Brief Notes

A Method for the Study of Cultured Cells by Thin Sectioning and Electron Microscopy.*

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Some years before thin sectioning techniques were developed, a number of investigators, notably Porter and his associates (6–8), made some new and interesting observations on cell structure by examining in the electron microscope fixed and dried preparations of whole cells grown on formvar-coated glass surfaces. The method depends on the fact that the peripheral regions of such cells are often extremely thin and consequently show fairly good detail in the electron microscope. The detail, however, does not compare favourably with that obtainable with the thin sectioning methods that were subsequently developed.

The present paper describes a procedure that allows cells grown *in vitro* to be sectioned in a plane parallel to the glass surface to which they are attached. The resulting micrographs have the high resolution associated with sectioned material and have the added advantage of presenting the cells in the aspect in which they are observed in the light microscope and in the whole-cell type of preparation referred to above. This facilitates comparison of the structures revealed by the present method with those observed by other techniques. Moreover, the method is applicable to the study of small numbers of cells or even to single cells, and promises to be very useful in studying the effects of different agents on cells growing *in vitro* or of variations in the procedures used for preserving the cells.

The method is essentially a modification of the methods described by Borysko and Sapranauskas (1), and by Gay (2). The former authors, however, used a double embedding procedure and sectioned the cells in a plane perpendicular to the one in which they were growing. The latter author described a method suitable for studying smears.

Materials and Methods

Cells.—Cell strains derived from monkey kidney cortex formed the source of material described here, but the method is applicable to all cells growing in contact with glass surfaces. The cells were grown in Petri dishes of diameter 5 or 10 cm. in a medium consisting of 80 per cent solution CMRL-1066[1] and 20 per cent horse serum. A continuous flow of moist air containing 5 per cent CO_2 was maintained in the cabinet in which the cells were incubated.

The cells multiply while adhering to the surface of the Petri dish, forming isolated colonies or a more or less continuous sheet according to the size of the inoculum and the period of cultivation. To prepare cells for phase microscope study, ordinary microscope slides are laid on the bottom of the Petri dish. Some of the cells then settle on the slides and multiply thereon.

Fixation.—Good results were obtained by fixing in OsO_4 vapour. This was done by inverting the open Petri dish over a small quantity of 1 per cent buffered OsO_4 solution (5) in a closed glass container, and exposing the cells to the vapour for about 2 hours. Equally satisfactory fixation was obtained by the simpler procedure of adding about 0.25 ml. of buffered OsO_4 solution to the Petri dish, replacing the cover, and leaving at room temperature for the same period.

Dehydration.—After a brief wash in distilled water, the cells were dehydrated in the usual way by adding successively higher concentrations of ethanol at 10 minute intervals, followed by several changes of absolute ethanol.

Embedding.—The ethanol was replaced by a mixture of 50 per cent ethanol and 50 per cent methacrylate (9 parts butyl and 1 part methyl methacrylate) and

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[1] Solution CMRL-1066 is a modification of solution CMRL-858 (4); it differs from solution 858 in that it contains thiamine, riboflavin, niacin, niacinamide, and pantothenate in the concentrations specified for solution 703 (3), but does not contain cholesterol, tween 80, or vitamins A, D, K, E.

then by several changes of pure methacrylate containing 1 per cent benzoyl peroxide. Excess fluid was poured off, and the lower half of a gelatin capsule filled with partially polymerised methacrylate was inverted over each selected area of cells. There is usually no difficulty in finding suitable groups of cells or colonies as the colour imparted by the OsO_4 renders them visible under suitable lighting conditions to the unaided eye.

If the partially polymerised methacrylate is of the right viscosity, the inversion is easily carried out. An air bubble may be trapped in the capsule, but causes no trouble as it slowly rises to the end of the capsule remote from the embedded cells.

Cells growing on slides for examination by phase microscopy are fixed, dehydrated, and infiltrated with methacrylate in the same way, but the final step consists in applying a few drops of partially polymerised methacrylate to the slide and mounting a coverslip on it.

