not be ascribed to a direct toxic effect in any of the cases where this was studied.

3. The compounds that proved to be most effective in reducing the amount of the incorporation of amino acids into the bacterial protein were also, in general, those most effective in inhibiting the growth of the Walker rat carcinoma.

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Some Physicochemical Properties of Human Fibrinogen

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Among the numerous studies of the physicochemical properties of fibrinogen the majority of investigators have confined themselves to the more readily available bovine protein. A preparation of human fibrinogen made by salt fractionation was studied by Holmberg (1944); sedimentation velocity and diffusion measurements gave a molecular weight of 600 000 and a frictional ratio of 3; this leads to an axial ratio of 50, assuming zero solvation. Human fibrinogen prepared by the ethanol fractionation method of Cohn and co-workers had a molecular weight of 400 000 and a frictional ratio of 1·98 from sedimentation-velocity and intrinsic-viscosity determinations, and a molecular weight of 580 000 by osmotic-pressure measurements (Oncley, Scatchard & Brown, 1947). Studies on the double refraction of flow by Edsall, Foster & Scheinberg (1947) led to the conclusion that the fibrinogen molecule could be approximated in shape by a prolate ellipsoid 700 Å long with an axial ratio of 18. The material prepared by further fractionation of fraction '1' (Morrison, Edsall & Miller, 1948) always contained a small proportion of a fast component in both the ultracentrifuge and electrophoresis apparatus. Electrophoretic studies by Avery & Munro (1948) of fractions of human fibrinogen prepared by various salt-fractionation methods showed that only one of the materials prepared by precipitation with phosphate consisted of a single electrophoretic component. In a preliminary investigation of purified human fibrinogen prepared by ether precipitation (Kekwick, Mackay, Nance & Record, 1955) a molecular weight of 340 000 and a frictional ratio of 2·34 were found from sedimentation velocity and diffusion measurements (Caspary & Kekwick, 1954). This gives an axial ratio of 28 on the assumption of an unhydrated prolate ellipsoid. The preparations examined had no additional components either on electrophoresis or in the ultracentrifuge. The present paper deals with more extensive sedimentation and diffusion measurements on similarly purified human fibrinogen. The

accuracy of the measurements is discussed and the electrophoretic and ultracentrifugal boundaries are analysed. The deviations of the free diffusing boundary from the ideal are also described. Some further evidence for the suggested disaggregation of the fibrinogen molecule at low concentration (Caspary & Kekwick, 1954) is also given.

MATERIALS AND METHODS

Fibrinogen. This protein, prepared by the method of Kekwick *et al.* (1955), had clottable-nitrogen values between 97 and 98% and was essentially free from plasmin and plasminogen. The clottable nitrogen usually fell by about 1% on freeze-drying. It should be noted that a peptide is split off during the clotting process (Lorand, 1952); it accounts for about 3–3·5% of the fibrinogen nitrogen and implies that the clottability of 100% fibrinogen should be assessed as 96·5–97%. For work on freshly prepared material the product was dialysed immediately in the cold and not allowed to reach room temperature until all traces of ether had been removed; this procedure greatly increases the stability of solutions during storage at 2–4°. The total protein concentration was determined either by micro-Kjeldahl nitrogen estimation or refractometrically.

Refractive index. Refractive index was measured with a Zeiss dipping refractometer at λ 546 mμ and 25°. The specific refractive increment was arbitrarily assumed to be 0·200 ml./g.

Micro-Kjeldahl nitrogen. This was estimated in the apparatus of Markham (1942) by using the mixed bromocresol green–methyl red indicator of Ma & Zuazaga (1942).

Buffers and solutions. Citrate–saline pH 7·7: NaCl 8·8 g./l., trisodium citrate 3·7 g./l.; phosphate buffer, pH 8·0, I 0·2: Na_2HPO_4 0·0654M, KH_2PO_4 0·0044M; phosphate buffer, pH 7·0, I 0·1: Na_2HPO_4 0·0271M, KH_2PO_4 0·0188M; phosphate buffer, pH 6·2, I 0·1: Na_2HPO_4 0·0127M, KH_2PO_4 0·0616M; acetate buffer, pH 5·5, I 0·1: sodium acetate 0·10M, acetic acid 0·0112M; acetate buffer, pH 4·3, I 0·1: sodium acetate 0·1M, acetic acid 0·25M; glycine buffer, pH 8·8, I 0·1: glycine 0·0950M, NaCl 0·0988M, NaOH 0·0050M.

