SCANNING MICROSCOPY OF DISSOCIATED TISSUE CELLS

JUAN VIAL and KEITH R. PORTER

From the Instituto de Ciencias Biologicas, Universidad Catolica, Santiago, Chile, and the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302

ABSTRACT

A method is described for studying by scanning electron microscopy (SEM) all the surfaces of fully differentiated cells from intact tissues. Thus, cell faces normally hidden from view are exposed and made available for SEM examination. This is achieved by fixing the tissue in OsO₄ and then soaking it in a 1% solution (in water) of boric acid. After different periods of time, varied according to particular tissue, slight mechanical pressure will cause the fixed tissue to dissociate into its component cells. These are then made to adhere to a substrate and are taken through critical point drying, etc., for examination.

Observations are reported on the topography of whole hepatocytes, adsorptive cells of the intestinal epithelium, proximal tubule cells of the rat kidney, mammary tumor cells of the mouse, and rat sarcoma cells. Several other tissues are reported to dissociate when similarly treated, but for each the procedure must be slightly modified.

Information on the surface fine structure of tissue cells has been obtained mainly from the study of sections. This fact has imposed certain restraints on the study of cell surface features which, because of their size, are accessible only by such time-consuming and tedious procedures as serial sectioning. It is perhaps for this reason that little attention has been given to the patterns of distribution and the functional changes of the numerous ruffles and processes observed in thin sections of many kinds of epithelial and connective tissue cells.

The scanning electron microscope (SEM) is an important tool for the study of these structures, because it gives a direct overall picture of the outside of cells and allows a ready detection of many experimentally induced changes. Thus far, its use has been restricted mainly to the observation of free surfaces, either of cultured cells or of cells lining organs or body cavities (2, 3, 15, 16). A few reports have appeared of SEM studies of teased or freeze-fractured organs (3, 4, 12, 13), and the results obtained suggest that a considerable amount of valuable information could be gathered if a procedure were devised to expose the faces of the tissue cells that normally lie hidden by the adjoining cells or tissue components.

Goodrich (9) reported that solutions of boric acid were capable of dissociating unfixed tissues into their constituent cells and that these retained their shape unaltered as judged from light microscopy. The application of this procedure to SEM requires the finding of a suitable fixative. The present report shows that fixation in OsO₄, followed by treatment in a boric acid solution weakens cell adhesion to the point where mild mechanical forces are sufficient to induce tissue disassembly and the release of individual cells. This procedure can be successfully applied to a variety of tissues. The cell surfaces obtained appear clean of extraneous material, and a comparison of their characteristics with information available from transmission microscopy of sections indicates that most of the surface structural features are well preserved (19).
MATERIALS AND METHODS

Tissues were obtained from adult mice and rats and treated as given below.

Fixation

The tissues, excised from the anesthetized animal, are placed in a drop of osmium fixative on a plate of denal wax and cut into tiny blocks with a razor blade. The fixative employed is 1% OsO₄ in 0.2 M cacodylate buffer pH 7.3. This choice of fixative is important because tissue maceration is not successful after glutaraldehyde or formaldehyde fixation. These reagents seem to make the cells firmly adhere to one another.

The time of fixation may be varied. After periods of 12 h or more the cells of many tissues tend to separate away from one another when subjected to mild mechanical stresses without any further chemical treatment. However, the membranes are much more fragile, and the proportion of cells that are well preserved, and thus available for observation, is low. On the other hand, if the fixative is allowed to act only for a short time, the cells remain firmly adherent and cannot be isolated without extensive damage.

The optimum time of fixation seems to depend on the type of tissue being studied. The absorptive epithelium of the small intestine, which is exposed to the fixative as a single layer of cells, falls apart after much shorter fixation than that necessary for liver or pancreas. An average fixation time might be set at 3-5 h at 4°C, but it is clear that the optimal time for each new tissue studied should be established by experimentation.

