THE UPPER CELL SURFACE: ITS INABILITY TO SUPPORT ACTIVE CELL MOVEMENT IN CULTURE

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ABSTRACT

A variety of epithelial cells and fibroblasts fail to move over one another's upper surfaces in culture, resulting in monolayering. The failure of seeded fibroblasts to adhere to and spread on epithelial cell surfaces suggests that monolayering in culture is due to the lack of adhesion of the upper cell surface, at least of epithelial cells. Seeded fibroblasts and postmitotic, rounded fibroblasts likewise fail to spread on the upper surfaces of spread fibroblasts, suggesting that the inability of the upper cell surface to support spreading may be a general phenomenon. Inert particles and cell processes do not adhere directly to the upper cell surface. However, they can initiate adhesions to the surface at a cell's free margin, suggesting a variation of adhesive properties over a cell's surface.

INTRODUCTION

The term "contact inhibition" (of movement) has been defined by Abercrombie (9) as the failure of a cell to continue moving in a direction that would carry it over the surface of another cell following contact. Fibroblasts will migrate over a glass or Falcon plastic substratum, but, upon meeting another fibroblast (2, 4) or epithelial sheet (6, 28, 36), fail to crawl over its upper surface. Barski and Belehradek (11) found that the movement of mouse sarcoma cells is obstructed by coherent endothelial sheets. As a result of this restricted movement, cells in culture tend to form "monolayers" with negligible "nuclear overlap."

Abercrombie (9) has proposed several possible explanations for the failure of cells to move over one another's upper surfaces. First, one cell may act as a simple mechanical obstacle to the continued movement of the other. Second, firm, lateral, intercellular adhesion (23, 31) and/or the passage of a signal between cells upon lateral contact might locally arrest locomotory surface activity (e.g., ruffling, protrusion of the flattened leading edge), and consequently, cell movement. Third, the adhesion of a cell to the inanimate substratum may be relatively stronger than its adhesion to the upper surface of another cell, with the result that cells preferentially adhere to the former (17, 22, 26, and 35). Finally, it is possible that the upper cell surface is totally nonadhesive, in contrast to the lateral edges, preventing other cells from adhering to it and moving on it.

In this study, we have investigated the ability of cells, once on the upper surface[1] of spread cells, to adhere, spread, and move. This was done by examining in detail with time lapse cinemicrography (*a*) the behavior of cells seeded *directly on to the upper surfaces of other cells* in culture, and (*b*) the behavior to protruding edges of cells on the upper surfaces of neighboring spread cells. Additional observations were made on the spreading behavior of postmitotic cells found resting on the upper surfaces of other cells.

[1] The upper surface of cells refers to that side of the cell away from the artificial substratum, regardless of its position with respect to gravity.

Part of this work has already been reported in preliminary form (19).

MATERIALS AND METHODS

Culture Methods

Epithelial Cells

The following three classes of epithelial cells were derived from 6.5–7-day (stage 30) chick embryos and isolated in the following ways.

EPIDERMIS: Embryos were removed aseptically and immediately placed in ice-cold Tyrode's Solution with 20% heat-inactivated fetal calf serum (4°C, pH 7.3). The skin was removed surgically from the mid-dorsal region of the embryo and transferred with a Pasteur pipette to a 0.1% trypsin solution with 0.1 mg/ml DNase (0°C, pH 7.3) for 5 min. The tissue was then transferred back to cold Tyrode's (4°C, pH 7.3) with 20% heat-inactivated fetal calf serum and the epidermis was carefully separated from the dermis with forceps and then pipetted into the culture medium. The tissue was minced into 1-mm^2 pieces with forceps, and the medium was carefully withdrawn to the extent that the tissue clumps were still wet but no longer capable of floating freely. Small drops of medium were placed around the drop containing the tissue to prevent it from drying. The cultures were incubated at 37.5°C and gassed with a mixture of 5% CO_2 in air. After 12 h, prewarmed medium was added to the cultures. Observations were made between 24 and 72 h after culturing.

CORNEAL EPITHELIUM: Corneas were excised from the eyes of 6.5–7-day chick embryos and carried through the same procedure as for epidermis.

GUT EPITHELIUM: Gizzards were removed and explants of epithelial tissue were obtained in the same way as above, except that no trypsin treatment was required. Mesenchymal tissue was completely removed with forceps. The epithelial tissue could be easily distinguished by its clear, glassy appearance. Observations were made after 48 h in culture. All epithelial cells were maintained in baby hamster kidney (BHK) medium supplemented with 10% fetal calf serum and 10% tryptose phosphate broth (Grand Island Biological Co., Grand Island, N. Y.).

Chick Heart Fibroblasts

Chick heart fibroblasts from 6.5–7-day (stage 30) embryos were obtained according to the method of Abercrombie et al. (5) and maintained in the same medium used for epithelial cells.

Cell Lines

The KB cell line and the sarcoma 180 cell line were kindly provided by the laboratory of Dr. F. H. Ruddle (Yale University). KB cells were maintained with diploid growth medium and the sarcoma 180 cell line was maintained in BHK medium (both media from Grand Island Biological Co.), supplemented with 10% fetal calf serum and 10% tryptose phosphate broth. Polyoma-transformed 3T3 cells (Py 3T3) and 3T3 cells were generously provided by Dr. H. K. Green (Massachusetts Institute of Technology, Cambridge, Mass.), and murine sarcoma virus-transformed BALB/c 3T3 cells were kindly provided by Dr. George Todaro (National Institutes of Health, Silver Spring, Md.). These lines were maintained in Dulbecco's modified Eagle's medium-high glucose supplemented with 10% calf serum (Grand Island Biological Co.). Cells were routinely transferred with Viokase (Grand Island Biological Co.), supplemented with 0.05 M EDTA. Primary cultures of sarcoma 180 cells were derived from tumors of BALB/c mice (Jackson Laboratories, Bar Harbor, Me.) and maintained in Dulbecco's modified Eagle's medium with 10% calf serum.

Tissue Culture Media

All media contained 50 U per ml of potassium penicillin G and 50 U per ml of streptomycin. The pH was adjusted to 7.2. All media were filtered and stored at 4°C.

Seeding Experiments

All seeding experiments were performed in BHK medium supplemented with 10% fetal calf serum and 10% tryptose phosphate broth. Gut epithelial cells were cultivated for 48 h and corneal and epidermal epithelial cells for 24 h before seeding. Single cell suspensions were added to the appropriate epithelial cell cultures 0.5 h after dissociation by Viokase solution (10 min, 37°C, pH 7.3) at a concentration of 5×10^5 cells per ml (2 ml in 25 × 10-mm Falcon plastic petri dishes [Falcon Plastics, Div. of D-D Laboratories, Inc., Los Angeles, Calif.] 0.5 ml in aluminum chambers). Filming was begun within 3 min after seeding.

Time-Lapse Cinemicrography

Time-lapse films were made with an Arriflex 16S movie camera, an Arriflex intervalometer, and an Arriflex DOM animation camera motor (Arriflex Co. of America, Woodside, N. Y.). Cells were filmed either directly in plastic culture dishes or on cover slips attached to aluminum or glass time-lapse slides (Bellco Glass, Inc., Vineland, N. J.). Phase-contrast optics were used throughout. Films were analyzed with a data-analyzer projector (L-W 224A, L-W Photo, Inc., Van Nuys, Calif.).

Preparation of Particles and Red Blood Cells for Surface Movement Studies

LATEX PARTICLES: An alcohol suspension of latex particles 0.5 μm in diameter (Ernest Fullam, Inc., Schenectady, N. Y.) was washed and suspended

in a × 10 volume of culture medium. This suspension was added directly to cell cultures. Alternatively, a small drop of the suspension of particles in alcohol was added directly to a cover slip, which was allowed to air dry and then used for culturing cells.

RABBIT RED BLOOD CELLS: Rabbit red blood cells were obtained by standard techniques, washed in Tyrode's, and resuspended in BHK or diploid growth medium. The concentration was adjusted to 5×10^6 cells/ml, and a total of 5×10^7 cells were incubated with 10 ml of 100 μg/ml concanavalin A (Con A) (Calbiochem, Los Angeles, Calif., A grade) at 37°C for 0.5 h. The red cells were then washed and resuspended in normal medium to give a concentration of 5×10^6 red blood cells per ml. Cultures were exposed to 2 ml of this suspension per 5 ml of medium for 1 h at 37°C before decanting the medium.

RESULTS

Failure of Epithelial Cells and Fibroblasts to Move Over One Another's Upper Surfaces in Culture

Embryonic chick epithelial cells (gut, corneal, and epidermal) spread as coherent sheets across a plane Falcon plastic or glass substratum (Fig. 1). Locomotory surface activity (e.g., ruffling, fluctuation of the flattened leading edge), is, for the most part, limited to the marginal cells of the sheet (see also reference 32). Time-lapse films show that, as the sheet advances, cells behind the margin do not crawl actively over one another's upper surfaces. The cells maintain their positions with respect to one another as the sheet moves.

