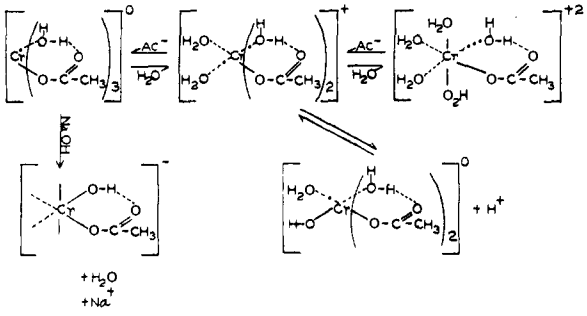

Scheme 1.

This scheme satisfactorily explains the long stability we observed for violet chromic acetate solutions in the neutral zone as well as the predominance of neutral and negative complexes observed in the electrophoresis experiments.

Volshtein[33] has shown that very stable complexes are formed between Cr^{III} and amino acids. Neuberg and Mandl[34] have demonstrated the solubilization of "insoluble" inorganic compounds by widespread biological compounds. In view of the many agents known to complex chromium (carboxylic acids, amino acids, peptides, hydroxy-carboxylic acids) found in the mammalian body, attention is directed to the possible role of these agents in the metabolism of trivalent chromium compounds which are considered insoluble at the *p*H of blood.

(33) L. M. Volshtein, *Izvest. Akad. Nauk. S. S. S. R., Otdel, Khim. Nauk.*, 248 (1952); *C. A.*, **46**, 10035h (1952).

(34) C. Neuberg and J. Mandl, *Arch. Biochem.*, **23**, 499 (1949).

BETHESDA, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICAL CHEMISTRY, HARVARD UNIVERSITY]

The Non-clotting Component of the Human Plasma Fraction I-1 ("Cold Insoluble Globulin")[1]

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Fraction I-1 of human plasma has previously been shown to contain a non-clottable globulin, very insoluble at temperatures near 0° and much more soluble above 20°. Studies on the physical properties of Fraction I-1 and of the cold insoluble globulin are reported. Electrophoresis, sedimentation and clotting studies indicate that reversible complex formation occurs, in Fraction I-1, between fibrinogen and the cold insoluble globulin. The latter showed well marked double refraction of flow in glycerol–water mixtures, with a rotary diffusion coefficient, Θ, slightly less than that of fibrinogen, the product $\eta\Theta/T$ being approximately 0.8. Electrophoretic studies, carried out at 20° to avoid precipitation of the protein, gave a mobility of -7×10^{-5} cm.²/volt sec. at *p*H 8.6, for 90% of the protein. The sedimentation constant of the principal component ($s_{20} = 15\ S$) was nearly twice as great as that of fibrinogen; while its intrinsic viscosity (0.15) was considerably lower than that of fibrinogen (0.25). The relation of this cold insoluble globulin to that of other reported plasma proteins with similar solubility properties is discussed.

Previous observations[3] on the sub-fractionation of Fraction I from human plasma have indicated the presence of a non-clottable globulin, characterized by its great insolubility at temperatures in the neighborhood of 0°, its solubility at temperatures of 20° or above being relatively high. This protein has been known as "cold insoluble globulin." Previous measurements of double refraction of flow[4] on Fraction I from which fibrinogen had been removed by clotting with thrombin had suggested that its rotary diffusion coefficient was not very different from that of fibrinogen. The cooling of solutions of Fraction I led to precipitation of the non-clotting protein in the form of a complex containing approximately 50% of fibrinogen by weight. On removal of the fibrinogen by clotting with thrombin, the cold insoluble non-clottable protein was obtained free of fibrinogen.

Studies on the characterization and partial purification of this non-clotting component of Fraction I are reported here. Some information concerning the complex formed between fibrinogen and the non-clotting component is also included. Since the studies here reported were completed a number of years ago (1947), the starting materials used for the sub-fractionation were those obtained by the earlier method of fractionation reported from this laboratory.[5]

Experimental

In the procedure previously described for the purification of fibrinogen from Fraction I,[3] Fraction I-1 was removed at *p*H 6.3 in citrate solution of ionic strength of 0.3 and 0.5% ethanol by cooling to 0°, since this fraction was found to be very insoluble at that temperature. Approximately 20% of the total protein of Fraction I precipitated in this fraction and approximately two-thirds of the fraction was clottable with thrombin.

