References and Notes

- 1. B. M. Bristol-Roach, Ann. Botany 40, 149
- B. M. Bristol-Roach, Ann. Bolany 40, 149 (1926); 41, 509 (1927); 42, 317 (1928).
 J. B. Petersen, Dansk Bot. Ark. 8, 1 (1935).
 G. W. Saunders, Botan. Rev. 23, 389 (1957).
 G. von Ernst and O. Pringsheim, Biol. Zentr.

- G. von Ernst and O. Pringsheim, Biol. Zentr. 78, 937 (1959).
 J. C. Lewin and R. A. Lewin, Can. J. Microbiol. 6, 127 (1960).
 J. H. Belcher and J. D. A. Miller, Arch. Mikrobiol. 36, 219 (1960).
 F. Trainor and H. C. Bold, Am. J. Botany 40, 758 (1953).
 R. C. Starr, Comparative Study of Chlorococ-cum Meneghini and Other Spherical, Zoo-spore-Producing Genera of the Chlorococ-cales (Indiana Univ. Press, Bloomington, 1955); W. R. Herndon, Am. J. Botany 45, 298 (1958); T. R. Deason, ibid. 46, 572 (1959). (1959)
- (1939).
 9. G. Arce and H. C. Bold, Am. J. Botany 45, 492 (1958).
 10. B. C. Parker, dissertation, Univ. of Texas, Austin (1960). 10. B.
- 11. The composition of B-1 medium is as fol-The composition of B-1 medium is as fol-lows: The concentration of macronutrients is identical to that described by H. C. Bold [Bull. Torrey Botan. Club 76, 101 (1949)]; to each liter of this solution was added 1.0 ml of each of the following micronutrient stock solutions: EDTA stock: 50 g EDTA plus 31 g KOH/lit. H-Boron stock: 11.42 g H₃BO₃/lit. H-Fe stock: 4.98 g FeSO₄ •7H₃0/lit. acidified water (acidified water = 999 ml distilled water plus 1.0 ml concentrated sulacidified water (acidified water = 999 ml distilled water plus 1.0 ml concentrated sul-furic acid). H-H₂ stock (per liter of acidified water): ZnSO₄•7H₂O, 8.82 g; MnCl₂•4H₂O, 1.44 g; MoO₆, 0.71 g; CuSO₄•5H₂O, 1.57 g; Co(NO₃)₂•6H₂O, 0.49 g. We acknowledge the financial assistance of the National Science Foundation (grant G-6373) which has greatly facilitated these in-vestigations.
- 12. vestigations.
- Present address: Department of Botany, University College London, London, England.

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Shear Rate Dependence of the Viscosity of Whole Blood and Plasma

Abstract. The analysis of the shear stress/shear rate relationship, and thus the viscosity/shear rate relation, of blood and plasma shows that (i) freshly drawn whole blood has a large shear rate dependence on viscosity (viscosity falls as shear rate increases), and (ii) the shear rate dependence of viscosity of whole blood, or plasma, that has not been treated to prevent clotting is substantially greater than that of whole blood or plasma treated with anticoagulants. The influence of this phenomenon upon the fluid mechanics of the microcirculation is commented upon.

In most of the studies (1) reported in the literature on whole blood or on blood plasma, the viscosity has been measured by a capillary viscometer. Among these studies are a number (2)in which the non-Newtonian or anomalous nature of the viscosity of whole blood was recognized, since the viscosity was observed to vary with the diameter of the capillary, the pressure gradient, the flow rate, and so on. On the other hand, possibly on account of the formidable difficulty in the interpretation of capillary data for non-Newtonian fluids, we have not as yet found

17 MARCH 1961

an instance in which the apparent viscosity (defined as shear stress/shear rate) has been analyzed as an explicit function of shear rate.

In what appears to be a unique example of viscometry by means of a coaxial cylinder viscometer, Brundage (3) tested both whole blood and blood plasma and found the viscosity of blood to be mildly shear dependent (decreasing as shear rate increases), whereas the viscosity of plasma was found to be Newtonian (not dependent on shear rate). As the coaxial cylinder viscometer permits unequivocal determinations of shear rate as well as shear stress, it is possible to recalculate Brundage's data originally given as a function of rotational speed, in terms of absolute shear rate. Describing viscometric data in terms of shear stress versus shear rate, or apparent viscosity versus shear rate, seems highly desirable because it permits intercomparison of data from different instruments and by different workers, and it offers an absolute basis for the analysis of the circulation by fluid mechanics.

