Thirty minutes after anoxia was induced (when the maximum binding of [^{3}H]diazepam was observed after electrically or chemically induced seizures), the animals were killed for determination of cortical benzodiazepine receptors.

8. Specific [^{3}H]diazepam binding was determined as described (*5*) with the following minor modifications. Synaptosomal suspensions were incubated in a total volume of 1.5 ml. Each preparation consisted of 1.0 ml of tissue suspension (crude synaptosomal fraction), 0.4 ml of tris-HCl buffer (0.05*M*, *p*H 7.4), 0.0375 ml of distilled water or nonradioactive diazepam (3 μM), and [^{3}H]diazepam (specific activity, 39.08 Ci/mmole, New England Nuclear) diluted to the appropriate concentration with distilled water. The preparations were incubated for 15 minutes in an ice bath at 0° to 4°C. Incubation was terminated by filtering through Whatman GF/B filters and washing the filter with 10 ml of ice-cold tris-HCl buffer (0.05 *M*, *p*H 7.4). Filters were suspended in 10 ml of Aquasol (New England Nuclear) and the radioactivity measured with a Beckmann LS-355 liquid scintillation counter.
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11. Diazepam is recommended as the drug of choice in the treatment of status epilepticus (*1*). A dose of 2 to 20 mg administered intravenously is effective in most cases. This compares to dosages of 200 to 400 mg and 300 to 500 mg for phenobarbital and diphenylhydantoin, respectively. Diazepam is 50 to 100 times more potent than the other commonly used anticonvulsants in this disorder [F. A. Elliott, *Clinical Neurology*, (Saunders, Philadelphia, 1971), pp. 140–141.]
12. Scatchard and saturation analyses (see Fig. 1) indicate seizure-induced alterations in receptor number rather than affinity. This enabled us to use a range of ligand concentrations in these experiments, since the relative changes in receptor number observed would be independent of the concentration used.
13. S.M.P. is a research associate in the Pharmacology Research Associate Training Program, National Institute of General Medical Sciences. P.S. was a guest worker at the Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, during these studies.

22 February 1978; revised 8 May 1978

The Red Cell as a Fluid Droplet: Tank Tread-Like Motion of the Human Erythrocyte Membrane in Shear Flow

Abstract. *When whole human blood is subjected to viscometric flow, individual red cells are seen to be elongated and oriented in the shear field. In addition, a tank tread-like motion of the membrane around the cell content occurs. In dilute suspensions of erythrocytes in viscous media, the same behavior is better observed and can also be measured quantitatively.*

The peculiar shape and unusual deformability of mammalian red blood cells (RBC's) have been the subject of much scientific debate. In the last century conflicting concepts of the structure of the red cell were advocated; hypotheses ranged from the assumption of an elastic internal matrix to the model of a flexible membrane shell filled with a fluid (*1*). In the first part of this century the low viscosity of RBC suspensions led to the model of blood as an emulsion (*2*). This idea was taken up by Dintenfass (*3*), who stated that the fluidity of concentrated RBC suspensions can only be explained if the cytoplasm of the red cell is liquid and participates in shear flow. By measuring the viscosity of RBC suspensions in which the viscosity of the suspending phase and the volume concentration of RBC's were varied and comparing the results with a theoretical equation for the viscosity of concentrated emulsions, he estimated the viscosity of the cytoplasm (*4*). Furthermore, by using a theory for the viscosity of emulsions, he postulated a membrane that becomes an extremely inviscid liquid surface film when the RBC suspension is sheared (*4*). Alternatively, Schmid-Schönbein and Wells (*5*) explained the viscometric data in terms of a tank tread-like motion of the whole membrane around the cell content. This concept was based on microscopic observation of RBC suspensions under shear. Füredi and Ohad (*6*) had reported a similar membrane motion of RBC's subjected to an alternating electromagnetic field. We present here an improved technical approach and a closer analysis of this phenomenon.

