dyes, is about the same as that in which the main rise in Δ values occurs. This concentration range extends up to the value at which dye and chromotrope are approximately equivalent. At chromotrope concentrations above this point there is little change in Δ values except with methyl green and methylene blue in the presence of potassium chloride. Band extinction values above the equivalence point differ rather markedly in behavior depending on whether the chromotrope is chondroitin sulfate or hyaluronate. The course of the curves relating band intensities to chromotrope concentrations of the present work differs somewhat from similar curves presented previously.[8] This difference is an important part of the whole phenomenon of metachromasy and shows the effect of the dyestuff concentration on the course of these curves. On the basis of the present work, as well as of our previous work, it seems possible to adopt tentatively the view that the metachromatic color is due to binding of dye by chromotrope and is not due to dimerization or polymerization of the dye.

The fact that methylene green and methyl green, dyes which have been thought to obey Beer's law and to be unaffected by chromotropes,[11] give values of Δ as large as, or even larger than, those given by the metachromatic dyes methylene blue and crystal violet, has raised a suspicion that perhaps the band intensities of these dyes are not as constant as was thought. Work on these two and other non-metachromatic dyes is in progress to examine this point.

NEW YORK 16, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Size and Shape of Bovine Fibrinogen. Studies of Sedimentation, Diffusion and Viscosity[1]

BY SIDNEY SHULMAN[2]

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Sedimentation and diffusion measurements have been made on solutions of bovine fibrinogen, using several experimental methods. The resulting sedimentation constant, extrapolated to infinite dilution and corrected to standard conditions, was 7.80×10^{-13} sec.$^{-1}$ in the Spinco ultracentrifuge and 7.95×10^{-13} sec.$^{-1}$ in the oil-turbine ultracentrifuge. The diffusion constant at a concentration of 4.0 g./l. was 2.02×10^{-7} cm.^{2} sec.$^{-1}$, using a schlieren optical system, and 2.01×10^{-7} cm.^{2} sec.$^{-1}$, employing a Gouy interferometer optical system. Appropriate combination of these results gives a molecular weight of 330,000 ± 10,000 and a frictional ratio of 2.34. The intrinsic viscosity was found to be 0.25. Deductions as to the molecular shape and dimensions have been made by use of several hydrodynamic theories.

Introduction

The sedimentation and diffusion constants of fibrinogen were first studied by Holmberg.[3] The resulting molecular weight was 700,000. No other diffusion results have been reported, but several additional sedimentation studies have been made.[4-8] In some of these investigations the results were combined with viscosity and flow birefringence data to give a molecular weight of 500,000 and, later, 400,000. Other experimental methods have been applied to the study of this protein, including osmotic pressure[5,9,10] and light scattering.[11] These have generally given values of the molecular weight in the range of 400,000–500,000, or a little higher. Very recently, a light scattering investigation in this Laboratory gave a result[12] of 340,000. It was felt that a new investigation of the molecular weight as determined by the method of sedimentation and diffusion would be of considerable interest.

Materials and Methods

The fibrinogen was prepared from Armour bovine fraction I, preparation 128–163, following two different procedures, previously described.[12] The older method, a freeze–thaw procedure, gave a product called fraction I-C, containing 88–90% clottable protein. This material was used in the earlier sedimentation experiments. The other method, an ammonium sulfate procedure, gave a product called fraction I-L, generally containing 94–98% clottable protein. This material was used in the other sedimentation experiments and in all the diffusion experiments. Because of reports[13,14] that 3 to 4% of the initial fibrinogen is split off during conversion to fibrin, the practice will be followed of adding about 3 units to all designations of per cent. clottability when the actual percentage of fibrinogen in the protein is to be stated.

Stock solutions of fibrinogen were adjusted to the desired ionic strength and *p*H by dialysis at 0° for 24 hours against several changes of buffer, and clarified by filtration through washed filter pads. The solvent was a buffer of 0.45 ionic strength, of which 0.40 was contributed by sodium chloride and 0.05 by sodium phosphates. The *p*H was always 6.2. Fibrinogen concentration was determined by gravimetric assay,[15] and total protein concentration by Kjeldahl or by dry weight.

The sedimentation studies were made in both the Sved-

(1) This is Paper No. 26 of a series on "The Formation of Fibrin and the Coagulation of Blood" from the University of Wisconsin, supported in part by research grants from the National Institutes of Health, Public Health Service.

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(3) C. G. Holmberg, *Arkiv Kemi, Mineral. Geol.*, **17A**, No. 28 (1944).

(4) E. J. Cohn, J. L. Oncley, L. E. Strong, W. L. Hughes, Jr., and S. H. Armstrong, Jr., *J. Clin. Invest.*, **23**, 417 (1944).

(5) J. L. Oncley, G. Scatchard and A. Brown, *J. Phys. Colloid Chem.*, **51**, 184 (1947).

(6) V. L. Koenig and K. O. Pedersen, *Arch. Biochem.*, **25**, 97 (1950).

(7) S. Shulman and J. D. Ferry, *J. Phys. Colloid Chem.*, **55**, 135 (1951).

(8) V. L. Koenig and J. D. Perrings, *Arch. Biochem. Biophys.*, **36**, 147 (1952); **40**, 218 (1952).

(9) G. S. Adair, in K. Bailey, *Advances Protein Chem.*, **1**, 308 (1944).

(10) L. B. Nanninga, Thesis, Amsterdam (1947).

(11) (a) R. F. Steiner and K. Laki, THIS JOURNAL, **73**, 882 (1951); *Arch. Biochem. Biophys.*, **34**, 24 (1951); (b) C. S. Hocking, M. Laskowski, Jr., and H. A. Scheraga, THIS JOURNAL, **74**, 775 (1952).

(12) S. Katz, K. Gutfreund, S. Shulman and J. D. Ferry, *ibid.*, **74**, 5706 (1952).

(13) L. Lorand, *Nature*, **167**, 992 (1951).

(14) K. Laki, *Science*, **114**, 435 (1951).