In the present studies the procedure was modified in one important respect. Fraction I-1 was found to be very susceptible to surface denaturation, and some preliminary experiments indicated that this susceptibility might be enhanced by the precipitation at *p*H 5.0 which was employed as the first step of the previous process. This degree of acidification was avoided in the studies reported here, and Fraction I-1 was precipitated by cooling Fraction I to 0° at *p*H 6.1, leaving the albumin and globulin impurities and most of the fibrinogen in solution.

Source of Material.—The greater part of the experimental work was carried out on dry Fraction I powder which had been prepared by the Lederle Laboratories according to methods developed here[5] and then stored *in vacuo* for three

(1) This paper has been supported by funds of Harvard University and the Eugene Higgins Trust, by grants from the Rockefeller Foundation and the National Institutes of Health, and by contributions from industry.

(2) (a) Department of Chemistry, The University, Birmingham, 15, England. (b) Department of Chemistry, Cornell University, Ithaca, New York.

(3) P. R. Morrison, J. T. Edsall and S. G. Miller, THIS JOURNAL, **70**, 3103 (1948).

(4) J. T. Edsall, J. F. Foster and I. H. Scheinberg, *ibid.*, **69**, 2731 (1947).

(5) E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, *ibid.*, **68**, 459 (1946).

years. Approximately 300 g. of protein from lots No. 416B, 431A, 416A, B, C were fractionated with a recovery of 13 g. of Fraction I-1. Half of this latter material was further fractionated to give 2 g. of the non-clotting protein. Some relatively fresh Fraction I, which had been stored as a paste at −5° for one month, was made available through the Massachusetts Biologics Laboratories and was similarly fractionated. The amounts of Fraction I-1 complex and non-clotting protein in Fraction I are estimated to be approximately 10 and 5%, respectively.

Fractionation Procedure.—Fraction I (powder or paste) was dissolved in sodium citrate buffer of ionic strength 0.3 and *p*H 6.1 at room temperature and filtered. After the protein concentration had been adjusted to 0.8–1.0% and the ethanol concentration to 0.5% the solution was cooled to 0°. Because of the difficulty encountered in redissolving Fraction I-1 precipitates, and also to avoid their gelation during freezing preparatory to freeze-drying, the Fraction I-1 precipitate was allowed to settle[6] to the bottom of a large suction flask, after which the synerized gelatinous skin was frozen, and dried *in vacuo* from the frozen state. Such dried preparations redissolved almost completely in about two hours on very gentle agitation with solvent.

Successive reprecipitation of Fraction I-1 under these conditions failed to yield a product with less than 50% of clottable protein. Moderate variations of *p*H, between 6 and 7.3, or of ionic strength, did not yield further separation. These findings suggested a stoichiometric complex rather than a non-specific adsorption of fibrinogen by the non-clotting protein. Accordingly, the clottable protein was removed by conversion to fibrin with thrombin as in the Morrison procedure[7] for the assay of fibrinogen. The remaining non-clotting material was then precipitated under the same conditions used previously for the complex. Under these conditions, the solubilities of the Fraction I-1 complex and of the non-clotting protein were both found to be approximately 0.2 mg./ml. In either case, solubility increased rapidly with temperature, and at room temperature both were soluble to at least 20 mg./ml.

Electrophoresis measurements, which were carried out for the routine control of the fractionation at each stage, were made at 20° because of the small solubility of these proteins at low temperatures. For these runs the protein solutions were dialyzed against a sodium diethylbarbiturate buffer of *p*H 8.6 and ionic strength 0.1. The electrophoretic boundaries were found to show satisfactory stability under the conditions employed. Concerning the problems of making electrophoretic measurements at temperatures well above 4°, the paper of Johnson and Shooter[8] may be consulted.