A suspension of RBC's was placed in a counterrotating transparent cone-and-plate chamber (Fig. 1) adapted to an inverted interference contrast microscope (*7*). When focusing the stationary layer of the shear field, it was possible to observe RBC's under shear without translational movement. Cinematographic records were taken with a high-speed camera driven to a maximum of 500 frames per second (*8*) and synchronized to strobe illumination (*9*). The cone angle was 1.5°. Observations were made at distances of 0.5 to 1.5 mm from the axis of revolution. The observed shear rate ($\dot{\gamma}$) for each experiment was obtained from the difference between the peripheral speeds of cone and plate divided by their distance. Erythrocytes were suspended either in autologous plasma or in viscous solutions of dextran (*10*) in phosphate-buffered saline. Dextran solutions were adjusted to isotonicity within a 10 percent error by measuring the vapor pressure (*11*). The viscosities of the dextran solutions (η_0) were measured with a capillary viscometer (*12*) and corrected for the temperature of the room in which the experiments were done. To prevent crenation, human albumin (0.1 to 0.3 g per 100 ml) was added. Latex particles (diameter, 0.8 μm) (*13*) were used as external membrane markers, but only in experiments with dextran. Heinz bodies (hemoglobin precipitates), produced by incubation of RBC's (hematocrit, 2.5 percent) in acetylphenylhydrazine (2 mg/ml) in phosphate-buffered saline at 30°C for 4 hours, were used as internal membrane markers and also as markers of the cytoplasm.

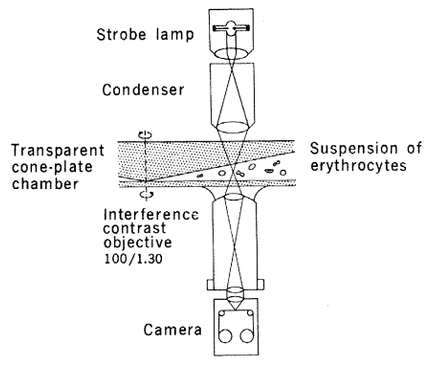

Fig. 1. Schematic drawing of the setup (for details see text).

When whole human blood—that is, red cells in plasma (hematocrit, 40 to 45 percent)—is subjected to shear rates above 500 sec^{-1} the RBC's are elongated flat ellipsoidal bodies (*14*), which are irregular in shape and not stationary. When one observes an individual cell, however, it becomes evident that for most of the time the main axis of the ellipsoid is oriented approximately parallel to the direction of flow and that its flat faces are parallel to the planes of shear. When $\dot{\gamma}$ or the hematocrit or both are elevated, the cells become more elongated, assume a more stationary orientation, and show less change of shape. In the oriented state of the RBC, the tank tread motion of the membrane can be observed through the Heinz bodies bound to the cytoplasmic side of the membrane.

To simplify the fluid mechanical boundary conditions of an individual cell, RBC's were suspended at a low hematocrit in viscous solutions. In these suspensions, the behavior mentioned above is not complicated by cell-cell interactions and is therefore more regular. Figure 2, A and B, shows a resting biconcave red cell. In Fig. 2C the same cell is elongated and oriented in the shear field.

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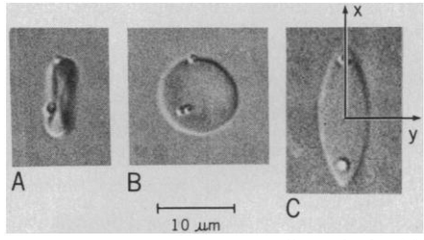

Fig. 2 (above). Red blood cell marked with three latex particles suspended in dextran solution (η_0 = 23 cP): (A and B) at rest, photographed at right angles, and (C) elongated and oriented in the shear field ($\dot{\gamma}$ = 240 sec^{-1}; x, flow direction; y, radial direction in the cone-and-plate chamber).