(15) P. R. Morrison, THIS JOURNAL, **69**, 2723 (1947).

berg oil-turbine ultracentrifuge, through the kindness of Professor J. W. Williams, and in the Spinco ultracentrifuge, through the kindness of Professor P. P. Cohen of the Department of Physiological Chemistry. In using the former, temperature corrections were applied in accordance with currently applicable calibrations obtained through the melting of phenyl ether.[16] In using the Spinco, the refrigerator was left off and the average of the initial and final temperature readings was used; the difference was generally about 0.5°. The photographic plates obtained with either instrument were enlarged and traced. The sedimentation constant was calculated from the slope of a plot of the logarithm of the distance of the boundary from the axis of rotation against time. In the Spinco measurements a single average correction was applied for the temperature influence on solvent viscosity, whereas in the oil-turbine experiments each time value in the plot was cumulatively corrected for this effect. In both cases, final corrections were made in the customary manner to a solvent with the density and viscosity of water at 20.0°. For this purpose, a partial specific volume of 0.71 was employed.

The diffusion measurements were made in both schlieren and Gouy interferometric optical systems. The schlieren experiment was carried out at 1.9° in the Tiselius electrophoresis apparatus, using an inclined slit (0.50 mm. wide), a cylindrical lens, and a schlieren lens on each side of the thermostat so that the light passed through the cell in a parallel beam. The boundary was formed in a standard cell and was brought into view by the gentle evolution of gas in a connecting vessel. Since the initial boundary was quite sharp in each experiment, no sharpening procedure was used. The photographic plates were enlarged and traced. Heights and areas (traced by planimeter) for each edge of the band of light were measured and averaged.

The single experiment using the Gouy optical system was carried out at 25.0° in the high-precision apparatus recently used by Dr. L. J. Gosting and collaborators in the study of several low molecular weight substances.[17-21] In preparation for this experiment, dialysis was continued for 36 hours, following which the protein solution and final dialysis bath were stoppered and allowed to warm before being put in the cell. The boundary was formed in the usual way, and was shifted to the center of the cell and sharpened at the optic axis by withdrawing liquid through a capillary inserted to this level. A total of 60 ml. was removed at rates of 1–3 ml. per minute, with several interruptions allowing brief diffusion in order to aid in washing the protein solution down the walls. The moment when the last sharpening period ended was taken as the start of diffusion. The photographic plates were measured with a comparator and the required computations were made. The method gives a height–area average diffusion coefficient.

The viscosity measurements were made at 25.0° with a pair of similar capillary viscosimeters having flow times for water of 120 and 124 sec. and average velocity gradients of 950 and 947 sec.$^{-1}$. The specific viscosity per unit concentration *of fibrinogen* was plotted against the concentration *of fibrinogen* to give, on extrapolation, the intrinsic viscosity.

Results

Sedimentation.—The data for several concentration series in both ultracentrifuges are shown in Fig. 1. The values, in the customary Svedberg units, at infinite dilution are 7.80 in the Spinco (Sp) and 7.95 in the oil-turbine (OT) machine. The OT data were obtained at a rotor speed of 50,400 r.p.m., while the Sp series—with one exception—were studied at a speed of 59,780 r.p.m. The exception was a concentration series studied at 42,040 and this gave an extrapolated result of 7.72, only 2% lower than the other Sp results, 7.82 and 7.83. When these are all plotted together, they agree well on a line extrapolating to 7.80. The three OT series gave somewhat more scattering, *viz.*, 8.19, 7.97 and 7.87; the best graphical average has been selected as 7.95.

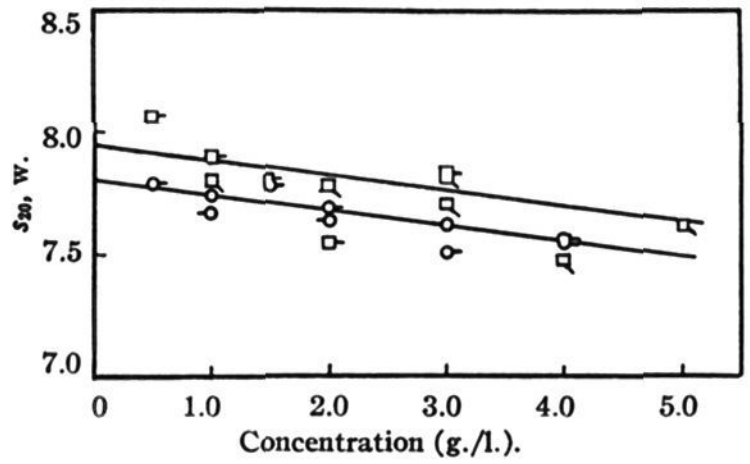

Fig. 1.—Sedimentation constant plotted against concentration of fibrinogen. Upper line (squares) is for the oil-turbine ultracentrifuge; lower line (circles) is for the Spinco ultracentrifuge.

Diffusion.—The schlieren study at 4.0 g./l. concentration was computed by the height–area method. Sixteen pictures had been taken over a total time of 46 hours. Figure 2 shows the first six pictures, illustrating the excellent initial sharpness and symmetry of the peak. The values of (area)^2/(height)^2 are plotted against time in Fig. 3. The slope of the line can be estimated quite reliably and, when multiplied by the optical factors, gives a

