Red Cell Rheology in Stomatocyte-Echinocyte Transformation: Roles of Cell Geometry and Cell Shape

By Walter H. Reinhart and Shu Chien

The influence of the shape of the red blood cell during stomatocyte-echinocyte transformation on its deformability was studied by microsieving through pores with diameters of 2.6, 4.5, and 6.9 μm. A stomatocytic transformation was produced by chlorpromazine (0.02, 0.1, and 0.5 mmol/L) and an echinocytic transformation by sodium salicylate (7.5, 30, and 120 mmol/L). For spherostomatocytes, an increase in filtration resistance through 2.6 and 4.5 μm pores was observed, whereas for spherochromeinocytes, a decrease in filtration resistance through 2.6 μm pores was found. Larger pores (6.9 μm) were not sensitive to those shape changes. The changes in deformability can be explained by the fact that the surface area of (sphero-)stomatocytes decreased, whereas that of (sphero-)echinocytes increased; the cell volume remained essentially constant. Echinocytes produced by 24-hour adenosine triphosphate depletion differed from drug-induced echinocytes: they had an increased cell volume at constant surface area and consequently an increased filtration resistance through 2.6- and 4.5-μm filter pores. Shape changes with spicule formation are therefore not a homogeneous entity, and cell geometric factors (e.g., surface area and volume) must be assessed with care. The viscosity of red cell suspensions at a hematocrit level of 45% was higher for drug-induced echinocytes than discocytes or stomatocytes at all shear rates tested. We conclude that the normal discocyte represents an optimum shape for the flow in vivo since a stomatocytic transformation could impair the passage through the microcirculation (decrease in cell filterability) and an echinocytic transformation could impair the flow in larger vessels (increase in blood viscosity).

THE SHAPE of a normal red blood cell (RBC) is that of a biconcave discocyte. It is well recognized that this shape represents an equilibrium state between two opposing extremes of red cell shapes, i.e., the stomatocyte and the echinocyte.1 Red cells can easily undergo either transformation in vitro under the influence of certain agents.1 These changes are reversible upon the removal of the causative agent or the addition of an antagonist.1 A stomatocytic transformation is characterized by a tendency of membrane internalization and echinocytic transformation by membrane externalization. It has been suggested that stomatocytic transformation could be caused by a preferential incorporation of the causative agent in the inner hemileaflet of the membrane bilayer and echinocytic transformation by a predominant incorporation in the outer hemileaflet.2 The red cell volume remains virtually unaltered3 in both types of transformation.

This stomatocyte-discocyte-echinocyte equilibrium provides a unique in vitro model system to study the influence of RBC shape on the rheologic behavior of blood. The flow properties of blood in large vessels are governed primarily by the hematocrit level, plasma viscosity, RBC aggregation, and RBC deformability, which are the major determinants of whole blood viscosity.4 The flow of blood in capillaries is primarily dependent on the deformability of the blood cells, which allows RBCs with a diameter of 8 μm to pass through channels with a diameter of 4 to 5 μm. The deformability of RBCs in such narrow channels is determined by the cell geometry, i.e., the surface area/volume ratio or sphericity of the RBCs, the viscosity of the cytoplasm, and the biophysical properties of the cell membrane (which can be assessed by micropipette technique).4 An in vitro test that can be used to assess the ability of red cells to pass through narrow channels is the filterability measurement by microsieving.5,6

We used filters with different pore sizes to study the influence of stomatocyte-echinocyte transformation on RBC deformability. The ultimate aim of the study was to obtain results from this in vitro model that allow further investigation of hematologic disorders with an abnormal RBC shape, eg, acanthocytosis, elliptocytosis, spherocytosis, etc.