Electrophoresis. Electrophoretic analyses were carried out in the Tiselius apparatus at 0°, with the edge version of the diagonal schlieren optical system and monochromatic light, λ 546 mμ. Solutions of fibrinogen, 5–10 mg./ml., were made up in buffer and dialysed against buffer at 2–4° for several days, with frequent changes. The buffers used were phosphate (pH 8, I 0·2, and I 0·1) and, on the acid side of the isoelectric point, acetate (pH 4·3, I 0·1). The average duration of an experiment was 40 mA hr. but several experiments were continued for periods up to 300 mA hr. to ensure maximal separation of any impurities.

Boundary-spreading experiments were made at the isoelectric point, pH 5·5 (Seegers, Nieft & Vandenbelt, 1945; Mihalyi, 1950). The fibrinogen was made up in and dialysed against acetate buffer, pH 5·5, I 0·1 or 0·15, and then centrifuged at 0–2° to remove any residue; the protein is not very soluble under these conditions and the maximum concentration attainable was of the order of 4 mg./ml. In order to obtain greater accuracy in tracing the electrophoretic patterns the schlieren straight edge was replaced by a slit 0·5 mm. wide. The form of the boundary was checked by a plot of log dn/dx against $(x-\bar{x})^2$, where x is the distance in the boundary and $\bar{x}$ the mean distance; non-linearity and divergencies between the two sides of the boundary curve indicate non-ideality. The apparent diffusion coefficient, D', was computed by the method of moments, and a heterogeneity constant calculated from the slope of the graph of D' against the time of electrophoresis (Alberty, 1948).

Sedimentation. All ultracentrifugal examinations were made in the Svedberg oil-turbine machine at 54 000 rev./min. (240 000 g) with the diagonal schlieren optical system (Philpot, 1938) and monochromatic light, λ 546 mμ. The rotor thermocouple of this machine was calibrated by observation of the melting of phenyl ether under standardized operating conditions (Cecil & Ogston, 1948). Photographs were taken on Ilford Rapid Process Panchromatic plates and measured with a travelling microscope (R. and J. Beck Ltd.), with a special adaptor for aligning the plates. The measurements were reproducible to $\pm$0·002 mm. Sedimentation coefficients were computed by the method of Cecil & Ogston (1948). The slope and standard deviation were calculated from the regression of the distance moved against the reduced time including the correction for viscosity, by the method of least squares. Correction to a liquid of the density and viscosity of water at 20° was made in the customary manner (Svedberg & Pedersen, 1940). The range of the mean temperatures of the ultracentrifuge rotor for the whole series of experiments was from 21 to 27°.

Two different assessments of the sedimentation homogeneity were made. First, the form of the boundary gradient curve was tested by plotting log dn/dx against $(x-\bar{x})^2$, where x is the position in the boundary measured from the centre of rotation, $\bar{x}$ the position of the maximum and dn/dx the gradient of refractive index obtained from the ordinates of the curve. For a Gaussian boundary this plot is linear and identical for both limbs of the curve. Secondly, the second moments, σ, of the boundary-gradient curve were computed from the measured ordinates of the sedimentation diagrams. The values of σ were corrected for the non-uniformity of the centrifugal field and for the concentration dependence of the sedimentation coefficient by the equation (Baldwin, 1953*a*):

$$\sigma^2_{corr.} = \sigma^2(1-\bar{\omega}^2St) + 4\omega K\Delta c' \int r\sigma\,dt,$$

where ω is the angular velocity, S the sedimentation coefficient, t the time measured from the estimated beginning of sedimentation, K is the constant in the linear sedimentation–concentration function, $\Delta c'$ is 0·34 times the concentration and r is the distance of the boundary from the centre of rotation. The integration is carried out graphically. A plot of σ^2 against t is linear for a homogeneous solute; heterogeneity is manifested as an upward curvature (Baldwin, 1953*a*, *b*). The standard deviation of the sedimentation-coefficient distribution was computed by fitting a quadratic equation to the σ^2–t plot by the method of least squares and then determining the slope of a plot of the apparent diffusion coefficient D' against a finally corrected time t''. To improve the accuracy of measurement in these experiments the schlieren straight edge was replaced by a 1 mm. wire.

