

# Adhesiveness and Stickiness: Two Independent Properties of the Cell Surface\*

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## SUMMARY

For the purposes of this report, adhesiveness was defined as that force which resists the mechanical separation of attached pairs of cells, and stickiness as the tendency of cells to cling to a foreign substrate; in the present experiments the substrate employed was glass. The cells selected for study were from the normal rabbit epiderm and the V<sub>2</sub> rabbit carcinoma. Adhesiveness was determined by measuring the bend produced in a calibrated microneedle when subjected to the strain of pulling apart an attached pair of cells. Stickiness was measured by calculating the percentage of cells clinging to a glass surface after being dipped 3 times in salt solution.

It was found that the normal epidermal cells were strongly adhesive but not very sticky, whereas the cancer cells were extremely sticky but poorly adhesive. Thus, it was concluded that the two properties are different and independent.

Possible explanations and implications of the results are discussed and further studies suggested.

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The strong adhesiveness of normal skin and parenchymatous cells has been demonstrated previously, as well as the reduced adhesiveness of neoplastic cells (1, 4). However, recognition that two independent properties exist, here designated as stickiness and adhesiveness, has not been presented previously as far as the author can determine. The possibility that this might be the case suggested the experiments described in the present paper.

## MATERIALS AND METHODS

The normal epidermal cells of the rabbit and V<sub>2</sub> rabbit carcinoma cells were selected for the purpose of these experiments. Keratinized cells were eliminated from consideration, only viable nucleated cells being studied. The possibility arose that the flat polygonal cells of the superficial layers might behave differently from the cells of the basal layer. However, when these two normal cell types were compared, they showed no remarkable differences in either stickiness or adhesiveness, so, thereafter, no attempt was made to separate the

two forms and their intermediates in the suspensions used.

Suspensions of normal cells were prepared by scraping the rabbit skin and gathering the cells in balanced salt solution. The V<sub>2</sub> cells were scraped from freshly excised tumors and similarly suspended. The number of cells was adjusted by dilution to arrive at convenient concentrations for counting. Transfer of the cells through several washes in salt solution was done by a wire loop to avoid contact with glass whereby the stickier cells would be lost.

Stickiness was measured by placing films of cell suspensions over circles, 3 mm. in diameter, cut in a glass slide. The cells were transferred by means of a wire loop. A 15-minute period was allowed to elapse while the cells settled onto the glass surface, and then the cells within the circles were counted. Thereafter, the slide was dipped 3 times into a beaker of salt solution. The excess fluid was drained off, and then the cells that remained stuck to the glass were counted. Small clusters of two to four cells were counted as one, whereas the occasional large clump was not counted at all. The percentage of cells that clung to the glass was calculated. In this way, normal epidermal cells and V<sub>2</sub> carcinoma cells were compared.

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Adhesiveness was measured by a method devised in this laboratory in 1944 (1). This procedure depends upon measuring the bend produced in a microneedle when subjected to the strain of pulling apart a pair of attached cells. Briefly, microneedles were drawn from glass rods having a uniform diameter of 0.85 mm. The needles were calibrated by hanging a series of microweights, made of fine wire, on their tips and measuring the resulting deflection with an ocular micrometer. In the experiments, a pair of cells was selected, and a holding needle, stiff and blunt, affixed one of the cells securely. The tip of the calibrated needle was inserted into the other cell of the pair and then moved away, eventually separating the cells. The movement of the needle tip was followed with an ocular micrometer, while the total excursion of the shaft was measured on a mm. scale that recorded the movement of an amplifying arm connected to the needle carrier. By subtracting the movement of the tip from the movement of the shaft, the bend of the needle was obtained. This value, interpolated in the calibration curve for the needle, yielded the force in mg. needed to separate the pair of cells.

A possible source of error was the difference in cell size. In general, the  $V_2$  cells were somewhat smaller than the epidermal cells, though a few were larger. One might suppose that cells having broader surfaces for contact would stick to the glass better. In any event, the result was in the opposite direction, so that if such an error existed it was masked by the excessive stickiness of the cancer cells. Also, the difference in the size of the cells might affect their adhesiveness, since the latter would presumably depend to some degree upon area of contact. There was no easy way to rule out this possible error except to note that variations in the degree of adhesiveness between cells of any one type did not seem to be correlated with the moderate differences observed in their size. In other words, some large cells separated relatively easily, and some smaller ones were strongly adherent to one another. No attempt was made to refine this determination further, since the result was consistent with those obtained in other similar studies where difference in cell size was not a factor (1).

A third variable might be the rate at which the two cell types settled out of the fluid and onto the glass surface. After many trials, it was found that after 15 minutes most of the cells of both types had settled, and waiting for  $\frac{1}{2}$  hour did not alter the results. While settling, the preparations were kept in a moist chamber to avoid drying out.

## RESULTS

**Stickiness.**—As shown in Table 1, a greater percentage of the  $V_2$  cancer cells (63.1 per cent) remained stuck to the glass surface after the dipping procedure than was true of the normal epidermal cells (16.6 per cent). The difference between the two, 46.5 per cent, is statistically significant. The probability of a difference of this size, or greater, occurring by chance alone is less than one in a thousand.