MATERIALS AND METHODS

Blood was drawn from healthy young volunteers into vacutainer tubes containing 20 United States Pharmacopeia U/mL sodium heparin. After centrifugation at 1,000 g for ten minutes the plasma, buffy coat, and the uppermost RBC layer were removed. The RBCs were washed three times in Ringer’s solution containing 0.85 g/dL NaCl, 0.03 g/dL KCl, 0.033 g/dL CaCl2, 0.1 g/dL dextrose, and 0.5 g/dL bovine serum albumin, buffered to pH 7.4 with 0.1 N Tris (final Tris concentration about 0.005 N). Ringer’s solution was prefilled through 0.2-μm Millipore membranes before use. The washed RBCs were resuspended in Ringer’s solution at a hematocrit value of 10.0%. The hematocrit value was determined in duplicate by the microhematocrit method. RBC and residual WBC counts were determined with an electronic particle counter (Coulter Counter Model ZB, Coulter Electronics, Hialeah, Fla). For the assessment of hemolysis, the RBC suspensions were centrifuged and the light absorption of the supernatant measured in a spectrophotometer at 540 nm. Reference values were obtained from the same amount of RBCs in distilled water (100% hemolysis) and from cell-free Ringer’s solution (0% hemolysis).

Fresh stock solutions of chlorpromazine hydrochloride (CP) and sodium salicylate (SA) were prepared for every experiment. The stock CP solution contained 1.5 mmol/L CP in Ringer’s solution, with an osmolality of 300 mosm/kg H2O and pH adjusted to 7.1 with 0.1 N Tris (at higher pH CP was not well soluble); the stock SA solution contained 160 mmol/L SA in distilled water, with an osmolality of 290 mosm/kg H2O and pH adjusted to 7.4 with 0.1 N Tris. The RBC suspension (hematocrit, 10.0%) was divided into
several aliquots. The aliquots were centrifuged (1,000 g for two to three minutes) to sediment RBCs and generate Ringer's solution supernatant. A calculated amount of Ringer's solution was replaced by an equal volume of either stock solution to obtain CP concentrations of 0.02, 0.1, and 0.5 mmol/L (final pH, 7.3) and SA concentrations of 7.5, 30, and 120 mmol/L. These RBC suspensions were then incubated for 20 minutes at 37 °C and used immediately afterwards for all experiments.

For adenosine triphosphate (ATP) depletion experiments, blood was drawn into sterile, heparinized vacutainer tubes. One half of the sample was used immediately for control measurements on fresh RBCs; the rest was incubated at 37 °C for 24 hours. A 24-hour incubation at 37 °C has been shown to deplete the RBC completely of ATP. Fresh and incubated blood samples were centrifuged, the plasma and buffy coat removed, and the RBCs washed three times and resuspended in Tris-buffered Ringer's solution without glucose and albumin (hematocrit, 10%).

The RBC morphology was assessed by scanning electron microscopy (SEM). The RBC suspensions were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.4, 4 °C), postfixed in 2% O3O4 dehydrated in ascending ethanol series, air-dried, and coated with gold-palladium. The RBC suspension was placed between a glass slide and a coverslip. By adding water from one side, an osmotic gradient was generated in the suspension; as a result, RBCs ranging from the initial shape to various degrees of swelling and hemolysis could be observed along the osmotic gradient created. There existed a sharp interface between maximally swollen cells and cells already hemolyzed; the diameter of these spherical cells along that line of interface was measured and the surface area calculated. Using the mean corpuscular volume measured prior to swelling, the sphericity index was calculated (sphericity index = 4.84 × V0.75/A, where V and A are the volume and surface area of the cell, respectively). A perfect sphere has a sphericity index of 1.0.

The RBC osmotic resistance was measured with a routine procedure. The packing capacity of stomatocytes and echinocytes was studied by ultracentrifugation of RBC suspensions at 100,000 g for 15 minutes. This supernatant was replaced by 1% glutaraldehyde, followed by another centrifugation of 15 minutes at 100,000 g. After overnight fixation, the RBCs were processed for TEM.