Diffusion. The diffusion coefficients were determined by the Gouy interferometric method in a modified form of the apparatus of Gosting, Hanson, Kegeles & Morris (1949) at 25$\pm$0·01°. This apparatus has a parallel light beam through the cell with an optical lever arm of 115 cm. A standard

short-section electrophoresis cell specially selected for plane surfaces was used in all experiments. Boundaries were formed and sharpened by siphoning through a fine capillary (Kahn & Polson, 1947). To obtain higher sensitivity the blue Hg line, λ 436 mμ, isolated by an interference filter, was used; this has the additional advantage of making it possible to use fine-grain 'process' plates. Diffusion coefficients were computed from the first interference minimum by applying the Airey integral correction of Gosting & Morris (1949); this approximates to the conventional height–area diffusion coefficient, D_A. For several experiments values of the ideal maximum ordinate C_t were computed for all interference minima (Kegeles & Gosting, 1947) to give a qualitative picture of 'homogeneity'. Experiments were also analysed by the method of Akeley & Gosting (1953). Some experiments were also made by using the schlieren optical system and calculating the diffusion coefficient by both the height–area and moment methods.

Partial specific volume. The value of 0·725 determined for human fibrinogen by Armstrong, Budka, Morrison & Hasson (1947) was used in all experiments.

Viscosity. The viscosity measurements were made at 25±0·01°, with an Ostwald viscometer having a flow time for water of 130 sec. The specific viscosity/unit concentration was plotted against the protein concentration to give the intrinsic viscosity on extrapolation.

RESULTS

Electrophoretic behaviour. The electrophoresis diagram of purified human fibrinogen after migration at pH 8·0, *I* 0·2, for a period of 40 mA hr. had a single symmetrical peak on the ascending side migrating towards the anode; the descending boundary was more spread out and slightly skew (Fig. 1). On continuing an experiment of this type for 300 mA hr. the ascending peak remained as a single component with considerable asymmetry, and a very complex picture appeared on the descending side. By lowering the ionic strength to *I* 0·1 the complex form of the descending picture appeared within a much shorter period. On the acid side of the isoelectric point in acetate buffer, pH 4·3, *I* 0·1, the complexing phenomenon was observed within a very short period, 30 mA hr., with a very sharp ascending peak and the spread-out complex picture on the descending side. Migration at this pH was towards the cathode with a greatly increased mobility.

The method of reversible boundary spreading at the isoelectric point (Alberty, 1948) was used to determine the degree of heterogeneity, the value of pH 5·5 determined for the isoelectric point of bovine fibrinogen (Seegers *et al.* 1945; Mihalyi, 1950) being taken. In acetate buffer, pH 5·5, *I* 0·1, the boundary formed by human fibrinogen remained stationary, justifying examinations at this pH value. At *I* 0·1 the solubility of the protein was only 2·5 mg./ml. and on electrophoresis at 5° a heterogeneity coefficient of $0{\cdot}58 \times 10^{-6}$ was obtained (Fig. 2). A more stable solution was obtained at *I* 0·15, and in these circumstances the heterogeneity coefficient was $0{\cdot}33 \times 10^{-6}$. The experimental boundaries were slightly skew, owing either to an inherent skew distribution or more probably to boundary distortion during compensation.

Behaviour in the ultracentrifuge. The appearance of preparations of purified human fibrinogen in the ultracentrifuge was that of a sharp symmetrical peak with a slight tendency to skewness at higher concentrations (Fig. 3). In several preparations a small quantity of a heavier component (*S* 18) was present; such preparations were not used for definitive physical measurements.

The sedimentation coefficients over a range of concentrations from 2 to 10 mg./ml., in phosphate buffer, pH 8, +NaCl to give a total ionic strength of 0·35, for three preparations, are shown in Fig. 4. Extrapolation to infinite dilution by the method of least squares gives $S^0_{20,w}$ $7{\cdot}63 \times 10^{-13}$, standard error $0{\cdot}07 \times 10^{-13}$ with a correlation coefficient of 0·974. The slope of this line is equivalent to the factor *K* in the equation for the linear concentration dependence of the sedimentation coefficient ($S = S_0 + Kc$) and has the value $1{\cdot}2 \times 10^{-14}$ for this

