

# A STUDY OF TISSUE CULTURE CELLS BY ELECTRON MICROSCOPY

## METHODS AND PRELIMINARY OBSERVATIONS

BY KEITH R. PORTER, PH.D., ALBERT CLAUDE, M.D., AND ERNEST F. FULLAM

*(From the Laboratories of The Rockefeller Institute for Medical Research, and The  
Research Laboratories, Interchemical Corporation, New York)*

PLATES 10 TO 14

(Received for publication, November 11, 1944)

Cells, as they appear in tissue cultures and especially those spread out against the supporting glass surface, have been a popular material for cytological studies (1 to 5, and others). They often extend to extreme dimensions and thus effectively exhibit all the details of organization and composition that the light microscope, with its limited power of resolution, is able to reveal. It occurred to us that thinly spread cells of this sort might be suitable for electron microscopy and the following studies were made to examine this possibility. The problem was to devise methods by which cells could be grown and ultimately displayed on the object screen of the electron microscope and not in the process suffer excessive distortion.

In this report evidence will be presented to demonstrate that the extended cultured cell can be prepared for electron microscopy by relatively simple means and that such cells possess a degree of thinness which permits the requisite differential penetration of electrons. The electron micrographs so far obtained show in the unstained cell all the major structures demonstrable with the light microscope and elaborate cytological techniques. Beyond this they picture structural details not heretofore noted. In view of these findings it seems probable that this new technique will make possible the study of the morphology, composition, and behavior of cell components which, because of their small size, are beyond the resolving power of the ordinary light microscope. In this latter connection particular interest attaches to the presence and distribution of "submicroscopic," cytoplasmic components previously isolated by centrifugal methods (6), and to the demonstration of viruses.

The observations are preliminary and make no pretense of completeness. It is proposed to (a) describe the procedures, (b) evaluate the results by comparing electron micrographs with photomicrographs, and (c) present observations on cellular details revealed by the technique and the great resolving power of the electron microscope.

### *Materials and Methods*

The technique for preparing materials for electron microscopy has been repeatedly described (7). Essentially, it consists in covering small discs of

fine wire mesh with a thin film of collodion or plastic (about 10  $m\mu$  thick) and then placing upon this the material to be examined. This usual procedure was of necessity modified in order to obtain preparations of cells extended as in growing cultures, and it is really this change in technique that has made possible the observations to be described. The alteration consisted in principle in coating the coverglass used to support the cultures with a thin film of plastic so that the cells in growing might adhere to the film and later be transferred with it from glass slide to wire screen. The technical details are described below.

*Origin of Cells.*—The cells for study have been derived for the most part from chick embryo tissues. Additional types from normal adult mouse tissues and  $S_{37}$  mouse tumor have been examined, but they will not be described in this report.

*Culture Methods.*—The cells were cultured either in hanging drops of physiological salt solution or in nutrient plasma clots. Following essentially the procedure of Lewis and Lewis (2), portions of 8 to 12 day old chick embryos were isolated under aseptic conditions and cut up in Locke's solution. The small pieces of tissue were then mounted in shallow hanging drops of Locke's solution on coverglasses on the side coated with a thin film of plastic. To produce this plastic film, a clean glass coverslip was flooded with a 0.3 per cent solution of "formvar"<sup>1</sup> in ethylene chloride, then drained and dried. Other fragments of embryonic tissue were mounted on film-coated slides in clots of chick plasma and nutrient solution<sup>2</sup> according to procedures recommended by Parker (8). After 36 to 72 hours at 38°C. both types of cultures generally showed enough growth to supply the required cells. The explant, or clot and explant together, were then removed; the cells left behind, adherent to the "formvar" film on the coverglass, were those used for preparation and study.

In order that electron microscopy of cultured cells should not be confined to those that can be obtained during the short life of a hanging drop preparation, another culturing technique of some usefulness has been devised. It involves the utilization of a culture flask now in general use in our laboratory, and especially designed to fit into a rack for roller tubes (9). As shown in Text-fig. 1, it is simply a flask with two parallel flat sides, blown from a regular soft glass or pyrex tube measuring 1.5 × 16 cm.<sup>3</sup> The body of the flask is about 35 mm. in diameter.

When employed for the electron microscope studies the roller flask was used in conjunction with a small (11 × 22 mm.) film-coated glass slide,<sup>4</sup> which was introduced through the long

---

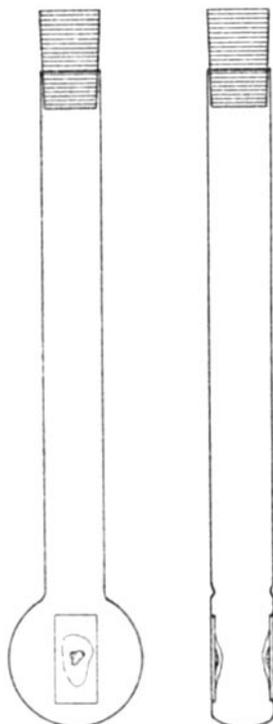
<sup>1</sup> The plastic used was "formvar" (grade No. 15-95) a polyvinyl formal sold by the Shawinigan Products Corporation, New York. Since the early experiments showed the plastic to be hydrophobic all the coated slides at first used were covered with a layer of horse serum applied under aseptic conditions from a water-spread film. It was later found, however, that the cells extended equally well over the plastic in the absence of the molecular layer of serum.

<sup>2</sup> The nutrient was made up of Tyrode's solution, horse serum, and chick embryo extract in the ratio of 6:4:2. The clot was produced by mixing 5 parts of nutrient with 3 parts of plasma.

<sup>3</sup> The sides of the flask portion of the culture tube are thin enough to permit examination of the cultures with the low power of the light microscope and even the high power in some cases. The large flat surfaces supply room for extensive growth from large explants. The volume, the double clot, the long large neck, and the sturdy structure of the flask as well as its adaptability to the roller tube rack for turning make it particularly useful.

<sup>4</sup> Ordinary 22 × 22 mm. coverglass cut in two.

neck of the tube and attached firmly on one side of the culture flask by means of a thin clot of plasma (Text-fig. 1). The tissue supplying the cells was planted in a small clot on the exposed surface of the slide. After 24 hours and then every 2 days for as long as necessary the cultures were fed 1.0 cc. of nutrient solution. When the growth of cells beneath the clot or beyond it was satisfactory for the desired preparations the slides were taken from the flasks and handled thereafter as ordinary coverglass preparations.



TEXT-FIG. 1. Two views of roller flask, diagrammed at one-half size. The cultured cells grow out over the surface of the coverglass insert and can be removed with it.

#### *Preparation of Cells for Electron Microscopy*

As already stated, specimens to be examined in the electron microscope are displayed on an extremely thin plastic or collodion film with no other electron-scattering material between the source of electrons and the fluorescent viewing screen or the photographic plate. The film is supported on a small disc (3 mm. diameter) of fine wire mesh. Ordinarily, a suspension or other preparation of the specimen is placed upon this film-coated disc and allowed to dry; the portions of it displayed over the spaces in the mesh ( $80 \mu$  square) are those examined. It was necessary in these studies to modify this regular procedure

by culturing the cells directly on the plastic film, itself spread on glass. There remains to be described, therefore, the method of fixation and the procedure whereby the film with attached cells is transferred from coverglass to wire screens.

