GEOMETRY OF THE HUMAN ERYTHROCYTE

I. EFFECT OF ALBUMIN ON CELL GEOMETRY

ALFRED W. L. JAY

From the Division of Medical Biophysics, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4

ABSTRACT The effects of albumin on the geometry of human erythrocytes have been studied. Individual red cells, hanging on edge from coverslips were photographed. Enlarged cell profiles were digitized using a Gradicon digitizer (Instronics Ltd., Stittsville, Ontario). Geometric parameters including diameter, area, volume, minimum cylindrical diameter, sphericity index, swelling index, maximum and minimum cell thickness, were calculated for each cell using a CDC 6400 computer. Maximum effect of human serum albumin was reached at about 1 g/liter. Studies of cell populations showed decreases in mean cell diameter of up to 6%, area 6%, and volume 15%, varying from sample to sample. The thickness of the rim was increased while that at the dimple was decreased. Studies of single cells showed that area and volume changes do not occur equally in all cells. Cells with lower sphericity indices showed larger effects. In the presence of albumin, up to 50% of the cells assumed cup-shapes (stomatocytes). These cells had smaller volumes but the same area as biconcave cells. Mechanical agitation could reversibly induce biconcave cells to assume cup shapes without area or volume changes. Experiments with de-fatted human albumins showed that the presence of bound fatty acids in varying concentrations does not alter the observed effects. Bovine serum albumin has similar effects on human erythrocytes as human serum albumin.

INTRODUCTION

The deformability of the normal human erythrocyte and therefore its ability to survive in the microcirculation are dependent on the geometry of the cell (Canham, 1969*a*; Jay, 1973). The normal biconcave human red blood cell has approximately 25% more membrane surface area than the minimum required to enclose its volume. This, together with the very high flexibility of the membrane (Rand and Burton, 1964) allows the cell to change its shape readily in response to external mechanical forces. Ponder (1930) was the first to study the biconcave shape of the red blood cell quantitatively. The areas and volumes of individual cells were calculated from cell profiles photographed edge-on. In recent years, the interest in red cell geometry has been revived because of the observed interrelationship between erythrocyte deformability, geometry, packing rate, cell survival, and rheological properties of blood (Jay, 1973; Jay et al., 1972; Rampling and Sirs, 1972; LaCelle, 1970; Canham, 1969*a,b;* Chien et al., 1964*a,b*). Rand and Burton (1963), using Ponder's method, studied the areas and

volumes of hemolyzing erythrocytes, while Canham and Burton (1968) using a similar method studied populations of cells and hypothesized geometric restrictions as a filtering mechanism for selective removal of the older red cells from the circulation. More recently, other methods have been developed for analysis of red cell geometry. Eden (1972) used image processing techniques to study the areas of erythrocytes while Evans and Fung (1972) used image holograms to analyze cell geometry. These techniques require sophisticated technology and expensive equipment. For this reason, the method of analysis using photographed cell profiles is still attractive. The procedure as used by Ponder (Ponder, 1930; Canham, 1967; Canham and Parkinson, 1970) however, involves tedious collection of raw data. Each cell profile has to be traced and divided into parts of equal width along the cell diameter. Equidistant points, about 30 for each cell, are marked along the cell profile and the sum of the perpendicular distances from each point to the axis of rotation of the cell is calculated. These, together with the thicknesses of the cell measured at several locations, provide the raw data for calculation of cellular volume and membrane area. Even with the aid of the computer for subsequent calculations (Canham and Burton, 1968), the time consuming procedure of data acquisition makes the method impractical. In our present study, the procedure is automated through the use of a digitizer, and the analysis of each cell profile takes less than 1 min.

There are several reasons for our interest in the effect of albumin on erythrocyte geometry. Firstly, albumin maintains the biconcave shape of the normal erythrocyte (Ponder, 1971). In slide preparations of cell suspensions, crenation often occurs in cells which come in contact with the glass surfaces. The presence of albumin in small quantities eliminates crenation, and ensures the biconcave shape. Many investigators whose work involves microscopic observation of erythrocytes have used albumin in various concentrations for this reason without much regard for the mechanism of action of albumin on the cell membrane, (Evans and Leblond, 1973). On the other hand, since albumin is normally present in plasma at rather high concentrations, it may be unjustified to carry out investigations in its absence. Thus, a quantitative study of albumin effect on cell geometry is desirable. Secondly, albumin decreases both osmotic and mechanical fragilities of red blood cells (Katchalsky et al. 1960; Blas and Whaun, 1973; Williams, 1973). The observed protection of albumin against cell lysis may be related to the ability of the protein to stabilize the biconcave cell shape. Finally, albumin density gradients are used for separating red cells (Leif and Vinograd, 1964; Kneece and Leif, 1971). If albumin affects cell geometry, it may alter the cell volume and therefore also its density.

EXPERIMENTAL

Blood Samples

Normal human erythrocytes were obtained, by finger-prick, from seven apparently healthy subjects (male and female), and drawn into micro-haematocrit capillaries coated with sodium heparin. About 0.05 ml of the blood was added to 5 ml of an

isotonic Tris-HCl-buffered Ringer solution (10 mM Tris, pH 7.40 ± 0.02, 310 ± 2 mosmol) with or without albumin. The human serum albumin used in most of our experiments was supplied by the Canadian Red Cross and is used clinically for blood supplement. The supply consisted of 25 g normal human serum albumin in 100 ml of salt-poor diluent containing 0.02 M sodium acetyltryptophanate and 0.02 M sodium caprylate as stabilizers. Aliquots of this albumin solution were added to the prepared Ringer solution to make up the required final concentrations of up to 10.0 g/liter. At a concentration of 10.0 g/liter, the osmotic pressure of the Ringer solution was altered by less than 2 mosmol (Osmette osmometer).

The manufacturer[1] describes the albumin as containing a small amount of bound fatty acids. No assay report was given. Since it is very difficult to have completely fatty acid-free albumin, two grades of de-fatted albumin were obtained from Kabi[2] containing 0.2–1.6% and from Sigma[3] containing less than 0.005% bound fatty acids. These came in crystalline form and were added to our Ringer solution to make up the required concentrations. The bovine serum albumin (Fraction V) used was supplied in dry power form by Pentex Biochemicals Co. (Kankakee, Ill.), and was added to the Ringer solution without further preparation.