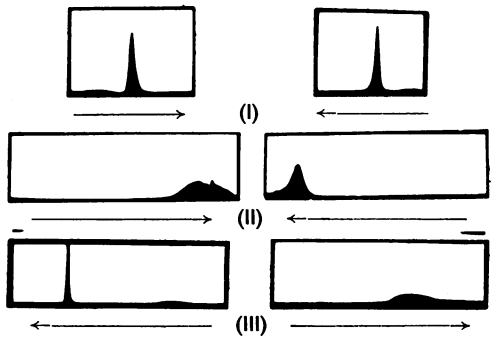

Fig. 1. Electrophoresis diagrams of human fibrinogen. (I) pH 8·0, *I* 0·2; 40 mA hr. (II) pH 8·0, *I* 0·2; 300 mA hr. (III) pH 4·3, *I* 0·1; 30 mA hr. Arrows indicate direction of migration.

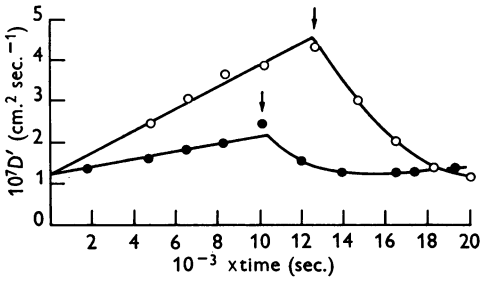

Fig. 2. Isoelectric boundary spreading of human fibrinogen. Plot of apparent diffusion coefficient against time of electrophoresis. ○, *I* 0·10; ●, *I* 0·15. Arrows indicate reversal of current.

combined experiment with a standard deviation of $0{\cdot}06 \times 10^{-14}$. Table 1 shows the values of K for the individual preparations and the combined experiment. When the 't' test was applied to these results the differences were not significant (P 0·5). No significant variations in sedimentation coefficient were found over the range of experimental temperatures (21–27°). The speed of the ultracentrifuge was held at 54 000 rev./min. and the effect of varying this was not examined.

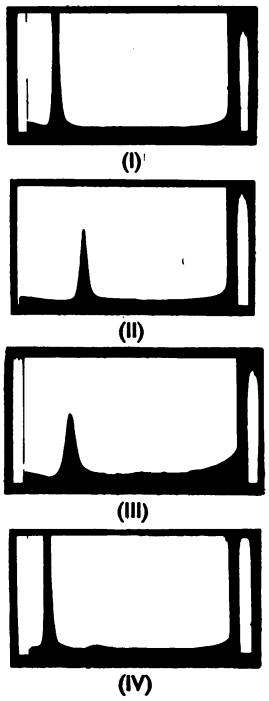

Fig. 3. Sedimentation diagrams of purified human fibrinogen. (I) 10 mg./ml., 20 min.; (II) 6 mg./ml., 30 min.; (III) 3 mg./ml., 20 min.; (IV) sample showing heavy component ($S = 18$ s), 10 min. Times indicate period at 240 000 g.

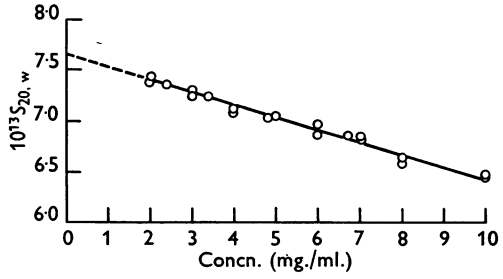

Fig. 4. Sedimentation coefficient of human fibrinogen as a function of concentration.

Bovine fibrinogen, prepared by the ether-fractionation method, in phosphate buffer, pH 8·0, with NaCl added to give a total ionic strength of 0·35, had a sedimentation coefficient of $7{\cdot}63 \times 10^{-13}$ at infinite dilution and a value for K of $0{\cdot}94 \times 10^{-14}$. It should be noted that this value of K is significantly different from that for human fibrinogen under the same conditions.

At concentrations below 1·5 mg./ml. the sedimentation coefficient of human fibrinogen preparations tended to decrease with dilution. Fig. 5 exemplifies the extremes of this type of behaviour for two preparations (F 33, F 38). Even at these low concentrations the decrease in sedimentation coefficient was well outside the limits of experimental error. The effect was eliminated or even reversed after freeze-drying or after allowing the dilute solutions to stand at 4° for 2 weeks or more (Fig. 5); it was not demonstrable with preparations having a trace of heavy component.