In mixed cultures of chick epithelial cells and various types of fibroblasts, epithelial cells were never observed to move actively from the substratum onto the upper surfaces of fibroblasts, and vice versa. Fig. 2 shows a typical interaction between a marginal epidermal epithelial cell and an approaching sarcoma 180 cell. The sarcoma 180 cell advances toward the epithelial sheet with a fluctuating flattened leading edge as described by Abercrombie et al. (7). After contact is made with the edge of the epithelial sheet, the confronted cells do not continue to move in a direction that would carry them over one another's upper surfaces. Instead, they either remain in their positions, move away in the opposite direction, or veer to the left or

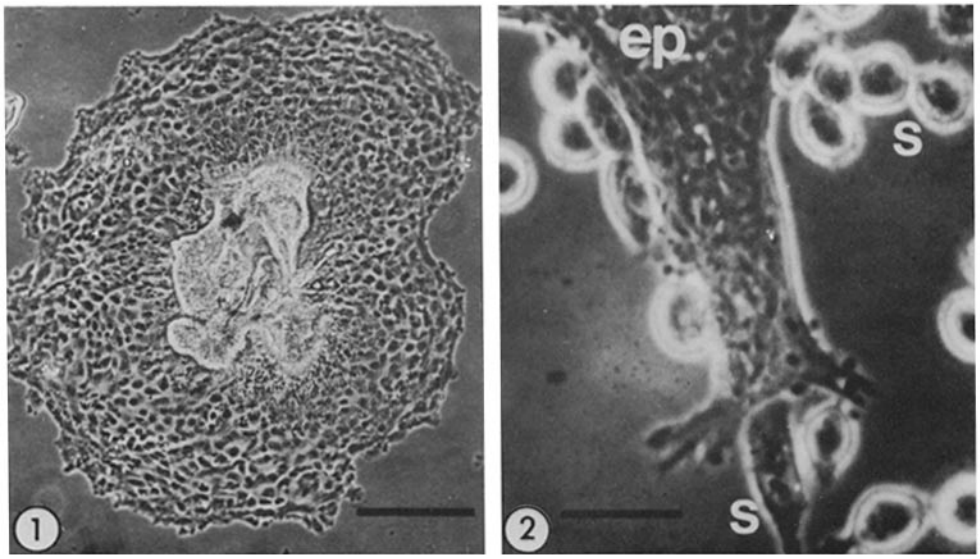


FIGURE 1 Explant of embryonic chick epidermal epithelium, 24 h in culture, spreading on a glass substratum. Phase contrast. Bar represents 300 μm. × 50.

FIGURE 2 Confrontation of epidermal epithelial sheet (*ep*) and sarcoma 180 cells (*s*). Cells do not move over one another's upper surfaces. Epithelial cells were cultured for 24 h on a glass cover slip, at which time sarcoma 180 cells were seeded as single cells. 6 h later, the cover slip was inverted and sealed in an aluminum chamber (see Materials and Methods) for filming. Note that the large sarcoma 180 cells did not adhere to the upper surface of the epithelial sheet but did stick firmly to the glass substratum. Phase contrast. Bar represents 100 μm. × 150.

right of the epithelial cell lamellipodium and eventually move *under* the epithelial sheet in a concave region of the epithelial margin not in contact with the substratum. Similar interactions observed in confrontations between the cell types listed in Table I support the general conclusion that fibroblastic cells fail to move on to and over the upper surfaces of epithelial sheets.

TABLE I

Interactions between Various Cells and Chick Epithelial Cells

Combinations	No. of contact events observed	No. of cells observed moving over upper surfaces of marginal epithelial cells
Chick heart fibroblast-chick epidermis	89	0
Chick heart fibroblast-chick corneal epithelial cells	25	0
Chick heart fibroblast-chick gut epithelial cells	34	0
Sarcoma 180 (cell line)-chick epidermis	15	0
Py 3T3-chick epidermis	21	0
Py 3T3-chick gut epithelial cells	19	0
KB cells-chick gut epithelial cells	13	0

In no case were cells seen to migrate over the marginal cells of the sheet and vice versa. Observations were made over periods as long as 48 h. At the start of the observation period, gut epithelial cells had been in culture for 48 h, and corneal and epidermal epithelial cells had been in culture for 24 h. Fibroblasts had been in culture from 1 to 24 h.

Failure of Seeded Fibroblasts to Spread on the Upper Surfaces of Epithelial Sheets

To test the possibility that the upper surfaces of epithelial cells in culture are actually nonadhesive, suspensions of single cells were seeded directly on to the upper surfaces of coherent epithelial sheets and their behavior was followed with time-lapse cinemicrography.

Fig. 3 shows the results of two typical seeding experiments. Py 3T3 cells were seeded on to gut epithelial cell sheets in culture, as described in Materials and Methods. All suspended Py 3T3 cells are initially spherical with no prominent protrusions or blebs. 5 min after seeding, the rounded cells contact the upper surface of the epithelial cells and the adjacent clear glass substratum.

BEHAVIOR OF SEEDED CELLS ON GLASS: For the next 10 min, the cells on the glass do not behave differently from those in suspension. Approximately 15 min after seeding, the Py 3T3 cells extend and retract rounded protrusions known as blebs which give the lateral edges of the cells a bubbly appearance. About 20 min after seeding, the cells begin to elaborate broad (5–25 μm), flattened protrusions. 30 min after seeding, the majority of Py 3T3 cells are flattened and spread on the glass. Subsequently, these cells migrate actively about but never move on to the upper surfaces of the epithelial cells.

BEHAVIOR OF SEEDED CELLS ON THE UPPER SURFACES OF EPITHELIAL CELLS: Those Py 3T3 cells on the upper surfaces of the epithelial cells remain rounded for the duration of the experiment (5 h). Occasionally, rounded blebs extend from two of the cells in the field, but no flattened protrusions emerge. When Py 3T3 cells happen to contact neighboring Py 3T3 cells during the 5-h period after seeding, they apparently adhere firmly to one another, since groups of two or three of these cells jostle about in the medium as units. In the experiment shown in Fig. 3 *a–d*, three Py 3T3 cells initially resting on the epithelial sheet floated off it 3 h after the filming was started. The first cell to contact the glass flattened (as previously described) within 15 min. The other two cells came to rest on its upper surface and remained rounded. One of these then slid off on to the glass and, like the cell that preceded it, spread and flattened on the glass within 15 min. The cell that never obtained access to the glass substratum remained rounded on the upper surface of the first Py 3T3 cell that spread for a full 2 h, at which time filming was stopped. It is clear that those Py 3T3 cells that remain on the upper surface of the epithelial sheet for the full 5 h do not adhere firmly to it; they sway freely in the liquid medium when the culture dish is moved.

Similar results were obtained in seeding experiments with the combinations of cells listed in Table II.

Fig. 4 shows the result of a "seeding experiment" in which primary explants of chick heart fibroblasts rather than suspensions of single cells