For ultracentrifuge runs the protein solutions were dialyzed against a *p*H 6.1 buffer containing 0.14 *M* sodium chloride and 0.01 *M* sodium citrate.

Determination of Protein Concentrations.—Concentrations were determined by means of a Beckman quartz spectrophotometer, using quartz cells 1.00 cm. in thickness and light of a wave length of 280 mμ (band width 3.2 mμ) corresponding to the maximum absorption of the tyrosine and tryptophan components of the protein.

The instrument was calibrated by determining the optical density and dry weight of a solution of the non-clotting protein. Using the Lambert–Beer equation

$$\log I_0/I = kcd$$

where $\log I_0/I$ is the optical density, k the extinction coefficient, c the concentration in g./100 ml. and d the thickness of the cell in cm. The value of k was found to be 13.5 at *p*H 6.1.

Viscosity.—The viscosities of solution of Fraction I-1 and the non-clotting protein in sodium citrate buffer (*p*H 6.1, ionic strength 0.3) were measured at 20° in an Ostwald viscometer (12 cm. head, 2 min. flow time with water). Measurements were made at several concentrations, c, expressed in g./100 ml. The function $H = (\ln \eta/\eta_0)/c$ was extrapolated to zero concentration to obtain the intrinsic viscosity.

Double Refraction of Flow.—Double refraction of flow measurements were carried out at 20.0°. The apparatus previously described[9] consisted of concentric cylinders of stainless steel, of radius 2.5 cm. and height 7.0 cm., the velocity gradient across the 0.25 mm. gap being numerically equal to 10.5 times the speed of the rotating inner cylinder in r.p.m. The cylinders were mounted between two Nicol prisms which could be coupled or rotated separately for measurements of the extinction angle or double refraction, respectively.

The double refraction was measured by means of a Sénarmont compensator; details of the procedure have been previously described. The quantity, Δ, in degrees, as determined by the compensator for the light emerging from the flowing solution is related to the amount of double refraction $(n_e - n_0)$ by the equation

$$\Delta = \frac{180S}{\lambda_0}(n_e - n_0)$$

where S is the length of the light path in the solution and λ_0 the wave length of the light *in vacuo*.

The extinction angle, χ, which defines the position of the optic axis in the flowing solution relative to the direction of the stream lines, was measured as previously described.[4,9,10]

χ and Δ are functions of the dimensionless parameter $\alpha = G/\Theta$, G being the velocity gradient and Θ the rotatory diffusion constant, both being expressed in sec.$^{-1}$. Tables have recently been compiled for the evaluation of Θ from extinction angle measurements for ellipsoidal particles.[11]

As previously pointed out,[4,9] one of the main sources of error arose from parasitic reflected light from the walls of the narrow gap between the cylinders. This error was most serious at low velocity gradients where the magnitude of the double refraction was small. With the new light source used here, which is described below, accurate observation could be made at gradients for which Δ was approximately 5°. If Δ was less than 5°, the double refraction inherent in the system due to strains in the windows became serious. For the data described here, this residual double refraction is approximately 1°.

The light source employed, which was effectively a point source and thereby greatly reduced the divergence of the beam, was a J-10 Western Union concentrated arc lamp[12] (10 watt), with a zirconium arc, used in conjunction with a simple power supply (resistor, coil and lamp in series with 110V d.c.). A Tesla coil was used to start the lamp. The diameter of the light source was 0.40 mm. and its brightness more than adequate for convenient observation. When the lamp was placed at the focus of a corrected convex lens, the emerging light was essentially parallel over the distance of the optical bench (approximately one meter). A lens mount provided with three-dimensional adjustments enabled the lens setting to be made easily and accurately. In aligning the optical system, it was found advantageous to make the light very slightly convergent so that it was brought to a focus at the exit slit of the cylinders. The observation telescope, instead of being focused at this exit slit, was focused several centimeters below, on the lower face of the analyzing nicol, to separate the residual fringes due to reflected light in the telescope field. This procedure was equivalent to the long optical path method described previously,[10a] but was found to be more precise. A Farrand interference filter was used to isolate a narrow band of green light, with maximum intensity at 552 mμ, from the white light source. The band half-width of the filter was 9 mμ.

