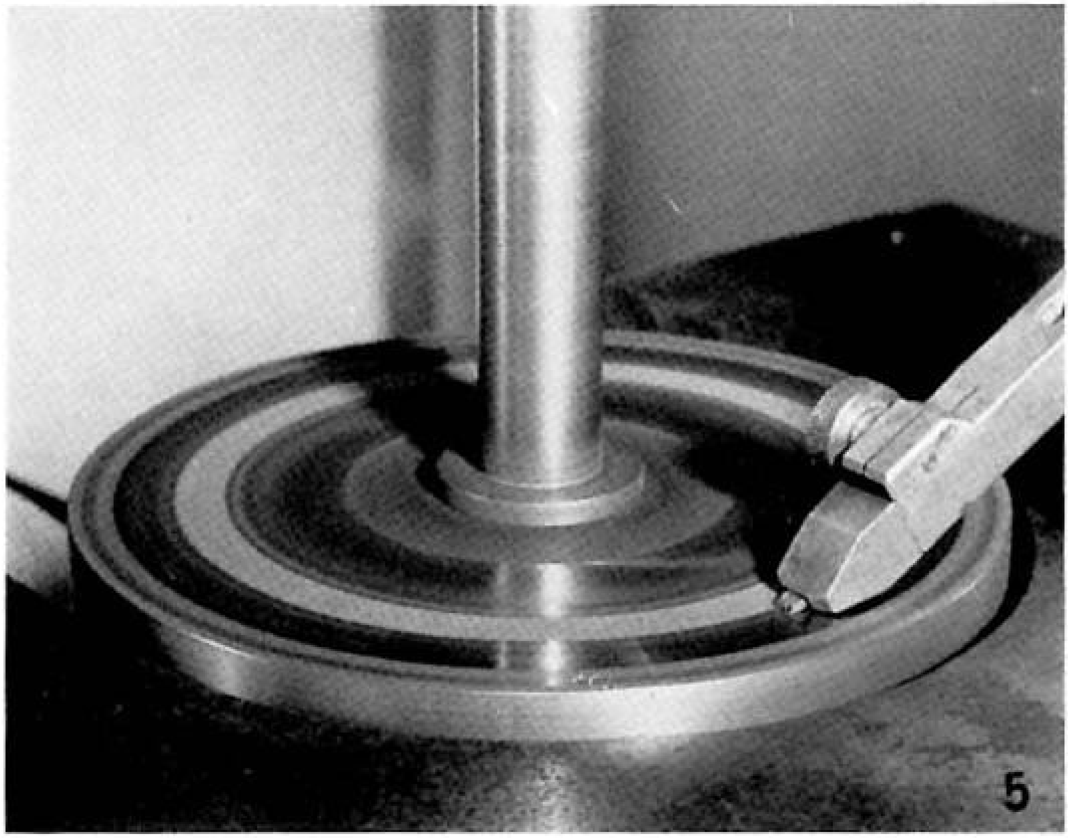
* The results of the investigations presented in this survey report, and the techniques for the production of the diamond knife, are described in detail in a publication now being prepared. The essential technical collaboration of Mr. A. Trommer, J. Weibel, J. Sutter, S. Liendo, and W. Rawyler in the development and testing of the diamond knife is gratefully acknowledged.