After suitable cultures have been selected and the explants or coagula and explants have been removed, the cultured cells remaining upon the film-coated coverglass are fixed with any one of a variety of fixatives. They can be exposed to vapors as over osmium tetroxide, or placed directly in contact with a solution of the fixative. During fixation, the duration of which is varied depending on type of fixative and results, the cultures are examined under the lower power of the light microscope, and regions of growth showing extremely thin cells or other items of interest are selected for later use.

Where fixatives other than formalin are used, the cells are washed with distilled water. During the washing, which can be adequately done in a 6 cm. Petri dish, the previously selected group or groups of cells are identified under the dissecting microscope, and an area of the film surrounding these, a little larger than the small mesh disc, is marked out. This area of film is then cut from the surrounding film with a fine sharp instrument or a pair of watchmaker's forceps. Thus freed the bit of film with cultured cells is gently peeled away from the glass until only a small corner remains attached. Kept under water in this way the thin sheet of plastic retains its smooth extended form so that adhering cells are not distorted. The small wire mesh disc, immersed beforehand in the washing bath, is now slipped under the film and the two are so manipulated that the film is spread over the screen's surface. They are then lifted from the bath, drained of water, and placed to dry over phosphorus pentoxide. Before studying with the electron microscope, the preparations are examined with the light microscope and their probable value estimated.

All the observations and micrographs presented in this report were made with an RCA, type B, electron microscope. Since the area of the field of this microscope is not great enough to include in most cases, even at low magnifications, the entire spread out cell, it was generally necessary to take micrographs of separate parts and later join these to form a single image.

#### OBSERVATIONS

The majority of the cultures providing cells for this study were derived from explants of chick embryo heart or fore-gut. The general appearance of such cultures and cultured cells is well known and excellent descriptions and illustrations can be found in the literature (3, 4, 10). As a rule, a migration and multiplication of cells could be observed in less than 24 hours of incubation, and these continued for another 36 or more hours depending on the method of culturing. As far as could be noted, no degenerative changes had set in at the time the cultures were used.

The cells most frequently encountered were either fibroblast-like or endothelial. The former are scattered loosely over the culture area, and have irregular outlines. The latter are usually aggregated to form sheets, with the individual elements in intimate contact except along the margins of the growth. Such cells as these have less irregular outlines than the fibroblasts.

When either type of cell comes in contact with the surface of the slide, it may spread to extreme dimensions. The margin may be so thin as to be almost

imperceptible. Shown in Fig. 1 is a *photomicrograph* (taken by ordinary light) of a moderately extended chick embryo fibroblast which had been fixed with osmium, dried, and stained with Giemsa. The nucleus, nucleoli, cell outline, and mitochondria are all visible. It is possible to obtain better photomicrographs (4, 10), but even the best show little more detail, and without exception possess at the magnification used here ( $\times 1250$ ) a fuzziness that results in part from the low resolving power of the microscope using visible light.

Fig. 2 is an *electron micrograph* of a cell cultured, fixed, and treated like that shown in Fig. 1 except that it has not been stained. It too came from a chick embryo and it too is only moderately spread. It has not been greatly magnified ( $\times 1600$ ) and hence comparison with the light microscope image of the stained cell (Fig. 1) can be more readily made. The outline is distinct in every detail, and mitochondria and Golgi bodies are readily observed. The image of the nucleus is without improvement over that shown in Fig. 1 because the central or nuclear portion was too thick for good electron microscopy, but the adequacy of the electron micrograph technique for the demonstration of the finest structural features in other areas is beyond question. It is not at present possible to do more than point out the more obvious details that appear in the various parts of the cell.

*Cell Outline.*—The margin of the cultured cell is thin, sharply defined, and is well suited to electron microscopy. Its most prominent feature is a variety of irregularities or extensions all of which are probably associated in one way or another with the cell's migration. The finest of these extensions are the scarcely perceptible fibers which look as though they had been drawn out fine as the cell pulled away from a surface to which it was attached (Fig. 10 (a)). They are not more than a few  $m\mu$  in width and appear to be derived from the plasma membrane and possibly the ectoplasm just beneath. It is conceivable that by means of these fine filaments a migrating cell could contribute to the formation of connective tissue fibers (see reference 11, footnote, p. 86).

The larger jagged points (Fig. 2 (a), and Fig. 10), which are prolonged into the filaments, are so straight as to be under tension and are probably associated with a retracting margin. Whatever their origin they are unlike and therefore to be distinguished from certain finger-like processes that are blunt and not apparently under tension. These latter can be identified along the upper and lower right margins of the cell in Fig. 2 (b) and more readily in Fig. 18. These, it is assumed, are the processes, resembling tentacles, which have been described as active in advance of migrating cell borders (12).

Another type of extension from the body of the cell is an extremely thin membranous process that gives the impression of flooding out from the margin over the slide's surface (Fig. 17 (a), also in Figs. 3 and 9). These, in life, are said to display undulating motion and are the accompaniment of a spreading or expanding cell (12).

Finally, in connection with the cell outline, it can be noted that there is considerable variation in the apparent thickness of the margin or limiting line of the cell, representing the plasma membrane and possibly an ectoplasmic layer. This is well illustrated in Figs. 5 and 17 as well as by other osmium-fixed cells (Figs. 2 to 4, 10, and 16). In Fig. 17, for example, it is observed that the limits of the thin membranous extensions mentioned above are denoted by a line of no greater density than the body of the extension itself, whereas the edge of the cell proper shows an outline of pronounced density and width. Whether this latter represents a piling up of the cell's margin along a retracted region or whether it indicates the presence of an ectoplasmic layer of greater density cannot be determined from these electron micrographs.

*Ground Substance.*—In several of the electron micrographs, that part of the cell commonly referred to as the ground substance is represented by a material which, on close inspection, appears to be finely granular. This appearance is especially pronounced in those cells subjected the longest to osmium fixation and is most evident in the thinnest portions of the cell. The condition is illustrated in Figs. 4 and 10, where the fringe of the cell looks like a loose mosaic of discrete particles, and in the triangular portion of Fig. 5. From measurements on enlarged micrographs the size of the apparently particulate elements varies from 30 to 150  $m\mu$  in diameter, with the majority in the range of 80 to 100  $m\mu$ . Small densities of this size range are likewise visible in the extended portion of a cell fixed with chromic acid (Fig. 15).

It is not known whether the particulate elements just mentioned pre-exist in the living protoplasm, or whether they are artifacts arising from the cell body or the cell wall, as a result of fixation or drying. In this connection, however, it is of interest to recall that experiments in this laboratory have shown that the chromophilic ground substance is sedimentable and, therefore, probably particulate in nature (13). Touching on this problem also is the fact that small particles, or microsomes, estimated to average about 70  $m\mu$  in diameter have been previously isolated from extracts of normal chick embryos and Chicken Tumor I (6).

Another formation appearing in the ground substance of cells fixed with osmium is a lace-like reticulum. This can be readily seen at relatively low magnification ( $\times 1600$ ) in the lower right corner of the cell in Fig. 2. It looks as if the reticulum had extended into the thin margin and even into the fine processes of the cell from the more dense center where possibly its three dimensional dispersal and overlapping act to confuse the details of its organization. This reticulum if not an artifact is possibly the homologue of the "kinoplasm" described more especially as a cytoplasmic differentiation of plant cells (14).

