IN VITRO
16(6):461-468; 1980

EFFECT OF CELL-SUBSTRATUM INTERACTION ON HEMICYST FORMATION BY MDCK CELLS

CARLOS A. RABITO,[1] RUY TCHAO, JOHN VALENTICH, AND JOSEPH LEIGHTON

Department of Pathology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

(Received June 22, 1979; accepted October 19, 1979)

SUMMARY

On impermeable substrata MDCK cells, a cell line derived from normal dog kidney, forms a confluent monolayer that is studded with numerous hemicysts. Previous studies with this cell line suggest that these hemicysts develop as a result of active fluid accumulation between cell sheet and substratum. However, the formation of hemicysts as a multifocal phenomenon is still unexplained. The results presented here show that the hemicysts are not only expressions of active transport of solutes and water, but also of cell-substratum interaction. The increase in number and size of the hemicyst produced by dbcAMP may be explained by a decrease in the adhesive strength to substrata produced by this compound. Moreover, when the strength of the cell-substratum adhesion was increased the number of hemicysts was reduced or abolished. On the contrary, when this strength was reduced, larger hemicysts occurred, covering practically all the area available for growth. Results from cinematographic time lapse studies, showing that 90% of the area of the monolayer is able to produce hemicysts, also suggest that hemicyst formation as a multifocal phenomenon is more an expression of local variations in cell-substratum interaction than of regional changes in transepithelial active transport.

Key words: MDCK cells; cell-substratum interaction; hemicyst formation; transepithelial active transport; dbcAMP.

INTRODUCTION

The MDCK cell line is an epithelial cell line derived from normal dog kidney (1). On solid and impermeable support these cells grow as a confluent monolayer that is studded with numerous "hemicyts" also termed "domes" or "blisters" (2). Since a variety of epithelial cell cultures have also been found to produce multicellular hemicysts (3-10) the formation of hemicysts seems to be a common phenomenon among epithelial cells in culture. Previous studies indicated that hemicyst formation is associated with active transport of solutes and water, resulting in a local accumulation of fluid between the cell sheet and the substratum (11).

MDCK cells grown on a permeable support provide a cell culture model that exhibits several characteristics of in vivo epithelial membranes (12-14). The simplicity of the model and the possibility to reverse from isolated cells to a complete monolayer makes it an ideal tool for studying several problems of the physiology and cell biology of epithelial membranes.

Since the presence of hemicysts on an impermeable support is an expression of active transport of solutes and water, their scattered appearance in a monolayer introduces some uncertainty of the homogeneity of the transport properties of this monolayer. Some factors that may determine why hemicysts appear only in localized sites of the monolayers are: interruption of zonula occludens in areas without hemicysts, local variations in the active transepithelial transport, differences in adhesion between cells and substratum, or differences in cell density. Previous studies have shown

[1]To whom all correspondence should be addressed at: Department of Medicine, Renal Biophysics Laboratory, Massachusetts General Hospital, Boston, MA 02114.

that the zonula occludens encircling MDCK cells can provide an effective permeability barrier with characteristics similar to those of a leaky epithelium (12,13,15). We also have shown that these zonula occludens have the same continuity and properties in hemicyst and nonhemicyst areas (15). In spite of 300% stimulation in the number of hemicysts produced by dibutyryl cyclic AMP (dbcAMP), the specific binding of [^{3}H]ouabain is the same in control and dbcAMP-treated cultures (14). The correlation found between [^{3}H]ouabain binding and transepithelial active transport (16), together with the inhibition of the active transport of solutes and water produced by cyclic AMP (cAMP) in leaky epithelia (17–20), suggest that factors other than the variations in the active transepithelial transport would be involved in the localized hemicyst formation. In this paper we describe the formation and abolition of hemicysts by altering the interaction between MDCK cells and the solid substratum.

Materials and Methods

Maintenance of cell line. MDCK cells were maintained by serial passage in stoppered 32-oz prescription bottles at 36° C. The cultures were fed with Eagle's minimal essential medium (Microbiological Associates, Bethesda, MD). The medium was supplemented with 15% bovine serum, *L*-glutamine (2 m*M*), penicillin (100 U/ml) and streptomycin (50 μg/ml). When cell growth reached saturation density, subcultures were prepared using 0.02% EDTA:0.05% trypsin (GIBCO, Grand Island, NY).