Polymerisation is completed by transferring the Petri dishes with capsules or microscope slides to an oven held at 50–55°C. for 16 to 24 hours. When polymerisation is complete the capsules are firmly attached to the glass surface. They are removed by placing them on a block of solid carbon dioxide for about 2 minutes, after which time they are easily detached. The surfaces of contact usually break cleanly, leaving the cell layer embedded at the surface of the block. It is important to prevent water condensing on the cooled block at this stage as this appears to cause swelling and distortion of the cells. Condensation can be avoided by transferring the blocks immediately to a desiccator and leaving them there until they have regained room temperature.

Trimming and Sectioning.—Rather more care than usual is required in trimming and sectioning the block since the cells extend at most to a depth of a few microns below the surface. The block is trimmed in the form of a truncated pyramid with the area of interest forming the flat upper surface. If desired, several such pyramids can be formed on the surface of a single block, the positions of the pyramids being so chosen that in cutting one the knife edge does not interfere with the others. The cutting and mounting of the sections is carried out in the same way as for ordinary tissue blocks. The sections were cut with a Porter-Blum microtome and examined in an RCA EMU 3-b electron microscope.

OBSERVATIONS AND DISCUSSIONS

The advantages of the method are particularly obvious in the case of cells that are spread very thinly on the glass surface. These have an almost two-dimensional distribution of their components, and sections cut parallel to the surface show to advantage the main structural features of the cells. Such, for example, are the giant cells that form in cultures that have been x-irradiated. These have recently been studied by this method. Fig. 1 shows a comparatively large field in a preparation consisting of cells from monkey kidney that had received *in vitro* several days previously, 5000R of x-radiation. Portions of two cells are shown. The one in the upper right has a long cytoplasmic process that extends to the adjacent cell. Processes of this type are common in cells growing on glass surfaces and are particularly noticeable in unhealthy cultures. The chance of observing such a process over its entire length in a section would be remote except by a procedure such as the one described. Other features of the cells which are shown to advantage in Fig. 1 are the long slender mitochondria, the dense lipide bodies in the upper cell, and the peripheral region devoid of cytoplasmic components such as vesicles and mitochondria in the lower. This region doubtless corresponds to the particulate-free cortical zone described by Porter (8) in whole cell preparations. It is of interest to note that the upper cell does not exhibit this feature.

Another example of the type of information obtained by this method is illustrated in Fig. 2, which shows a group of mitochondria in a non-irradiated cell growing on glass. The mitochondria have the familiar double-layered enveloping and transverse membranes, and, in addition, exhibit clearly the great length and complex shapes (including numerous side branches and even closed loops) that are characteristic of the mitochondria in thinly spread areas of cells growing *in vitro*. Figs. 1 and 2 are typical of the standard of preservation obtained routinely by the method described.

The slide preparations, examined by phase contrast microscopy, cover a larger field of view and provide useful supplementary information about the general characteristics of the cultures.

SUMMARY

A method is described which allows cells growing on a glass surface to be sectioned parallel to the surface. Micrographs of the sections show cell components in the aspect in which they have been visualized by other techniques, but in much greater detail.

We wish to thank Dr. Gordon Whitmore who provided us with the cells.

