Decreased Mutual Adhesiveness, a Property of Cells from Squamous Cell Carcinomas*

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In the course of microscopic observations on living cells from a lip carcinoma it was found that these cells could be separated one from another by needles with the greatest ease. When similar observations were made on normal lip epithelium the cells seemed to be far more strongly adherent, and were pulled apart only after considerable resistance had been overcome. This suggested the possibility that a decrease in adhesiveness might be characteristic of malignant cells. Malignancy consists essentially in local invasiveness and distant metastasis, both of which would be favored if malignant cells could break away readily from one another, but hampered if they were strongly adherent in sheets.

Accordingly, experiments were planned to measure quantitatively the forces necessary to separate carcinomatous squamous cells, as compared with normal squamous cells and squamous cells from benign tumors.

METHOD AND MATERIAL

The method used for measuring the adhesiveness of cells was a modification of that employed by Sichel (9) for determining the elasticity of muscle fibres, and by Norris (6) for measuring the surface tension of erythrocytes. This method, as adapted to the present problem, depends upon the amount of bend in a needle when it pulls apart a pair of cells.

Two glass microneedles were attached to a Chambers micromanipulator. One (the holding needle), stiff and blunt, served to hold one of an attached pair of cells firmly against a coverslip, and remained stationary. The second (the pulling needle), flexible and sharp-pointed, was inserted into the other cell of the pair and, on being moved by the micromanipulator, pulled the cells apart. As the cells resisted separation because of their mutual adhesion the pulling needle bent, and its bending reached a maximum just before the cells had been pulled apart. The maximum bend was measured, and the value thus obtained was expressed as the force necessary to separate the pair of cells.

The bend of the needle was found by subtracting the excursion of the tip from that of the shaft. For example, at maximal bending (just before the cells separated) it might be found that the shaft had been displaced 0.20 mm. by the micromanipulator, but that the tip had moved only 0.03 mm.; hence the bend of the needle was 0.17 mm.

The excursion of the needle tip was measured under a microscope provided with a filar micrometer eyepiece. The excursion of the shaft was measured as follows. To the shaft was attached a glass rod 25 cm. long, which served as a lever to amplify the movement of the shaft. The distal end of the rod was provided with a fine tip. This tip was focused by a microscope on a mirror, which reflected the image on a ground glass millimeter scale. On the scale, the excursion of the needle shaft was amplified about 50 times.

The microneedles were drawn from Pyrex glass rods having a uniform diameter of 0.85 mm. A Livingston microneedle puller (5) was used for this purpose. Each pulling needle was calibrated by hanging microweights on its tip in such a position that the force was applied in the same direction as it would act when the needle was pulling a pair of cells apart. The bend in the needle thus produced by hanging a weight on its tip was read on a filar micrometer attached to a horizontal microscope. By using a series of microweights a calibration curve was constructed for each pulling needle.

The microweights were cut from platinum wire of uniform diameter weighing 1 mgm. per 0.565 cm. Lengths of wire were measured and their weights calculated; they ranged from 0.25 mgm. to 2.00 mgm.

The cells to be examined were placed upon a coverslip in physiological salt solution, and the coverslip was then inverted over a moist chamber. It was not necessary to consider bending of the needle by surface forces since the force required to separate a pair of cells was great enough so that relatively stiff needles could be used.

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The calculations in a typical experiment were as follows:

Ocular micrometer reading of pulling-needle tip at start = 3.5

" " " " " " " " " " " " " end = 6.0

2.5 × 0.012 mm. (micrometer factor) = movement of needle
tip = 0.030 mm.

Scale reading of pulling-needle shaft at start = 30 mm.
" " " " " " " " " " " " movement " = 40 mm.

10 mm. ÷ 50 (amplification factor of lever) = movement of
shaft of pulling needle = 0.200 mm.

0.200 mm. (movement of shaft of pulling needle)
0.030 " " " " " " " " " " " tip of pulling needle

0.170 " = bend in pulling needle

The calibration curve of this needle showed that it bent
0.2 mm. with 2 mgm.
0.1 " " 1 "
0.05 " " 0.5 "

Therefore, by interpolation, the force required to separate the
cell of pairs = 1.70 mgm.