Preparation of hemicysts and monolayer. Cultures of hemicysts and monolayer were prepared in large Leighton tubes containing a sterile standard glass microscope slide. Each tube was inoculated with 10 ml of cell suspension of approximately 10^6 cells/ml to reach a cell density of 5×10^5 cells/cm^2. The cultures were fed every 2 or 3 days with 10 ml of medium during 10 days prior to the electrical measurements.

Monolayers on a permeable support were prepared using a polycarbonate filter membrane with 5-μm pore size and 25-mm diameter (Nucleopore Corp., Pleasanton, CA), both faces of which had been covered with a very thin film of 1% collagen dispersion (Ethicon, Somerville, NJ) and applied to a glass slide. The methods for collagen aggregation into native bundles and sterilization of the collagen-coated membrane are described elsewhere (15).

Solutions. Hanks' solution was used for dilution potential determination. The standard Hanks' solution has the following composition (m*M*): 136.8 NaCl, 5.63 KCl, 1.26 $CaCl_2$, 0.49 $MgCl_2$, 0.45 $MgSO_4$, 4.16 $NaHCO_3$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 5.5 glucose, pH 7.42. Isosmotic dilutions were prepared by mixing equal volumes of Hanks' complete solution and Hanks' solution in which all of the NaCl was substituted isosmotically by sucrose. Bi-ionic potentials were measured by replacing 136.8 m*M* NaCl with 136.8 KCl, LiCl, RbCl, CsCl, or choline Cl. All these solutions were supplemented with 15% bovine serum of known electrolyte composition. The final ionic concentration was calculated taking into account the serum contribution. All solutions were prepared from reagent grade chemicals. The electrolyte composition was checked using simultaneous multiple analyzer model SMA-6 (Technicon Instruments Corp., Tarryton, NY).

Electrical measurements. The collagen-coated filter with the monolayer was removed from the culture tube and mounted as a flat sheet between two Lucite half chambers with a window area of 3.14 cm^2. Transepithelial potential differences were measured as described before (15) using an Orion model 701 pH meter. The current was measured with a CSC microammeter model 320 G (Triplett Corp., Bluffton, OH) and was conducted by AG/AgCl electrodes on opposite sides of the membrane and at the rear of the chamber. All experiments were performed at 25° C. The inside solution was Hanks' complete solution. To obtain the dilution or bi-ionic potentials, changes in the composition were made in the outside solution even though the same numerical values were obtained by changes in the inside solution. For conductance measurements, an outside solution identical in composition to the inside solution was used. The potential difference values ($\Delta\psi$) were obtained every 30 s until a steady or quasi-steady state was achieved.

Changes in cell-substratum interaction. To study the role of the adhesive strength in different cellular functions, two methods have been commonly used: by modification of substratum adhesiveness (21) or by isolation of cell attachment mutant (22). We have used the first method in our study.

To increase the adhesive strength, MDCK cells were grown on glass cover slips, half of which had been treated with glutaraldehyde-activated 3-aminopropyltriethoxysilane and the other half

used as control. This compound is a protein-linking reagent and has been demonstrated to increase cell-glass adhesion without change in the cell viability (23). On the other hand, the strength of the cellular adhesion to the substratum was reduced by growing MDCK cells on a standard microscope slide whose surface had been coated with a very thin film of collagen. Using a spreader, a 0.30-mm-thick collagen layer was deposited on a microscope slide from 1% collagen dispersion (Ethicon). Collagen was precipitated and aggregated into native bundles as described before (15).

Measurement of the adhesive strength. The assay in this study using silicone-coated substratum is a modification of the procedure of Walther et al. (24). Glass liquid scintillation vials were treated for 5 min with Dricote (Fisher Scientific Co., Pittsburgh, PA). The vials were then rinsed in distilled water and dried overnight in an oven at 100° C. MDCK cells were labelled by the addition of [^{3}H]thymidine (0.2 μCi/ml) to growth medium for 48 hr prior to the experiment. The labeled cells were trypsinized and maintained in suspension overnight in spinner medium. At the start of the experiments, the cells were resuspended in Eagle's complete medium at approximately 5×10^4 cells/ml and a 3-ml aliquot was added to Si-treated scintillation vials that were centrifuged at 600 $\times g$ for 1 min. An aliquot of the cell suspension was taken for determining the total cellular radioactivity. At intervals thereafter, sets of vials were removed and the medium was aspirated to remove nonadherent cells. The adhered cells were removed mechanically with a jet of Hanks' solution. The radioactivity of the remaining cells was determined by the addition of scintillation fluid (Amersham-Searle Corp., Arlington Heights, IL) and measured in a liquid scintillation counter (Intertechnique model SL30). The data were expressed as a percent of total cells adhering during a given time interval.

