Brief Notes

The Use of Carbon Films to Support Tissue Sections for Electron Microscopy.

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Since the earliest days of electron microscopy one of the most irksome technical problems has been that of specimen drift. With the application of high-resolution microscopy to thin sections of biological material this problem has become even more acute. Sections mounted on collodion or formvar-coated grids show a persistent tendency to drift which may be minimized by reducing the thickness of the sections as much as possible, by relatively long exposure to vacuum, and by stabilization with the electron beam. The last mentioned procedure is rather effective but is also destructive in that it reduces contrast considerably by adding a layer of comtamination. Finally, exposure to the electron beam usually results in shrinkage of formvar- or collodion-supported sections amounting to perhaps 10 per cent in one dimension. This may serve to enlarge preferentially, those spaces between tissue-laden areas, as between membranes, thus introducing a complex, small-scale distortion of fine structure. In order to minimize sublimation of the embedding material, low beam intensities were used by Sjöstrand (1) and by Rhodin (2) to produce excellent micrographs. While such a procedure appears to be effective, it is inconvenient because of the difficulty at low intensities of scanning for suitable areas and of focusing.

The use of carbon films as support for thin sections has in this laboratory resulted in an almost complete solution to the problems described above. Shrinkage and the specimen drift which accompanies it have been so reduced that excellent micrographs may be taken, as soon as focusing has been accomplished, of sections freshly introduced into the microscope. The strength of the carbon film is such that it is essentially impossible under normal conditions of exposure to the electron beam to tear any area of a section, whether thick or thin, which is mounted on a suitably prepared carbon film.

Carbon films were prepared using essentially the methods first described by Bradley (3). A commercially available attachment[1] for mounting graphite rods in the evaporator was used in this work. At least for purposes of supporting thin sections, 99 per cent pure graphite rods appear satisfactory. Spectroscopically pure graphite may provide a more structureless film desirable for some purposes, but is considerably more expensive. The tips of the electrodes are prepared for evaporation by sharpening to fine points with a pencil sharpener. They are then faced on No. 600 carborundum paper so that one tip has a final diameter of about 0.25 mm. and the other, of about 1.5 mm. This keeps the contact area small and at the same time increases the amount of misalignment which can be tolerated.

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[1] Supplied by Ernest F. Fullam, Inc., Post Office Box 444, Schenectady.

Evaporation is carried out at a pressure of 0.1 μ Hg or less and takes place with some sparking when an alternating current of about 30 amperes at 15 volts is passed through the electrodes. We have not been able to float satisfactory films from glass coated with a parting layer such as the detergent, victawet,[2] or glycerine. Since carbon has higher density (*ca.* 2) than collodion (*ca.* 1.4) it has higher scattering power and should therefore be used in the thinnest films compatible with stability. Accordingly, the carbon is cast on formvar- or collodion-coated grids. Formvar appears to be superior to collodion since there is a tendency with the latter for the film to tear during evaporation of the carbon. The grids, following evaporation, are placed film-side up on a wire screen immersed in ethylene dichloride (or amyl or butyl acetate in the case of collodion). The wire screen is elevated from the bottom of the container by bending the corners down so that circulation of the solvent is facilitated. At the end of 2 or 3 hours, the screen bearing the grids is lifted with great care from the solvent and put in a dust-free place to dry. It may be blotted from beneath to remove most of the solvent. About 10 per cent of the squares in grids thus prepared will be torn, most of the breakage, perhaps, being due to sparks which fly from the carbons during evaporation.

Using the porcelain plate–oil drop method of Bradley for detecting the amount of carbon deposited, we have found that the best film thickness is that which has a rather marked tan, but not brown, color against the porcelain surface placed at the level of the grids during evaporation.

Carbon films are now used as routine in this laboratory for the support of thin sections. The additional work involved in their preparation is more than compensated for by the striking increase in the number of usable micrographs. In one series of twenty-five fields taken of sections mounted on carbon films, only three showed drift which was significant at a final magnification of 50,000.

[2] Victawet 35-B supplied by Victa Chemical Works, 141 West Jackson Boulevard, Chicago.

Bibliography

1. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.* 1953, **42**, 15.
2. Rhodin, J., Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954, 14.
3. Bradley, D. E., *Brit. J. Appl. Physics*, 1954, **5**, 65.