**A New Technique for Carbon Films.*** By Étienne de Harven.‡ (*From the Cell Growth Section, Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York.*)§

In electron microscopy, the films supporting the thin sections should have the following characteristics: (1) a maximum transparency to electrons, (2) a maximum stability when exposed to electron beam, (3) a minimum contamination with dust particles. Formvar films lack stability. If reinforced with evaporated carbon, the transparency to the electron beam is reduced. Removal of the formvar, in order to get a naked carbon film, increases the fragility of the film, and increases also the possibility of contamination, because it requires supplementary manipulations and treatments (*e.g.* passage through ethylene dichloride, drying, final checking with the light microscope, etc.) Fragility and contamination of the films make some grid squares useless, and this may present a problem, especially when one looks for infrequently occurring components of cells (centrioles, for example), or for rare cytopathologic features (virus particles). The technique recently described by Sjöstrand (3) appears difficult to apply to such material, because the chances of finding the desired structure over a hole in metallized net are small.

For these reasons, a new technique, offering good conditions for microscopical observation over the largest possible surface appeared necessary, and the following procedure, deriving partially from that indicated by Watson (4), has been devised.

Thin sections are cut with a Porter-Blum microtome equipped with a diamond knife[1] (2). Under these conditions, it is easy to get sections over 0.5 millimeters square in size. (Such large sections are also obtainable with a glass knife, but not so easily.) A short ribbon of around 6 sections is prepared and allowed to float on diluted acetone for 2 or 3 minutes, the last section being still attached to the knife edge. A clean grid (Athene, new 200) *without any film coating it*, is used to take up the ribbon. The grid coated with sections is dried by placing it on a filter paper in the bottom of a small petri dish. This petri dish is then placed, open, in the carbon evaporation unit, and in a vacuum of $10^{-4}$ mm. of Hg, a film of carbon is evaporated in the usual way, the grids being at approximately 15 cm. from the contact point between the two tapered carbon rods. Afterwards, the preparations are ready for electron microscope study.

This technique has been tested in various cytological materials (1) and has proved generally reliable.

The advantages of the technique described can be listed as follows: the procedure is simple and quick, and the preparations are perfectly stable when exposed to the electron beam.[2] The transparency is very satisfactory and permits one to take micrographs of satisfactory contrast. Since the number of manipulations and treatments of the grids is greatly reduced, contamination by dust particles is extremely rare. The preparations are much cleaner than those prepared by the older procedure, and it becomes possible to take pictures of all interesting fields. No distortion in the section due to sublimation of the methacrylate has been observed.

It is necessary to note, however, that a new artifact may occur if the carbon is not evaporated in a direction normal to the grid's surfaces, and if the sections are not perfectly smooth. Knife marks must, therefore, be carefully avoided. If one uses a diamond knife and allows the sections to float on the diluted acetone, these marks are

* This investigation was supported in part by a research grant from the National Cancer Institute, of the National Institutes of Health, United States Public Health Service, and in part by the Lillia Babbitt Hyde Foundation.

‡ Fellow of the Belgian-American Educational Foundation, and of the Damon Runyon Memorial Fund. Present address: Department de Microscopie Electronique, Institut d'Anatomie de l'Université Libre de Bruxelles, Brussels, Belgium.

§ *Received for publication, September 16, 1957.*

[1] We are grateful to Prof. H. Fernández-Morán for supplying the diamond knife.

[2] A Siemens Elmiskop I electron microscope was used in this study.

quite infrequent. Nevertheless, it is advisable to put the grids close together directly below the carbon source, in order to obtain normal or nearly normal exposure and avoid oblique deposition. Finally, one must mention that shrinkage of sections may occur when a square of the grid is not entirely covered with a section. Large surface sections, obtainable regularly with a diamond knife, tend to overcome this difficulty.


BIBLIOGRAPHY

1. Edwards, G. A., Ruska, H., and de Harven, E., in preparation.
2. Fernández-Morán, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 29.
3. Sjöstrand, F. S., *Exp. Cell Research*, 1956, **10**, 657.
4. Watson, M. L., *J. Biophysic and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 31.