To check the alignment of the optical system, extinction angle and double refraction measurements were made on ethyl cinnamate.[9] With these modifications, such a calibration indicated that measurements were reliable at velocity gradients for which Δ was approximately 5° or greater.

It should be mentioned that prolonged rotation of the cylinders caused enough surface denaturation of protein to lead to the production of foam over the windows and a deterioration in the field. Occasionally such disturbances developed rapidly and prevented completion of the run.

(6) If the protein concentration did not exceed 1%, the precipitate settled overnight as a gelatinous mass from which the supernatant liquid could easily be decanted.

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

(8) P. Johnson and E. M. Shooter, *J. Colloid Sci.*, **3**, 539 (1948).

(9) J. T. Edsall, C. G. Gordon, J. W. Mehl, H. Scheinberg and D. W. Mann, *Rev. Sci. Instr.*, **15**, 243 (1944).

(10) (a) J. F. Foster and J. T. Edsall, THIS JOURNAL, **67**, 617 (1945); (b) J. T. Edsall and J. F. Foster, *ibid.*, **70**, 1860 (1948).

(11) H. A. Scheraga, J. T. Edsall and J. O. Gadd, Jr., *J. Chem. Phys.*, **19**, 1101 (1951).

(12) This was obtained by us from the Central Scientific Co., Cambridge, Massachusetts.

Results

Electrophoretic and Ultracentrifugal Analysis. (a) **Cold Insoluble Globulin.**—Electrophoretic analysis of the non-clotting protein (cold insoluble globulin) derived from the dried Fraction I indicated that 90% of it had a mobility of -7×10^{-5} cm.^{2}/sec. volt at 20°, in barbiturate buffer at *p*H 8.6. The remaining 10% was not resolved, but formed a tail to the main peak.

Ultracentrifugal analysis of the non-clotting protein (cold insoluble globulin) showed a less homogeneous product, some 75% of the protein moving with sedimentation constant $s_{20} = 15$ *S*, 13% with $s_{20} = 22$ *S* and 12% with $s_{20} = 8$ *S*. The latter value is practically identical with that of human or bovine fibrinogen.[13] Non-clotting protein derived from fresh Fraction I showed substantially the same behavior. The relative areas of the observed peaks do not necessarily correspond to the relative amounts of the ultracentrifugal components because of the Johnston–Ogston effect,[14] but a detailed critical analysis of the problem does not seem warranted here.

(b) **Fraction I-1 Complex.**—Electrophoresis of the complex under conditions similar to those above revealed three components which remained practically unchanged in relative amount after attempted purification of the complex by reprecipitation. The results of a typical run are shown in Fig. 1, the analysis of the figure being given in Table I.

TABLE I

FRACTION I-1 COMPLEX

Electrophoretic mobilities[a] (6 hr. run; No. 3196) $\mu \times 10^5$ cm.^{2}-volt^{-1}sec.$^{-1}$ in barbiturate buffer, *p*H 8.6, 20°

Ascending			Descending		
−7.4	−6.1	−5.0	−6.6	−5.4	−4.3
(34.4)	(17.4)	(45.3)	(60)	(17.5)	(21)

[a] Relative areas in parentheses.

Comparison of these mobilities with those of non-clotting protein (approximately -7×10^{-5}) and fibrinogen (-3 to -4×10^{-5}) under the same conditions would suggest the identification of the fast peak as non-clotting protein and the slow peak as fibrinogen. However, the considerable difference between the patterns on the ascending and descending sides requires a modification of this view. The middle peak has the same area on both sides and may perhaps be considered a separate component, but protein which appears in the leading boundary on the descending side is partly transferred to the slow boundary on the ascending side.

This behavior is what might be expected from a formation of a reversible complex between the fibrinogen and the non-clotting protein.[15] The formation of the complex, and its dissociation at some of the moving boundaries, lead to modification of both the mobilities and the areas of the observed boundaries, as compared with the values for the separate components without complex formation. The augmentation of the area of the slow component on the ascending side, and of the fast component on the descending side (see Table I) are qualitatively the effects that would be predicted from the theory. The present system is so complex, however—as illustrated, for instance, by the ultracentrifugal analysis discussed below—that a quantitative comparison between theory and experimental results will not be attempted here.