Apparatus

Fig. 1 *A* shows the type of slide arrangements used for our cell population studies. Two no. 1 glass coverslips were mounted on a glass slide with Corning silicone vacuum grease (Corning Scientific Products Div., Corning, N.Y.), these supported a third making a thin chamber which was closed on two sides. The chamber was filled by touching a small drop of the cell suspension on one of the open sides. Surface tension drew the sample in and filled the chamber. The open sides were sealed with vacuum grease. The slide was inverted for about 1 min to allow the cells to settle on the coverslip. Upon reinversion, many cells were hanging on edge from the underside of the coverslip. A Leitz Dialux microscope (E. Leitz, Inc., Rockleigh, N.J.) with a bright-field 100-power oil immersion objective lens and 25-power eyepieces provided the optics. A Nikon AFM automatic exposure control unit with a half-frame 35 mm camera were used for photography. 50–100 cells in Ringer solution, and about an equal number of cells in Ringer containing 3 g/liter serum albumin were photographed from each of 14 blood samples taken from seven apparently healthy adult subjects. Fig. 1 *B* shows a Lucite cell used to study single erythrocytes. It consisted of a shallow chamber about 1 mm deep, 6 mm wide, and 5 cm long. About half of its length was covered with a glass coverslip and a small hole at the end of the slide connected to a micrometer syringe provided a means of drainage. The covered half of the chamber was filled with a sample of the cell suspension and cells were allowed to hang from the coverslip by inversion as described above. A cell in Ringer solution was observed and

[1] Connaught Laboratories Ltd., Willowdale, Ontario, Canada.
[2] Kabi, Stockholm, Sweden.
[3] Sigma Chemical Co., St. Louis, Mo.

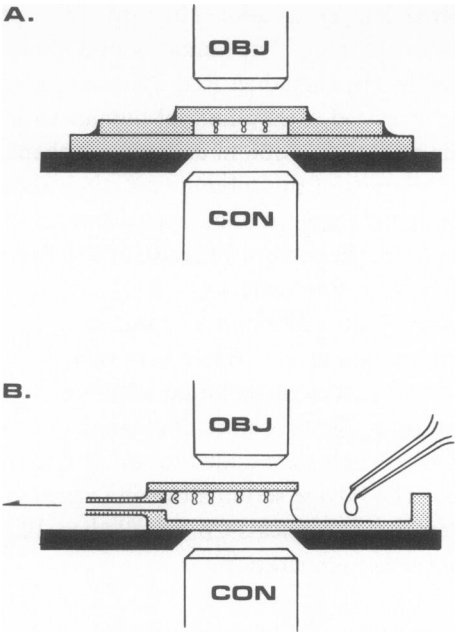

FIGURE 1 Diagrams of the microscope chambers. See text for explanation.

three photographs were taken. The open half of the chamber was then filled with Ringer solution containing albumin. The micrometer syringe was slowly operated until the air-fluid interface returned to the position shown in Fig. 1 *B*. The washing procedure which took about 1 min was repeated twice to ensure complete change of the medium. The cell was again photographed three times.

ANALYSIS

At the beginning of each experiment, a pair of etched-lines on a calibrated stage micrometer was photographed. This photograph and those of the cells were printed at a final magnification of about 15,000 X so that the cell profiles were about 10 cm in length. Photographs of four cells are shown in Fig. 2 *A*. A line was traced along each cell profile such that it followed the outer edge of the dark emulsion at the rims and the inner edge of the dark emulsion at the dimples. The basis for this method of tracing cell profiles has been discussed elsewhere (Ponder, 1930; Canham, 1967). Fig. 2 *B* shows the lines traced along the profiles of the same four cells.

A Gradicon digitizer[4] was used to read the coordinates of points relative to a preset origin, and to record them on magnetic tape or on punched computer cards. The coordinates were recorded to an accuracy of better than ± 0.02 cm (precision of digitizer) by placing the cross hair of the sensing device over the point and pressing a micro-

[4] Instronics Ltd., Stittsville, Ontario.

