

## SHORT COMMUNICATIONS

Measurements of the Apparent Interfacial Viscosity  
of the Red Blood Cell<sup>1</sup>

A great deal of rheological data on the flow behavior of the red blood cell have appeared in the literature (1-4) and studies of its shear-dependent behavior have characterized blood as a non-Newtonian fluid (5). Red cell deformation has been expressed in terms of stress and strain occurring as a response to a deforming stress acting on the cell surface (4-8). To date, no data have been reported on measurement of the apparent interfacial viscosity of the red blood cell.

The concept that the surface layer of a solution, such as red blood cells in 0.9% isotonic NaCl solution, may have mechanical properties different from the bulk solution is not a new one, having been frequently tested in examining the surface viscosity of surfactant solutions. Hence, it is assumed that the liquid-air interface of red blood cells in solution may have properties which are a counterpart to the bulk viscosity, particularly a greater resistance to shear stress. Using the interfacial viscometer, it is possible to determine the ratio between shear stress and shear rate in the plane of the interface, primarily to examine adhesion, adsorption, and coagulation properties of the red blood cell at the interface.

Such an interfacial viscometer was constructed in our laboratory based on a modification of the design of Brown *et al.* (9) and Karam (10), as shown in the accompanying schematic diagram (Fig. 1). The instrument consists of a turntable, a rotating dish, and a knife-edged bob suspended by a torsion wire.  $R_b$  is the radius of the knife edge of the bob, 2.0 cm;  $R_c$  is the radius of the cup, 2.5 cm;  $R_d$  is the diameter of the turntable, 20.7 cm; while  $e = 3$ ,  $f = 5$ , and  $h = 10$  mm. The torsion constant of the wire,  $K$ , was determined by measuring the period of oscillation time,  $t$ , with an object of known moment of inertia,  $I$ , suspended by the wire:  $K = 4\pi^2\Delta I/t^2 - t_0^2$ , where  $t_0$  is the first measure of the chuck and damping ring.

Evaluation of the apparent interfacial viscosity (in surface poise, dyne-sec/cm) for an ideal Newtonian flow from the angular displacement of the bob corresponding to the angular velocity (shear rate) was formulated by Reiner (9),

$$\eta_s = \frac{K(\theta - \theta_\omega)}{4\pi\omega} \left( \frac{1}{R_b^2} - \frac{1}{R_c^2} \right),$$

<sup>1</sup> This work was supported by the College of Medicine and, in part, by NSF grant BMS 71-00850-A03.

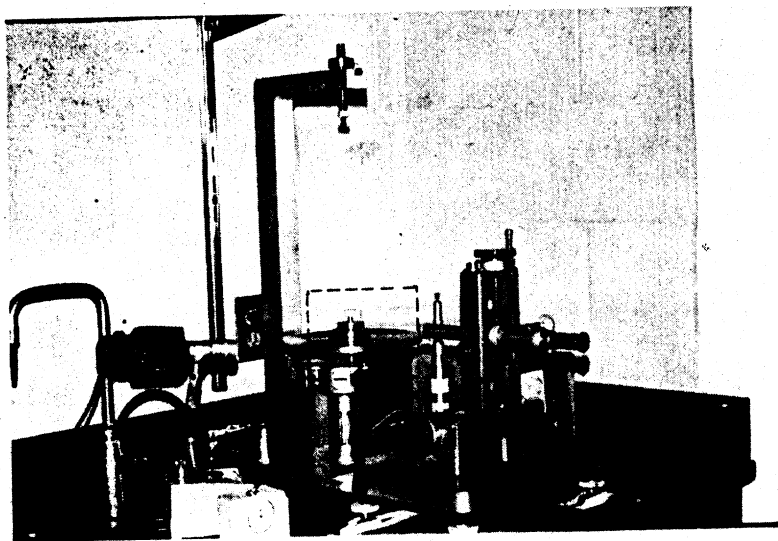
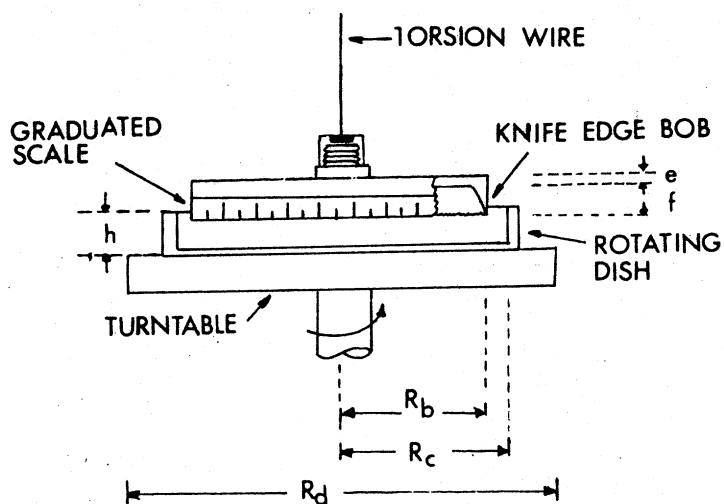


