ULTRASTRUCTURAL AND HISTOCHEMICAL DIFFERENCES IN CELL SURFACE PROPERTIES OF STRAIN-SPECIFIC AND NONSTRAIN-SPECIFIC TA3 ADENOCARCINOMA CELLS

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ABSTRACT

Transmission and scanning electron microscopy and histochemical and biochemical methods were used to investigate differences in cell structure and cell surface properties between the strain-specific TA3-St and nonstrain-specific TA3-Ha ascites sublines of the TA3 murine mammary adenocarcinoma. The TA3-St subline is lethal only to the syngeneic strain A mouse (the strain of origin), whereas the TA3-Ha subline is lethal even to foreign species. In contrast to the TA3-St cell surface, which has numerous folds and irregular microprojections, the TA3-Ha cell has abundant long microvilli of uniform dimensions. An extensive cell surface coat which resembles the "fuzz" coat found on microvilli of normal epithelium was present on the TA3-Ha, but not on the TA3-St cells. After routine fixation, the surface coat of the TA3-Ha cell usually appeared as a filamentous network extending 30–50 nm from the plasmalemma; occasionally, longer filamentous or rod-like structures were found extending 200–400 nm from the plasmalemma. The cell coat material was more extensive on the microvilli than on the intermicrovillous membranes. Free virus-like particles associated with TA3-Ha cells have a similar-appearing surface coat on their outer membranes. The density of surface anionic sites, determined with polycationic ferritin, was greater on the TA3-Ha than on the TA3-St cell surface, consistent with the presence at the TA3-Ha cell surface of several-fold more neuraminidase-susceptible sialic acid groups. The observed surface features of the nonstrain-specific TA3-Ha cell, in comparison to the strain-specific TA3-St cell, are consistent with the suggestion that sialic acid-rich glycoproteins at the TA3-Ha cell surface mask histocompatibility antigens and enhance the ability of malignant cells to invade foreign species.

Transformed cells demonstrate a variety of physiological and morphological changes which have been implicated in the altered behavior and malignant growth of these cells (12, 18). However, these cells generally behave like normal cells with regard to transplantation rejection by incompati-

ble hosts and, indeed, it has been found that the major histocompatibility antigens are present and expressed at their cell surfaces (13). Thus, most transformed or neoplastic cells cannot survive in allogeneic or xenogeneic hosts, as they are rejected by the immune system of the foreign hosts'. There are, however, some tumor cell lines in which the surface histocompatibility antigens are absent or not expressed, and these cells are lethal to foreign as well as syngeneic strains. Investigations of such tumor cell lines are of interest because the cellular events associated with loss of strain-specificity, i.e., the ability to survive in a hostile immunological environment, are relevant to our understanding of cell surface biology and may also shed light on late recurrence and metastasis in human cancer (3, 13, 19).

The most extensively investigated example of closely related strain-specific and nonstrain-specific cells are the two ascites sublines of the TA3 murine mammary adenocarcinoma, a tumor which arose spontaneously in a female strain A mouse (20, 25). The TA3-St subline is strain-specific and is lethal only to the syngeneic strain A mouse, the strain of origin, whereas the TA3-Ha subline is nonstrain-specific and is lethal to foreign strains and species (13, 30). The history and transplantibility of the two sublines have been described (20).

Some marked differences have been noted regarding the cell surface biology of these two adenocarcinoma sublines. The strain-specific TA3-St cells are readily agglutinated by the plant lectin concanavalin A (Con A), whereas the nonstrain-specific TA3-Ha cells are not (15, 17). In this respect the latter are unique among murine ascites tumor cells (15). The H-2 histocompatibility antigens have been detected on the surfaces of both the TA3-St and the TA3-Ha cell lines, but these antigens are poorly expressed on the nonstrain-specific TA3-Ha cells (14, 16, 30). Moreover, a large molecular weight glycoprotein, epiglycanin, which is not present at the TA3-St cell surface, has been found to be present in large quantity on the TA3-Ha cell (8, 4, 10). It has been suggested that this cell surface glycoprotein may be mainly responsible for the unusual transplantation properties of the TA3-Ha cell (3, 8, 30).

In the present report, we describe these two cell types for the first time with transmission and scanning electron microscopy and we also investigate the distribution of net anionic sites on their surfaces with polycationic ferritin. The demonstration in this study of a distinct cell surface ultrastructure and sialic acid-rich surface coat on the nonstrain-specific TA3-Ha cell subline compared to the strain-specific TA3-St subline is to our knowledge the first report of altered cell surface and cell coat morphology associated with the loss of strain-specificity in neoplastic cells.

MATERIALS AND METHODS

The strain-specific TA3-St and nonstrain-specific TA3-Ha tumor cells were maintained by intraperitoneal serial transplantation in A/HeJ male mice (Jackson Laboratory, Bar Harbor, Maine). Cells were harvested on day 7 after inoculation of 1×10^5 TA3-Ha cells or 1×10^6 TA3-St cells. The procedures for harvesting and washing cells were similar to those previously described (7). Cells were prepared for electron microscopy on the day of harvest.

Cells to be used for transmission electron microscopy were prepared in several different ways.

Method A

Cells were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 2 h at 4°C followed by 1 h in 1% osmium tetroxide in the same buffer. These cells were either dehydrated in ethanol or stained en bloc with uranyl acetate and then dehydrated in ethanol. The cells that were stained en bloc with uranyl acetate were washed 4 times in 0.2 N sodium maleate buffer, pH 5.15, and placed in 1% uranyl acetate in 0.2 N sodium maleate buffer, pH 6.0, for 10 to 60 min before dehydration.

Method B

Cells were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 2 h at 4°C and then dehydrated in ethanol without the use of osmium tetroxide as a post-fixative.

Method C

Cells were fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.4, without prior aldehyde fixation. All cells prepared for electron microscopy were dehydrated in ethanol and embedded in Spurr's low viscosity embedding medium (32).

For scanning electron microscopy, the suspensions of viable (>95%) TA3-Ha or TA3-St cells in phosphate-buffered saline (PBS), pH 7.4, were allowed to settle on clean glass coverslips for 10 min at room temperature. The cover slips were then gently flooded with 2.5% glutaraldehyde in PBS and allowed to fix 1 h at room temperature. The cover slips were then placed for 1 h in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4. Cells were also fixed in suspension in 2.5% glutaraldehyde in PBS for 1 h at room temperature and then allowed to settle after fixation for several minutes on

polylysine-coated substrata (27). All specimens prepared for scanning electron microscopy were dehydrated in ethanol, transferred to liquid CO_2, and critically-point dried in a BRC (Biodynamics Research Corp., Rockville, Md.) Samdri drying apparatus (2).

Dried specimens were coated with a thin layer (100 Å) of carbon followed by a layer (200 Å) of gold:palladium (60:40) in a Denton DV 502 vacuum evaporator equipped with a rotary stage (Denton Vacuum Inc., Cherry Hill, N. J.). Specimens were examined in a JEOL JSM U-3 scanning electron microscope. Random samples were prepared on four different occasions for each cell type. These presumably included cells in all phases of the cell cycle. The description to follow applied to the general characteristics of the TA3-Ha and TA3-St cell regardless of phase of the cell cycle.

Cell surface sialic acid was removed by incubating the cells for 80 min at 24°C with neuraminidase (from *Vibrio cholerae*, Behring Diagnostics, American Hoechst Corp., Somerville, N. J.) at a concentration of 200 U/ml. The manufacturer claims the neuraminidase to be free of proteases, and this was confirmed in our laboratory (6). The amount of released sialic acid was determined by the thiobarbituric acid method (34). These cells, as well as untreated cells, were labeled with polycationic ferritin (Miles Laboratories Inc. Miles Research Corp., Elkart, Ind.) as follows: the cells were fixed for 0.5 h in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, then washed 3 times in the same buffer. 1 ml of a cell suspension containing 1×10^7 cells/ml was added to 0.5 ml of a cationized ferritin solution to give a final concentration of 0.35 mg/ml polycationic ferritin. After the cells were allowed to react for 0.5 h with the polycationic ferritin, they were washed several times in buffer and post-fixed for 1 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.4, and prepared for electron microscopy as described earlier. Thin sections cut for transmission electron microscopy were stained with uranyl acetate and lead citrate and examined on a RCA-G or a JEOL 100 B electron microscope.

RESULTS

General Description of TA3-St and TA3-Ha Cells

Low power transmission electron micrographs of the strain-specific TA3-St cell (Fig. 1) and the nonstrain-specific TA3-Ha cell (Fig. 2) demonstrate that these cells are generally spherical in shape when fixed in suspension. The TA3-St cells tend to be slightly larger than the TA3-Ha cells. Both cell types are covered with numerous microprojections; on the TA3-St cell (Fig. 1) these cell processes are curved and project from an irregular cell surface. The projections extending from the TA3-Ha cell (Fig. 2) are uniform in distribution and resemble microvilli in diameter (0.1 μ) and length (1–2 μ).

Cells from both sublines have large pleomorphic nuclei which are usually eccentric in location and contain a moderate amount of heterochromatin. Nucleoli are large and irregular in shape (Figs. 1 and 2). As many as three nucleoli may occur in any given nucleus. The Golgi apparatus is located adjacent to the cell center, and the Golgi complex contains the usual smooth-surfaced lamellae, vesicles and small vacuoles. Mitochondria are distributed throughout the cytoplasm and are of various sizes, but are generally elongated in shape. Sparse amounts of rough endoplasmic reticulum can be found in the cytoplasm, and abundant membrane-free ribosomes are one of the conspicuous features of these cells. Lipid droplets are common (*L* in Figs. 1 and 2) and occasional lysosome-like bodies are seen. Both cell sublines are found to contain abundant virus particles (arrows, Figs. 1 and 2), which appeared to be concentrated in the region of cytoplasm immediately surrounding the Golgi membranes.

