Is the surface area of the red cell membrane skeleton locally conserved?

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ABSTRACT The incompressibility of the lipid bilayer keeps the total surface area of the red cell membrane constant. Local conservation of membrane surface area requires that each surface element of the membrane skeleton keeps its area when its aspect ratio is changed. A change in area would require a flow of lipids past the intrinsic proteins to which the skeleton is anchored. In fast red cell deformations, there is no time for such a flow. Consequently, the bilayer provides for local area conservation. In quasistatic deformations, the extent of local change in surface area is the smaller the larger the isotropic modulus of the skeleton in relation to the shear modulus. Estimates indicate: (*a*) the velocity of relative flow between lipid and intrinsic proteins is proportional to the gradient in normal tension within the skeleton and inversely proportional to the viscosity of the bilayer; (*b*) lateral diffusion of lipids is much slower than this flow; (*c*) membrane tanktreading at frequencies prevailing in vivo as well as the release of a membrane tongue from a micropipette are fast deformations; and (*d*) the slow phase in micropipette aspiration may be dominated by a local change in skeleton surface.

INTRODUCTION

From a continuum mechanical point of view, the red cell membrane can be considered to be laminated from two layers: the lipid bilayer with its embedded intrinsic proteins, and the membrane skeleton, a network of extrinsic proteins lining the cytoplasmic face of the bilayer. Relative motion between skeleton and bilayer can lead to changes in skeleton density. The influence of such changes on the shape of the red cell membrane in static equilibrium has been studied in model calculations (Stokke et al., 1986*b*; Markin and Kozlov, 1988). Conflicting results were obtained by both groups when the micropipette experiment was analyzed.

In the present work, a continuum mechanical approach on the diffusion of intrinsic proteins in membranes (Saffman, 1976) is used to estimate the relative motion of bilayer and skeleton under dynamic red cell deformations. The main conclusion is that such motion is likely to be slow compared to typical time scales of red cell deformation, but that it may be significant under static or very slowly varying conditions. Qualitative arguments are presented with respect to the conflict mentioned above.

PROPERTIES OF THE RED CELL MEMBRANE

Shear and areal stiffness

A striking property of the membrane is its comparatively low shear modulus, being five orders of magnitude smaller than its isotropic modulus. This can be explained by the fact that the red cell membrane is a composite material in which shear and isotropic tensions are supported by different structures. The bilayer being liquid can not contribute to the shear elasticity of the red cell membrane which by exclusion is ascribed to the membrane skeleton. The shear modulus is designated μ.

The molecular design of the skeleton makes it unlikely that its isotropic modulus is five orders of magnitude larger than μ. It can, therefore, not contribute significantly to the observed areal elasticity of the whole membrane which by exclusion is ascribed to the bilayer. Additional evidence comes from measurements on the isotropic modulus of vesicle membranes made from lipids extracted from red cell membranes (Needham and Nunn, 1990). The isotropic modulus of the bilayer and thus of the whole membrane is designated K_b.

The isotropic modulus of the skeleton is designated K_s. It cannot be measured in a straightforward manner because K_b is much larger. Therefore we resort to considerations on a molecular level. The shear stiffness of the membrane skeleton appears to result from the springlike elasticity of spectrin tetramers joined into a network by actin filaments and protein 4.1 (Stokke et al., 1986*a*; Liu et al., 1987; Vertessy and Steck, 1989). This mechanism can be expected to provide for a K_s of the same order of magnitude as μ.

But there may be additional contributions by other mechanisms. Spectrin under physiological conditions is a charged molecule which may endow the skeleton with areal elasticity due to electrostatic interactions (Stokke

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et al., 1986*a*). This mechanism does not necessarily contribute to the shear elasticity. Consequently the total K_s could become appreciably larger than μ.

Global and local conservation of surface area

The bilayer with its large isotropic modulus resists changes in the total surface area of the red cell membrane. In pipette experiments, an increase of $<5\%$ was observed before hemolysis occurred (Evans et al., 1976). The maximum isotropic membrane tensions estimated for red cells flowing through capillaries (Halpern and Secomb, 1989) or flowing in the shear field of a rheoscope (Tran-Son-Tay et al., 1987) are more than an order of magnitude smaller than the tensions at hemolysis. Accordingly, the total surface area of the red cell is constant within fractions of a percent under these flow conditions. In other words, the surface area can be considered to be globally conserved.