At higher magnifications (Figs. 4 and 16), vesicle-like bodies, *i.e.* elements presenting a center of less density, and ranging in size from 100 to 150  $m\mu$ , can be seen arranged along the strands of the reticulum just mentioned. In

chromic acid-fixed cytoplasm, granules can be noted within the strands of shrunken cytoplasm (Fig. 15). It is conceivable that these, and the "vesicles" of the osmium-fixed reticulum, are identical.

*Mitochondria.*—These cytoplasmic components have appeared with impressive clarity in most of the cells after osmium tetroxide fixation and the majority of the characteristics observable are the same as those that have been reported from light microscope studies. In the chick embryo cells that have been examined the mitochondria were found to be mostly of the filamentous type (Figs. 7, 9, and 10); occasionally spherical or vesicular forms were observed (Figs. 3 and 17).

The electron microscope has revealed morphological details in mitochondria not hitherto detectable with the ordinary light microscope. In some cases, inclusions in the form of masses of greater density and variable size appear distributed along the body of the mitochondria (Figs. 8 to 10). Other formations, visible only at high magnification (Fig. 8) appear as extremely minute bodies, not more than 10 to 20  $m\mu$  in diameter. The relatively high density of these smaller elements suggests that they may be composed mainly of inorganic salts, or that they represent centers where reduced osmium has accumulated.

The minute elements just mentioned are found distributed throughout the body of the mitochondrion and in places serve to outline sharply its contour (Fig. 8). The latter observation indicates that the limiting surface between mitochondria and the surrounding cytoplasm is well defined although in none of the micrographs is there distinct evidence of a differentiated cortex.

*Golgi Bodies.*—There have consistently appeared in these micrographs dense bodies characterized by rather angular outlines. Sometimes, as shown in Figs. 2, 6, and 12, they are grouped together near the nucleus; in other cells they are scattered about and fewer in number (Fig. 9). That they are Golgi bodies is strongly suggested by their size, distribution, and seemingly osmiophilic properties, and their subdivided, dispersed state in no way discourages such a view (4, 5, 15). Their density after osmium has prohibited an exploration of their internal structure if any exists.

*Cell Nuclei.*—Among the cultured cells so far examined the nucleus has been too thick, as a rule, for a detailed electron microscopic study. It is of some interest to note, however, that after osmium the nucleus appears of uniform density except for the nucleoli which are usually discernible (Figs. 2 and 9). Following the use of such fixatives as Flemming's fluid, chromic acid, or formaldehyde, the conventional precipitation patterns appear with more prominent nucleoli (Figs. 11 to 13).

*Intercellular Relationships.*—A few relationships between cells have been observable in the preparations thus far studied. Fig. 14, for example, shows lines of contact between three squamous endothelial cells fixed with formaldehyde. Along the line of fusion each cell border is thickened and in spite of

considerable cytoplasmic shrinkage and tearing (probably due to drying) the cohesiveness of the contiguous cell margins has been sufficiently strong to maintain contact except at certain spots. Fibroblasts in contact (Fig. 3) have been noted to display claw-like processes which are not characteristic of the attachment between such cells and the plastic-coated surface of the slide. The fibrocyte in Figs. 2 and 4 is in contact with nerve endings, but the visible details are not at present interpretable.

*Nerve Ending.*—Of some special interest in these electron micrographs is the image of an ameboid nerve ending or growth cone of the sort initially described by Cajal (Figs. 2 and 4). It is an ending of a sympathetic nerve fiber grown from chick embryo fore-gut and in general outline is similar to the growing nerve endings depicted at a much lower magnification in the *drawings* of Harrison (1) or Lewis and Lewis (2). The finest ramifications of probable significance so far seen have been between 200 and 300  $\mu$  in diameter. The long one shown in Fig. 4, for example, extending toward the top of the micrograph is about 300  $\mu$  across. It has a much finer short lateral extension off to the right, but this is probably the remains of a larger branch that has been retracted. Thus far, nerve endings too small to be seen with the ordinary microscope have not been encountered.

The defining margins of the long extension (Fig. 4) are smooth in comparison with those of the body of the growth cone. Why the latter should be so irregular is not clear. Possibly it is due to the existence of innumerable small pseudopodia, or possibly to fixation. The edge of the adjacent fibrocyte is by contrast quite smooth. The density of the growth cone and of some of its branches is greater than that of the extended fibroblast, owing perhaps to a greater thickness, greater density of molecules, or to a greater capacity of its plasma membrane and cytoplasm to reduce osmium tetroxide. The finer branch of the ameboid ending is seen to have a central axis, which gives the impression of having granules scattered along it (2). Close examination reveals some similarities between the structure of this axis and the structure of the strands which constitute the reticulum of the adjacent fibrocyte. Observations as preliminary as these serve mainly to give direction to further studies on this and other cell types.

*Cell Thickness.*—An unexpected opportunity for making a direct measurement of cell thickness was provided by one preparation. As shown in Fig. 18, a portion of an extended cell has been caught within a crease in the plastic film and where folded over the edge of the crease its thickness is revealed. In this dry state it varies between 30 and 60  $\mu$ .

#### *The Effects of Various Cell Fixatives*

Since materials to be examined by electron microscopy must be dry, it was necessary to find fixatives that would keep the cell relatively life-like in spite

of desiccation. To this end, a small number of common fixatives have been tested, some more thoroughly than others. Fixation was continued in most cases for a relatively short time (5 to 15 minutes), because the thinness and exposed character of the cells made longer treatment seem unnecessary. It is possible that some of the configurations observed are referable to insufficient fixation.

*Formaldehyde*, 4 per cent (pH 3.7), was first used, and after drying the formalin-fixed cytoplasm had the appearance of a coarse spongework (Figs. 11 and 14). Since shrinkage, like that pictured, was not detected during fixation (3), it is assumed that this structural configuration is largely the product of subsequent dehydration. Formed bodies of the cytoplasm such as mitochondria are not visible and may be obscured by the strands of the shrunken cytoplasm. Otherwise formalin-fixed cells are conventional in showing a typical shape and a clear segregation of nucleus and cytoplasm.

*Osmium tetroxide* (osmic acid, pH neutral). This was applied in vapor form from a 2 per cent water solution for a short period after which the fixed cells were washed in water. Examination with the light microscope during fixation detected blackening of some intracellular bodies but no shrinkage or change in outline.

The ground substance of the cytoplasm in cells receiving only a brief treatment with osmium tetroxide (15 minutes) appears quite uniform and inclusions stand out against it with striking clarity (Figs. 6 to 9 and 17). The nucleus is generally homogeneous, and the nucleoli appear as bodies of slightly greater density.

Cells fixed with osmium for a longer period (45 minutes) present a somewhat different picture (compare Figs. 2 and 10 with Figs. 6 or 9). Mitochondria and nucleus show increased density and in the otherwise undifferentiated cytoplasm there now appears a definite fine reticulum and what may be granules. These have been described in preceding paragraphs. The best technique of osmium fixation has not yet been worked out.

