BRIEF COMMUNICATION

ON THE ENERGY DISSIPATION IN A TANK-TREADING HUMAN RED BLOOD CELL

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ABSTRACT The energy dissipation in the membrane (ED_{mem}) and in the cytoplasm (ED_{cyt}) of tank-treading human red blood cells is estimated. The tank-tread motion of the membrane occurs when the cells in a sheared suspension assume a steady-state of orientation (Fischer et al., 1978, *Science* [*Wash. D. C.*], **202**:894). The kinematic data used are from red cells suspended either in a dextran-saline solution at a low hematocrit, or in plasma at a hematocrit of 45%. The viscosities of the cytoplasm and the membrane are taken from the literature. The cell in dextran was subjected to seven different shear rates. Both ED_{mem} and ED_{cyt} showed a strong increase with shear rate. Their ratio, however, was always of the order of 1. From this value and the value which was given by Hochmuth et al. (1979, *Biophys. J.*, **26**:101) for a shape recovery of a red cell, it is concluded that the range of ED_{mem}/ED_{cyt} for all possible geometries is 1–100.

From viscosity measurements of red blood cell suspensions it has long been known that the cytoplasm of the human red cell must be liquid and participate in flow (Hatschek, 1920; Bingham and Roepke, 1944). The viscosity of the cytoplasm of the red cell (η_{cyt}) has been estimated by theoretical modeling by Dintenfass (1968) and measured by viscometry by Cokelet and Meiselman (1968). Because the volume of the cytoplasm is two orders of magnitude greater than the membrane volume, the tacit assumption has generally been made that most of the energy dissipation in a flowing red cell occurs in the cytoplasm. The shear viscosity of the red cell membrane (η_{mem}) was recently estimated by Evans and Hochmuth (1976) and later measured by Chien et al. (1978) and Hochmuth el al. (1979). The energy dissipation in the cytoplasm (ED_{cyt}) and the membrane (ED_{mem}) can be estimated from the two viscosities, η_{cyt} and η_{mem}, and the kinematic data of individual red cells in sheared suspensions, as measured in the rheoscope (Fischer et al., 1978*a*).

The method of rheoscopy is described in detail elsewhere (Fischer et al., 1978*a*). Briefly, a suspension of human red cells is subjected to a simple shear flow in a transparent counter-rotating cone-and-plate chamber. In the stationary fluid layer midway between cone and plate, individual red cells are observed through a microscope directed along the gradient of the shear field. Films are taken with a high speed camera. Motion of the membrane is monitored by observing the motion of membrane-bound particles (latex spheres 0.8-μm diam or Heinz bodies produced by treatment with acetylphenylhydrazine). Fischer et al. (1978*a*) have shown that red cells in sheared suspensions become oriented and elongated above a

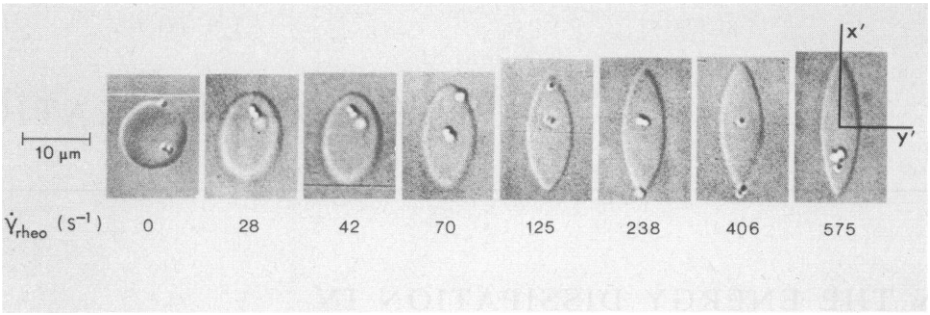

FIGURE 1 Red blood cell marked with three latex spheres suspended in a dextran-saline solution (23 cP) in simple shear flow at different shear rates ($\dot{\gamma}_{rheo}$). x', flow direction; y', direction of the vorticity vector.

certain threshold shear rate (for a suspending medium of a given viscosity). In these states the membrane is driven into a motion around the red cell, generating a shear flow in the cytoplasm. This membrane motion has been dubbed tank-tread motion. The tank-tread motion was shown to have the same frequency for all parts of the membrane, consistent with the notion of a membrane having elastic properties. The kinematic data of tank-treading red cells, together with membrane and cytoplasmic viscosities, are used in this brief communication to establish a lower bound for the energy dissipation ratio ED_{mem}/ED_{cyt}.

