Here H_1 or E_1 represents the energy of desorption of the first monolayer, and H_V or E_L the energy of vaporization of a mole of liquid.

The Rheology of the Blood. II. The Effect of Fibrinogen on the Fluidity of Blood Plasma*

By Eugene C. Bingham and Raymond R. Roepke

Treffers[1] has shown recently that the fluidity–concentration relation is linear for solutions of a number of different proteins over a relatively wide range of concentrations. The fluidity–concentration relation may be expressed as

$$\varphi_{rel} = \varphi/\varphi_0 = (1 - kC) \tag{1}$$

where φ_{rel} = the relative fluidity of the solution, φ = the fluidity of the solution, φ_0 = the fluidity of the solvent, C = the concentration of the solute in grams per 100 ml. of solvent, and k = a constant which is dependent on the nature of the solute. This equation is similar to that found by Bingham[2] to hold for various suspensions. The constant, k, in the above equation will be referred to as the fluidity lowering constant (FLC) of the solute and is equal to the fractional lowering of the fluidity of the solvent brought about by unit concentration of the solute

$$kC = (\varphi_0 - \varphi)/\varphi_0 = 1 - \varphi/\varphi_0 \tag{2}$$

Treffers[1] has expressed the fluidity of a mixture of two proteins which do not interact by the relation

$$\varphi_{mix}/\varphi_0 = 1 - k_1C_1 - k_2C_2 \tag{3}$$

where φ_{mix} = the fluidity of the mixture, φ_0 = the fluidity of the solvent, and k_1C_1 and k_2C_2 are the FLC's and the concentrations of the two proteins. If this relation holds true for all of the constituents of blood plasma, then the effect of fibrinogen on the fluidity of plasma may be expressed by the relation

$$\varphi_p/\varphi_0 = \varphi_s/\varphi_0 - k_fC_f \text{ or } k_fC_f = (\varphi_s - \varphi_p)/\varphi_0 \tag{4}$$

where k_f and C_f are the FLC and concentration, respectively, of the fibrinogen; φ_p is the fluidity of the plasma and φ_s is the fluidity of the serum (plasma less the fibrinogen).

The FLC was determined for blood fibrinogen in solutions of oxalated sodium chloride. An attempt was also made to determine the value of the FLC of fibrinogen in blood plasma by measuring the fluidity of heparinized plasma and of the serum obtained by coagulation of the plasma either by the addition of protamine, to "neutralize" the effect of the heparin,[3] or by the addition of thrombin.

Experimental

Fibrinogen was separated from ox-blood plasma according to the procedure described by Smith, Warner and Brinkhous[4] and modified by Warner, Brinkhous and Smith.[5] The fibrinogen preparations were dissolved in a solution containing 0.855% sodium chloride and 0.092% potassium oxalate, dialyzed against oxalated saline and adjusted to approximately pH 7.5 with 0.1 N sodium hydroxide using phenol red as the indicator. The various concentrations were obtained by diluting the original solutions with oxalated saline.

The heparinized plasma was obtained by using a sterile heparin solution (Connaught Laboratories, Toronto) in the proportion of 0.5–0.7 ml. to 100 ml. of whole blood. The heparin solution, containing approximately 17 mg. of dry solids per ml., was evaporated to dryness on the wall of the tube before collection of the sample. Coagulation of the heparinized plasma was brought about by the addition of 0.5–1.0 mg. of a thrombin preparation (250 units of activity per mg.) or by the addition of 0.25 mg. of protamine (salmine) sulfate per ml. of plasma. We are indebted to Dr. Walter H. Seegers, State University of Iowa for the thrombin preparation and to E. R. Squibb and Sons, New Brunswick, N. J., for the protamine sulfate.

Fibrinogen was estimated by nitrogen determination on the washed clot, using the micro-Kjeldahl method of Keys.[6] The centrifugalized clot was washed once with 0.9% sodium chloride and twice with distilled water, allowing an hour or more for each washing. Fibrinogen was calculated from the nitrogen content by use of the factor 6.25, and the concentration of fibrinogen was expressed in grams per 100 ml. of solution.

(1) H. P. Treffers, This Journal, **62**, 1405 (1940).

(2) E. C. Bingham, "Fluidity and Plasticity," McGraw-Hill Book Co., New York, N. Y.

(3) E. Chargaff and K. B. Olson, J. Biol. Chem., **122**, 153 (1938).

(4) H. P. Smith, E. D. Warner and K. M. Brinkhous, J. Exp. Med., **66**, 801 (1937).

(5) E. D. Warner, K. M. Brinkhous and H. P. Smith, Am. J. Physiol., **125**, 296 (1939).

(6) A. Keys, J. Biol. Chem., **132**, 181 (1940).