Boric Acid Treatment

After several rinses in distilled water, the blocks are further reduced in size with a razor blade under the dissecting microscope and are immersed in a 1% boric acid solution in distilled water at room temperature. The time required for the boric acid to act varies with the type of tissue studied, but if immersion is prolonged beyond 12 h, damage to the cell surface may result. An average of 5 h may be considered acceptable. In each instance it is important to check the degree of maceration every 1 or 2 h in the following manner: a very small piece is cut off from one of the blocks and placed in a drop of water on a slide. A coverslip is then laid upon the drop and gently moved around in the plane of the slide. If the tissue has reached an adequate degree of fragility, it will break up into its constituent cells.

Mechanical Dissociation

The blocks are then submitted to mild shearing forces by whatever procedure may seem to be the most adequate for the particular tissue studied. Good results can be obtained with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.) run at slow speed. However, the following procedure, even though apparently crude, has proved to be very effective. The blocks are placed in water in the well of a depression slide. The rounded bottom of a 2-inch diameter centrifuge tube is dipped in the water, and, under the dissecting microscope, it is brought into gentle contact with the tissues, with care taken not to exert more pressure than that provided by the weight of the tube. This procedure has the advantage that the cells released from the tissue blocks, by the contact with the tube, float away into the bulk of the water and are not submitted to any further mechanical pressures. This procedure has been very valuable for obtaining large numbers of cells from organs of homogeneous composition such as liver, pancreas, bone marrow, etc.

Once the cell suspension has been obtained, it is advisable to remove from it the larger clumps of tissue, which are usually damaged and which may prevent the layering of the cells on the gelatin support (see below).

Whatever method of dissociation is chosen, care should be taken not to expose dissociated cells to an air-water interface.

Preparation for the SEM

Small flat-bottomed aluminum dishes are useful during the succeeding steps in the preparation of cells for SEM. These dishes, about 3/4 inches in diameter (Fig. 1), can be fashioned out of aluminum foil. The bottoms of the dishes are heated gently with an alcohol burner until they lose their brightness (usually a few seconds of heating suffices). When the dish has colored, one or two drops of warm 3% gelatin solution are spread over its bottom. The dishes are then cooled to 4°C for 10 min to harden the gelatin and then filled with 3% glutaraldehyde which is left to act on the gelatin for periods of 5 min to 1 h. Before putting the cell suspension into them, the dishes are briefly rinsed in distilled water. The cell suspension then added to the dish is left to sediment for 5-10 min. At
FIGURE 2 Lateral aspect of a group of cells from the epithelium of the small intestine of the rat. The free surface is at the upper left. Just below the villous border a shallow zone (arrows) is occupied by various components of the junctional complex. The lateral cell surface is folded into numerous plicae. Where these are viewed at the edge of a cell, their orientation, with longer axis parallel to the vertical axis of the epithelium, becomes more evident. $\times 8,000$.

the end of this period, most of the fluid is pipetted out and replaced with 3% glutaraldehyde, which augments adhesion between the cells and the gelatin. This is left to act for a few minutes, but no damage is observed if the cells remain in glutaraldehyde for several days in the cold. The preparations are then dehydrated by increasing concentrations of acetone (14) and submitted to drying by the CO$_2$ critical point method of Anderson (1). The bottom of each dish (sides having been trimmed away) is then affixed to an aluminum stud (specimen holder for SEM) by means of silver paint, and the preparations are coated with carbon and gold. It is sometimes necessary to recoat the specimens with gold in order to rid the images of charging effects. This is especially true where a cuboidal cell is attached to the gelatin by only a small facet of its surface.

The observations reported here were made in a Cambridge Stereoscan Microscope (Model S4) at accelerations of 5–20 kV, with apertures of 200 and 100 $\mu$m and at magnifications ranging from 100 to 25,000.

RESULTS

Figs. 2–13 provide evidence that the technique is capable of yielding good images of the topography of both the free and “hidden surfaces” of cells in a variety of tissues.

