ADHESIVE PROPERTIES OF MDCK CELL MEMBRANES: POSSIBLE ROLE IN EPITHELIAL HISTOGENESIS

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SUMMARY

MDCK cells in culture form a functional transporting epithelium. Apical surfaces of MDCK monolayers are not adhesive to free MDCK cells that fail to grow attached to existing monolayers. In contrast, when plated at high density (10⁶ cm⁻²) MDCK cells form multilayered structures in which only the outermost layer shows a typical apical surface (microvilli, tight junctions, etc.). In the lower layers cell-cell contacts (desmosomes, etc.) are made, suggesting that the basolateral surfaces remain adhesive.

Using a novel, quantitative, heterotypic cell adhesion reaction with aged human red cells, we have measured the adhesive properties of the apical surfaces of MDCK cells in a variety of conditions. It is found that the adhesion of red cells is dependent on cell density in the monolayer and falls to a low value at confluence. The reaction is considerably stronger at 6 °C compared to 22 °C (the temperature of most studies), while at 37 °C the reaction is expressed less strongly. MDCK cells with adherent red cells fail to divide, suggesting that the cell surface receptors involved might mediate growth inhibition by a similar homotypic reaction at confluence. The expression of the adhesive property is associated with cell division. This was shown by inducing synchrony in cultures either by refeeding starved cultures or by removal of a thymidine 'block'. Peaks in adhesiveness were related to peaks in thymidine incorporation in synchronized cultures.

Because of the precise quantitative way in which adhesion can be measured it is considered that this is an ideal system in which to identify the membrane components responsible for the reaction. Furthermore, it may prove possible to identify signals that arise as an immediate consequence of the adhesion reaction.

INTRODUCTION

Cells in secretory and transporting epithelia show a striking polarization of their plasma membranes into 2 domains, apical and basolateral (Louvard, 1980). The differential localization of membrane proteins in these 2 domains presumably underlies the functional asymmetry of epithelia.

The MDCK cell line has been used extensively as a model system for the study of some aspects of epithelial polarity. This line, established in 1958, retains a close resemblance to transporting epithelia present in the kidney (Madin & Darby, 1965). Monolayers exhibit density-dependent inhibition of growth with confluence at 3 x 10⁵ cells cm⁻². Transporting activity is demonstrated by the formation on

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impermeable substrates of multicellular hemicysts as a result of vectorial transport and trapping of salts and water (Leighton, Estes, Mansukhani & Brada, 1970). Monolayers grown on porous supports show transepithelial potentials and time-dependent resistance changes (Cereijido et al. 1978; Misfeldt, Hamamoto & Pitelka, 1976). Furthermore, hormonal responses typical of kidney epithelia have been reported (Rindler, Chuman, Shaffer & Saier, 1979; Simmons, 1977). Finally, several viruses bud asymmetrically from MDCK cell monolayers (Rodriguez Boulan & Sabatini, 1978; Roth, Fitzpatrick & Compans, 1979).

In this paper we describe an adhesion reaction between human red cells and MDCK cells in situ, which reveals at least some of the changes occurring in the apical surfaces of MDCK cells as they grow to confluence. Moreover, this reaction may provide some insight into the mechanisms underlying the density-dependent inhibition of growth in this cell line.

**MATERIALS AND METHODS**

**Cell line**

All the experiments reported here were made with MDCK cells (NBL-2 Flow Laboratories) obtained as monolayer cultures at passage numbers 62-65. For the entire experimental period less than 10 subcultivations were made at a split ratio of 1:10. The cells were grown on either plastic dishes (Sterilin) or Millipore filters (pore size 0.45 μm, type HA).

**Solutions**

MDCK cells were grown in plastic dishes (usually 7 cm²) containing 2 ml Eagles' Minimum Essential Medium (Flow Laboratories) with 2 g/l NaHCO₃ added plus 10% (v/v) foetal calf serum, 2 mM-glutamine, 1% (v/v) non-essential amino acid concentrate (Flow Laboratories) and Garamycin (140 μg/ml). Plates were incubated at 37 °C in an atmosphere of 95% O₂, 5% CO₂, (v/v).

