

The Preparation of Carbon Films for Electron Microscopy. BY M. SPENCER. (*From the Medical Research Council Biophysics Research Unit, King's College, London.*)*

Carbon films prepared by the method of Bradley (1) are commonly used as specimen supports in preference to plastic films because they are more stable under electron bombardment. Their preparation involves, however, a number of operations, in each of which there is some risk of contamination. Techniques have been developed which reduce the number of operations, but they are useful only in the study of particular types of specimen. de Harven (2), for example, has described a method of depositing carbon on sections which are to be studied, instead of on a plastic film which is later removed. He stated that certain precautions were necessary if artefacts were to be avoided. Davies and Wallace (3) have reported that a tissue culture may be grown on a carbon film deposited on glass, the film with its culture being floated off when ready for study and picked up on grids. They found it necessary to deposit films 250 to 300 Å thick in order to float them off without damage. This Note describes a method of preparing carbon films with a measured thickness of about 50 Å, which are suitable for all normal purposes, without the intermediate step of depositing on a plastic film.

The principle of the method is that carbon is deposited on a freshly cleaved mica surface; the resulting film is then floated off and picked up on grids. The use of mica has already been described by Hall (4), who used it in preparing preshadowed replicas. When freshly cleaved it has a surface which is more hydrophilic than that of glass, and a film will usually float off it extremely readily. A carbon film is much more fragile than one of plastic, but by following closely the technique described below it has been found possible to prepare grids carrying practically undamaged films. The average thicknesses of typical films have been measured to within 20 Å by multiple-beam interferometry (5) and found to lie in the range 30 to 70 Å. Shadowed films have been found to show large areas with no structure other than that attributable to granularity in the shadowing metal deposit. The films have been found to be suitable for picking up sections from the surface of a liquid.

Squares of best quality ruby mica¹ about 0.05 mm. thick and 2 cm. square were cleaved in air by

bending down one corner and inserting a needle parallel to the surface; care was taken to avoid scratching the surface, and cleaved squares showing numerous faults were discarded. The squares were transferred as soon as possible to a vacuum chamber, and carbon deposited on them at a pressure of 10^{-4} mm. Hg. It was found best to deposit the carbon as soon as the pressure had fallen to this value. The carbon electrodes were made from $\frac{1}{2}$ -inch diameter rods,² one being shaped to a sharp point and the other ending in a cylindrical section of diameter 3 mm. The evaporation was carried out with a current of approximately 30 amps, and lasted 3 seconds. The mica surfaces were 14 cm. from the arc. The film was then floated off a mica square on the cleaned surface of a solution containing one volume of acetone to nine of water. The acetone was found to reduce slightly the risk of breakage of the film by surface tension forces during the next operation. If a higher proportion of acetone was used the films would not readily float off. One or two cracks corresponding with faults in the mica were normally visible at this stage; if the film was badly cracked, or had only partially floated off, it was swept off the surface and a new square chosen. An average of half the squares made had to be discarded at this stage. The floating film was guided with a blunt instrument until it was above a submerged holder carrying grids (Athene, new 200). The holder was then very slowly raised by a rack and pinion device to pick up the film. The holder was left under cover to dry before the grids were removed. The grids were inspected in a light microscope giving $\times 40$ magnification, and those carrying cracked films were discarded.

The grid holder was specially designed for this technique, and gave better results than a number of other types. Its base was a sheet of phosphor-bronze 0.1 mm. thick and 2.5 cm. square. It was drilled with sixteen holes 2 mm. in diameter over which the grids were laid. It had a raised rim 4 mm. high which held the phosphor-bronze rigid and prevented the film from slipping sideways while it settled on the grids. The thin base prevented large drops of liquid from adhering to the grids on removal from the dish, so reducing the risk of con-

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¹ Mica and Micanite Supplies Ltd., London.

² LINK WLI, made by Morgan Crucible Co., London.

tamination and breakage of the film during drying. It also enable the grids to dry off as rapidly as possible.

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