At higher magnification (Fig. 3), the large numbers of free ribosomes which are present in the cytoplasm can be better visualized. The virus particles, which are common in the cytoplasm of both cell lines, are seen to vary tremendously in shape and size, but are generally crescent shaped or circular with electron-transparent cores (arrows, Fig. 3). These particles are often seen within membranes of the endoplasmic reticulum. However, smaller virus particles about 80 nm in diameter with an electron-dense core can sometimes be found, usually adjacent to large vacuoles which contain debris similiar in density to the virus cores (*vac*, Fig. 3). Virus particles budding from the cell surface are, commonly seen in both cell lines (inset, Fig. 3). There were no significant differences that we could judge in the morphology or quantity of intracytoplasmic viruses between the two cell types.

Cell Surface Morphology

The differences in cell morphology between the TA3-St and the TA3-Ha cells that were visible in transmission electron micrographs (Figs. 1 and 2) are even more striking when cells are examined by scanning electron microscopy (Figs. 4–6). The strain-specific TA3-St cells have numerous filopodia which are bent, branched, and highly irregular (Fig. 4). The surface membrane is thrown into folds from which the irregular filopodia project. In

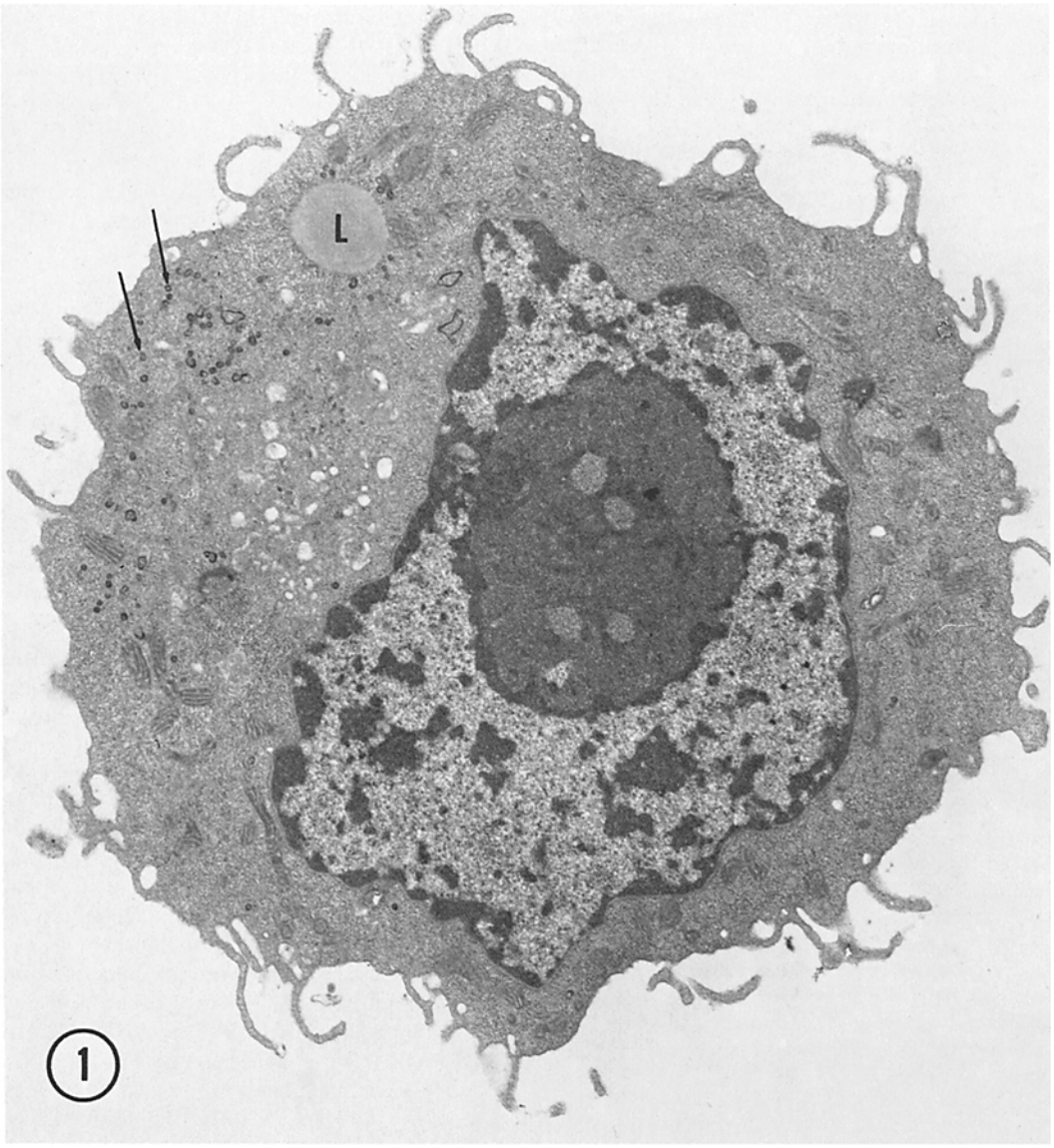


FIGURE 1 Low-power transmission electron micrograph of a section of a strain-specific TA3-St cell showing the characteristic irregular surface contour and large eccentric nucleus with prominent nucleolus. The cytoplasm contains elongated mitochondria, abundant free ribosomes, lipid droplets (one of which is labeled *L*), virus particles (arrows) and, to the left of the nucleus, a Golgi zone. The numerous cytoplasmic processes projecting from the cell tend to be bent and folded. Glutaraldehyde-osmium tetroxide fixation. × 9,400.

contrast, the surface of the nonstrain-specific TA3-Ha cells is much more regular in appearance. The most striking feature of the surface architecture of the TA3-Ha cell is the presence of long microvilli (Figs. 5 and 6), which are more numerous and more uniform in diameter than the projections coming from the surface of the TA3-St cell. These microvilli, unlike the projections found on the TA3-St cells, rarely branch and are fairly straight. The intermicrovillous spaces on the TA3-Ha cells are generally smooth, and the surface lacks the membrane folds which are characteristic

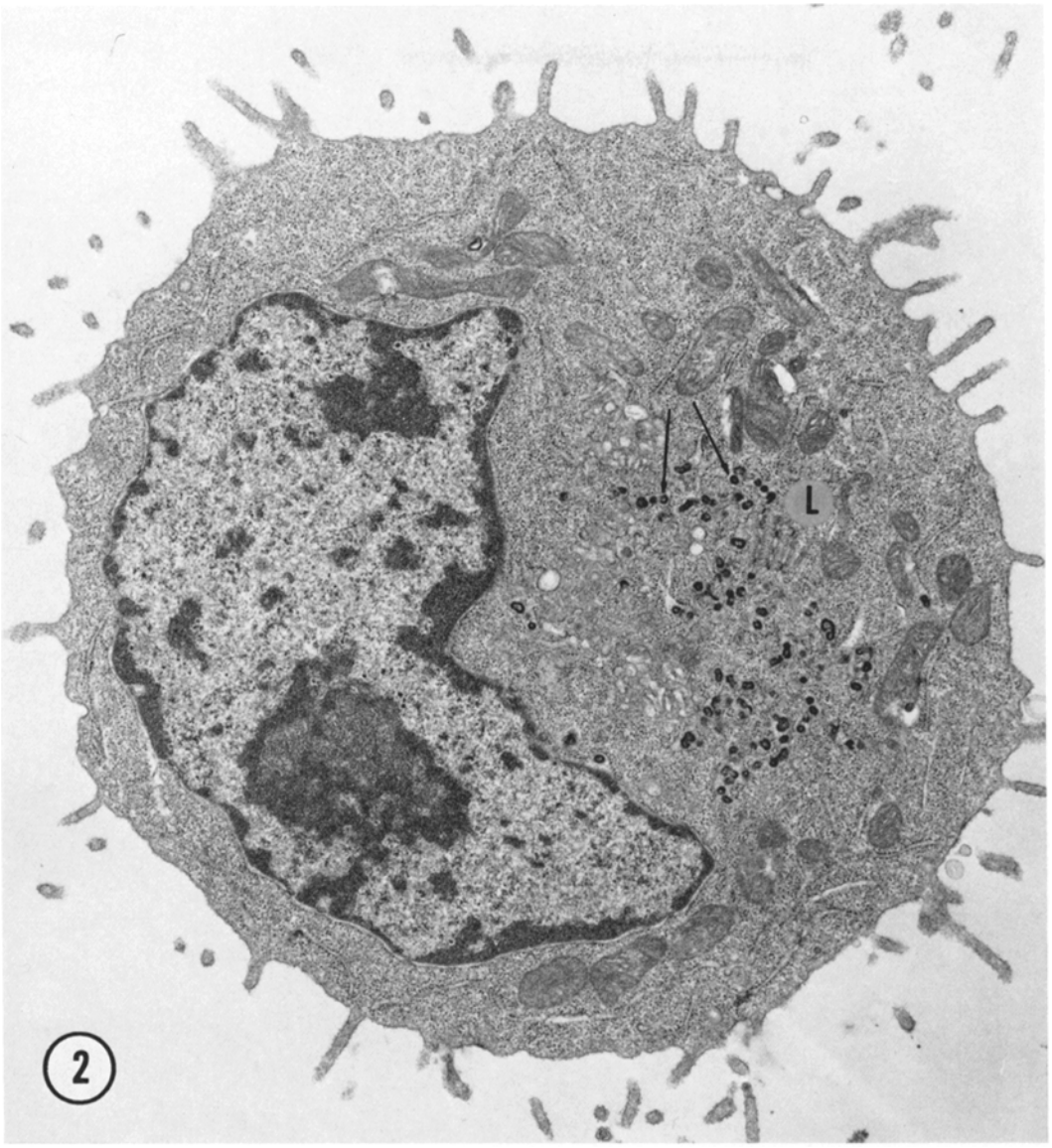


FIGURE 2 Low-power electron micrograph of a section of a nonstrain-specific TA3-Ha cell. The internal features of this cell are similar to those of the TA3-St cell (Fig. 1), i.e. eccentric nucleus, Golgi zone, abundant free ribosomes, lipid droplet (*L*), and virus particles (arrows). The processes projecting from the cell surface are generally straighter and more uniform in diameter than those found on the TA3-St cell. They appear to be true microvilli. Glutaraldehyde-osmium tetroxide fixation. × 12,200.