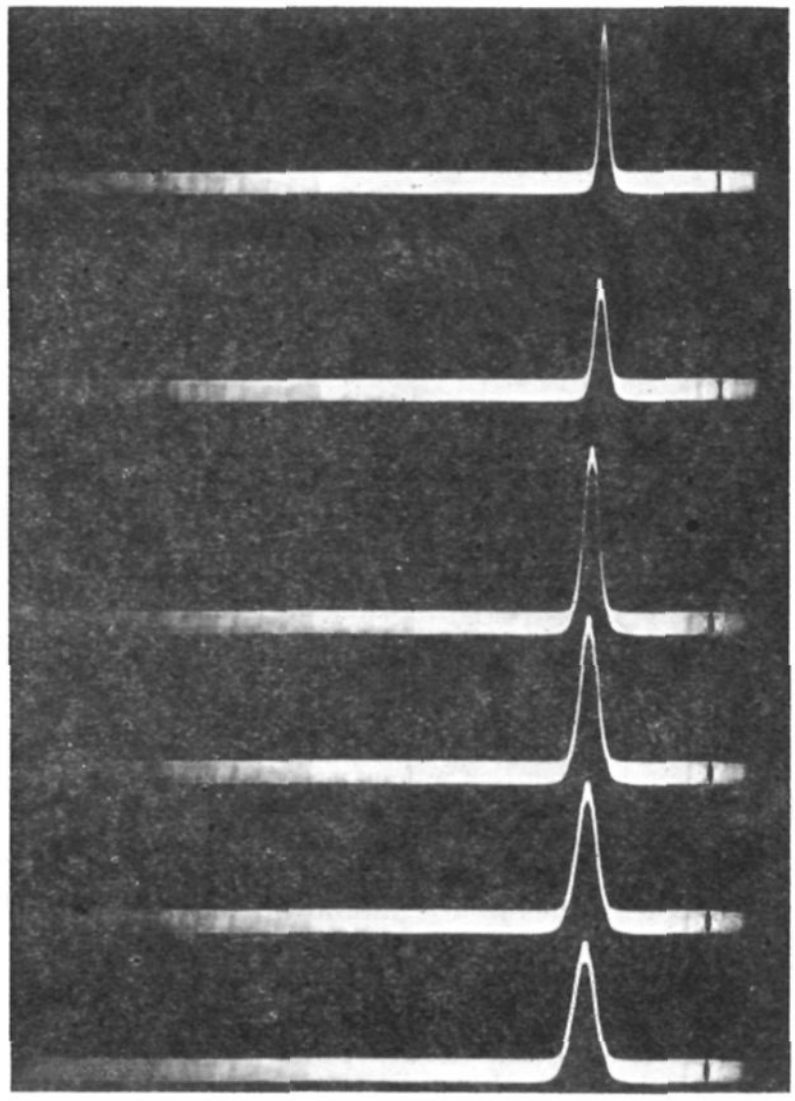

Fig. 2.—Diffusion of fibrinogen as seen by means of the schlieren optical system. The six exposures are in descending order. Each time interval is three hours.

(16) S. Shulman, *Arch. Biochem. Biophys.*, **44**, 230 (1953).

(17) L. J. Gosting, E. M. Hanson, G. Kegeles and M. S. Morris, *Rev. Sci. Instruments*, **20**, 209 (1949).

(18) L. J. Gosting and M. S. Morris, THIS JOURNAL, **71**, 1998 (1949).

(19) L. J. Gosting, *ibid.*, **72**, 4418 (1950).

(20) M. S. Lyons and J. V. Thomas, *ibid.*, **72**, 4506 (1950).

(21) L. J. Gosting and D. F. Akeley, *ibid.*, **74**, 2058 (1952).

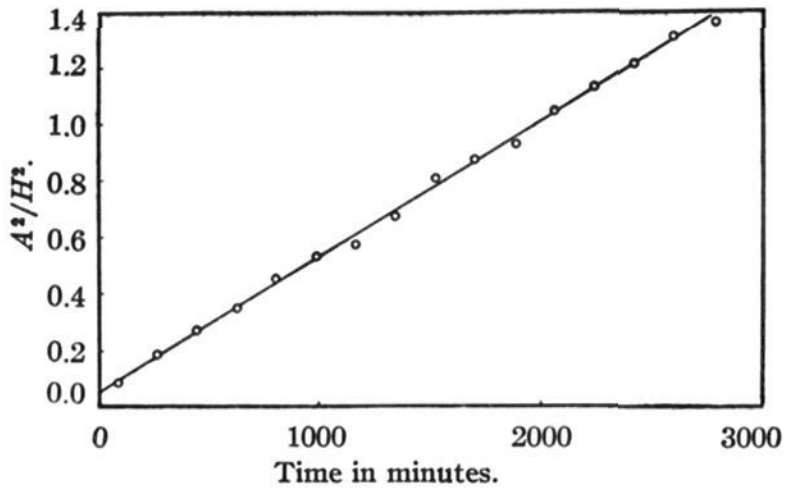

Fig. 3.—Values of (area)²/(height)² for fibrinogen schlieren peaks plotted against time.

diffusion constant of 1.09 in the customary units of 10^{-7} cm.² sec.$^{-1}$. To correct this to a solvent with the viscosity and density of water at 20.0°, a factor of 1.856, involving the effect of temperature on kinetic energy and the influence of temperature and buffer salts on the viscosity of water, is used. This gives a final result of 2.02.

The Gouy diffusion experiment, also at 4.0 g./l., lasted a total of 30 hours, and utilized ten photographic exposures. The total number of fringes, j_m, was 37.17. The first four records are shown in Fig. 4. Table I illustrates some computations for