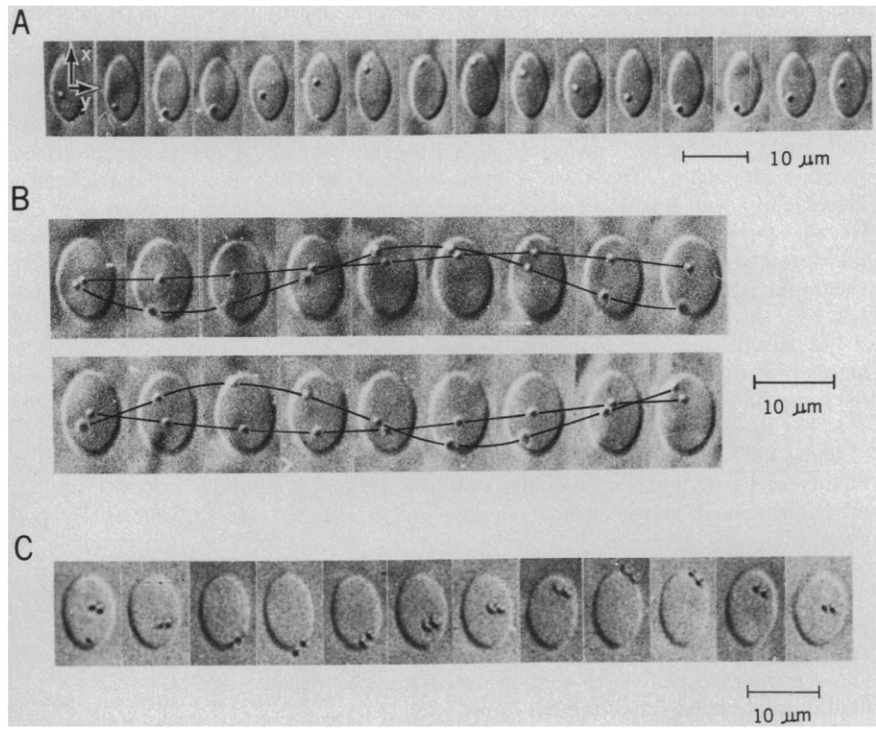

Fig. 3 (right). Photomontages from a motion picture of red blood cells in dextran solution subjected to viscometric flow (x, flow direction; y, radial direction in the cone-and-plate chamber). (A) Tank tread motion of a membrane-bound Heinz body (η_0 = 35 cP; $\dot{\gamma}$ = 46 sec^{-1}; time interval, 40 msec). (B) Motion of a membrane-bound and a cytoplasmic Heinz body (η_0 = 35 cP; $\dot{\gamma}$ = 22 sec^{-1}; time interval, 120 msec). Heinz bodies of consecutive frames are connected by lines. (C) Tank tread motion of two latex particles adhering to the RBC membrane (η_0 = 22 cP; $\dot{\gamma}$ = 46 sec^{-1}; time interval, 83 msec).

The elongation increases when $\dot{\gamma}$, η_0, or both are elevated. When the shear flow stops the cells immediately relax to their resting shape. Figure 3A shows the motion back and forth of a membrane-bound Heinz body. In three dimensions we interpret it as a continuous motion around the cell. In addition to the membrane-bound markers, freely suspended markers are occasionally seen moving in the cytoplasm of the cell. With their help it is possible to directly demonstrate the existence of cytoplasmic shear flow in addition to the membrane tank tread motion. A cytoplasmic Heinz body reverses its direction of linear motion before actually reaching the edges of the cell and has a lower frequency of motion than does a membrane-bound one (Fig. 3B). If one observes the motion of a cytoplasmic Heinz body for several periods, it usually changes its y position in the cell or its maximum x position; that is, it moves across planes of shear.