Phosphate-buffered saline (PBS) used here had the following composition (g/l): NaCl, 8.6; KCl, 0.2; CaCl₂·2H₂O, 0.13; MgCl₂·6H₂O, 0.10; KH₂PO₄, 0.2; and Na₂HPO₄, 1.15.

**Standard adhesion reaction assay procedure**

Groups of plates were removed from the incubator and the medium was removed by aspiration. Each plate was washed twice with 2 ml PBS. The cultured cells were overlaid with 2 ml red cell suspension (0.5% (v/v) in PBS, red cells previously washed 3 times with PBS immediately prior to assay) at room temperature (22 °C) for 20 min. The red cell suspension was removed by aspiration and the plates washed 3 times with 2 ml PBS. To the monolayers of MDCK cells with adherent red cells 1.5 ml of trypsin solution was added (trypsin, 1 mg/ml containing 10,000 BAEE units/ml dissolved in sterile calcium- and magnesium-free PBS, containing 0.2 mg/ml EDTA, disodium salt) and the plates were reincubated at 37 °C for 30 min. Afterwards the cells were dispersed by drawing the contents of the dish up and down into a pipette several times. A sample (100 μl) of the cell suspension was mixed with 100 μl trypan blue solution (4 mg/ml in PBS) and live MDCK cells were counted using a standard haemocytometer. To the remaining 1.4 ml of suspension sodium dodecyl sulphate (SDS) solution was added (either 1.5 ml 4% (w/v) or 0.5 ml 12% (w/v)) and lysis was allowed to proceed overnight. The haemoglobin content of the lysate was read at 418 nm in a spectrophotometer, using a culture without red cells as a blank. The number of red cells originally present was read off from a standard curve relating absorbance at 418 nm to red cell concentration.

Generally, groups of 5 plates were used for each measurement. The results are given as the adhesion ratio, which is the mean number of red cells bound per MDCK cell.
In some instances a group of 5 plates was taken to determine the density of MDCK cells and the mean value was considered to apply to all plates that had been cultured under identical conditions.

**[3H]thymidine incorporation procedure**

Cultures were washed twice with 2 ml of fresh medium, after which 1 ml medium containing 1 μCi [3H]thymidine (91 μCi/μg) was added and the plates were incubated at 37 °C for 30 min. The medium was then removed by aspiration and the plates were washed twice with 2 ml PBS. One ml ice-cold trichloroacetic acid (TCA) (10 %, w/v) was added to the dishes, which were then stored at 4 °C for 30 min. After a further wash with 1 ml cold TCA solution, a solution of SDS (1 ml, 4 %, w/v) was added and the dishes were kept at room temperature overnight. The fluid was transferred to vials, scintillant was added and the radioactivity counted by the usual procedures. [3H]thymidine incorporation into DNA is given as d.p.m. [3H]thymidine per 10^6 cells, the cell counts being obtained from other identical plates used to measure the adhesion ratio.

**Human red cells**

Samples of human red cells were obtained from sterile blood samples collected into sodium citrate (38 %, w/v) as anticoagulant. Before use the blood samples were stored at 4 °C. Washed red cell suspensions (0.5 %, v/v) were prepared by resuspending and centrifuging in PBS 3 times. For washing, 50 μl samples of blood were mixed with 10 ml PBS and centrifuged at 600 g.

Occasionally fixed red cells (and MDCK cells) were used. The procedure for fixation was as follows: red cells were fixed as a 0.5 % (v/v) suspension in 2.5 % (w/v) glutaraldehyde in PBS for 60 min at 22 °C. Cells were then resuspended at 0.5 % (v/v) in 0.2 M-glycine in PBS for 10 min at 22 °C. Finally, they were washed 3 times in PBS before use. MDCK cell monolayers were fixed in a similar way.

**Morphological studies**

Samples for electron microscopy were prepared by the usual procedures. After fixation with glutaraldehyde in cacodylate buffer, tissues were stained with osmium tetroxide, embedded, sectioned and examined with a Philips 300 electron microscope. Photomicrographs of live cells were made using phase-contrast optics.