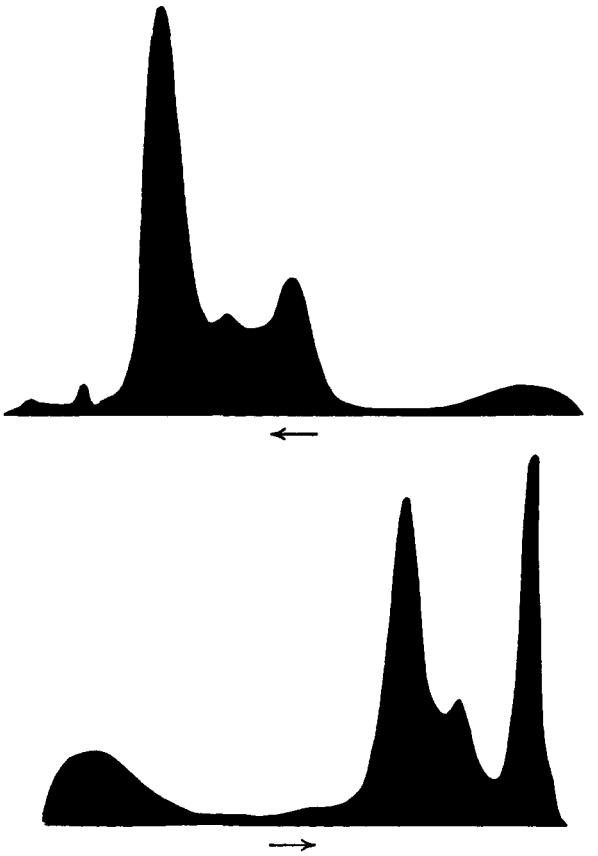

Fig. 1.—Electrophoretic diagrams of Fraction I-1 in barbiturate buffer at 20°, *p*H 8.6: upper diagram, descending boundaries; lower diagram, ascending boundaries.

The ultracentrifugal analysis of the complex gave evidence of polydispersity, and of partial association, since the peak corresponding to fibrinogen was found to be present, but only to the extent of 15% of the total protein, although about 50% could be removed as a clot by treatment with thrombin. One typical preparation analysed as

$$s_{20} = 14\ S\ (63\%)\ s_{20} = 8\ S\ (16\%)\ s_{20} = 11\ S\ (12\%)\ s_{20} = 19\ S\ (8\%)$$

Adsorption or occlusion of non-clotting protein by fibrin formed during the fibrinogen assay cannot account for the high figure for clottable protein. This was demonstrated by clotting synthetic mixtures of fibrinogen and the non-clotting protein with no significant differences, beyond the moder-

(13) S. Shulman, THIS JOURNAL, **75**, 5846 (1953) (bovine fibrinogen); for human fibrinogen, see ref. 16 below.

(14) J. P. Johnston and A. G. Ogston, *Trans. Faraday Soc.*, **42**, 789 (1946); W. F. Harrington and H. K. Schachman, THIS JOURNAL, **75**, 3533 (1953). Where reversible complex formation occurs, as is apparently the case in Fraction I-1 (see below), additional complications in the sedimentation patterns are to be expected, due to dissociation at the boundaries. See G. A. Gilbert, *Discs. Faraday Soc.*, **13**, 159 (1953).

(15) Concerning electrophoresis of complexes, see L. G. Longsworth and D. A. MacInnes, *J. Gen. Physiol.*, **25**, 507 (1942); R. A. Alberty and H. M. Marvin, Jr., *J. Phys. Colloid Chem.*, **54**, 47 (1950); R. V. Rice, M. A. Stahmann and R. A. Alberty, *J. Biol. Chem.*, **209**, 105 (1954); and G. A. Gilbert, *Disc. Faraday Soc.*, **13**, 239 (1953).

ate occlusion effects already described by Morrison,[7] between the fibrin yields of these mixtures and those of pure fibrinogen.

