Variability in erythrocyte deformability among various mammals

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SMITH, JOSEPH E., NARLA MOHANDAS, AND STEPHEN B. SHOHET. *Variability in erythrocyte deformability among various mammals.* Am. J. Physiol. 236(5): H725–H730, 1979 or Am. J. Physiol.: Heart Circ. Physiol. 5(5): H725–H730, 1979.— Deformability is an important aspect of erythrocyte physiology and has been extensively studied using human red cells. We have studied erythrocytes from 25 different animals using a viscometric technique. Erythrocyte diameters ranged from 3.3 μm in the goat to 11.4 μm for the elephant seal. Erythrocytes from most species deformed readily when a fluid shear stress was applied. A deformability index of the stressed cell defined as (length − width)/(length + width) correlated with cell size. The erythrocytes of four animals (pygmy goat, goat, Batanga horse, and miniature horse) deformed less than most species. Camel and llama erythrocytes, which were ellipsoidal, did not deform but oriented in the stress field.

ektacytometry; camel; llama; goat; elephant seal; deformability index

HUMAN ERYTHROCYTES must regularly squeeze through capillaries that are 4 μm or larger in diameter as well as through extremely narrow endothelial slits of the spleen (0.5–1 μm wide). Because they are 7–8 μm in diameter, they must deform significantly in order to pass through these small vessels. Human erythrocytes that do not possess normal deformability usually have a shortened life span (1, 14).

Erythrocytes from other mammals vary in diameter from 2.1 to 9.2 μm (20), yet capillary size appears to be relatively constant with a mean diameter of 4 μm (10, 22). Because the erythrocyte's normal life span does not correlate with the erythrocyte size in different species they must be able to deform sufficiently to travel through the capillaries. In fact, the larger erythrocytes must be more deformable than the small ones (5).

In the present study we have examined erythrocyte deformability in mammals with varying cell size using a viscodiffractometric technique (3). We found that regardless of cell size most species showed considerable red cell deformability in low shear fields suggesting that this was an important basic erythrocyte property. Exceptions in the cameloid family were noted.

MATERIALS AND METHODS

Blood was collected from the various species, with EDTA used as an anticoagulant. The ektacytometer was used as previously described (3) to measure erythrocyte deformability. In brief, this technique measures the ability of a red cell to deform in a shear field by monitoring the laser-generated diffraction patterns of red blood cells maintained in suspension between stationary and rotating transparent cylinders. The transformation of the diffraction pattern from a circle to an ellipse is related to the deformability of the individual cells in suspension. Whole blood (20 μl) was suspended in 10 ml of a media consisting of 25% dextran (avg mol wt, 40,000) dissolved in a phosphate-buffered saline (0.12 M NaCl, 0.02 M Na_2HPO_4, and 0.005 M KH_2PO_4; pH 7.4). The gap between the inner and outer cylinders was filled with this suspension. The diffraction patterns at various shear stress were photographed, and cellular dimensions were calculated from the diffraction patterns with a calibration standard of 8 μm for human erythrocytes. The applied shear stress was calculated in dyn/cm^2 using the formula $\tau_s = \mu \times$ shear rate $= 2\pi\mu NR/60h$, where μ is the suspending media viscosity, N is the rpm of the outer cylinder, R is the radius of the inner cylinder (2.5 cm) and h is the gap between the inner and outer cylinders (0.05 cm) (3). All experiments were done within 3 h after phlebotomy.

RESULTS

The basis for obtaining the deformability index (length − width/length + width) for red cells of different mammalian species with the ektacytometer involves measuring red cell sizes calculated from diffraction patterns created by varying the shear stress applied. To validate the calculations, we compared the deformability index calculated from the diffraction patterns for human red cells with the deformability index calculated from actual measured, deformed ellipsoids photographed in a rheoscope similar to the one described by Schmid-Schönbein and Wells (21). The two measurements agreed well (Fig. 1) even though the deformability index from rheoscopic measurements is calculated for individual cells whereas that from ektacytometric measurements represents a population of cells.