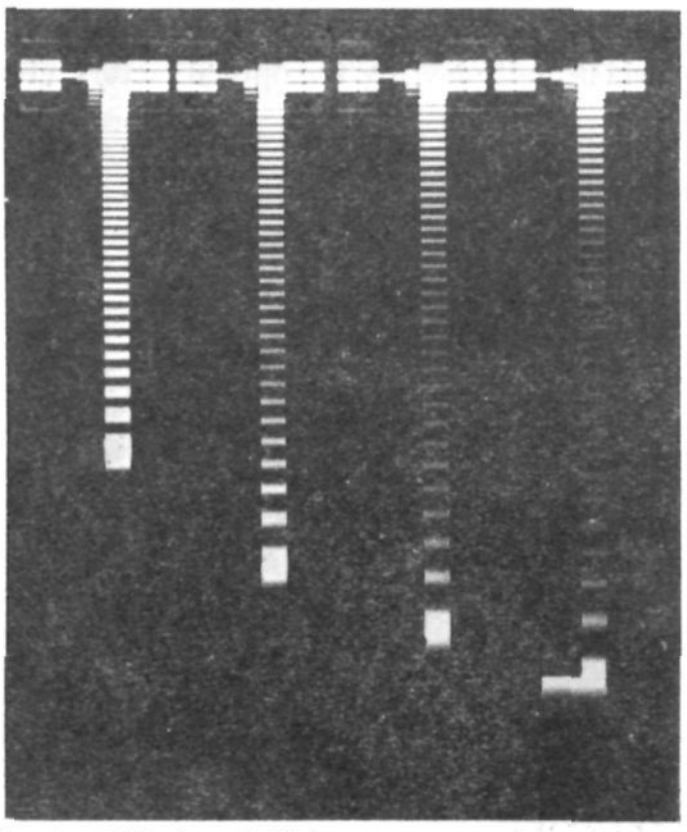

Fig. 4.—Diffusion of fibrinogen as seen by means of the Gouy optical system. The four exposures are in right-to-left order.

three of the pictures. In the case of Gaussian diffusion, the values of C_t' should not change with j, the fringe number. In experiments with highly purified, two-component systems, this is observed to be strictly true,[17,18] and the average value for each set of fringes is used to compute the apparent diffusion constant for that elapsed time, D'. The values presented here show a slight steady decrease with increasing fringe number. To obtain the characteristic value of C_t' for a given time, the values are plotted against j, and extrapolated to $j = -3/4$; *i.e.*, to $z_j = 0$. Figure 5 shows such plots for the first three exposures. The apparent diffusion constant at each time (t') is calculated from

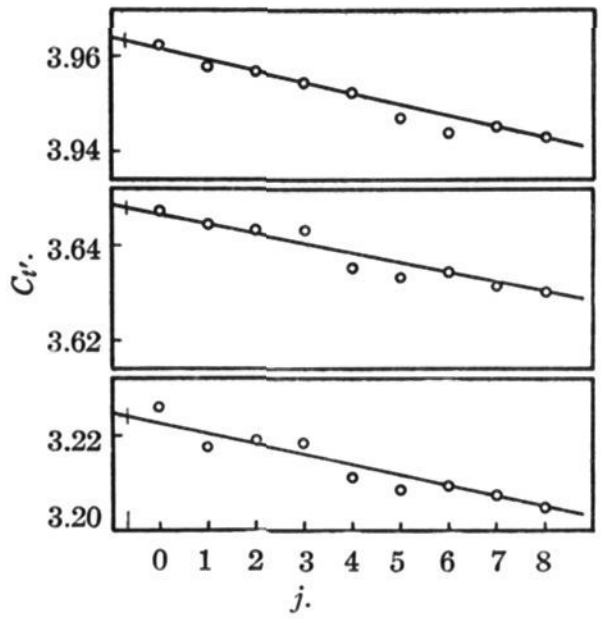

Fig. 5.—Values of $C_{t'}$, plotted against fringe number for the first three Gouy diffusion pictures in the study of fibrinogen.

$$D' = \frac{(j_m \lambda b)^2}{4\pi C_t'^2 t'} \qquad (1)$$

where λ is the wave length of the light, 5461 Å., b is the optical distance, and the other symbols have been explained. The diffusion constant, D, is obtained by plotting D' against the reciprocal of the time. This is shown in Fig. 6. The result is $D =$

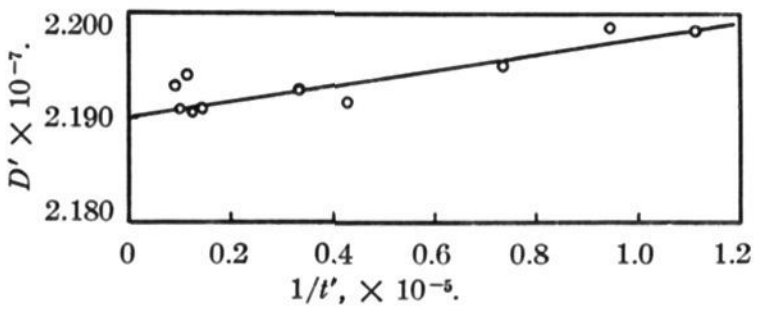

Fig. 6.—Values of apparent diffusion constant of fibrinogen as determined by the Gouy system plotted against reciprocal of time.

2.190×10^{-7} cm.² sec.$^{-1}$. To correct this to the viscosity and density of water at 20.0°, a factor of 0.918 is used. The resulting corrected diffusion

TABLE I

COMPUTATIONS ON THE FIRST THREE PHOTOGRAPHIC RECORDS IN THE DIFFUSION STUDY OF FIBRINOGEN

Picture	1		2		3	
time, sec.	9000		10620		13620	
j	Y_j	C_t'	Y_j	C_t'	Y_j	C_t'
0	3.6080	3.9624	3.3212	3.6474	2.9375	3.2260
1	3.3430	3.9577	3.0785	3.6446	2.7179	3.2177
2	3.1321	3.9571	2.8822	3.6413	2.5480	3.2191
3	2.9463	3.9543	2.7132	3.6415	2.3981	3.2186
4	2.7794	3.9524	2.5566	3.6356	2.2580	3.2110
5	2.6229	3.9473	2.4145	3.6337	2.1319	3.2084
6	2.4785	3.9441	2.2840	3.6346	2.0170	3.2097
7	2.3452	3.9454	2.1587	3.6317	1.9067	3.2078
8	2.2168	3.9431	2.0411	3.6306	1.8017	3.2047
20	1.0185	3.9111	0.9383	3.6032	0.8292	3.1842
30	0.3179	3.848	0.2922	3.537	0.2591	3.136

constant is 2.010×10^{-7} cm.^{2} sec.$^{-1}$. The excellent agreement between these two experiments is all the more remarkable in view of the fact that one was conducted at 1.9°, while the other was at 25.0° requiring considerably different correction factors.

