sorption by DS in the case of EMC virus. The action of DEAE-dextran in removal of inhibition by binding of the sulfated polysaccharides is probably entirely extracellular and unrelated to the action of L-cystine.

Summary. DEAE-dextran was shown to increase plaque diameter of poliovirus *m* mutants m_{alpha}, m_{pi}, and $m_{epsilon}$, and slow Mahoney and wild-type echoviruses 5, 8, 11, and 26, but it did not affect plaque diameter of wild-type polioviruses tested. DS and agar extract slowed completion of CPE by poliovirus *m* mutants and echoviruses; however, wild-type polioviruses were not affected. The increase or decrease in plaque diameters of poliovirus mutants and echoviruses was dependent on increased concentrations of DEAE-dextran and DS, respectively. The effect of DEAE-dextran on e.o.p. of poliovirus mutants and echoviruses was related to their plaque development period. L-cystine did not affect plaque size of poliovirus *m* mutants nor wild-types of Mahoney, Theiler's Lansing, and Charbonneau; however, it increased plaque diameters of Brunhilde and Akron wild-types and echoviruses 5, 8, 11, and 26.

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Intercellular Forces and Separation Energy of Red Cells Sludged by Contrast Medium.* (30060)

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(Introduced by J. S. Lee)
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Knisely and his coworkers showed that sludging of red blood cells occurs in over 100 pathological conditions, or, alternatively, in the presence of excess of a contrast medium such as sodium diatrizoate(1). Bernstein and his coworkers enumerated possible intercellular forces of repulsion and attraction which could be altered with the occurrence of such red cell sludging(2), while Bloch and his coworkers reported that this sludging involves the formation of a sticky coating around the sludged red cells(3). This coating is observable with the electron microscope but usually not with the light microscope. The data given here were obtained by applying Berwick and Coman's method(4) to measurement of the adhesion forces and energy of sludged red cells, and these data are combined with microelectrophoresis findings to show that viscosity

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and/or surface energy of the sludge are more important than ionic forces.

Methods and results. Both heparinized and citrated dog blood was tested in both micromanipulation and in microelectrophoresis studies. A standard 75% diatrizoate solution was added to produce cell sludges, and both clumped and unclumped cells were studied.

For micromanipulation an Aloe Co. pneumatic micromanipulator (of De Fonbrune type) and a semimicromanipulator, made by W. R. Price and Co., Ltd., were used in conjunction with a binocular microscope. Eyepieces with grids in one and 2 orthogonal directions, respectively, were inserted in the microscope, and its oil immersion lens was removed for convenience. The microneedles were prepared as described by Jones(5), and were calibrated with microweights at several positions on each needle. The microweights were made of high-grade steel wool and human hair (which was sometimes split). The microweights were weighed to less than 0.002 mg on a torque balance, and the calibration was reconfirmed after several days of experimentation.

The microelectrophoresis was done with instrumentation like that of Bernstein *et al* (2), except that a plastic cell-holder was added to introduce 4 bolts that could be adjusted for levelling. For a simpler calculation of the results than that of Bernstein *et al* one compares the electrophoretic motion (horizontal in the electric field), of a given cell or clump, to the settling motion of the same cell or clump (vertical and gravitational with the electric field turned off). The steady settling rate can be observed in a relatively short time by measuring the rate at which the tube of the microscope must be lowered in order to keep the cell or clump in focus. Since both the electrophoretic and settling rates depend on the orientation of the moving cell or clump, which may be unsymmetric, an attempt was made to obtain and use data for cases in which either both motions occurred with similar orientations or else the cell or clump was approximately spherical. In such cases the charge can be calculated as:*

$$q = V_{cl}(\rho_{cl} - \rho_{pl})\, g\, v_{el}/E_x\, v_{se} \qquad (1)$$

in Coulombs per cell or clump, if

V_{cl} = cell or clump vol. in cm^3,
ρ_{cl} = cell or clump density in g/cc,
ρ_{pl} = plasma or surrounding liquid density in g/cc,
g = 980.6 in cm/sec.^{2},
v_{el} = electrophoretic velocity in μ/sec.,
v_{se} = settling velocity in μ/sec., and
$E_x = 10^7 \times$ the potential gradient in volts/cm.

In these studies there was much indirect evidence for a clump-enveloping sludgy phase like that demonstrated by Bloch and his coworkers with the aid of electron microscopy (3). Although our microneedles had much smaller diameters than did any of the red cells, it was never possible to intentionally pierce a red cell, and it was done accidentally only once. In spite of this it was possible to interpose either the shorter-tipped (and pulling) needle, or the longer-tipped (and force-measuring) needle between adjacent cells of a group and still have them move or stand together.

Even in a rouleau formation there was a nearby intercellular attachment that was weak enough to separate without breaking the somewhat stronger attachment between the cells on the two sides of the needle, and when the neighboring intercellular attachment did break it broke in a stepwise fashion that turned the adjacent parting cells as if they had been attached by bits of sticky material at discrete points on their contacting convex surfaces. The force required to break a rouleau string is less than 10^{-4} dyne, which is the smallest force we could have measured.

As mixtures of blood and 75% diatrizoate were made up with increasing fractions of diatrizoate, the red cell clumps that appeared were first approximately round and were of increasing size and number of cells. Later, with at least 9% by volume of diatrizoate, the clumps were of both the round type and a stringy type. Each stringy-type clump of

* In the works of Bernstein and coworkers (ref. 2 and earlier articles) the charge values were too high by a factor of 10^7 because one of us (R.L.E.) supplied a formula that neglected the conversion factor between ergs and Joules.

red cells contains a string of cells which move and are pulled as if they are attached by a string consisting of the sticky transparent phase. Often several loops of a single string were caught by the needle. As each successive red cell was pulled around the needle the pulling force would increase slightly. For both types of clumps the red cell separation forces and energies have been measured, but only with the round type of clump has it been possible to measure the net excess of electric charge (because of ambiguity in the observed settling).