*Chromic acid*, 1 per cent water solution (pH 1.5), was a third fixative tried. It produced in the cytoplasm a sharply defined spongework and the nucleus shows one that is similar though less well defined (Figs. 13 and 15). Observed during fixation the cell was noted to shrink considerably immediately upon contact with the fixative. Presumably the strong protein-precipitating powers of chromic acid, resulting from its acidity, cause the cytoplasm to draw up into clumps and strands and pull away from the margin of the cell (Fig. 15). The density of this precipitated network may derive in part from a combination between chromic acid and protein (16). The only formed bodies of the cytoplasm to appear in the micrographs were what seem to be very small granules caught within the fine strands of cytoplasm (Fig. 15).

It is of interest to note that the electron micrographs of the chromic acid-

fixed cells have a three dimensional appearance. This is doubtless real and results from the large depth of field (conservatively 5 micra) possessed by the electron microscope. If the material being examined is sufficiently open and thin then structures in different planes over the entire depth of the field are reproduced in sharp outline. A stereoscopic study of such material is possible and would show strands of the spongework in their true relationship.

*Flemming's solution*,<sup>5</sup> (pH 1.2), was tried in an attempt to improve, in the sense of making more conventional, the image of the nucleus. In the hope that the mitochondria and cytoplasm in general might also be plainly seen, the cells were treated first with osmium tetroxide vapors for 7 minutes and then with Fleming's solution for 15 minutes. The result is shown in Fig. 12. The nucleus is more finely granular and in an ordinary stained preparation might be said to show good fixation. The cytoplasm, in spite of the initial osmium treatment and the presence of osmium in the Fleming's solution, shows the type of spongework seen in chromic acid preparations. Mitochondria can be made out, but the coarseness of the cytoplasm background confuses their outline.

Alcohol, acetic acid, freezing and drying, and simple drying were also used as fixation procedures. The images were on the whole unsatisfactory in that they failed to resemble closely those of living cells as observed by transmitted or reflected light.

#### DISCUSSION

The observations reported in this paper, though preliminary, seem adequate to show that the detail of cell outline, cytoplasmic ground substance, mitochondria, etc., revealed in electron micrographs is in excess of that which can be seen with the light microscope. In part responsible for this is the far greater resolving power of the electron microscope. Micrographs taken at the conservative magnification of 1600 are so packed with detail that the smaller clearly resolved structural features are not easily seen until the pictures are enlarged two or three times (compare Figs. 2, 4, and 5). But also important is the fact that the cells are thin enough for differential penetration of electrons with a minimum of multiple scattering and a consequent fair definition of cellular components. It appears, therefore, from these early observations that the cultured cell, when selected for thinness, lends itself well to electron microscopy.

It is not at present possible to interpret relative to cell morphology or composition all the details revealed in these micrographs. Some of them may be artifacts of fixation and drying, and to distinguish between these and those

<sup>5</sup> Chromic acid 1 per cent (15 cc.), osmium tetroxide 2 per cent (4 cc.), acetic acid, glacial (0.25 cc.).

that represent genuine formed structures of the cell will require further study.

Of the fixatives so far used osmium tetroxide has been the most satisfactory in that it yields what impresses one as being the most truthful picture of the cell. It seems not to distort the cells and it largely prevents subsequent dehydration effects. It may serve also as a differential stain, for by its action some cellular structures are apparently rendered more electron-scattering than others. Presumably this latter effect results from a differential reduction of the tetroxide and a consequent differential deposition of the lower oxides of osmium. If not treated with osmium or another fixative of similar properties the magnitude of variation in the molecular densities which determine electron scattering and absorption may not be sufficient to outline distinctly some of the formed bodies present. This may help explain the apparent absence of such bodies from electron micrographs of formalin-fixed cells. For electron microscope preparations the properties of osmium as a fixative are therefore excellent.

The electron micrographs of cells treated with fixatives other than osmium tetroxide provide excellent illustrations of alterations that can arise from fixation. The loose spongework shown is neither present in the living cell nor is it the same with the different fixatives. Abnormal as these configurations are, they are not, however, without interest; they result from a reaction between fixative and cytoplasm and may be indicative of the latter's composition. Certainly the electron microscope through its greater powers of resolution reveals major differences between fixation artifacts which formerly were barely detectable.

The operation of the electron microscope requires that the object for study be placed in a high vacuum, so making it necessary for the material to be thoroughly dry. Although this has been considered to be one of the chief obstacles to the study of biological material, the present work shows that it can be overcome through osmium fixation. Probably other ways will be found to cope with this same difficulty.

Finally in connection with artifact production it is important to mention that the electron energy absorbed, whatever the amount, seemed not to alter the material greatly. The preparations were studied with 60 kv. electrons and only after some minutes of exposure did the outlines of structures show any loss of distinctness. There was vacuolation of portions of the cells in some of the earliest preparations, possibly resulting from the high vacuum or energy absorption, but this was never observed in dry, osmium-fixed cells. The thicker parts of a preparation have been noted to shrink and develop cracks under electron exposure, presumably because of heating. Since such portions in any case were not especially suitable for electron microscopy, this was not a serious fault.

## SUMMARY

By means of a tissue culture technique, cells from chick embryos were procured in a state which proved to be suitable for electron microscopy. The electron micrographs disclosed details of cell structure not revealed by other methods of examination.

It is a pleasure to acknowledge the helpful assistance of Mary Schuster and Vincent Salines in the preparation of micrographs.

## BIBLIOGRAPHY

1. Harrison, R. G., *J. Exp. Zool.*, 1910, **9**, 787.
2. Lewis, W. H., and Lewis, M. R., *Anat. Rec.*, 1912, **6**, 7.
3. Strangeways, T.S.P., and Canti, R. G., *Quart. J. Micr. Sc.*, 1927, **71**, 1.
4. Richardson, K. C., *Arch. exp. Zellforsch.*, 1934, **16**, 100.
5. Luford, R. J., *Proc. Roy. Soc. London, Series B*, 1927, **101**, 409.
6. Claude, A., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 398; *Science*, 1943, **97**, 451.
7. Marton, L., *J. Bact.*, 1941, **41**, 397.
8. Parker, R. C., *Methods of tissue culture*, New York, Paul B. Hoeber, Inc., 1938.
9. Gey, G. O., *Am. J. Cancer*, 1933, **17**, 752.
10. Lewis, W. H., and Lewis, M. R., Behavior of cells in tissue cultures, in *General cytology*, (E. V. Cowdry, editor), Chicago, The University of Chicago Press, 1924, 383.
11. Danielli, J. F., The cell surface and cell physiology, in *Cytology and cell physiology*, (G. Bourne, editor), Oxford, Clarendon Press, 1942, 68.
12. Wilbur, K. M., and Chambers, R., *J. Exp. Zool.*, 1942, **91**, 287.
13. Claude, A., in *Frontiers in cytochemistry*, (N. L. Hoerr, editor). *Biological symposia*, Vol. **10**, Lancaster, The Jaques Cattell Press, 1943, 111.
14. Scarth, G. W., *Protoplasma*, 1927, **2**, 189.
15. Worley, L. G., *J. Morphol.*, 1944, **75**, 261.
16. Baker, J. R., *Cytological technique*, London, Methuen and Co. Ltd., 1933.