It appears that the clottable protein of Fraction I-1 consists of much more material than can be accounted for by the $s_{20} = 8$ *S* component which is commonly identified with fibrinogen; indeed at least 70% of the clot must be formed from material of higher sedimentation constant. This suggests that the fibrinogen is in the form of a complex or a low polymer in these solutions or that material other than fibrinogen is incorporated in the clot. The Johnston-Ogston effect, and the effects of dissociation of the complex by dilution at the boundaries,[14] must undoubtedly modify the quantitative relations to be inferred from the sedimentation diagrams; but any correction made on the basis of these effects must increase still further the contribution of the components of higher sedimentation constant. Hence the qualitative conclusions drawn above are not likely to be affected.

Viscosity.—Studies on the intrinsic viscosity of the non-clotting protein yielded a limiting value of 0.15 for the limiting ratio $[\ln (\eta/\eta_0)]/c$, extrapolated to zero c (c = concentration in g./100 ml.). The higher value of 0.25 has been reported for fibrinogen, both human[16] and bovine.[13] The value for the Fraction I-1 complex was found to be 0.23.

Double Refraction of Flow.—The results of measurements of birefringence and extinction angle measurements for the non-clotting protein and for Fraction I-1 are summarized in Tables II and III, respectively. $(\Delta/G\eta C)_0$ is the limiting slope of the Δ/C *vs.* $G\eta$ curve at low gradient. Calculated values of the product $\eta\Theta/T$, where Θ is the rotary diffusion constant of the protein, are also given. These are limiting values for low gradient.

The data show that in the concentration range employed here, interaction effects are small except possibly in run No. 1 on the non-clotting protein, there being little distinguishable concentration dependence evident in the other runs. Where it does appear, it is in the expected direction, the recorded values of $\eta\Theta/T$ tending to diminish with increasing concentration. Run No. 7 on the non-clotting protein in 38.3% glycerol gave substantially the same value of $\eta\Theta/T$ as other results in 53.4% glycerol, indicating that the size and shape of the protein are not noticeably affected by such a change in solvent composition.

The data are plotted in Figs. 2–5, inclusive. In the χ *vs.* $G\eta$ graphs the curves are theoretical ones, constructed from the tabulations of Scheraga, Edsall and Gadd[11] using the assumed rotary diffusion coefficients, and assumed percentages of the various components, listed in the legends to the figures.

TABLE II

DOUBLE REFRACTION OF FLOW DATA NON-CLOTTING PROTEIN—20.0°

Runs 1–9 Lederle, Run 10 Massachusetts Biologics Laboratories preparations. Runs 1–7 not corrected for viscosity change on evacuation to remove bubbles. G denotes velocity gradient in sec.$^{-1}$; Δ is double refraction expressed as phase difference at 540–550 m.; θ is rotary diffusion constant.

Run no.	Solvent, wt. % glycerol	Protein, g., %	Viscosity of solvent η, poise	Range of $G\eta$	$\left(\frac{\Delta}{G\eta C}\right)_0$	$\eta\Theta/T$
1	53.4	0.65	0.073	277–692	..	0.68
2	53.4	.29	.073	277–1382	0.14	.79
3	53.4	.29	.073	277–1382	.12	.85
4	53.4	.15	.073	277–1382	.15	.85
5	53.4	.073	.073	277–1382	.13	.82
6	53.4	.15	.073	277–923	..	.90
7	38.3	.29	.035	132–660	.12	1.02
8	53.4	.34	.073	277–1382	.13	0.88
9	53.4	.17	.073	277–1382	.11	0.97
10	53.5	.36	.074	278–696	.14	0.83

TABLE III

DOUBLE REFRACTION OF FLOW DATA FRACTION I-1—20.0°

Runs 1 and 2 not corrected for viscosity change of solvent on evacuation to remove bubbles.