**RESULTS**

**Morphological evidence for asymmetry**

When cells are allowed to grow to confluence on Millipore filters the structure formed resembles a typical tight transporting epithelium (Fig. 1). The apical surface is characterized by short microvilli and adjacent cells are joined at their apical edges by tight junctions. The lateral intercellular spaces are complex and inter-digitated, and extend from the tight junctions to the surface of the substrate. The basolateral surfaces of the cells form an intimate contact with the Millipore filter and small projections invade the pores of this surface. The usual collection of intracellular organelles including endoplasmic reticulum and Golgi assemblies are seen. Close association of the basolateral surfaces of adjacent cells occurs at many places, which may represent low resistance pathways; there are, in addition, typical desmosomes. Confluence is reached at a density of around 3 x 10^6 cells cm^-2 at which the whole of the growth surface is covered, but there is still a slow increase in cell number after this time (see Fig. 8) with an increase in cell packing.
Although there is some overlap between cells (Fig. 1) they are strongly monolayered, as found with contact inhibition of locomotion in other cell types (Middleton, 1973). Even in compacted monolayers cell division still occurs at a slow rate, but in this situation the extra cells are found free in the medium.

When a suspension of free-living MDCK cells is added on top of an already confluent monolayer and the whole reincubated, the free cells fail to adhere. Thus we were unable to form multilayered structures by addition of cells to existing monolayers. As with cells budded off from confluent monolayers, exogenously added MDCK cells are unable to interact with apical surfaces of established cells. When MDCK cells are plated at 3 times the confluent density \((10^6 \text{ cm}^{-2})\) the resultant structures are somewhat uneven, with thin monolayer sections alternating with areas 5–6 cells deep. In sections of these structures the epithelial cells are joined by tight junctions at their apical edges in only the outermost layer of cells and many cells have only a small proportion of their total surface exposed at the apical side. It appears, however, that some cells are apposed to the surface of the filter and have no apical surface at all (Fig. 1). However, we have not examined an extensive series of sequential sections and it remains possible that long filamentous projections may reach the apical surface. Cell–cell contacts are plentiful between the cells of all the layers of the composite epithelium.

It appears, therefore, that MDCK cells plated at high density can form a multilayered structure in which cell–cell contacts allow a functional syncytium to be established. On the other hand, cells are unable to associate with the apical surface of an established monolayer while, apparently, there is no impediment to advancing cells on a substrate meeting and establishing the contacts that ultimately delimit the apical and basolateral domains. It would seem necessary for the upper surfaces of MDCK cells grown \textit{in vitro} to undergo radical changes in surface properties during histogenesis. The heterotypic adhesion reaction described below may monitor the predicted changes referred to above.

The red cell adhesion reaction

It became apparent almost immediately that the adhesion reaction was dependent on the developmental stage of growth (Fig. 2). The adhesion reaction is pronounced at 16 and 27 h post-plating, compared to 4 h, but most striking of all is the disappearance of adhesion at 72 h when the cells are near to confluence. Careful examination shows that at 72 h most of the adherent red cells are found in fault lines, where the edges of adjacent clones have only just come together and where the regular packing pattern of a fully confluent structure has not yet developed.

The adhesion ratio was followed for 96 h with 4 different initial plating densities. The results are shown in Fig. 3A. When MDCK cells were plated at an initial low density of \(1 \times 10^4 \text{ cm}^{-2}\) the adhesion reaction was very marked within 1 h, but increased still further to reach a maximal value at 24 h, following which it declined. However, when cells were plated at a density of \(3 \times 10^6 \text{ cm}^{-2}\), that is sufficient to form a confluent monolayer without division, there is, remarkably, only the most minimal indication of an increase in adhesiveness. Plating at intermediate densities gave an
Fig. 1A, B and C. For legend see p. 261.
intermediate response, where both the strength and duration of the increased adhesiveness was reduced, compared to the response following lower initial plating densities. Whatever the initial plating density the final value for the adhesion ratio was approximately the same.

