The Use of Carbon Films in the Culture of Tissue Cells for Electron Microscopy. BY MAURICE C. DAVIES AND R. WALLACE.* (*From the Biochemical Research Section and Viral and Rickettsial Research Section, Research Division, American Cyanamid Company, Pearl River, New York.*)‡

Cells in tissue culture are particularly suited to electron microscopy because of their tendency to flatten out to extreme thinness on a flat surface. Porter *et al.* (1) were the first to recognize this and to develop a technique, using plastic-coated cover-slips as a substrate, by which undisturbed cultures of tissue cells could be fixed and transferred to specimen grids of the electron microscope. These basic procedures have since been used by a number of investigators in the study of many different cell types (2–6).

The polyvinyl formal, "formvar" films used in these procedures, though giving satisfactory end results, have some disadvantages. Slides or cover-slips coated with plastic cannot be sterilized by autoclaving or dry heat, but require exposure in special containers to ultraviolet radiation or sterilizing vapors. The use of alcoholic solutions for preservation or fixation of the cells is limited, since they sometimes cause the films to adhere so firmly to the glass that subsequent removal without damaging the cells becomes difficult. In addition, the films often break or develop holes which can be seen in the micrographs. Attempts to use silicone vapor and other films in place of the plastic have generally been unsuccessful (5).

Although thin sectioning has almost entirely superseded the observation of cultured cells *in situ*, in some types of experiments the latter technique is still desirable, for example in the study of the cytotoxic effect of antibodies. The use of carbon film as a specimen support has become widespread since its introduction by Bradley (7). The results of our investigations on the use of the carbon film as a substrate for the electron microscopy of cultured cells form the basis for this report.

New, 3 x 1 inch glass slides were polished with lens tissue and coated with carbon essentially according to Bradley (7), but with no parting layer. A commercial evaporator and carbon holder were used[1]. After they were coated, the slides were placed, carbon side up, on 7 mm. glass rod supports in 145 mm. covered culture dishes—approximately 4 slides to a dish. The dishes containing the slides were then sterilized in an oven at 170°C. for 1 hour.

In order to obtain slide cultures of thinly spread cells, selected stock cultures of a strain of human bone marrow cells (8) were initially treated with a solution of 0.05 per cent trypsin[2] and 0.02 per cent versene ethylene diamine tetra acetic acid[3] in Earle's balance salt solution (9). This treatment allowed the gentle removal of the cells from the glass of the stock culture flask and served to separate them from one another. The dispersed cells were then centrifuged, washed free of trypsin and versene with balanced salt solution, and resuspended in a nutrient composed of Eagle's synthetic basal medium (10) supplemented with 5 per cent horse serum.

One to 1.5 ml. of this suspension, adjusted to approximately 150,000 cells per ml. as determined by direct cell count in a hemocytometer, was spread over the upper surface of each carbon-coated slide as close to the edge as possible. Thirty to 40 ml. of balanced salt solution were pipetted into each dish between the 7 mm. rod supports underneath the slides in order to provide a moist atmosphere during incubation. The covered dishes were then placed in an 8 x 12 inch desiccator jar; the jar was flushed out with 5 per cent CO_2 in air, immediately sealed, and placed at 37°C.

At the end of 18 to 20 hours, the slide cultures were removed from the desiccator and examined briefly under a light microscope and washed in 2 changes of balanced salt solution warmed to 37°C. The cultures were fixed by placing the slides in direct contact with 2 per cent osmium tetroxide in isotonic acetate-veronal buffer at pH 7.2 (11) for 20 to 30 seconds. The optimum time of fixation depended to a large degree on the structure to be observed. For the cytoplasm, in particular the filamentous mitochondria, the longer fixation time proved better. For the nucleus a shorter time gave more detail. We have begun to observe another cell line, and it appears that the optimum fixation conditions may be different. After they were fixed, the cultures were washed for 3 hours in 8 to 10

* Present Address: Laboratoire de Recherches d'Immuno Pathologie, Hôpital St. Antoine, Paris, France.

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[1] Vacuum Equipment Division. New York Air Brake Company, Camden, New Jersey.

[2] Crystalline, lyophiled; Worthington Biochemical Corporation, Freehold, New Jersey.

[3] Dow Chemical Company, Midland, Michigan.

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changes of distilled water; water changes were made with care to avoid premature separation of the carbon from the glass. The washing and fixing of the cultures were carried out in standard staining Coplin jars.

The fixed slide cultures were then removed from the staining jars of distilled water and, after a final examination under the light microscope, were ready for removal of the films.

The films were scored off in small squares with a razor blade, floated off onto a distilled water surface, and picked up directly on 200 mesh copper specimen grids for electron microscopy. The specimens were examined in an RCA EMU-3C electron microscope with a 40 to 50 micron objective aperture and an accelerating voltage of 50 kv.

