ing the branch of the inverted "Y" tube that is shown perfusing the central end of the left subclavian artery in Fig. 1. Utilizing the central ends of the common carotid and left subclavian arteries, the isolated aortic arch around the outside of the cannula can be perfused and its pressure recorded. Many experiments requiring such a preparation were suggested by Aviado and Schmidt(7).

Summary. In anesthetized open-chest dogs, a relatively simple method is described for isolating total coronary artery inflow without coronary dissection by using a specially designed double lumen cannula. The small lumen is used for injecting pharmacologic agents into the coronary artery inflow; the large lumen permits direct measurement of left ventricular output. The method can be modified for studying the aortic arch receptors.

1. Hufnagel, C. A., *Bull. Georgetown Univ. Med. Center,* 1951, v4, 128.

2. Wang, H. H., Blumenthal, M. R., Wang, S. C., *Circulation Research,* 1960, v8, 271.

3. Rayford, C. R., Khouri, E. M., Lewis, F. B., Gregg, D. E., *J. Appl. Physiol.,* 1959, v14, 817.

4. Ross, J., Jr., Mosher, P. W., Shaw, R. F., *Circulation,* 1961, v24, 1025.

5. Wang, H. H., Katz, R. L., Nahas, G. G., Wang, S. C., *Fed. Proc.,* 1962, v21, 106.

6. Blumenthal, M. R., Wang, H. H., Markee, S., Wang, S. C., *Proc. XXII Internat. Cong. Physiol. Sci.,* Leiden, 1962, Abst. 64.

7. Aviado, D. M., Jr., Schmidt, C. F., *Physiol. Rev.,* 1955, v35, 247.

Received April 24, 1963. P.S.E.B.M., 1963, v113.

Critical Point Preparations of Intact Cells for Electron Microscopy.* (28468)