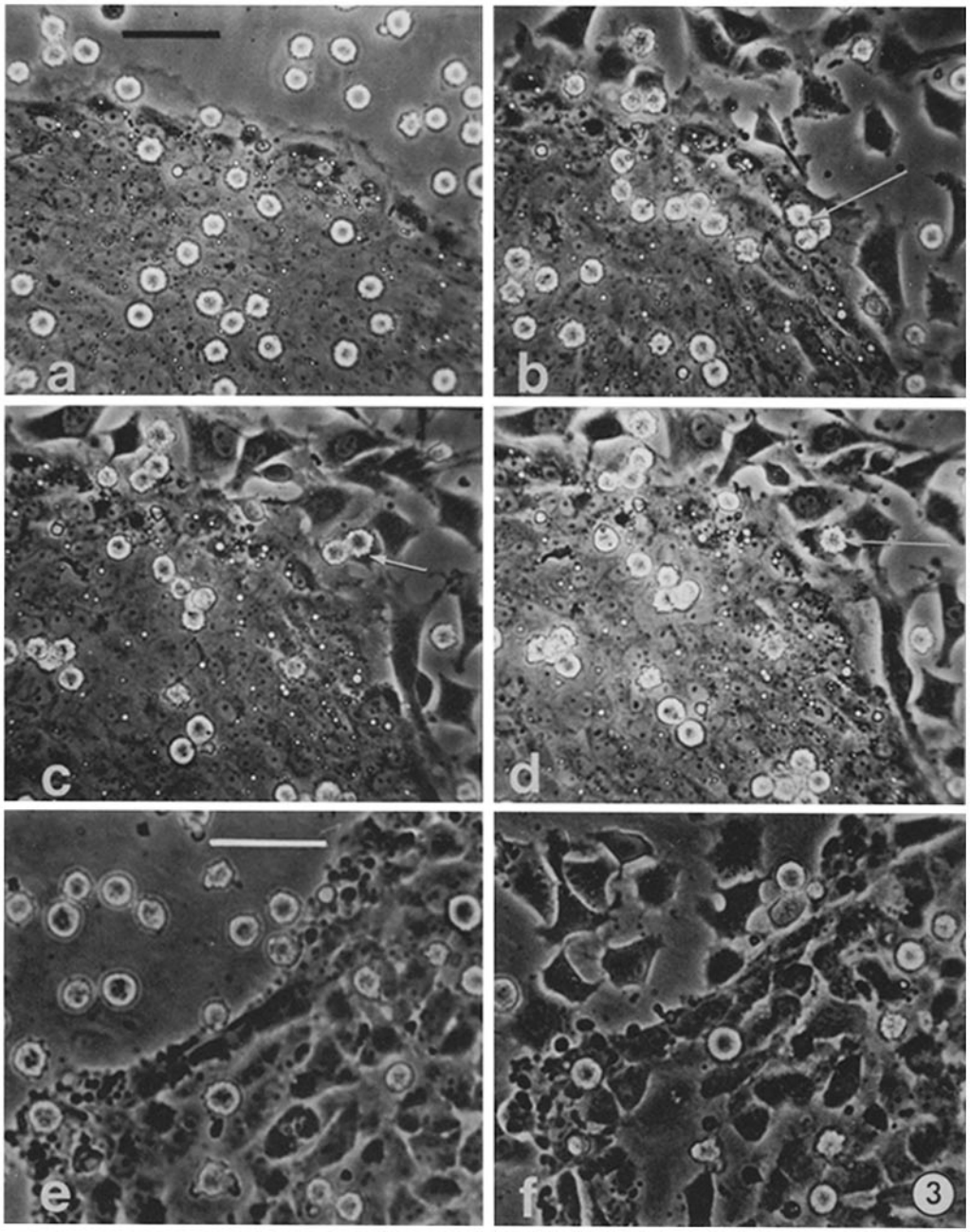


FIGURE 3 Seeding experiments (*a–d*, Py3T3 seeded on to chick gut epithelial sheet; *e–f*, Py3T3 seeded on to chick corneal epithelial sheet). (*a*) Gut epithelial sheet cultured on glass 48 h. Rounded Py 3T3 cells out of focus are falling by gravity toward the glass substratum. Frame was taken 1 min after seeding. Phase contrast. Bar represents 50 μm. × 130. (*b*) Same field as (*a*), 111 min later. Py 3T3 cells on the glass have spread, while those on the upper surface of the gut epithelial sheet have not. The Py 3T3 cells that have not spread are starting to cluster. Note triplet near margin of sheet (arrow). (*c*) Same field as (*a*), 151 min later than (*a*). One of the cells of the triplet in (*b*) is now on the glass substratum and has spread (arrow). (*d*) Same field as (*a*), 162 min later than (*a*). Another of the triplet cells has spread on the glass. The one cell remaining on top (arrow) of the two spread cells has not spread, nor have any of those remaining on top of the sheet. (*e*) Corneal epithelial sheet (*ep*) cultured on Falcon plastic for 24 h. Rounded Py3T3 cells out of focus are falling by gravity toward the sheet and the plastic substratum. Frame was taken 3 min after seeding. Phase contrast. Bar represents 50 μm. × 130. (*f*) Same field as (*e*), 90 min later. Py 3T3 cells on the plastic have spread, while those on the upper surface of the epithelial sheet have not.

TABLE II

Seeding Experiments

Cells seeded (5×10^5 cells/ml)	Artificial substratum	Cellular substratum	Duration of filming (hs)	Total no. of seeded cells in field		Total no. of seeded cells spread at end of experiment	
				On artificial substratum	On sheet	On artificial substratum	On cellular substratum
CHF	Glass	Epidermal epithelial sheet	7	21	9	14	0
CHF	Glass	Corneal epithelial sheet	7	14	16	11	0
CHF	Falcon plastic	Gut epithelial sheet	5	18	12	10	0
Sarcoma 180	Glass	Epidermal epithelial sheet	6	15	13	14	0
Polyoma 3T3	Falcon plastic	Epidermal epithelial sheet	5	17	10	12	0
Polyoma 3T3	Falcon plastic	Gut epithelial sheet	5	17	24	15	0
KB	Falcon plastic	Gut epithelial sheet	5	23	18	17	0
KB	Falcon plastic	Epidermal epithelial sheet	5	23	35	20	0

Experiments were carried out as described in Materials and Methods.

were used. Explants of chick heart fibroblasts were placed on both the Falcon plastic substratum and the upper surface of a gut epithelial sheet which had been in culture for 48 h. After 24 h, fibroblasts had spread from the explant on to the plastic, but none spread on to the upper surface of the epithelial sheet.

Failure of Seeded Fibroblasts to Spread on the Upper Surface of Spread Fibroblasts

When fibroblasts were seeded on to fibroblast monolayers, no clear case of a seeded cell *spreading* on the upper surface of another cell was seen. However, the fibroblasts used in this study fail to form stable, coherent sheets in culture. Rather, they form loose networks with numerous gaps between cells. Because seeded cells were able to gain access to and spread on the inanimate substratum, it was not always possible to discern clearly whether seeded cells were able to adhere to the upper surfaces of spread fibroblasts.

Failure of Single Cells in Culture to Move Over Each Other

In the course of our studies of the behavior of cells in culture, we have observed numerous instances of cell-to-cell contact on a plane substratum. In no case has a cell been observed to migrate actively on to the upper surface of another cell. The combinations of cell types observed in time-lapse films are listed in Table III.

Failure of Rounded Postmitotic Cells to Spread on the Upper Surfaces of Other Cells

During mitosis, cells become rounded and following cytokinesis the two daughter cells appear similar to cells freshly plated out. They are more or less spherical and bleb vigorously. Occasionally, one or both of these rounded daughter cells comes to lie on the upper surface of a nearby spread cell, making it possible to follow the course of spreading of single cells on other cells. Figs. 5 and 6 illustrate such situations.

In Fig. 5, one Py 3T3 cell is seen to bridge the upper surface of another Py 3T3 cell. Following mitosis of the bridging cell, both daughter cells lie on the upper surface of the lower cell. Neither rounded daughter cell spreads on the surface of the lower cell. Eventually one of the daughter cells spreads on the glass substratum to the right of the spread cell, pulling the other daughter cell behind

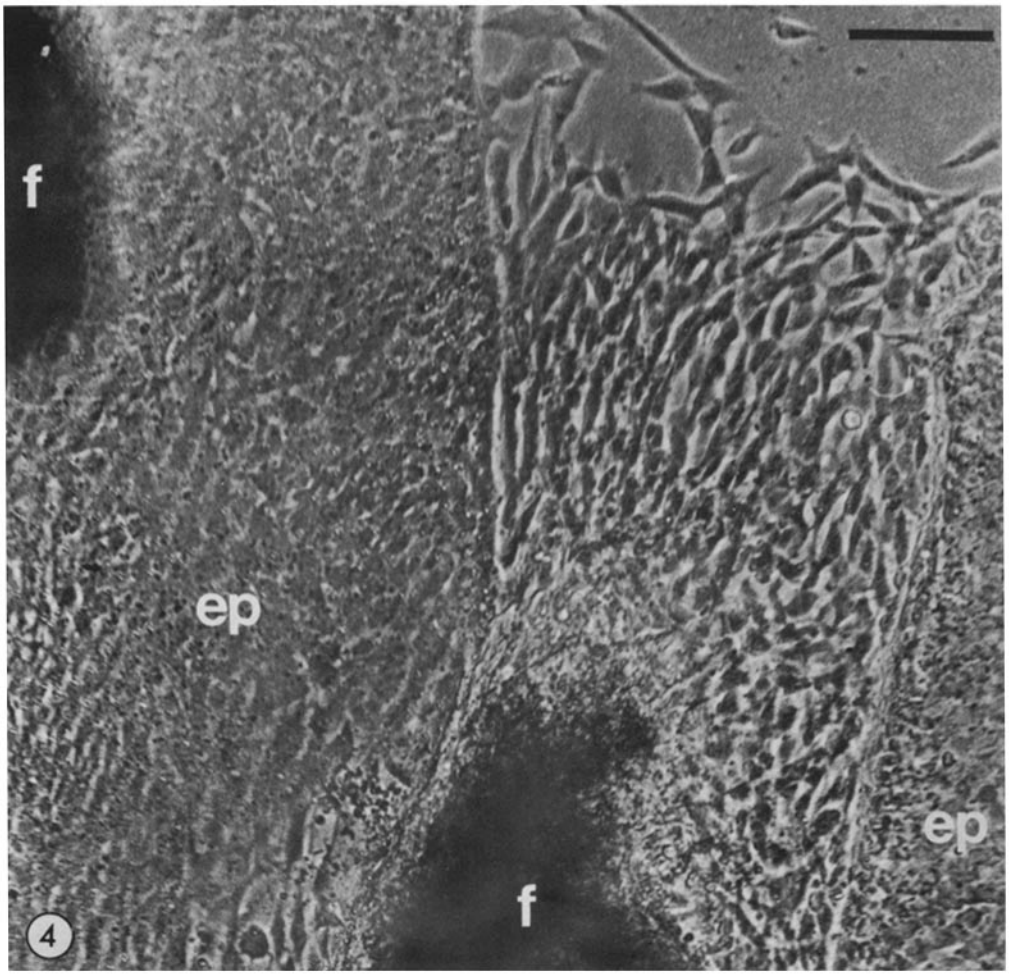


FIGURE 4 Gut epithelial sheets (*ep*) spreading on glass, 72 h in culture. Chick heart fibroblast explants (*f*), one placed on the glass between the two epithelial sheets and one placed on top of the epithelial sheet, 24 h in culture. Fibroblasts have spread profusely on to the glass between the two epithelial explants. None was detectable spreading on top of the epithelial sheet. Phase contrast. Bar represents 100 μm. × 190.

it. Finally both daughter cells rejoin into a single spread binucleated cell.