The effect of varying the ionic strength at pH 8·0 is shown in Table 2. The values of the sedimentation coefficient at infinite dilution are not significantly

Table 1. *Values of K in the sedimentation coefficient–concentration function for different preparations of fibrinogen*

Preparation	$10^{14}K$
F36	−1·24
F37	−1·31
F38	−1·17
F36 + F37 + F38	−1·21

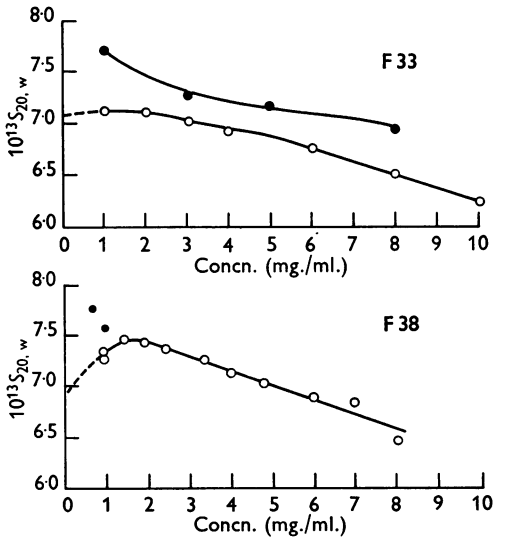

Fig. 5. Sedimentation coefficient as a function of concentration, showing anomalous behaviour at low concentration. F 33: ●, Freeze-dried; ○, fresh preparation. F 38: ●, After storage; ○, fresh preparation.

different at the lower ionic strengths, but are on the border line of significance at I 0·60. The values of K tend to decrease slightly with increasing ionic strength.

The effect of pH on the sedimentation coefficient of fibrinogen was also examined at constant ionic strength. The buffer ionic strengths were held constant at I 0·1 and 0·15 M-NaCl was added to give a total ionic strength of 0·25. The buffers used were acetate, phosphate and glycine. The variation of the sedimentation coefficient over the range pH 5·5–8·8 was barely significant; the values of K varied between 0·77 and 1·3, tending to increase with increase in pH.

It is generally considered that freeze-drying leaves the properties of proteins unchanged, but a slight increase in the sedimentation coefficient of fibrinogen occurred after freeze-drying (Fig. 5, F 33). A comparison experiment showing this effect is illustrated by Fig. 6. The values computed for the fresh material were $S_0 = 7{\cdot}65 \times 10^{-13}$ and $K = 1{\cdot}27 \times 10^{-14}$, and for the freeze-dried material $S_0 = 7{\cdot}71 \times 10^{-13}$ and $K = 1{\cdot}20 \times 10^{-14}$. The values of K were not significantly different (P 0·5) and the difference in S_0 was on the border line of significance. However, the fact that the alteration occurred with all the preparations examined tends to substantiate these findings. The sedimentation coefficient of a solution of fibrinogen (5 mg./ml.) stored at 4° and tested at intervals showed no change over a period of 30 days.

Table 2. *Effect of ionic strength on sedimentation in phosphate, I* 0·1, pH 8, *+sodium chloride*

I	$10^{13} S_0$	$10^{14} K$
0·175	7·68	−1·40
0·350	7·65	−1·26
0·600	7·76	−1·20

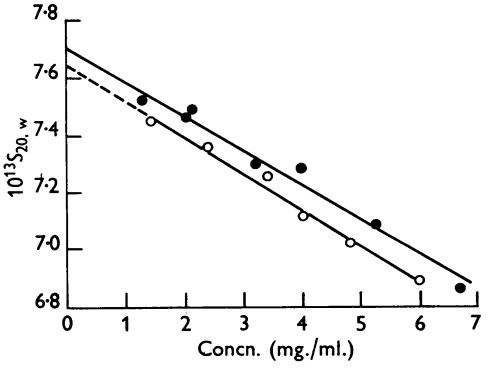

Fig. 6. Effect of freeze-drying on the sedimentation coefficient of human fibrinogen. ○, Fresh preparation; ●, freeze-dried.

On storage, fibrinogen proved to be more stable in a citrate–saline solution, pH 7·4, I 0·225, than in phosphate–saline. In citrate–saline S_0 was $7{\cdot}60 \times 10^{-13}$ and K was $1{\cdot}44 \times 10^{-14}$.