Time lapse studies. Cultures were grown in Falcon flasks (25 cm^2) or on glass slides in large Leighton tubes. The equipment used was a Zeiss inverted microscope connected to a stage instrument.

Electron microscopy. For electron microscopy, cultures were fixed with 2.5 glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.4) followed by postfixation with cacodylate-buffered 1% OsO_4. Cultures were dehydrated in graded ethanols, treated with propylene oxide, and embedded in Epon 812. For light microscopy, thick sections were stained with Toluidine Blue. Thin sections cut perpendicular to the plane of the monolayer were stained with uranyl acetate and Reynold's lead citrate and examined at 60 to 80 kv with a Zeiss RM 10A electron microscope.

RESULTS

Effects of dbcAMP on the permeability properties of MDCK monolayer. We have shown previously that dbcAMP increased the number and size of hemicysts. On the other hand, studies using different epithelia showed that cAMP has an inhibitory effect on the active reabsorption of solutes and water in "leaky" epithelia (17-20). One possible explanation of these apparent contradictory observations is that even though there is a reduction in the active influx, there is also an increase in the "tightness" of the zonula occludens. This increase brought about by dbcAMP would improve the efficiency of the active pathway by reducing the backflux through the passive pathway. This possibility makes the study of the effect of dbcAMP on the permeability properties of an MDCK monolayer highly pertinent. The results shown in Table 1 indicate that dbcAMP (2 m*M*) does not modify the dilution

TABLE 1

EFFECT OF dbcAMP ON THE FUNCTIONAL PROPERTIES OF ZONULAE OCCLUDENS IN MDCK MONOLAYER

Cation	Dilution Potential		Bio-ionic Potential	
	Control	dbcAMP	Control	dbcAMP
	mV		*mV*	
K^+			+1.60 ± 0.26 (14)	+1.28 ± 0.17 (11)
Na^+	−12.9 ± 0.30 (14)[a]	−12.7 ± 0.23 (11)		
Li^+			−3.59 ± 0.26 (14)	−3.37 ± 0.16 (11)
Rb^+			−4.95 ± 0.27 (14)	−5.04 ± 0.21 (11)
Cs^+			−9.28 ± 0.35 (14)	−9.14 ± 0.28 (11)
$Choline^+$			−23.1 ± 0.52 (14)	−26.2 ± 0.56 (11)

[a] Results are given as mean ± standard error (number of observations). The sign of the difference potential correspond to the collagen side.

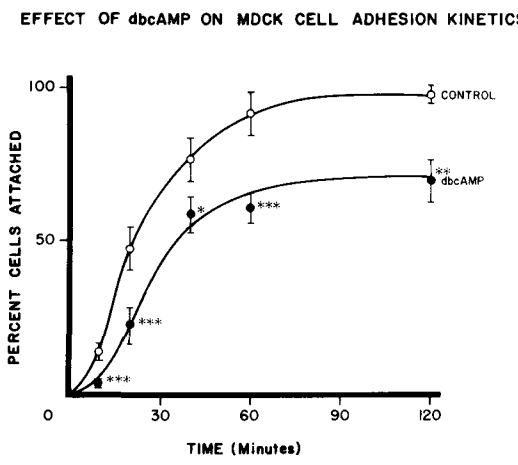

FIG. 1. [^{3}H]thymidine-labeled MDCK cells in Eagle's minimal essential medium were pretreated in suspension with 2 m*M* dbcAMP for 30 min. At the start of the experiment a 3-ml aliquot of the control and dbcAMP-treated cell suspension was added to Dricote-treated scintillation vials. At time zero the vials were centrifuged at 600 ×*g* for 1 min. The percent of adherent cells was determined after wash by measurement of the radioactivity that became bound to the vials compared with the radioactivity of a 3-ml aliquot added to the vials. Each point represents the mean of six determinations ± SE. ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.2$.

potential for NaCl. The same is also true for the bi-ionic potential obtained with other alkali cations. The bi-ionic potential obtained with choline+ in control and dbcAMP conditions are also similar and very close to the equilibrium potential calculated from the imposed concentration gradients. However, the electrical resistance in dbcAMP-treated monolayer is 159 ± 1.09 Ω cm^2, slightly less than the value 172 ± 1.32 Ω cm^2 obtained in control condition ($P < 0.01$).