Fig. 6 is a series of tracings from a time-lapse film of a Py 3T3 cell culture. Again, rounded mitotic daughter cells fail to spread so long as they are blocked from access to the glass substratum by other cells. In this case cytokinesis was complete.

In similar events in other films of Py 3T3 cells, 3T3 cells, and KB cells, rounded mitotic daughter cells were never observed to spread on the upper surfaces of other cells upon which they rested. These observations show that the upper surfaces of fibroblastic cells as well as those of epithelial cells fail to support spreading and movement of other cells. Thus the inability of the upper cell surface to support the spreading and active migration of other cells may be a general phenomenon.

Initiation of Adhesions of Particles and Cell Processes only at the Free Margins of Spread Cells

Although the above findings imply that the upper surfaces of cells in culture are not adhesive, we have found that cell processes as well as inert objects may come to adhere to the upper cell surface, but only after initially adhering in the region of the fluctuating leading edge.

TABLE III

Confrontations of Single Cells observed in these Studies

Isotypic Cultures
3T3
Py 3T3
KB
Chick heart fibroblast
Sarcoma 180 (cell line)
Sarcoma 180 (primary culture from tumor)
Murine sarcoma virus transformed BALB/c 3T3
Heterotypic Cultures
Py 3T3–3T3
Py 3T3–chick heart fibroblast
Sarcoma 180 (primary culture)–mouse connective tissue fibroblast (primary culture)

Latex Particles and Con A-Treated Red Blood Cells

Both latex particles and con A-treated red blood cells adhere to the leading edge of marginal epithelial cells. Fig. 7 shows tracings of sequential frames from a time-lapse film of a marginal epidermal epithelial cell spreading on a glass substratum. The leading edge advances in the form of flattened protrusions 2–30 μm in breadth. Localized regions of the edge occasionally withdraw while remaining flattened. When the protruding lamellipodium contacts latex particles (black circles), these objects adhere to the leading edge and subsequently move away from it on the upper cell surface (see also 8, 10, 21, and 24). Latex particles were determined to be on the upper surface by focusing at high magnification and by the failure to observe them within cells in thin sections of epithelial cell cultures containing latex particles. Con A-treated rabbit red blood cells (hatched circles, Fig. 7) behave the same way as latex particles. When these cells are not exposed to con A or when they are exposed to con A in the presence of 0.1 M sucrose, they do not adhere to the leading edge. It seems likely that cross linking between con A binding sites on the epithelial cell surface and the red blood cell surface is responsible for the adhesion.

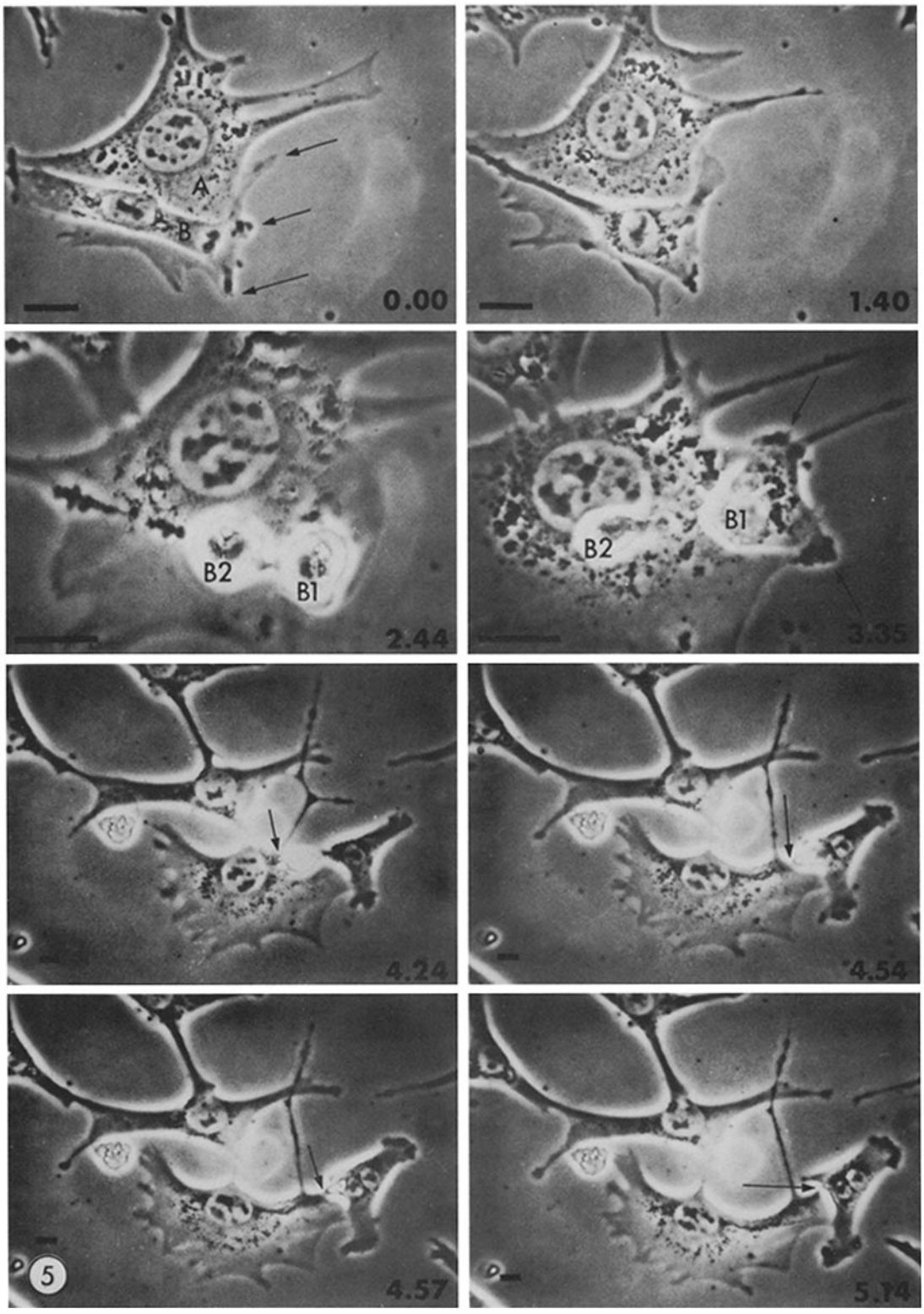
Although latex particles and con A-treated red blood cells adhere at the fluctuating leading edge, they do not adhere directly to other regions of the upper cell surface. Latex particles were allowed to fall by gravity on to cultures of epidermal epithelial cells spreading on glass. After 1 h, the culture dish was shaken. Only one latex particle was found to adhere to the upper surface of a submarginal epithelial cell in a culture vessel with seven epithelial sheets roughly the size of that shown in Fig. 1. None adhered to the glass substratum either. In contrast, the marginal cells of the sheets were covered with particles. Time-lapse films show that latex particles in contact with the substratum are picked up by the advancing epithelial cell lamellipodium at the leading edge and subsequently move backwards on its upper surface. But none adheres initially to the upper surfaces of the marginal cells. Similar results were obtained with con A-treated red blood cells (Fig. 8 *a*, *b*).

Cell Processes

Cell processes have also been observed adhering to the upper surface of other cells in the region of their leading edge. This might appear to be inconsistent with the upper cell surface's being nonadhesive, but time-lapse films show that these adhesions are always initiated at the leading edge before being moved on to the upper cell surface. Fig. 9 shows a typical case involving two 3T3 cells on a glass substratum. The ruffling lamellipodium of cell A at the bottom of the picture advances toward the narrow extended process of cell B. Contact between the two lamellipodia is made in a small area about 1 μm in breadth (arrow). Focusing during filming revealed that the leading edge of cell A advances and underlaps the process of cell B. The tip of the process of cell B is left adhering to the *upper* surface of cell A, since otherwise the process would have retracted into the cell body. Other cases have been observed in which retraction fibers were drawn out when such processes pull away from upper cell surfaces, indicating that firm adhesions are present.

Significantly, the tip of the process of cell B is moved back over the upper surface of cell A with reference to the substratum in a manner similar to latex particles and con A-treated red blood cells. Indeed, the whole process of adhesion to the leading edge with subsequent centripetal movement up on to the upper surface is superficially the same as for latex particles and con A-treated red blood cells.