Red cell filtration was performed as described elsewhere. In brief, the RBC suspensions were placed in a glass syringe and pumped at a constant flow rate of 0.82 mL/min (Model 975, Harvard Apparatus Co, Inc, Millis, Mass) and a temperature of 37 °C through polycarbonate filters (Nucleopore Corp, Pleasanton, Calif) with different pore sizes. The filters had a diameter of 13 mm, a thickness of 13 μm, and nominal pore sizes of 3 μm (lot no. 62C1A70, mean ± SD pore diameter determined by SEM to be 2.6 ± 0.2 μm), 5 μm (lot no. 54C1D7, pore diameter 4.5 ± 0.6 μm) and 8 μm (lot no. 51C9A29, pore diameter 6.9 ± 0.8 μm). The filtration pressure during constant flow was measured on the upstream side close to the filter with a pressure transducer (model MP 45-14, Validyne Engineering Corp, Northridge, Calif) connected to an amplifier (Validyne, model MC 1-3) and a recorder (model 385, Linear Instruments Corp, Costa Mesa, Calif). Prior to the filtration of RBC suspensions, Ringer’s solution containing the same concentrations of CP or SA was filtered to obtain the filtration pressure for suspending medium (Pm); the presence of CP or SA did not affect the Pm value. The initial pressure rise (P1), which reflects the deformability of the RBCs in suspension, was determined for the RBC suspensions. The relative filtration resistance of an individual red cell, β, was calculated as follows: β = 1 - [(P1/Pm) - 1]V/δh, where V is the fraction of the pore volume occupied by the red cell and δ is the fractional volume of RBCs in suspension. This dimensionless parameter β, developed by theoretical modeling, is the ratio of resistance in a pore bearing a RBC to that filled with buffer alone.

The viscosity of suspensions of RBCs in Ringer’s solution containing the drug (CP or SA) was measured with a Couette viscometer, which is a rotational cylinder viscometer, at various shear rates from 0.1 to 208/s.

<table>
<thead>
<tr>
<th>Salicylate (mM)</th>
<th>7.5</th>
<th>30</th>
<th>120</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>SA, 7.5 mmol/L</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>SA, 30 mmol/L</td>
<td>G</td>
<td>H</td>
<td>I</td>
</tr>
<tr>
<td>SA, 120 mmol/L</td>
<td>J</td>
<td>K</td>
<td>L</td>
</tr>
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Fig 1. Scanning electron micrographs of RBCs undergoing echinocytic transformation induced by SA. (A) Normal discocytes. (B) SA, 7.5 mmol/L, with discocytes and echinocytes I. (C) SA, 30 mmol/L, with primarily echinocytes II. (D) SA, 120 mmol/L, with spheroechinocytes.
For statistical analysis a one-way analysis of variance was used. Data on ATP-depleted RBCs were analyzed by Student's t test.

RESULTS

The morphologic changes of red cells after incubation with different doses of SA are shown in Fig 1. A concentration of 7.5 mmol/L SA had a discernible effect on red cell morphology (Fig 1B, discocytes and echinocytes I), 30 mmol/L SA induced a clear echinocytic transformation with predominantly echinocytes II (Fig 1C), and 120 mmol/L SA transformed the vast majority of red cells into spherocytic cells (Fig 1D). The dose-dependent stomatocytic transformation obtained with chlorpromazine is shown in Fig 2. CP at 0.02 mmol/L generated mainly cup-shaped RBCs (Fig 2A), 0.1 mmol/L CP predominantly stomatocytes II and III (Fig 2B), and 0.5 mmol/L CP spherostomatocytes (Fig 2C). Spherostomatocytes had multiple intracellular vacuoles as seen by TEM (Fig 2D). When the extracellular marker ruthenium red was added to the cell suspensions prior to embedding, the vast majority of the vacuoles (more than 95%) were ruthenium red-negative, indicating that these vacuoles were not in communication with the cell surface and the extracellular fluid. By morphometric analysis it was calculated that 3% of the total cellular volume was occupied by vacuoles. The degree of transformation was assessed in three to four different experiments by classifying the shape of 150 to 200 RBCs on SEM pictures. As shown in Table 1, the concentrations of the two antagonistic drugs chosen in this study cover the whole range of transformation from spherostomatocytes to spherocytic cells. Spherostomatocytes and spherocytic cells were not completely reversible, but lesser degrees of transformation fully recovered upon removal of the drug or by adding the antagonist.