Fig. 1 shows one single red cell suspended in an isotonic dextran-saline solution (viscosity 23 cP) subjected to different shear rates in the rheoscope ($\dot{\gamma}_{rheo}$). The outline of the cell $x'(y')$ was traced from the photographs and digitized in ~ 15 points for each $\dot{\gamma}_{rheo}$. From the motion picture the x' coordinate of a latex sphere on the centerline ($y' = 0$) was measured and plotted as a function of time. Fig. 2 shows such a graph for the highest shear rate. The curves are essentially straight between their extrema. This means that the membrane in the centerline travels at essentially constant velocity over the flat face of the cell. This property was used in the approximations to be described. From the graphs the tank-tread frequency f and the projected velocity v' were determined (Table I). Both show a linear increase with $\dot{\gamma}_{rheo}$. The

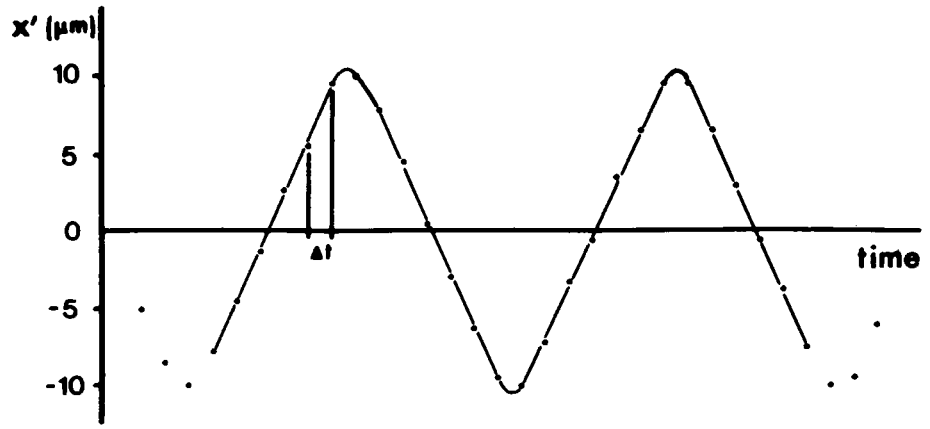

FIGURE 2 x'-Position of a latex sphere on a tank-treading red cell ($\dot{\gamma}_{rheo}$ = 575 s^{-1}) versus time; time interval between two frames Δt = 2.08 ms.

TABLE I

ENERGY DISSIPATED IN THE MEMBRANE (ED_{mem}) AND IN THE CYTOPLASM (ED_{cyt}) AND THE RATIO (ED_{mem}/ED_{cyt}) OF TANK-TREADING RED CELLS SUBJECTED TO A SHEAR RATE γ_{rheo}

γ_{rheo}	f	v'	$2c$	θ	ED_{mem}	ED_{cyt}	ED_{mem}/ED_{cyt}
(s^{-1})	(s^{-1})	($\mu m/s$)	(μm)	(degrees)	(10^{-8} erg/s)	(10^{-8} erg/s)	
28*	1.11	33.7	2.55	20	1.7	1.6	1.0
42*	1.62	51.7	2.51	18	4.8	4.0	1.2
70*	2.47	79.3	2.73	15	14.3	8.1	1.8
125*	4.01	161.3	2.81	9	42.0	30.9	1.4
238*	7.24	324.7	2.74	8	151.7	133.3	1.1
406*	12.2	547.8	3.17	7	365.9	269.4	1.4
575*	17.3	816.6	2.95	5	650.5	661.6	1.0
700‡	3.2	56	2.6	0	6.6	2.4	2.8

Input parameters are tank-tread frequency f, projected centerline velocity v' of the membrane, volume V of the cell, and the viscosities of membrane and cytoplasm. Intermediate results are the maximum thickness $2c$, the surface area A of the cell, and its inclination θ to the flow direction.

*Cell suspended in dextran-saline solution (23 cP). $V = 130\ \mu m^3$; $A = 178\ \mu m^2$.

‡Cell suspended in plasma. $V = 85\ \mu m^3$; $A = 120\ \mu m^2$.

volume V of the red cell was assumed to be 130 μm^3. This value was chosen according to the correlation between diameter and volume established by Canham and Burton (1968) and the diameter of the cell (9 μm, see Fig. 1). From V and the traced outline $x'(y')$ of the cell, its thickness $2c$, its surface area A,and its inclination θ to the x' axis were estimated iteratively for each shear rate (Table I). The coordinate system describing the cell geometry was then rotated about the y' axis through the angle θ; the new axes x, y, and z were then aligned with the major axes of the cell at each shear rate. For the estimation of $2c$ and A the y-z cross section was assumed to be an ellipse. The outline in the x-z plane was assumed to be of the form $z = ky(x)$, where k was chosen to give the assumed value of 130 μm^3 for the volume of the cell. $y(x)$ is the outline of the cell in the x-y plane. The inclination θ was estimated using observations of the positions at which the latex spheres moved in and out of focus. The calculated thickness $2c$ showed a small increase from 2.6 μm for $\dot{\gamma}_{rheo} = 28\ s^{-1}$ to 3 μm for $\dot{\gamma}_{rheo} = 575\ s^{-1}$. The calculated surface area A varied between 171 and 183 μm^2, with a mean of 178 μm^2.