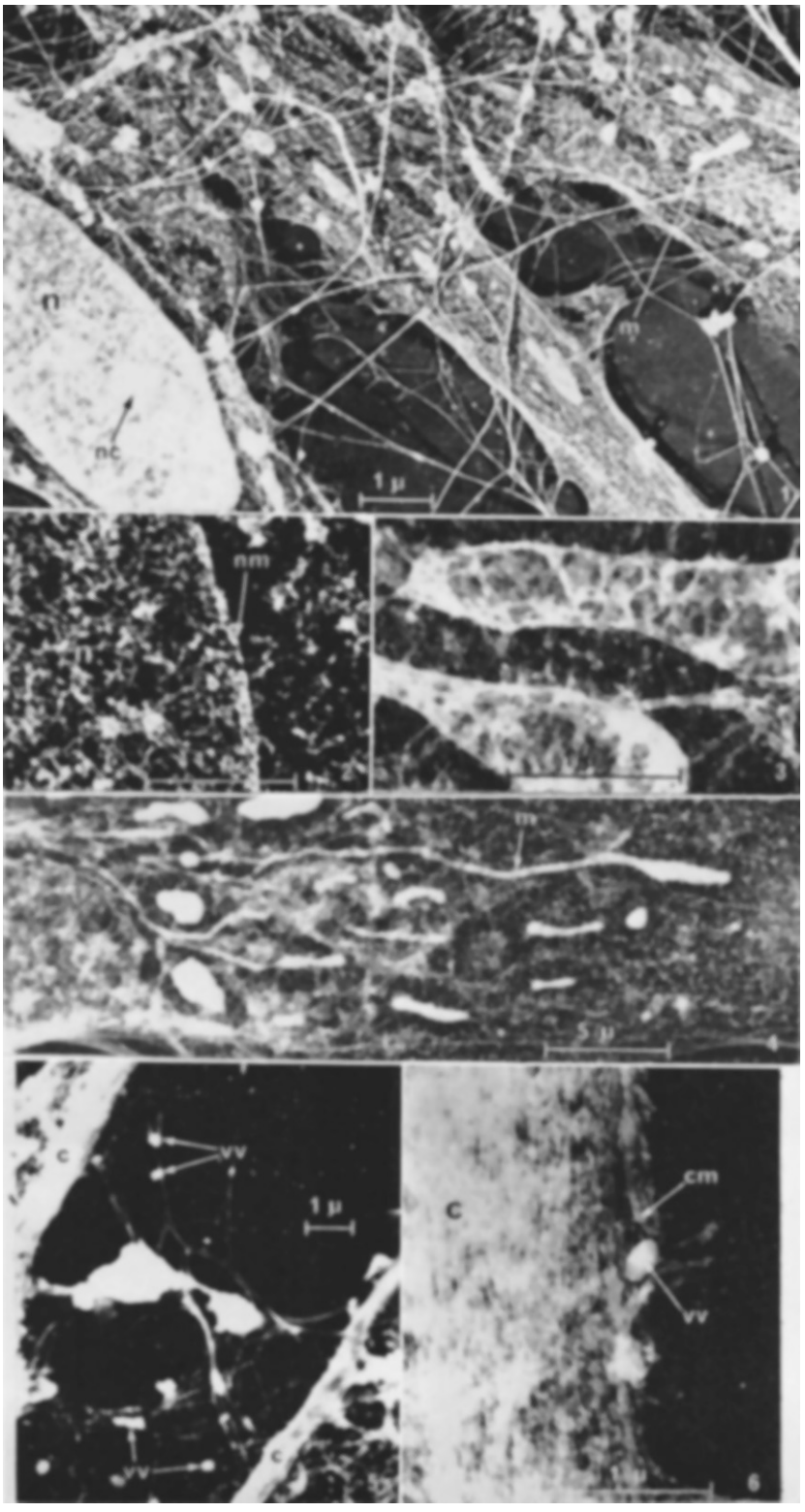
JOHN R. OVERMAN

Departments of Microbiology and of Medicine, Duke University, Durham, N. C.

The use of intact cells for study with the electron microscope has been limited by the structural distortion produced by drying and the thickness of the unsectioned cell which blocks penetration by the electron beam. The desirability under certain circumstances of observations on the intact cell, however, is obvious. The area of cell surface is vastly greater than in cell sections and, although 3 dimensional reconstructions of cells can be made from serial thin sections, the overall relationship of some of the major cell components could be determined more readily in the whole cell. Finally, possible variations in structural dimensions may not be apparent in sections but may be perfectly obvious in the intact cell if the structures in question are visible and identifiable. The present report demonstrates the application to tissue cells of the critical point method of Anderson previously applied to viruses and bacteria(1,2). The critical point of a fluid, as defined by Ramsey and quoted by Anderson is "that point at which the liquid, owing to expansion, and the gas, owing to compression, acquire the same specific gravity and consequently mix with each other." Essentially, then, by this method liquid (water) can be removed from a cell at the critical temperature with the surface tension at zero and drying without distortion or flattening is possible.

Materials and methods. In the method used by Anderson and followed here, water is replaced by alcohol, this replaced by amyl acetate and the latter replaced by liquid CO_2 whose critical temperature of 31.1 C is more convenient than the very high critical temperature of water. Chicken fibroblasts were grown on coated electron microscope grids as described previously(3,4). After the cells had attached to the supporting film, the grid mounts were removed, washed and fixed with veronal-buffered osmium for one minute. The fixed preparations were then re-washed in acetate-veronal buffer of Michaelis and passed

* This work was supported by a U. S. Public Health Service grant.


n
nc
m
1 μ
nm
3
m
5 μ
c
vv
1 μ
vv
c
cm
c
vv
6

through the replacement series outlined and into the apparatus designed by Anderson for using CO_2 at the critical point. Preparations were lightly shadowed with platinum and the micrographs made in an RCA EMU-3F Electron Microscope.

Results. The overall absence of cell flattening during drying and the excellent preservation of morphologic detail resulted in a 3-dimensional aspect of the cultured tissue cell having many features in common with drawings or reconstructions. As is apparent in Fig. 1, some portions of the cell were fixed relatively large distances above the film and measurements of the highest portions of the individual cell structures to the supporting membrane gave a distance of 25 μ or greater. Interconnecting many of the cells was a delicate fibrillar network distinct from cell microvilli and from cell bridges. This network was not present in air dried preparations made in this laboratory, nor has it been found in thin sections of these cells. Note that a few fibrillar strands in Fig. 1 held together long enough to cast a shadow but then disappeared, probably disrupted by the electron beam. Considering the delicacy of these fibrils and the fact that they lie unsupported so far above the membrane, it is remarkable that these structures persist at all. The different levels which a single structure may occupy is demonstrated in most of the micrographs which show both in- and out-of-focus detail. Thus, cell components can be examined at the different levels by changing focus.

Preservation of the nucleus (Fig. 2) and mitochondria (Fig. 3) was good but the amount of flattening and visualization varied from cell to cell and in parts of a single cell. The mouse fibroblast is much thicker than the chicken fibroblast and visualization of its nucleus was not comparable to that in Fig. 2. Of further interest was the length, diameter and distribution of the mitochondria in relation to the ectoplasm and the endoplasmic reticulum, dimensions and relationships which are difficult to assess in thin sections. Although the greater number of mitochondria were in the endoplasmic reticulum, many were found close to the plasma membrane and some even extended into cell processes. None was found in microvilli. Marked variations were found in mitochondrial length, shape, and diameter. One mitochondrion (Fig. 4) measured more than 20 μ in length and varied in diameter from .5 to .1 μ.

Studies in this laboratory have been concerned with the uptake of virus particles by cells and active vaccinia virus was added to cells which were then preserved by the critical point method. The fibrillar network described above appeared to enmesh virus (Fig. 5) but obviously is not a specific response to vaccinia since it is present in uninfected cells. The preliminary studies indicate, however, that this network is more extensive in the virus-infected cultures. Various stages of virus entry into the cell were found as exemplified by Fig. 6.