Effect of dbcAMP on MDCK cell-substratum interaction. Since dbcAMP did not alter the electrical properties of a monolayer of MDCK, we examined the possibility that dbcAMP could affect the detachment of cells from the substratum. Figure 1 shows the results of an experiment on the effect of dbcAMP treatment in cell-glass interaction. The number of radiolabeled MDCK cells that remain attached after their treatment in suspension with 2 m*M* dbcAMP for 30 min is smaller than the number of cells in control condition. After 2 hr of incubation, 100% of cells in control condition and only 70% of dbcAMP-treated cells remain attached to the substratum following their mechanical removal with a jet of Hanks' solution ($P<0.01$).

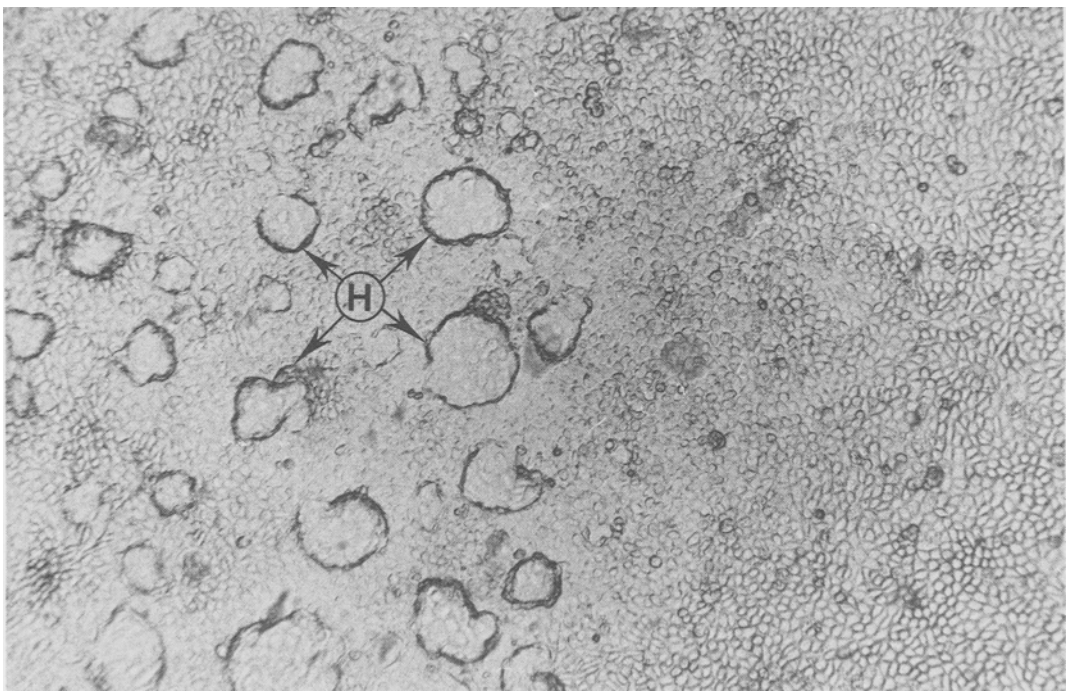

FIG. 2. MDCK monolayer grown on glass cover slips, half of which (*right side* of the figure) had been treated with 3-aminopropyltriethoxysilane, and the other half (*left side* of the figure) used as control. Notice the presence of hemicyst (H) in the control but not in the treated side. Phase contrast. ×36.