The observation of constant membrane velocity at $y = 0$ indicates that there is almost no membrane stretching or shear strain on the centerline. This is even more so at the other $\dot{\gamma}_{rheo}$, where the maximum stretch ratio is smaller. To model this nonuniform shear strain, the following method of estimating the energy dissipation in the membrane was used. It was assumed that the membrane motion on each face of the cell could be approximated by a planar motion, with suitable adjustment at the rim. The membrane velocity was taken to be

$$\dot{x} = 4fx(y)$$

$$\dot{y} = 0,$$

where f is the tank-tread frequency and $x(y)$ the coordinates of the rim of the cell. Fig. 3 shows a sketch of successive positions of a line fixed in the membrane at equal time steps. It also shows two examples of the deformation of the membrane, on and off the centerline,

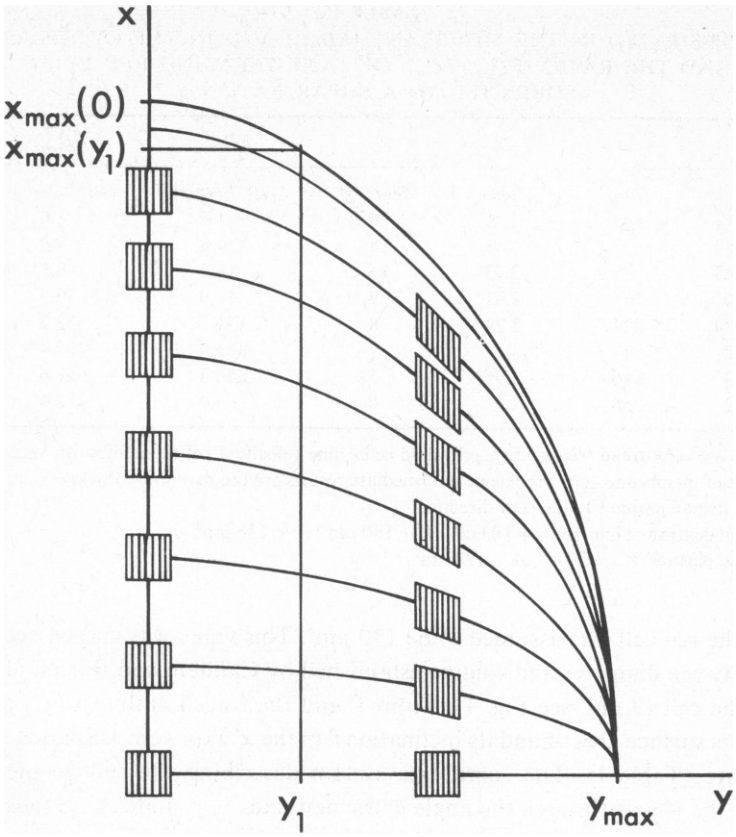

FIGURE 3 Schematic drawing of a segment of a red cell, showing successive positions at equal time intervals of a line fixed in the membrane. Also depicted is the successive deformation of a pair of small squares fixed in the membrane. The contour $y(x)$ is for $\dot{\gamma}_{rheo} = 28\ s^{-1}$.

respectively. The energy dissipation in the membrane per surface area for this motion is given by Evans and Skalak (1979):$\eta_{mem}(d\dot{x}/dy)$.[2] This expression depends only on y. After integration the energy dissipation in the membrane (ED_{mem}) is obtained:

$$ED_{mem} = 8\,\frac{178\ \mu m^2}{S}\int_0^{y_{max}} \eta_{mem}(y)\left(\frac{d\dot{x}}{dy}\right)^2 x(y)\,dy.$$

Here η_{mem} was taken from the data of Chien et al. (1978), who observed a shear rate dependence of the membrane viscosity. The range of η_{mem} used was 1.5–5.4 10^{-4} dyn s/cm. y_{max} is the width of the cell (Fig. 3). The integration was carried out numerically using the trapezoidal rule. As a consequence of the planar model the membrane shear rate $d\dot{x}/dy$ diverges when y approaches y_{max}, that is, near the rim. To give more realistic behavior, $d\dot{x}/dy$ was assumed to stay constant beyond the point where z-coordinate (at $x = 0$) was equal to $y_{max} - y$. The ratio 178 $\mu m^2/S$ corrects for the smaller surface area of the flat model (surface area S) as compared with the three-dimensional cell (surface area 178 μm^2). The values of ED_{mem} for the different shear rates are given in Table I. They show, as expected, a strong increase with $\dot{\gamma}_{rheo}$.