The chemically inert, non-toxic surface provided by the carbon films proved to be an ideal substrate for the culture of tissue cells. The cells used in these studies adhered and migrated onto the films equally as well as on a clean pyrex glass surface. Also, in contrast to plastic films, the carbon films provided a strong, stable support under the electron beam.

It was necessary to use a thick carbon film (on the order of 250 to 300 A) to facilitate removal of the cultures from the slide. On very thin films, the areas of cell growth adhered to the slides, so that sometimes the films could not be removed. With thick films, it was often possible to float an entire film off the slide, and on an average we obtained sufficient squares from one slide to provide 15 to 20 grids for electron microscopy.

Figs. 1 and 2 illustrate the type of pictures we have obtained with this technique.

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Explanation of Plate 124

Fig. 1. Human leukemic bone marrow cell grown on carbon film and photographed with low intensity electron beam. This shows a peripheral portion of the cytoplasm containing filamentous mitochondria. × 12,600.

Fig. 2. Same cell as above photographed with high intensity electron beam. The center of the field is occupied by the nucleus in which large nucleoli and other structures, probably chromatin masses, can be seen. Numerous spherical and some filamentous bodies are present in the surrounding cytoplasm. × 6,000.

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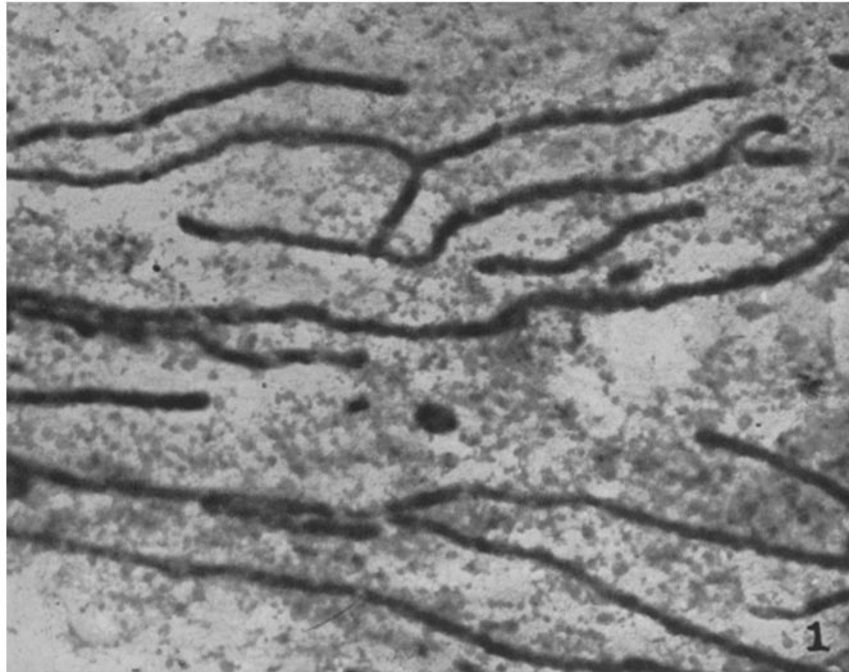

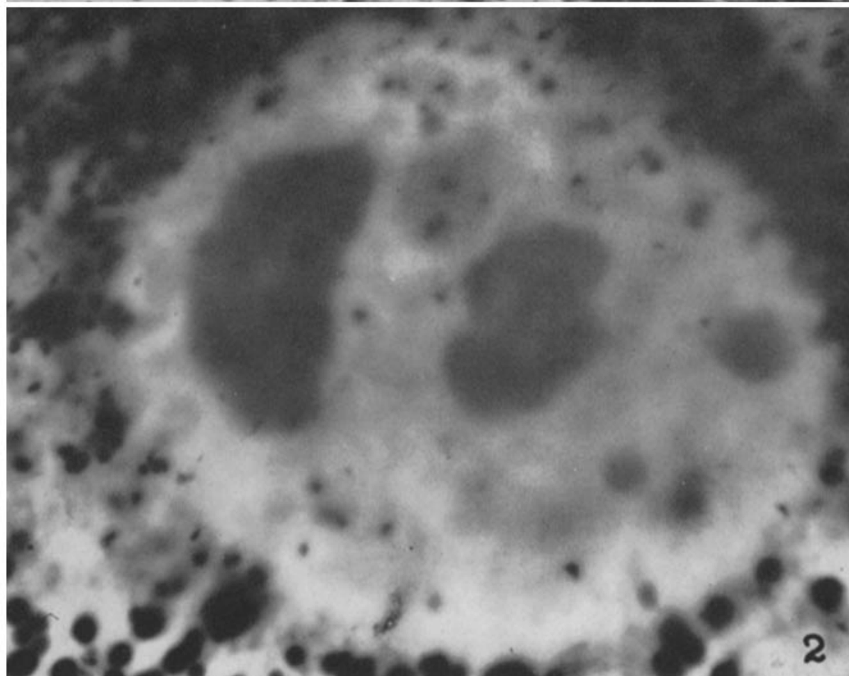

(Davies and Wallace: Carbon films in tissue cell culture)

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