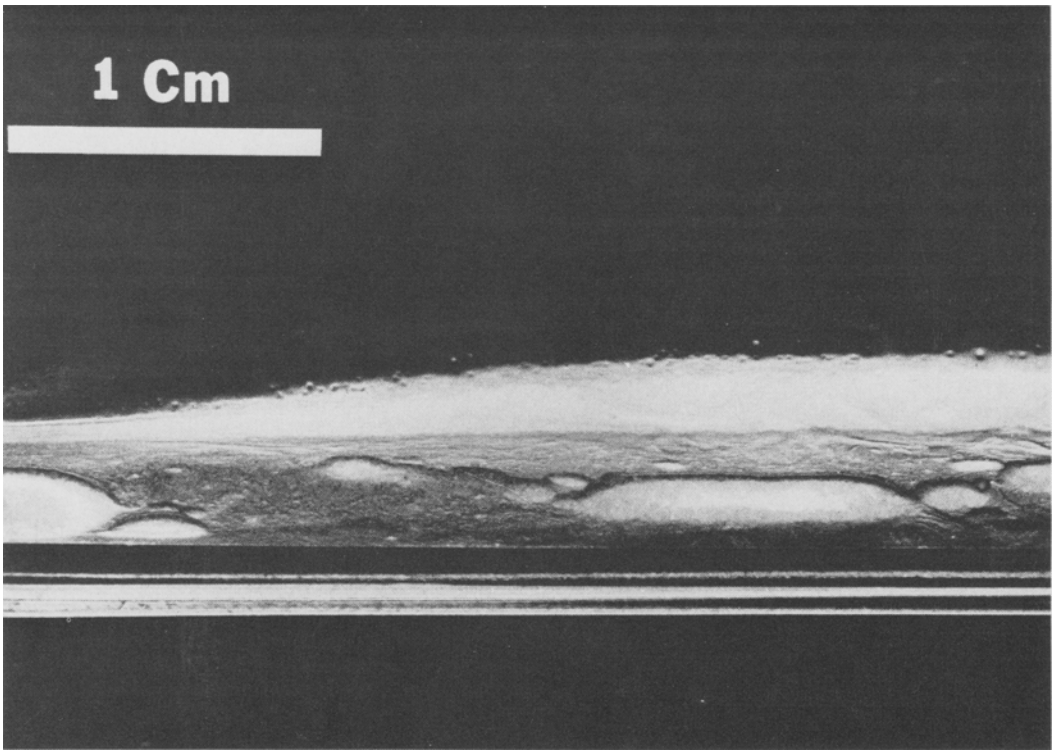

FIG. 3. Lateral view of a MDCK monolayer grown on a collagen-coated glass microscope slide for 10 days. Notice the large size of the hemicysts compared with those of Fig. 2.

Effects of altering cell-substratum interaction on hemicyst formation. In order to confirm the role of the cell-substratum interaction in the localized hemicyst formation, MDCK cells were grown on glass cover slips, half of which had been treated with 3-aminopropyltriethoxysilane and the other half used as control. This is a compound that increases the strength of the cell-glass adhesion (23). MDCK cells grew on the treated glass surface as they did on the control. By 3 days after plating, the cells had formed a confluent monolayer, and the first hemicysts began to appear on the control side. Ten days after plating, there were numerous hemicysts on the uncoated glass. However, on the treated glass, hemicyst formation was much reduced or totally abolished (Fig. 2). No difference in the cell morphology and cell density, as counted by the number of nuclei per unit area in stained preparation, were found between the cultures on the treated and the uncoated glass.

As a counterpart of this experiment, we reduced the strength of the cellular adhesion to the glass substratum by growing MDCK cells on a standard microscope slide whose surface had been coated with a very thin film of collagen. The rationale behind this experiment is that if cells adhere strongly to collagen layer (25), and that

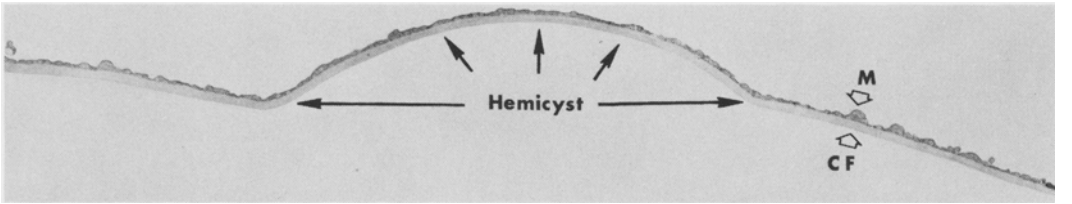

FIG. 4. A thick-section cut through a big hemicyst shows in Fig. 3. The ensemble formed by MDCK monolayer (M) and the collagen film (CF) was detached as a unit at the hemicyst level indicating that the surface of cleavage occurs between the collagen film and glass. Toluidine Blue stain. ×50.

collagen-glass adhesion is weaker than cell-collagen adhesion, they should detach together from the glass by hydrostatic pressure developed from the transported fluid. Figure 3 shows that, as we expected, MDCK cells grown in this condition form very large hemicysts. The area covered by these cysts was in excess of 500 times the area covered by control cysts. The size of these big hemicysts increased with the time after plating. At the end of the 2nd week, they had grown to