It is clear from Fig. 9 that the margin of cell B is inactive where it adheres to the upper surface of cell A. In contrast, the leading edge of cell A, which is on the glass substratum, continues to fluctuate, forming broad, flattened protrusions


A
B
0.00
1.40
B2
B1
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B1
B2
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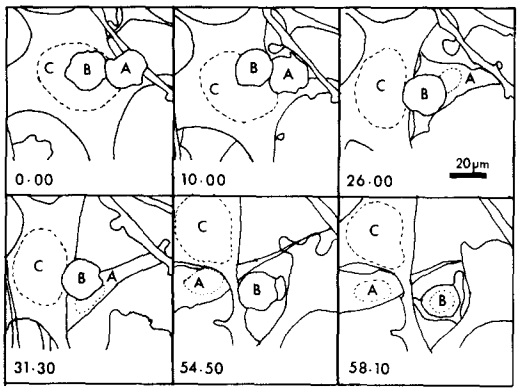

FIGURE 6 Failure of two rounded Py3T3 cells to utilize the upper surface of another Py3T3 cell as a substratum for spreading. Time is in minutes and seconds. Cell outlines traced from projected single frames of a time-lapse film. Broken lines indicate the outline of the cell nucleus. At the beginning of this sequence two mitotic daughter cells, labeled *A* and *B*, are rounded up and resting on the upper surface of spread cell *C*. Rounded cells are highly refractile and their nuclei are not visible. At 10 min, cell *C* has withdrawn slightly and cell *A* has begun to spread onto the glass substratum in the space between cell *C* and a process from another cell. By 26 min, cell *A* is well spread and its nucleus has become visible. Cell *C* has withdrawn further and cell *B* has come to lie on the upper surface of cell *A*. At 31.30, cell *A* has migrated downward somewhat while cell *B* has remained rounded above it. By 54.50, cell *B* has come to lie on the glass substratum between the cells, and the first sign of spreading is seen to the right of the still mostly rounded cell body. 3 min and 20 s later, at 58:10, cell *B* is rapidly spreading on the glass and its nucleus is becoming visible. The bar represents 20 μm. × 500.

that extend the cell margin over the substratum. In numerous cases of cytoplasmic overlap between 3T3 cells and between Py 3T3 cells, in one case between two chick epidermal epithelial cells, and in one case between a chick heart fibroblast and a chick epidermal epithelial cell, similar events were observed. A cell process adhering directly to the upper surface of another cell consistently fails to spread on it, while the *underlapping* lamellipodium continues to spread on the glass or plastic substratum, at least for a short distance.

Fig. 10 shows a contact event between two Py 3T3 cells. The margins of both cells A and B have been actively extending across the glass substratum reaching the positions shown in Fig. 10 *a*. Fig. 10 *b* covers a period of 5 min and 20 s during which the margins of cells A and B first contact and then overlap. Cell B extends itself under cell A, while cell A adheres to the upper surface of cell B. All of the sites of adhesion between cell A and the upper surface of cell B appear to be initiated as marginal contacts. These points of adhesion are subsequently propagated backward over the uppersurface of cell B away from the margin for a short distance, just as are latex particles, con A-treated red blood cells, microspikes, and retraction fibers. The scalloped pattern of the margin of cell A along the region of contact with the upper surface of cell B, with attenuated adhering processes separated by concave areas, is evidence of tension exerted at the adhesions (see reference 23). The tension probably results both from the elastic and/or contractile pull by cell A, and the backward pull of the surface of cell B. Thus it seems

FIGURE 5 Sequences from a time-lapse movie showing the division of one Py3T3 cell while stretched over the upper surface of a second Py3T3 cell. Numbers indicate elapsed time in hours and minutes. At the beginning of this sequence, cell *B*, while adhering to the glass substratum (arrows), extends over the upper surface of cell *A*. At 1:40 cell *B* has withdrawn partly from its adhesion to the left, drawing out a thick retraction fiber. By 2 h 44 min, cell *B* has completely rounded up and has almost completed cytokinesis. The two daughter cells, now labeled *B*1 and *B*2, are connected to each other by a narrow cytoplasmic bridge. *B*1 and *B*2 lie completely on top of cell *A* while still adhering to the glass substratum at the far left and at the right. At 3 h 35 min, *B*1 has begun to spread on the glass substratum to the right of cell *A* (arrows) while cell *B*2 remains rounded but still connected to *B*1. At 4 h 24 min *B*2 is fully spread on the glass and has moved away from cell *A* to the right. *B*2 is still rounded and connected to *B*1. By 4:54 *B*2 has begun to be pulled toward *B*1 and has partly flowed into it. 3 min later (4:57) the nucleus of *B*2 can be seen within the spread cell body of what was the daughter cell *B*1. By 5 h 14 min, *B*1 and *B*2 are completely rejoined into a binucleated cell spread on the glass and moving away from cell *A* to the right. Note in the last four frames that cell *B*2 seems to be adhering to cell *A* by a fine process (arrow). This is a remnant of the old retraction fiber to the left which was apparently partly adhering to the margin of cell *A*. As cell *B*2 was pulled toward the right by *B*1, the adhesion point between *B*2 and *A* persisted and eventually appeared on the upper surface of cell *A*. The bar in each Figure represents 20 μm. × 425, × 650, × 150.

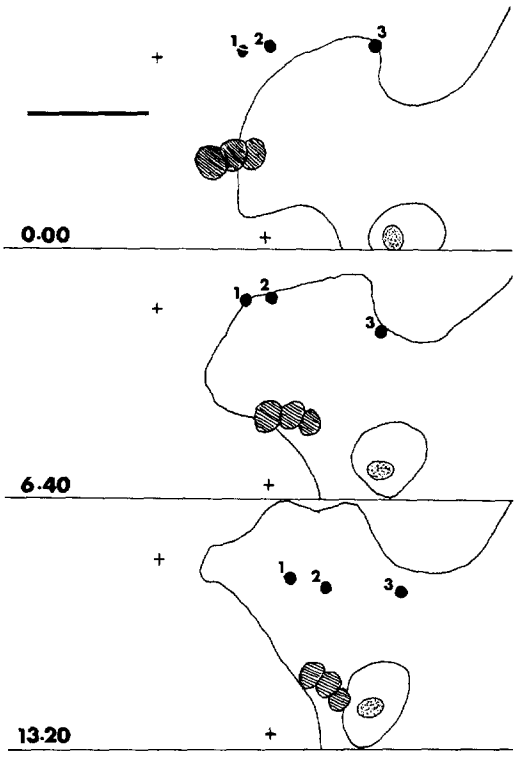

FIGURE 7 Adhesion of latex particles (black circles) and con A-treated red blood cells (hatched circles) to the fluctuating leading edge of advancing epithelial cells. Particles and red blood cells were prepared as described in Materials and Methods and added to a culture of corneal epithelial cells spreading on glass. Once the particles and red blood cells contact the leading edge, they move backward toward the nucleus of the marginal cell with respect to both the leading edge and the substratum. Small crosses are fixed reference points on the substratum. Time is in minutes and seconds. Tracings from a time-lapse film. Bar represents 20 μm. × 1650.

probable that the *formation* of adhesions occurs at the cell margins and that one cell subsequently comes to adhere to the upper surface of another cell by the centripetal migration of marginal adhesion sites onto that surface.

Microspikes—small, rigid, sticklike extensions of the lamellipodium that are actively thrust out of the cell at the leading edge—behave in much the same way as broad processes that overlap the lamellipodium of another cell (see Fig. 11).

The results of our seeding experiments are also consistent with the notion that only a free margin can initiate adhesions. Only the cells at the perimeter of epithelial sheets have free margins. The margins of all other cells are locked in lateral adhesions with neighboring cells. Seeded cells would therefore be expected to adhere only to the cells at the perimeter of the sheet and then only to their free margins. It is difficult to observe such cases, because cells initially adhering to the peripheral cells would quickly spread on the adjacent inanimate substratum. However, Fig. 12 shows a case in which a seeded cell has been detected adhering to the margin of a cell at the perimeter of a gut epithelial sheet. The suspended cell, A, contacts the leading edge of a peripheral cell and subsequently is moved back on its upper surface as a rounded cell in a manner similar to particles or cell processes. Thus it appears that seeded cells can adhere to the *free* margins of epithelial sheets, and once adhering, they may subsequently come to adhere to the upper surface of the epithelial cell.

DISCUSSION

Our observations lead us to conclude that the upper surfaces of the cultured spread cells used in this study are nonadhesive, in the sense that other cells and inanimate objects are unable to initiate adhesions with them. This conclusion is based on several lines of evidence. (*a*) Several types of fibroblasts and epithelial cells in culture do not crawl on top of one another. Any cell crisscrossing that occurs is produced by *under*lapping (see also 13, 14, and 23). (*b*) When seeded onto the upper surfaces of epithelial sheets, various trypsin-suspended cells fail to adhere to or to spread on the top of the sheets. Meanwhile, the rounded, suspended cells do adhere to each other and form floating clumps. Those cells which fall on the glass substratum adjacent to the sheets adhere to and spread on the glass. Trypsin-suspended cells also fail *to spread* on the upper surfaces of spread fibroblasts when seeded onto them. (*c*) Rounded fibroblasts which come to lie fortuitously on the upper surfaces of other fibroblasts likewise do not spread. Spreading is delayed until the rounded cells gain access to the inanimate substratum, at which time they readily spread. (*d*) Small protrusions of cells, microspikes, whole cells, latex particles, and red blood cells treated with con A do not adhere directly to the upper surfaces of spread cells. They do adhere, however, at the free (often ruffling) margins of cells and are subsequently transported centripetally on to the upper surface of the cells. At this time they are indeed adhering to the upper cell surface, but the adhesion was initiated at the cell margin.