To establish that dextran per se did not significantly affect the deformability, measurements were carried out with dextran of various molecular weights (20,000, 40,000, 80,000, and 500,000), and polyvinylpyrrolidone (PVP) with molecular weights of 40,000 and 360,000. In each instance the deformability was identical when viscosity

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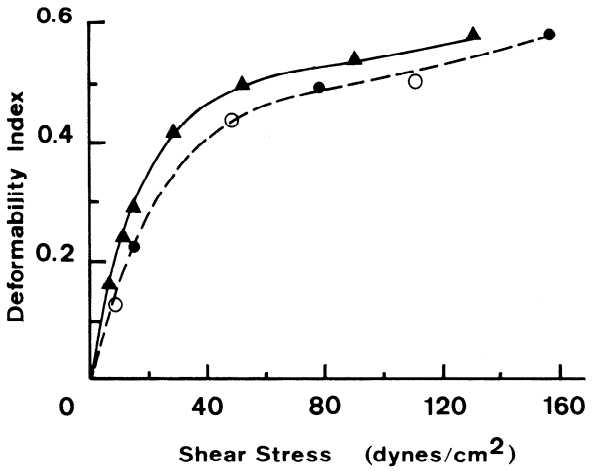

FIG. 1. Deformability index of human red cells as a function of applied shear stress using *a*) the ektacytometer (●, ○) and *b*) the rheoscope (▲).

of the suspending medium was the same, even over wide ranges of concentration of the different polymers necessary to obtain the desired viscosities. Figure 2 shows the deformability index at different suspending media viscosities with dextran 40. The deformability index is insensitive to suspending media viscosity up to 100 dyn/cm^2. At higher values of applied shear stress, as the ratio of internal to suspending medium viscosity (λ) approaches unity, the deformability index becomes moderately sensitive to suspending media viscosities. These experiments showed that the technique is valid and demonstrated that dextran only minimally influences deformability.

In the absence of applied stress, the diffraction patterns of erythrocytes from most of the species studied were circular, and their sizes were inversely related to the erythrocyte diameter (Fig. 3). Erythrocyte diameter varies from 3.3 μm in the goat to 11.4 for the elephant seal (Table 1). However, two species, the llama and camel, had ellipsoidial patterns at conditions of no stress. With the exception of these cameloid cells all the erythrocytes deformed into ellipsoids under the action of fluid stresses (Figs. 3 and 4). A minimum erythrocyte dimension was reached in all species at low values of applied shear stress (100 dyn/cm^2). The largest minimum erythrocyte dimension was 3.5 μm for the elephant seal, and cells from most species were below 3 μm (Table 1). Maximum erythrocyte length was significantly correlated ($r = 0.96$; df, 21; $p < 0.01$) with the undeformed cell diameter. The maximum cell length can be estimated as 2.5 × cell diameter − 4.7 with both length and diameter in micrometers.

The llama and camel erythrocytes did not show this deformation sequence. The diffraction pattern at low stresses was a vertical ellipse (Fig. 5*A*). At higher shear stresses, the patterns remained ellipsoidal, but the major axis had rotated 90° (Fig. 5*B*). This suggests that the camel ellipsoidal red cells are oriented in the direction of flow at low values of applied stress, but as the applied stress was gradually increased they began to orient in a direction perpendicular to the direction of flow. The dimensions of the diffraction patterns were the same at low and high applied stresses, suggesting that these ellipsoidal cells are not deformable in the ektacytometer. If the erythrocytes from these species were fixed with glutaraldehyde, the ability to orient in the stress field was abolished and circular diffraction patterns were observed at all shear stresses indicating random orientation (Fig. 5*C*).

The deformation of normal human erythrocytes in a shear field has been compared to that of a fluid drop (21). The deformation of fluid drop has been characterized by an index D which is related to dimensions of the deformed drop as follows: $D = (L - W)/(L + W)$ where L and W represent the drop length and width, respectively (23). When this deformability index was calculated and plotted as a function of the shear rate, the values for most animals were remarkably similar (Fig. 6) and significantly correlated ($r = 0.78$; df, 17; $P < 0.01$) to cell size. The deformability index could be predicted by the formula 0.0313 × cell diameter + 0.439.

Although these generalizations applied to most of the species we studied, erythrocytes from four different kinds of animals had deformability significantly different from the other animals. The lowest deformability occurred in the smallest cell studied, i.e., the goat and pygmy goat (Fig. 6*D*). The miniature horse and Batanga horse had deformability values between that of the goats and the rest of the species. Although the cells of both horses are smaller than human cells, they are larger than those of several of the other animals studied. Erythrocytes from the miniature horse and Batanga horse could, however, decrease their minimum dimensions to less than 3 μm.