As opposed to this, local area conservation requires that each membrane element keeps its surface area when its aspect ratio is changed. To define a membrane element on a molecular level the time scale has to be considered. Within the observation time, the molecules used for this definition must not loose their neighbor relation by diffusion. Therefore, we choose to define integral proteins connected to the skeleton as "fenceposts" lining the border of membrane elements, or more specifically, of surface elements of the membrane skeleton. The membrane skeleton has been shown to behave as a solid, on time scales, much larger than the ones considered in this paper (Markle et al., 1983).

Poisson ratio

The Poisson ratio ν constitutes a measure of the local change in surface area of a two-dimensional elastic continuum. We consider a strip of this material with an external force acting on it in x direction. At right angles (y direction), the strip is unsupported. In linear elastic theory, ν is defined as the negative quotient of the strains in y and x direction that prevail in static equilibrium. $\nu = 1$ when the surface area of the strip is conserved. $\nu = -1$ when the aspect ratio of the strip is conserved. The last value corresponds to the maximum change in area (increase when the strip is stretched or decrease when it is shortened by the external force). At $\nu = 0$, the width of the strip is conserved. This corresponds to a medium change in surface area, the sign of which depending again on the sign of the external force.

Besides, as a geometrical measure, ν is used as an elastic constant of the material. Because two constants suffice to describe the elastic behavior of an isotropic continuum, ν is a function of shear and isotropic modulus. It can be shown easily that

$$\nu = \frac{K_s - \mu}{K_s + \mu}. \tag{1}$$

As expected, the surface area is essentially conserved ($\nu \simeq 1$) when $K_s \gg \mu$ and the aspect ratio is essentially conserved ($\nu \simeq -1$) when $\mu \gg K_s$. Practically, it is very rare that $\nu < 0$ because the mechanisms responsible for the shear stiffness provide for an areal stiffness of the same order of magnitude.

Bounds to the local values of surface area

At extreme changes in local skeleton area, molecular mechanisms additional to the ones discussed above come into play. First, the resistance against area dilation would increase strongly if the spectrin tetramers were stretched completely. However, uniaxial extension is limited for the same reason. In this paper, only extension ratios within the elastic regime are considered. Therefore this upper bound is not reached.

Second, $\sim 15\%$ of the surface area of the bilayer of the red cell membrane consists of intrinsic proteins as can be calculated from the diameter of the hydrophobic portion of these proteins and their surface density. This puts a lower bound to the local skeleton surface area because these proteins cannot escape from the meshes formed by the spectrin actin network (Sheetz, 1983). For a conservative estimate we assume that in the worst case (if there is no time for a lateral motion to reach a close packed configuration) the intrinsic proteins can be packed with an area fraction of only 50%. This means the local surface area of the skeleton cannot decrease to $<30\%$ of its normal value. To reach this lower bound the extension ratio would have to drop at least to 0.3 assuming $\nu = 0$. Such small extension ratios, however, are neither imposed in micropipette aspiration (within the elastic regime) nor in extremely large red cell elongations as observed in the rheoscope by Fischer and Schmid-Schönbein (1977).

CONSIDERATION OF POSSIBLE CASES

Because the value of K_s is unknown, the red cell behavior is discussed for two extreme values within the possible range. In addition, the influence of time is considered. The reason for the latter being that the skeleton is anchored to intrinsic proteins which in turn are embedded in the bilayer. Therefore, a local change in surface area of the skeleton involves a relative motion between

lipid molecules and intrinsic proteins. Because of friction, this motion requires time. A deformation is called slow when there is ample time for the rearrangement of lipids and intrinsic proteins. A special case is static equilibrium. A deformation is called fast when there is no time for a relative motion of lipids and intrinsic proteins. In the intermediate range the two velocities, that of propagation of shear strain on the membrane and that of relative movement between lipids and intrinsic proteins are comparable.

Behavior of the membrane when $K_s \gg \mu$

When $K_s \gg \mu$, ν is practically 1. This means the surface area of the skeleton proper is locally conserved irrespective of the time scale of the deformation. Please note that K_s can still be much smaller than K_b because of the five orders of magnitude difference between μ and K_b.