Examples of pressure-time curves obtained for discocytes, stomatocytes, and echinocytes are shown in Fig 3. The filtration pressure curves obtained with 4.5-μm pores were not significantly affected by SA, even with the formation of spherocytic cells in 120 mmol/L SA (Fig 3A). CP at concentrations of 0.02 and 0.1 mmol/L also did not have a significant effect on the filtration pressure curves with 4.5-μm pores, but at 0.5 mmol/L, CP elevated the pressure-time curve approximately fivefold. With 2.6-μm pores (Fig 3B), the slope of the pressure-time curve was more flat for echinocytes (30 and 120 mmol/L SA) and steep for stomatocytes (0.1 and 0.5 mmol/L CP). It is possible that the RBC passage did occur primarily through pores with a diameter larger than 2.6 μm (about 12% of the pores are >2.8 μm, ref 6). No differences were found for 6.9-μm pore filters (not shown). The initial pressure (P1) was ascertained on these pressure tracings (Fig 3). For 2.6-μm pores and 0.5 mmol/L CP, the transition of the pressure-time curve (P) was not always clearly definable (Fig 3B); P was therefore defined for 2.6-μm pores as the pressure reading obtained 2 seconds after the onset of the pressure rise, at a time when P was normally found for other CP and SA concentrations.

The ratio β of the resistance in a pore bearing an RBC in transit to that in a pore with Ringer's solution alone was calculated. The results are shown in Figure 4. For the narrow

<table>
<thead>
<tr>
<th>Table 1. Percentage of Red Cell Shapes</th>
<th>Chlorpromazine (mmol/L)</th>
<th>SA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Spherostomatocytes</td>
<td>92 ± 14</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Stomatocytes III</td>
<td>8 ± 14</td>
<td></td>
</tr>
<tr>
<td>Stomatocytes II</td>
<td>44 ± 8</td>
<td>8 ± 8</td>
</tr>
<tr>
<td>Stomatocytes I</td>
<td>28 ± 13</td>
<td>52 ± 28</td>
</tr>
<tr>
<td>Discocytes</td>
<td>6 ± 4</td>
<td>40 ± 22</td>
</tr>
<tr>
<td>Echinocytes I</td>
<td>4 ± 5</td>
<td></td>
</tr>
<tr>
<td>Echinocytes II</td>
<td>53 ± 7</td>
<td>2 ± 4</td>
</tr>
<tr>
<td>Echinocytes III</td>
<td>28 ± 7</td>
<td>9 ± 16</td>
</tr>
<tr>
<td>Spherocytic cells</td>
<td>88 ± 20</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± SD.
STOMATOCYTE-ECHINOCYTE TRANSFORMATION

Fig 3. Pressure-time curves obtained for the filtration of suspensions with a fixed number of RBC \( (0.98 \times 10^8/\mu L) \) at a flow rate of 0.82 mL/min (resulting in a cell flux of \( 8 \times 10^8 \) cells/min) through filters with a pore diameter of 4.5 \( \mu \)m (A) and 2.6 \( \mu \)m (B). Controls, stomatocytes (0.1 and 0.5 mmol/L CP), and echinocytes (30 and 120 mmol/L SA) are shown. \( P_0 \) is the pressure reading for Ringer’s solution alone. \( P_t \) is the initial pressure for the RBC suspension.

filter pores with a diameter of 2.6 \( \mu \)m, the increasing degree of (sphero) stomatocytosis induced by CP tended to increase the filtration resistance (\( \beta \)) of these red cells, whereas the increasing degree of (sphero)-echinocytosis induced by SA tended to decrease \( \beta \); the changes being statistically significant at the highest concentrations of SA and CP used. Spherostomatocytes (0.5 mmol/L CP) also caused a significant increase in \( \beta \) for 4.5-\( \mu \)m pores, but filtration through 6.9-\( \mu \)m pores was insensitive to these shape changes.

Hemolysis in the suspensions before and after filtration was less than 1% for all shape transformations except spherostomatocytes (0.5 mmol/L CP) where hemolysis was 0.5% before filtration and 80% in the filtrate.

The geometric characteristics of stomatocytes and echinocytes are shown in Fig 5. The MCV showed a trend to be slightly higher in 0.5 mmol/L CP and slightly lower in 120 mmol/L SA as compared with discocytes; the difference was not statistically significant. The surface area of normal RBCs measured with our swelling method was 135 ± 12 \( \mu m^2 \); this agrees with the results of Linderkamp et al. who obtained 134 ± 14 \( \mu m^2 \) with the micropipette method.

