not by splanchnicectomy. The plasma clearance of insulin was decreased by splanchnicectomy but the remaining renal circulation was able to maintain the consumption rate of insulin at a normal level.

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Electron Microscope Studies of Intact Epithelial and Fibroblast Cell Surfaces.* (26764)

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The earliest electron microscopic studies of tissue cell morphology utilized whole cells grown in tissue culture on formvar-coated copper wire grids. This technic, as described by Porter *et al.*(1), allowed visualization of some cell structures not seen by light microscopy, but did not permit the extensive studies of cellular morphology afforded by ultrathin sectioning. Consequently, the above method has had limited use and only a few additional studies(2,3,4) have been reported. Almost all of these appeared prior to the present widespread use of tissue culture as an experimental approach to various problems.

Although whole cell preparations are too thick to allow resolution of most structures in the nucleus and cytoplasm, the cell surface is clearly seen. Microvilli and other structures which project from the cell surface at various angles usually appear in thin sections as disconnected parts while in the whole cell mounts their structural integrity is retained. For certain studies in progress in this laboratory it was desirable to determine: (a) Whether the surface features of different tissue culture cell types are similar or vary significantly from one cell line to another, and (b) whether the surface morphology of one cell line studied over a prolonged period of time remains constant or varies. The present paper compares the surface morphology of 5 cell types commonly used in tissue culture and describes certain modifications of technic affording reproducible whole cell preparations in which microvilli and other surface structures are well preserved and remain free of most extracellular debris.

Materials and methods. Tissue culture. Cell types used included chicken fibroblasts, mouse fibroblast ("L"), HeLa, Chang's liver and chicken monocytes. These cells were grown and maintained in stock flasks of various sizes and after suitable sheets had been obtained were trypsinized into suspension. The cell suspensions were then adjusted to contain the following cell concentrations: 1-2 million chicken fibroblasts or 600,000 "L",

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150,000 HeLa cells or 125,000 Chang's liver cells. HeLa and Chang's liver strains were obtained initially from Microbiological Associates. Chicken fibroblast cultures were prepared in this laboratory from embryonic tissue. Chicken monocytes were obtained as follows. The buffy coat was separated from adult chicken whole blood, washed and the total cell suspension of 4-5 million cells/ml inoculated directly into the Leighton tubes, because monocyte sheets are not readily removed and resuspended from flask cultures. All cultures contained penicillin 100 units/ml, streptomycin 100 μg/ml and mycostatin 50 units/ml.

Electron microscope grid mounts. Stainless steel 200 mesh grids were glued (scotch tape glue in xylene) to a 10 $\times$ 50 mm #1 glass cover slip. This surface was then used to pick up a 0.4% collodion membrane which was then air dried. Thus, a collodion film coated both the grid surface and the surrounding glass area overlapping to cover part of the opposite side of the cover slip. After drying, the entire grid-cover slip preparation was placed in the vacuum evaporator and coated with a thin carbon film.

Tissue culture cells growing on the coated grid were observed by light microscopy during the culture period and removed when cells were well spaced over the membrane but, of course, before continuous sheets were formed. At this point the glass cover slip was removed from the tube, washed in buffered salt (Hanks') solution and fixed for 90 seconds in 1% veronal-buffered osmium, pH 7.4(5). After fixation the cover slip grid preparation was washed in distilled water, air dried and metal shadowed with chromium. Micrographs were made with an RCA-EML electron microscope.

Results. Comparison of the microvilli and other surface features of the cells studied revealed striking morphologic differences and the specific structural features associated with a particular cell line remained constant unless variations were induced deliberately. Variations in age of culture, time-length of fixation or per cent of osmium used induced no significant morphologic changes. This does not mean that each cell was precisely like all others of its type but the differences observed were quantitative rather than qualitative.

TABLE I. Growth of Tissue Culture Cell Lines on Grids Coated with Formvar, Collodion and Carbon-Collodion Membranes.

Cell line	Type of membrane		
	Formvar	Collodion	Carbon-collodion
Chicken fibroblast	3+	3+	4+
Mouse fibroblast (L)	0	2+	4+
HeLa	2+	2+	4+
Liver (Chang)	0	2+	4+
Chicken monocyte	2+	2+	4+

4+ = normal growth, no rounded or degenerating cells.
0 = no normal cells, all cells rounded and/or degenerating.