In most previous studies, the blood or blood plasma was rendered nonclotting prior to viscometry by defibrination, addition of heparin, addition of oxalate, and so forth. Brundage reports that his attempts to test whole blood were unsuccessful because clotting commenced during the viscometry; consequently he added oxalate. Pirofsky (4) obviated the use of anticoagulants by the use of an intravenous needle functioning as a capillary, but he was limited by the nature of this experiment to a one-point determination on the shear rate viscosity curve. The studies reported here resulted from a twofold objective: (i) to determine the rheology of human blood and blood plasma directly in absolute values of shear stress and shear rate; and (ii) to determine the rheology in so short a time after withdrawal from the subject as to be able to work directly with unmodified, untreated whole blood and plasma. As is shown below there appear to be substantial differences, respectively, between freshly analyzed blood or plasma and the same modified by anticoagulants.

The samples of blood obtained in this study were collected by venepuncture through a No. 18 needle (1-mm inside diameter) gently aspirated by a silicone-coated syringe. Anticoagulants when used consisted of 200 units of heparin of 20 mg of potassium oxalate per 10-cm³ volume of whole blood. The sample was placed in a plastic cup and analyzed at once by means of a cone and plate viscometer (5, 6). In the experiments with plasma, the blood sample was first centrifuged in a plastic tube (3 minutes at 3400 rev/min), and the supernatant plasma was tranferred to the plastic cup and tested at once. The viscometer (6)used in this study was adapted from an industrial viscometer in which a cone was attached to a spindle projecting from the drive mechanism. The spindle was linked to the drive shaft by a calibrated beryllium copper spring to which a pointer was attached and was operable at four different speeds. The degree of deflection caused by the cone rotating in the fluid was read by the position of the pointer over a dial rotating in phase with zero position of the spring. All observations were carried out in a constant-temperature water bath at 37°C. A 3-ml sample of blood without anticoagulants could be fully analyzed within 3 minutes, and plasma within 6 minutes, from the time of collection. Clotting could easily be determined by sudden erratic movements of the pointer. Reproducible values could be obtained up to the instant of clot development, which appeared therefore to be a sudden rather than a gradual phenomenon, as determined by this technique. The relation of shear stress to shear rate of a National Bureau of Standards viscometer calibration oil (H-oil), with a viscosity of 4.635 centipoise at 37.0°C, is plotted in Fig. 1 to demonstrate that for a Newtonian fluid the viscometer correctly gives a linear relation between shear stress and shear



Fig. 1. (Top) Rheological diagram of whole blood with three different hematocrits; H, Bureau of Standards calibration oil. Fig. 2. (Bottom) Rheological diagram of three samples of plasma derived as noted. Serum protein, 6.9 g/100 g.

763

Table 1. A comparison of the coaxial cylinder data of Brundage on oxalate-stabilized whole blood (3) with our data for fresh, untreated whole blood, at equivalent hematocrits; cp, centipoise.

Hematocrit	Shear rate (sec ⁻¹)	Viscosity			
		Brundage		Present work	
		ср	A/B	cp A/	'B
28	23 (A)	2.8		3.7	
			1.00	1.	12
28	46 (B)	2.8		3.4	
44	23 (A)	6.0		8.4	
			1.00	1.1	15
44	46 (B)	6.0		7.3	
56	23 (A)	16.6		12.6	
	(*-)	2010	1.12	1.3	38
56	46 (B)	14.8		9.1	

rate, and that the instrument value agrees precisely with the stated viscosity.

A cone and plate viscometer is particularly appropriate for the study of blood rheology, because a small sample can be rapidly analyzed to obtain unequivocal and absolute values for shear stress and therefore viscosity for every value of shear rate imposed on the sample. The validity and derivation of the shear stress and shear rate with this type of instrument have been given elsewhere (6). In the present studies the full-scale deflection of the viscometer spring equaled 673.7 dy-cm, while the cone had an angle of 1°33' and a radius of 2.4 cm. The four shear rates recorded, therefore, were 23.18, 46.36, 115.92, and 231.84 sec⁻¹.

In Fig. 1 (top) the curves of apparent viscosity of whole blood versus shear rate are presented for three hematocrit levels as indicated. The characteristic shear thinning type of curve for pseudoplastic fluids is apparent. The higher the hematocrit the more pronounced is the rise of apparent viscosity at low rates of shear. Values of serum protein and cholesterol were normal in all samples studied. To clarify the role of anticoagulants, the coaxial cylinder data of Brundage (3)on oxalate-stabilized whole blood are compared with our data (Table 1) for fresh, untreated whole blood, at equivalent hematocrits.

There appears to be a consistent trend in these data of substantially greater shear dependence of viscosity in fresh whole blood as compared to oxalate-treated whole blood, as indicated by the viscosity values at rates of 23 sec⁻¹, (A), and 46 sec⁻¹, (B).