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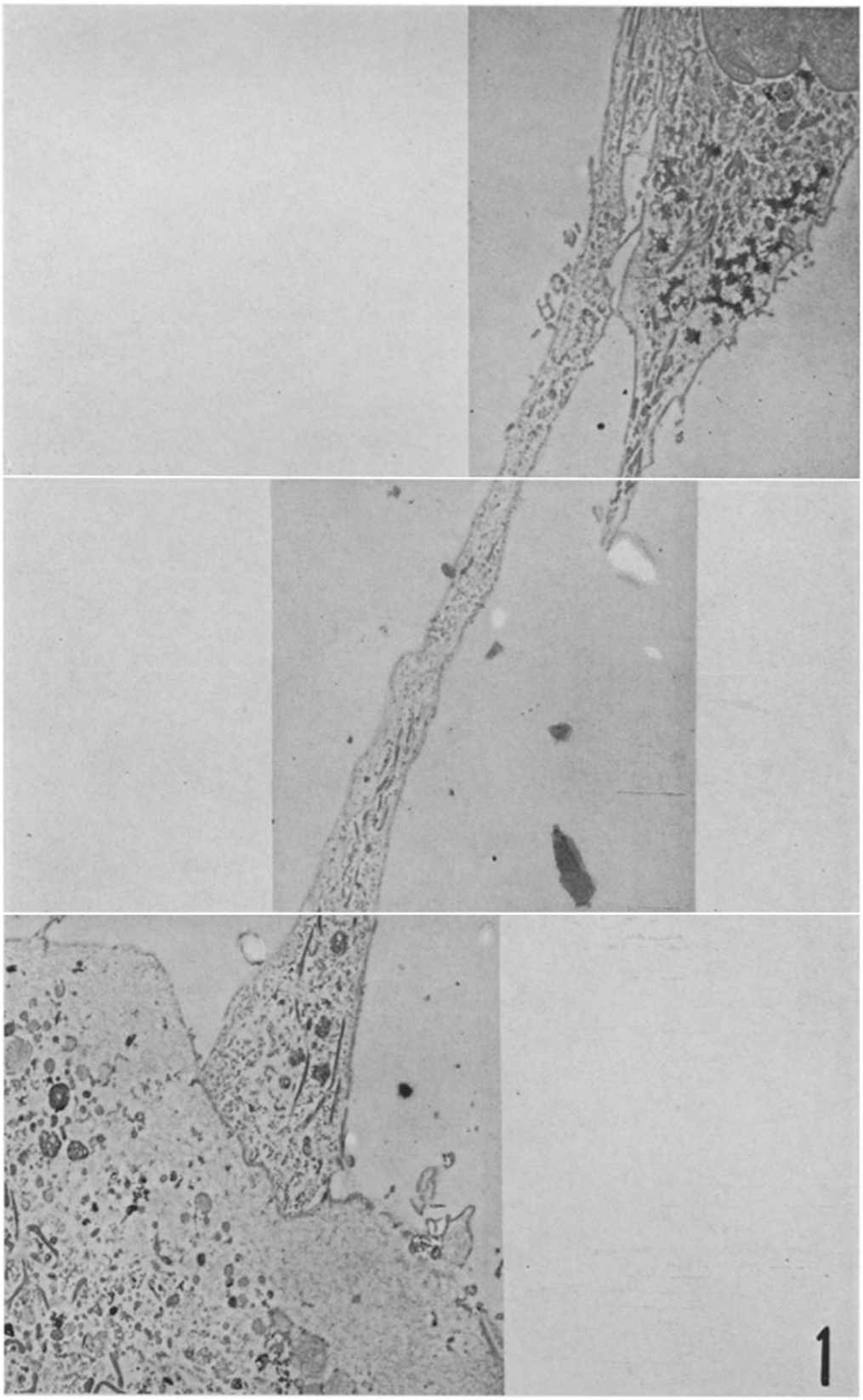
Explanation of Plates

