STUDIES ON CELL DEFORMABILITY

I. Effect of Surface Charge

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ABSTRACT

The deformability of the surface membranes of Sarcoma 37 and Ehrlich murine ascites tumor cells was assessed by the pressure required to suck a hemispherical bulge from these cells into a micropipette. It was shown that treatment with neuraminidase allowed the cells to be deformed with significantly less suction, and that enzymatic treatment also produced a significant reduction in surface charge as determined by measurement of cellular electrophoretic mobility. It is suggested that the increase in cellular deformability may be related to charge reduction, and that the charge at the cell periphery may affect not only the magnitude of the potential energy barriers hindering contact between cells, but also the ease with which cells can form low radius of curvature probes in order to help overcome these barriers.

Observations on living cells show their peripheries to be in a constant state of movement resulting in activities ranging from pinocytosis to phagocytosis, and from pseudopod formation to translation of the whole cell. These various types of movement depend on a balance of extracellular and intracellular deforming forces on the one hand, and the deformability of the cell periphery on the other. The present communication deals with the effect of enzymatic removal of the negatively charged carboxyl groups of *N*-acetylneuraminic acid on the deformability of murine ascites tumor cells. It will be suggested that the findings are relevant, with qualifications, to cell peripheries in general.

MATERIAL AND METHODS

Sarcoma 37 and Ehrlich tumors were grown in ascitic form in an inbred strain of Swiss mice, and harvested 9 to 11 days after inoculation. The cells were washed three times in Hanks' saline (pH 7.2), and resuspended in this solution in a concentration of 50,000 cells per milliliter.

Six milliliters of the cell suspensions were incubated at 37°C for 30 minutes with 0.5 ml of neuraminidase (Behringewerke, Marburg-Lahn, West Germany, 500 units/ml), dissolved in 0.05 M of sodium acetate–acetic acid buffer, pH 5.5, with the addition of 9.0 mg/ml sodium chloride and 1.0 mg/ml $CaCl_2$. One unit of neuraminidase activity corresponds to a release of 1 μg of *N*-acetylneuraminic acid in 15 minutes at 37°C from orosomucoid in 0.05 M sodium acetate–acetic acid buffer at pH 5.5. Controls were incubated with either boiled enzyme or Hanks' saline alone, as it was subsequently shown that no difference was detectable between these two control groups.

The deformability of the cells was assessed by determining the pressure required to suck a hemispherical bulge of cell into micropipettes of 5–6 μ internal diameter. Cell suspensions were placed under a coverslip on a ridged slide; pressures were measured with a water manometer; the pipettes were made on a de Fonbrune microforge, held in a de Fonbrune micromanipulator, and observed under a magnification of 430 times.

Electrophoretic mobility was measured in the type of apparatus described by Bangham *et al.* (1), with the cells suspended in half strength Hanks' saline which was brought up to "physiological" tonicity

with 5 per cent sucrose when it had a final pH of 7.85. The cells were timed over distances of 25 μ at 28.5°C, with 50 volts applied over a distance of 16.5 cm, when 0.9 milliamperes were flowing.

The experiments reported here were made on three different days, using cells from different animals, and the results were pooled.

RESULTS

Deforming Pressures

The results and their statistical analysis are summarized in Table I, and show that treatment with neuraminidase produces significant increase in cellular deformability.

DISCUSSION

Although the actual measurement of the pressure required to produce a standard deformation in a cell is relatively simple, the precise mathematical analysis of such measurements is complicated, and has been discussed by Cole (2), Mitchison and Swann (3), Wolpert (4), Rand and Burton (5), and Rosenberg (6) among others. Quite apart from the question of such measurements being a complex parameter of membrane tension, elasticity, and rigidity, the problem is further complicated by the possible presence of a higher hydrostatic pressure within the cell than outside, as in the case of the *Arbacia* egg (2) and the human erythrocyte (5).

In this communication, no mathematical analysis of the pressure measurements will be attempted, and the various factors contributing

TABLE I

Effect of Neuraminidase Treatment on Cellular Deformability

	Mean pressure ± SE	No. of observations	Results of t test
	cm H_2O		
Sarcoma 37 ascites tumor cells			
Control	14.15 ± 0.55	40	t = 5.17 with 78 degrees of freedom
Neuraminidase-treated	10.28 ± 0.52	40	$P < 0.001$
Ehrlich ascites tumor cells			
Control	17.57 ± 0.55	60	t = 3.24 with 118 degrees of freedom
Neuraminidase-treated	15.29 ± 0.45	60	$0.01 > P > 0.0$ O1

Electrophoretic Mobilities

The results and their statistical analyses are summarized in Table II, and show that treatment with neuraminidase produces a significant decrease in cellular electrophoretic mobility.

TABLE II

Effect of Neuraminidase Treatment on Cellular Electrophoretic Mobility

	Mobility ± SE	No. of observations	Results of t test
	μ *.sec.$^{-1}$ volts^{-1} .cm*		
Sarcoma 37 ascites tumor cells			
Control	−1.119 ± 0.052	40	t = 21.43 with 69 degrees of freedom
+ Neuraminidase	−0.616 ± 0.051	31	$P < 0.001$
Ehrlich ascites tumor cells			
Control	−1.206 ± 0.066	32	t = 15.94 with 75 degrees of freedom
+ Neuraminidase	−0.793 ± 0.051	45	$P < 0.001$

to the ease or difficulty with which part of a cell periphery may be drawn up into a micropipette will be covered by the blanket term "deformability," which is considered to have physiological significance in spite of its deliberate mathematical vagueness. The experimental results summarized in Table I indicate that after treatment with neuraminidase both types of ascites tumor cell show a highly significant increase in ease of deformability.