of the TA3-St cell. Cells from either subline, when allowed to settle on glass cover slips before fixing, attach to the glass by means of slender filopodia like those seen in Fig. 5. Other than these anchoring filopodia, there were no differences in surface morphology when live cells were allowed to settle on glass before fixation as opposed to being fixed in suspension and then picked up on polylysine-coated glass or filter.

When viewed by the scanning electron microscope, cells from either subline were sometimes closely associated with one another, as if they were joined by close contacts or junctions (Fig. 4). Indeed, junctional complexes are occasionally

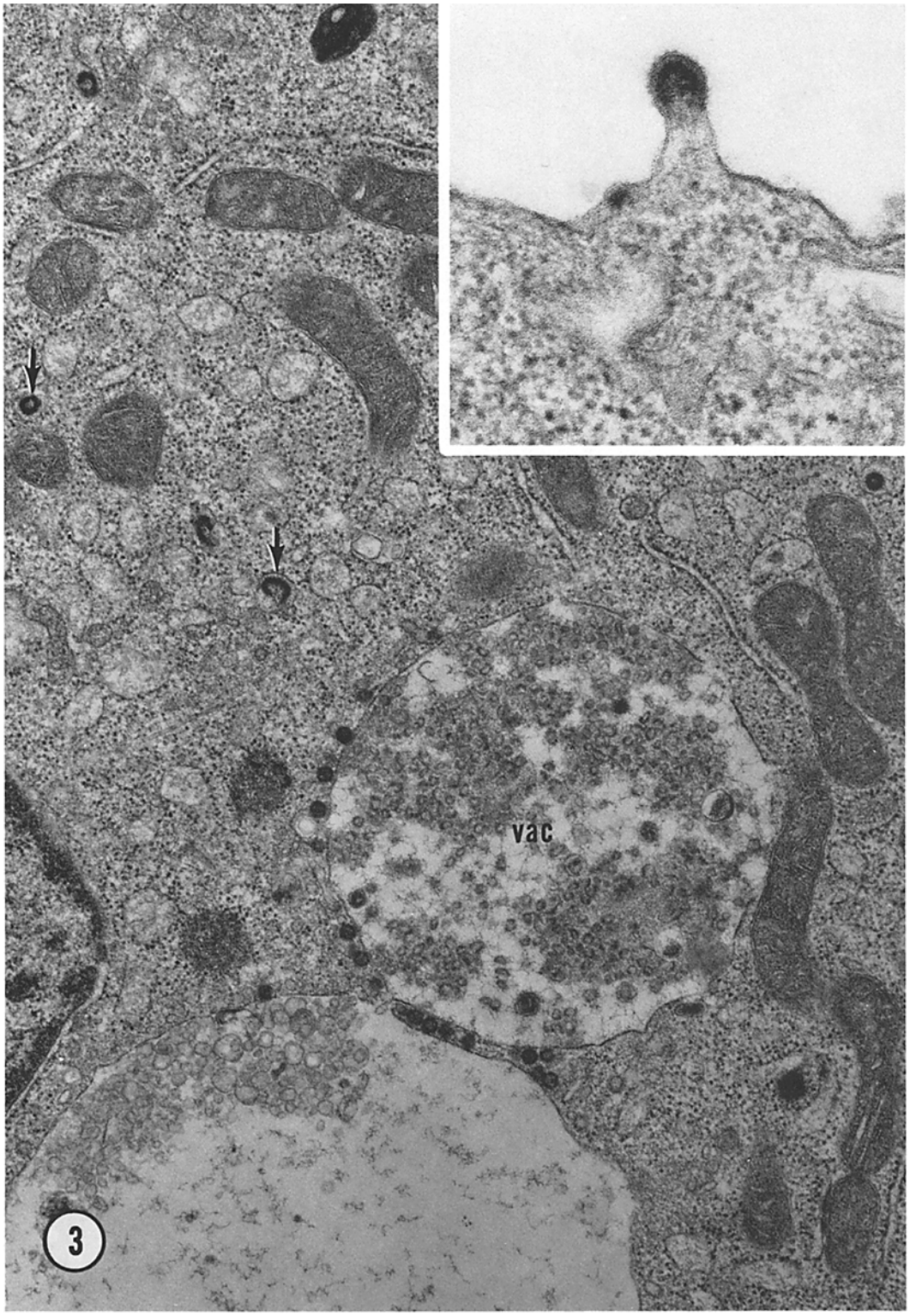


FIGURE 3 Higher power transmission electron micrograph showing cytoplasmic detail in the TA3-Ha cell. Numerous free ribosomes are conspicuous, and occasional profiles of granular reticulum can be resolved. Irregular virus particles with electron-transparent cores are present (arrows). Smaller virus particles with a dense central core surround a large vacuole (vac) containing virus-like particles and other amorphous material. In the inset, a virus particle is seen budding from the surface of a TA3-St cell. Glutaraldehyde-osmium tetroxide fixation. × 28,800. Inset, × 89,000.

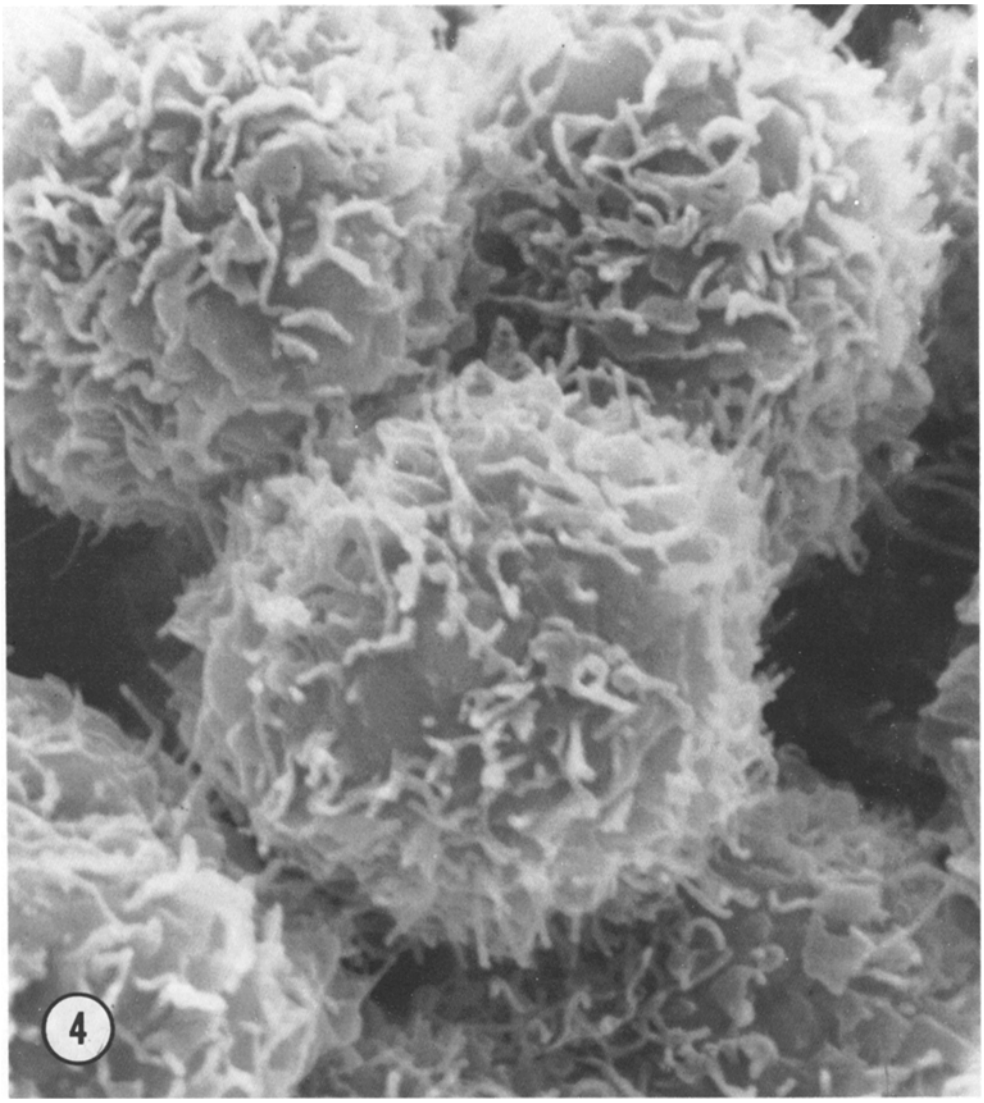


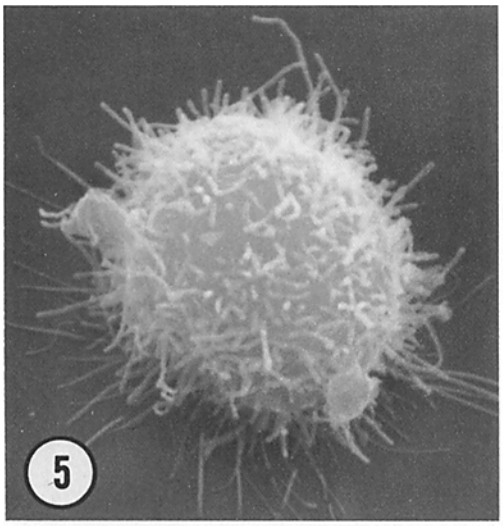
FIGURE 4 Scanning electron micrograph of a strain-specific TA3-St cell. The cell processes seen in thin section (Fig. 1) have the form of folds from which one or more filopodia project. The filopodia tend to fall back onto the surface of the cell. × 9,000.

seen between these cells when thin sections are viewed in the transmission electron microscope. Some junctions are desmosome-like (*ma*, Fig. 7), while others resemble the zonula adherens of normal epithelial cells (*za*, Fig. 7). Tight junctions or zonulae occludentes are also found (Fig. 8), but gap junctions are seldom, if ever, seen in thin sections.