Fluidity measurements were made at a temperature of 37.0° by means of a Bingham viscometer, no. 1-28, the characteristics of which have been described by Bingham and Thompson.[7] The fluidity was expressed as rhes (the inverse of the viscosity in poises). The viscometer was calibrated with water at 20° (the fluidity of water being taken as 99.50 rhes[8]) and the fluidity of water at 37° was determined to be 144.3 rhes.

The fluidity of serum obtained by adding protamine to heparinized plasma was corrected for the direct effect of protamine on the fluidity which was determined by adding protamine to normal serum containing heparin and noting the change in fluidity. Similarly, the fluidity of serum obtained by adding thrombin to heparinized plasma was corrected for the direct effect of the added thrombin on the fluidity, the correction factor being determined by adding thrombin to normal serum.

Results and Comment

The relative fluidity–concentration relation of two different preparations of ox-blood fibrinogen is shown in Fig. 1, which is obtained from the data given in Table I. The fluidity–concentration relationship is shown to be linear up to the highest concentration investigated, 1.063%. The FLC calculated from the data by the method of least squares is found to be 0.2358 and the average deviation of the calculated from the observed fluidities, using Eq. 1, is 0.08%, the maximum deviation being 0.24%.
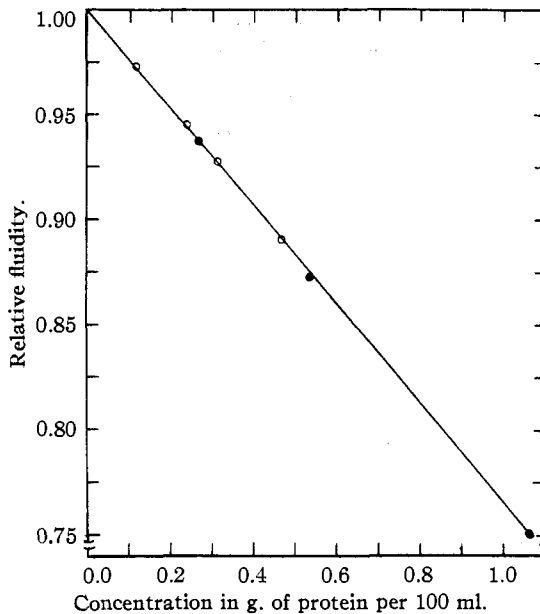
TABLE I

THE RELATIVE FLUIDITY $\left(\dfrac{\varphi}{\varphi_0}\right)$ OF BLOOD FIBRINOGEN IN OXALATED SODIUM CHLORIDE SOLUTIONS[a] AT 37.0°

Concentration of fibrinogen, g. per 100 cc.	Relative fluidity	Concentration of fibrinogen, g. per 100 cc.	Relative fluidity
Preparation No. 1		Preparation No. 2	
0.116	0.9734	0.266	0.9373
.232	.9456	0.531	.8728
.310	.9276	1.063	.7509
.465	.8905		

[a] The fluidity of the oxalated sodium chloride solution at 37.0° was determined to be 142.2 rhes.

The FLC of fibrinogen in preparation no. 1 was also determined by coagulation of the fibrinogen with thrombin, adding 1.0 mg. of the thrombin preparation to each ml. of the solution containing 0.465% protein. From the concentration of fibrin removed and the fluidities of the original solution and the solution removed from the clot, the FLC was calculated to be 0.259. In this calculation the fluidity of the solution separated from the clot was corrected for the direct effect of thrombin on

(7) E. C. Bingham and T. R. Thompson. J. Rheol., 1, 418 (1930).
(8) E. C. Bingham, and R. F. Jackson, Bull. U. S. Bur. of Standards, 14, 59 (1917).

Fig. 1.—The relative fluidity (φ/φ_0) of blood fibrinogen in oxalated sodium chloride solutions: O, preparation no. 1; ●, preparation no. 2.

the fluidity as determined by adding thrombin to blood serum and noting the change in the relative fluidity. Although the active thrombin could be removed along with the fibrin and hence would require no fluidity correction, at least three-fourths of the thrombin preparation may be composed of inactive substance since Seegers[9] has succeeded in obtaining a thrombin preparation with an activity of 950 units per mg. There is a possibility, however, that much of the inactive portion may also be removed with the fibrin, thus making the calculated FLC too large.

Of the total protein in the two fibrinogen preparations, 88.5% and 87.6% respectively, could be removed as fibrin by coagulation with thrombin. Although approximately the same percentage of the total protein was separated as fibrin in the two cases, a relatively greater amount of thrombin was added to the solution of the second preparation. Whether this is an indication of the degree of purity of the fibrinogen preparations or is due to incomplete coagulation with thrombin is not known. As shown by the data given in Table II, the amount of fibrin obtained by the coagulation of heparinized plasma by the addition of thrombin is usually less than that obtained by the addition of protamine shortly after collection of the sample or by normal coagulation of the non-heparinized plasma.

(9) W. H. Seegers, J. Biol. Chem., 136, 103 (1940).