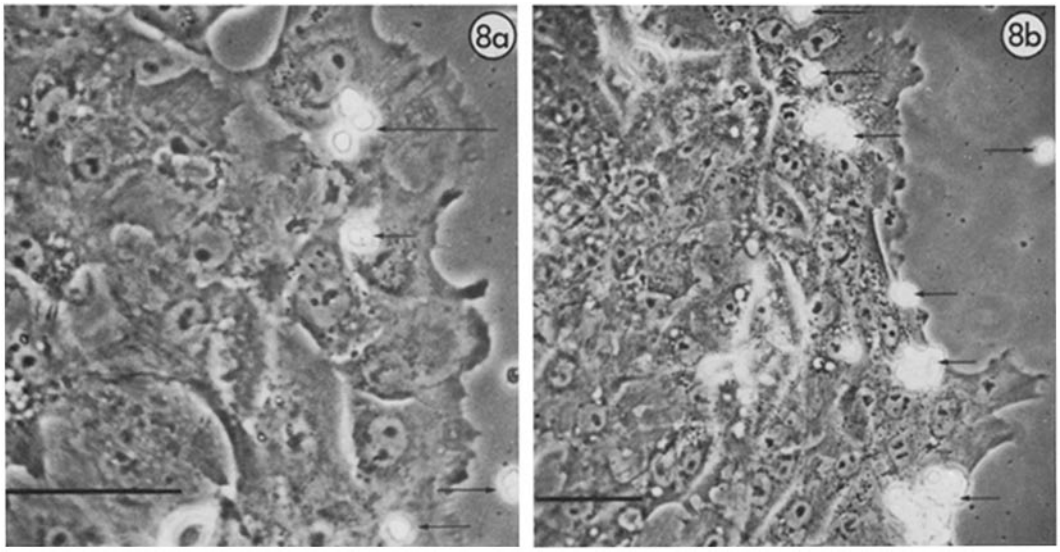

FIGURE 8 *a* Binding of con A-treated red blood cells limited to the marginal cells of an epithelial sheet. Red blood cells prepared as described in Materials and Methods were seeded into 48 h cultures of gut epithelial cells spreading on Falcon plastic. After 1 h, the cultures were rinsed with Tyrode's (37°C) twice, and normal medium was added to the dishes. Con A-treated red blood cells (arrows) were found only on the upper surfaces of the marginal cells. Time-lapse films show that red blood cells do not adhere initially to the upper surfaces of marginal cells. All red blood cells on the upper surfaces of marginal epithelial cells arrive in this position only after first contacting the leading edges. Phase contrast. Bar represents 50 μm. × 480.

FIGURE 8 *b* Same conditions as Figure 8 *a*. Arrows denote clumps of con A-treated red blood cells. Bar represents 50 μm. × 280.

In order to explain these observations it seems necessary to postulate that only the free marginal areas of spread cells are capable of initiating adhesions, either with other cells or with inanimate objects. The upper surfaces of spread cells are totally nonadhesive in the sense of being unable to initiate adhesions, but other cells and particles may come to adhere to the tops of cells by virtue of the centripetal movement of adhesion sites established first at the cell margin.

It must be emphasized, however, that we limit our conclusions only to the cell types used in this study and only to in vitro conditions. The nonadhesiveness of the upper cell surface may not apply to all cell types nor to all combinations of cells in culture. Indeed, recent evidence (P. C. Letourneau and N. K. Wessels, personal communication), suggests that nerve cells may be able to adhere to and spread on the upper surfaces of glial cells in culture. Furthermore, the cell types used in this study could conceivably migrate over one another's surfaces in vivo, particularly if some extracellular material associated with the plasma membrane can provide a suitable substratum for spreading.

Possible Mechanisms Responsible for Adhesion Differences

The centripetal movement of objects adhering to the cell surface may provide a clue to the mechanisms responsible for the differences in adhesive properties of different parts of the surface of the same cell. Carbon particles (8, 21, 24, and 25), ion exchange resins (24), and con A-treated red blood cells (20) have all been observed to adhere to and move away from the leading edge on to the upper surface of a variety of moving cells in culture. This has been interpreted as evidence that new surface material is added at the leading edge of advancing cells and that this postulated new surface moves centripetally from the edge (8, 24). Our observation would be explained if (*a*) the newly added surface material is adhesive, and (*b*) the adhesiveness of this surface material is lost or reduced as it moves backward from the cell margin, except at

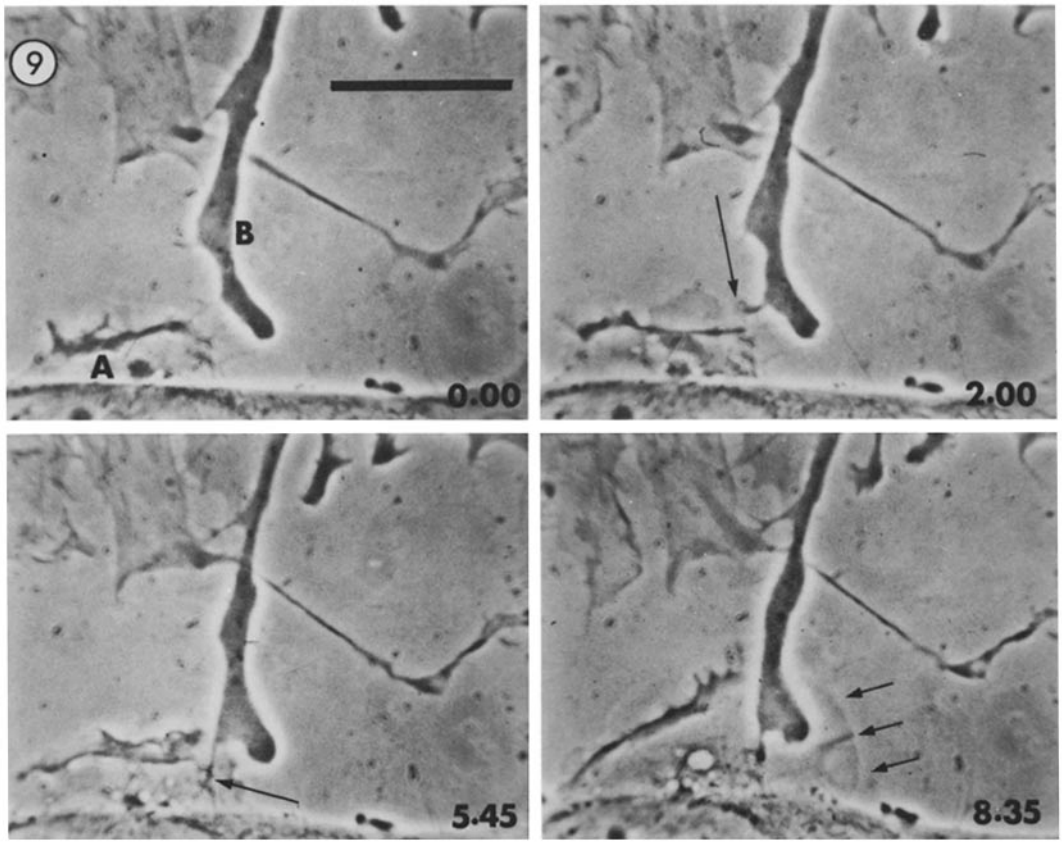

FIGURE 9 Contact event between two 3T3 cells. Single frames from a time-lapse movie. Time is in minutes and seconds. At the beginning of this sequence, the actively ruffling process of cell *A* advances toward the narrow extended process of cell *B*. At 2 min, contact between the two processes occurs over a region of about 1 μm (arrow). By 5.45, the margin of cell *A* has continued to advance over the glass substratum. The initial point of contact between cells *A* and *B* has been maintained as a point of adhesion on the upper surface of cell *A* (arrow). In addition, this point of adhesion has *moved backward* relative to the substratum over the upper surface of cell *A*. At 8.35 the tip of the process of cell *B* is still adhering to the upper surface of cell *A* (arrow), while the margin of cell *A* (arrows) continues to advance on the glass substratum, undercutting the process of cell *B*. The bar represents 10 μm. × 2400.

loci already involved in adhesions initiated at the margin.

How this loss of adhesiveness could occur is not known, but several possibilities may be suggested. The postulated newly added surface molecules might be altered by exposure to the medium. Medium components, for example, might bind to the newly inserted molecules responsible for adhesion and render them nonadhesive. On the other hand, adhesive molecules might have to be clustered to function in forming adhesions; surface molecules initially clustered when inserted at the cell margin may subsequently diffuse in the lipid bilayer and become scattered and thus nonadhesive (see reference 29). It is also possible that adhesive molecules at the margins are selectively removed from the cell surface, either being taken back into the cell, or shed into the culture medium as they flow back from the leading edge.