Although MDCK cells show remarkable changes in adhesive properties with time there is, of course, a further complication in that the cell density is changing during the experiment. The change in the adhesion ratio with time together with the total cell number is shown in Fig. 3B. It can be seen that the maximal rate of decrease of the adhesion ratio corresponds approximately in time to that when the total number of cells is increasing rapidly. If indeed there is contact inhibition of the adhesion reaction, then as the cell density increases the number of cells that are not surrounded by other cells will also fall. As the monolayer becomes confluent a more regular packing pattern develops and each cell is spread out less on the dish. A small part of the fall-off of the adhesion ratio may be due, therefore, to a reduction in cell area. Nevertheless, the total number of red cells adsorbed per dish decreases after 48 h while the area of the dish covered by cells is increasing, indicating a loss of adhesiveness unrelated to cell size.

Throughout these experiments, the standard assay conditions for measuring the adhesion reaction have been to incubate the cultured MDCK cells with red cells suspended in PBS (0.5%, v/v) for 20 min at 22 °C. Some justification for the choice of time and temperature is given in Fig. 4. Adhesion of red cells was measured for periods of up to 1 h at 3 different temperatures. At all temperatures binding of red cells had reached a maximal value at 60 min, but there was a striking difference in the number of red cells attached at different temperatures, the adhesion reaction being stronger in the cold. At 22 °C the adhesion reaction had not quite reached steady state at 20 min and we were concerned that removal of the cultures from the incubator for periods longer than this, especially with the medium replaced by PBS, might

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Fig. 1. Electron micrographs of MDCK cells cultured on Millipore Filters (type HA, pore size 0.45 μm).
A. Cells incubated overnight at 37 °C and allowed to form a monolayer. Section shows 2 cells meeting at their apical surfaces to form a well-defined tight junction. A projection from a third cell is inserted below the other two.
B. Cells plated at 10⁶ cm⁻² and incubated for 19 h at 37 °C. Section shows 3 cells, 2 of which are in contact with the substratum. Again tight junctions delimit the apical surfaces of the 3 cells. The central cell appears to have a restricted amount of its total membrane in the apical surface. Complex interdigitated basolateral spaces occur between the cells, which are joined at typical desmosomes.
C. Cells plated at 10⁶ cm⁻² as in B. The cell shown is in contact with the substratum and small finger-like projections enter the pores of the filter. The cell forms contacts with overlying cells but would appear to make no contacts with the apical surface.
D. High-power section of a tight junction between 2 epithelial cells; note, too, the well-defined Golgi apparatus.
E. Section showing prolific collection of apical microvilli.
F. High-power section of a desmosome between 2 MDCK cells.
Bars A–E, 1 μm; F, 0.1 μm. m, Millipore filter; mv, apical microvilli; arrows, tight junctions; circled areas, desmosomes; g, Golgi apparatus; n, nucleus.
Heterotypic cell adhesion

Fig. 3. Time dependence of the adhesion reaction at different plating densities. MDCK cells were plated into plastic dishes at different initial densities. They were removed at different times in groups of 5 and the standard adhesion reaction carried out as described in Materials and methods and using a freshly prepared red cell suspension. Results are shown as the adhesion ratio (mean number of red cells adsorbed per MDCK cell) versus time. In A results are given for 4 different initial plating densities of $10^4$ (curve A), $5 \times 10^4$ (curve B), $10^5$ (curve C) and $3 \times 10^5$ (curve D) MDCK cells/cm$^2$. In B the adhesion ratio for an initial plating density of $10^4$ cells/cm$^2$ is plotted versus time (○—○) together with the total number of MDCK cells per plate (●—●) and haemoglobin absorbance at 418 nm (— — —) (proportional to total number of red cells adhered). Means ± s.e. for 5 measurements are given. Where error bars are not shown they lie within the symbol.