RBC surface area was lower for 0.5 mmol/L CP and higher for 120 mmol/L SA. The intracellular vacuoles seen in spherostomatocytes (Fig 2D) may account for the loss in surface area. Spheroechinocytes, incubated at 135 mosm/kg H\(_2\)O for maximum swelling, were examined by TEM. It was found that all spicules were smoothened out by that procedure (not shown). The sphericity index was significantly higher for RBCs in 0.5 mmol/L CP and lower for RBCs in 120 mmol/L SA (Fig 5). This was confirmed by morphometric analysis of TEM pictures of spherostomatocytes and spheroechinocytes (not shown).

The difference in sphericity between spherostomatocytes and spheroechinocytes is illustrated in Fig 6. Spheroechinocytes showed a capacity to deform in centrifugal packing, with the disappearance of spicules (Fig 6B), and the transmission electron micrographs of centrifugally packed spheroechinocytes are similar to those of the packed discocytes. Packed spherostomatocytes were composed of undeformable spheres, and a significant degree of centrifugal packing was attained by the intercalation of less severely transformed cells that still had sufficient deformability to fit between the spheres (Fig 6A).

The relationship between cell surface area and volume was further tested by determining the osmotic resistance (Fig 7). The osmotic resistance was increased for echinocytes (30 and 120 mmol/L SA) and decreased for stomatocytes (0.1 mmol/L CP). For spherostomatocytes (0.5 mmol/L CP) complete hemolysis occurred above 0.6% saline.

The RBC morphology observed after metabolic depletion by incubating whole blood for 24 hours at 37 °C is shown in Fig 8A. The cell shape ranged from discocyte to echinocyte III. When these cells were washed and incubated in Ringer’s solution containing 0.5 g/dL albumin, they regained a discoid shape (Fig 8B), which has also been observed by Ferrell and Huestis. Sometimes the shape reversal even showed an overshoot, resulting in cup-shaped RBCs. Depleted RBCs washed and resuspended in buffered saline remained echinocytes (Fig 8C); the degree of echinocytic transformation became even more pronounced when the depleted RBCs were transferred from plasma to saline (Fig 8A). Fresh cells incubated in buffered saline for 20 minutes at 37 °C showed no echinocytic transformation (Fig 8D).
The results of filtration studies on 24-hour-depleted RBCs washed and resuspended in Tris-buffered saline are shown in Fig 9. The relative filtration resistance ($\beta$) in 2.6-$\mu$m pores for the echinocytes induced by 24-hour depletion was more than twice as high as that for the discocytes from the same donor studied immediately after blood withdrawal. $\beta$ was slightly but significantly increased for 4.5-$\mu$m pores also, whereas 6.9-$\mu$m pores were insensitive.

The geometry of the depleted RBCs is shown in Fig 10. The 24-hour depletion did not affect the surface area, slightly increased the cell volume, and therefore increased significantly the sphericity index ($P < .05$). These geometric results, as the filtration data, are in contrast to those obtained with drug-induced echinocytosis.

The morphology of RBCs was unaffected by the filtration procedure for stomatocytes as well as echinocytes (Fig 11). This indicates that despite the differences in filtration resistance there was no noticeable selective retention of cells with a higher filtration resistance by the filter and that the shape transformation is not altered after the mechanical shearing during filtration.

Neither drug affected the viscosity of the suspending medium alone (the same value of 0.75 CP for control, 0.5 mmol/L CP, and 120 mmol/L SA). Viscosity measurements of RBC suspensions in Ringer's solution (Fig 12) showed that spheroechinocytes (120 mmol/L SA) had a significantly higher viscosity than discocytes (control) and spheroematocytes (0.5 mmol/L CP). The difference was more pronounced at low shear rates.

**DISCUSSION**

Stomatocytic and echinocytic transformations of red blood cells are dose dependent. The degree of transformation for a given drug concentration found in the present study agrees with previous reports. The concentrations used for shape transformations are higher than the therapeutic plasma concentrations, which are about 0.001 mmol/L for chlorpromazine and 2 mmol/L for salicylate.

The stomatocyte-echinocyte transformation of RBCs affected their filterability through narrow pores. The sensitivity of the filtration test increased with decreasing filter pore size, which is in agreement with a previous study. For a pore size of 2.6 $\mu$m, spheroematocytic transformation (0.5 mmol/L CP) increased the filtration resistance, ie, decreased RBC deformability, whereas spheroechinocytic transformation (120 mmol/L SA) increased RBC deformability in this system. These changes in deformability can be explained by differences in surface area and cell volume. With spheroematocytic transformation (0.5 mmol/L CP) membrane vacuoles were formed, which became detached from the cellular membrane towards the cell interior. The formation of intracellular membrane vacuoles reduced the membrane surface area and increased the cell volume because of such vacuole formation combined to cause the increase in sphericity, resulting in an increase in the sphericity index to 0.93.