Viscosity.—The results of two viscosity studies are shown in Fig. 7. Both preparations of fibrinogen had clottabilities of 96%. The resulting intrinsic viscosity is 0.250.

Discussion

Sedimentation.—The results obtained in this work are compared with those of other investigators in Table II. It is apparent that earlier values —including some from this Laboratory—have been too high, probably because of unappreciated temperature errors in the oil-turbine ultracentrifuge. This has been critically discussed in another publication.[16] The work of Koenig and Perrings (using a Spinco) claims variation in corrected sedimentation constant as a function of experimental temperature and rotor speed, though a later paper indicates that the influence of experimental temperature is much less than originally believed.[22] Though it is difficult to make a definite comparison between the several (Sp) results of Koenig and Perrings and the one reported here, it is clear that, no matter how their numbers are selected for averaging, they find the sedimentation constant of fibrinogen to be about 0.2–0.4 unit smaller than the value given in the present work.

TABLE II

SEDIMENTATION CONSTANT[a] OF FIBRINOGEN

Investigators	Ref.	$s_{20,w}$	Species	Ultracentrifuge
Holmberg	3	8.5	Human	OT
Koenig and Pedersen	6	8.4–8.6	Bovine	OT
Shulman and Ferry	7	8.4	Bovine	OT
Koenig and Perrings	8	7.27–7.87[b]	Bovine	Sp
This work	..	7.80	Bovine	Sp
This work	..	7.95	Bovine	OT

[a] At infinite dilution. [b] Values lie in this range; see text.

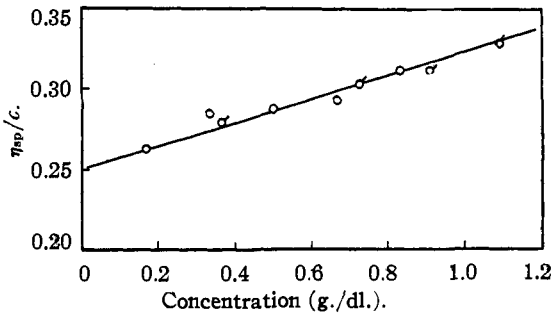

Fig. 7.—Values of the specific viscosity per unit concentration (*of fibrinogen*) plotted against concentration *of fibrinogen*.

In a very recent study by Carroll and Laki,[24] a rather extensive series of sedimentation studies was conducted on bovine fibrinogen, using a Spinco machine. Nineteen runs were made, utilizing a large number of protein concentrations, ranging from 0.05 to 1.82%, and the solvent was 0.3 *M* potassium chloride plus either 0.02 *M* phosphate or 0.02 *M* borate, to give a *p*H of 7.2 or 8.2, respectively. All but one run were at a rotor speed of 59,780 r.p.m.; the exception was at a speed of 39,460 r.p.m. The partial specific volume used in the calculations was 0.710. A plot of these corrected sedimentation constants against concentration gave a good straight line, extrapolating to a value of 7.72, only 1% different from the results of the present work. The one value at a lower speed of rotation, it should be noted, was 6.99, whereas the value interpolated at this concentration from the plot was 6.95.

Diffusion.—Apparently, the only diffusion constant for fibrinogen that can be found in the literature is the study by Holmberg[3] in 1944. This investigation gave a corrected constant of 1.1×10^{-7} cm.^{2} sec.$^{-1}$. The fibrinogen was admittedly not homogeneous, and the possible occurrence of molecular aggregation was suggested by some of the sedimentation studies. The present determination gives a diffusion constant practically double the former value. The new result would seem to be reliable for several reasons: (1) the high degree of protein purity, (2) the good agreement between two entirely different experimental procedures, and (3) the good internal agreement in both experiments in plotting the computations as a function of time. It is regrettable that studies were not made at several concentrations, but the 0.4% solution that was used is quite dilute for diffusion measurements, and the result should be satisfactorily close to the value at infinite dilution.

Viscosity.—The results obtained in this work are compared with earlier findings in Table III. Since the intrinsic viscosity of fibrinogen is much greater than that of albumin or globulin, it follows that the measured value will be affected by the presence of small amounts of these other blood proteins. The previous studies from this Laboratory were performed on preparations in which the protein was

(22) The extrapolated results, as given in the second paper, in 0.2 *M* sodium chloride at 59,733 r.p.m. were 7.66, 7.48 and 7.87 for operating temperature of 30, 25 and 20°, respectively, while in 0.1 *M* disodium phosphate at 59,733 r.p.m., the results were 7.28, 7.60, 7.57 and 7.27 at 30, 25, 20 and 15°, respectively. It might be suggested that this behavior, showing none of the monotonic trend with temperature that was originally claimed (9.64, 8.46, 7.92: 9.37, 8.74, 7.71, 6.64), may reflect nothing more serious than experimental error. It would be enlightening, for example, to know the reproducibility in performing several repeated identical concentration series at the same experimental temperature.

No real influence of experimental temperature can be demonstrated from the data of the present work. For example, two runs at 4.0 g./l. concentration which happened to be made at average temperatures of 25.3 and 22.4° gave corrected sedimentation constants of 7.55 and 7.57, respectively, and another at 26.3° gave 7.56. *Conversely*, a similar run at 25.8° gave 7.16, a result obviously in considerable error when compared to the other three and to the graph drawn for the whole concentration series. As for the effect of rotor speed, Koenig and Perrings observed extrapolated values of 7.57, 7.52 and 7.28 at speeds of 59,733, 50,733 and 39,466 r.p.m., respectively, in 0.1 *M* disodium phosphate at 20° and concluded that a significant drop occurred at the lowest speed, indicating molecular orientation during sedimentation. In the present work, one concentration series was studied at 42,040 r.p.m., almost as low as the lowest speed of these other workers. No significant difference from the results at 59,780 r.p.m. could be seen. It might further be suggested that since tobacco mosaic virus shows no molecular orientation in the ultracentrifuge, it would seem highly unlikely that fibrinogen would do so. In the study by Schachman,[23] rotor speeds extending over a 6-fold range were employed, but there was no significant variation in the sedimentation constant of the virus.