Run no.	Solvent, wt. % glycerol	Protein, g., %	Viscosity of solvent η, poise	Range of $G\eta$	$\left(\frac{\Delta}{G\eta C}\right)_0$	$\eta\Theta/T$
1	53.4	0.29	0.073	277–1382	0.16	0.55
2	53.4	.15	.073	277–1382	.13	.61
3	53.6	.31	.074	280–1400	.15	.59
4	54.4	.21	.077	293–1170	.13	.68
5	53.8	.090	.075	283–1415	.13	.68

Discussion

A number of plasma proteins have been described —generally obtained from pathological plasmas— which were characterized by insolubility at very low temperatures, and a high temperature coefficient of solubility. In the terminology of Lerner and Watson[17] they are cryoglobulins; and the physical properties of those which have been studied differ greatly from one individual protein to another. A useful comparative tabulation of the data reported by various authors has been given by Abrams, Cohen and Meyer,[18] who have also reported a particularly thorough study of a cryoglobulin from the serum and lymph nodes of a case of lymphosarcoma. The sedimentation constants for various reported cryoglobulins range from s_{20} = 7 to 23 *S*, and the estimated molecular weights from near 200,000 to more than 1,000,000.

Unlike the proteins reported by other authors, the non-clotting cold insoluble globulin, studied in this paper and previously,[3] was obtained by fractionation of pooled plasmas from many different individuals. Since the blood from which the pooled plasma was derived was taken from healthy donors, anyone who was obviously ill being rejected, it seems unlikely that the cold insoluble globulin studied by us is related to proteins derived from patients suffering from grave diseases. However, it should be noted that the sedimentation constant (s_{20} = 14 to 15 *S*) found for the principal component of the non-clotting protein in our studies is quite close to the value of 16 *S* reported by Abrams, Cohen and Meyer[18] for their protein from a lymphosarcoma patient. It has already been pointed out, in an earlier discussion,[3] that the non-clotting cold

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

(17) A. B. Lerner and C. J. Watson, *Am. J. Med. Sci.*, **214**, 410, 416 (1947).

(18) A. Abrams, P. P. Cohen and O. O. Meyer, *J. Biol. Chem.*, **181**, 237 (1949).

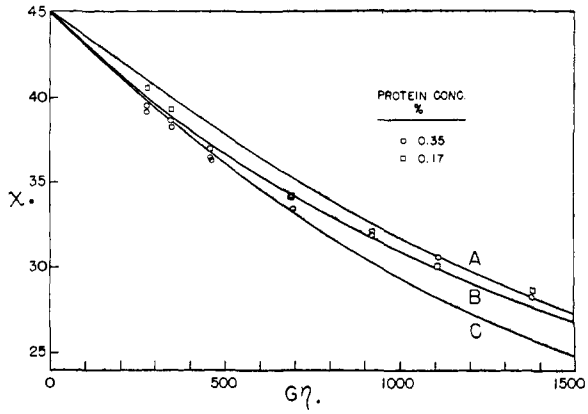

Fig. 2.—The extinction angle χ as a function of $G\eta$ for the non-clotting protein. The symbols are experimental data while the curves are theoretical ones. Curve A is calculated for particles with $\eta\Theta = 312$, curve B is calculated for a mixture of particles 98% of which have $\eta\Theta = 312$ and 2% have $\eta\Theta = 53$. Curve C is for particles with $\eta\Theta = 252$.

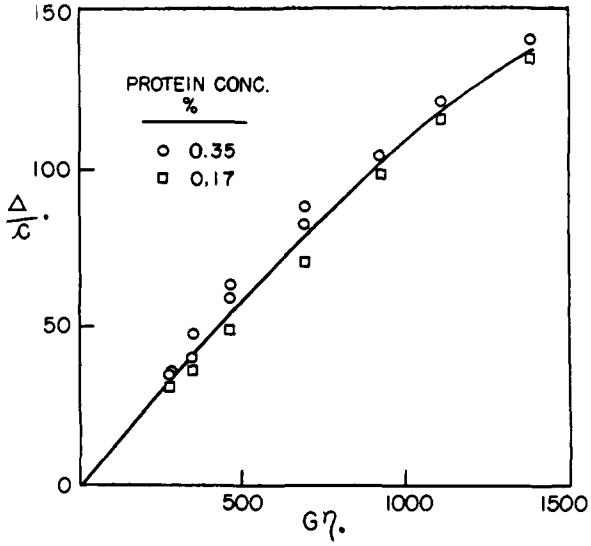

Fig. 3.—Birefringence data for the non-clotting protein.