PLATES

EXPLANATION OF PLATES<sup>6</sup>

## PLATE 10

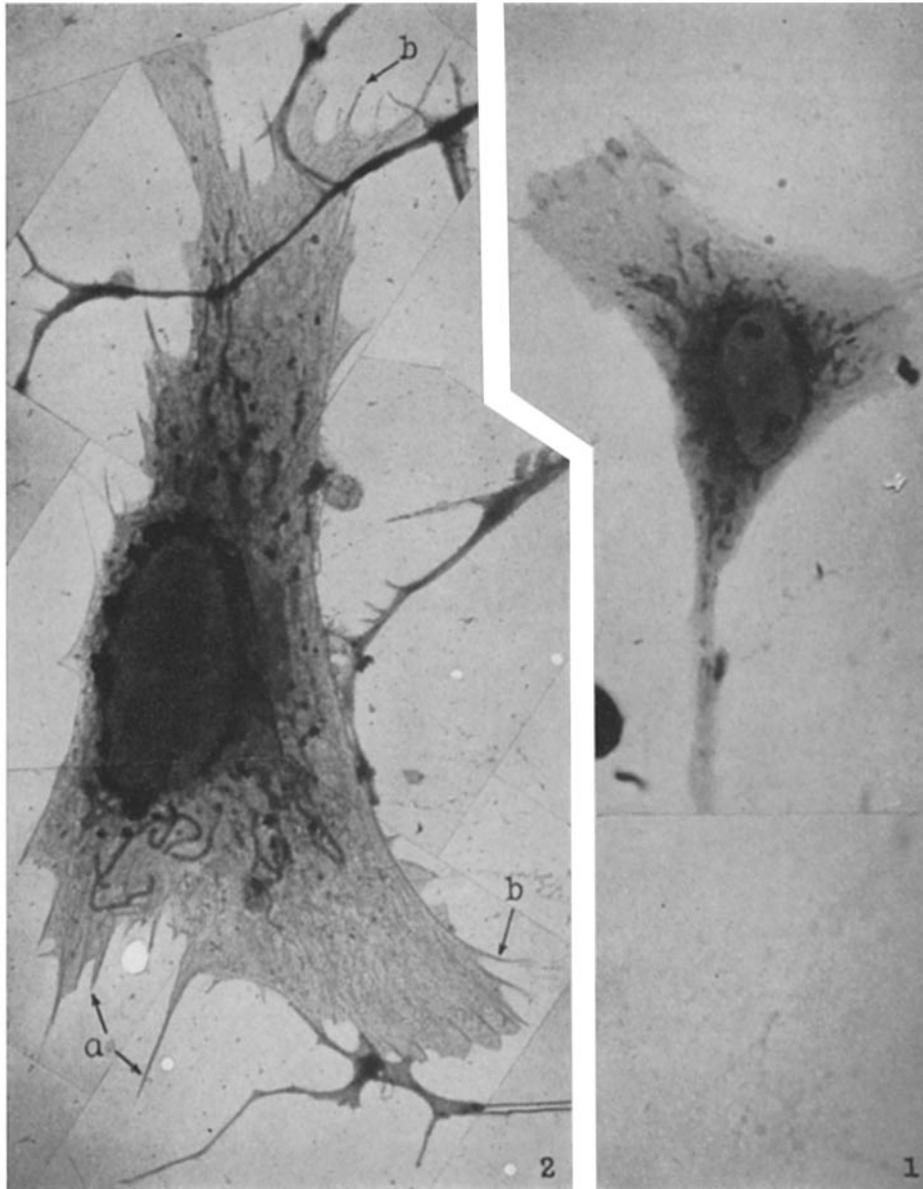
FIG. 1 (on the right). Photomicrograph of a fibroblast-like cell cultured from chick embryo tissue. The cell was osmium-fixed and treated like those prepared for electron microscopy with the single exception that it was stained with Giemsa to permit photography with the light microscope. *Culture*: chick embryo fore-gut grown 48 hours on slide coated with formvar film, in hanging drop of Locke's solution. *Fixation*: vapors from a 2 per cent osmium tetroxide solution for 45 minutes. Stained with Giemsa. Photomicrograph taken at 900  $\times$  and enlarged here to 1250.

FIG. 2. Electron micrograph of a fibroblast-like cell, and nerve fibers cultured from chick embryo tissue. Differential absorption and scattering of electrons by the cytoplasmic area has silhouetted a number of structural details among which are: filamentous mitochondria of various lengths and fairly constant width; scattered, small elements of high density especially abundant around the nucleus and presumably representing Golgi bodies; and a delicate lace-work extending throughout the cytoplasm. The nucleus is visible but multiple scattering of electrons due to excessive thickness results in considerable blurring. Three nerve fibers can be seen: one crossing the upper part of the picture and having no connection with the cell; one ending in contact with the cell surface at the right; and one at the lower part of the picture also in contact with the cell surface. This latter has the appearance of a growth cone. Details of the cell's margin and extensions are clearly defined. The arrows point to extensions mentioned in the text as "jagged points" (a) and "finger-like processes" (b).

Comparison of the similar cells in Figs. 1 and 2 shows the present relative values of the two different microscopic techniques. For further comparison better photomicrographs can be found in papers by Richardson (4) and by Lewis and Lewis (10).

*Culture*: chick embryo fore-gut grown 48 hours on slide coated with formvar film in hanging drop of Locke's solution. *Fixation*: vapors from a 2 per cent osmium tetroxide solution for 45 minutes followed by a 30 minute washing in distilled water during which the cells and supporting film were transferred to the screen. Electron micrograph taken in five parts. Magnification 1600.

<sup>6</sup> A hand-lens may be useful for examining figures. Unfortunately methods of reproduction are not able to duplicate all the interesting fine detail of the original micrographs.



(Porter *et al.*: Electron microscopy of cultured cells)