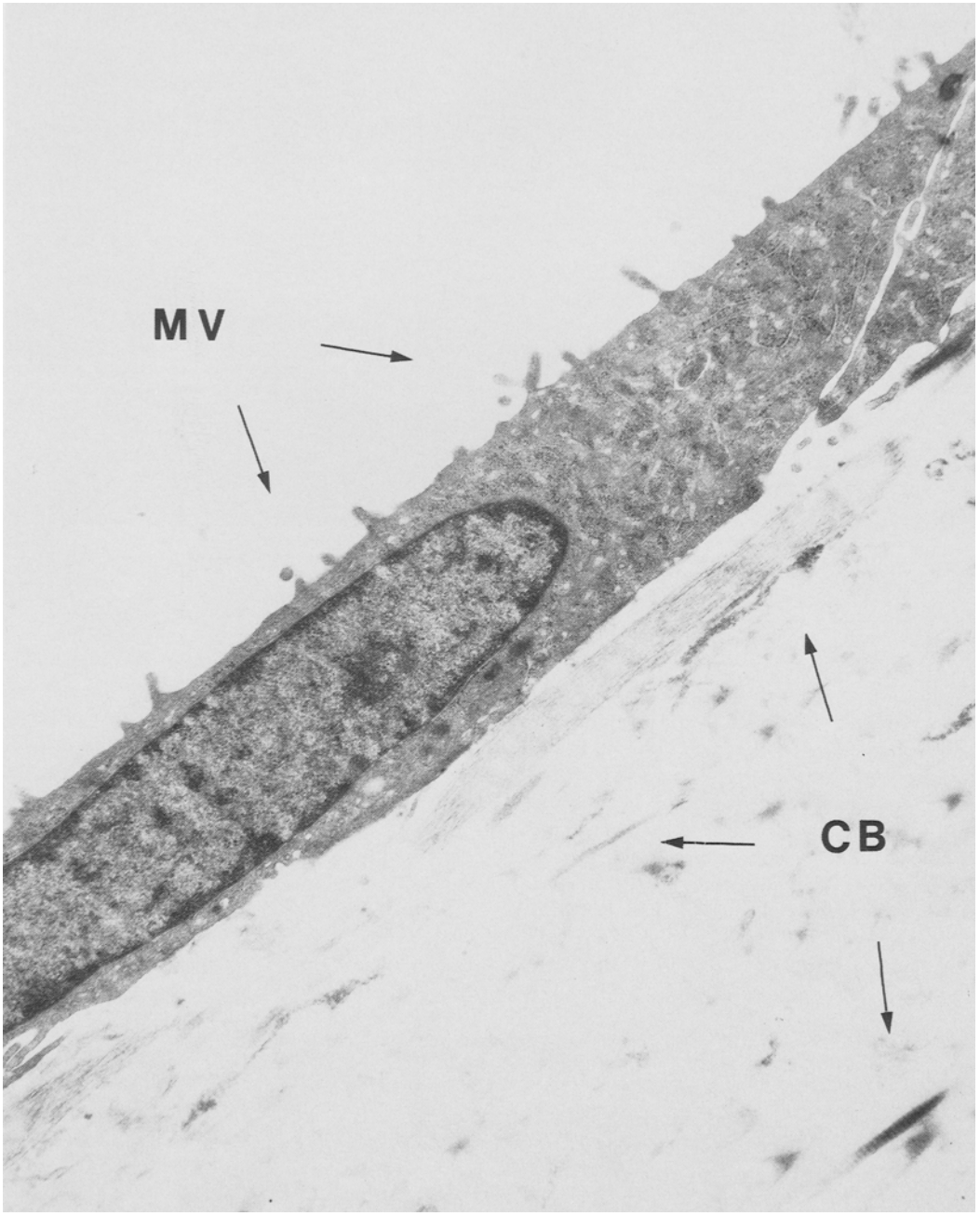

FIG. 5. Electron micrograph of hemicyst's roof shows in Fig. 3. The outer surface of MDCK cell is covered with microvilli (MV). No separation between cell sheet and collagen film was observed. The collagen bundles (CB) show the typical lateral periodicity. ×7700.

reach a diameter similar to the width of the microscope slide (2.5 cm). When they reached the slide border they collapsed, probably due to escape of the contents of the hemicyst. Figure 4 shows the ensemble formed by the MDCK monolayer and the collagen film, which detached as a result of hemicyst formation. The cleavage surface occurs between the glass and the collagen film. Figure 5 shows in more detail the roof of a hemicyst. No separation between cell sheet and collagen film was observed.

Time lapse studies and analysis. In order to determine if hemicyst formation was restricted to certain cells and areas of the culture, we examined the cultures under time lapse cinematography over several days. The film was then projected on to a piece of paper and hemicyst formation was traced. The area of the frame was taken as 100%. Over a period of 3 days, more than 90% of the filmed area had hemicysts at one time or another. This result indicated that all the cells in the culture were capable of forming hemicysts on all of the substratum surface.