Cell Surface Coat

One of the most striking differences between the strain-specific TA3-St cell and the nonstrain-specific TA3-Ha cell which was revealed by this study is the presence of a morphologically distinct cell surface coat on the nonstrain-specific TA3-Ha cell that is lacking on the strain-specific TA3-St cell. As viewed by transmission electron microscopy, the appearance of the TA3-Ha surface coat was found to depend upon the fixation and staining methods employed.

With routine glutaraldehyde and osmium tetroxide fixation (Method A from Materials and



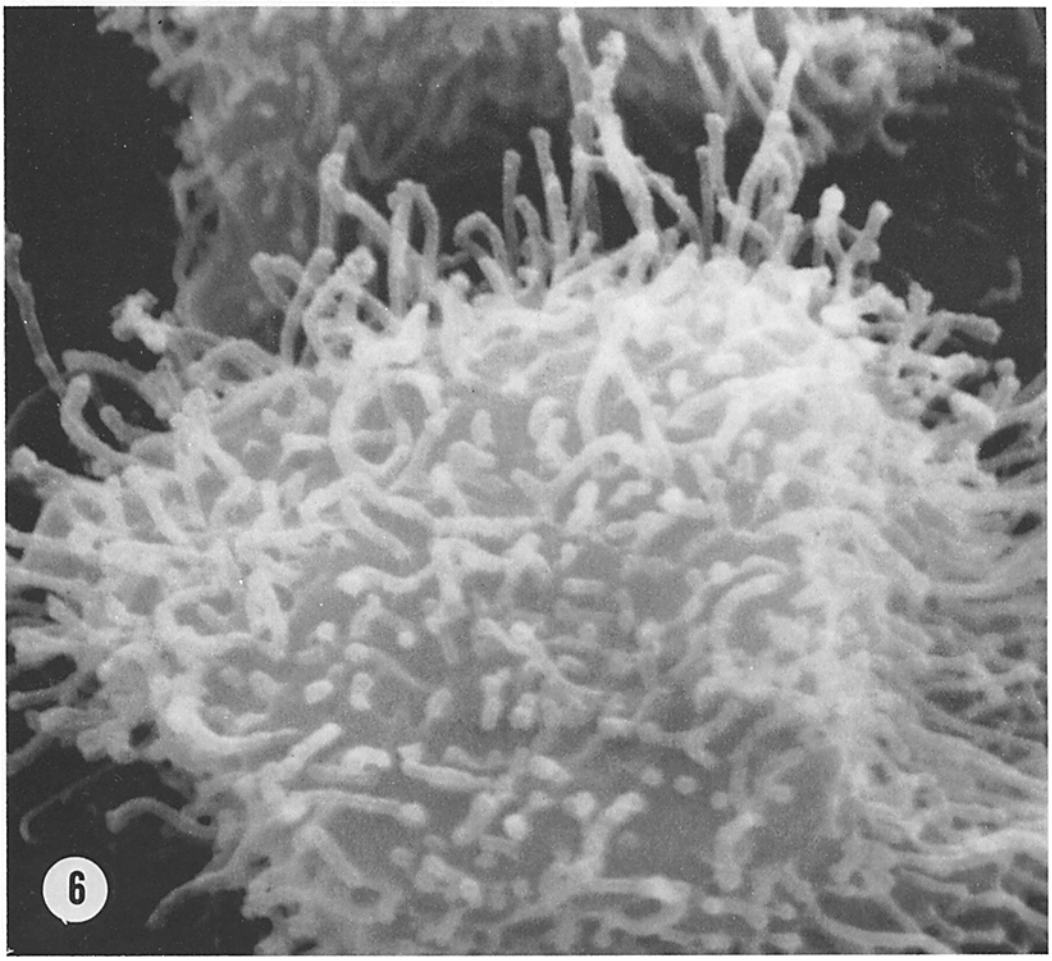
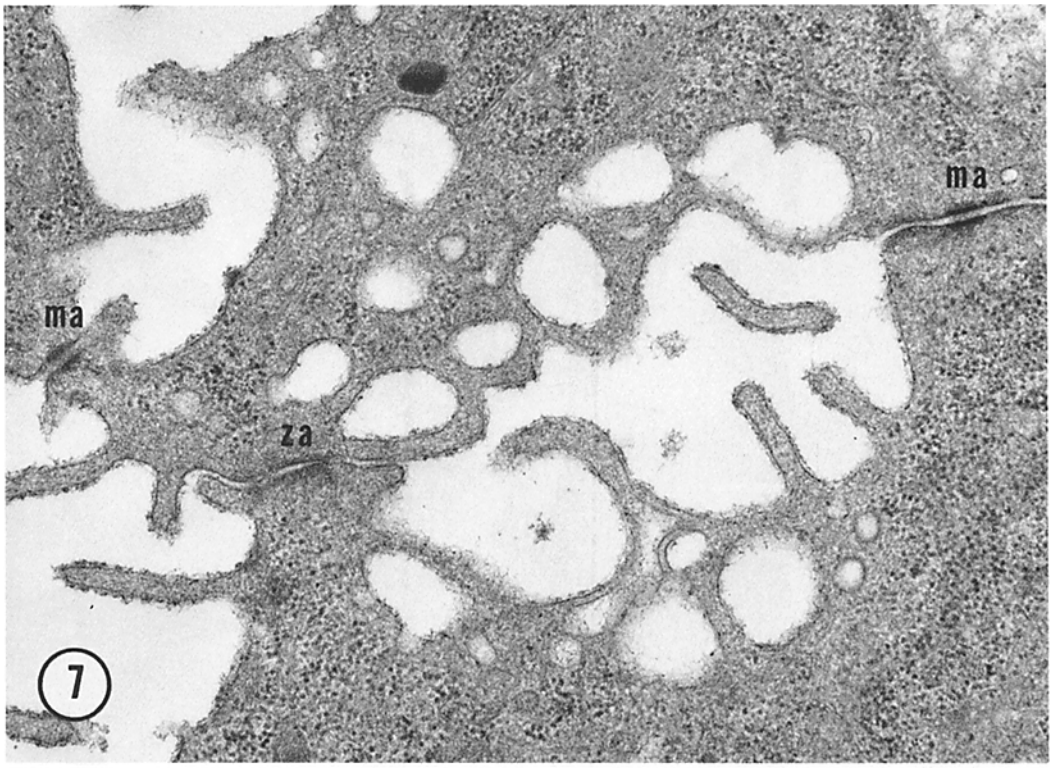


FIGURE 5 Scanning electron micrograph of a nonstrain-specific TA3-Ha cell which is attached by slender filopodia to a glass coverslip. Long, regular microvilli are characteristic of the surface of this cell. × 5,000.

FIGURE 6 Higher power scanning electron micrograph demonstrating the detail of the surface morphology of the nonstrain-specific TA3-Ha cell. The cell has a dense population of long, uniform microvilli. The intervillous space is quite smooth, and the cell lacks the membrane folds which are characteristic of the TA3-St cell (Fig. 4). × 16,000.



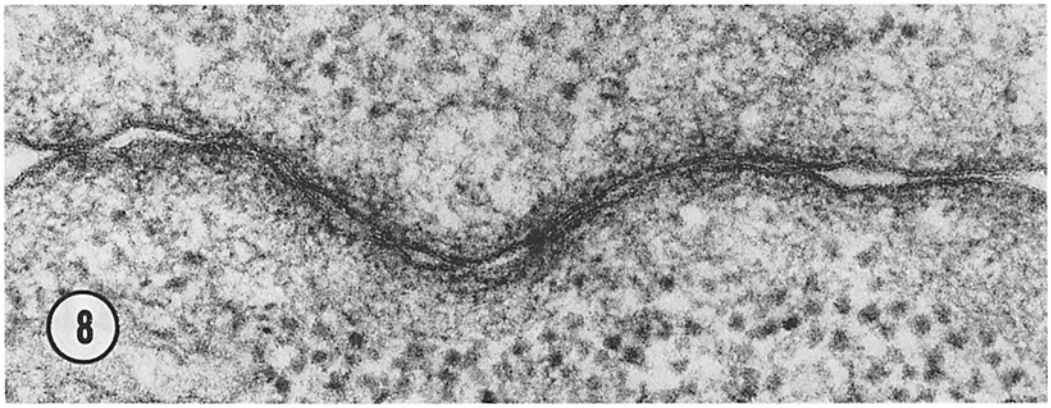


FIGURE 7 Transmission electron micrograph illustrating regions of cell contact and junctions between adjacent TA3-Ha cells. Epithelial-type cell junctions such as the macula (*ma*), zonula adherens (*za*), and zonula occludens are found on cells from either TA3-Ha or TA3-St sublines. Glutaraldehyde-osmium tetroxide fixation. × 37,000.