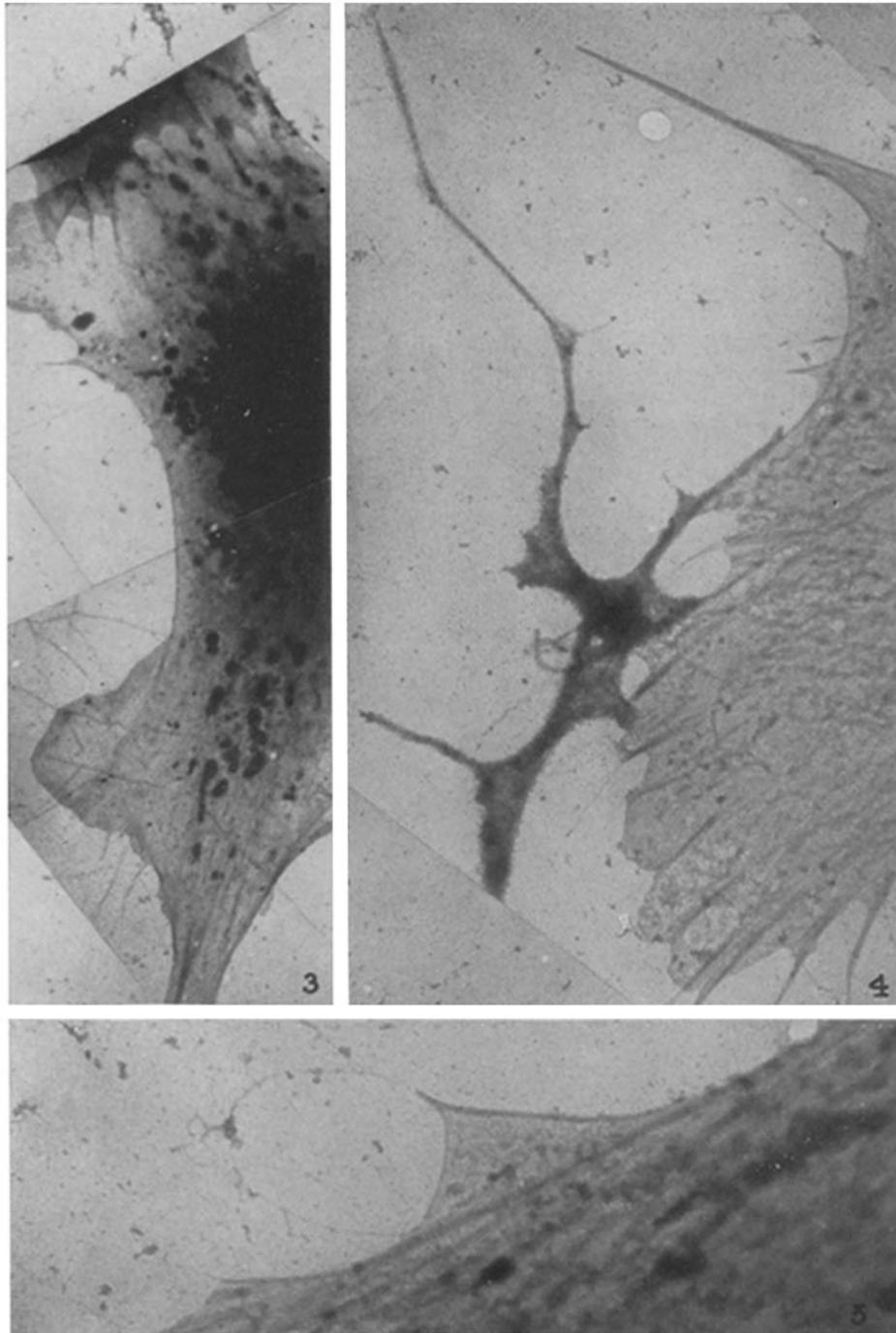
PLATE 11

FIG. 3. *Electron micrograph* of a portion of a cultured cell. Details of outline and cytoplasmic content are clear except in central portion where density and thickness of the cell have prevented penetration of electrons. For some reason possibly related to condition of the cell at time of fixation, the majority of the mitochondria have rounded up. The denser bodies showing angular outlines are probably Golgi bodies. The upper end of the cell is connected to an adjacent fibroblast by claw-like extensions, and on the left hand margin of the lower extension the cell is apparently developing a new pseudopodium. *Culture*: chick embryo fore-gut grown in a hanging drop of Locke's solution for 48 hours. *Fixation*: with vapors from 2 per cent osmium tetroxide for 10 minutes, then rinsed in Locke's solution and washed 15 minutes with water. Micrograph taken in 2 sections on high resolution plates.<sup>7</sup> Taken at 1600  $\times$ , enlarged to 2500.

FIG. 4. Enlargement of a portion of Fig. 2, to show the finer structure of the nerve cell growth-cone, especially that of its most delicate extension. To be noted as well are (1) the cytoplasmic reticulum along the right hand margin of the field, and (2) what appears to be a mosaic of granules constituting the ground substance. The figure also illustrates the importance of enlargement for showing to best advantage the details of the original electron micrograph negatives. Taken at 1600  $\times$ , enlarged to 4000.

FIG. 5. Enlargement of another section of Fig. 2 to show in greater detail than elsewhere the granular appearance of the ground substance of an osmium-fixed cell (especially in the triangular extension). Also to be observed is the apparent variation in thickness of the line bounding the cell's edge (see also Figs. 3 and 17). Taken at 1600  $\times$ , enlarged to 6000.

<sup>7</sup> Eastman Kodak No. 548-G.



(Porter *et al.*; Electron microscopy of cultured cells)

#### PLATE 12

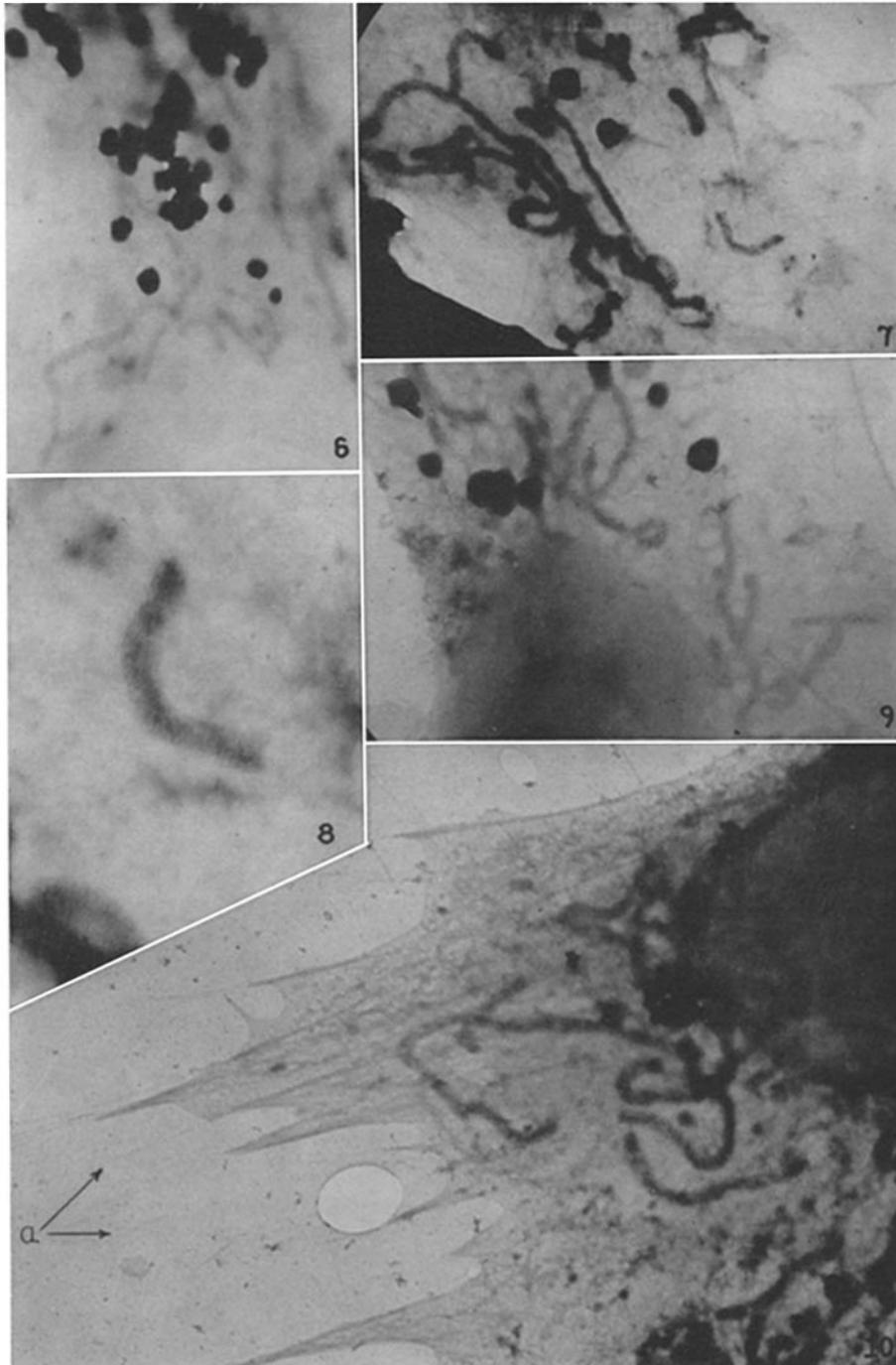
FIG. 6. Electron micrograph of portion of cell from culture of chick embryo tissue. The intensely black structures with *angular outlines* (seen also in Figs. 2, 3, 7, 9, and 12) are probably Golgi bodies. From their density it appears that they have retained large quantities of osmium. *Culture*: chick embryo fore-gut grown for 48 hours in a plasma clot in a roller flask. *Fixation*: by vapors from 2 per cent osmium tetroxide for 15 minutes followed by 25 minutes of washing in distilled water. Magnification 4500.