Spheroechinocytes produced by 120 mmol/L SA also had an overall spherical appearance. But they were not spherocytic in a strict sense since the abundant number of little spicules lead to an increase of surface area by about 10%. This excess surface area was available for different types of
cellular deformation, eg, during osmotic swelling of the cells (Fig 7), RBC packing (Fig 6), and filtration. It has been reported that the membrane expansion produced by amphiphilic drugs that are incorporated into the membrane is several times greater than the pure volume of occupation within the membrane. This has been explained on the basis of disordering and expansion of lipid regions, conformational changes, rearrangement of membrane proteins, and/or a change in membrane calcium content. It is possible that these changes, in addition to the incorporation of SA into the membrane, contributed to the 10% membrane expansion found in our study. The volume of spherocytinocytes decreased, although it did not reach statistical significance. This is probably the result of the Gardos effect; salicylate increases the permeability for calcium, which activates calcium-sensitive potassium channels, leading to a loss of potassium and water from the RBC.

Stomatocyte-echinocyte transformation has not been studied systematically with the RBC filtration test. It has been reported briefly that stomatocytes produced by chlorpromazine had a higher filtration pressure than normal discocytes. Other methods that test the deformability of the whole cell have been used. Leblond measured the pressure required to aspirate whole echinocytes into micropipettes with a diameter of 2.8 to 3.0 μm. This approach can be compared with the 2.6-μm pore filters of our study. It was found that echinocytes produced by lysolecithin and pH 8.5 required less pressure than discocytes. This finding is consistent with the lower filtration resistance following SA treatment found in our experiment. Leblond also found that echinocytes produced by oleate required the same pressure as discocytes; echinocytes produced by ATP depletion required pressures ten times higher.

Another approach to the measurement of RBC deformability is to use a system without geometric constraints on individual RBCs as in narrow pores, eg, ektacytometry, high speed centrifugation, and the counterrotating rheoscope. Using these methods it was found that stomatocytes produced by chlorpromazine are less deformable than discocytes and that spherostomatocytes are undeformable, which is in agreement with our results with filter pore sizes of 2.6 and 4.5 μm. Echinocytes III had a similar deformability in ektacytometry as discocytes, but spherocytinocytes were less deformable. With high-speed centrifugation and the rheoscope, echinocytes were less deformable than discocytes. The discrepancy between these findings and our results might be due to differences in methodology in the...
for suspensions of red blood cells treated with 0.1 mmol/L through Nucleopore filters with a nominal pore diameter of 3 gm dyn/cm²) and do not explain the differences. Spicules have slight increase in the mean corpuscular hemoglobin concentration in the case of spheroechinocytes, although study; in volume, the other determinants of red blood cell deformability in a laminar flow field studied the surface/volume ratio,9 those without geometric constraints (eg, deformation in a laminar flow field studied by ektacytometry and rheoscopy). The shear stresses applied in ektacytometry9 and filtration6 are similar (about 100 dyn/cm²) and do not explain the differences. Spicules have been found to disappear at shear stresses above 10 to 15 dyne/cm².10

Although the changes in RBC deformability are explained by the changes in the relationship between surface area and volume, the other determinants of red blood cell deformability, ie, the cytoplasmic viscosity and the viscoelastic properties of the membrane, need be considered also. The corpuscular hemoglobin concentration, which determines primarily the cytoplasmic viscosity, did not change significantly in our study; in the case of spheroechinocytes, although there was a slight increase in the mean corpuscular hemoglobin concentration and an expected increase in cytoplasmic viscosity, the filtration resistance was reduced rather than increased. We are not aware of any study on the influence of chlorpromazine and salicylate on membrane deformability. It has been shown, however, that the filtration of RBCs through small pores is primarily affected by the cell geometry.19 It is therefore likely that the increased sphericity of spherostomatocytes causes the increased filtration resistance and that direct effects of CP on the cell membrane are less important. Leblond28 found that echinocytes produced by oleate or pH 8.5 required twice the negative pressure for a penetration of 1 μm into a micropipette with a diameter of 1.5 μm, indicating a reduced membrane deformability of echinocytes.