FIGURE 8 Greater detail of a zonula occludens between two TA3-St cells. Similar junctions can be found between adjacent TA3-Ha cells. Glutaraldehyde-osmium tetroxide fixation, en bloc uranyl acetate stain. × 133,000.

Methods), but without en bloc uranyl acetate staining, the surface coat of the TA3-Ha cell most often appears as a network of fine filaments about 5 nm in width extending 30–50 nm, sometimes farther, from the plasma membrane (Fig. 9). Individual filaments may extend several hundred nm from the plasma membrane, bridging adjacent microvilli. The surface coat of the TA3-Ha cell gen-

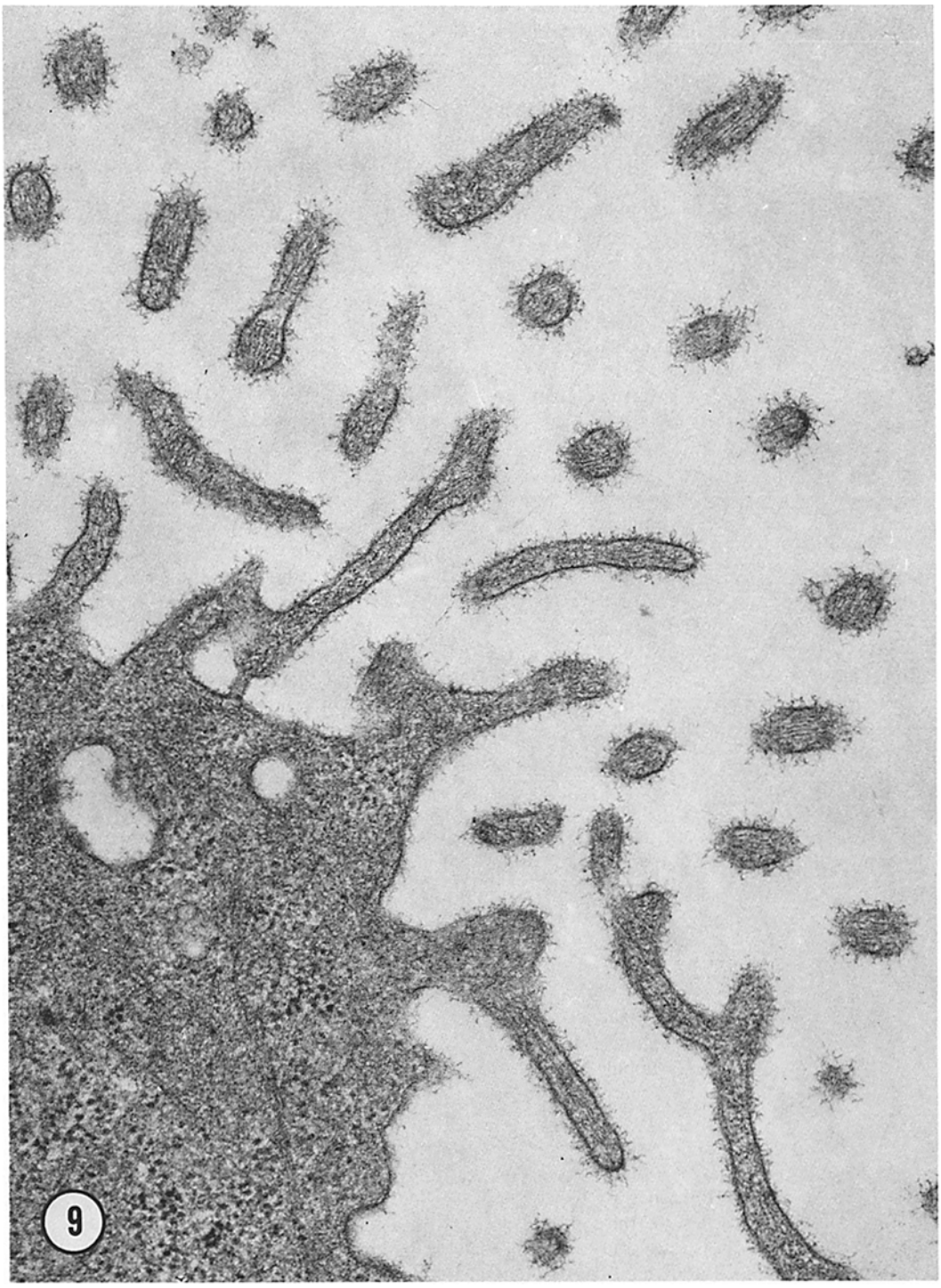


FIGURE 9 Electron micrograph of the surface of a nonstrain-specific TA3-Ha cell showing the cell surface coat as it appears on the plasma membranes of these cells after routine fixation. This fuzz coat is especially evident on the microvilli. Glutaraldehyde-osmium tetroxide fixation. × 62,400.

erally appears to be most extensive on the long, regular microvilli (Figs. 9 and 10). Such microvilli contain "actin-like" intracytoplasmic filaments (Figs. 9 and 11). Free virus particles have a surface coat at their outer membrane which appears to be similar to that found on the microvilli of the TA3-Ha cells (arrow, Fig. 11). A distinct surface coat was not found on free virus particles or virus buds of the TA3-St cell (Fig. 3, inset). The intermicrovillous cell surface has a similar surface coat (Fig. 12), which is not quite so extensive as that on the microvilli (Fig. 10). No morphologically distinct cell surface coat can be observed on similiarly fixed and stained TA3-St cells (Figs. 13 and 14). In addition, the cell processes of the TA3-St cells lack the actin-like filaments observed in the microvilli of the TA3-Ha cell (Figs. 9–11).

In some cases with glutaraldehyde and osmium tetroxide fixation, the long filamentous material present on the TA3-Ha cells appears to aggregate into thicker more rigid-appearing rod-like structures lower in electron density than the adjacent cytoplasm (Fig. 15). These rod-like structures are about 25 nm wide and generally extend 200–400 nm from the plasma membranes, and are most conspicuous in regions containing numerous microvilli. The rod-like structures extend freely from the membranes or bridge adjacent microvilli. At higher magnifications, find banding patterns may be seen on these extracellular cell surface structures (inset, Fig. 15).

The size and visible detail of the TA3-Ha cell surface coat are greatly diminished when the fixed cells are stained en bloc in aqueous uranyl acetate before embedding. This staining method did, however, increase the contrast of the plasma membranes (Fig. 8).

The surface coat of the TA3-Ha cells fixed in glutaraldehyde and dehydrated in ethanol, without osmium tetroxide as a post-fixative (Method B), takes on an unusual configuration. Regular semispherical electron-dense globules about 30 nm in diameter are present on the plasma membranes of these cells (Fig. 16). These globules are most conspicuous on the microvilli and probably represent ethanol-coagulated cell surface glycoprotein. The plasma membranes of these cells appear as negative images since the lipids either were extracted by ethanol or were unstained due to the absence of osmium tetroxide in the fixative. No surface coat structures were apparent on similarly treated TA3-St cells.