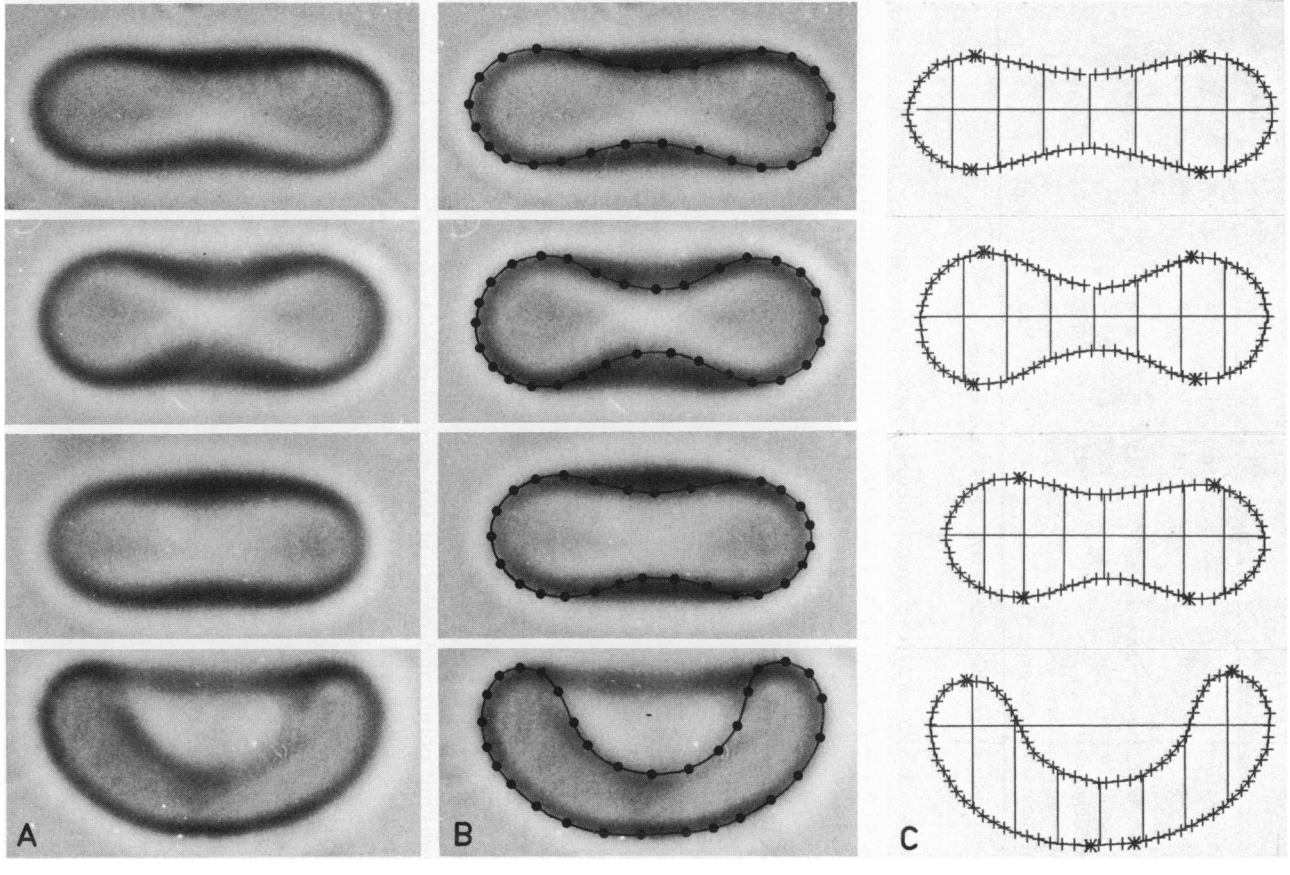

FIGURE 2 First column (*A*), photographs of four cells; Second column (*B*), the same cells outlined and digitization points inserted. Third column (*C*), computer plot of cell showing the generated equidistant points along the outline.

TABLE I

CALCULATED VALUES OF GEOMETRIC PARAMETERS FOR THE FOUR CELLS SHOWN IN FIG. 2

	Cell 1	Cell 2	Cell 3	Cell 4
Diameter, μm	8.04	7.64	7.00	7.48
Area, μm^2	134	129	106	143
Volume, μm^3	99	95	76	89
Minimum cylindrical diameter, μm	3.21	3.19	3.22	2.62
Sphericity index	0.771	0.776	0.822	0.675
Swelling index	47.7	46.3	34.1	80.3
Maximum thickness, μm	2.49	2.76	2.53	3.65
Minimum thickness, μm	1.59	1.35	1.83	1.33

For sphericity index and swelling index see text. Cell 4 is cup-shaped.

switch. In a similar way the prependicular distance between the lines of the stage micrometer was digitized to give a scale factor. The points were digitized in sequence along the traced outline of the cell. The distances between points vary from 0.5 to 1 cm, and were arbitrary. Where the curvature of the cell membrane appeared to be greater, the points were taken closer together. Fig. 2 *B* shows the points digitized for the four cells.

Computer programs were written in Fortran IV for the CDC 6400 computer to do the following: (*a*) Calculate the scale factor. (*b*) Locate and measure the maximum cell diameter, divide it into eight equal parts and measure the thickness of the cell at the seven junctions between the parts. (*c*) Calculate the minimum and maximum thickness of the cell, the maximum being the mean of the two maxima measured on opposite sides of the cell profile. (*d*) Generate a series of equi-distant points along the cell outline, and sum the perpendicular distances measured from each point to the axis of rotation of the cell shape. (*e*) Generate a print-out of the digitized cell outline, showing the equi-distant points and points at the maximum thickness as a check for correct digitization (Fig. 2 *C*), and store the coordinates of these points for future analysis. (*f*) Use the results obtained and assuming cylindrical symmetry, calculate the surface area and the volume of the cell together with all other shape parameters.[5]

Computer programs were also written for statistical analysis, calculation of correlation of correlation matrices, and for graphics. The values of the various parameters for the four cells shown in Fig. 2 are given in Table I.

RESULTS

The results of the observations on populations of cells are shown in Table II. A total of 788 cells in Ringer solution and 843 cells in albumin were analyzed. Values given show the mean value and the standard error of the mean. The percent difference for each parameter between the cells in Ringer and cells in albumin are given in the fifth

[5] The original computer program for part *f* of this procedure was kindly supplied by Dr. P. B. Canham.

TABLE II

EFFECT OF ALBUMIN ON GEOMETRY IN ERYTHROCYTE POPULATIONS

	Canham and Burton (1968)	Cells in Ringer	Cells in albumin	Percent difference	Maximum SEM
N	1,016	788	843		
Percent cup	—	4	21		
Diameter, μm	8.06	8.03	7.72	−3.8	±0.02
Area, μm^2	138.1	136.9	133.4	−2.5	±0.5
Volume, μm^3	107.5	104.2	98.1	−5.9	±0.6
Minimum cylindrical diameter, μm	3.33	3.32	3.19	−4.0	±0.01
Sphericity index	0.773	0.772	0.769	−0.4	±0.001
Swelling index	—	45.0	48.9	8.7	±0.4
Maximum thickness, μm	—	2.65	2.88	8.9	±0.02
Minimum thickness, μm	—	1.60	1.51	−11.7	±0.01

All differences statistically significant, $P < 0.0005$. For sphericity index and swelling index see text.

column. All differences shown are statistically significant, $P < 0.0005$. For comparison, the results of Canham and Burton (1968) are shown in column 2. The distributions of these parameters are shown in Fig. 3. The histograms in rows 1 and 3 are for cells in Ringer solution, the corresponding ones in rows 2 and 4 are for cells in albumin. All the parameters are normally distributed. Scatter diagrams (computer printout) depicting area-diameter, volume-diameter, and volume-area relationships, without and with albumin, are given in Fig. 4. A greater spread is apparent in the albumin-treated cells. Fig. 5 shows examples of the dependence of area and volume on albumin concentration. Fig. 5 on the left shows the change in area and volume of a single cell in response to repeated washings with various concentrations of albumin. Fig. 5 on the right shows a population study of the dependence of mean area and volume on albumin concentration. As in most cases studied, maximum effect is reached at a concentration of about 1 g/liter. In a few instances, up to 3 g/liter was required for maximum changes to occur. Fig. 6 *A* shows a cell in Ringer solution, *B* to *H* show the same cell in albumin at concentrations of 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 g/liter. The same cell was then washed three times with Ringer solution. The shape changes did not reverse, Fig. 6 *I*.