Squamous epithelium obtained from scrapings of
fresh tissues was used in these experiments. Scrapings
were made from the normal lip and the cervix uteri,
from squamous cell papillomas of the skin, and from
squamous cell carcinomas of the lip and of the cervix.
Much of this material was obtained from the Radiology
Department of the Philadelphia General Hospital.
Material was also obtained from the Surgical Depart-
ment of the Lankenau Hospital. The tissues were
scraped with a blunt-pointed instrument and the cells
thus removed were placed on a coverslip in Gey's
solution (3). In selecting cells for an experiment only
those were used that were nonkeratinized and appar-
ently viable. Since there did not appear to be any
obvious differences in cell size in comparable series,
and since there was no apparent correlation between
cell size and adhesiveness, cell size was not considered
in the calculation.

RESULTS

Qualitative differences between normal and
carcinomatous cells.—Striking differences were observed
between normal and carcinomatous cells from the lip
when pairs of cells were pulled apart. In normal
squamous cells, tension lines developed in the cyto-
plasm, the nuclei often became elongated, and the
cells separated only after considerable distortion; when
they finally parted the cells snapped back into ap-
proximately their original shape. These observations
indicated that the cells were firmly adherent to one
another. Similar observations on squamous epithelium
are recorded by Chambers and Renyi (2). In contrast,
the cells from squamous cell carcinoma of the lip
appeared to separate with much less resistance, show-
ing less distortion and less prominent tension lines.

These observations are recorded in a series of photo-
micrographs (Figs. 1 to 6).

Quantitative differences.—The forces required to
separate pairs of cells derived from normal and car-
cinomatous lips, normal and carcinomatous cervixes,
and skin papillomas were measured by the method
described above. In each of these groups the cells
were obtained from 5 individuals, and from each
specimen 10 pairs of cells were examined, a total of
50 pairs of cells in each group. The results (Table 1)
are expressed as the mean number of milligrams (with
the standard error of the mean) required to separate
different pairs of cells. Values for carcinoma of the lip
and cervix are seen to be distinctly lower than those for
normal cells of these organs and for skin papilloma.
When carcinomatous and normal cells from the lip
were thus compared an average of only 0.47 mgm.
was required to separate pairs of carcinomatous cells,
as compared with 1.42 mgm. to pull apart normal
cells, a three-fold difference. An even greater contrast
was found between cells from carcinoma of the cervix
and from the normal cervix. Here the average force
required to separate pairs of carcinomatous cells was 0.18
mgm., whereas for normal cells it took a force of 1.11
mgm., a difference of 6 times. It is interesting that
the adhesiveness of skin papilloma cells ranks this
lesion with normal epithelium rather than with
malignant cells, the average for the papilloma cells
being 1.25 mgm.

The same results are represented graphically in
Figs. 7 and 8, which show the distribution of values for
adhesiveness. The distribution for carcinomatous and
normal cells of the lip is represented in Fig. 7. Though
there is slight overlapping carcinoma cells (black rect-
angles) lie mostly on the left side of the graph,
indicating low values of adhesiveness, while normal
cells (hatched rectangles) are mostly grouped on the
right side of the figure, indicating their greater ad-
hesiveness. Even more striking separation of malig-
nant and normal cells is shown in Fig. 8, which
represents the distribution of values for adhesiveness
in cells from the cervix uteri. The malignant cells

TABLE I: FORCES REQUIRED TO SEPARATE PAIRS OF CELLS BY
MICROMANIPULATION

<table>
<thead>
<tr>
<th>Derivation of cells</th>
<th>Mean and its standard error, mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lip</td>
<td>1.42 ± 0.041</td>
</tr>
<tr>
<td>Carcinoma, lip</td>
<td>0.47 ± 0.051</td>
</tr>
<tr>
<td>Papilloma, skin</td>
<td>1.25 ± 0.032</td>
</tr>
<tr>
<td>Normal cervix</td>
<td>1.11 ± 0.039</td>
</tr>
<tr>
<td>Carcinoma, cervix</td>
<td>0.18 ± 0.022</td>
</tr>
</tbody>
</table>

Each figure is based upon 50 pairs of cells and represents the
mean (with its standard error) of the force in mgm. required to
separate the cells by micromanipulation. It is seen that the values
for carcinomatous cells are much lower than those for normal cells
and for cells from papillomas.
Figs. 1 to 3.—A pair of living squamous epithelial cells from a normal lip being separated from each other by microneedles. In Fig. 1 a needle has been placed in each cell. In Fig. 2 the needles have been moved apart, stretching the cells, which are thereby distorted. Tension lines appear in the cytoplasm as the cells cling to each other tenaciously. In Fig. 3 the cells have finally separated and have retracted into approximately their former shape. The needles are widely separated.

