Method for Carbon Film Preparation.* (30640)

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Bradley(1) reported a method for preparing carbon films for use as specimen substrates in electron microscopy. Since then, Bradley's technic has been improved and presently carbon films may be prepared by deposition of carbon on the surface of freshly cleaved mica. Other methods have also been developed for making carbon films, including Humphries' technic(2) and the method of Kafig(3).

The main advantage in the method presented here for the preparation of carbon films lies in its simplicity and convenience as compared to other methods. The quality of the carbon films appears to be comparable to those made by other technics.

Materials and methods. Soft glass rods approximately 4 mm in diameter were scored completely around the circumference with the scoring device shown in Fig. 1 (Mergel & Sons, San Leandro, Calif.). This instrument was developed to shear the glass rod as evenly as possible. It consists of a rubber-covered metal drum in front of which a glass rod is positioned on a stage, and against which a glass cutter, oriented perpendicular to the drum and glass rod, can be advanced. The length of glass rod to be scored is regulated by a small bent metal arm located on the righthand side of the instrument. This is attached firmly to a metal rod which can be moved laterally in the base of the device and which is secured by a wing bolt. The glass rod is scored by placing it on the stage so that one end butts against the pre-set regulating arm. The glass cutter is then driven firmly against the glass rod at a distance of 1.5 to 2 cm from the end by means of a rotating knob and screw mechanism. The rubber-covered drum is rotated so as to press the glass rod against its supporting stage, causing it to also rotate. This motion produces a deep score in the glass rod which usually causes it to fracture before it is removed from the instrument.

The small pieces of glass rod produced in this fashion were placed in suitable holes drilled in an aluminum block with the fractured surfaces up.

Carbon was then evaporated onto the broken surface of the glass at a pressure of about 5×10^{-5} μ Hg. During the period of carbon deposition the pieces of glass rod were either rotated through 360° on a turntable or placed directly under the carbon arc in a stationary position. They were then removed individually from the aluminum block and immersed in water at an angle of about 45° to the water surface, causing the carbon to float off onto the surface of the water in intact discs. When occasional difficulty was encountered in removing carbon discs from a glass surface, the edge of the surface was scored. This usually caused the carbon to be released from the glass when introduced into water. Two hundred mesh copper grids (held at their edges with fine-pointed forceps) were soaked in approximately 0.1 M HCL for about 15 seconds, dipped in distilled water to rinse and brought under the carbon discs.

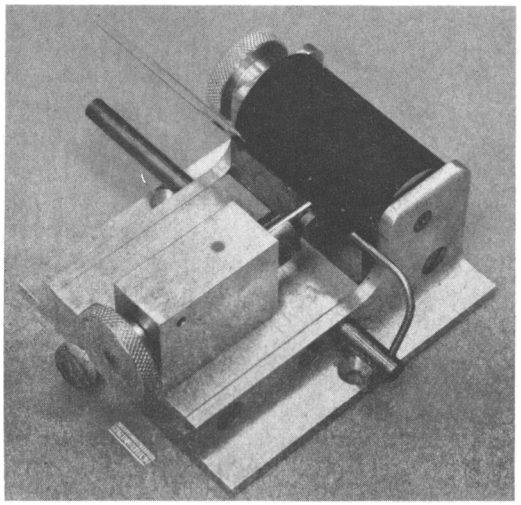

FIG. 1. Instrument designed for cutting glass rod.

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Grids were then lifted to coat them with carbon. Excess water was removed from the grid and forceps by blotting with filter paper from the under side of the grid. Coated grids were then placed on a piece of filter paper, gridside down, and allowed to dry for at least an hour prior to use.

Results. The carbon disc substrates were made slightly larger than the standard grid on which they were deposited by employing 4 mm glass rod. This had the advantage of permitting the carbon film to fold around the edge of the grid as it emerged from the water and thus assist in banding carbon to grid.

Although the surfaces of the fractured glass rods were slightly contoured, this was not reflected in the surface of the carbon films when floated on water or when viewed in the electron microscope. As is the case with carbon films that are stripped from a mica surface, films made as described above appear rather rough to the naked eye as they float on the surface of the water.

Experiment showed clearly that only soft glass could be used as a substrate from which carbon could be floated off after deposition. Attempts to remove carbon from pyrex rod by flotation were completely unsuccessful. If sections of soft glass rod were made by cutting with a glass saw, these also proved unsatisfactory as a base on which to evaporate carbon.

The thickness of the carbon film deposited on the glass rods was evaluated by noting the color of the carbon deposited on a piece of white porcelain on which was placed a small drop of vacuum oil. Films could be successfully removed from the glass rods when the porcelain indicator showed a faint brown color indicating a thickness of about 100 Å of carbon.

In addition to the simplicity and convenience of this method of making carbon substrates it should also be pointed out that after deposition of carbon on glass rods these may be held for a period of at least one to two months before removing the carbon without any apparent deterioration in the properties of the film. It is important, however, to evaporate carbon on the glass rods shortly after they are cut. If rods are permitted to stand for about a day prior to evaporation of carbon on their surfaces, the films tend to shred and adhere to the glass.

1. Bradley, D. E., Brit. J. Appl. Phys., 1954, v5, 65 and 96.

2. Humphries, W. J., J. Cell. Biol., 1963, v19, 634.

3. Kafig, E., J. Appl. Phys., 1958, v29, 1624.

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Urine and Serum Lysozyme Measurement in Renal Homotransplantation.* (30641)

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The major cause of failure in renal homotransplantation, aside from technical difficulties, is rejection of the homograft. Although several signs of ascertaining rejection of the transplant may be present, none is specific and the diagnosis usually is made on the basis of cumulative laboratory and clinical evidence. Recently, several complicated and time-consuming procedures for detection of homograft rejection have been advocated (1-3) but most have proved to be impractical.

Several investigators(4-6) have demonstrated that increased urinary lysozyme activity occurs in patients with a variety of types of kidney disease. Because of the rapidity (20 minutes) and ease of lysozyme assay and since such measurements have been proposed as "an important tool for estab-

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