DISCUSSION

The results of 788 cells in Ringer solution are compared in Table II with those of Canham and Burton (1968) for 1,016 cells. The experiments were carried out under identical conditions. Except for cell volume, the results from the two studies are not different. Our results for a mean cell volume is smaller than Canham and Burton's figure by 3%. While part of this difference may have resulted from biological variations, this difference may also be attributed to a slight difference in our method of tracing out the cell profiles on the photographs. While Canham and Burton (1968) traced the cell profiles at the dimple region according to Ponder's method (i.e. by tracing the line at

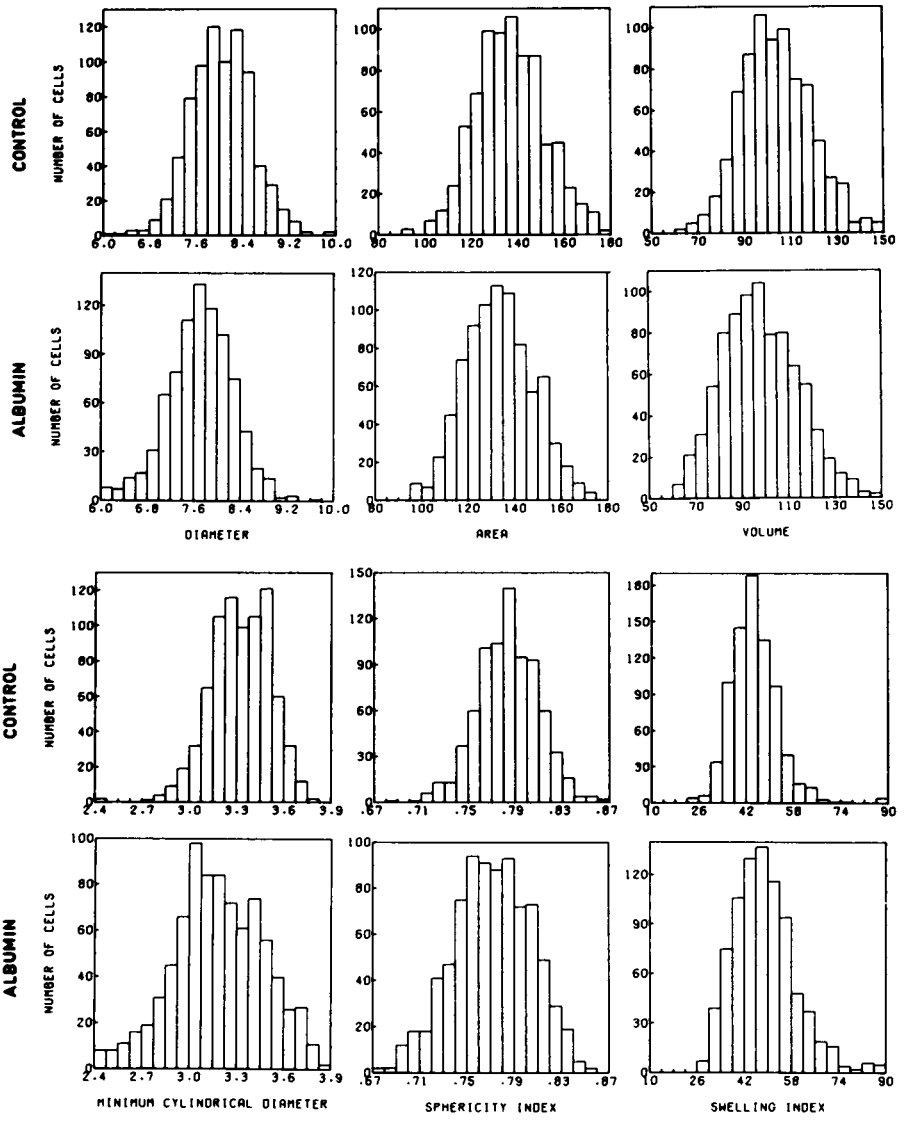

FIGURE 3 Distribution for the first six parameters from Table II. Rows 1 and 3, controls in Ringer solution. Rows 2 and 4, albumin-treated cells.

about 2/3 of the way into the image at the dimple), we traced the cell profiles along the innermost boundary of the image at the dimple.

Concentration Dependence

The effect on most cells reached maximum at an albumin concentration of about 1 g/liter. This suggests saturation of sites of action of albumin on the membrane. Williams (1973) reported similar saturation effects in a study of increased resistance to

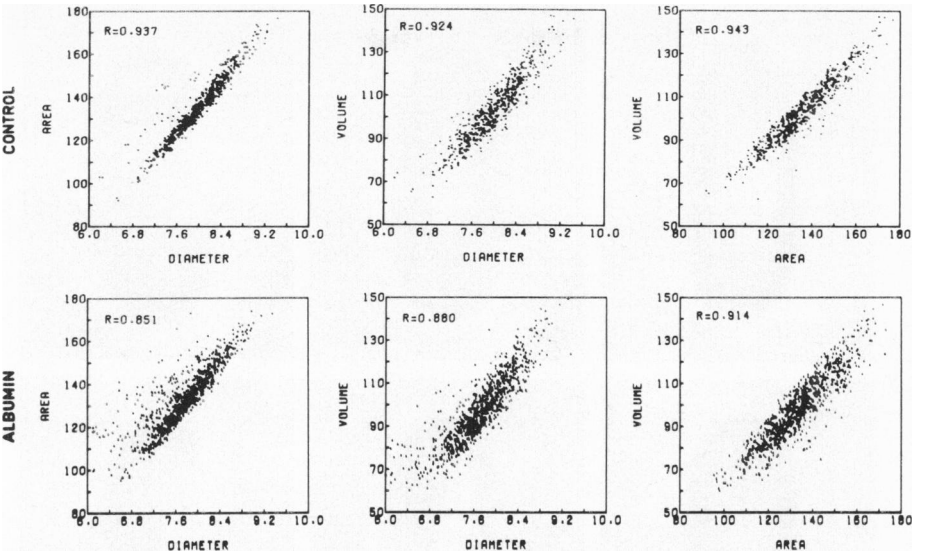

FIGURE 4 Computer plots for the populations of cells of Table II showing relations between area and diameter, volume and diameter, and volume and area. Row 1 controls in Ringer. Row 2, albumin-treated cells.