FIG. 7. Electron micrograph of part of a cultured cell to show form and detail of mitochondria. The dark shadow across the lower left hand corner represents the edge of a wire strand in the screen. *Culture*: chick embryo spinal column grown 48 hours in a hanging drop of Locke's solution. *Fixation*: by vapors from 2 per cent osmium tetroxide for 15 minutes followed by a 30 minute washing in water. Micrographed on high resolution plate.<sup>7</sup> Magnification 4500.

FIG. 8. A section of Fig. 7 enlarged. Shown within the single mitochondrion are large areas of increased density as well as a great number of what appear to be extremely fine granules. Magnification 15,000.

FIG. 9. Electron micrograph of a segment of a cell from the same preparation that supplied the subject for Fig. 7. All the mitochondria in the electron micrograph of the entire cell were as clearly visible as these in this portion. They are uniformly filamentous but of variable lengths and contours. Golgi bodies are also shown. Taken at 4100  $\times$ , enlarged to 4500.

FIG. 10. Enlargement of another portion of Fig. 2. Filamentous mitochondria appear to show variations in density. In addition there are to be noted: (1) the sharply pointed extensions of the cell, which, at the time of fixation, were probably retracting and leaving behind extremely fine threads of plasma membrane and ectoplasm, indicated by arrows (*a*), (2) granular structure of ground substance. Taken at 1600  $\times$ , enlarged to 4100.



(Porter *et al.*: Electron microscopy of cultured cells)

### PLATE 13

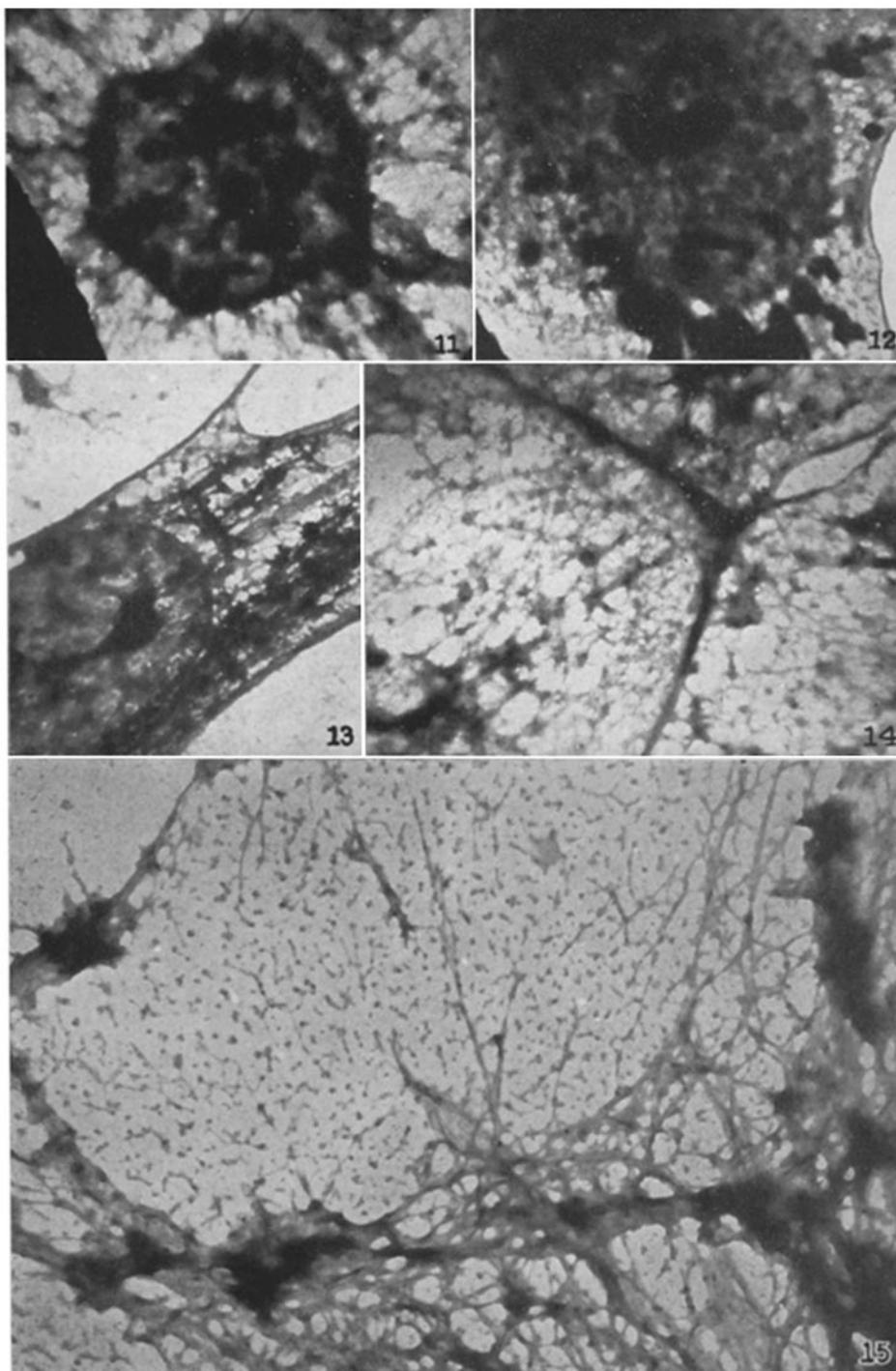
FIG. 11. Electron micrograph of nucleus of a cultured mesothelial cell. It shows the vacuolation of formalin-fixed nucleus and cytoplasm, a result probably of shrinkage due to drying. The micrograph should be compared with the others showing fixation artifacts following the use of Flemming's solution (Fig. 12), chromic acid (Fig. 13), and osmium tetroxide (Figs. 9 and 10, etc.). *Culture*: heart of chick embryo grown for 72 hours in plasma clot in roller flask. *Fixation*: 10 per cent formalin (pH 3.7) for 15 minutes. Magnification 4100.