Boundary-spreading experiments in the ultracentrifuge on a fibrinogen solution in citrate–saline showed, first, that the boundary is not Gaussian, because the graph of dn/dx against $(x-\bar{x})^2$ (Fig. 7*a*) is not linear and the lines for both limbs are not coincident. Secondly, the plot of the corrected standard deviation against time (Fig. 7*b*) is not linear. By fitting a quadratic equation to derive a finally corrected time the weight-average diffusion coefficient (D_w) and the standard deviation of the sedimentation coefficient distribution (p) were computed; the values were $p = 0{\cdot}53 \times 10^{-13}$ and $D_w = 2{\cdot}4 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. Analysis to derive the actual distribution was not made in view of the complications likely to arise from the disaggregation observed (Caspary & Kekwick, 1954) and the converse tendency to form a heavy component on ageing. This may also explain the relatively high

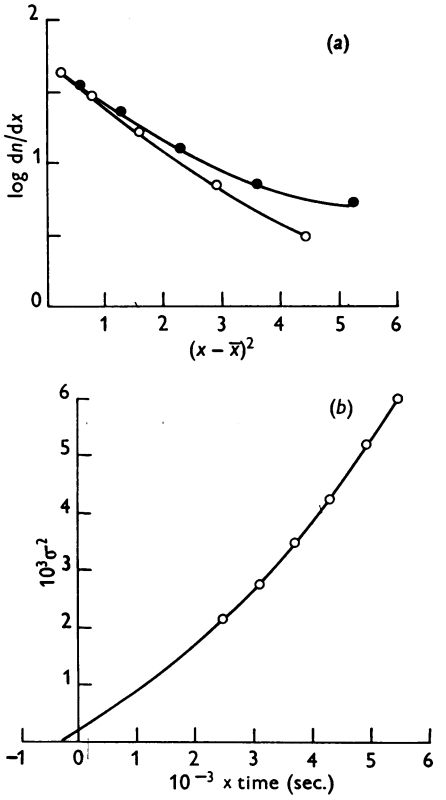

Fig. 7. Ultracentrifugal boundary spreading of human fibrinogen. (*a*) Test of the Gaussian form of both limbs of the boundary; (*b*) plot of the corrected standard deviation against time.

value of p when compared with other proteins (Baldwin, 1954). Attempts to analyse the boundaries from experiments made at low concentrations in the range 1–2 mg./ml. were unsuccessful.

Diffusion-coefficient measurements. The diffusion coefficient of human fibrinogen preparations was measured over a range of concentrations, 2–7 mg./ml. At higher concentrations accurate measurements could not be made as the magnification of the optical system was insufficient to resolve the larger numbers of fringes produced; the results for three preparations are shown in Fig. 8. The value of the diffusion coefficient D extrapolated to infinite dilution was $1{\cdot}98 \times 10^{-7}$ cm.^{2} sec.$^{-1}$, with a standard deviation of $0{\cdot}06 \times 10^{-7}$.

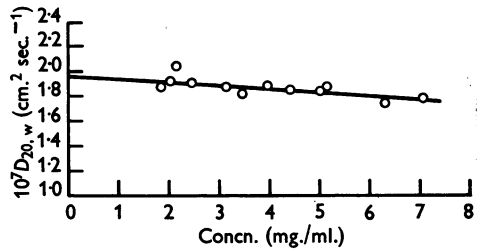

Fig. 8. Diffusion coefficient of human fibrinogen as a function of concentration.

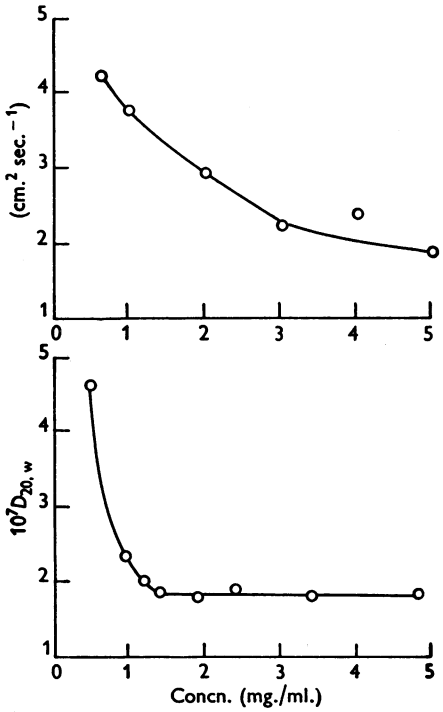

Fig. 9. Diffusion coefficient as a function of concentration, showing anomalous behaviour at low concentrations for two preparations.