(23) H. K. Schachman, THIS JOURNAL, **73**, 4808 (1951).

(24) W. R. Carroll and K. Laki, personal communication.

TABLE III

INTRINSIC VISCOSITY OF FIBRINOGEN

Investigators	Ref.	Intrinsic viscosity	Species
Nanninga	10	0.27	Bovine
Oncley, Scatchard and Brown	5	.23	Human
Shulman and Ferry	7	.34	Bovine
Hocking, Laskowski, and Scheraga	11[b]	.25	Bovine
Ehrlich, Shulman and Ferry	25	.30	Bovine
This work	...	.250	Bovine

about 75% fibrinogen,[7] and about 90% fibrinogen,[25] and since the computations were made using the *fibrinogen* concentration rather than the concentration of total protein, it was anticipated[7] that the resulting intrinsic viscosities would be too large. In the study by Oncley, *et al.*,[5] the fibrinogen was about 95% pure, and the measured intrinsic viscosity was 0.23. Because these computations utilized the total protein concentration, this value should be slightly too low, and a probable correct value for fibrinogen of 0.25 was, in fact, estimated by these workers.

The Size of the Fibrinogen Molecule.—The molecular weight of fibrinogen can now be calculated, using the usual Svedberg ultracentrifuge equation

$$M = \frac{RTs}{D(1 - \bar{v}\rho)} \qquad (2)$$

One additional datum is required—the partial specific volume, $\bar{v}$. This recently has been determined for bovine fibrinogen in several laboratories. Koenig[26] obtained a value of 0.706, and Carroll and Laki[24] recently obtained 0.710. A value of 0.717 also has been calculated[27] from the amino acid analysis.[28] Using $s = 7.80 \times 10^{-13}$, $D = 2.02 \times 10^{-7}$ and $\bar{v} = 0.71$, a molecular weight of 328,000 is computed. If $s = 7.95 \times 10^{-13}$ were used, 334,000 would be the result. This can be compared with the original sedimentation–diffusion study,[3] in which Holmberg calculated a molecular weight of 700,000. He assumed a $\bar{v}$ of 0.75. If a value of 0.71 is used with his data, a molecular weight of 604,000 is deduced. The major source of disagreement is the diffusion constant, as has already been discussed.

The molecular weight derived from the present work compares remarkably well with the value of 340,000 recently obtained in this Laboratory by means of light scattering.[12]

The Shape and Hydration of the Fibrinogen Molecule.—The present data also permit the calculation of the frictional ratio, f/f_0, as shown in the equation

$$f/f_0 = 10^{-8}\left(\frac{1 - \bar{v}\rho}{D^2 s\bar{v}}\right)^{1/3} \qquad (3)$$

Again using $s = 7.80 \times 10^{-13}$, $D = 2.02 \times 10^{-7}$ and $\bar{v} = 0.71$, the resulting frictional ratio is 2.34, as contrasted with the value of 3 obtained by Holmberg.[3,31] By making the customary assumption that the molecule is a rigid, impenetrable, unhydrated ellipsoid of revolution, the axial ratio of such an ellipsoid can be obtained from either the frictional ratio or the intrinsic viscosity, using graphical expressions of the Perrin or Simha relations, respectively.[32] To interpret the viscosity data, it is convenient to express the results in terms of the viscosity increment, ν, which may be computed from the intrinsic viscosity, $[\eta]$, by the relation, $\nu = (100/\bar{v})[\eta]$. Using a partial specific volume of 0.71, a viscosity increment of 35.2 is calculated for fibrinogen. A choice between prolate and oblate ellipsoids only need be made. The prolate model has become firmly established for fibrinogen, largely because of flow double refraction,[11b,33] light scattering,[11b,12] and electron microscope[34,35] studies, as well as the needle-shaped appearance of microscopically-visible aggregates formed in the conversion to fibrin. With this choice of model, one readily derives an axial ratio of 18.9 from the viscosity data and a value of 29.5 from the frictional ratio. The axial ratio, assuming no hydration, obtained from sedimentation and diffusion studies is 56% larger than the value obtained from viscosity measurements. This is somewhat surprising in light of the generally good agreement that has been found between these two methods.[36] The axial ratio of 18.9 is in excellent agreement with that deduced from light scattering studies, wherein a molecular weight of 340,000 and a major dimension of 650 Å. was deduced for a thin ellipsoid. Using a specific volume of 0.71 with these data, an equatorial diameter of 34.4 Å. was calculated. These dimensions correspond to an axial ratio of 18.9. Since light scattering would be insensitive to a bound layer of solvent (unless there were a significant difference in refractive index between bound and bulk solvent), this agreement suggests a very small degree of hydration.

The actual dimensions for these ellipsoid models can be computed from the molecular weight of 328,000 and specific volume of 0.71, corresponding to a molecular volume of 38.7×10^{-20} cm.^{3}. The results are 34 × 640 Å. and 29 × 860 Å. for the viscosity and the sedimentation–diffusion data, respectively.

The discrepancy in computed axial ratio between these two hydrodynamic studies is not appreciably decreased by consideration of possible hydration.

(25) P. Ehrlich, S. Shulman and J. D. Ferry, THIS JOURNAL, **74**, 2258 (1952).

(26) V. L. Koenig, *Arch. Biochem.*, **25**, 241 (1950).

(27) K. Bailey and F. Sanger, *Ann. Rev. Biochemistry*, **20**, 118 (1951).

(28) For the sake of completeness, it may be noted that a value of 0.725 has been measured for human fibrinogen,[29] and 0.723 has been computed from analytical data.[30]

(29) S. H. Armstrong, Jr., M. J. E. Budka, K. C. Morrison and M. Hasson, THIS JOURNAL, **69**, 1747 (1947).

(30) T. L. McMeekin and K. Marshall, *Science*, **116**, 142 (1952).