FIG. 1. Schematic diagram of the interfacial viscometer, a modification of the design of Brown *et al.* (9) and Karam (10), showing the turntable, rotating dish, and knife-edged bob suspended by a torsion wire. The instrument itself is shown in the accompanying photograph (dotted line defines area shown in diagram).

where  $\omega$  is the angular velocity in degrees per second and  $(\theta - \theta_\omega)$  is the angle of deflection of bob in degrees. For non-Newtonian fluids, the apparent interfacial viscosity is a function of the shear rate; hence, a rheogram of mean shear stress versus mean shear rate conveys more information about the red blood cell system.

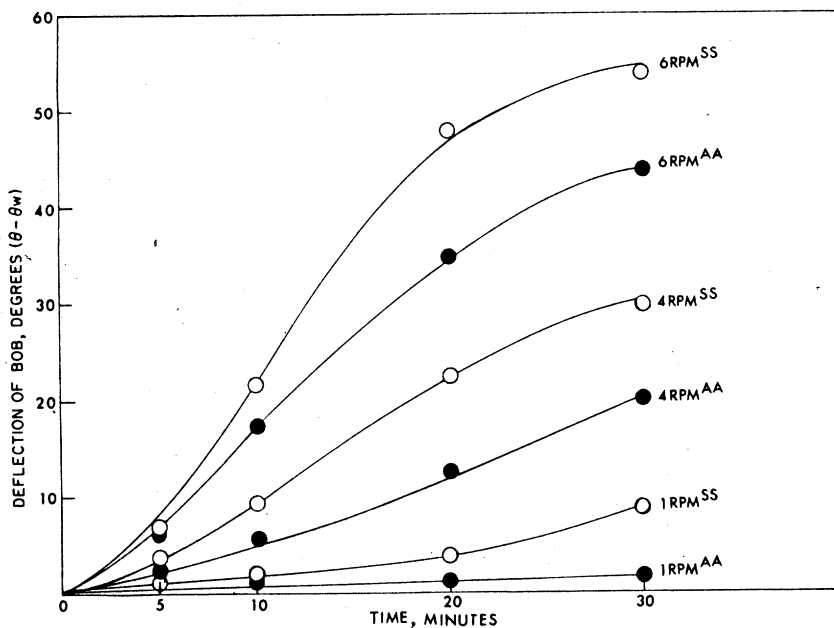


FIG. 2. Plot of deflection of the bob in degrees ( $\theta - \theta_\omega$ ) versus elapsed time at a given velocity of the turntable, for samples of normal (AA) and sickling (SS) red blood cells.

Karam's expression (10) for apparent interfacial viscosity as a function of the mean shear rate,  $\bar{D}$ , is

$$\bar{D} = \frac{2R_b R_c \omega}{R_c^2 - R_b^2}$$

The apparent interfacial viscosity is then found by calculating the ratio of the coordinates. The mean shear stress,  $\bar{\tau}$ , is given by (10)

$$\bar{\tau} = \frac{K(\theta - \theta_\omega)}{2\pi R_b R_c}$$

The interfacial viscosity curves for normal and sickling red blood cells are shown in Figs. 2 and 3. Figure 2 shows the time-dependent deflection of the bob at various rates of rotation of the cup for samples of normal (AA) and sickling (SS) red blood cells. At 30 min, differences of 10–15° are observed between normal and sickling erythrocyte samples.