DISCUSSION

Cellular deformability is crucial for normal erythrocytes to function. It allows passage of red cells through capillaries, reduces bulk viscosity of blood flowing in large vessels, and may control removal of "nonfunctional" and aged erythrocytes by the reticuloendothelial

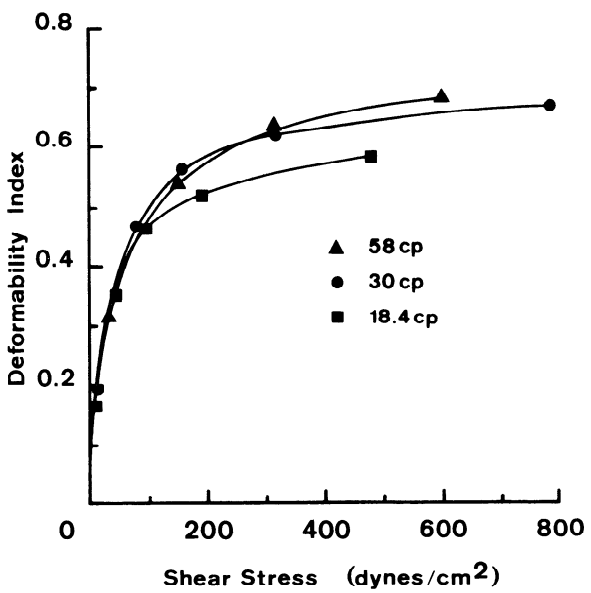

FIG. 2. Deformability index of human red cells as a function of applied shear stress using different suspending media viscosities (▲, 58 cP; ●, 30 cP; ■, 18.4 cP). Ratio of red cell internal viscosity to suspending medium viscosity (λ) is 0.147, 0.283, and 0.462 for suspending media viscosities of 58, 30, and 18.4 cP, respectively.