(31) A value of 1.98 has also been quoted as the frictional ratio of fibrinogen,[5] but this is really a fictitious value, deduced from viscosity data by assuming that the two types of measurement would agree on a value for the axial ratio.

(32) See, for example, E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, pp. 406 and 519.

(33) J. T. Edsall, J. F. Foster and H. Scheinberg, THIS JOURNAL, **69**, 2731 (1947).

(34) C. E. Hall, *J. Biol. Chem.*, **179**, 857 (1949).

(35) P. Kaesberg and S. Shulman, *ibid.*, **200**, 293 (1953).

(36) In the study by Mehl, Oncley and Simha,[37] the (prolate) axial ratios of 9 out of 13 proteins, as deduced from the two methods, agreed to within 15%.

(37) J. W. Mehl, J. L. Oncley and R. Simha, *Science*, **92**, 132 (1940).

If one assumes a hydration of 0.3 g. water/g. protein, these axial ratios become 15.1 and 22.1, respectively, using the theoretical approach suggested by Oncley.[38] Larger hydration values do bring these numbers still closer together, while making each smaller, but an unreasonably large 3.0 g. water/g. protein is needed to give as small a difference as 12%, the respective values being 5.7 and 6.4. Yet these very same data provide a molecular weight agreeing to within 4% with the value obtained from light scattering. Clearly, for fibrinogen at least, these hydrodynamic theories prove inadequate.

There are at least two possible alternatives to the ellipsoid model that may be of interest. One of these is the interpretation of the hydrodynamic behavior of protein solutions recently provided by Scheraga and Mandelkern.[39] In this approach a hypothetical particle which *is* a rigid ellipsoid of revolution and which exhibits the observed hydrodynamic behavior is considered in place of the molecule, and the axial ratio and volume of this effective hydrodynamic ellipsoid is calculated. No assumptions are made as to the rigidity or hydration of the original molecule or as to the degree of permeation by the solvent. A function, β, is derived, which may be defined as

$$\beta = \frac{Ns[\eta]^{1/3}\eta}{M^{2/3}(1 - \bar{v}\rho)} \qquad (4)$$

where η is the solvent viscosity, N is Avogadro's number, and the other symbols have been defined.

Substituting the values utilized above in computing the molecular weight of 328,000 a magnitude of 2.14×10^6 is obtained, corresponding to an axial ratio of 2.5. A similar expression for β, involving the diffusion constant in place of the sedimentation constant also can be used, and the result is 2.18×10^6, corresponding to an axial ratio of 3.5. The solvent viscosity that was used in these calculations was that of water at 20°, *viz.*, 0.0100, since both s and D had been corrected to such a solvent. As is readily apparent, computations by this theory require very accurate experimental data. A 2% difference in β in this range gives a 33% difference in axial ratio. It should be pointed out, for example, that if a value of 0.72, rather than 0.71, were used for $\bar{v}$ in eq. 4, a result for β of 2.22×10^6 would be obtained, instead of 2.14×10^6. This larger value corresponds to an axial ratio of 5. Hence, an increase of 1.4% in the partial specific volume leads to an increase of 100% in the equivalent ellipsoid axial ratio. According to some recent studies in Dr. Scheraga's laboratory,[40] a value for β of 2.23×10^6 was indicated, providing again an axial ratio of 5, and this may be tentatively accepted as the computed parameter. This value is so much smaller than the 18 to 20 that has usually been assigned in the past because the effective volume of the particle is no longer taken as being the partial specific volume. A much larger effective volume, V_e, is in fact derived, and accordingly a much smaller axial ratio will, together with the larger V_e, explain the observed viscosity and diffusion characteristics. To obtain this volume one may use the length of 560 Å., as derived from flow birefringence.[22,41] Using the axial ratio of 5, the thickness of this effective ellipsoid is 112 Å., and thus the volume is 368×10^{-20} cm.^{3}. This is 9.5 times larger than the 38.7×10^{-20} cm.^{3} computed from the partial specific volume, sedimentation–diffusion molecular weight, and Avogadro's number.[42] The smaller value corresponds, of course, to the volume occupied by the polypeptide portion of the molecule. Taking the polypeptide density to be 1.4 g./ml. and the water density, 1 g./ml., this would be a hydration of 4.5 g. water per g. protein.[43] If this description of the molecule is to be accepted as depicting its actual state, it must mean that the structure is a rather open and swollen one, with immobilized solvent permeating the entire mass. At present, we cannot establish the degree of similarity of the structure of the actual molecule with that of this *equivalent* hydrodynamic model. It seems most unlikely for fibrinogen, at least, that the molecule is a swollen structure, for such a unit would be very ill-fitted for the polymerization to fibrin that these molecules undergo. Studies that have been reported from this Laboratory, and particularly the electron microscope observations that have been made on intermediate polymers in the reaction,[35] have led to the conclusion that fibrinogen units become aligned in an end-to-end and overlapping pattern to form smooth and compact larger units that constitute the fibrils of the network. Such considerations lead one to favor a molecular structure with a rod-like shape.

As a direct result of this conclusion, the third possibility for describing the molecule that will be discussed is the string-of-beads model of Kuhn.[44] This model was originally suggested as a means of obtaining an approximation to the hydrodynamic behavior of a rod (with rounded ends). It is a string of beads of radius r with centers separated by distances equal to $4r$, so that the separation between surfaces of adjacent spheres is equal to a sphere diameter. This was taken as equivalent to a rod with diameter equal to the sphere diameter and length equal to the over-all length of the string of beads, but for the present purpose the model may be taken for its actual form. The viscosity increment and the frictional ratio can be expressed in terms of the axial ratio, S/d, where S is the total

(38) J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941).

(39) H. A. Scheraga and L. Mandelkern, THIS JOURNAL, **75**, 179 (1953).

(40) H. A. Scheraga, personal communication.