Plate 56

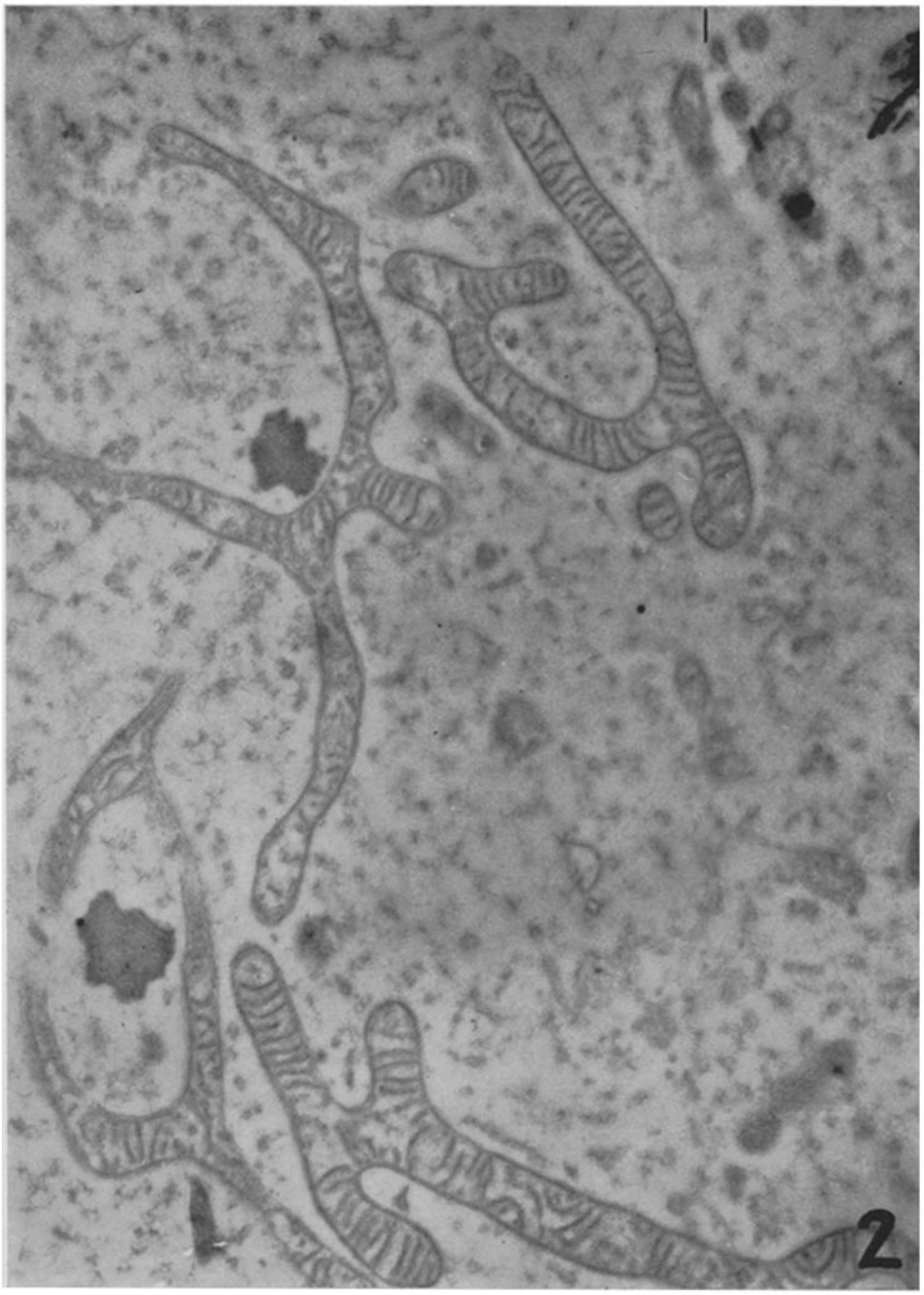
Fig. 1. Electron micrograph showing portions of two cells in a culture derived from monkey kidney; the culture had received 5000 R of x-radiation several days previously. The entire length of the long cytoplasmic process extending from the upper cell is included in the section which was cut parallel to the surface on which the cells were growing. Long slender mitochondria are visible especially in the cytoplasmic process, and dense irregularly shaped fat granules are present in the upper cell. The peripheral regions of the lower cell are practically devoid of cytoplasmic components apart from a fine granular material, but in the upper cell cytoplasmic vesicles, mitochondria, etc. extend to the cell border. The difference in the cytoplasmic contents is evident at the junction of the two cells (lower left). Magnification, 2,200.



(Howatson and Almeida: Study of cultured cells)

PLATE 57

FIG. 2. Electron micrograph of part of the cytoplasm of a monkey kidney cell growing on glass, showing a group of mitochondria. The transverse internal membranes (cristae) are prominent, and the unusual length and multiple branching of the mitochondria are shown to advantage in this section. Magnification, 22,000.



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