At concentrations below 2 mg./ml. there was a sharp rise in the diffusion coefficient, deviating from the linear behaviour with respect to concentration; Fig. 9 shows the extremes of this effect for two preparations. The increase does not occur with freeze-dried preparations and disappears when fresh solutions are stored for several weeks.

The reproducibility of the Gouy diffusion experiments was of the order of 2–3 %. The value of D calculated from the first minimum is a close approximation to the height–area diffusion coefficient.

The boundary characteristics were checked by two methods. Values of the computed maximum ordinate, C_t, were calculated for interference minima covering the complete pattern. The results at two time intervals are given in Table 3. The values of C_t are more nearly constant at the longer

Table 3. *Computation of the theoretical maximum ordinate, C_t, after two different time intervals of diffusion*

Preparation F38, 5·5 mg./ml. j_m (total no. of interference fringes) = 57·24.

	C_t	
j (fringe no.)	9000 sec.	21600 sec.
0	1·6055	1·0370
2	1·6036	1·0360
4	1·5993	1·0333
6	1·5952	1·0330
8	1·5941	1·0317
10	1·5910	1·0307
12	1·5884	1·0299
14	1·5874	1·0305
16	1·5850	1·0292
18	1·5831	1·0276
20	1·5796	1·0285
22	1·5775	1·0260
24	1·5759	1·0266
26	1·5748	1·0254
28	1·5695	1·0253
30	1·5702	1·0252
34	1·5676	1·0236
38	1·5607	1·0222
42	1·5514	1·0231
46	1·5409	1·0329
50	1·5106	1·0451
Max. variation ...	0·095	0·015

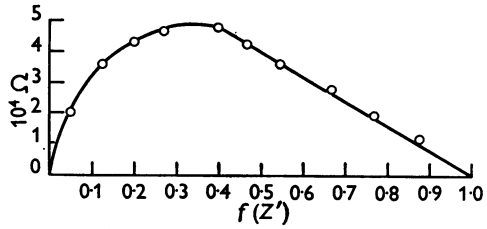

Fig. 10. Analysis of the diffusing boundary of human fibrinogen. Plot of the relative fringe deviation, Ω, against a function of the fringe number, Z'.

interval, an effect probably due to the smoothing-out of some slight boundary disturbances. The final analysis of the diffusing boundary was made by the application of the method of Akeley & Gosting (1953); Fig. 10 shows the plot of the relative fringe deviation against a function of the fringe number; the curvature of this plot may be interpreted as indicating heterogeneity.

Measurements of the diffusion coefficient with the schlieren optical system gave $D_A = 2{\cdot}4 \times 10^{-7}$ and $D_m = 2{\cdot}7 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. The difference between the two average values suggests some heterogeneity; the absolute values of the coefficients tend to be high, because of greater boundary distortion caused by the method of boundary formation and compensation in this apparatus, in comparison with the interferometric apparatus.

In view of the limited accuracy of the methods and the low values of the constant in the linear concentration function for diffusion, no investigations of the concentration dependence of the diffusion of fibrinogen under various conditions were made.

Molecular weight. The molecular weight, calculated from the sedimentation and diffusion coefficients (Gouy values) extrapolated to infinite dilution, by using the Svedberg equation and a partial specific volume of 0·725, was 341 000 with a standard deviation of 10 000. The frictional ratio was 2·34. The values of the molecular weight calculated from the coefficients at corresponding concentrations over the range studied show a sharp drop in molecular weight below a concentration of 1·5 mg./ml. (Fig. 11).

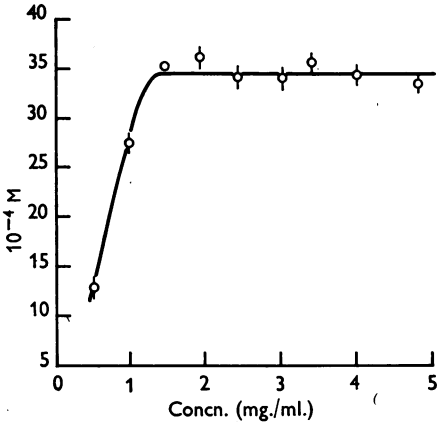

Fig. 11. Molecular weight of human fibrinogen as a function of concentration.