The importance of present modification of methods for this type of preparation must be emphasized since there was marked variation in the ability of cells of different cell lines to grow on surfaces suitable for electron microscopy. Thus, formvar membranes allowed reasonably good growth of chicken fibroblasts as reported by Porter(1) but were completely unsuitable for the "L" cells which did not put out processes on this surface and remained in a rounded form. Under these latter conditions few microvilli were seen projecting from the cell surface, and degeneration of most cells occurred within 24 hours. The growth of the various cell lines with various membranes is presented in Table I.

The following paragraphs summarize the morphologic observations of several hundred cells of each type. These specimens were prepared in many separate experiments over a period of several months. In no instance did the prototype cell morphology vary significantly. For example, "L" cell preparations studied months ago and new preparations studied recently were entirely comparable as was true with all other cell types.

Strain "L", mouse fibroblast (Earle). This cell was characterized by its elongated appearance and a profusion of microvilli projecting from all surfaces (Fig. 1 and 2). No nuclear or cytoplasmic structure was ever visible. The microvilli were 1-10 μ long and 0.1-0.3 μ wide without a terminal bulb structure. Microvilli were more profuse in the

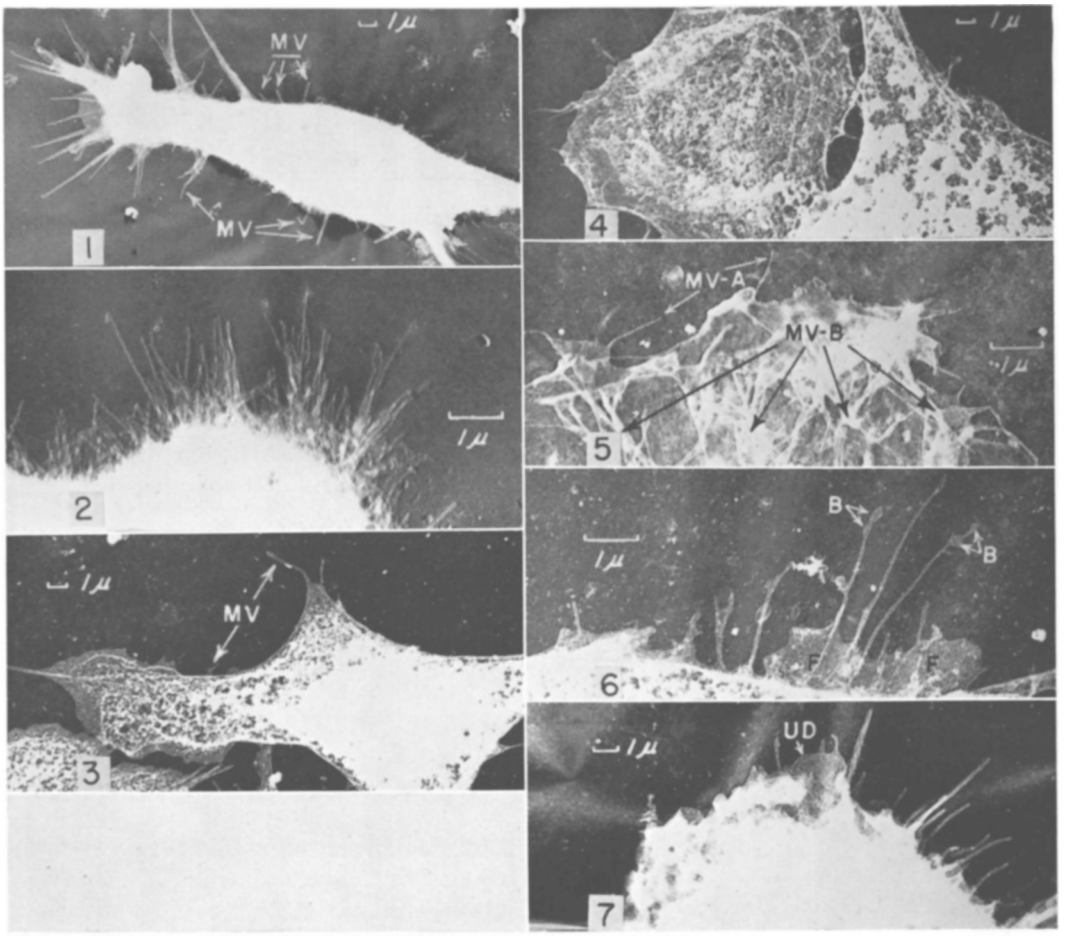

FIG. 1. Mouse fibroblast strain "L" (Earle). Only a few of the larger microvilli (MV) are specifically indicated. The cell body is always thick and opaque to the electron beam. Chromium shadowed. × 1600.

FIG. 2. A higher magnification of the surface of another "L" cell. The profusion of villi projecting from the surface is characteristic. Chromium shadowed. × 6400.

FIG. 3. Chicken fibroblast which has flattened so that some cytoplasmic structures are visible. The cell shape resembles the "L" cell but microvilli are numerically few. Chromium shadowed. × 1600.

FIG. 4. Two HeLa cells showing a few intracellular bridges. At this magnification only a few microvilli appear to be present. Chromium shadowed. × 1600.

FIG. 5. Higher magnification of a HeLa cell surface. MV-A indicates typical bulbless microvilli extending out from cell surface; MV-B locates masses of villi folded back onto cell body. Chromium shadowed. × 6400.

FIG. 6. Typical Chang's liver epithelial cell surface showing bulbous (B) microvilli which extend through or arise from a flap (F) of cell cytoplasm. Chromium shadowed. × 6400.