Critical point drying of cultured cells provides an adjunctive method for the electron microscope study of cell structure and, indeed, presents some cell structures in aspects not possible by any other method. Thus, this method may have applicability to many problems involving cell structure. Further studies of normal cell structure as found in critical point dried cells as well as further study of cell-virus relationship are needed.

Summary. Cultured chicken fibroblasts and

FIG. 1. Parts of 3 chicken fibroblast cells interconnected by a delicate fibrillar network. The fibrils in many instances lie a considerable distance above the supporting film. A cell nucleus (n) with visible nucleolus (nc) and mitochondria (m) are identifiable (×9,360).

FIG. 2. Nucleus (n) and nuclear membrane (nm) of chicken fibroblast. The thinnest nuclear fibrils measure about 100 Å in this and other micrographs (×20,000).

FIG. 3. Two mitochondria with internal structure undoubtedly representing cristae. Note the marked narrowing of mitochondria in some segments (×24,000).

FIG. 4. A mitochondrion (m) measuring about 20 μ in length. Marked variation in length and shape is common (×3,360).

FIG. 5. Vaccinia virus (vv) particles apparently trapped in fibrillar network between 2 cells (c). Although this network is present in normal cells as seen in Fig. 1, it appears to be greatly increased in virus-infected cells (×6,450).

FIG. 6. Vaccinia virus (vv) in an opened area of the cell membrane (cm) of a cell (c) (×24,800).

other cells retain their normal 3-dimensional morphology when dried by the critical point technique yet nuclear structure and cytoplasmic components are well visualized in the electron microscope. A delicate intercellular fibrillar network is described as are some aspects of virus uptake by these cells.

1. Anderson, T. F., *Trans. N. Y. Acad. Sci.*, 1951, v13, 130.

2. ———, in *Physical Techniques in Biological Research*, G. Oster and A. W. Pollister, Eds., Academic Press, N. Y., 1956, III, 178.

3. Overman, J. R., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 895.

4. Overman, J. R., Eiring, A. G., *ibid.*, 1961, v107, 812.

Received April 26, 1963. P.S.E.B.M., 1963, v113.

Susceptibility of Conventional and Germfree Mice to Lethal Effects of Endotoxin. (28469)

S. BORGLUM JENSEN, STEPHAN E. MERGENHAGEN, ROBERT J. FITZGERALD AND HAROLD V. JORDAN

National Institute of Dental Research, National Institutes of Health, U. S. Department of HEW, Bethesda, Md.

Recent studies have indicated that the composition of the intestinal flora greatly affects the reaction of mice to bacterial endotoxins(1,2,3). Utilizing a strain of mice with a predominantly gram-positive intestinal flora, Schaedler and Dubos(2) found that colonization of the intestinal tract by certain gram-negative bacilli rendered the mice susceptible to the lethal effect of endotoxin. This altered reactivity suggested a sensitization by bacterial products absorbed through the intestinal wall.

To obtain further evidence for this hypothesis, it was thought of interest to investigate the response of germfree mice, reared and maintained in an environment without contact with living bacteria, to challenge with endotoxin, and to compare the lethal effect of endotoxin in germfree mice with the effect in conventional mice of the same genetic stock. Additional information on the influence of the intestinal flora on the reaction of mice to endotoxin challenge was provided through experiments with monoinfected and conventionalized ex-germfree mice, and by utilizing a strain of specific pathogen-free mice (SPF mice) with a known and controlled intestinal flora.

Materials and methods. Conventional animals. Conventional mice of the General Purpose (G.P.) and NIH stock (NIH) derived from the albino Swiss-Webster colony were obtained from the NIH Animal Production Section.

Conventional Lobund mice (CVN) derived from the Univ. of Notre Dame germfree stock were obtained originally from Dr. W. L. Newton, Laboratory of Germfree Animal Research, Nat. Inst. of Allergy and Infectious Diseases. These animals were bred in clean, but not germfree, quarters which had not previously housed animals and were provided with sterilized food, water and bedding(4).

Germfree animals. Germfree mice of the NIH and Lobund stocks were supplied by Dr. C. E. Miller of the Germfree Unit, Laboratory Aids Branch, NIH, and housed in plastic isolators of the type described by Trexler(5).

Specific pathogen-free animals. The SPF mice were derived from the NIH germfree stock. They were maintained under the same conditions as their germfree counterparts but harbored an intestinal flora composed of 2 strains of lactobacilli, an enterococcus and a pseudomonad. These bacteria were obtained from Dr. R. W. Schaedler. They were isolated from the NCS mice of Dubos and Schaedler.

Bacterial strains. Earlier experiments indicated that increased susceptibility of mice to endotoxins induced by parenteral injection