Unfortunately, this explanation is itself based on hypothesis rather than established fact. The backward movement of particles away from the leading edge may not necessarily result from the addition and subsequent flow of new surface at the leading edge. DePetris and Raff (18) have suggested that the adhesion of particles would cross link surface receptor molecules which in turn might induce a backward movement of only these discrete patches of cross-linked membrane molecules, accompanied by a counter-current flow of other molecules

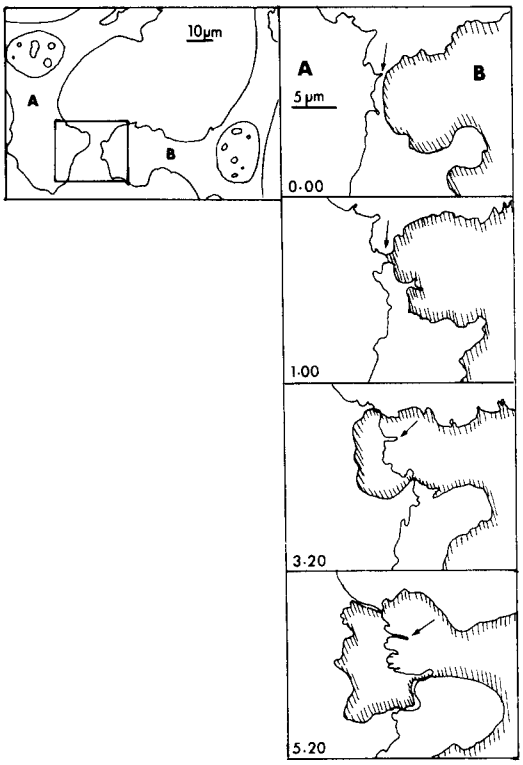

FIGURE 10 A contact event between two Py3T3 cells. 10 *a* A tracing of a low power field showing two Py3T3 cells, labeled *A* and *B*. The outlines of the cell margin, nucleus, and nucleoli are shown for each cell. The small rectangle shows the area included in the high power sequences shown in Fig. 11 *b*. Tracing from a time-lapse movie. The bar represents 10 μm. × 750. 10 *b* A sequence of tracings from a time-lapse movie showing contact between the advancing margins of two Py3T3 cells. The marginal outline of cell *B* is shaded for the sake of clarity. Time is in minutes and seconds. The bar represents 5 μm. × 2600. 0.00. Cells *A* and *B* about to contact. The margin of *B* is advancing toward cell *A*. First contact will occur where cell *A* has a point adhesion to the glass substratum (arrow). 1.00. The two cells have contacted and there seems to be an apposition of about 0.8 μm in width. 3.20. Cell *B* has extended its margin *under* the margin of cell *A*, undercutting any adhesions cell *A* may have had with the substratum in that region. There are at least three points where cell *A* is adhering to what is now the upper surface of cell *B*. One of these adhesions (arrow) seems to be the remnant of the initial adhesion between cell *A* and *B*. 5.20. Cell *B* has extended its margin further under cell *A*. More points of adhesion between the margin of cell *A* and the top of cell *B* are now apparent. The concave areas between the adhesion sites indicate that there is tension exerted away from the adhesion sites toward the body of cell *A*. In addition, the points of adhesion identifiable from the previous frame have moved to the right over the upper surface of cell *B*.

forward. Such a model, however, does not of itself explain why the margins of cells would be adhesive and the upper surfaces not.

Contact Inhibition of Movement

Despite the lack of knowledge concerning the mechanisms underlying the observed adhesive differences, our observations have important implications for understanding the social behavior of cells. The failure of various types of fibroblastic cells (see Table I) to move onto the surfaces of epithelial cell sheets could be explained by any of five hypotheses (see Introduction). The mechanical obstacle and differential adhesion hypotheses both postulate a barrier existing at the cell margin which prevents one cell from gaining access to the upper surface of another. Both hypotheses predict that seeded cells, which are not subject to such barriers, should adhere to and move freely about on the upper surfaces of the other cells. The firm adhesion and signal hypotheses would both be

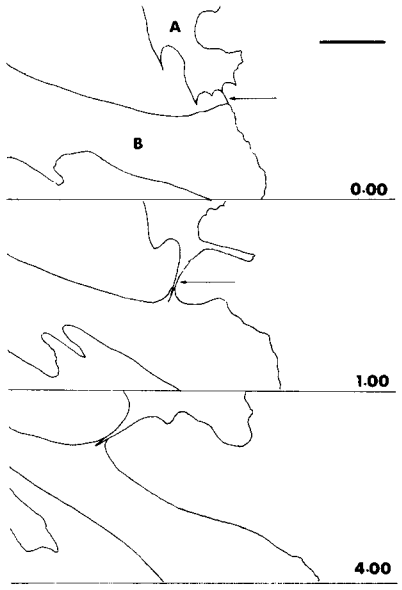

FIGURE 11 Adhesion of a microspike (arrow) of an epidermal epithelial cell to the lamellipodium of a neighboring marginal epithelial cell, *B*, at its leading edge. The microspike of cell *A* apparently adheres to the margin of cell *B*, since it seems to exert tension on the surface of cell *B*, pulling it toward cell *A*. As it moves backward with reference to both the leading edge and the substratum, the microspike does not spread over the upper surface of cell *B*. Small crosses are fixed reference points on the substratum. Time is in minutes. Bar represents 10 μm. × 2300. Tracings from a time-lapse film.

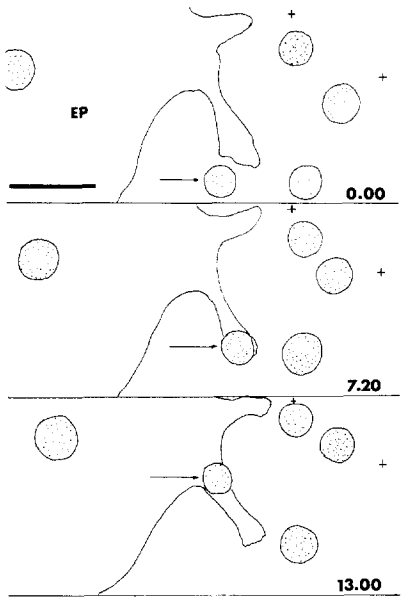

FIGURE 12 Sequential tracings from a time-lapse film of a typical seeding experiment. First frame (0.00) was taken 1 min after seeded cells were added to the dish. The epithelial sheet (EP) is gut epithelium. Stippled cells are KB cells falling onto the sheet and the Falcon plastic substratum. Note that one KB cell (arrow) near the lamellipodium of a marginal epithelial cell contacts the upper surface in the region of the leading edge (7.20). It subsequently moves directly backward away from the leading edge (13.00). Although the other suspended cells show random movement indicative of a lack of adhesion to the cellular or plastic substratum, this particular cell displays a backward, nonrandom movement, suggesting that it adheres to the upper surface just as latex particles and con A-treated red blood cells. Time is in minutes and seconds. Small crosses represent fixed points on the substratum. Bar represents 30 μm. × 1000.

consistent with the seeded cells' adhering to the upper surface of the sheet but failing to spread or migrate on it. The nonadhesive hypothesis, on the other hand, would predict that the seeded cells would neither adhere nor spread. That seeded cells without exception fail both to adhere and to spread supports the nonadhesive hypothesis of contact inhibition. In other words, cells fail to move over the surfaces of other cells because the latter do not provide suitable substrata to support adhesion and spreading.

Of course, cell contact behavior is quite complex, consisting of several separate phenomena. Inhibition of ruffling, the formation of lateral adhesions, and contact contraction have all been described as being components of contact inhibition (9, 26, 31, and 35). Although the inability of the upper cell surface to support the adhesion and spreading of other cells in culture is sufficient to account for the failure of overlap, other aspects of cell contact behavior, such as ruffling inhibition, reduction of velocity, lateral adhesion formation, crisscrossing, and contact contraction require additional explanation.

The significance of contact inhibition was accentuated by reports that cells derived from tumors or transformed by tumorigenic agents show less of this property than normal cells. Abercrombie et al. (3) observed that mouse sarcoma cells infiltrated outgrowths of normal chick heart fibroblasts and mouse muscle cells and that the nuclei of the former came to overlap the nuclei of the latter in a random distribution. They concluded that a failure of contact inhibition permitted invasive behavior. Later, Abercrombie and Ambrose (4) reported that sarcoma cells move freely over the exposed upper surfaces of fibroblasts. Likewise, Vogt and Dulbecco (33) reported that polyoma virus-transformed hamster cells crawl over one another's surfaces and thus "pile up" in culture. In light of these observations it is of interest that we have failed to detect any differences between transformed or tumor cells and "normal" cells in their ability to spread upon or crawl over other cells. Polyoma-transformed 3T3 cells and KB cells fail to spread either on the tops of epithelial sheets or on spread cells of their own types. These and MSV-transformed BALB/3T3 and sarcoma 180 tumor cells were never observed to crawl over each other in culture.

Observations consistent with ours have been reported by Barski and Belehradek (11). They found that the movement of malignant murine fibroblasts was obstructed by coherent sheets of embryonic mouse heart cells. On the other hand, these same cells were able to infiltrate areas where the heart cells were loosely arranged, and individual malignant cells were observed working their way through gaps in the heart cell network. Barski and Belehradek (12) further reported that suspended KB cells dropped on to sheets of human kidney or human amnion cells failed to spread on top of the normal cells. Only when they gained access to the substratum, either through gaps between kidney cells or by penetrating the amnion sheet, did the KB cells spread.