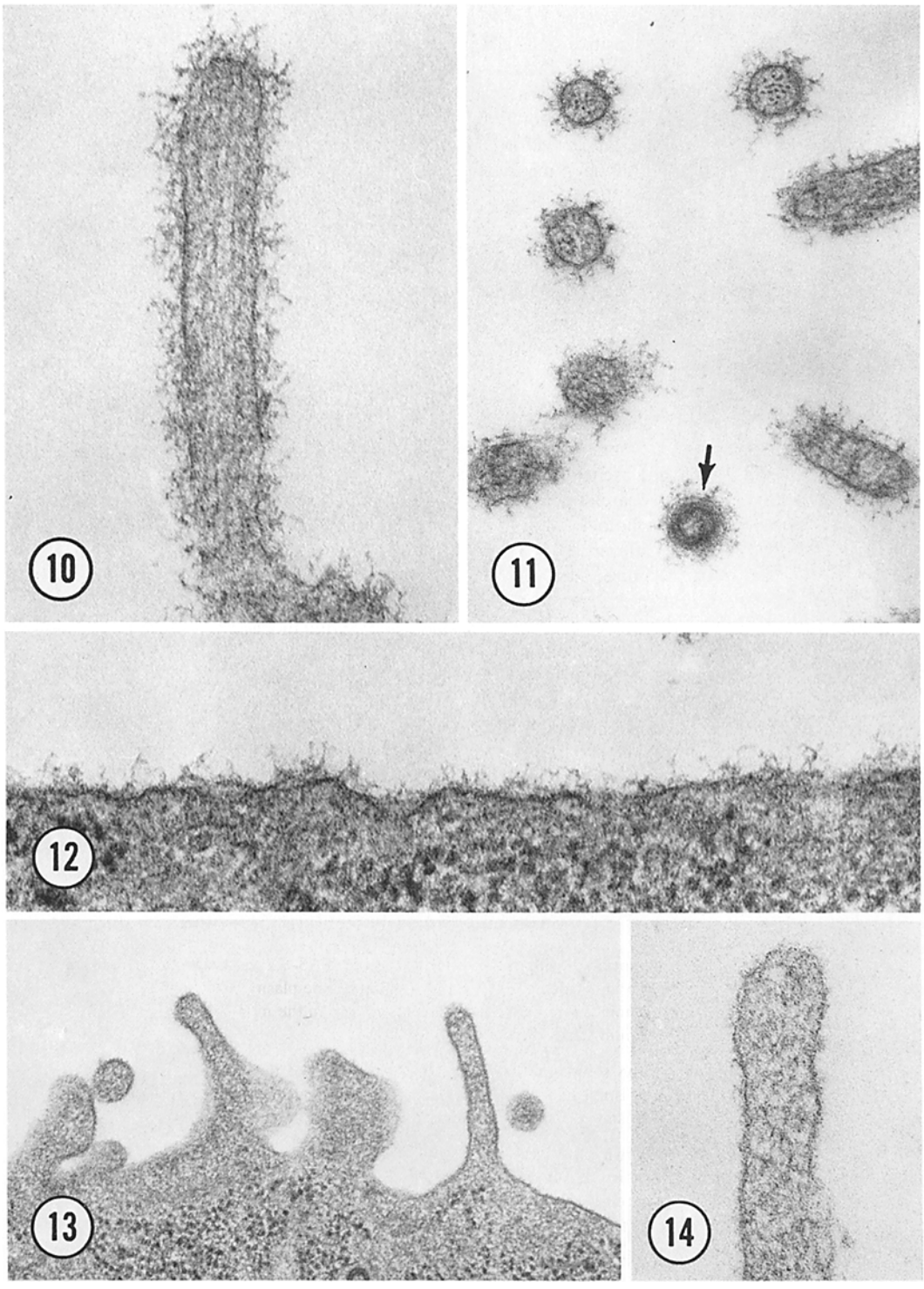
On the other hand, there is generally no distinguishable surface coat on either the TA3-Ha cell or the TA3-St cell if osmium tetroxide is used as the primary fixative without previous aldehyde fixation (Method C).

In summary, transmission electron microscopy did not reveal a distinct cell surface coat on the TA3-St cells, but a prominent surface coat is demonstrated on the TA3-Ha cells following identical aldehyde-osmium tetroxide fixation and lead and uranyl acetate staining procedures.

Characterization of Anionic Sites on the Cell Surface

It has been demonstrated that the TA3-Ha ascites cell possesses over twice as much neuraminidase-removable sialic acid at its surface as the TA3-St cell under all conditions of growth (8, 9). It was therefore of interest to compare the reactions of polycationic ferritin with the two cell types before and after neuraminidase treatment. Even though no surface coat is visible on routinely fixed and stained (Method A) preparations of TA3-St cells, one or two layers of ferritin particles bind to the surface at any given location (Figs. 17 and 18). Treatment of the TA3-St cells with neuraminidase removes more than 90% of the $\sim 270 \pm 30$ $\mu g/10^9$ cells of total neuraminidase-sensitive sialic acid, as determined by the thiobarbituric acid method (34). After this treatment, the density of ferritin particles present on the plasma membrane was reduced (Figs. 19 and 20), an indication that ferritin binding was due in some part to surface sialic acid residues.

Far more polycationic ferritin was bound to nonstrain-specific TA3-Ha cells (Figs. 21 and 23) than to strain-specific TA3-St cells (Fig. 17), as would be expected from the fact that 1×10^9 residues of sialic acid are present on the TA3-Ha surface compared to 4×10^8 for the TA3-Ha cell (8, 9). Although the reaction of the cell with polycationic ferritin obliterates much of the detail of the surface coat, the filamentous strands of the TA3-Ha cell coat can be observed in favorable areas with polycationic ferritin particles attached to them (arrows, Fig. 21). The surface coat is quite irregular and is thickest near these strands. If the TA3-Ha cells are treated with neuraminidase, in the same fashion as the TA3-St cells just described, the density of polycationic ferritin particles present on the plasma membrane is greatly reduced (Figs. 22 and 24). It had been previously shown that incubation of TA3-Ha cells with neur-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aminidase under these conditions removes more than 90% of the approximately 580 ± 40 μg per 10^9 cells of the neuraminidase-sensitive sialic acid from the TA3-Ha cell surface (9), and these results were confirmed in this experiment by determination of liberated sialic acid by the thiobarbituric acid method (34). The great reduction in polycationic ferritin binding after neuraminidase treatment suggests that much of the net anionic-charge of the TA3-Ha cell surface can be attributed to neuraminidase-sensitive sialic acid. Ferritin binding to the remaining (neuraminidase-insensitive) cell surface coat of the TA3-Ha cell (Figs. 22 and 24) is similar to that of the TA3-St cell (Figs. 19 and 20).

DISCUSSION

This study demonstrates distinct topographical differences between the cells of a nonstrain-specific and a strain-specific malignant ascites subline of the murine mammary adenocarcinoma, TA3. While the general internal morphology of both cell sublines is similiar, dramatic differences were observed in cell surface architecture, surface coat ultrastructure, and density of neuraminidase-sensitive anionic sites expressed at the cell surface.

An extensive surface coat reminiscent of the fuzz coat of the intestinal brush border (21, 22) was found on the immunologically protected, nonstrain-specific TA3-Ha cell; this cell coat covers the numerous microvilli characterizing the TA3-Ha cell and possibly contributes to their regular arrangement. The TA3-Ha cell is able to transgress both strain and species histocompatibility barriers (3, 5). By comparison, the strain-specific ascites cell, TA3-St, from the same mammary tumor was not found to have a morphologically distinct surface coat. The TA3-St cell surface is thrown into folds from which irregular cell processes project.

After glutaraldehyde and osmium tetroxide fixation, the surface coat of the TA3-Ha cells generally appeared as a network of fine filamentous material extending 40 nm from the plasma membrane. Sometimes, more rigid-appearing, rod-like structures as long as 400 nm were observed. This thick cell coat stains intensely with polycationic ferritin, most of which does not attach if the cells are pretreated with neuraminidase. No TA3-St cell coat was visible after routine preparation, but polycationic ferritin revealed a thin layer of exposed anions on the plasmalemma of TA3-St cells.

Visualization by transmission electron microscopy of an extensive and highly-charged surface coat on TA3-Ha cells invites comparison with previously reported biochemical (8, 10) and immunological (3, 16, 30) data, which demonstrated that a large glycoprotein is present at the TA3-Ha cell surface. This glycoprotein, called epiglycanin, has a molecular weight of about 500,000 (5) and is

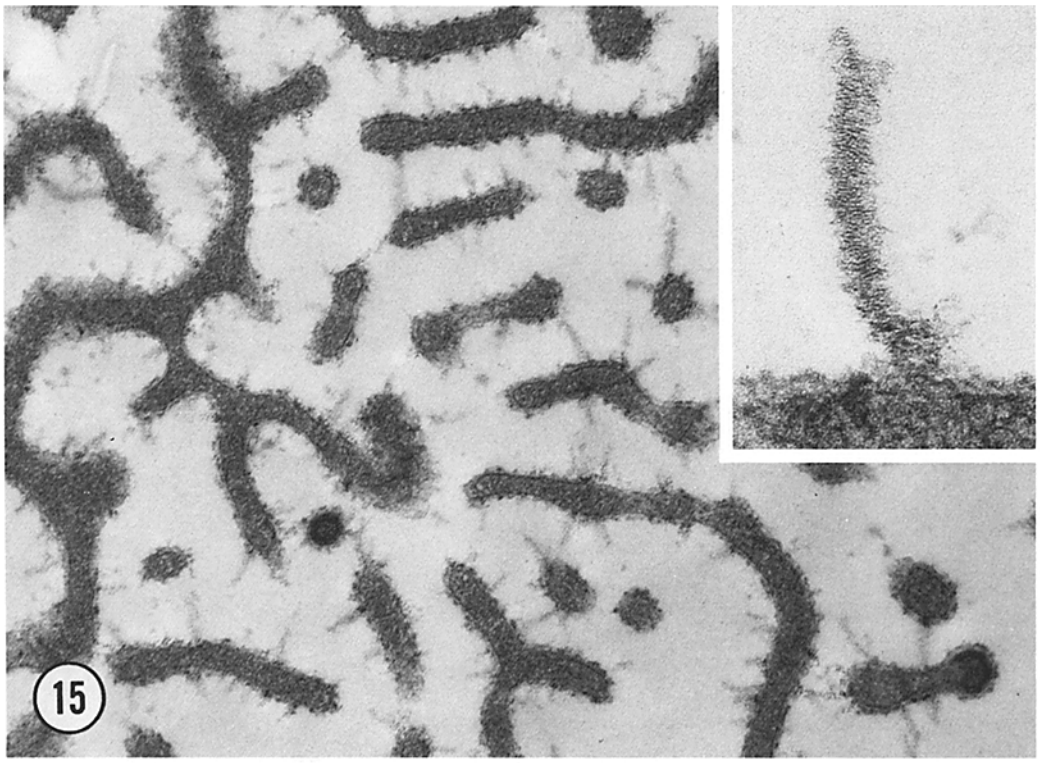
FIGURE 10 Greater detail of the surface coat on a microvillus of a nonstrain-specific TA3-Ha cell. The cell coat appears as a network of fine filaments which extends about 40 nm from the plasma membrane. Actin-like filaments run longitudinally within the microvillus. Glutaraldehyde-osmium tetroxide fixation. × 128,800.