Figs. 4 to 6.—A pair of living squamous epithelial cells from a carcinoma of the lip being separated from each other by microneedles. In Fig. 4 a needle tip has been placed in each cell. In Fig. 5 the needles have been moved only slightly apart and the cells begin to separate. Note the lack of distortion in these cells as compared to Fig. 2. In Fig. 6 the cells have been completely separated by only a slight additional movement of the needles. Notice that the needles in this whole series of manipulations have remained relatively close together as compared with the movements of the needles required to separate the normal cells in Figs. 1 to 3. It is apparent that these carcinomatous cells were far less mutually adherent than the pair of normal cells.
Fig. 7.—Distribution of the forces required to separate, by micromanipulation, pairs of squamous epithelial cells derived from the lip. The solid black columns represent cells from carcinomas of the lip, the crosshatched columns cells from normal lips. With few exceptions the carcinomatous cells are shown to be separable by distinctly smaller forces than are necessary to separate normal cells.

Fig. 8.—Distribution of the forces required to separate, by micromanipulation, pairs of squamous epithelial cells derived from the cervix uteri. The solid black columns represent cells from carcinomas of the cervix, the crosshatched columns, cells from normal cervixes. With few exceptions the carcinomatous cells are shown to be separable by distinctly smaller forces than are necessary to separate normal cells.
are mostly grouped at the lowest values, indicating that they were pulled apart with hardly any resistance, in contrast with the much higher values obtained from normal cervical cells.

It is therefore concluded from these experiments that the assumption of malignancy by squamous epithelial cells of the lip and cervix uteri is accompanied by decrease in the adhesive forces that normally hold these cells together.

**DISCUSSION**

The decreased adhesiveness observed in carcinoma cells of the lip and cervix, as compared with that of normal cells, is of interest chiefly because of its possible bearing on the nature and mechanism of malignancy.

As long as squamous epithelium is held together firmly in sheets there is little likelihood of free penetration of the surrounding tissues, or of cells breaking into vessels and being transported to distant organs. On the other hand, should the adhesive forces that normally bind squamous epithelium together be greatly lessened, then presumably cells might break loose from one another and be free to move off by amoeboid motion into tissue spaces, lymphatics, and blood vessels. These conditions would favor both local invasiveness and distant metastasis.

Thus lessened adhesiveness in these carcinomatous cells suggests a physical basis for the property of malignancy.

In this connection, it is of interest to cite observations of Rous, Beard, and Kidd (7) on the relation of adhesiveness to metastasis. In their studies on the virus papilloma of rabbits they state that: "The virus-induced papillomas frequently penetrate into the blood and lymph vessels, but their cells adhere to one another, retaining the tenacious association that is so evident in the high, peaked surface growths. Instances of unaided metastasis formation have yet to be observed, but slight operative interferences are followed not infrequently by the development of secondary nodules in the lungs."

No attempt has been made in the present study to ascertain the cause of decreased adhesiveness in cells. However, it is well known (4) that decreased adhesiveness may be associated with lack of calcium in the fluid medium. Further, a remarkable decrease in calcium content has recently been found by Carruthers and Sunzteff (1) in chemically induced squamous cell carcinomas of mice. They found that the tissue calcium shows an initial drop soon after application of the carcinogen, and later a second drop when the cells become carcinomatous. Also, reduction in calcium and magnesium was demonstrated by Scott (8) in hyperkeratosis, warts, and in human breast and skin cancers. These observations suggest a possible chemical basis (decreased calcium content) for the lessened adhesiveness of cells from squamous cell carcinomas found in the present study.

**SUMMARY AND CONCLUSIONS**

1. The mutual adhesiveness of normal and of neoplastic squamous epithelial cells from the lip and from the cervix uteri was measured in milligrams by a method dependent upon the bend produced in a microneedle when a pair of cells was pulled apart.

2. Normal squamous epithelial cells from the lip and from the cervix were found to have relatively high values of adhesiveness.

3. Benign neoplastic squamous cells from skin papillomas had values of adhesiveness in the same range as did normal squamous cells.

4. Malignant neoplastic squamous cells from carcinomas of the lip and from carcinomas of the cervix showed mean values of adhesiveness far below that of the normal cells.

5. Decrease in mutual adhesiveness in cells from carcinoma of the lip and cervix uteri may constitute the physical basis for the malignancy of these cells. Such cells, no longer strongly adherent to each other in sheets, would presumably break loose and then be free to penetrate tissue spaces and vessels. Local invasiveness and distant metastasis would thus be promoted.

6. It is suggested that decreased mutual adhesiveness in cells of squamous cell carcinoma may be related to a lowered calcium content of these cells.

**REFERENCES**


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