Table 4. *Analysis of fractions obtained by preparative ultracentrifuging of a solution containing the heavy component*

Material	Percentage of fraction	
	$S = 7$ s	$S = 18$ s
Initial	90	10
Supernatant	93	7
Sediment	62	38

Some observations on the $S = 18$ s component. A heavy component (S 18 s) forms when solutions of fibrinogen are held at 2–4° for about 10 days, without altering the clottability of the preparation. It was concluded that this component might be one of the intermediate polymers in the fibrinogen–fibrin transformation. After addition of sufficient urea to bring the concentration to 3 M (Ferry, Katz & Tinoco, 1954) or NaBr to M (Sturtevant, Laskowski, Donnelly & Scheraga, 1955), this component could no longer be detected in preparations originally containing it. Differential ultracentrifuging at 105 000 *g* (Spinco Model L) for 150 min. of a preparation containing the heavy component followed by ultracentrifugal analysis of the separated materials gave the results shown in Table 4. Calculations from the proportions of the $S = 7$ s and $S = 18$ s in the starting material and in the fractions indicated that all the protein originally present as heavy component should have been recovered in the precipitate; this, however, contained less heavy component than was expected; when the analysis was repeated after the solution had been kept for 3 days an even smaller proportion of the heavy component was detected. This suggests that the system is in a slowly reversible equilibrium and that the quantities of the components will tend to reach a state of constant proportion.

Viscosity. The value for the intrinsic viscosity determined at 25° over the concentration range 1–9 mg./ml. was 0·245.

DISCUSSION

It is clear from the experimental results that the molecular kinetic behaviour of the most highly purified preparations of human fibrinogen is extremely complex. Fresh preparations when subjected to electrophoresis for a period adequate for the resolution of the major constituents of human plasma display a single peak only, but the descending boundary eventually becomes very complex in contour when the electrophoresis is continued for six times this period. Similarly, such preparations in the ultracentrifuge give rise to a single peak only; however, at fibrinogen concentrations below 2 mg./ml. the sedimentation coefficient drops sharply away from the linear relationship with concentration observed at higher concentrations. Correspondingly, the diffusion coefficient rises sharply and by combining the two sets of results the molecular-weight values obtained suggest that a disaggregation may be occurring involving a rapid readjustment of equilibrium.

When such fibrinogen solutions are stored for two to three weeks, or are freeze-dried and then reconstituted, this characteristic disappears and there is a tendency for a polymer of sedimentation coefficient approximately 18 S to be formed. That this is also probably in equilibrium with the major component ($S = 7$ S) is shown by the analysis of the supernatant and sediment from the differential preparative ultracentrifuge experiment, and also from the effect of adding urea or NaBr to solutions exhibiting heavy component. In this instance, however, the rate at which equilibrium is attained appears to be much slower than in the possible disaggregation discussed above.

The heterogeneity shown by electrophoretic and ultracentrifugal boundary-spreading measurements could be explained either by the intrinsic heterogeneity of the protein molecules (Colvin, Smith & Cook, 1954; Ogston, 1955) or as a manifestation of the disaggregation and aggregation phenomena. It has been shown by immunological methods (Caspary, 1956) that the purified fibrinogen preparations are contaminated with trace amounts of other plasma proteins, but it is unlikely that these could account for the heterogeneity demonstrated.

If the values of the sedimentation and diffusion coefficients observed at low concentrations in fresh fibrinogen preparations are accepted as indicating a disaggregation of the molecule, weight 341 000, the number of subunits formed from each molecule must be at least three and could possibly be four.

SUMMARY

1. On prolonged electrophoretic migration purified human fibrinogen showed complex behaviour. Slight heterogeneity was demonstrated by isoelectric boundary spreading.

2. Ultracentrifugal examination of human fibrinogen was made under various conditions of protein concentration, pH and ionic strength. The fall of the sedimentation coefficient at low concentrations suggests a disaggregation in rapid equilibrium. Some heterogeneity was demonstrated by boundary analysis.

3. The diffusion coefficient of fibrinogen was determined. Its increase at low concentrations suggests disaggregation. The diffusion boundaries deviate from the ideal form.

4. The molecular weight of the undissociated molecule was found to be $341\,000 \pm 10\,000$.

5. Bovine fibrinogen showed a difference in variation of the sedimentation coefficient with concentration from that of human fibrinogen, although the sedimentation coefficients at infinite dilution were the same.

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