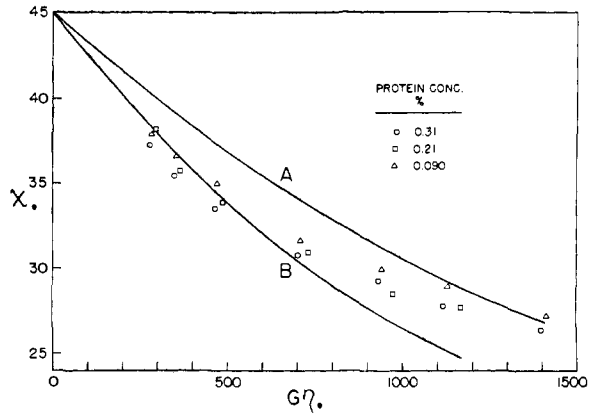

Fig. 4.—χ as a function of $G\eta$; data for Fraction I-1. The experimental data lie within the range indicated by the theoretical curve A, for particles with $\eta\Theta = 282$, and the curve B, for particles with $\eta\Theta = 195$.

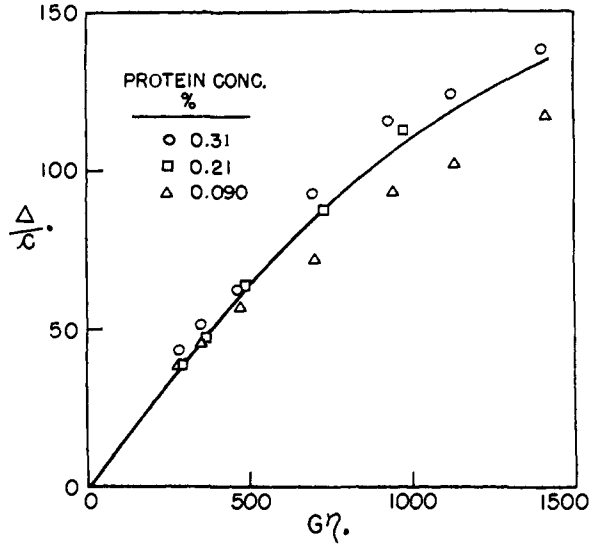

Fig. 5.—Birefringence data for Fraction I-1.

insoluble globulin may well be present in only a relatively small number of donors from whom the plasma was derived. Further studies on the individual plasmas of a wide variety of normal individuals will be required to clarify the matter further.

The cold insoluble globulin shows well marked double refraction of flow, comparable to that of fibrinogen. Its intrinsic viscosity is higher than that of most plasma proteins and the sedimentation constant of the principal component is considerably higher than that of all but a very small fraction of plasma proteins. These data give a strong indication that the molecule is large and of relatively high asymmetry. The product $\eta\Theta/T$ derived from the measurements of extinction angle is approximately 0.8, as compared with an average value in dilute solution of approximately 1.1 for human fibrinogen.[4] If, as in previous calculations,[4,10] we assume the molecule to be a hydrated ellipsoid of revolution and calculate the axial ratio from the intrinsic viscosity, the ellipsoid derived from these calculations is approximately 700 Å. long and about twice as large in cross-section at the center as the equivalent ellipsoid calculated for the fibrinogen molecule. If this model is assumed, the length is approximately the same as that of fibrinogen and the molecular mass approximately twice as great. This might suggest that the non-clotting protein is a dimer composed of two fibrinogen molecules joined together side by side with their long axes parallel, in such a way that the dimer is no longer susceptible to the action of thrombin. However, we believe that all such models should be viewed with the greatest reserve at the present time—on the one hand, because the non-clotting protein has been shown to consist of more than one component in sedimentation and electrophoresis; on the other hand, because of recent modifications in our underlying conceptions of the inferences that may be drawn from experimental information on intrinsic viscosities, rotary diffusion constants, sedimentation constants and related quantities.[19]

BOSTON, MASS.

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