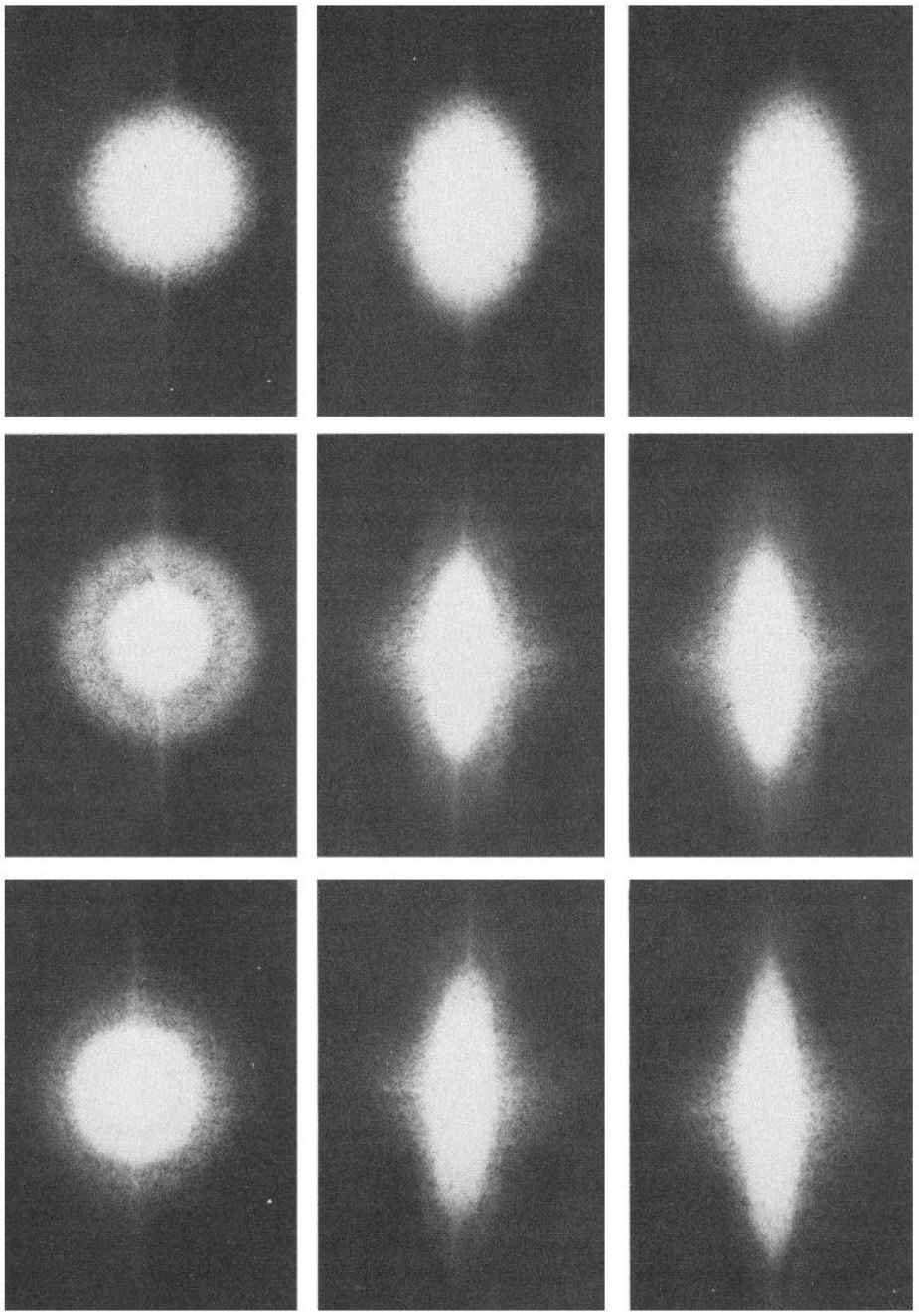

FIG. 3. Diffraction patterns of erythrocytes photographed at 0, 100, and 590 dyn/cm^2 (*left to right*). Top row shows pygmy goat, middle row, shetland pony, and bottom row, elephant seal.

system (4, 14). Erythrocyte deformability is a function of the viscoelastic properties of the membrane (7), the viscosity of the intracellular hemoglobin, and cell geometry (including the ratio of surface to cell volume) (24). Deformability of human erythrocytes has been measured by various techniques including the monitoring of deformation induced by fluid stresses on erythrocytes stuck to flat surfaces (11), aspiration of red cells into micropipettes (24), measurement of red cell filtration time (17), resistive pulse spectroscopy (16), and ektacytometry (3).

Measurements on other mammalian erythrocytes are limited. When goat and sheep erythrocytes were examined by viscometric techniques, they were less deformable than those of other mammalian red cells (5). This low deformability was hypothesized to be due to either the low surface-to-volume ratio, low cell membrane lecithin, low adenosine-5′-triphosphate (ATP), or all three (5). The latter two possibilities seem unlikely because cattle erythrocytes also contain low lecithin (18) and low ATP (2), yet are fully deformable. When filtration techniques are applied to mammalian erythrocytes, goats had the lowest deformability, and as the erythrocyte size increased, deformability also increased (5). In our study, using a much greater variation in cell sizes, erythrocyte size and deformability were similarly related.

The failure of camel and llama erythrocytes to deform is unusual among erythrocytes from normal mammals. Their shape (flat, thin elliptocytes) apparently allows them to transverse the circulatory system without the usual problems of reduced deformability. Although the

exact reason for their rigidity is unknown, they do have a high protein-to-lipid ratio in the membrane (6). The membrane rigidity may be functionally related to their unusual osmotic resistance. Camels are able to withstand long periods of dehydration, then drink large quantities of water in a short time without ill effects (20). None of the species with deformable erythrocytes can tolerate such a rapid rehydration without hemolysis (13). Although not tested in the current study, other species in the family camelidae (guanacos, alpacas, and vicunas) may have similarly reduced deformability because they also have elliptical erythrocytes (19).