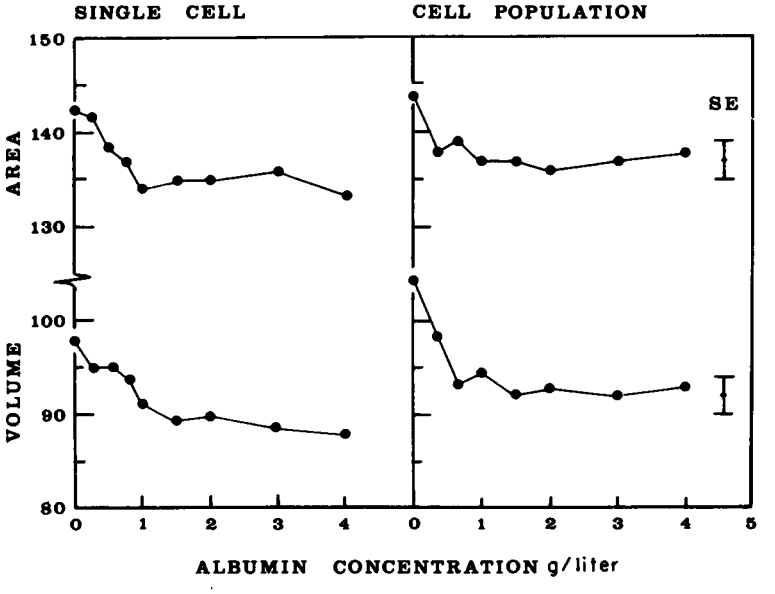

FIGURE 5 Change in area and volume of a single cell (left) and a population of cells (right) in response to changes in the concentration of albumin in the suspending medium.

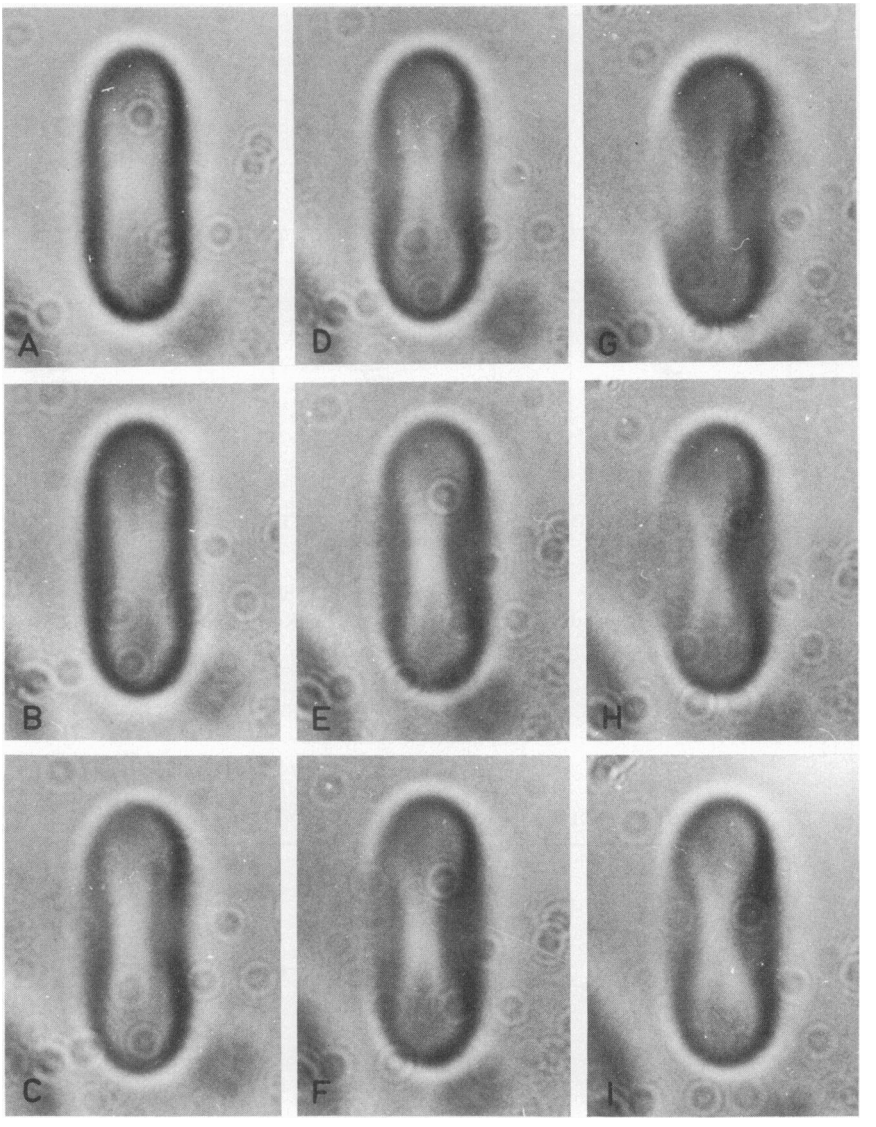

FIGURE 6 Single cell in (*A*) Ringer solution; *B* to *H* in Ringer solution containing 1.0, 1.5, 2.0, 4.0, 6.0, and 8.0 g/liter of albumin, respectively. *I*, same cell washed three times in Ringer solution.

mechanical hemolysis by albumin, and has discussed the mechanism of action as being a replacement of membrane protein by albumin. This may account for the observed irreversibility of albumin effect (see Fig. 6).

