The Hyperviscosity Syndrome

I. IN IgG MYELOMA. THE ROLE OF PROTEIN CONCENTRATION AND MOLECULAR SHAPE

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ABSTRACT The hyperviscosity syndrome is an uncommon complication in IgG myeloma. Its occurrence has been ascribed to the presence in the serum of high molecular weight polymers of the IgG proteins. Three patients with IgG myeloma and the clinical hyperviscosity syndrome were investigated, none of whom had IgG polymers in the serum by analytical ultracentrifugation. Relative serum viscosity in these patients ranged from 10 to 17.4 (normal 1.4–1.8). The total serum proteins ranged from 14 to 19 g/100 ml of which 10 to 17 g/100 ml was IgG globulin. Physicochemical studies of two of the isolated myeloma proteins indicated that they were of normal molecular weight (near 158,000 and 162,000). Protein Ca had a normal molecular radius (52.2 A) and configuration, (intrinsic viscosity of 5.5 cc/g, frictional ratio 1.48), but was present in very high concentration in the serum. Protein Pur had an increased molecular radius (58.2 A) and was asymmetrical (intrinsic viscosity 10.2 cc/g, frictional ratio 1.63). These results indicate that the concentration and molecular configuration of the myeloma protein are important determinants of the presence or absence of the hyperviscosity syndrome.

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INTRODUCTION

The hyperviscosity syndrome (mucous membrane bleeding, tinnitus, central nervous system abnormalities, blurred vision, dilated retinal veins, retinal hemorrhages, and occasional gastrointestinal hemorrhage associated with a marked increase in serum viscosity) is a common complication of Waldenström's macroglobulinemia. These manifestations have been attributed to the presence in the serum of such patients of large quantities of asymmetrical molecules of high molecular weight. Although unusual, the hyperviscosity syndrome may also occur in multiple myeloma of the IgG type. In two such cases recently reported by Smith, Kochwa, and Wasserman (1) the increased viscosity of the serum was attributed to the presence of circulating aggregates of IgG globulins.

This report describes an investigation of three patients with IgG myeloma associated with mild bleeding, central nervous system abnormalities, and hyperviscosity of the serum. Detailed studies of the myeloma proteins were undertaken when analytical ultracentrifugation failed to reveal evidence of high molecular weight polymers in the patient's sera. For comparative purposes, a fourth patient with IgG myeloma associated with a mild increase in relative serum viscosity but no clinical manifestations of the hyperviscosity syndrome was also studied.

In these investigations, special attention was given to the physical properties of the isolated myeloma proteins and in particular their intrinsic viscosity. Intrinsic viscosity, a value derived from extrapolation of the

specific viscosity of a protein in solution at a given concentration to infinite dilution, is related to the size and shape of the protein and, under ideal conditions, is independent of concentration and molecular interaction (2).

METHODS

Total serum proteins were determined by the biuret method. Serum electrophoresis was performed at pH 8.6 in 0.1 M barbital buffer on cellulose acetate.

Myeloma proteins were isolated by sequential precipitation with sodium sulfate at 18 and 14% saturation (3), followed by zone electrophoresis on starch block (4) and gel filtration on Sephadex G-200 columns, 2.5 × 100 cm. The columns were equilibrated with 0.02 M Tris-HCl–0.15 M NaCl buffer, pH 8.0. The sera and isolated proteins were characterized by immunoelectrophoresis (5) and by Ouchterlony analysis (6). Quantitation of the myeloma components on cellulose acetate was performed using an analytical densitometer in standard fashion.

Analytical ultracentrifugation was performed in a Spinco model E ultracentrifuge at 20° and 37°C at 59,780 rpm. These determinations were carried out on serum samples undiluted and diluted 1:4 in normal saline, and on serial dilutions of the isolated proteins in unbuffered 0.15 saline, pH 6.5, or potassium phosphate buffer ionic strength 0.2, pH 7.4. Sedimentation constants were calculated at infinite dilution from plots of S against concentration.

Diffusion constants were calculated by first determining the Stokes radius by gel diffusion chromatography on a Sephadex G-200 column 2.5 × 100 cm equilibrated with 0.02 M Tris-HCl–0.13 M NaCl buffer, pH 8.0, calibrated with bovine serum albumin by the method of Ackers (7). Proteins were studied at a concentration of 20 mg/ml, values were corrected for the viscosity of solvents, and temperature to standard conditions.