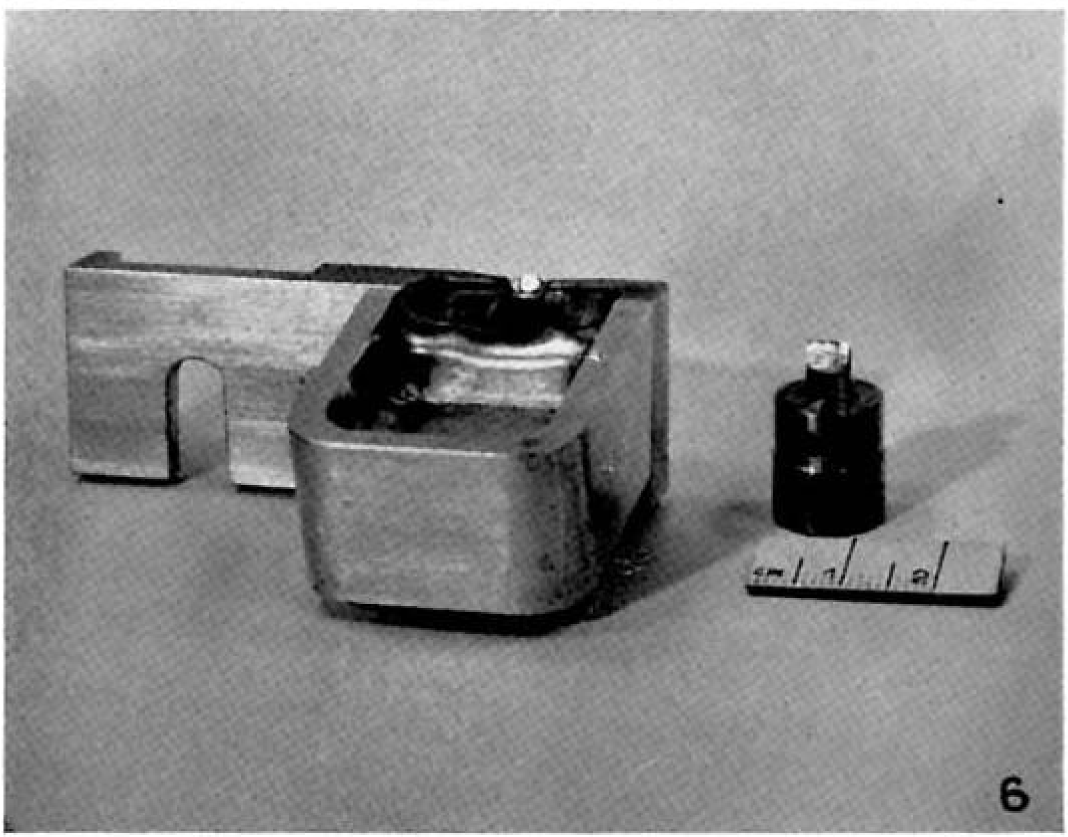
PLATE 10

FIG. 5. Special high speed precision grinding machine for producing the extremely regular diamond knife cutting edge of molecular dimensions. The device consists essentially of a metal disc (scaife) of adequate hardness rotating at high speeds (10,000 R.P.M. or more) on a vertical spindle journalled in special bearings. The optically flat and highly polished upper surface of the disc carries a uniform layer of ultrafine abrasive particles, with an average diameter of 20 to 50 A, which are obtained by a special differential ultracentrifugation process. Careful balancing of the rotating disc together with other design features of the machine eliminates the high frequency vibrations which have hitherto been one of the main limiting factors in obtaining perfect edges. By means of a magnetically supported holder the rough diamond edge (see Fig. 3 *a*) is applied to the rotating surface in such a way that the ultrafine particles carry out their submicroscopic abrading and polishing action along definite crystallographic planes, taking care to orient the "preferred" grinding directions of the diamond parallel to the desired cutting edge. Although grinding along these optimal directions often involves working on the exceptionally hard natural octahedron faces of the diamond, this polishing procedure will result in bringing out a stable array of the unit crystallographic layers (10 to 20 A thick) to form a perfectly regular cutting edge of molecular dimensions. Moreover, since the fine abrasion tracks run parallel to the cutting edge, they will not show up in the ultrathin sections, in contrast to the inevitable knife tracks found in steel and other knives requiring sharpening perpendicular to the cutting edge. Even with this specialized equipment the production of each diamond knife edge requires exceptional skill and usually takes 2 to 3 days because of the frequent occurrence of irregular crystalline inclusions or intrinsic twinning which abruptly changes the selected grinding direction. During the polishing process periodic controls of the diamond edge quality are carried out by means of darkfield observations (see Fig. 3 *b*), microscopic interferometry (Figs. 3 *c*, 3 *d*), and examination of ultrathin sections of plastic-embedded specimens and of aluminum with the electron microscope. Only diamond knives producing perfectly smooth serial sections of large specimen blocks (0.3 to 1 $mm.^2$ face area), which are equivalent to the best sections cut with glass knives, are now considered satisfactory. However, the effort expended is more than compensated for by the subsequent saving in time over a period of nearly a year, considering that several million reproducible sections of soft and hard specimens can be obtained before resharpening is necessary, the latter being readily performed within a few hours. ×

FIG. 6. Diamond knife mounted in the special holder for ultrathin sectioning, and (to the right) the finished diamond cutting edge embedded in its standard metal base. The polished diamond edge is embedded in a special alloy which plays an important role in absorbing vibrations, and this in turn is firmly soldered into a rectangular stainless steel piece. The cylindrical base to which the diamond knife is fastened carries a plastic hood which adequately protects the knife when shipping it abroad. The knife holder shown was originally designed for the Morán ultramicrotome used in this laboratory, but it can be easily adapted to any other type of microtome. It is important to carefully insert the rectangular knife carrier of stainless steel into the special slot of the holder, ensuring a perfect fit in order to obtain a water-tight connection and to prevent vibrations of the knife during sectioning, which would rapidly damage the cutting edge. Although the diamond knife is extremely durable, it is quite sensitive to sharp blows and to the deleterious effect of dust or abrasive particles rubbed against the edge. Bearing in mind that we are after all dealing with a precision edge of molecular dimensions, the diamond cutting edge should be treated with the same alert care bestowed upon the glass knives, if prolonged usefulness is expected. The choice of the optimal cutting angle is critical and must be determined for each type of specimen, after which it remains constant for long periods while using the same specimen. For sectioning plastic-embedded (methacrylate) material a knife facet angle of 40–45° is recommended and a clearance angle similar to the one used with glass knives can be taken as a basis for adjustment. Sectioning hard materials and metals requires diamond knife facets of 55–65° with a correspondingly larger clearance angle.

Concluded on reverse of Plate 10





(Fernández-Morán: Diamond knife and ultrathin sectioning)