Alteration of Cell Shape

While it may be possible that the presence of albumin might slightly alter the imaging properties of the cell membrane, it is assumed that a significant, consistent error has

not occurred. Cells 1 and 3 shown in Fig. 2 were in Ringer solution, cells 2 and 4 were in albumin-Ringer. In the presence of albumin, the shape of the red blood cell changed. The minimum thickness of the cell at the dimple decreased by 12% and the maximum thickness at the rim increased by 9%. The average diameter decreased by 4%. At least part of the diameter decrease came as a result of some cells assuming the cup-shape (named stomatocyte by Bessis, 1974). In samples without albumin, 0–2% of the cell population are cup cells (2 of 12 samples studied had 20% cup cells), whereas in 3 g/liter albumin, the percentage of cup cells ranged from 15 to 50. Crenation of cells is common when they are suspended in Ringer solution. Careful cleaning of the slides and coverslips with ethanol reduces this effect but, even then, up to 2% may be crenated. In the presence of albumin, crenated cells are not observed.

Area

The collected data in Table II show a decrease of mean cell area by 2.5%, from 136.9 to 133.4 μm^2, by albumin. Although the difference appears to be small, it is statistically significant, $P < 0.0005$. A decrease in membrane surface area suggests a condensation of membrane material perhaps to a tighter configuration. This is consistent with our observation (Jay and Rowlands, in preparation) that albumin increases membrane viscosity and decreases red cell deformability.

The change in membrane area in response to albumin varied from sample to sample, ranging from +3 to −6%. In the study of single cells, the change in membrane area ranged from +2.5 to −7.5%. It is puzzling that the membrane area decreases in some cells and remains unchanged or actually increases in others. The direction and magnitude of the change may be related to the original geometry of the cell. Fig. 7 shows

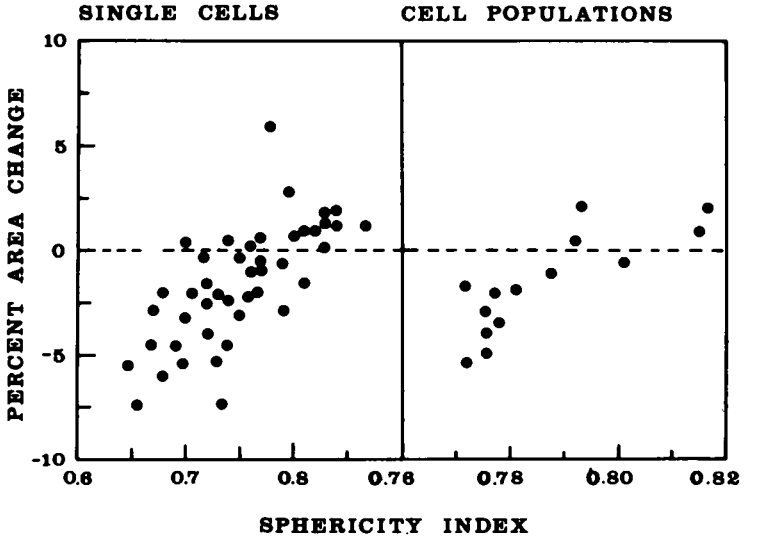

FIGURE 7 Percentage change in cell area after treatment with albumin as a function of the sphericity index of cells before treatment. Left: single cells. Right: cell populations.

plots of percent area change after albumin treatment against the initial sphericity index for single cells and for cell populations, respectively. Although correlation is low, there is some dependence of area change on sphericity index of the cell. The cells which increase in area have a higher sphericity index, on average, than those which decrease their area.

Volume

The mean cell volume for all cell populations decreased by 6% (Table II), while individual populations and single cells recorded decreases as high as 15%. That the volume changes in response to increasing concentrations of albumin show saturation effects (Fig. 5) is evidence that the changes are not the result of osmotic pressure changes. This is further supported by the observation that the addition of 10 g/liter albumin only increased the osmotic pressure by less than 2 mosmol, a change which will not account for as much as 1% reduction in cell volume.

A possible mechanism for this volume change is an alteration of membrane permeability by albumin, such that the normal pump-leak balance of the $Na^+ - K^+$ pump is modified, resulting in an altered set point for volume regulation. In another investigation in which the rate of swelling of single red blood cells was studied (Jay et al., 1974), we have observed membrane permeability to change in the presence of albumin. Fig. 8 shows plots of percent volume change against initial sphericity index for single cells and for cell populations. Again, although correlations are low, there appears to be some dependence of volume change on sphericity index. A decrease in volume of the red cell, presumably due to a loss of cell water without loss of hemoglobin, will

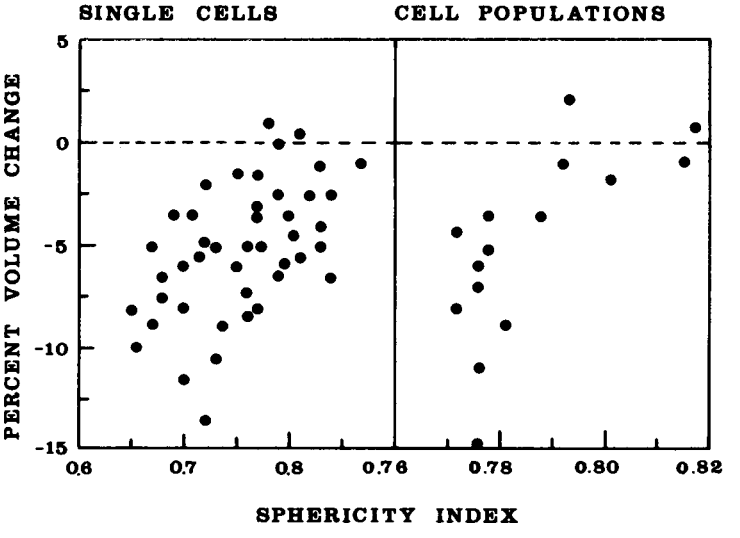

FIGURE 8 Percentage change in cell volume after treatment with albumin as a function of the sphericity index of cells before treatment. Left: single cells. Right: cell populations.

undoubtedly result in an increase in cell density. Furthermore, since the decrease in cell volume is dependent on cell shape, the increase in cell density is not the same in all cells.