In brief, the  $V_2$  cancer cells are sticky, whereas the normal epidermal cells are far less so.

**Adhesiveness.**—With respect to adhesiveness, Table 1 shows that more force is required to sepa-

TABLE 1  
"STICKINESS" AND "ADHESIVENESS" OF NORMAL AND CARCINOMATOUS EPIDERMAL CELLS

Cell characteristic	$V_2$ carcinoma	Normal epiderm	Difference ( $V_2$ -Nor- mal)	$P_{diff}$
Stickiness				
Mean of % of cells sticking to glass* (25)†	63.1 ( $\pm 9.2$ )	16.6 ( $\pm 5.1$ )	+46.5	< .001
Adhesiveness				
Mean force re- quired to sepa- rate attached pairs of cells‡ (25)†	0.20 ( $\pm 0.18$ )	1.67 ( $\pm 0.56$ )	- 1.47	< .001

\* The number of cells originally deposited in each experiment ranged from 40 to 210 for the normal epiderm and from 63 to 200 for the  $V_2$  carcinoma. A total of 2904 normal cells and 4717  $V_2$  cells were tested for stickiness.

† The numbers in parentheses refer to the number of determinations.

‡ Force is expressed as the number of mg. required to bend the glass needle to the curvature observed at the time of separation.

rate pairs of normal cells (1.67 mg.) than pairs of  $V_2$  cells (0.20 mg.). The forces required are consistent with previous demonstrations of the relatively poor adhesiveness of neoplastic cells as compared with their normal prototypes. Indeed, the values obtained are comparable to those recorded for carcinomatous and normal epidermal cells from man (1). Once again, the probability of a difference this large or larger is less than one in a thousand.

## DISCUSSION

It is at once apparent from these experiments that stickiness and adhesiveness, as defined for the purposes of this paper, are two different and independent properties of the cell surface. The cancer cells tested had poor adhesiveness but were

quite sticky. The normal cells were strongly adhesive but not very sticky. This implies that the underlying physico-chemical factors responsible for these two properties are probably different also.

Stickiness no doubt confers a certain degree of adhesiveness, indeed it may account for most or even all of the adhesive force found in the V<sub>2</sub> cells. It is of interest, too, that after separation when the cancer cells are re-approximated, they again cling to one another. In other words, the cells are sticky in relation to one another as well as to foreign substrates. On the other hand, re-approximated normal cells show no tendency whatsoever to cling to one another. An analogy to set and unset glue might be drawn. Unset glue obviously is sticky when two surfaces covered with it are re-approximated after separation, whereas fracture of the set glue produces hard, smooth surfaces that are not in the least sticky.

Previous studies have indicated that the calcium ion is essential to the maintenance of cellular adhesiveness and that cancer cells, which are deficient in adhesiveness, are for some unexplained reason incapable of binding adequate amounts of calcium at their surfaces (3). It has been suggested that calcium normally forms a chemical bridge between cells by linkage to carboxyl, phosphate, or other such groups and that an adequate number of these groups is not available at the cancer cell surface (2).

Stickiness, as here defined, may be independent of calcium but related instead to the presence of such substances as polysaccharides, mucins, or lipides that form a tenacious coat around the cells.

One is tempted to speculate that perhaps with the assumption of the malignant state some cells elaborate mucopolysaccharides at their surfaces. The presence of the acidic groups in these mucopolysaccharides (sulfate, carboxyl, hydroxyl) might then compete for calcium at the cell surfaces, complexing or combining with it. This would reduce the adhesiveness of the cells by breaking the calcium linkages and at the same time would render the cells sticky. The reduced adhesiveness would facilitate separation and allow the motile cancer cells to invade adjacent tis-

ues and vessels, whereas stickiness would favor their chances of lodging at distant sites to initiate metastases when transported through vascular channels.

The fact that the two qualities are independent paradoxically clarifies the problem somewhat by complicating it. Each of the qualities may now be studied separately, and the combined results may lead to a better understanding of the structure and function of cell surfaces in these regards. For instance, if the above speculations come anywhere near the truth of the situation, then the removal of calcium by chelation should reduce adhesiveness but have little, if any, effect on stickiness. On the other hand, removal of the polysaccharide, mucinous, or lipide coat should diminish stickiness. Experiments along these lines are planned for the future.

Since it is clear that adhesiveness and stickiness are two different and independent properties, care must be taken to distinguish between the two when interpreting the results of experiments. Otherwise, confusion is certain to arise in that stickiness may erroneously be interpreted as adhesiveness, and adhesiveness as stickiness, leading to false conclusions.

For the purposes of this report, it was expedient to define the two properties under consideration as stickiness and adhesiveness, granting that these are neither very satisfactory nor elegant terms. It is hoped that further elucidation of these properties, with a clearer understanding of their physico-chemical background, will suggest better terms by which to designate them.

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