In the pulling apart of several red cell clumps a diminution of the pulling force was followed by a slight reversal of the separating motion—an elastic type of response. For this reason we believe that the sticky cell coating is an elastoviscous material.

In separating single red cells from round-type clumps the maximum force was always about 0.001 dyne, and this maximum pulling force was attained within a movement distance of about 2 μ. To estimate the energy consumption of this separation one notes that this energy, in ergs, is given by

$$\Delta E = \int_{x_i}^{x_f} F(x)\, dx \qquad (2)$$

if F(x) is the force in dynes as a function of distance x and if x_i and x_f are the respective initial and final distances of needle separation in cm. In the above case of a red cell being separated from a round-type clump we assume the force F(x) to increase linearly with separation x, which would imply that a maximum value for the energy consumption of the separation is 10^{-7} erg.

In Table I the force and distance values are given for the breaking of a single strand and of 2 parallel strands among the stringy-type clumps that were produced in a sample of heparinized dog blood. These illustrate the 2 shapes of force function, F(x), that were observed, as well as the range of forces and distances that were found. The energy changes in these 2 experiments were about 3×10^{-6} erg and 5×10^{-5} erg, respectively, according to calculation by means of equation 2 and the assumption of linear functions F(x) between the successive pairs of points given in Table I.

TABLE I. Separation Forces in Two Cases as a Function of Distance Moved.*

	Force $\times 10^4$, dynes, as a fct of dist, μ						
Distance, μ:	24	72	120	168	192	264	312
Exp 1	–	1	–	1	–	*1.4*	
" 5	2	10	13	20	24	–	*24*

* Exp 1 broke one string and Exp 5 broke 2 parallel strings, but in breaking either one or two strands the force function can be either of the plateau type or of the increasing type. The maximum force is italicized in each case, and in the case of another single strand in the same mixture the maximum force was 10^{-3} dyne—which occurred at a breaking separation of 144 μ.

In a few experiments with stringy-type clumps in citrated blood, some larger forces were observed at somewhat shorter breaking distances. These differences, however, could have resulted from slower manipulation and consequent drying in earlier experiments.

These measured separation forces and energies should be compared to those that could be expected to arise from either ionic bond changes or from the energy requirements of increasing the interfacial surface area during the separation of neighboring cells.

To test the possibility of ionic bond changes during cell separations the electrophoretic and settling rates of red cells were measured. These cells were present in both the unclumped state and within round-type clumps. (Meaningful settling motions of stringy-type clumps were not observable.) Using a voltage gradient of 6.0 volts/cm, it was found that with a density difference of 0.04 g/cc the electrophoretic velocities of single cells averaged 17 times greater than the respective settling velocities of the same cells. The equivalent average ratio for round-type clumps was about half as large. Since the volume of each of the red cells studied was almost 100 μ^3, it can be shown with the aid of equation 1 that each such cell carries a net negative charge of about 10^{-15} coulomb. Similarly, each cell component of a round-type red cell clump has about half as much net negative charge. Since the sign of the net charge is the same for both clumped and unclumped red cells the net ionic forces cannot account for the energy

changes associated with clumping. Furthermore, the magnitude of the net red cell charge is very small and was never reduced by more than about half when the red cells clumped. To show the smallness of the net charge magnitude one has only to assume a cell count of 5×10^6 cells/mm^3 and to calculate that if the excess cellular charges were distributed uniformly throughout the solution then they would have a concentration of only about 50 nanomoles/liter.

Although the ionic effects are thus of a minor magnitude compared to the observed energy requirements for separating sludged red cells, the roles of surface energy and viscous frictional losses remain to be considered. To test the possible role of surface energy changes the above energy and force data can be combined with Norris' observations of the surface energies of large nucleated red cells (about 1 erg/cm^2)(6), and with reasonable estimates of the surface area changes that accompany sludging. This consideration shows that surface energy changes could be large enough to account for major portions of at least some of our observed total energy expenditures. The surface energy of a given interface, however, is usually proportional to the area of that interface, while the observed energy consumptions did not appear to vary with pulling distance in a way that would be consistent with that proportionality.

Specifically, if we assume that the intracellular sludge strings remain cylindrical during stretching, then the surface area and surface energy should increase as the square root of the growing intercellular distance ($x_f - x_i$ in equation 2. Actually, though, the observed forces were all like those of Table I in increasing monotonically with that distance—so that the energy requirement always increased as either the first power, or a higher power, of that intercellular separating distance. Hence it follows that at least a major part of the observed energy requirements for separating sludged cells is still unexplained and may later be shown attributable to more complicated phenomena, such as those of viscous flow.

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In vivo Effects of Ouabain on Calcium Metabolism in Rabbit Hearts and Plasma.* (30061)

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There is considerable *in vitro* evidence suggesting that the cardiac glycosides produce their effects on cardiac muscle by altering calcium (Ca) metabolism(1). The present study was undertaken to determine if an effect of the cardiac glycosides (ouabain) on Ca metabolism of the heart could be demonstrated under *in vivo* conditions.

Methods. Rabbits weighing between 1 and 2 kg were used. Ca determinations were done as follows: The rabbits were anesthetized with pentobarbital and the ear vein

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