Minimum Cylindrical Diameter

Canham and Burton (1968) defined the term "minimum cylindrical diameter" as the calculated diameter of the smallest cylindrical channel which a red cell of a given area and a given volume could pass without area or volume change. In the presence of albumin, the mean value of this parameter decreased by 4% from 3.32 to 3.19 μm. This means that theoretically, the cells would be able to enter smaller passages. The deformability of the red blood cell depends not only on its geometry, but also on the resistance to bending as well as the permeability and the viscosity of the cell membrane (Jay, 1973). The observed decrease in minimum cylindrical diameter by albumin is not paralleled by an increase in cell deformability, as demonstrated by results from other studies (Jay and Rowlands, in preparation), probably because of increased resistance to bending and increased membrane viscosity. In fact, cell deformability is significantly decreased.

Sphericity Index

A parameter which Canham and Burton (1968) have used to describe the shape of the erythrocyte is the "sphericity index." It is equal to $4.84\ V^{2/3}/A$ and has the value of unity for a sphere. These authors have calculated a mean value of 0.773. In our present study, the mean value for cells in Ringer solution was 0.772. In the presence of albumin, the mean value decreased to 0.769. The difference between our control and albumin studies, although small, was statistically significant, $P < 0.0005$. The lower mean sphericity index came mainly as a result of decreased cell volume.

Swelling Index

While the mechanical fragility of the red cell is dependent on the mechanical strength of the cell membrane, the osmotic fragility of the cell, an often used clinical laboratory test, is dependent mainly on the ability of the cell to accommodate volume increase in response to hypotonic environment. We have defined the term "swelling index" equal to the percent swelling required by a cell to reach the spherical shape. It is the difference between the hemolytic and isotonic volumes of a cell expressed as a percent of the initial or isotonic volume. Cells in Ringer solution have swelling indices ranging from about 20 to 70 with a mean of 45.0. That is to say some cells require only as little as 20% volume increase to become spheres while others may require as much as 70%. Thus, placed in a hypotonic solution, some cells would have completely sphered and hemolyzed while others might have been only moderately swollen. In the presence of albumin, because of a reduction in isotonic volume, the percent volume increase required to sphere red cells is increased. The swelling indices of individual albumin-treated cells ranged from 25 to 80 with a mean of 48.9. This is an increase of 8.7% in swelling index, providing a protection of 8.7 percent against osmotic hemolysis.

Osmotic fragility studies on normal human erythrocytes (Beck et al., 1972) showed that 50% of cells hemolyzed at 125 mosmol. The mean swelling index of 45.0% implies that, on reducing the tonicity of the suspending medium, half the cells could accommodate a swelling of 45% and the rest would hemolyze. Hence a 125 mosmol solution will, on the average, cause a swelling of 45%. In the presence of albumin the swelling index increased to 48.9% and in order to hemolyze half the cells the tonicity of the solution would have to be lower. By simple proportion the further decrease is calculated to be 15 mosmol and so the osmotic fragility curve would be shifted (towards lower osmotic pressures) by approximately 15 mosmol. This figure compares well with the results of Blas and Whaun (1973) who reported a similar shift of the osmotic fragility curve in response to albumin treatment.

The Cup Shape

When the cells are in Ringer solution, usually less than 2% of the cells in a sample are cup-shaped. A cell is considered cup-shaped if one face is concave while the other is either flat or convex. When albumin was added to the samples, the number of cup cells increased into the range of 15–50%, varying from sample to sample. Since we have observed changes in area and volume of the cells placed in albumin, it would be interesting to see if these parameters are different between biconcave and cup cells. Table III shows a comparison between biconcave and cup cells. Of 843 cells in albumin, there were 173 cup cells. The mean diameter of cup cells was 8% smaller than in biconcave cells. The mean surface areas for biconcave cells and for cup cells were not significantly different, $P > 0.05$. Biconcave cells had a mean volume of 100.0 μm^3 compared with 90.8 μm^3 for cup cells. The smaller mean cellular volume, with the same mean membrane area, resulted in lower sphericity and minimum cylindrical diameter for cup cells. The large difference in volume suggests that the cup shape is a characteristic of small cells. This is a different phenomenon from that described by Canham (1970) who reported cup shapes resulting from osmotic swelling. With a decrease in cellular volume, some cells assume the cup shape (plano-concave or convexo-concave), instead of just a thinner biconcave shape possibly because of a differ-

TABLE III
GEOMETRICAL PARAMETERS FOR CUP CELLS

	All cells	Biconcave	Cup shape	Percent difference	*P*
N	843	670	173		
Diameter, μm	7.722	7.849	7.232	−7.9	<0.0005
Area, μm^2	133.4	133.8	132.0	−1.3	>0.05
Volume, μm^3	98.1	100.0	90.8	−9.2	<0.0005
Minimum cylindrical diameter, μm	3.186	3.247	2.950	−9.1	<0.0005
Sphericity index	0.769	0.777	0.739	−4.9	<0.0005
Swelling index	48.9	46.5	58.3	25.3	<0.0005

All cells in 3 g/liter albumin.