FIG. 12. Electron micrograph of a portion of a cultured cell fixed with vapors of osmium tetroxide (2 per cent) for 7 minutes and then in Flemming's solution for 15 minutes. This was done in an attempt to obtain conventional nuclear configuration and at the same time retain the mitochondria of osmium fixation. The resulting nucleus shows more pronounced precipitation than is characteristic after osmium alone and the cytoplasm resembles a fine spongework; both have resulted from the treatment with Flemming's solution. The dark elements are presumably Golgi bodies. *Culture*: fore-gut of chick embryo grown for 48 hours in plasma clot in roller flask. Magnification 2900.

FIG. 13. Electron micrograph of a portion of the nucleus and cytoplasm of chick fibroblast to show the precipitation pattern characteristic of chromic acid fixation. Also to be noted is the three dimensional aspect, a product of the relatively great depth of field possessed by the electron microscope. *Culture*: fore-gut of chick embryo grown in roller flask for 72 hours. *Fixation*: 1 per cent chromic acid (pH 1.5) for 90 minutes followed by a 30 minute washing in distilled water. Magnification 4100.

FIG. 14. Electron micrograph of portions of three adjoining mesothelial cells found in the same preparation as the cell and nucleus shown in Fig. 11. The sponge-like structure of cytoplasm is typical of formaldehyde fixation and drying. The contiguous cell margins are slightly thickened. The cell edges have pulled apart in one section. Taken at 4100  $\times$ , enlarged to 4500.

FIG. 15. Electron micrograph of a cell margin found in the same preparation (screen) that provided Fig. 13. Of particular interest are (1) the pattern of cytoplasmic precipitation and shrinkage following chromic acid fixation, (2) the apparent depth of field displayed, (3) the small granule-like bodies along the strands of the spongework in the lower right hand section of the picture, and (4) the irregular fragments of material associated with what appears to be granules (over upper half of picture), which, before fixation, constituted together an extremely thin layer of cytoplasm plus cell membrane. Taken at 4500  $\times$ , enlarged to 9000.



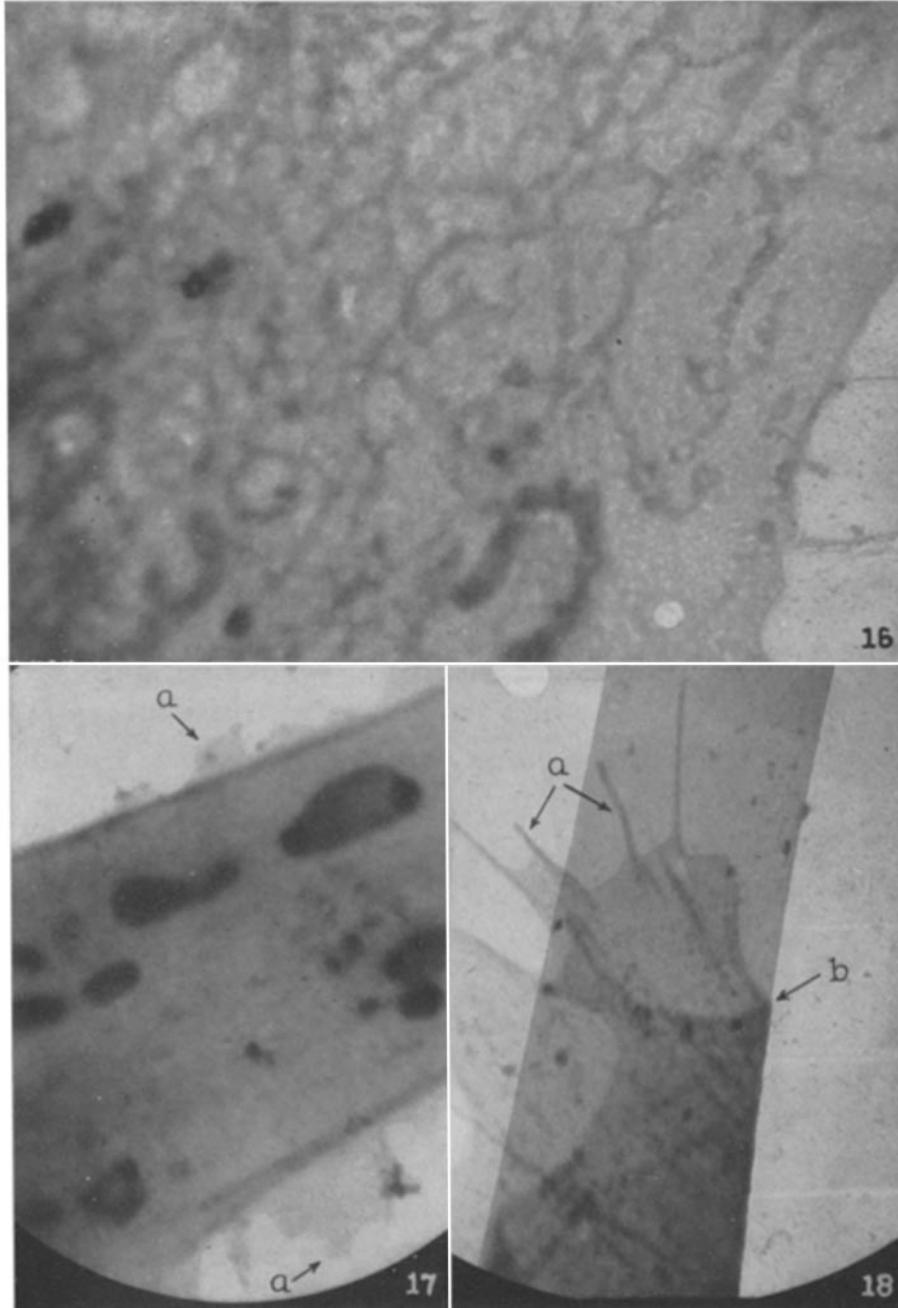
(Porter *et al.*: Electron microscopy of cultured cells)

PLATE 14

FIG. 16. Electron micrograph of small section of cultured chick fibroblast. Thinner portion of cell at right of figure shows a granular background and details of a darker lace-like reticulum which in places appears to be made up of chains of "vesicles." Culture and fixation same as for cell in Fig. 2. Taken at 4500  $\times$ , enlarged to 10,000.

FIG. 17. Electron micrograph of section of cell from same preparation (screen) as cell in Fig. 7. Presented to show extremely thin membranous processes indicated by arrows (*a*). To be noted as well are (1) the thickened cell membrane and possibly ectoplasm along the edge of the cell body and (2) the vesicular mitochondria. Taken at 6600  $\times$ , enlarged to 7800.

FIG. 18. Electron micrograph of a portion of a cell from the same preparation as Fig. 2. While being placed on the screen the plastic film folded and the cell lying thereon was doubled upon itself. Where the cell extends over the edge of the fold its thickness (indicated by arrow (*b*)) is displayed and measures from 30 to 60  $\mu$ . This is the thickness of a marginal portion of a thinly spread *dry* cell. Also of interest are the finger-like extensions from the edge of the cell indicated by arrow (*a*). Taken at 4500  $\times$ , enlarged to 6300.



(Porter *et al.*: Electron microscopy of cultured cells)