The membrane in static equilibrium assuming $K_s \simeq \mu$

When K_s is of the same order of magnitude as μ, ν is < 1. Without the bilayer, the skeleton would change locally its surface area as a response to imposed shear strains, the extent of this change being the greater the smaller the value of ν. With the bilayer, the total surface area of the skeleton is constant. Therefore, its local change in area depends besides ν on this global boundary condition.

As an example we first consider the deformation of an infinite flat membrane under aspiration into a micropipette. Imposed is a shortening in azimuthal direction of all surface elements of the skeleton except the one at the tip of the tongue. For $\nu < 1$, the area of these surface elements decreases. Consequently, the elements are stretched less in radial direction than if $\nu = 1$.

If the dimple region of a red cell is aspirated, the surface area of the skeleton cannot decrease everywhere because of the restriction of constant total surface area. Because the stretch ratios enter the expression for the strain energy in a quadratic fashion, a minimum in total elastic energy is attained when the surface area decreases where the shear strain is large and as a compensation increases at locations where it is small. For the micropipette experiment this means that the surface area decreases at the pipette rim and increases far away from the rim, e.g., at the tip of the tongue. This plausibility consideration is in contrast to the theoretical model of Stokke et al. (1986*b*) and in agreement to that of Markin and Kozlov (1988).

The membrane under dynamic deformations assuming $K_s \simeq \mu$

To estimate the relative velocity of lipids and intrinsic proteins, let us assume that the membrane is deformed suddenly, according to a step function. This is a fast deformation where the bilayer provides for local conservation of skeleton surface area. Subsequently, the local surface area of the skeleton relaxes to its final value determined by ν and the static boundary conditions. The relaxation is opposed by the viscous drag in the lipid bilayer experienced by the intrinsic proteins that are connected to the skeleton. The drag can be estimated from the force (F) necessary to move a circular cylinder embedded in a thin liquid sheet of finite size (Saffman, 1976).

$$F = \frac{4\pi\eta v}{\ln (R/\rho) - \frac{1}{2}}. \tag{2}$$

η is the two-dimensional viscosity of the sheet. v is the velocity and ρ the radius of the cylinder. R is the radius of the circular sheet to which the flow is restricted.

We consider a small membrane element of trapezoidal shape. Its line of symmetry is along the y axis. Its converging sides coincide with the direction of skeleton flow (v_r) relative to the bilayer. $\Delta x(y)$ is the distance of these sides. From the force balance in y direction we obtain,

$$F\sigma = -\frac{dT_y}{dy} - \frac{T_y}{\Delta x}\frac{d\Delta x}{dy}, \tag{3}$$

where $T_y(y)$ is the normal tension exerted by the skeleton on the border of the element (outward direction positive), and $\sigma(y)$ is the surface density of cylinders, intrinsic proteins, respectively. F is calculated from Eq. 2 where $v_r(y)$ the velocity of the intrinsic proteins relative to the lipids is inserted for v.

As to the intrinsic proteins, glycophorins were neglected for simplicity. This is justified because they are smaller in number and diameter than band 3 and 4.5. These two sum up to 19×10^5 copies per red cell. All copies whether connected to the skeleton or not were treated alike because the nonconnected ones appear to be confined within the meshes of the skeleton (Sheetz, 1983). It is not settled whether band 3 and 4.5 exist as dimers or tetramers. The radius (ρ) of the hydrophobic portion of dimers or tetramers of both proteins was taken to be 3 or 4 nm, respectively. R was estimated assuming circular patches of equal size to be close packed on the red cell surface (taken to be 135 μm^2). The denominator in Eq. 2 turned out to be practically identical for dimers or tetramers. This is not the case for the number of oligomers per red cell. 7.1×10^5 which

constitutes the average between the dimeric and the tetrameric state was taken to calculate the mean density σ. For η a value of 5×10^{-6}dyn s/cm was used (Waugh, 1982).

It might be objected that diffusion was an alternate mechanism for the flow of lipids past the proteins. To estimate its contribution, a complementary view is adopted. The skeleton induces (via the intrinsic proteins to which it is anchored) a macroscopic gradient in tension (dT_y/dy) within the bilayer. This gradient in tension besides driving the viscous flow of lipids past the intrinsic proteins induces a gradient in surface concentration of lipids which in turn could cause a diffusive flow.