Since the preparation of Heinz bodies changes the mechanical behavior of RBC's (*15*), latex particles adhering to the outer surface of the membrane were used as membrane markers in the quantitative experiments. That latex particles are good membrane markers was shown with cells marked with both Heinz bodies and latex particles; observation of the tank tread motion for up to 30 periods did not show any change in the phase relation between these two types of markers. Moreover, the tank tread motion of

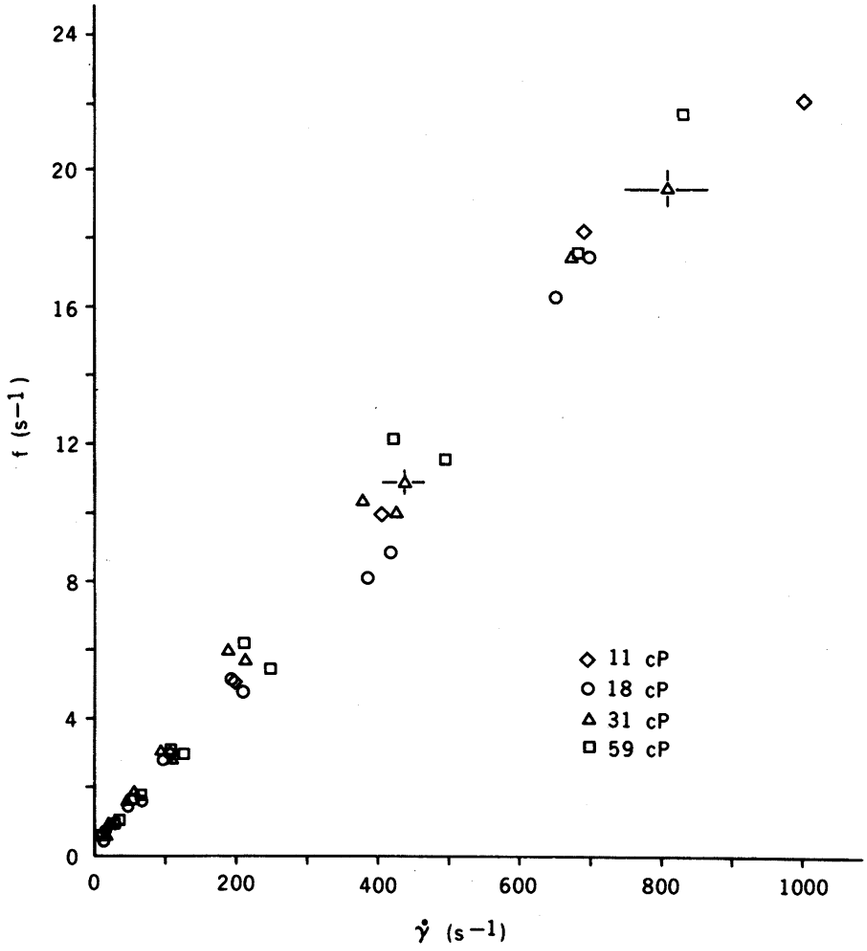

Fig. 4. Tank tread frequency f plotted against $\dot{\gamma}$ for four different values of η_0. Each point represents the mean for 5 to 20 cells. Error bars show representative standard errors of the means.

the membrane can be characterized by a single frequency, since multiple marker particles on a single RBC membrane moved with the same frequency during the motion (Fig. 3C). Figure 4 shows the tank tread frequency (f) measured as a function of $\dot{\gamma}$ and η_0. There was a linear increase of f with $\dot{\gamma}$ with the same slope for all values of η_0 tested. In contrast, the observed elongation for a particular shear rate strongly depends on η_0 (*16*, *17*).

The results reported here lead to a number of observations about red cell micromechanics:

1) The demonstration of the existence of shear flow within the cell proves the fluidity of the cytoplasm and rules out the assumption of elastic elements in the cytoplasm.

2) The constant phase relationship between multiple membrane markers during tank tread motion makes it unnecessary to postulate an extremely inviscid liquid membrane film (*4*) to explain the fluid droplike behavior of the entire red cell. In contrast, our observations show that despite the well-established shear elasticity of the membrane (*18*), continuous shear deformations are tolerated and allow the generation of stationary flow within the cytoplasm. Other results (*19*) show that tank tread motion can occur even after the normally low shear modulus of the membrane has been strongly increased on modification of membrane proteins by reagents with SH groups.