Fig. 2. Development and decline of MDCK cell adhesive capability. MDCK cells were initially plated at $10^4$ cells cm$^{-2}$ in 3-cm diameter plastic dishes in 2 ml growth medium and incubated at 37 °C for periods up to 72 h. On removal from the incubator, plates were subjected to the standard adhesion assay procedure. Photomicrographs are of live, unfixed preparations photographed with phase-contrast optics. MDCK cells appear as large, flattened islands of cells, red blood cells as white discs. The adhesion reaction is illustrated at 4 h, 16 h, 27 h and 72 h post-plating. Bar, 50 μm.
Exposure time (min)

Fig. 4. Effect of temperature on the kinetics of the adhesion reaction. Cells were plated at a density of $5 \times 10^4$ cells/cm$^2$ and incubated at 37 °C for 20-24 h before carrying out the standard adhesion reaction. Groups of 5 plates were exposed to a standard red cell suspension (0.5, v/v in PBS) for different times and at different temperatures. As the whole experiment was carried out over a short period (4 h), the number of MDCK cells per plate was taken as the average from 5 plates chosen at random from those used for each of the 3 temperatures. The figure shows the adhesion ratio versus the duration of exposure to the red cell suspension at 6, 22 and 37 °C. Means ± S.E. for 5 measurements are given. Where no error bars are shown the values are within the dimensions of the symbol.

provoke changes in the apical surface of the MDCK cells not associated with normal histogenesis in vitro. The choice of 22 °C for measuring the adhesion reaction was simply one of convenience, while it must be emphasized that the strength of the reaction measured at this temperature is twice that for the adhesion reaction carried out at 37 °C.

The relative adhesiveness of different types of red cells was investigated using MDCK cell cultures at a stage of maximal adhesiveness. Red cell adhesiveness was found to increase with ageing (Fig. 5A). There was no relationship between adhesiveness and ABO blood grouping. Fig. 5B shows the more rapid increase in adhesiveness that could be obtained by storing red cells in sterile PBS.

Fixation of red cells with glutaraldehyde enhanced their adhesiveness (results not
Fig. 5. Change in adhesiveness of red cells with time. MDCK cells were plated at 5 x 10⁴ cells/cm² in 7 cm² dishes and incubated at 37°C. A standard adhesion reaction was carried out at 23-25 h. Afterwards the contents of the dishes were lysed in SDS (4%) and the absorbance read at 418 nm. All blood samples were sterile and stored in their own plasma at 4°C. Sodium citrate (3.8%) was used as anticoagulant. In A blood samples were tested at 5, 41 and 69 days after collection. Six each of the samples were of groups A, B and O. In B group O blood samples were used. A suspension of red cells (0.5%, v/v in sterile PBS) was prepared and stored for 1 week at 4°C. The adhesion of these samples (O) was compared with cell suspensions prepared immediately before the measurement of adhesion (●).

Inhibition of growth by adherent red cells

The heterotypic adhesion reaction we have described may mimic a similar homotypic interaction between MDCK cells. To investigate if there was a response following red cell recognition and adhesion to the apical surface, we have followed the growth pattern of MDCK cells with adherent red cells.

Two types of experiment were carried out, both of which are illustrated in Fig. 6. In the first we used both live and fixed red cells from the same batch, knowing already that fixation increased the adhesive properties of the red cells and, further, that the normal reaction was greatly weakened at 37°C. When monolayers at low density (around 1 x 10⁵ cm⁻²) with adherent red cells were returned to the incubator for a further 70 h there was a very significant reduction in growth (P < 0.001) when fixed red cells were used, although viability was not altered as measured by dye exclusion.
Microscopic examination showed that many MDCK cells still had adherent red cells after 70 h. These had continued to grow in size although they had not divided. In those dishes exposed to live red cells, most of the latter were found to be released from the MDCK cells upon incubation at 37 °C. In this experiment the number of live cells present was smaller than the number of fixed cells, as more of the latter remained attached after exposure to the red cell suspension and subsequent washing before returning to the incubator. Clearly the experiment would be better controlled if equal numbers of adhesive and non-adhesive red cells were added to identical cultures. Fixed, but trypsinized and poorly-adherent red cells, therefore, provide a better comparison with fixed adherent red cells. It was found that even fixed trypsinized non-adherent red cells are able to inhibit growth when added in large excess to the medium (300 red cells per MDCK cell) but this non-specific effect is of no interest. Thus in the second type of experiment the adhesion of fixed red cells was measured at 24 h when around 30 red cells adhered to each MDCK cell. This number of fixed
adherent or fixed and trypsinized red cells was added to different groups of plates and their effects were assessed after 72 h. It is evident that (Fig. 6A) fixed trypsinized red cells cause some growth inhibition, while the inhibition produced by fixed adherent cells is significantly greater.