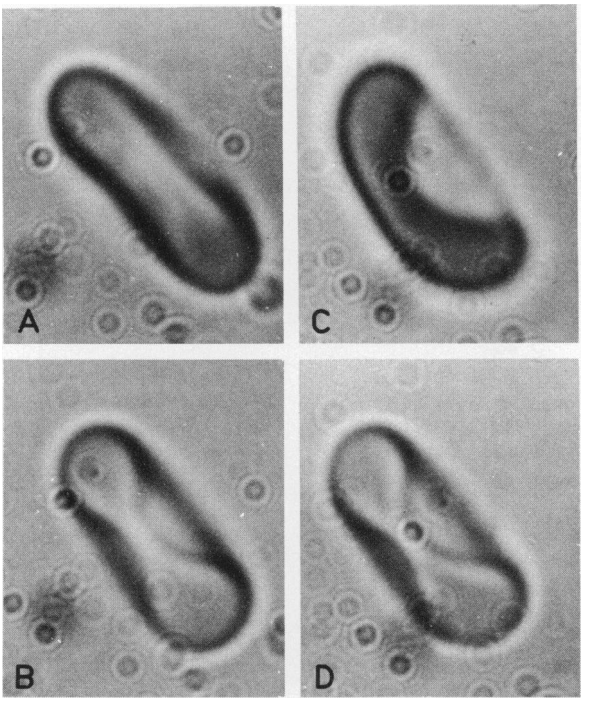

FIGURE 9 *A*, cell in Ringer solution; *B*, same cell in Ringer–albumin 3 g/liter; *C*, after agitation by washing with Ringer–albumin; *D*, after still more washing.

ence in resistance of the membrane to bending on opposite sides of the cell. The smaller volumes in cup cells also resulted in a much higher swelling index, 58.3 compared with 46.5 for biconcave cells. This means that cup cells can accommodate 12% more volume increase in response to osmotic swelling without hemolysis. This is equivalent to a shift of the osmotic fragility curve by as much as 45 mosmol, and suggests that in the presence of albumin, the cells which assume the cup shape, amounting to about 20% of the cell population are osmotically the least fragile.

During the experiments with single cells, it was observed that agitation could induce biconcave cells to assume the cup shape, and that this transformation could be reversed by further agitation. Canham (1970) in his explanation of the biconcave shape by the theory of minimum bending energy has described the cup shape as a metastable shape for the red cell. It would be interesting to see how the area and the volume of the cell behave in such a shape transformation. Four photographs of the same cell are shown in Fig. 9. Initially, the cell was in Ringer solution (Fig. 9 *A*). The medium was then changed by washing albumin over the cell. The cell responded with the usual shape changes as shown in Fig. 9 *B*. Further washing produced a cup shape, Fig. 9 *C*, which was reversed during continued washing, Fig. 9 *D*. The profiles of five cells were analyzed and results are shown in Fig. 10. The diameter, area, and volume of the cells decreased in response to albumin. Transformation to the cup shape involved a further

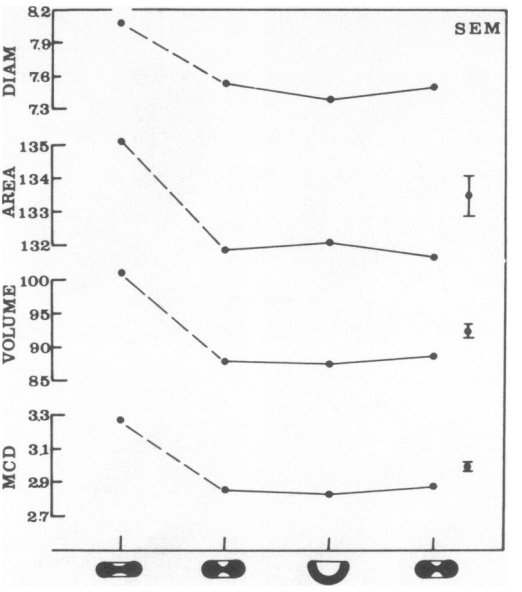

FIGURE 10 Effects on the diameter, area, volume, and minimum cylindrical diameter of a single cell. First column, cells in Ringer. Second column, in albumin-Ringer. Third and fourth columns, in albumin-Ringer during repeated agitation. The cell shapes are depicted below. SEM for five repeated measurements are shown. The SEM for diameter is less than the diameter of the solid circles.

reduction in cell diameter but there were no significant changes in area nor volume. The criterion for a cell to take on the cup shape may be sufficient assymetry in bending resistance characteristics of the membrane between opposite faces of the cell.

Bovine Serum Albumin

Three experiments of population studies were performed with bovine serum albumin instead of human albumin. Concentration dependence was not studied, but using the same concentration, 3 g/liter, cells responded similarly to bovine serum albumin as to human serum albumin, in all parameters.

Effects of Bound Fatty Acids

It has been reported that free fatty acids induce crenations in red blood cells (Bessis, 1974), an effect which is qualitatively opposite to that which we have observed for albumin. Fatty acids bind readily to serum albumin, and the de-fatting processes only remove bound fatty acids from albumin to varying degrees, while complete removal is virtually impossible. For this reason, it is important that any effect due to fatty acids present in the albumin be evaluated. Table IV shows our results of such a study. The three human serum albumins used contained different amounts of bound fatty acids, one of which, containing less than 0.005%, is essentially fatty acid free. The results are statistically consistent with those of the collected data of Table II and demonstrate that

TABLE IV

EFFECT OF DIFFERENT ALBUMINS ON CELL GEOMETRY

	Control	Albumin 1	Albumin 2	Albumin 3	Percent change*
N	47	70	62	69	
Diameter, μm	8.36	7.67	7.59	7.94	−5.0
Area, μm^2	144	138	140	140	−2.8
Volume, μm^3	110	102	103	104	−5.5
Minimum cylindrical diameter, μm	3.33	3.18	3.16	3.20	−3.9
Sphericity index	0.772	0.761	0.751	0.762	−1.3
Swelling index	48	51	52	51	+6.3
Percent cup cells	2	40	50	12	

Concentration of albumins, 5 g/liter.

*Percent change between control and albumin 3 (essentially fatty acid free).

Albumin 1, Red Cross, normal concentration of bound fatty acids; albumin 2, Kabi, Stockholm, Sweden, 0.2–1.6% bound fatty acids; albumin 3, Sigma Chemical Co., St. Louis, Mo., < 0.005% bound fatty acids.

All percent differences are significant, $P < 0.0005$.

no difference can be ascribed to variations in the fatty acid content of the three albumin solutions used.

It is clear from these results that fatty acids bound to albumin do not affect the cell-shape stabilizing effects of albumin, and that the presence of albumin prevents cell crenation by fatty acids.

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