3) For better definition of individual RBC's, the tank tread motion in whole blood was observed at a small distance (5 μm) between cone and plate. This gives rise to the objection that the proximity of the cone and plate is responsible for the tank tread motion. However, elongation and orientation could be observed at a much greater distance (15 μm), where wall effects can be neglected. From this we conclude that the tank tread motion also occurs in the bulk flow of whole blood at sufficiently high shear rates. We postulate that the participation of the RBC cytoplasm in the shear flow of the whole suspension is an important factor in the low-bulk viscosity of whole blood and its shear thinning behavior at high shear rates.

4) Although an emulsion is a better model for blood than a suspension, there are decisive differences in the behavior of red cells and liquid droplets when the viscosities of continuous and dispersed phases are matched in both systems. In liquid droplets the frequency of motion of the boundary face depends on the viscosity ratio of the dispersed and the continuous phase, whereas in RBC's the tank tread frequency is independent of the suspending media investigated and thus of the viscosity ratio. This difference could be attributed either to a favorable ratio of the volume to the surface area of the RBC, allowing large deformation of the cell without an increase of the membrane area, or to the predominance of the mechanical properties of the membrane over the viscosity ratio of the cytoplasm and the suspending phase (*20*).

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20. The comparison of RBC and liquid droplet behavior is extensively dealt with in (*17*).
21. We thank H. Malotta for measuring the viscosities of the dextran solutions, F. J. Kaiser for photographic work, and M. Brendt for preparing the manuscript. The work was supported by the Deutsche Forschungsgemeinschaft, SFB 109, C 5.

10 May 1978; revised 6 July 1978

Human Flicker Sensitivity: Two Stages of Retinal Diffusion

Abstract. *A well-known solution of the diffusion equation gives an exponential square-root function as the frequency response for a one-dimensional diffusion or transmission process. When two or more such processes are cascaded, the result is still an exponential square-root characteristic, but with a longer time constant. This seems to explain why flicker thresholds obey the Kelly-Veringa diffusion model at high frequencies, even though the psychophysically inferred diffusion process is much slower than the first stage of visual transduction measured by, for example, late receptor potentials. Two such stages in tandem are sufficient to account for the psychophysical data, because the psychophysical time constant is proportional to the square of the number of stages involved. In addition, the nonlinear behavior of flicker thresholds under intense light adaptation can be explained if the loss factor in the first stage is proportional to the amount of the photopigment bleached. Apparently the flicker thresholds are governed by first- and second-order retinal neurons.*

An old problem in psychophysics is the question of whether the thresholds for certain classes of simple stimuli are controlled primarily by retinal mechanisms or by higher visual centers. For photopic flicker thresholds, some insight into this question is provided by the photoreceptor diffusion model proposed by Veringa (*1*). As modified by Kelly (*2*), the diffusion model describes the flicker sensitivity as a function of frequency by the expression

$$G(\omega) \sim \exp(-\sqrt{\omega\tau}) \qquad (1)$$

where G is the amplitude sensitivity, ω is the flicker frequency multiplied by 2π, and τ is the time constant of the diffusion process. At relatively high flicker frequencies, this exponential square-root function fits the sinusoidal flicker data from many sources (*2*–*8*), both psycho-

Table 1. Comparison of receptor and psychophysical time constants from several sources.

Receptor time constants			Psychophysical time constants	
τ_1	Reference	$4\tau_1$	τ	Reference
0.19	(*5*)*	0.76	0.82	(*6*)‡
0.19	(*3*)†	0.76	0.74	(*8*)§
0.15	(*6*)*	0.60	0.50	(*2*, *7*)‖

*Late receptor potential. †Electrical phosphene. ‡10° field. §7° field. ‖Ganzfeld.

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