Epithelia

ABSORPTIVE EPITHELIUM OF THE SMALL INTESTINE: The microvillous border (brush border) is well preserved (Fig. 2), although it exhibits a tendency to be torn away from the cell (Fig. 3). In
FIGURE 3 Apical surface of cells of the same intestinal epithelium seen from "below." During its preparation for SEM the villous border adhered to the gelatin, and the major part of the cells was torn away. The cleavage plane revealed, which is just beneath the brush border, is remarkably smooth and uniform in texture. It is perforated by numerous small openings. Careful inspection reveals that these are situated at the bases of the individual villi. × 24,000.

Some specimens one can see on the lateral surface of the cell body, immediately underneath the base of the microvilli, a shallow furrow limited by two indistinct ridges (Fig. 2). The location of this structure coincides with that of the junctional complex. The most striking features of the cell surface are the *plicae*. This name is applied in the present report in a generic sense to flap-like, lamellipodial protrusions of the cell surface that are present on a wide variety of tissue cells, even though they show special characteristics in each type. In the intestinal cell they measure approximately 0.1 µm in thickness and from 1 µm to 4 µm in length and height. Their free edges are curved and often undulated. They do not project perpendicularly outwards from the cell surface but rather tend to be flattened against it. The orientation of their long axes is not random but tends to follow a definite pattern parallel to the long axis of the cell in such a way that successive laminar expansions contribute to the formation of longitudinal valleys (Fig. 2). These features are observed to best advantage in stereoscopic pairs. Near the base of the cell the plicae are thinner and randomly oriented. Where it contacts the basal lamina, the cell spreads out to form a broad foot in such a way that neighboring cells are separated in this region by only narrow clefts.

Some of these features undergo important changes during the absorption and transport of fat.
(Figs. 4 and 5). 1 h after intragastric injection of corn oil, the cells appear swollen in their apical third, while the regions nearer the base have become considerably narrower than in the fasting controls (Fig. 5). In this way large intercellular spaces are created around the basal half of the cell. These spaces are sometimes found empty, but in other cases they are filled by large aggregates of small spherical particles held together by a tenuous network of delicate filaments. The cell surface is also much changed. The laminar expansions are more sparsely distributed, they appear flattened against the cell body and detectably thinner than in the controls. The particles, from 0.03 μm to 0.05 μm in diameter, adhere to the cell surface and are especially numerous under the free edges of the laminar expansions (Fig. 4). This appearance of the cell and intercellular spaces corresponds very closely to the images obtained with transmission electron microscopy (5) and with the SEM after glutaraldehyde fixation (12). The large accumulations of chylomicra in spaces formed between the basal ends of the epithelial cells suggest a certain damming up of the lipid particle flow, possibly at the level of the basal lamina (Fig. 5).

Liver: This tissue is easily disassembled, and high yields of isolated cells can be obtained (Fig. 6). In good preparations the cells are cleanly separated from each other and from the sinusoidal linings; bile canaliculi appear open, and their entire villous cover becomes visible. Practically no collagen can be found on the cell surface. However, if
FIGURE 5  Intestinal epithelium, 1 h after intragastric administration of corn oil. The apical third of the cells appears swollen, with a surface considerably smoother than in the fasting animal. The middle and basal portions of the cells (at the lower left and center) are separated by large spaces which appear partially filled with clusters of chylomicra. It is probable that the preparative procedure removed large numbers of chylomicra present in these spaces. × 3,800.
FIGURE 6 Hepatocyte. The intratrabecular surfaces are marked by the bile canaliculi (arrows), with terminal branches included (18), and the sinusoidal surface (ss) by the presence of large numbers of short processes which give it its rough appearance. × 3,500.

the OsO₄ fixation or boric acid treatment has been prolonged, the cells tend to be torn along the edges of the bile canaliculi.

As shown by Motta and Porter (13), the surface of the liver cell underlying the sinusoid shows marked differences from the face contiguous to the next hepatocyte (which will be referred to here as "intratrabecular surface") (Fig. 7). The sinusoidal face sometimes appears as a wide shallow depression, but more often it has the form of a small polygonal face near an edge or vertex of the cell. It is covered by short and thick microvilli. Even though these often have a finger-like aspect, they are shorter and thicker than the typical microvilli encountered on the adsorptive cells of the intestinal epithelium, mesothelial cells of the peritoneum, etc.