FIG. 7. Chicken monocyte. In this cell type villi appeared in patches and most of the visible surface was occupied by a membranous structure, seen here in part, probably corresponding to the undulating membrane (UD). Chromium shadowed. × 1667.

regions of cell processes but were present to some extent on all surfaces.

Fig. 1 shows the typical spindle shape which is similar to that of the chicken fibroblast in Fig. 3. The number of microvilli (MV) in Fig. 1 represent the minimum found with the "L" cell. Most cells of this type showed the profusion of microvilli seen in Fig. 2.

Chicken fibroblast. As previously observed by Porter *et al.*(1), this cell tended to flatten considerably so that internal cell

structure could be seen (Fig. 3). Microvilli were absent in most areas and only a few per cell were seen, usually concentrated in the region of the cell processes.

HeLa epithelial cell (Gey). This cell (Fig. 4 and 5) had an irregular shape exhibiting more variation in this regard than any of the cells studied. Internal structures of the cell, the nucleus and cytoplasm, were visible in about half of the cells. The surface was characterized by large numbers of microvilli which varied greatly in length but had a fairly uniform width of 0.05 to 0.2 μ. Occasionally HeLa microvilli terminated in a bulb but enlarged areas of the body of the villus have not been found similar to those noted for Chang's liver cells. The distinguishing feature of this cell was the folding back of the cell edge onto the cell body proper as seen in Fig. 5. This configuration was present in all the cells examined and conforms in general to the "ruffle edge" described by Lewis(6). Superficial examination of HeLa surfaces at low magnification (Fig. 4) would suggest that very few microvilli are present. A higher magnification micrograph (Fig. 5) shows that a few villi, MV-A, project out from the surface but many more, MV-B, are seen folded back onto the cell body. Although this surface configuration was found occasionally in the Chang's liver cells (described below) it was far less prominent than in the HeLa cells. The folding back of the microvilli was not found with the chicken fibroblast, the mouse fibroblast or the chicken monocyte.

Liver epithelial cell (Chang). In shape and amount of internal structures visible this cell resembled HeLa but examination of the surface at high magnification revealed two distinct differences. As indicated above, the microvilli did not tend to fold back onto the cell body although this was occasionally seen. More characteristic was the appearance of bulbous enlargements of the villi (Fig. 6B). Usually a single terminal bulb was present but in some instances 2 such enlargements were found. In addition, villi appeared to project through thin flaps or extrusions of the cytoplasm (Fig. 6,F).