Cell division and the adhesion reaction

It seemed important to investigate if the red cell adhesion reaction also varied during the cell cycle. This was approached in 2 ways, both designed to synchronize cell division. In the first, cells were blocked in the S-phase by incubation with a high concentration of thymidine added to 8 h cultures of MDCK cells. After a further 28 h the thymidine was washed away and both control and thymidine-treated cultures were refed. Cell density, the adhesion reaction and [3H]thymidine incorporation were then followed for a further 24 h (Fig. 7). In the control cultures, and in spite of refeeding, the number of red cells adhering to each MDCK cell and [3H]thymidine incorporation fell gradually over 24 h as the MDCK cell number rose towards the confluent density. At the time of refeeding the density of cells in the thymidine-treated group was only

Fig. 7. Removal of thymidine block and the adhesion reaction. MDCK cells were added to dishes at a density of $5 \times 10^4$ cells/cm$^2$ and incubated at 37 °C for 8 h. Afterwards all dishes were refed either with medium alone or medium containing 2 mM-thymidine followed by incubation for a further 28 h. All dishes were then washed with medium, refed and returned to the incubator. Measurements of the standard adhesion reaction and of [3H]thymidine incorporation were made at intervals, each using groups of 4 dishes. The adhesion ratio ($\bigcirc$) and total number of cells per dish ($\blacktriangle$) are shown against time. Results shown in A are for control cells while B shows results for cells that were thymidine-blocked between 8 and 36 h. [3H]thymidine incorporation is given as d.p.m. [3H]thymidine per 10$^6$ cells, the cell counts being derived from the plates used to measure the adhesion ratio.
one-third of those in the control group. The cells had continued to grow in size during thymidine application, which may be the reason for the higher red cell/MDCK cell ratio in this group. [³H]thymidine incorporation increased after the inhibiting concentration of the base was removed, and an increase in intensity of the adhesion reaction and in cell number then followed. The increase in cell number was only small, suggesting that only some 40% of the cells had divided 16–20 h after the block was removed. In spite of this the increase in red cell adhesion does suggest that this property is strongly expressed during cell division.

Fig. 8. The effect of refeeding on the adhesion reaction. MDCK cells, plated at 5 x 10⁴ cells/dish (each dish 7 cm²), were incubated at 37 °C in 2 ml growth medium for 5 days without refeeding. The red cell adhesion reaction and [³H]thymidine incorporation were then measured, after which all the remaining dishes were refed with 2 ml growth medium. Further measurements of the adhesion ratio and [³H]thymidine incorporation were made at intervals for a further 36 h. Mean values ± standard errors for the adhesion ratio (O, n = 5) and for [³H]thymidine incorporation (●, n = 4) are shown. The mean value of the total number of cells per dish (▲) is also given, the broken line indicating full confluence (3 x 10⁶ cells/cm²).

The peak in the adhesion response precedes the peak in cell number but it must be emphasized that the red cell/MDCK cell ratio is an average value for a large number of cells. Cells that lie in the centre of a clone are, in some respects, confluent and may be less adhesive than those at the edges. The relative expression of the adhesive property in different parts of subconfluent monolayers has yet to be investigated but it is not amenable to the methods used here. In addition, the cell number declines at 60 h, suggesting that viability is reduced in thymidine-blocked cultures.

A second way to produce synchrony in MDCK cells is as follows. Cells are grown and allowed to form confluent monolayers and then starved. When such cultures are refed there is a synchronous burst of cell division in which the excess of cells are shed
into the overlying medium. In this way the adhesion reaction can be followed under conditions of confluence in which, normally, the adhesion reaction is rather weak, with the difference that the cells are actively dividing. The result, given in Fig. 8, shows that the number of cells in the monolayer changes very little during the experiment yet there is a peak in [PH]thymidine incorporation, which is mirrored by a similar peak in the adhesion response. This finding also supports the view that the expression of that property of the apical surface that is responsible for the adhesion reaction is related to the cell cycle.