The intratrabecular surface is distinctly smoother. It has a very limited number of laminar expansions (or plicae), in aspect reminiscent of the ones found in intestinal epithelia. Their number increases after bile duct ligation.¹ The cell surface shows scattered pits and small blebs. The pits are

¹ Vial, J. D., F. R. Simon, and M. MacKinnon. Manuscript in preparation.
FIGURE 7  Higher power micrograph of the same hepatocyte shown in Fig. 5. The sinusoidal surface (ss) is covered with broad and blunt processes of variable shapes and sizes which are not typical microvilli. The canaliculus on the upper left shows a row of microvilli projecting from its margin. The intrablobular surfaces show a few scattered blebs and some evidence of pits.
more numerous in the neighborhood of the bile canaliculi.

The structure of the bile canaliculi is well preserved. The canicular floor is covered with microvilli which are sometimes densely packed, while on other occasions they appear more sparsely distributed (6). One interesting feature is the presence along the canicular margins of a row of regularly spaced microvilli, which project from a low ridge (Fig. 6). When the bile duct is ligated, the lumen of the widened canaliculus appears covered by these ridges, and the villi implanted on their margins do not suffer the same process of atrophy as the rest of the villous lining. These findings are reported in detail elsewhere.

OTHER EPITHELIAL CELLS OF THE DIGESTIVE TRACT: Good preparations were obtained also of parietal cells (oxyntic cells) and pancreatic acinar cells. The lateral cell surfaces in both instances show a few plicae similar to the ones already described. It is quite striking that the lateral surfaces of these cells are very similar in their general aspect to the intercellular faces of hepatocytes.

KIDNEY: An interesting contrast in response to dissociation is offered by the behavior of kidney tubules. Even after very prolonged treatment in boric acid, the basal lamina remained apparently intact. This circumstance seems to hinder the separation of individual cells, or even small groups of cells. Tubule fragments were frequently found in preparations of kidney, and these sometimes provided transverse views of fractured tubules. In such cases, the lateral surfaces of the cells were adequately exposed, but usually the apical portion was damaged and the brush border entirely torn away. As expected, the lateral faces of the cells beneath the junction are folded into laminar expansions of approximately triangular outline that extend from the apical zone to the basal lamina (Fig. 8). Most

![Figure 8](image.png)

**Figure 8** Kidney tubule of the chick. A portion of the lateral surfaces of cells in proximal tubule is shown with characteristic plicae. These form valleys that extend from the junctional zone to the basement membrane. This membrane presents a series of ridges (arrows) which are oriented parallel to the plane of the plicae and are probably related to their impingement on the membrane. × 7,500.
of these expansions are oriented with their planes perpendicular to the tubule axis and are extensively interdigitated with those of adjoining cells (7). In this way a system of grooves and channels is established which runs along the entire lateral aspect of the cell (Fig. 8). It is interesting to note that in all cases in which a view could be obtained of the inner aspect of the basal lamina, it showed a system of thin sharp ridges oriented circumferentially with respect to the axis of the tubules and apparently destined to fit with corresponding edges of the laminar expansions. In this instance the macerated tissue preparations were not appreciably better than those obtained by pulling the tissue apart after glutaraldehyde fixation (3, 12).

MAMMARY TUMOR OF (C3H)MICE: This tumor comprises tubules and cysts which are easily fragmented after the boric acid treatment. The luminal surfaces of the cells are covered with

2 Kindly supplied by Dr. Dan H. Moore, Institute for Medical Research, Camden, N. J.
FIGURE 10 Apical surfaces of cells from mouse mammary tumor. The microvilli are irregular in their distribution and size, and many show knoblike ends (arrows) which are thought to represent type B virus. The intervillar space contains numerous folds and is covered by small rounded prominences of variable size. $\times 18,000$. 