Although the ellipsoidal erythrocytes from llama and camel did not deform, they did orient in the shear field, indicating some effects of the shear stresses. Erythrocyte membranes are not stationary under fluid shear stress (21). Microscopically the cell membrane rotates around

TABLE 1. *Erythrocyte size and deformability*

Species	Erythrocyte Dimensions, μm		
	Normal	Deformed at 590 dyn/cm²	
		Minimum	Maximum
Goat	3.3	2.5	5.5
Pygmy goat	3.4	2.4	4.4
Sheep	4.4	2.2	8.5
Cow	5.8	2.5	10.0
Batanga horse	5.9	2.7	7.8
Antelope	6.0	2.7	9.9
Shetland pony	6.2	2.7	12.0
Water buffalo	6.2	2.7	9.4
Miniature horse	6.4	2.9	7.6
Burro	6.7	2.8	11.0
Mouse	6.8	2.4	13.0
Pig	7.0	2.6	12.0
Arctic fox	7.3	2.1	14.0
Hamster	7.3	2.5	14.0
Rat	7.5	2.4	16.0
Dog	7.6	2.7	16.0
Human	8.0	2.9	16.0
Rhesus monkey	8.0	2.9	15.0
Baboon	8.2	2.7	18.0
African elephant*	9.0	3.3	17.0
Indian elephant*	9.0	3.0	20.0
California sea lion	9.8	3.0	20.0
Elephant seal*	11.4	3.5	25.0
Llama	2.8 x 7.8	Did not deform	
Camel	3.5 x 6.8	but oriented	

* Measured at 220 mosM.

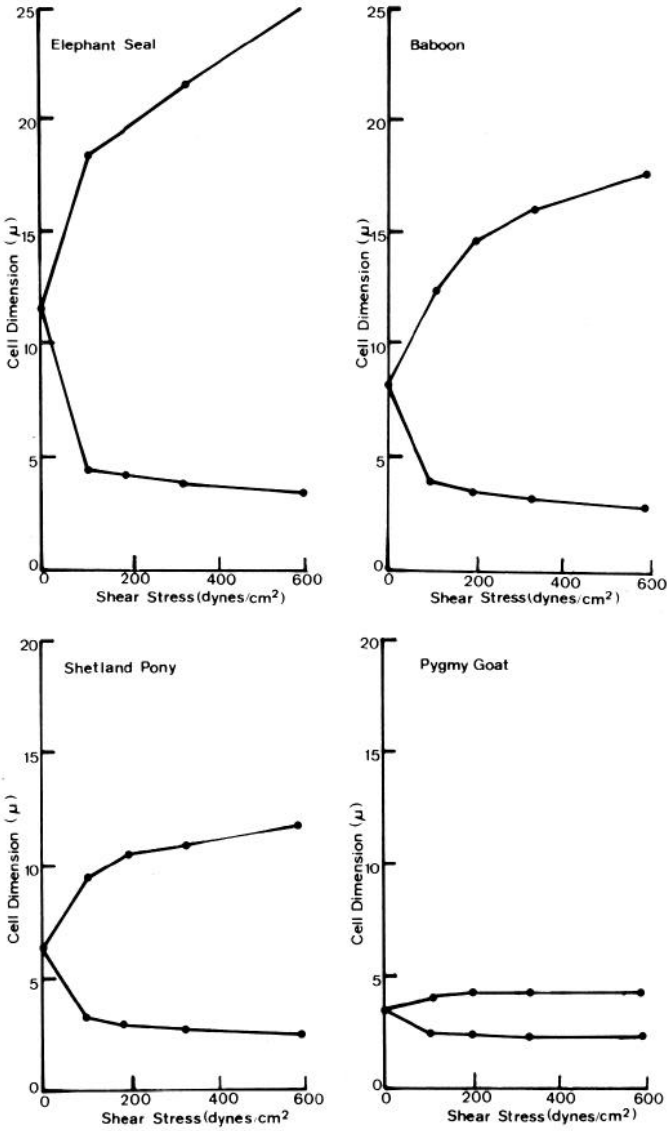

FIG. 4. Cellular dimensions calculated from diffraction patterns during shear stress of blood.