The non-Newtonian flow behavior exhibited by both normal and sickling red blood cells may be clearly seen in Fig. 3, plotting deflection of the cup after 20 min. have elapsed. In examining the polyamine content of blood samples from 24 donors, we found a 10-fold

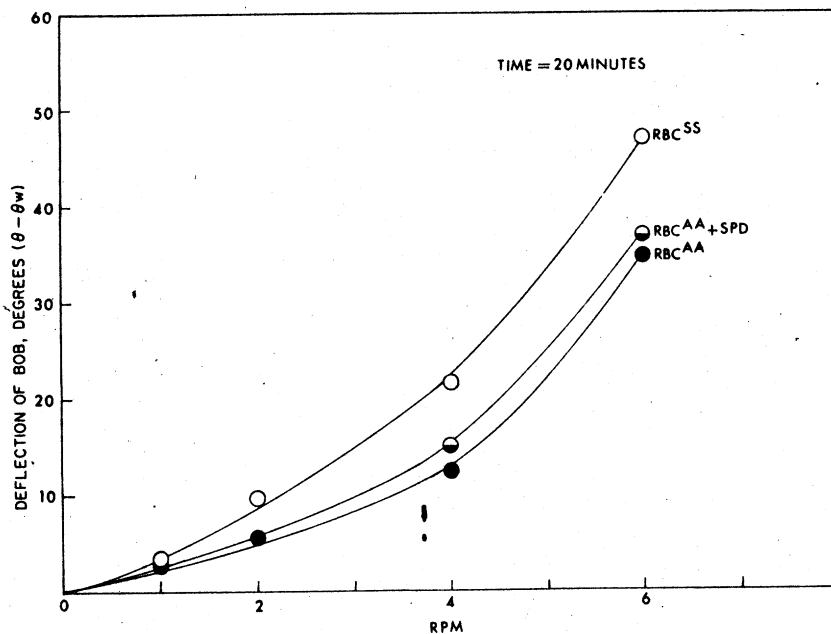


FIG. 3. Plot of deflection of the bob in degrees ( $\theta - \theta_0$ ) versus velocity of the turntable (rpm) at an elapsed time of 20 min, for samples of normal (AA) and sickling (SS) red blood cells, and normal red blood cells in the presence of spermidine (AA + spd). All samples exhibit non-Newtonian flow behavior.

difference in the polyamine content of normal and sickling erythrocytes (11–13). Addition of 300 nmol of spermidine per milliliter of normal red blood cells ( $1.89 \times 10^9$  cells/ml) caused a variation of 4–5° in the angle of deflection of the bob during interfacial viscosity measurements, as seen in this figure.

The increase in the interfacial viscosity with time under constant stress apparent from these results is rheopectic as opposed to another frequently observed example of non-Newtonian flow behavior under unsteady-state conditions, the thixotropic decrease in viscosity with time. Our measurements, then, would seem to support earlier reports (5) that the normal red blood cell exhibits non-Newtonian flow behavior. Our results show significant differences in the apparent interfacial viscosity of normal and sickling erythrocytes, as shown here in tabular form (Table 1).

Our findings represent the first application of the interfacial viscometer to studies on the red blood cell. The sensitivity of the instrument in determining differences between normal and sickling red blood cells, however, indicates that it might be applied in examining adhesion,

TABLE I  
INTERFACIAL VISCOSITY OF NORMAL AND SICKLING ERYTHROCYTES<sup>a</sup>

rpm	Normal red blood cell (AA)					Sickling red blood cell (SS)				
	$(\theta - \theta_\omega)$	$\bar{D}$	$\bar{\tau}$	$\log \bar{D}$	$\log \bar{\tau}$	$(\theta - \theta_\omega)$	$\bar{D}$	$\bar{\tau}$	$\log \bar{D}$	$\log \bar{\tau}$
1	0.20	26.6	0.13	1.43	-0.89	3.5	26.6	2.38	1.43	0.38
2	6.30	53.3	4.11	1.73	0.61	8.7	53.3	5.92	1.73	0.77
4	12.1	106.6	7.89	2.03	0.90	22.7	106.6	15.44	2.03	1.19
6	34.8	159.8	22.7	2.20	1.36	47.6	159.8	32.37	2.20	1.51