JUAN VIAL AND KEITH R. PORTER  Scanning Microscopy of Dissociated Tissue Cells  355
microvilli which vary in length and are irregularly distributed, with many patches devoid of them (Fig. 9). Both the microvilli and the cell surface between them show small (30-60 nm) rounded protuberances, with a distribution closely resembling that of B-type particles revealed in this cell type by transmission electron microscopy (10, 17). It is worth special note that some microvilli are of uniform diameter with rounded ends characteristic of these structures, whereas others show bulbous ends (forming B particles) and stronger secondary emission (arrows, Fig. 10). The lateral surfaces of these cells are also folded into laminar expansions or plicae.

**Connective Tissue**

**Rat Sarcoma no. 4337 (8):** This neoplasm affords an interesting example of an application of the technique. With transmission electron microscopy of thin sections, these cells are shown to have what appear to be long microvilli on their surfaces and to be surrounded by a network of collagen fibers. The maceration technique allows an easy separation of individual cells (Fig. 11). These show a great wealth of surface folds (plicae, not microvilli) which are disposed in complex patterns. Individual plicae often have a narrow insertion edge, while their free edges are somewhat ex-
FIGURE 12  Higher power of the same cell shown in Fig. 10. The close packing of the plicae is clearly seen. A few particles populate their surfaces. There is a contamination at C. \( \times \) 18,000.

panded and thickened. They tend to arrange themselves as stacks of folds that cover stretches of cell surface (Fig. 12). Small particles (ca. 30 nm) are sparsely scattered on the surface of the expansions (Fig. 12). Among several dozen cells that were studied carefully, there was only one instance in which particles with characteristics similar to those described were found completely covering the surface of the cell (Fig. 13).

**BONE MARROW:** This tissue affords an excellent material for the maceration procedure. Especially striking is the surface pattern of megakaryocytes, formed by laminar structures connected to the cell body by numerous fine stalks. These
FIGURE 13 Sarcoma no. 4337 of the rat. This cell surface was observed in the same preparation that contained many other surfaces similar to those illustrated in Figs. 10 and 11, and it is the only one observed to date that presents an aspect so densely studded with particles. \( \times 15,000 \).

Laminar structures present some knoblike thickenings and presumably correspond to platelets. All the cells isolated in the bone marrow had clean surfaces free from any extraneous material.

**Muscle and Peripheral Nerve**

The basal lamina could not be removed from these cells by the standard procedure described. Individual muscle and nerve fibers fell apart very easily, but their outer surfaces were covered by a thin adherent material, in some instances homogeneous and in others showing a finely filamentous texture.

**DISCUSSION**

No attempt has been made to study the mechanism whereby boric acid dissociates the cells of osmium-fixed tissues. However, some occasional observations may be relevant to this point. Other weak acid solutions, such as acetic acid, did not show the same property, even when used at a pH similar to that of the boric acid solution. Even after very prolonged maceration in boric acid, abundant collagen fibers can be found in pieces of tissue, and basal laminae are found resistant to the maceration procedure. These findings suggest that boric acid treatment operates by loosening the attachment between the cell surfaces, collagen, and basal lamina, rather than by actually dissolving away any of the latter two elements. It is conceivable that in calcium- and magnesium-free environments the cells would separate, especially before fixation (20). Since, however, the preservation of cell form as it exists in the intact tissue is our purpose, it would be defeating to isolate the cells while they are still alive.

The combination of OsO\(_4\) fixation and tissue disassembly in boric acid allows large quantities of isolated cells to be gathered from liver, intestine, stomach, pancreas, bone marrow, rat sarcoma no. 4337, and mouse mammary tumor. In this respect it seems that this technique has some advantage over that described by Miller and Revel (12) who use glutaraldehyde fixation and blunt dissection. Preliminary work indicated that separation of...
difficult after glutaraldehyde than after osmium fixation.