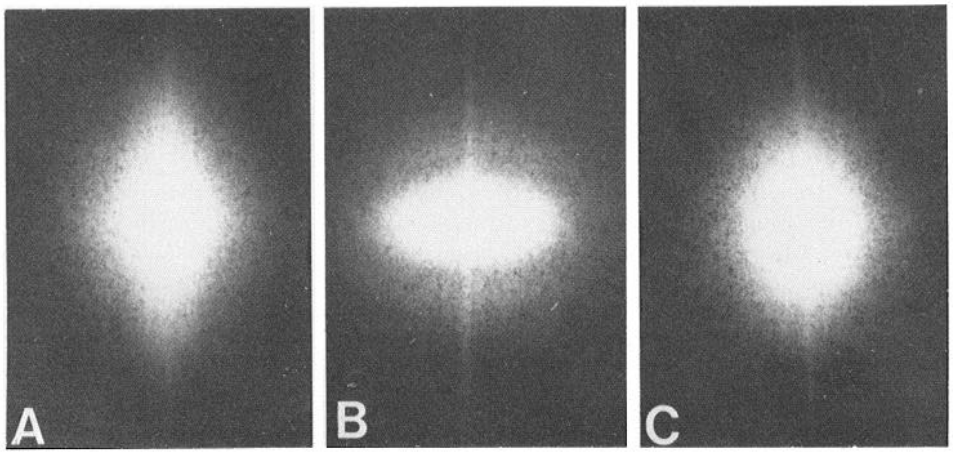

FIG. 5. Diffraction patterns of camel erythrocytes *A*: at 50 dyn/cm²; *B*: at 100 dyn/cm²; and *C*: glutaraldehyde-fixed at 100 dyn/cm².

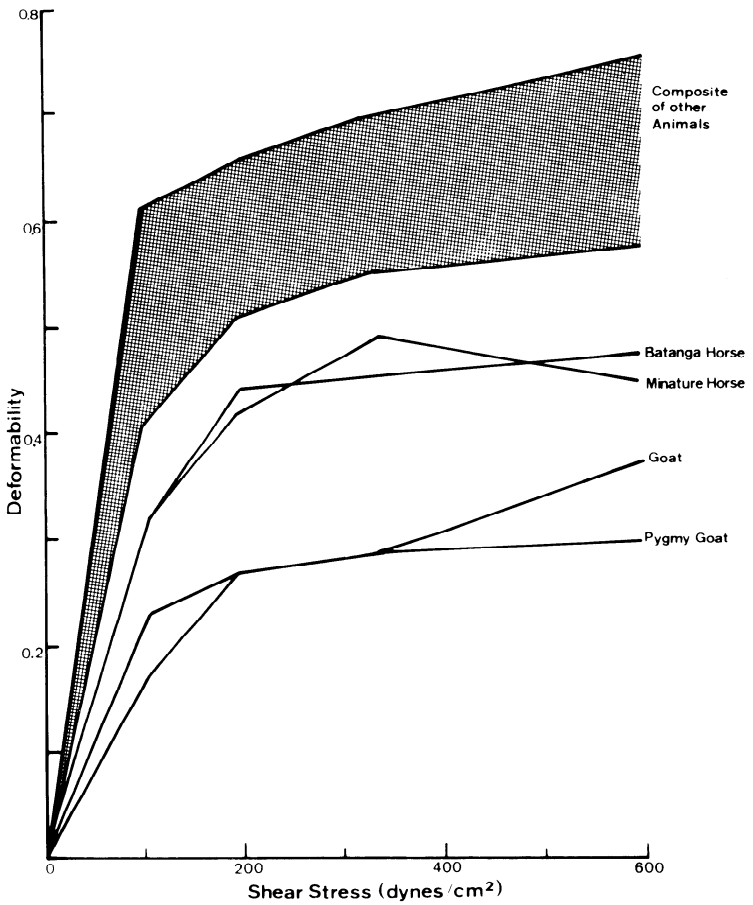

FIG. 6. Deformability index: (length − width)/(length + width).

its contents much as an army tank rotates its tread around the wheels. As the membrane rotates, it is probably accompanied by an inner circulation of hemoglobin. Fixation of the erythrocyte membrane and hemoglobin by glutaraldehyde stops the hemoglobin movement and thus prevents orientation of cells in the stress field.

The minimum cylindrical dimension of human erythrocytes was estimated to be 2.8 μm when thin polycarbonate filters (9) or long micropipette chambers (14) were used. This agrees well with the minimum cell dimension of 2.9 μm (14) observed at 590 dyn/cm^2 in the current study. Increasing the applied shear stress to 590 dyn/cm^2 leads to a time-dependent cell fragmentation, presumably related to an increase in the surface area. The fact that the erythrocytes of nearly all species could decrease to less than 3 μm suggests that this may be the size necessary to traverse the smallest capillaries (5).

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