(41) Dr. Scheraga has pointed out[40] that in computing the length of the major axis from the rotary diffusion constant, the axial ratio of the effective hydrodynamic ellipsoid should be used. The published[11b] length of 670 Å. was based on a ratio of 18. A recalculation, using a ratio of 5, as obtained from β, in conjunction with the published rotary diffusion constant of 39.4×10^3 sec.$^{-1}$ provides a length of 560 Å.

(42) Alternatively, a volume of 39.4×10^{-20} cm.^{3} is obtained from the ellipsoidal dimensions, 34 × 650 Å., derived from the light scattering length in conjunction with an axial ratio of 19. It should be emphasized that this computation belongs to the conceptual scheme representing the molecule as a rigid, solid and unhydrated ellipsoid of revolution.

(43) According to the Oncley treatment a hydration of this magnitude would correspond to axial ratios of 4.2 and 4.5, from viscosity and sedimentation–diffusion, respectively. This would be an unreasonably large hydration if it were taken to indicate a thick shell of solvent bound peripherally to a solid polypeptide core.

(44) W. Kuhn, *Z. physik. Chem.*, **A161**, 1 (1932); W. Kuhn, H. Kuhn and P. Buchner, *Ergeb. exakt. Naturwiss.*, **25**, 1 (1951).

length and d is the thickness or sphere diameter. The theoretical equations were given as

$$\eta_{sp} = \frac{\pi}{48} GS^3, \text{ and} \quad (5)$$

$$D = \frac{kT}{(3\pi/2)\eta_0 S} \quad (6)$$

where η_{sp} is the specific viscosity, G is the number of particles per ml., D is the diffusion constant, η_0 is the solvent viscosity, k is the Boltzmann constant, T is the absolute temperature and S has been defined. From these expressions we can derive the required relations. Since the viscosity increment, ν, is related to the specific viscosity and to the volume fraction, ϕ, by

$$\nu = \frac{\eta_{sp}}{\phi} = \frac{\eta_{sp}}{GV} \quad (7)$$

we obtain

$$\nu = \frac{\pi}{48} \times S^3 \times \frac{1}{V} \quad (8)$$

We note that the volume, V, of our string-of-beads model is

$$V = \frac{S}{2d} \times \frac{4}{3}\pi\frac{d^3}{8} = \frac{\pi}{12} d^2 S \quad (9)$$

since the number of beads is approximately $S/2d$. The volume of the corresponding rodlet is just three times as big, *viz.*, $\pi d^2 S/4$, but we shall choose to ignore this version of the model.

A length, S, of 595 Å. can be computed directly from eq. 8, but it seems preferable to obtain an expression involving the axial ratio. By combining eq. 8 and 9 one obtains[45]

$$\nu = \frac{1}{4}(S/d)^2 \quad (10)$$

To derive the diffusion equation, we require the expression for the diffusion constant, D_0, of a sphere of equal volume. This is

$$D_0 = \frac{kT}{6\pi\eta_0((3/4\pi)V)^{1/3}} \quad (11)$$

By combining eq. 6 and 11 one obtains the desired frictional ratio

$$\frac{f}{f_0} = \frac{D_0}{D} = (S^2/4d^2)^{1/3} \quad (12)$$

Applying eq. 10 and 12 to the fibrinogen study gives an axial ratio of 11.9 from the viscosity data and an axial ratio of 7.2 from the sedimentation–diffusion data. These ratios, in conjunction with the molecular volume of 38.7×10^{-20} cm.^{3}, give molecular dimensions of 50 × 590 Å. (six spheres plus an intraspheral segment) and 57 × 410 Å. (four spheres) from viscosity and sedimentation–diffusion, respectively. The two axial ratios are again not in very good agreement, but it is interesting to observe that the viscosity-derived value is the bigger one here, while it is the smaller one in the ellipsoid treatment. It is therefore suggested that the actual molecular shape (ignoring or minimizing the possibility of hydration) is something intermediate between an ellipsoid and a string of beads, perhaps a nodular rod with very approximate dimensions of 50 × 600 Å. This model is depicted in Fig. 8, along with the alternative possibilities. Electron microscope studies[34] have already described the (dehydrated) fibrinogen molecule as being a string of several similar spheres. This fact, along with the considerations already mentioned, would seem to provide some plausibility to this molecular description.

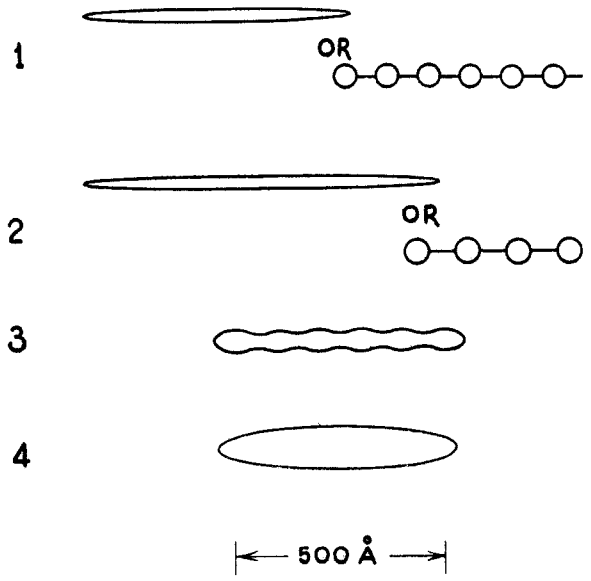

Fig. 8.—Schematic representation of various proposed molecular models for fibrinogen: 1, ellipsoid and string of beads, from viscosity axial ratio; 2, ellipsoid and string of beads from sedimentation-diffusion axial ratio; 3, compromise of ellipsoid and string of beads; 4, equivalent hydrodynamic ellipsoid.

It need only be noted in addition that the various physicochemical data that have thus far been assembled on fibrinogen of human and bovine origin fail to reveal any significant difference in size and shape between these two molecular species.

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(45) For the rodlet form, one would obtain $\nu = (S/d)^2/12$, which apparently supersedes the analogous equation derived in the earlier paper,[44] *viz.*, $\nu = 2.5 + (S/d)^2/16$.