Discussion

Our results indicate that passive permeability of the monolayer is not decreased by exposure to 2 m*M* dbcAMP; on the contrary, they suggest that if anything, the transepithelial conductance, which is largely attributable to the shunt pathway, is slightly increased. These results discard the possibility that the increase in the number and size of the hemicysts produced by dbcAMP could be due to an improvement in the efficiency of the active transport by a reduction in the backflux through the passive pathway. Studies using [^{3}H]ouabain also showed that dbcAMP did not increase ouabain binding to (Na^+-K^+) adenosine triphosphatase (14).

The results presented here confirm the idea that a hemicyst is not only an expression of active transport of solutes and water, but also of cell-substratum interaction. Our results showed that after dbcAMP treatment, the cell-substratum interaction is altered. The number of cells that remain attached following their mechanical removal with a jet of Hanks' solution is smaller in dbcAMP treated than in control cultures. This effect of dbcAMP can be the result of an increase in the cell detachment or a reduction in the number of cells attached to the substratum. Results obtained using other cell lines however indicate that cAMP does not affect the cell attachment to the substratum (26). Previous experiments using trypsin or EDTA showed that dbcAMP did not change the detachability of MDCK cells from glass or plastic (27). Enzyme treatment however does not measure the strength necessary to detach the cell from the substratum. Since an absolute measurement of this strength is not obtainable at present, our results suggest that dbcAMP could reduce it, thus allowing more and large hemicysts to form as a result of hydrostatic pressure developed between the cell sheet and the substratum.

The hemicyst shape is very close to a hemisphere, so the tension in the hemicyst's wall is directly proportional to the radius and to the hydrostatic pressure inside the hemicyst. In control conditions, i.e., bare glass surface, the maximum size of the hemicyst will reflect the maximum tension that this wall can support (28). The hydrostatic pressure due to the actively transported fluid inside the hemicyst is maintained at a constant value. The tension on the hemicyst wall, however, increases with the increase in the radius of the hemicyst. When this increase surpasses the maximum tension that the wall can support, the hemicyst bursts and promptly collapses. This explains why hemicysts in cultures of mouse mammary tumors reach an approximately constant maximum diameter (29), which we also confirm in cultures of MDCK cells (C. A. Rabito, *unpublished observation*). When cell-substratum adhesion is increased no hemicyst, or a low number of hemicysts, occurs (Fig. 2), because the hydrostatic pressure developed by the actively transported fluid is not enough to overcome the cell-substratum adhesion. On the contrary, if we reduce this adhesion, the hydrostatic pressure necessary to detach the cell sheet will be lower than in the control condition. This explains the observation that when we reduce the cell substratum adhesion a hemicyst reaches an area in excess of 500 times the area covered by a control hemicyst (Fig. 3). We have previously shown that a thin film of collagen does not modify the electrical properties of a MDCK cell sheet. Since cell-to-collagen adhesion is very strong, for practical purposes of this study, this collagen film can be regarded as a component of the cell membrane.

The evidence presented here suggests that hemicyst formation on solid substratum depends very much on the strength of cell adhesion. The multifocal phenomenon is an expression of local variation in the cell-substratum adhesion at any particular instant, rather than regional changes in

transepithelial active transport. Results from time lapse cinematographic study also confirm this idea in that more than 90% of the area of the monolayer is capable of producing hemicysts.