The measurements summarized in Table II show that after incubation with neuraminidase there was a highly significant decrease in the electrophoretic mobility of the tumor cells. In the Helmholtz-Smoluchowski (7) equation

$$\mu = \frac{\sigma}{K\eta} \tag{1}$$

the electrophoretic mobility, μ, is related to charge density at the cell periphery (σ), its Debye-Huckel parameter ($1/K$), and the viscosity of the suspending medium at the electrophoretic plane of shear η. Microscopical observation of the cells does not show a viscous exudate, following neuraminidase treatment, of the type seen, for example, following treatment with high pH salines (8). It is therefore assumed that changes in electrophoretic mobility are entirely due to changes in charge density.

The neuraminidase used was free of proteolytic activity, as determined by Anson's (9) and Kunitz' (10) methods of assay with hemoglobin and casein, respectively, as substrates. No aldolases are detectable by Bruns' (11) technique with fructose 1,6-diphosphate as substrate. No phospholipase (lecithinase C) activity was detectable with the technique described by Pillemer and Roth (12). Incubation of the neuraminidase with olive oil for 30 minutes at 37°C revealed no detectable lipase activity as evidenced by free fatty acids (13). It is therefore likely that the effects of the enzyme are due to its activity in removal of charged terminal sialic acid units.

In his studies of the physical properties of ovine submaxillary gland sialoprotein, Gottschalk (14) suggested that the presence of charged carboxyls on the terminal *N*-acetylneuraminic acids, which form part of the prosthetic groups of these mucoidal materials, would confer structural rigidity on the underlying protein core. It is well known that the sialic acids are structural components of every mammalian cell so far examined (15–17), whether they are linked to cellular protein or to lipid. Removal of the terminal *N*-acetylneuraminic acid at the cell periphery may be readily accomplished with neuraminidase, and this enzymatic cleavage may readily be verified by measurements of electrophoretic mobility. It must be emphasized that measurements of electrophoretic mobility only indicate the electrokinetic properties of cells at the plane of shear, and that "deeper" ionized carboxyl groups not electrically reflected at this plane may well be removed without change in mobility. As also pointed out by Simon-Reuss *et al.* (18), the neuraminidase-induced release of esterified sialic acids from the cell peripheral zone would also not be reflected in change of cellular electrophoretic mobility, although among cells so far characterized this does not appear to be a major consideration. Thus, although mobility measurements probably indicate less than the total loss of charge, and possibly do not indicate fully the loss of sialic acid moieties occurring through the full depth of the peripheral zone of cells, it still remains a distinct possibility that there is a causal relation between the reduced mobility and the increased deformability in these cells after treatment with neuraminidase.

It is not suggested that the only way in which charged carboxyl groups of terminal sialic acids act in this context is by conferring structural rigidity on tangentially oriented protein molecules at the cell periphery, since they may also be linked to lipids as in the horse erythrocyte (19), and not all lipid-bound sialic acids are removable by neuraminidase (20). Pilot experiments by Horwitz and Weiss (21) also suggest that reduction in surface charge is accompanied by increase in deformability of Sarcoma 37 cells and erythrocytes. However, the enzyme used was of doubtful purity and contained preservative, and we were unable to ascertain whether all the effects on deformability were simply due to charge reduction, or were in fact due to other degradative changes in the cell periphery.

As pointed out in a number of recent reviews, including those of P. Weiss (22), Curtis, (23), Steinberg (24), L. Weiss (25), and Moscona (26), the ability of cells to distinguish self from non-self, and to act on this information, depends on the ability of the cells to first make contact with one another. The resulting cellular activities may be

the rearrangements seen in organizing morphogenetic systems, the cellular infiltrations seen in inflammatory and neoplastic diseases, the adhesion of cells to vessel walls in immunological reactions and metastasis, or in phagocytosis. A potential energy barrier V_R, which is generally covered by equation (2), tends to prevent cells from making contact (27, 28):

$$V_R = \tfrac{1}{2} Da \psi_0^2 \log_e(1 + e^{-kd}) \qquad (2)$$

(where ψ_0 is small, and $kd \gg 1$) where D is the dielectric constant of the medium; a is the radius of curvature of the cells or cellular projections; ψ_0 is the surface potential; $1/k$ is the Debye-Huckel characteristic parameter; and d is the distance separating the approaching projections. The physicochemical considerations of this type of contact have been discussed by Bangham and Pethica (27–29). The suggestion was made by Bangham and Pethica that an effective reduction in the potential energy "barrier" to cell contact would take place if the radius of curvature of approaching surfaces was reduced. The biological relevance of this suggestion has received support from Lesseps' (30) and Taylor and Robbins' (31) electron micrographs of contacting cells, which show that cell contact is made *via* low radius of curvature probes in their systems. In an attempt to relate cellular locomotive pressure to cell contacts, Weiss (32) concluded that among the main physical factors determining whether or not contact between cells occurred was the magnitude of their locomotive pressure supplemented by their ability to put out low radius of curvature probes. The present communication suggests that if the experimental observations and the speculative conclusions drawn from them are generally applicable, then surface charge may well be an important factor regulating not only the magnitude of the potential energy barriers to contact (ψ_0 in equation (2)), but also the ease with which cells can deform their peripheries to form probes (a in equation (2)) to help overcome these barriers.

It should be made quite clear, however, that the speculations advanced here are at best relevant to only one aspect of cell contacts, and that they do not indicate the quantitative importance of potential energy barriers in cell contact phenomena under physiological conditions, where other factors are also thought to play a role (33, 34).

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