<sup>a</sup> Results represent the mean average of data for five blood samples, with an experimental error of  $\pm 3.2\%$ . Blood cells were washed eight times with 0.9% NaCl and centrifuged at 900 rpm for 20 min at 4°C. The measurements were made within a day at 24°C. Prolonged standing at 4°C produces an increase in  $(\theta - \theta_\omega)$ . Least-square analysis for  $\log \bar{D}$  versus  $\log \bar{\tau}$  gives

$$\eta_s^{AA} = 9.94 \times 10^{-3}(\bar{D})^{0.5} \text{ and } \eta_s^{SS} = 20.3 \times 10^{-3}(\bar{D})^{0.5},$$

$$\Delta\eta_s^{(SS-AA)} = 10.4 \times 10^{-3}(\bar{D})^{0.5}.$$

$K$  in these experiments was 20.48 dynes-cm for normal and 21.36 dynes-cm for sickle cells.

adsorption, and coagulation properties with good results. One potential drawback, that of performing all experiments in air-interface open system, may be easily overcome by enclosing the viscometer in a glove box.

## REFERENCES

1. Roscoe, R. (1952) *Brit. J. Appl. Phys.* **9**, 280.
2. Brinkman, H. C. (1952) *J. Chem. Phys.* **20**, 571.
3. Dintenfass, L. (1971) in *Blood Microrheology: Viscosity Factors in Blood Flow, Ischaemia and Thrombosis*, Appleton-Century-Crofts, New York.
4. Chien, S., Usami, S., Dillenback, R. J., and Gregersen, M. I. (1970) *Amer. J. Physiol.* **219**, 136.
5. Chien, S. (1975) in *The Red Blood Cell*, (MacN Surgenor, D., ed.), 2nd ed., p. 1031, Academic Press, New York.
6. Goldsmith, H. L. (1968) *J. Gen. Physiol.* **52**, 58.
7. Hochmuth, R. M., Marple, R. N., and Sutura, S. P. (1970) *Microvas. Res.* **2**, 409.
8. Batchelor, G. K., and Green, J. T. (1972) *J. Fluid. Mech.* **54**, 401.
9. Brown, A. G., Thuman, W. C., and McBain, J. W. (1953) *J. Colloid Inter. Sci.* **8**, 491.
10. Karam, H. J. (1974) *J. Appl. Poly. Sci.* **18**, 1693.
11. Chun, P. W., Rennert, O., Saffen, E. E., Shukla, J. B., Taylor, W. J., and Shah, D. O. (1976) Abstracts, 50th Colloid and Surface Science Symposium, B-2, San Juan, Puerto Rico.

12. Chun, P. W., Rennert, O., Saffen, E. E., and Taylor, W. J. (1976) ASBC Abstract, 392, San Francisco, Calif.
13. Chun, P. W., Rennert, O., Saffen, E. E., and Taylor, W. J. (1976) *Biochem. Biophys. Res. Commun.* **69**, 1095.

PAUL W. CHUN<sup>2</sup>  
SHIH Y. SHIAO  
EUGENE E. SAFFEN  
DINESH O. SHAH<sup>3</sup>  
W. JAPE TAYLOR<sup>4</sup>  
RICHARD J. DiTORE

*Departments of Biochemistry, <sup>3</sup>Anesthesiology and Chemical Engineering, and Medicine  
Colleges of Arts and Sciences, <sup>4</sup>Medicine, and <sup>3</sup>Engineering  
University of Florida  
Gainesville, Florida 32610*

*Received February 3, 1976; accepted July 14, 1976*

<sup>2</sup> Address all correspondence to P. W. Chun, Department of Biochemistry, College of Medicine, University of Florida, Gainesville, Florida 32610.