From this filtration study, which has a reasonable in vitro analogy of the flow through capillaries in vivo, it appears that echinocytes, at least those produced with SA, would pass more easily through the capillaries than normal discocytes. The question arises as to why the normal RBC is not echinocytic. Echinocytes have a rheologic disadvantage over discocytes, ie, suspensions of echinocytes have a higher viscosity (Fig 12). This is in agreement with an earlier communication by Meiselman1 who attributed the increased viscosity to an enhanced cell-cell interaction or tangling of spicules. The increase in viscosity was more pronounced at low shear rates than at high shear rates. At high shear rates the surface of the echinocyte is gradually smoothened out by the high shear forces, as documented by direct observation in a rheoscope.30 The high viscosity of echinocyte suspension would affect the bulk flow in larger blood vessels. It has recently been shown that echinocytes have also an impaired capacity of oxygen release.32 The decrease in the rate of oxygen release was pronounced at low flow velocities, probably because of a stagnant unstirred layer around the echinocyte. At high flow velocities when the surface was shown to smooth out, the oxygen-releasing capacity returned toward normal.

Probably the best known cause of echinocytic transformation is ATP depletion. When red blood cells are metabolically depleted in vitro for 24 hours or longer, they become echinocytes.7 The whole-cell deformability of those ATP-depleted echinocytes has been studied by several investigators, and there is general agreement that they are less deformable.8,31 This is opposite to our results on echinocytes induced by salicylates. Therefore we performed experiments with 24-hour–depleted RBCs, and the results confirmed the earlier observations of a reduced deformability of these cells (Fig 9). The reason for the discrepancy between the two types of echinocytes may be explained by the fact that the sphericity index increased in ATP-depleted cells, whereas it decreased in salicylate-induced echinocytes. RBCs with the same shape may therefore have different surface area–to-volume relationships and consequently different deformabilities.

The increase in sphericity following ATP depletion was mainly the result of a cell swelling while the surface area remained unchanged (Fig 10); this could be explained by a breakdown of the sodium pump after ATP depletion. Meiselman found the discocyte-echinocyte transformation by ATP depletion to be isovolumic.30 The difference may be due to the fact that he incubated washed RBCs in isotonic buffer solution, whereas we incubated unwashed RBCs in autologous plasma. Earlier communications support our result of an increased cell volume after metabolic depletion.34,35 After incubations of >30 hours, a Gardos effect is observed, ie, losses of potassium and water from the RBCs.8 The 24-hour
depletion in this study did not affect the membrane surface area. It is known, however, that a longer depletion period during blood storage leads to a loss of membrane because of exovesiculation of the membrane. This process would additionally increase sphericity and further decrease the filterability of these cells.

We conclude that echinocytic transformation can either be accompanied by an increase in sphericity, which leads to a decrease in deformability, or by a decrease in sphericity, which causes an increase in deformability. The sphericity and not the shape of a red cell appears to be the more important determinant of RBC passage through narrow channels. Stomatocytic transformation and ATP depletion cause an increase in sphericity and a decrease in red cell deformability. Salicylate-induced echinocytic transformation causes a reduction in sphericity and an increase in deformability. Red cell deformability determines not only the rheology of the circulating blood but also the life span of the individual red blood cell. Our results suggest that the discocyte or shapes close to it represent an optimum shape for the in vivo situation. Stomatocytes with their decreased deformability in narrow channels are probably removed more rapidly by the spleen. The same holds true for echinocytes with increased sphericity as seen after metabolic depletion. Echinocytes with unchanged or even decreased sphericity are probably not removed by the spleen more rapidly, but they impair blood flow because of cell-cell interactions and hence increase blood viscosity.

These results may serve as a basis to study other hematologic disorders with an altered red blood cell shape, eg, hereditary elliptocytosis, spherocytosis, acanthocytosis, as well as acquired disorders with spiculed RBCs.

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Red cell rheology in stomatocyte-echinocyte transformation: roles of cell geometry and cell shape

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