For simplicity, we assume the membrane element to be rectangular. Then the second term on the right hand side of Eq. 3 vanishes. Multiplying Fick's law by the surface area of a single lipid molecule gives after some rearrangement v_d, the average velocity of diffusive flow:

$$v_d = -\frac{D}{K_b}\frac{dT_y}{dy}, \tag{4}$$

where D is the diffusion coefficient of phospholipids in the red cell membrane. For the calculations the values 8.2×10^{-9} cm^2/s (Bloom and Webb, 1983) and 450 dyn/cm (Evans and Waugh, 1977) were used for D and K_b.

Inserting the numbers into Eqs. 2, 3, and 4 allows to compare both contributions under the same gradient dT_y/dy. For the ratio v_r/v_d, a value of 400 is obtained. This means that the lateral redistribution of lipid molecules is dominated by the viscous flow.

COMPARISON TO EXPERIMENTS

We now address the question whether v_r as calculated from Eqs. 2 and 3 is sufficiently fast to provide for a local change in surface area within realistic time intervals. To this end we estimate v_r for two typical experimental situations and compare the value to the velocity of propagation of shear strain on the membrane.

Tanktread motion

The example chosen has been used in a previous publication (Fischer, 1980). A red cell suspended in a dextran-saline solution is subjected to an average shear rate of 42/s in the cone-plate chamber of a rheoscope. The red cell is deformed into a shape resembling a triaxial ellipsoid. We assume the two-dimensional flow of the membrane (tanktread motion) to be a steady-state flow and consider the membrane elements on the flow line in the plane of symmetry. The surface coordinate along this flow line is called y. At right angles (x direction), a periodical shortening and stretching is imposed by the tanktread motion. Estimates for the minimum and maximum stretch ratio of the skeleton (λ_x[center] and λ_x[tip]) are obtained from the dimensions of the ellipsoid assuming $\lambda_x(\text{tip}) = 1/\lambda_x(\text{center})$. It follows $\lambda_x(\text{tip}) = 1.39$ and $\lambda_x(\text{center}) = 0.72$.

For the order of magnitude estimate to follow a convergence of the flow lines and consequently the second term on the right hand side of Eq. 3 was neglected. At first we assume that the velocity of the membrane v_m due to the tanktread motion is so fast that the bilayer keeps the surface area of the skeleton locally constant. The respective stretch ratio of the skeleton in y direction is called λ_y^f. It holds

$$\lambda_y^f = 1/\lambda_x. \tag{5}$$

The shape of membrane elements (for this fast case) at selected locations is shown schematically in thick outline in Fig. 1.

Next, we assume that v_m at time t_0 suddenly drops to such a small value that the membrane deformation becomes quasistatic. λ_y will then relax from λ_y^f to the equilibrium value which is called λ_y^s. For T_y at time t_0 we use the approximation,

$$T_y = \mu(\lambda_y^f - \lambda_y^s). \tag{6}$$

For μ we use a value of 6×10^{-3} dyn/cm. The value of λ_y^s depends on ν. For a conservative estimate we use $\nu =$

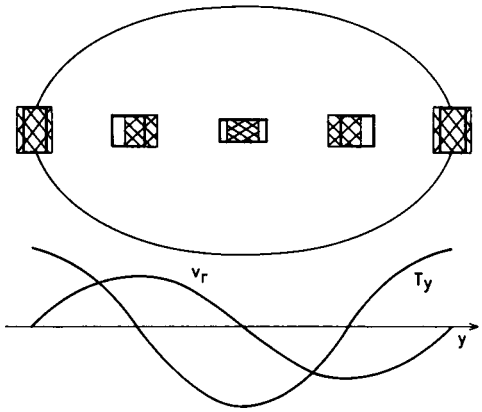

FIGURE 1 Slip between skeleton and bilayer in a tanktreading membrane. The schematic drawing shows in the upper portion the outline of an elongated red cell in projection onto the shear planes, the shape of surface elements of the membrane skeleton (for a fast deformation in thick outline and for the quasistatic case cross-hatched) at selected locations (center, tip, and the intermediate location where $\lambda_x = 1$) on the midline of the membrane. In the lower portion, the dependence of T_y and v_r on the surface coordinate along the midline (for convenience on a contracted scale) is shown. For details see text.