Chicken monocyte. This cell generally assumed an oval or round shape and the cell body was opaque to the electron beam so that no internal detail was seen. The cell surface was characterized by microvilli appearing in patches and varying greatly in number, length and width. Fig. 7 illustrates the typical appearance of this cell type. Microvilli project out from one segment of the surface and the remaining surface area consisted of a smooth, scalloped membranous structure corresponding to the undulating membrane (UD) observed by light microscopy. It is of interest that micrographs of this cell invariably showed folding of the underlying carbon-collodion membrane to an extent not found with other cell studies. The monocyte is well known for its strong adhesion to surfaces on which it grows, which may account for the folding of the grid membrane.

Discussion. The present study demonstrates that the surface morphology of the 5 cell lines examined varies considerably from line to line particularly in regard to number and configuration of the microvilli. On the other hand, the surface structure of any given one of these 5 cell lines remains quite constant and reproducible when the tissue culture preparations are made in the manner described. The original method for whole cell electron microscopy(1) utilized chicken fibroblasts grown on formvar-coated copper mesh grids. This technic provides reasonably good results with chicken fibroblasts but is of little or no value for some of the other cell types. The mouse fibroblast strain "L", for example, will not grow normally on formvar and the other cell lines used have shown varying degrees of toxic or abnormal changes apparently due to the formvar, the copper or both. Carbon-collodion films on stainless steel grids allow cell growth comparable to that observed on glass.

The reproducibility of the cell surface structures of any of the 5 lines examined was very good in contrast to the marked variations found when the same cells were grown on either formvar or plain collodion. Blind tests to determine cell type on the basis of the surface morphology have demonstrated that the distinction between the mouse fibroblast, chicken fibroblast and chicken mono-

cyte is extremely easy and accurate. The ability to distinguish between the liver epithelial cell (Chang's) and HeLa is more difficult but can be accomplished if one scans a number of cells. It is not proposed that all tissue culture cell lines can be distinguished in this manner but knowledge of their surface features may be of some help.

Some of the features of cell surfaces described here can be seen and have been photographed in the light microscope. The undulating membrane and the patchy distribution of the microvilli in the chicken monocyte and the folding back of the HeLa cell edge correspond to similar structures seen in light micrographs and reported by various investigators(7,8). It would appear, then, that the present technics have not introduced an inordinate amount of cell distortion.

Summary. An improved method for the electron microscopic study of intact tissue culture cells is based on the use of carbon-collodion coated stainless steel grids upon which the cells are grown. Cell preparations are fixed with buffered osmium and metal shadowed. This technic has been applied to the study of 5 cell lines including mouse fibroblasts strain "L" (Earle), HeLa (Gey), liver epithelial (Chang), chicken fibroblasts and chicken monocytes. Distinctive surface features of these cell lines are described.

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The Tumor Necrotizing Effect of Lipoid A Component of *Escherichia coli* Endotoxin.* (26765)

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The endotoxins of gram-negative bacteria cause a large variety of primary and secondary reactions in susceptible hosts, including fever, the local and generalized Shwartzman reactions, hemorrhagic necrosis in experimental tumors, as well as alterations in non-specific resistance, immunological response, properdin levels, and dermal reactivity to epinephrine(1-5). Chemically, the endotoxins are complexes made up of polysaccharide, protein or peptide, and lipoid. It is the polysaccharide moiety that is responsible for serologic specificity. Recent studies suggest that the lipoid A component of this complex is pyrogenic, alters nonspecific resistance to experimental infection, and is responsible for enhanced reactivity of the rabbit to epinephrine(6,7).

A trichloracetic acid extract from *Staphylococcus aureus* strain D was found to alter dermal reactivity of rabbits to epinephrine (8) in a fashion comparable to that of endotoxins from gram-negative bacteria and the lipoid A component of *E. coli* endotoxin(7). The same staphylococcal extract caused extensive hemorrhagic necrosis of mouse sarcoma 180(9). The present study was undertaken, therefore, to determine whether *E. coli* lipopolysaccharide and the corresponding lipoid A component also produce hemorrhagic necrosis of sarcoma 180 of mice, and to com-

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