In Fig. 1 (bottom) are shown curves of viscosity of plasma versus shear rate. The three curves represent values obtained with untreated plasma, and plasma obtained from anticoagulanttreated whole blood. All three were obtained from the same blood sample. It is evident that below approximately

50 sec⁻¹ shear rate, the curvature (and thus the shear rate dependence of viscosity) of fresh plasma is greater than that of heparinized or oxalate-derived plasma.

In respect to plasma, Brundage (3)working on oxalate-treated samples found no difference in viscosity between a 2.2 sec⁻¹ and a 5.14 sec⁻¹ shear rate, which is in essential agreement with the findings reported here. Bingham (7) makes no mention of having observed non-Newtonian viscosity in plasma in which the content of oxalatetreated fibrinogen was varied. In the light of his pioneering work in non-Newtonian rheology, it is unlikely that non-Newtonian viscosity effects would have escaped his notice, had they existed.

The above data seem clearly to indicate that more attention should be focused on the viscosity of fresh blood and fresh plasma, even though it is experimentally difficult, in order to detect rheological characteristics at different rates of shear.

The consequences of shear rate dependence of viscosity on hemodynamics may prove to be profound. For example, in the microcirculation, the procession of erythrocytes in "single file" through capillaries (8) may be strongly influenced by the high shear rate dependence of plasma viscosity, in that next to the wall where the shear rate is highest, the plasma viscosity would be low, whereas between any two erythrocytes where the shear rate is very low, the viscosity would be high. Such a situation would tend to stabilize single-file procession and minimize tumbling or rotation of the erythrocytes. This shear rate dependence of blood and plasma viscosity does not necessarily support or contradict the concepts of "plasma skimming" or "axial streaming" (9) as applied to flow in the smaller vessels. Continuing research is aimed at elucidating the effects of the shear rate dependence of viscosity upon these and related phenomena.

Freshly drawn whole blood and fresh plasma separated from the red cells show a high degree of shear rate dependence of viscosity, whereas the addition of oxalate or heparin reduces the degree of shear rate dependence of viscosity in whole blood and causes it virtually to vanish in plasma. The shear rate dependence of viscosity in whole blood, especially in regard to movement of erythrocytes through arterioles, capillaries, and venules, would appear therefore to be a critical factor in the dynamics of blood flow of the microcirculation.

ROE E. WELLS, JR.

Harvard Medical School and

Peter Bent Brigham Hospital, Boston, Massachusetts

EDWARD W. MERRILL Department of Chemical Engineering, Massachusetts Institute of

Technology, Cambridge

References

- See, for example, the review compiled by L. Bayliss in Deformation and Flow in Biological Systems, A. Frey-Wyssling, Ed. (Interscience, New York, 1952), chap. 6.
 R. Fahreus and T. Lindquist, Am. J. Physiol. 96, 562 (1931); N.A. Coulter, Jr., and J. R. Pap-penheimer, *ibid.* 159, 501 (1949); A. Muller, Helv. Physiol. et Pharmacol. Acta 6, 181 (1948).
 J. T. Brundage Am. J. Physiol. 110 (59) T. Brundage, Am. J. Physiol. 110, 659
- (1934/35).

- (1934/35).
 4. B. Pirofsky, J. Clin. Invest. 32, 292 (1953).
 5. R. McKennel, Anal. Chem. 28, 1710 (1956).
 6. R. E. Wells, Jr., R. Denton, E. W. Merrill, J. Lab. Clin. Med., in press.
 7. E. C. Bingham and R. R. Roepke, J. Am. Chem. Soc. 62, 1204 (1942).
 8. B. R. Lutz and G. P. Fulton, Proc. 3rd Conf. of Microcirculatory Physiol. (American Physiological Society 1958), p. 13.
- logical Society, 1958, p. 13. 9. L. E. Bayliss, J. Gen. Physiol. 149, 593 (1959).
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Faunal Remains on an

Antarctic Ice Shelf

Abstract. Fishes and benthic invertebrates discovered frozen in situ and exposed at the ablation surface of the Mc-Murdo ice shelf provide evidence both for the occurrence of a fauna, including large fishes, under the permanent ice shelf and for Debenham's hypothesis of the nourishment of an ice shelf by the freezing of sea water on its bottom.

On 8 November 1960 we found the remains of upwards of 50 partially decomposed fish and the remains of several kinds of benthic invertebrates on the surface of the floating ice shelf in McMurdo Sound, Antarctica (Fig. 1). The remains were scattered over a small area some 2 km from the ice front near the easternmost of the Dailey Island group (77° 52' S, 165° 18' E). The ice shelf is probably more than 30 m thick in this area, and the surface is at least 3 to 5 m above sea level.