0, the smallest realistic value. For simplicity we assume,

$$\lambda_y^s = 1, \tag{7}$$

although the boundary conditions for the elements of a tanktreading membrane are different from the one used to define ν. With these assumptions we obtain qualitatively a sinusoidal behavior for T_y and via Eq. 3 also for v_r. Both curves are depicted in Fig. 1. The way the skeleton is shifted with respect to the bilayer is sketched schematically (cross-hatched). To estimate v_r at time t_0 we use,

$$\Delta T_y = T_y(\text{tip}) - T_y(\text{center}). \tag{8}$$

If we insert the numbers into Eqs. 5 and 6, we obtain from Eqs. 5–8 a value of 4×10^{-3} dyn/cm for ΔT_y. The curvilinear distance (Δy) on the membrane between the cell center and the tip can be determined to be 6.68 μm. Inserting $\Delta T_y/\Delta y$ for $-dT_y/dy$ in Eq. 3 gives an order of magnitude estimate for v_r at time t_0. An absolute value of 4×10^{-3} μm/s is obtained. The average value of v_m is calculated according to

$$v_m = 4f\Delta y, \tag{9}$$

where f is the frequency of tanktread motion (1.62/s). A value of 44 μm/s is obtained for v_m which is four orders of magnitude larger than v_r. That means a local change in surface area does not form because it is exported from the location to which it is characteristic before it becomes appreciable. It follows that the deformation is fast in the sense introduced above, i.e., the local surface area is essentially conserved even when $\nu < 1$. The same applies to tanktreading in vivo because the tanktread frequencies involved are of the same order of magnitude as in our example (Fischer, 1978; Gaehtgens and Schmid-Schönbein, 1982).

Micropipette experiment

The second example is the dynamic micropipette experiment in which the time course of aspiration or release of a membrane tongue is observed. Characteristic times on the order of 100 ms have been reported for the rapid loading or the unloading phase. After the rapid loading phase, a slow phase has been observed with a characteristic time of 5 s (Chien et al., 1978).

We assume that at time t_0, the membrane is aspirated so fast that the bilayer keeps the surface area of the skeleton locally constant. Subsequently, the skeleton approaches exponentially its final location. From the tensions at time t_0 the initial velocity of the skeleton relative to the bilayer is estimated and compared to the velocity of the membrane during entry.

For the radial tension outside of the pipette at time t_0, the approximation for the infinitely large membrane plane (Evans and Skalak, 1979) is used. According to the sign convention defined above, we obtain for the radial tension (T_r) of the skeleton with respect to the bilayer:

$$T_r(R_p) = -\frac{\mu}{2}[\lambda_r^2(R_p) - 1 + \ln \lambda_r^2(R_p)], \quad \text{and} \tag{10}$$

$$\frac{dT_r}{dr}(R_p) = \frac{\mu}{r}[\lambda_r^2(R_p) - \lambda_r^{-2}(R_p)], \tag{11}$$

where R_p denotes the inner pipette radius. The radial stretch ratio at the pipette rim $\lambda_r(R_p)$ can be expressed by the tongue length (L) and R_p:

$$\lambda_r^2(R_p) = 2L/R_p. \tag{12}$$

For a conservative estimate we take $T_r(R_p)$ and $dT_r/dr(R_p)$ in static equilibrium ($t = \infty$) to be zero. We assume σ at time t_0 not to depend on r. Eq. 3 at time t_0 then reads:

$$\frac{4\pi\eta\sigma}{\ln(R/\rho) - \frac{1}{2}} v_r(R_p) = -\frac{dT_r}{dr}(R_p) - \frac{T_r(R_p)}{R_p}, \tag{13}$$

where Eqs. 10 and 11 have to be inserted on the right hand side. To estimate the initial v_r, we use numbers from an experiment published by Chien et al. (1978). R_p was 0.68 μm. L for the rapid phase was 1.4 μm and after completion of entry, 1.6 μm. From Eq. 13 we obtain $v_r(R_p) = -10^{-2}$ μm/s. Because there is an approximately equal but opposite flow towards the pipette rim from the other (tongue) side, the skeleton would pile up and decrease its surface area in the vicinity of the rim.