Most of the surface features described after the use of this technique can be identified in micrographs of thin sections of material prepared for transmission electron microscopy. This is true for the general shape and size of cells, and for such structures as plicae, microvilli, and the like. It is also true for some temporary phenomena affecting the cell surface, as exemplified by the secretion of chylomicra by the intestinal epithelium, and the exocytotic phenomena (virus production) of cells of the mammary tumor of (C3H) mice. That these details can be seen indicates that the technique preserves fine structural detail and is potentially useful for gathering new information on the structure of cell surfaces, especially when the resolution of the SEM is improved.

Even in its present state, however, the technique allows some relatively overlooked features of the cell surface to be better observed. It is evident that in the mammalian tissues employed, the preferred elaboration of the free cell surfaces takes the form of microvilli, whereas the folding of the "hidden (lateral) surfaces" appears most often in the shape of what we prefer to call plicae. The evidence presented shows further that these expansions are found in both epithelial and connective tissue cells. Their shapes vary according to the type of tissue studied. It is interesting to note that all of the several cell types in the digestive tract, such as intestinal epithelium, mucous cells of the stomach, parietal cells, pancreatic acinar cells, and hepatocytes, show laminar expansions that conform to the same general outline and are different from the thick laminar expansions of the kidney cells and from the irregular and possibly abnormal plicae expansions of the sarcoma no. 4337. When this is taken together with the observation that the general picture of the cell surface is closely similar in a variety of cells of the digestive system which have important structural and functional differences, it may be interpreted to suggest that there are surface features of cells which reflect their embryonic origin. Even though the evidence reported does not afford definite proof of this point, the idea is definitely suggested and deserves further exploration.

Notwithstanding their differences, plicae of tissues engaged in analogous functions may assume a correspondingly similar disposition. This can be observed in the transporting epithelia of the kidney and the small intestine. In both cases, and even though the shapes of the expansions are quite different, they become arranged in such a way as to provide the cell surface with elongated channels that run along the lateral surface of the cell down to the basal lamina. This disposition is quite different from the randomly oriented processes that one might have expected from the study of sections. It is likely that these longitudinal grooves are in some way connected with the direction of flow along the intercellular spaces in these transporting epithelia.

It is possible also that in transporting epithelia the plicae serve to increase the area of cell membrane lining the intercellular spaces, where active transport of solutes and osmotic equilibration are believed to take place. However, the fact that plicae are found in many cells not prominent for their transport activity, such as mucous cells of gastric epithelium, etc., does not allow one to present any definite conclusion on this point.

Miller and Revel (12) have presented evidence that in gall bladder epithelium the pattern of plicae may not be the same in all cells. It is clear that further experimental work is needed to try to evaluate the changes in pattern of the surface plicae that accompany different states of functional activity. In this connection it is worthwhile to stress that the lateral surface of the intestinal epithelium cells undergoes important changes during fat absorption. Such alterations in the pattern of distribution of the lateral expansions suggest that these surfaces are capable of altering their topography in relation to changes in cell function. There is then the further possibility that the arrangement and orientation of plicae may not be related solely to giving direction to flow along the cell surface, but that they may promote the flow by migrating from the apical to the basal poles of the cell in much the way ruffles are observed to migrate on cells grown in vitro (11).

We have reported on the separation of cells from tumors and the relative ease with which this is accomplished. This latter fact, perhaps related to the paucity of desmosomes between these cells, makes quite feasible the routine use of this procedure for the study and characterization of tumor cells. The whole procedure is simple and can be completed within a few hours if necessary; e.g., where virus involvement is known or suspected, it is an advantage to be able to examine large areas of cell surface in a few hours. The nature of the particles shown on the surfaces of the rat sarcoma
cells is not known but they serve to illustrate what 300-A particles would look like.

The authors are pleased to acknowledge the assistance of Ms. Virginia Fonte and Mr. Robert McGrew with the microscopy and manuscript preparation. Dr. Juan Vial was supported for the period of this study by a Fulbright Grant.

Received for publication 5 February 1975, and in revised form 16 June 1975.

BIBLIOGRAPHY

1. ANDERSON, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N. Y. Acad. Sci. 13:130-134.

2. ANDREWS, P. M., and K. R. PORTER. 1973. The ultrastructural morphology and possible functional significance of mesothelial microvilli. Anat. Rec. 177:409-426.