For the estimation of the cytoplasmic shear rate ($\dot{\gamma}_{cyt}$) only x-components of the velocity were considered. $\dot{\gamma}_{cyt}$ was assumed to be constant in z-direction. Observation of cytoplasmic and membrane-bound markers in the same cell indicate that this is a reasonable assumption (Fischer and Schmid-Schönbein, 1977). $\dot{\gamma}_{cyt}$ then becomes

$$\dot{\gamma}_{cyt}(x, y) = \frac{v'}{\cos\theta} \frac{x_{max}(y)}{x_{max}(0)} \Big/ z(x, y).$$

The energy dissipation in the cytoplasm (ED_{cyt}) was obtained by integration using the trapezoidal rule:

$$ED_{cyt} = 8\eta_{cyt} \int_0^{y_{max}} \int_0^{x_{max}(y)} \dot{\gamma}_{cyt}^2(x, y)\, z(x, y)\, dx dy.$$

See Fig. 3 for the nomenclature of y_{max} and x_{max} (y). Since the shear rate calculated with the above simplified formula rises strongly near the rim of the cell, it was assumed to stay constant in the integration when the z-coordinate became larger than the distance from the rim. For η_{cyt} a value of 10 cP (at room temperature) was used according to Cokelet and Meiselman (1968) and Chien et al. (1970). The results for ED_{cyt} are shown in Table I. ED_{cyt} also shows a strong increase with $\dot{\gamma}_{rheo}$. The ratio ED_{mem}/ED_{cyt}, however, is essentially constant and of the order of 1. This means that there is as much energy dissipated in the membrane as in the cytoplasm despite the two orders of magnitude difference in volume of the two components.

To show that this result is pertinent to whole blood as well, a similar estimation was done based on the results of an experiment with a whole blood-like suspension. The results have been published elsewhere (Fig. 6, Fischer, 1978). Red cells were suspended in autologous blood plasma at a hematocrit of 45% and sheared at $\dot{\gamma}_{rheo} = 700\ s^{-1}$. Such shear rates occur physiologically near the walls of small arteries and arterioles (Whitmore, 1968). Since the shape of red cells under these conditions is rapidly changing due to cell-cell interaction (Fig. 6, Fischer, 1978), only the average length and width of the cell under consideration were determined. Assuming a triaxial ellipsoid for the shape of the cell, the thickness $2c$ was determined so that volume and surface area were related according to a formula established experimentally by Canham and Burton (1968). The estimate for the energy dissipation ratio is 2.8. This is about twice as high as was found for the cell suspended in dextran. This difference could be attributed to one or more of the following facts: (*a*) The boundary conditions for the two cells are different. (*b*) The cell in plasma has altered properties due to the treatment with acetylphenylhydrazine (Lubin and Desforges, 1972). (*c*) The cell in dextran has an unusually low volume-to-surface area ratio.

In view of the approximations used, the basic conclusion of this study is that in a tank-treading red cell the energy dissipation in the membrane is of the same order of magnitude as in the cytoplasm. This corroborates the notion that the dynamic behavior of a normal red cell is dominated by its membrane, which was adopted on the basis of a comparison of the behavior of red cells and liquid droplets (Fischer et al., 1978*b*). The estimated energy dissipation ratio ED_{mem}/ED_{cyt} is probably an underestimate because (*a*) ED_{cyt} has probably been overestimated by taking a constant $\dot{\gamma}_{cyt}$ near the rim, whereas the actual flow field there looks more like a rigid body motion with much lower shear rates. (*b*)

ED_{mem} has probably been underestimated by assuming pure shear and neglecting stretching of the membrane.[1]

Hochmuth et al. (1979) estimated the ratio of energy dissipation in membrane and cytoplasm for a red cell which recovers freely from an elongated shape. This geometry can be considered to give a maximum ratio for ED_{mem}/ED_{cyt}. The ratio was estimated by these authors to be of the order of 100. The geometry of the tank-tread motion on the other hand should give approximately a minimum value for ED_{mem}/ED_{cyt}. Since the calculated value of 1 is probably an underestimate, one might expect ED_{mem}/ED_{cyt} for all other geometries, e.g., passage of a red cell through a small pore to lie in the range of 1–100 given by these two extreme values.

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[1] ED_{mem} has also been estimated under the assumption of pure stretching. The results are of the same order magnitude as those obtained by assuming pure shear.