FIGURE 11 Free virus particles associated with the nonstrain-specific TA3-Ha cells have cell coat material (arrow) on their outer membrane which appears similar to that on the plasma membranes of the TA3-Ha cell. Actin-like intracytoplasmic filaments are seen in cross section in the microvilli at the top of this micrograph. Glutaraldehyde-osmium tetroxide fixation. × 89,200.

FIGURE 12 This electron micrograph shows the intermicrovillous cell coat of the same TA3-Ha cell depicted in Fig. 10. The surface coat is not so extensive on the intermicrovillous plasmalemma as on the microvilli (Fig. 10). Glutaraldehyde-osmium tetroxide fixation. × 50,700.

FIGURE 13 Electron micrograph showing the surface of a strain-specific TA3-St cell fixed and stained in the same way as the TA3-Ha cell illustrated in Figs. 9–12. No cell surface coat is evident (cf. Fig. 9). Glutaraldehyde-osmium tetroxide fixation. × 50,700.

FIGURE 14 Higher magnification of a microvillus from a strain-specific TA3-St cell. A distinct cell surface coat is not evident at this magnification (cf. Fig. 10). The plasma membrane is not well contrasted in Figs. 10 and 14, due to omission of uranyl acetate staining en bloc. Glutaraldehyde-osmium tetroxide fixation. × 133,000.



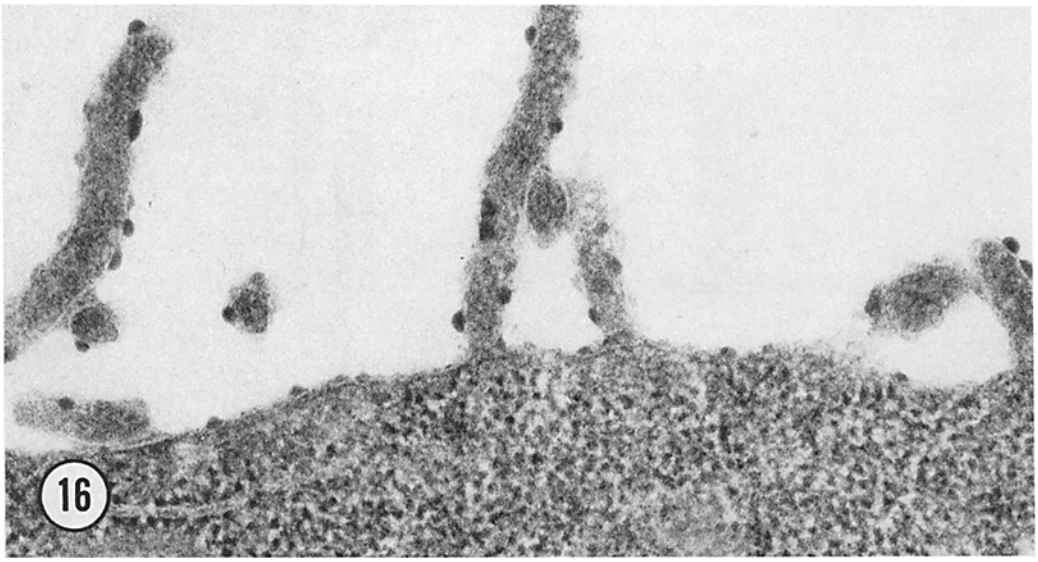


FIGURE 15 The cell surface coat of the TA3-Ha cell occasionally fixes in the form of rigid-appearing, rod-like structures generally 200 nm long but up to 400 nm in length. These surface structures are most evident in regions containing a high density of microvilli. The inset shows at high magnification one of the surface structures observed on the TA3-Ha cell in Fig. 15. A fine banding pattern is evident. Glutaraldehyde-osmium tetroxide fixation. × 44,700. Inset, × 195,000.

FIGURE 16 Semi-spherical globules are observed on the plasma membranes of TA3-Ha cells fixed in glutaraldehyde with no subsequent osmium tetroxide fixation. These structures are most evident on the microvilli of these cells and are not found on similiarly fixed TA3-St cell. Glutaraldehyde-osmium tetroxide fixation. × 70,000.