If, as we postulate, cells are in general incapable

of moving over each other, then the increased nuclear overlapping reported by Abercrombie et al. (3) or the crisscrossing reported by Vogt and Dulbecco (33) could be accounted for by underlapping, i.e., one cell passing between another cell and the substratum. The occurrence of underlapping has been demonstrated clearly by Boyde et al. (16), Harris (23), and Weston and Roth (35); and Bell (13, 14) has shown that the crisscrossed patterns produced by Py 3T3 cells in culture are due exclusively to underlapping. In light of all of these findings, we suggest that the ability of cell surfaces to serve as substrata for the invasive movements of malignant cells is open to question. Further investigation will be needed to settle this point, particularly with regard to invasion in vivo.

Adhesion to Cell Monolayers

A final area for which our findings have implications is the use of cell monolayers as substrata for the adhesion of suspended cells. Studies of growth inhibition have been reported which involve seeding transformed cells on to monolayers of other cells (15, 30). More recently, cell monolayers have been used in a quantitative assay for cell adhesion (34). In apparent contradiction to our observations, these workers reported that suspended cells adhered to monolayers of other cells when seeded onto them. However, considering that the cells which adhere to the monolayer remain rounded and do not spread (34), it is conceivable that, instead of adhering directly to the upper surfaces of the monolayer cells, the rounded cells established adhesions to their free margins in a manner similar to our Fig. 12. Cells in monolayers, particularly fibroblasts, would be expected to have at least parts of their margins free. In fact, chick heart fibroblasts (1) and 3T3 cells (27, 14) continue to move around as individual cells while in monolayers, and 3T3 cells in compact monolayers continue to extend ruffles up into the medium (14). Thus it is consistent with our hypothesis that cells seeded on to monolayers could adhere to the underlying cells at their free margins but would fail to spread onto the upper surfaces of these cells. Detailed examination of fibroblast monolayers by time-lapse cinemicrography during the attachment of seeded cells would allow this suggestion to be tested.

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REFERENCES

1. ABERCROMBIE, M., and J. HEAYSMAN. 1953. Observations on the social behavior of cells in tissue culture. I. Speed of movement of chick heart fibroblasts in relation to their mutual contacts. *Exp. Cell Res.* **5**:111.
2. ABERCROMBIE, M., and J. HEAYSMAN. 1954. Observations on the social behavior of cells in tissue culture. II. "Monolayering" of fibroblasts. *Exp. Cell Res.* **6**:293.
3. ABERCROMBIE, M., J. HEAYSMAN, and H. KARTHAUSER. 1957. Social behavior of cells in tissue culture. III. Mutual influence of sarcoma cells and fibroblasts. *Exp. Cell Res.* **13**:276.
4. ABERCROMBIE, M., and E. AMBROSE. 1958. Interference microscope studies of cell contacts in tissue culture. *Exp. Cell Res.* **15**:332.
5. ABERCROMBIE, M., M. LAMONT, and E. STEPHENSON. 1968. The monolayering in tissue culture of fibroblasts from different sources. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **70**:349.
6. ABERCROMBIE, M., and C. MIDDLETON. 1968. Epithelial-mesenchymal interactions affecting locomotion of cells in culture. *In* Epithelial-Mesenchymal Interactions. R. Fleischmajer, and R. Billingham, editors. The Williams & Wilkins Company, Baltimore, Md. 56.
7. ABERCROMBIE, M., J. HEAYSMAN, and S. PEGRUM, 1970 *a*. Locomotion of fibroblasts in culture. I. Movements of the leading edge. *Exp. Cell Res.* **59**:393.
8. ABERCROMBIE, M., J. HEAYSMAN, and S. PEGRUM. 1970 *b*. Locomotion of fibroblasts in culture. III. Movements of particles on the dorsal surface of the leading lamella. *Exp. Cell Res.* **62**:389.
9. ABERCROMBIE, M. 1970 *c*. Contact inhibition in tissue culture. *In Vitro.* **6**:128.
10. ABERCROMBIE, M., J. HEAYSMAN, and S. PEGRUM. 1972. Locomotion of fibroblasts in culture. IV.

Surface marking with concanavalin A. *Exp. Cell Res.* **73**:536.

11. BARSKI, G., and J. BELEHRADEK. 1965. Étude microcinematographique du mechanisme d'invasion cancéreuse en cultures de tissue normal associé aux cellules malignes. *Exp. Cell Res.* **37**:464.
12. BARSKI, G., and J. BELEHRADEK. 1968. *In vitro* studies on tumor invasion. *In* The Proliferation and Spread of Neoplastic Cells. The Williams & Wilkins Company, Baltimore, Md. 511.
13. BELL, P. B. 1972. Criss-crossing, contact inhibition, and cell movement in cultures of normal and transformed 3T3 cells. *J. Cell. Biol.* **55**:16 *a*.
14. BELL, P. B. 1974. Movement, contact behavior, and morphology of 3T3 and polyoma virus-transformed 3T3 cells in tissue culture: a comparative study. Ph.D. Thesis, Yale University, New Haven.
15. BOREK, C., and L. SACHS. 1966. The difference in contact inhibition of cell replication between normal cells and cells transformed by different carcinogens. *Proc. Natl. Acad. Sci. U. S. A.* **56**:1705.
16. BOYDE, A., F. GRAINGER, and D. JAMES. 1969. Scanning electron microscope observations of chick embryo fibroblasts *in vitro*, with particular reference to the movement of cells under others. *Z. Zellforsch. Mikrosk. Anat.* **94**:46.
17. CARTER, S. B. 1965. Principles of cell motility: the direction of cell movement and cancer invasion. *Nature (Lond.).* **208**:1183.
18. DEPETRIS, S., and M. RAFF. 1973. Fluidity of the plasma membrane and its implications for cell movement. *In* Locomotion of Tissue Cells. Ciba Foundation Symposium No. 14. Elsevier N. V. Uitgevers Mij., Amsterdam. 27.
19. DIPASQUALE, A., and P. BELL. 1972. The cell surface and contact inhibition of movement. *J. Cell Biol.* **55**:60 *a*.
20. DIPASQUALE, A. 1973. An analysis of the contact behavior and locomotion of epithelial cells in culture. Ph.D. Thesis. Yale University, New Haven.
21. HARRIS, A. K., and G. DUNN. 1972. Centripetal transport of attached particles on both surfaces of moving fibroblasts. *Exp. Cell Res.* **73**:519.
22. HARRIS, A. K. 1973 *a*. Behavior of cultured cells on substrata of variable adhesiveness. *Exp. Cell Res.* **77**:285.
23. HARRIS, A. K. 1973 *b*. *Dev. Biol.* In press.
24. HARRIS, A. K. 1973 *c*. Cell surface movements related to locomotion. *In* Locomotion of Tissue Cells. Ciba Foundation Sympsoium No. 14.d Elsevier N. V. Uitgevers Mij, Amsterdam, 3.
25. INGRAM. V. 1969. A side view of moving fibroblasts. *Nature (Lond.).* **222**:641.
26. MARTZ, E., and M. S. STEINBERG. 1973. Contact inhibition of what? An analytical review. *J. Cell Physiol.* **81**:25.
27. MARTZ, E. 1973. Contact inhibition of speed in 3T3 and its independence from post confluence inhibition of cell division. *J. Cell Physiol.* **81**:39.
28. SANTESSON, L. 1935. Characteristics of epithelial mouse tumor cells *in vitro* and tumor strains *in vivo*. *Acta Pathol. Microbiol. Scand.* (Suppl.) **24**:1.
29. SINGER, S., and G. NICOLSON. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Wash. D. C.).* **175**:720.
30. STOKER, M., M. SCHEARER, and C. O'NEILL. 1966. Growth inhibition of polyoma-transformed cells by contact with static normal fibroblasts. *J. Cell Sci.* **1**:297.
31. TRINKAUS, J. P., T. BETCHAKU, and L. KRULIKOWSKI. 1971. Local inhibition of ruffling during contact inhibition of cell movement. *Exp. Cell Res.* **64**:291.
32. VAUGHAN, R., and J. P. TRINKAUS. 1966. Movements of epithelial sheets *in vitro*. *J. Cell Sci.* **1**:407.
33. VOGT, M., and R. DULBECCO. 1962. Properties of cells transformed by polyoma virus. *Cold Spring Harbor Symp. Quant. Biol.* **27**:367.
34. WALTHER, B., R. OHMAN, and S. ROSEMAN. 1973. A quantitative assay for intercellular adhesion. *Proc. Natl. Acad. Sci. U. S. A.* **70**:1569.
35. WESTON, J. A., and S. A. ROTH. 1969. Contact inhibition: behavioral manifestations of cellular adhesive properties *in vitro*. *In* Cellular Recognition. R. T. Smith, and R. A. Good, editors. Appleton-Century-Crofts Inc., New York. 29.
36. WILBANKS, G., and R. RICHART. 1966. The *in vitro* interaction of intraepithelial neoplasia, normal epithelium and fibroblasts from the adult human uterine cervix. *Cancer Res.* **26**:1641.