3. ANDREWS, P. M., and K. R. PORTER. 1974. A scanning electron microscopic study of the nephron. Am. J. Anat. 140:81-116.

4. BROOKS, S. E. H., and G. H. Haggis. 1973. Scanning electron microscopy of rat's liver: application of freeze-fracture and freeze-drying techniques. Lab. Invesit. 29:60-64.

5. CARDELL, R. R. Jr., S. BADENHAUSEN, and K. R. PORTER. 1967. Intestinal triglyceride absorption in the rat. An electron microscopical study. J. Cell. Biol. 34:123-155.

6. CARRUTHERS, J. S., and J. W. STEINER. 1962. Fine structure of terminal branches of the biliary tree. II. Parenchymal cell cohesion and intracellular bile canaliculi. Arch. Pathol. 74:117-126.

7. DIAMOND, J. M., and W. H. BOSSERT. 1967. Standing gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelium. J. Gen. Physiol. 50:2061-2083.

8. DUNNING, W. F., M. R. CURTIS, and F. D. BULLOCK. 1938. A study of the growth of Cysticercus sarcomata 4337 and 4338 from grafts of each tumor simultaneously transplanted in the subcutaneous tissue of the same hosts. Amer. J. Cancer 32:90-113.

9. GOODRICH, E. S. 1942. A new method of dissociating cells. Quart. J. Microsc. Sci. 83:245-258.

10. HAIRSTONE, M. A., J. B. SHEFFIELD, and D. H. MOORE. 1964. Study of B particles in the mammary tumors of different mouse strains. J. Natl. Cancer Inst. 33:825-846.

11. HARRIS, A. K. 1973. Cell surface movements related to cell locomotion. In Locomotion of Tissue Cells. Ciba Found. Symp. 14:3-26.

12. MILLER, M. M. and J. P. REVEL. 1974. Scanning electron microscopy of the apical, lateral and basal surfaces of transporting epithelia in mature and embryonic tissue. In Scanning Electron Microscopy. O. Johari and I. Corvin, editors. IIT Research Institute, Chicago. 549-556.

13. Motta, P., and K. R. PORTER. 1974. Structure of rat liver sinusoids and associated tissue spaces as revealed by scanning electron microscopy. Cell Tissue Res. 148:111-125.

14. PORTER, K. R., D. KELLY, and P. M. ANDREWS. 1972. The preparation of cultured cells and soft tissues for scanning electron microscopy. In Proceedings of the Fifth Annual Stereoscan Scanning Electron Microscope Colloquium. Kent Cambridge Scientific, Inc., Morton Grove, III. 1-20.

15. PORTER, K. R., G. J. TODARO, and V. FONTI. 1973. A scanning electron microscope study of surface features of viral and spontaneous transformants of mouse Balb/3T3 cells. J. Cell Biol. 59:633-642.

16. REVEL, J. P. 1974. Scanning electron microscope studies of cell surface morphology and labeling, in situ and in vitro. In Scanning Electron Microscopy. O. Johari and I. Corvin, editors. IIT Research Institute, Chicago. 542-548.

17. SHEFFIELD, J. B. 1974. Membrane alterations which accompany MuMTV maturation. I. Studies by freeze-cleave techniques. Virology. 57:287-290.

18. STEINER, J. W., and J. S. CARRUTHERS. 1961. Studies on the fine structure of the terminal branches of the biliary tree. Amer. J. Pathol. 37:639-650.

19. VIAL, J., and K. R. PORTER. 1974. The surface topography of cells isolated from tissues by maceration. Anat. Rec. 178:502 a. (Abstr.).

20. WESTFALL, J. A., and P. D. ENOS. 1972. Scanning and transmission electron microscopy of isolated cells of Hydra littoralis. In Proceedings of the 30th Annual Meeting Electron Microscopy Society of America. C. J. Arceneaux, editor. Claitor's Publishing Division, Baton Rouge, La. 160-161.