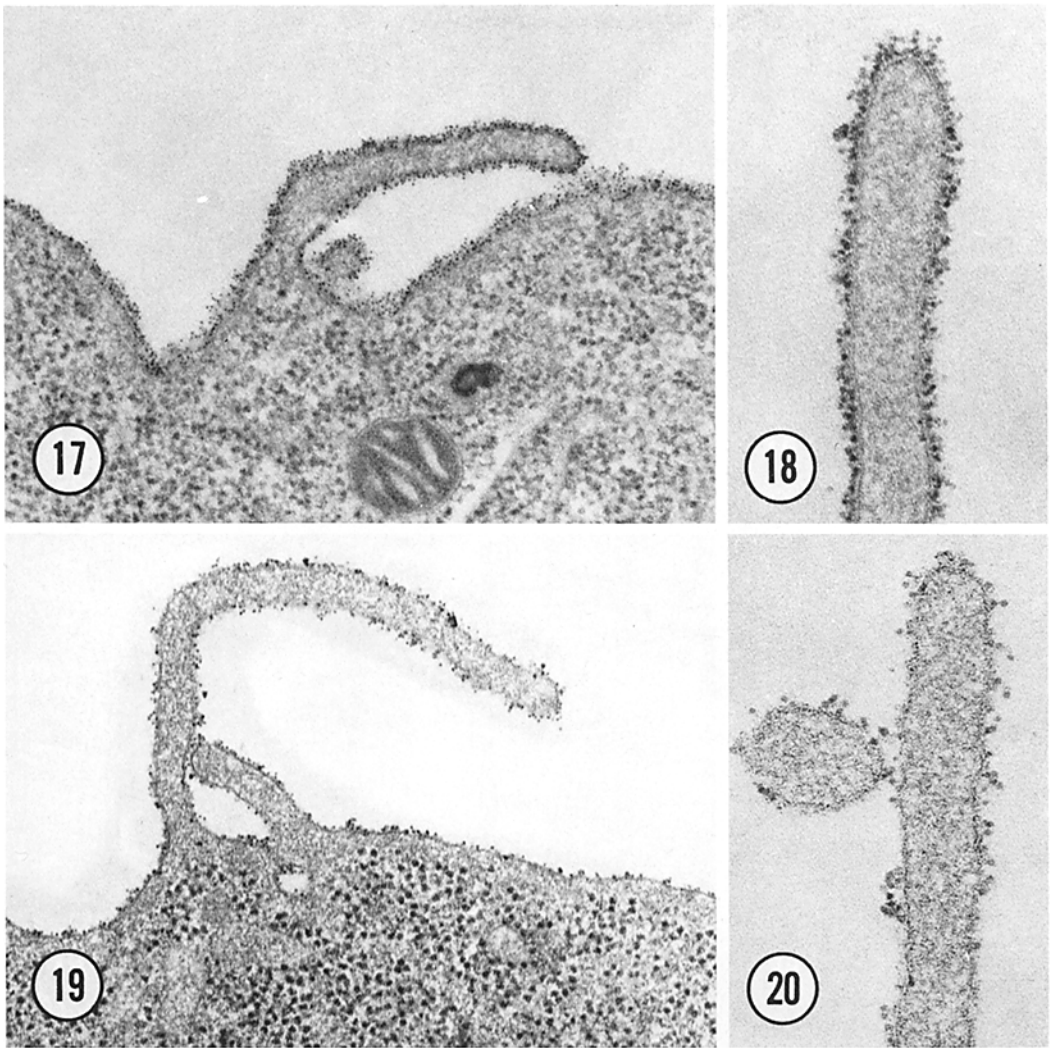


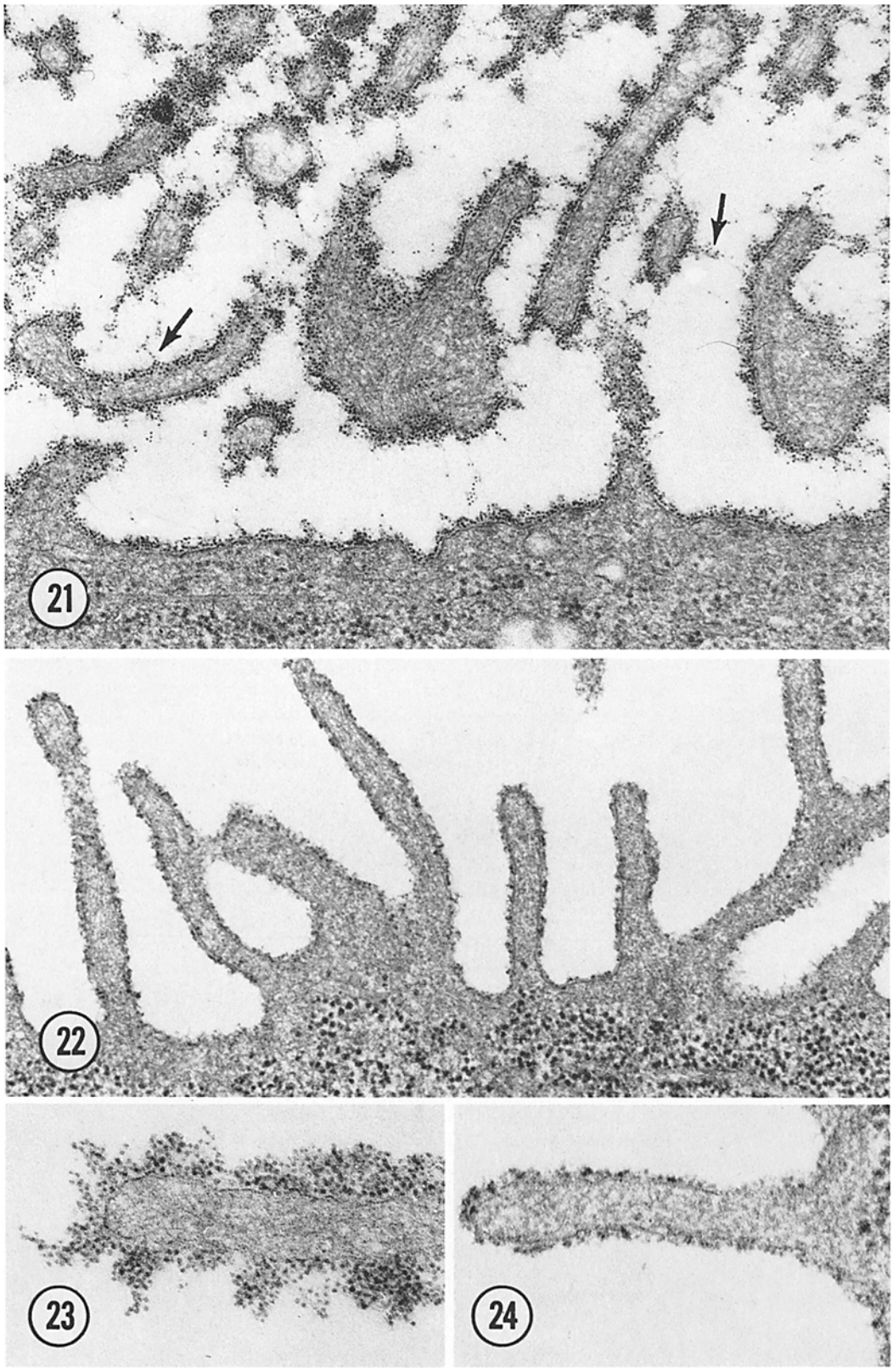
FIGURE 17 Electron micrograph showing the binding of polycationic ferritin to the surface of a previously fixed, strain-specific TA3-St cell. The ligand appears evenly distributed on the plasma membrane. Glutaraldehyde-osmium tetroxide fixation. × 52,400.

FIGURE 18 Higher magnification of the polycationic ferritin label on a cell process of a TA3-St cell. Glutaraldehyde-osmium tetroxide fixation. × 52,400.

FIGURE 19 Neuraminidase-pretreated TA3-St cell reacted with polycationic ferritin. Fewer ferritin particles are present on this cell compared to untreated TA3-St cells (Fig. 17). Glutaraldehyde-osmium tetroxide fixation. × 52,400.

FIGURE 20 Higher power view of neuraminidase-pretreated, polycationic ferritin-reacted TA3-St cell surface. Glutaraldehyde-osmium tetroxide fixation. × 121,000.

present on the cell surface in large quantity, approximately 5×10^6 molecules per cell. Epiglycanin consists of about 12% sialic acid, which is approximately half of the total surface acid that is removable from viable cells by neuraminidase (5). Purified epiglycanin has been found by electron microscopy to exist in an extended polypeptide configuration, often longer than 400 nm in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length and about 2.5 nm in width (31). No epiglycanin-like material can be isolated from the surface of the strain-specific, TA3-St, cell (8, 4, 10).

Because of the appearance and neuraminidase-sensitivity of the cell surface coat observed on the TA3-Ha cells in the transmission electron microscope, it is tempting to conclude that epiglycanin and related sialic acid-rich molecules are largely responsible for the surface coat structure which is observed in this study. These large, highly charged molecules, although probably aggregated and deformed by the fixation procedures used for electron microscopy, may exist in extended configurations in their natural hydrated environment. It is to be expected that different fixation procedures, as demonstrated here, as well as different methods of visualization would affect their appearance. (22, 33).

Several functions can be proposed for the thick glycoprotein coat that characterized the nonstrain-specific TA3-Ha cell. Because of the much lower absorption of histocompatibility (H-2) antibodies by intact TA3-Ha cells than by TA3-St cells (16, 30), it is believed that epiglycanin molecules may serve to mask histocompatibility (H-2) antigens at the TA3-Ha cell surface (8, 3, 30). This hypothesis is consistent with observations made in the present study. It appears plausible that the extensive glycoprotein coat demonstrated here to cover all the microvilli and exposed cell surface might serve to block antibody contact with glycoprotein antigens of smaller molecular weight at the cell membrane.

The presence of an extensive surface coat on the TA3-Ha, but not on the TA3-St cell, may also help to explain differences in agglutinability of these two cell lines to the plant lectin Con A (15, 17). Con A binds in equivalent amounts to both the TA3-St and the TA3-Ha cells, but the TA3-Ha cells, unlike all other murine ascites tumor cells which were tested, are not caused to agglutinate by Con A. This observation led to the suggestion that Con A-binding sites may reside in "crypts" on the cell surface (17). Thus, the surface coat of the TA3-Ha cell may physically interfere either with the migration of Con A receptor molecules in the cell membrane to form caps or with the crosslinking of Con A molecules between adjacent cells.

The nonstrain-specific TA3-Ha cell with its prominent glycoprotein surface coat was found to have a surface architecture different from that of the strain-specific TA3-St cell. While both cells were found to be covered by numerous cell processes, the processes on the TA3-Ha cell are generally straighter, more uniform, and often appear longer than those on the TA3-St cell. They resemble true microvilli, whereas the filopodia and lamellipodia on the TA3-St cell are too irregular to be called microvilli. Different methods of specimen preparation (1) and the attachment of viable cells to a solid substrate (29) are known to alter the surface morphology of cells examined in the scanning electron microscope. However, the surface features of the cells examined in this study were identical whether the cells were allowed to settle on glass cover slips for a short period of time (10 min) or were prefixed in cell suspension and picked up on polylysine-coated filters or cover slips.

Murine mammary adenocarcinoma may develop from interactions involving a B-type RNA

FIGURE 21 Electron micrograph showing the binding of polycationic ferritin to the surface of a previously fixed nonstrain-specific TA3-Ha cell. Substantially more ferritin particles can be seen on the cell surface compared to the label on the TA3-St cell (Fig. 17). The filamentous strands of the cell surface coat are labeled (arrows) with the ligand. Glutaraldehyde-osmium tetroxide fixation. × 51,600.

FIGURE 22 Neuraminidase-pretreated TA3-Ha cell reacted with polycationic ferritin. There is a great reduction in the binding of ferritin to the surface of these cells compared to untreated cells (Fig. 21). A thin mat of surface coat is still present. Glutaraldehyde-osmium tetroxide fixation. × 52,400.

FIGURE 23 Higher power view of polycationic ferritin label on a microvillus of a TA3-Ha cell. The ligand is densely and unevenly distributed compared to the label observed on the strain-specific TA3-St cells (Fig. 18). Glutaraldehyde-osmium tetroxide fixation. × 109,000.

FIGURE 24 Higher power view of polycationic ferritin label on a neuraminidase-pretreated TA3-Ha cell microvillus. The ferritin particles are enmeshed in the remaining cell surface coat. Glutaraldehyde-osmium tetroxide fixation. × 109,000.

virus, murine mammary tumor virus (MMTV) (26). The TA3 cell line was derived from a spontaneous murine mammary adenocarcinoma, but the role of viruses in the original transformation of this cell line is not known. Viruses are commonly found in murine ascites cell lines (11), and abundant virus-like particles were found in both the TA3-St and TA3-Ha cell in this study. The observation that an extensive surface coat is present on virus particles after budding from the TA3-Ha cell but not on virus particles in media containing TA3-St cells suggests that in the budding process the virus particles may accept host cell-surface glycoproteins on their surface. Certain glycoproteins have been found to be present on the surface of some C-type RNA viruses and on the host cell plasmalemma. However, these glycoproteins are of viral genome origin (23, 24). Large cell-surface glycoproteins such as those observed here have not been illustrated on virus particles. It is not known whether or not the viruses derived from the TA3-Ha cell that contain an extensive surface coat differ in infectivity from those viruses that are budded from TA3-St cells and contain no visible surface coat.

While it is tempting to emphasize the invasiveness of the TA3-Ha cell because of its ability to kill, nonspecifically, different strains and species of rodents, the property that prevents recognition of the foreign nature of these cells by the host immune system may not necessarily in itself be related to the malignant change. These adenocarcinomas are derived from an epithelial cell line, and the individual ascites cells may retain or reform remnants of the same intercellular junctions (maculae adherentes, zonulae adherentes and occludentes) that characterize the cells of origin, even though intercellular communication is probably limited and gap junctions are rarely seen. In their structure, the microvilli of the TA3-Ha cell are remarkably reminiscent of the normal microvilli of brush borders. Actin-lke filaments comprise the central cores (28), and the filamentous glycoprotein network on their surface inserts on the plasmalemma much as does the fuzz glycoprotein coat on normal microvilli (21, 22). Except that a basal surface and a true lateral surface are lacking, the TA3-Ha cell might pass for a normal epithelial cell, whereas the surface architecture of the strain-specific TA3-St cell is much more abnormal in appearance. The function of sialic acid-rich glycoproteins such as epiglycanin in the maintenance of the architecture of brush borders and the masking of recognition proteins on the apical (free) surfaces of normal epithelia should be considered before the role of epiglycanin is classified as strictly neoplastic in character. Thus, the present study not only provides a morphological basis for the surface localization of the sialic acid-rich masking protein of the TA3-Ha cell but also invites comparison of the coated surface of such a tumor cell and the normal free surface of exposed epithelial cells that possess microvilli.

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