TABLE II

THE FLUIDITY LOWERING CONSTANT OF FIBRINOGEN IN
HEPARINIZED BLOOD PLASMA

Sample no.	Source of plasma	Manner of removing fibrinogen	Concn. of fibrinogen g./100 ml.	φ of plasma rhes	FLC
9	Ox	Protamine	0.385	90.15	0.178
10a	Ox	Protamine	.411	81.40	.155
10a	Ox	Protamine	.411	81.40	.151
11	Ox	Protamine	.498	85.67	.162
13	Ox	Protamine	.377	91.81	.168
15	Ox	Protamine	.644	83.82	.156
14	Dog	Protamine	.304	85.32	.170
16a	Dog	Protamine	.312	78.90	.165
16b	Dog	Normal clotting[a]	.472	79.00[a]	.159
32a	Dog	Protamine	.197	89.26	.184
32b	Dog	Thrombin	.227	89.26	.176
32c	Dog	Normal clotting[a]	.240	89.40[a]	.171
18	Human (R. R.)	Protamine	.252	81.12	.148
26	Human (R. R.)	Protamine	.237	83.59	.153
27a	Human (E. B.)	Protamine	.355	76.23	.158
27b	Human (E. B.)	Thrombin	.289	76.23	.151
28a	Human (R. R.)	Protamine	.237	83.84	.155
28b	Human (R. R.)	Thrombin	.213	83.84	.148
29a	Human (R. G.)	Protamine	.259	76.75	.143
29b	Human (R. G.)	Thrombin	.228	76.75	.142
				Average	.160

[a] Untreated (non-heparinized) plasma obtained by use of paraffin lined centrifuge tube and allowed to clot naturally. The fluidity of untreated plasma was calculated from that of heparinized plasma by correcting for the effect of heparin on the fluidity.

The values for the FLC determined for fibrinogen in blood plasma are given in Table II. The values may be somewhat in error due to the difficulty of correcting for the direct effect of the added protamine and thrombin on the fluidity of serum. However, the results obtained with samples 16 and 32 show that the values for the FLC obtained with the aid of protamine and thrombin do not differ greatly from that obtained with untreated plasma which is allowed to clot naturally.

Although there is a slight variation among the values for the FLC of fibrinogen in the different samples of blood plasma shown in Table II, the values are nearly a third less than the value obtained for purified solutions of fibrinogen calculated from the data indicated in Fig. 1 to be 0.2358. Thus Eq. 4 does not hold for fibrinogen in blood plasma. The effect of fibrinogen in decreasing the fluidity of water is appreciably less in the presence of serum proteins than in purified solutions of fibrinogen. This may be due to an interaction between the fibrinogen and the serum proteins or perhaps to the fact that the linear fluidity–concentration relation does not hold at the higher concentrations of protein. Treffers has noted that the fluidity vs. concentration curve of a protein deviates from linearity at higher concen-

trations such that the calculated fluidity is less than the observed fluidity. That is, the FLC decreases with increase in protein concentration.

Calculations from the viscosity data of Starlinger and Winands[10] in 25 pathologic cases give values for the FLC of fibrinogen in blood plasma at 25° varying from 0.090 to 0.290 with an average of 0.170. These investigators determined the viscosity of the untreated plasma (without addition of an anti-coagulant) by keeping the plasma samples in paraffin-lined containers until the viscosity was measured in a rapid Ostwald viscometer. Starlinger and Winands explained the difference in the effect of fibrinogen on the viscosity of the different plasma samples as being due to differences in the physico-chemical state of the fibrinogen in the different plasmas. However, one would expect that it would be very difficult to measure the fluidity or viscosity of untreated plasma before the coagulation process is initiated.

The FLC of purified fibrinogen is appreciably greater than that of other fractionated blood proteins. From the viscosity data of Fahey and Green[11] the FLC of three different serum globulin fractions in water are found to be 0.103, 0.132, and 0.178 and the FLC of serum albumin is found to be 0.048. From the data of Lauffer,[12] the FLC of tobacco mosaic virus protein, which gives a linear fluidity–concentration up to a concentration of 0.1%, is found to be 0.550.

Summary

The fluidity–concentration relationship of solutions of blood fibrinogen prepared from ox plasma was found to be linear up to a concentration of at least 1.0%.

The fluidity lowering constant (fractional lowering of the fluidity per unit concentration) of purified blood fibrinogen was found to be greater than the fluidity lowering constants of other blood proteins.

The fluidity lowering constant of fibrinogen in heparinized blood plasma was determined by comparing the fluidity of the plasma and the serum obtained by the use of protamine and thrombin preparations and was found to be less than the fluidity lowering constant of saline solutions of purified fibrinogen.

(10) W. Starlinger and E. Winands. A. ges. Exp. Med., 71, 389 (1930).

(11) K. R. Fahey and A. A. Green. THIS JOURNAL, 60, 3039 (1938).

(12) M. A. Lauffer. Science, 87, 469 (1938).