To estimate the initial velocity of the membrane (v_m) relative to the pipette rim we divide the total distance travelled in radial direction by the characteristic time. Calculation of the distance involves the conversion of the surface of the tongue to that of a circular disk. Division by the characteristic time of this experiment (60 ms) gives $v_m(R_p) = -12$ μm/s. This is three orders of magnitude faster than $v_r(R_p)$ and indicates that the surface area of the skeleton does not change appreciably because any decrease is exported into the tongue by the membrane flow.

If, however, in a similar fashion $v_m(R_p)$ for the slow phase is calculated a value of -3.5×10^{-2} μm/s is obtained. This is of the same order of magnitude as $v_r(R_p)$. Therefore, it is likely that the slow phase is governed by the motion of the skeleton relative to the bilayer.

COMPARISON TO PREVIOUS WORK

Molecular models

Stokke et al. (1986*a*) were the first to account for a change in local surface area of the skeleton. They suggested the membrane skeleton to behave as an ionic gel and considered cases where K_s was much larger as well as much smaller than μ. The molecular basis for the case $K_s \ll \mu$, however, remained obscure.

Markin and Kozlov (1988) suggested two molecular models: first, the model of immobilized boundaries and second, the model of free boundaries. In the first, a strong interaction between the lipids and spectrin is assumed which prevents the escape of lipids from the meshes of the spectrin actin network. This, however, is not supported by experimental evidence because (*a*) lateral diffusion of lipids over distances of several microns has been observed with the photobleaching technique (Kapitza and Sackmann, 1980) and (*b*) the diffusion coefficient of lipids was not found to be influenced by the absence of the membrane skeleton (Bloom and Webb, 1983). In the model of immobilized boundaries the surface area of the skeleton is locally conserved. In the second model, no restriction of lateral lipid motion by spectrin was assumed which is in keeping with the experimental evidence. K_s was taken to be zero.

Both groups considered only states of static equilibrium. In the present paper, the dynamics of shape changes were considered. To this end the friction involved in a relative motion between skeleton and bilayer had to be taken into account. The molecular model suggested for this friction is in keeping with experimental observations. The two states represented by the two models of Markin and Kozlov (1988) are now represented by a single model at different time scales.

In contrast to both groups, K_s is assumed to be at least of the same order of magnitude as μ because the mechanisms responsible for the shear stiffness also cause an areal stiffness. A shear free deformation regime which resulted from the model of free boundaries and $K_s = 0$ (Markin and Kozlov, 1988) is inconsistent with this assumption.

Continuum mechanical models

In principle, it should be possible to determine ν besides μ from the static micropipette experiment. However, due to the microscopic nature of the experiment the data suffice just to fit one parameter. This parameter has been traditionally the membrane shear modulus (μ) where local conservation of surface area had been assumed (Evans and Skalak, 1979). A model calculation by Stokke et al. (1986*b*) shows that in an approximate fashion μ and ν compensate for each other, i.e., if the real ν was <1, the real μ would be larger than the published value. This result is also intuitively plausible considering that an isolated decrease of ν at constant tongue length would decrease the total elastic strain energy in the membrane. It is this difference in energy that drives the relaxation of local skeleton surface area towards its equilibrium value after a sudden deformation.

The rapid phase of tongue entry as well as the release cannot be used to determine ν because the bilayer provides for local conservation of skeleton surface area. The slow phase of tongue entry, on the other hand, may be an expression of a $\nu < 1$ as speculated above. Based on this speculation K_s can be estimated by comparing the experimentally observed increase in tongue length during the slow phase to the theoretical calculations of Stokke et al. (1986*b*). From the experimental values used above (Chien et al., 1978) the gain in tongue length during the slow phase was 13% at a final tongue length of 1.6 μm. According to Stokke et al. (1986*b*) this corresponds to $K_s/\mu \simeq 10$. This would indicate that other molecular mechanisms than those causing the shear elasticity dominate the areal elasticity of the skeleton.