REFERENCES

1. Madin, S.; Darby, N. B. American Type Culture Collection. Catalog of strains. 2: 47.
2. Leighton, H.; Brada, Z.; Estes, L. W.; Justh, G. Secretory activity and oncogenicity of a cell line (MDCK) derived from canine kidney. Science 163: 472-473; 1969.
3. Auersperg, N. Histogenic behavior of tumors. I. Morphologic variation in vitro and in vivo of two related human carcinoma cell lines. J. Natl. Cancer Inst. 43: 151-153; 1969.
4. Enami, J.; Nandi, S.; Haslam, S. Production of three-dimensional structures — "domes" — from dissociated mammary epithelial cells of mice (abstr.). In Vitro 8: 405; 1973.
5. Lissitzky, S.; Fayet, G.; Giraud, A. Thyrotropin-induced aggregation and reorganization into follicles of isolated porcine thyroid cells. I. Mechanism of action thyrotropin and metabolic properties. Eur. J. Biochem. 24: 88-89; 1971.
6. McGrath, C. M. Replication of mammary tumor virus in tumor cell cultures: Dependence on hormone-induced cellular organization. J. Natl. Cancer Inst. 47: 455-467; 1971.
7. Owens, R. B.; Smith, H. S.; Hackett, A. J. Epithelial cell cultures from normal glandular tissue of mice. J. Natl. Cancer Inst. 53: 261-269; 1974.
8. Sherman, M. I. The culture of cells derived from mouse blastocysts. Cell 5: 343-349; 1975.
9. Smith, R. E.; Ellis, L. E.; Hull, R. N. Light, scanning, and transmission electron microscopy of "dome-like" structure in a continuous pig kidney cell strain. J. Cell Biol. 63: 322a; 1974.
10. Soule, H. D.; Vasquez, J.; Long, A.; Albert, S.; Brennan, M. A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst. 51: 1409-1416; 1973.
11. Leighton, J.; Estes, L. W.; Mansukhani, S.; Brada, A. A cell line derived from normal dog kidney (MDCK) exhibiting qualities of papillary adenocarcinoma and renal tubular epithelium. Cancer 26: 1022-1028; 1970.
12. Cereijido, M.; Robbins, E. S.; Dolan, W. J.; Rotunno, C. A.; Sabatini, D. D. Polarized monolayers formed by epithelial cells on a permeable and translucent support. J. Cell Biol. 77: 853-880; 1978.
13. Misfeldt, D. S.; Hamamoto, S. T.; Pitelka, D. R. Transepithelial transport in cell culture. Proc. Natl. Acad. Sci. U.S.A. 73: 1212-1216; 1976.
14. Rabito, C. A.; Tchao, R.; Valentich, J.; Leighton, J. A tissue culture model to study transport functions in epithelial membranes (abstr.). J. Gen. Physiol. 70: 15a; 1977.
15. Rabito, C. A.; Tchao, T.; Valentich, J.; Leighton, J. Distribution and characteristics of the occluding junctions in a monolayer of a cell line (MDCK) derived from canine kidney. J. Membr. Biol. 43: 351-365; 1978.
16. Cala, P. M.; Cogswell, N.; Mandel, L. J. Binding of [^{3}H]ouabain to split frog skin. The role of the Na, K-ATPase in the generation of short circuit current. J. Gen. Physiol. 71: 347-367; 1978.
17. Agus, Z. S.; Gardner, L. B.; Beck, L. H.; Goldberg, M. Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium, and phosphate. Am. J. Physiol. 224: 1143-1148; 1973.
18. Gekle, D. Der Einflu von Parathormon auf die Nierenfuktion. I. Tierexperimentalle Untersuchungen. Pfluegers Arch. 323: 96-120; 1971.
19. Kuntziger, H.; Amiel, C.; Roinel, N.; Morel, F. Effects of parathyroidectomy and cyclic AMP on renal transport of phosphate, calcium, and magnesium. Am. J. Physiol. 227: 905-911; 1974.
20. Schultz, S. G.; Frizzell, R. A.; Nellans, H. N. Ion transport by mammalian small intestine. Annu. Rev. Physiol. 36: 51-91; 1974.
21. Folkman, J.; Moscona, A. Role of cell shape in growth control. Nature 273: 349; 1978.
22. Kleve, R. J.; Rosenberger, P. G.; Naylor, S. L.; Burns, R. L.; Novak, R. Cell attachment to collagen. Isolation of a cell attachment mutant. Exp. Cell Res. 104: 119-125; 1977.
23. Gottlieb, D. I.; Glaser, L. A novel assay of neuronal cell adhesion. Biochem. Biophys. Res. Commun. 63: 815-821; 1975.
24. Walther, B. T.; Ohman, P.; Roseman, S. A quantitative assay for intracellular adhesion. Proc. Natl. Acad. Sci. U.S.A. 70: 1569-1573; 1973.
25. Elsdale, T.; Bard, J. Collagen substrata for studies on cell behavior. J. Cell. Biol. 54: 626-637; 1972.
26. Grinnell, F.; Milam, M.; Srere, P. A. Cyclic AMP does not affect the rate at which cells attach to a substratum. Nature New Biol. 241: 82-83; 1972.
27. Tchao, R.; Valentich, J. D.; Leighton, J. Cyclic AMP does not affect the detachability of MDCK, an epithelial cell line (abstr.). J. Cell. Biol. 70: 76a; 1976.
28. Misfeldt, D. S. Transepithelial transport in cell culture. Berkeley, CA: Univ. of California; 1975. 71 p. Dissertation.
29. McGrath, C. Analysis of mammary tumor virus (MTV) replication in tumor. Berkeley, CA: Univ. of California; 1970. 218 p. Dissertation.