**DISCUSSION**

Two important, and perhaps related, consequences of the attainment of confluence by an MDCK cell monolayer are a reduction in the adhesiveness at the apical surface to free MDCK cells and a contact inhibition of cell division. These phenomena presumably depend upon the ability of each cell to respond appropriately to signals received from the cells around it via cell–cell contact. In this paper we have used red cell adhesion to MDCK cells to monitor the changes occurring in the MDCK cell membrane as monolayers grow to confluence, and to investigate the effects of cell–cell adhesion on MDCK cell growth and division. The heterotypic adhesion reaction may be a good indicator of processes normally occurring during the development of an MDCK cell monolayer. However, any conclusion as to the true significance of this adhesion reaction must await an elucidation of its biochemical nature.

Many examples of a differentially active cell surface component on a range of cultured cell types exist in the literature. These protein, glycoprotein and glycolipid components include complete and incomplete blood group antigens (Hakomori & Kubata, 1974), a variety of other immunologically active substances (Cikes & Friberg, 1971), a number of membrane-bound glycosyl transferases (Roth & White, 1972) and specific lectin receptors (Burger, 1971). The expression of these active components has been associated with neoplastic transformation (Brady & Fishman, 1973; Critchley, 1973). Others have been shown to be selectively active during particular stages of the cell cycle (Glick & Buck, 1973; Kuhns, 1968; Thomas, 1971), while others appear to be expressed only under specific conditions of cell density (Grooten, De Baetselier, Vercauteren & Hamers, 1980; Roseman, 1970). It is difficult to know with certainty if such substances are responsible for cell–cell adhesion. Even if cell adhesion is directly related to a cell surface component it is likely that changes in cell shape and mobility, in membrane fluidity and in membrane reorganization will make their individual contributions to the overall adhesion reaction.

Contact inhibition of growth, movement (Abercrombie, 1970) (or, more correctly, cell overlap), and phagocytosis (Vasiliev *et al.* 1975) have been described for various cell types including epithelia. In contact inhibition of phagocytosis the ability of epithelial cells to be adhesive to particles is limited to the periphery of the clone where cells have free edges. The inability of central cells to take up particles may be related to a lack of adhesiveness of the apical surface of confluent monolayers, a phenomenon that has been verified here for the haemadsorption reaction.
In our system we have evidence that recognition, at least of a red cell, is associated with inhibition of cell division. Furthermore, our results suggest that expression of adhesiveness is maximal when cells are actively dividing. A simple feedback system for the control of histogenesis can be envisaged: recognition leads to the establishment of cell contacts and to inhibition of cell division; following the establishment of permanent contacts and the formation of apical and basolateral membrane domains the need to express the adhesive property declines, disappearing altogether at confluence. This deceptively simple view is only of use in formulating new questions about mechanisms. Two that appear to be amenable to investigation are the nature of the adhesive moieties in the apical membrane and the nature of the signal that ensues from their activation.

We have shown that the relative adhesiveness of MDCK cells towards red cells varies through the cell cycle. A similar variation has been reported for several receptor sites in the cell membrane. For example, receptors for wheat germ agglutinin are 20 times greater in 3T3 cells in mitosis than at rest (Burger, 1971). Similarly, the expression of H-antigen in thymidine-synchronized HeLa cells is greatest during division (Kuhns, 1968). Various other blood group antigens on the surface of many cultured cell types have been shown to vary dramatically with the stage in the cell cycle (Fukuda, 1980; Thomas, 1971). In the case of the MDCK cell it is possible that adhesion is related to apical membrane mobility, a parameter that changes in rapidly dividing transformed cells (Inbar & Sachs, 1973) and during the cell cycle (Garrido, 1975; de Laat, van der Saag, Elson & Schlessinger, 1980).

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Heterotypic cell adhesion


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