To determine the value of K_s/μ more precisely, one needs observables that selectively vary with this ratio. One such observable could be the curvature of the streamlines on the membrane of a tanktreading red cell. In model calculations Secomb and Skalak (1982) assuming local conservation of surface area and minimizing the energy dissipation within the membrane came up with quite curved streamlines. With $\nu < 1$, the lateral variations in membrane shear strain would become smaller at the same red cell elongation. It can be conjectured that this allows for straighter streamlines. However, for a $\nu < 1$ to be expressed the tanktread frequencies should be orders of magnitude smaller than the ones presently applied (Fischer and Schmid-Schönbein, 1977; Sutera et al., 1983).

It is interesting to note that in the tanktreading experiment used above where it was shown that the surface area is locally conserved the observed streamlines are already less curved than the predicted ones. This is demonstrated in Fig. 2 where the path of membrane attached latex beads is shown. The red cell elongation is the same as in the theoretical example presented by Secomb and Skalak (1982). It is unlikely that for the discrepancy the neglection of the fluid flows inside and outside of the red cell is responsible because variations in the flow of the enclosing surface are not expected to change the three-dimensional flows much. It

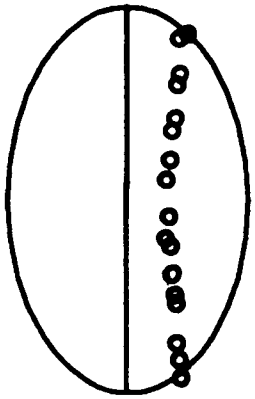

FIGURE 2 Two-dimensional flow on a tanktreading membrane. A red cell was subjected to simple shear flow (mean shear rate 42/s) in dextran-saline solution (23 cP, at room temperature). The drawing shows in projection onto the shear planes: the outline of the cell, a midline, and the consecutive positions of a latex bead for a full cycle of tanktread motion (time interval 41.6 ms). The asymmetry of the path is due to the fact that the ellipsoidal outline of the red cell showed some oscillation during the tanktread motion, which, in turn, is attributed to a slightly noncircular resting shape of this particular cell.

is more likely that shear thinning of the membrane viscosity (Chien et al., 1978) not taken into account by Secomb and Skalak (1982) is responsible. And indeed the two-dimensional membrane shear rate in this experiment is in a range where shear thinning is prominent.

DISCUSSION

Assumptions

The estimates made above are rather rough for several reasons. (*a*) The experimental determination of the viscosity of phosphatidylcholine bilayers in vesicles was for technical reasons an upper bound of the real value (Waugh, 1982). On the other hand, the presence of other lipids especially of cholesterol may increase the viscosity of the lipid phase in the red cell membrane over that of a pure phosphatidylcholine bilayer (Cooper et al., 1978; Bloom and Webb, 1983).

(*b*) The assumption of equidistance among intrinsic proteins may be wrong in light of the fact that only a fraction of all copies is connected to the skeleton. The free ones caught within the meshes of the skeleton may be dragged into the corners thus constituting a larger resistance to lipid flow than in an equidistant arrangement.

(*c*) The force on moving proteins was adopted from Saffman (1976). For the diameter of the circular sheet to which the flow is restricted the mean distance of the proteins was taken. Saffman and Delbrück (1975) used the diameter of the whole cell instead. It happens, however, that the choice made here gives the same result as when the force is obtained from calculations on flows past infinite periodic arrays of cylinders (Ishimi et al., 1987). With increasing density of cylinders the last model is to be preferred against the first one.

Consequences for model calculations

It was stated above that the real μ would be larger than the published value if $\nu < 1$. If the real values of ν and μ were known one could use them instead of the published values to model red cell behavior in situations governed by static equilibrium other than micropipette aspiration (shape change, aggregation, or centered flow of red cells through a capillary). This would require to include the boundary condition of constant total surface area of the skeleton into the model. The improvement by the increased computational effort, however, might not be large because a similar compensation as in modeling the micropipette experiment may be operative. In other words, using the published value of μ and assuming local conservation of surface area may give similar results as using the correct values plus the boundary condition.

In modeling dynamic red cell deformation (tanktread motion in simple shear flow, off axis flow of red cells through a capillary, or the dynamic micropipette experiment) local conservation of surface area can be assumed. However, the real membrane viscosity would be larger than the published value by the same factor as is the real μ because the measured membrane viscosity is calculated as the product of μ and a characteristic time.

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