The partial specific volume of protein Pur was measured at 20°C in a 5 ml Nicol pycnometer. Determinations were made at a protein concentration of 10 mg/ml in potassium phosphate buffer 0.2 ionic strength, pH 7.4. Protein concentration was determined by evaporation to dryness of weighed samples at 70°C over P_2O_5 under a vacuum.

Molecular weights were calculated from the sedimentation and diffusion constants (8), or by the method of Yphantis (9). Frictional ratios were calculated by the equation

$$\frac{f}{f_0} = \left[\frac{1 - \bar{V}\rho}{(D^\circ_{20,w})^2 s^\circ_{20,w}} \bar{V}\right]^{\frac{1}{3}} \cdot 10^{-8}$$

Relative serum viscosity was determined at room temperature and at 37°C with an Ostwald capillary viscometer with a solvent descent time of greater than 80 sec; distilled water was used as the reference solvent. Protein quantitation for intrinsic viscosity determinations was performed as described by Lowry, Rosebrough, Farr, and Randall (10), by use of a standard curve constructed with Cohn fraction II (IgG). The intrinsic viscosity of the isolated proteins in 0.15 M sodium chloride, pH 6.5 was determined in the Ostwald viscometer (total capacity 5 ml), with 0.15 M saline as the reference solvent. Determinations were performed in a controlled temperature bath at 25° ±0.05°C. Density measurements were done with a 5 cc Nicol pycnometer in the same water bath.

TABLE I

Initial Characterization of Sera and Proteins of Four Patients with IgG Myeloma

Patient	Relative serum viscosity	Serum total protein	IgG	Antigenic type: Heavy chain	Antigenic type: Light chain
		g/100 ml	*g/100 ml*		
Ca	17.4	19.0	17.0	γG_1	kappa
Pur	10.9	16.9	14.1	γG_1	lambda
Hu	10.0	14.0	12.0	γG_3	lambda
Cl	3.6	16.8	14.0	γG_1	lambda

Viscosity values were derived as follows. Relative viscosity was calculated as $\eta \text{ rel} = \frac{tp}{t_0 p_0}$, were t and p are the descent. time (p = rho) and density of the protein solution, and t_0 and p_0 are the descent time and density of the saline solvent. The specific viscosity was calculated as $\eta \text{ sp} = \frac{\eta - \eta_0}{\eta_0}$ where η is viscosity of the solution and η_0 the viscosity of the solvent. The intrinsic viscosity (η) was then determined from the intercept of a plot of η sp/C against C, where C equals the protein concentration in grams per cubic centimeter and is extrapolated to infinite dilution.

Human IgG was prepared from Cohn fraction II (American Red Cross or The Cutter Laboratories, Berkeley, Calif.) by diethylaminoethyl (DEAE) chromatography (11), followed by gel filtration as above to remove 19S and 10S aggregates. The 7S IgG was concentrated by pervaporation. To obtain concentrations of protein near 150 mg/ml for viscosity studies, solutions of 100 mg/ml were placed in dialysis bags and exposed to dry Sephadex G-200. All attempts to achieve such concentrations by lyophilization and reconstitution uniformly resulted in 10S aggregate formation.

For hexose determinations, the proteins were isolated by DEAE-cellulose chromatography (11). Total hexose content was measured by the orcinol method (12).

RESULTS

Results of initial studies on the sera and myeloma proteins of the four patients are shown in Table I. In the three patients with the hyperviscosity syndrome (Ca, Pur, and Hu), the relative serum viscosities ranged from 17.4 to 10.0. In the patient with no clinical manifestations of the hyperviscosity syndrome (Cl), the relative serum viscosity was 3.6. The total serum protein concentration ranged from 14 to 19 g/100 ml, of which 12–17 g was myeloma protein. In all four cases the abnormal protein was IgG globulin. On further subtyping, three proteins were γG1 and one was γG3; one contained kappa light chains and three contained lambda light chains.

The four sera, undiluted and diluted 1:4 with normal saline, were examined by analytical ultracentrifugation at 20° and 37°C. Under these conditions, none of the four contained significant amounts of high molecular

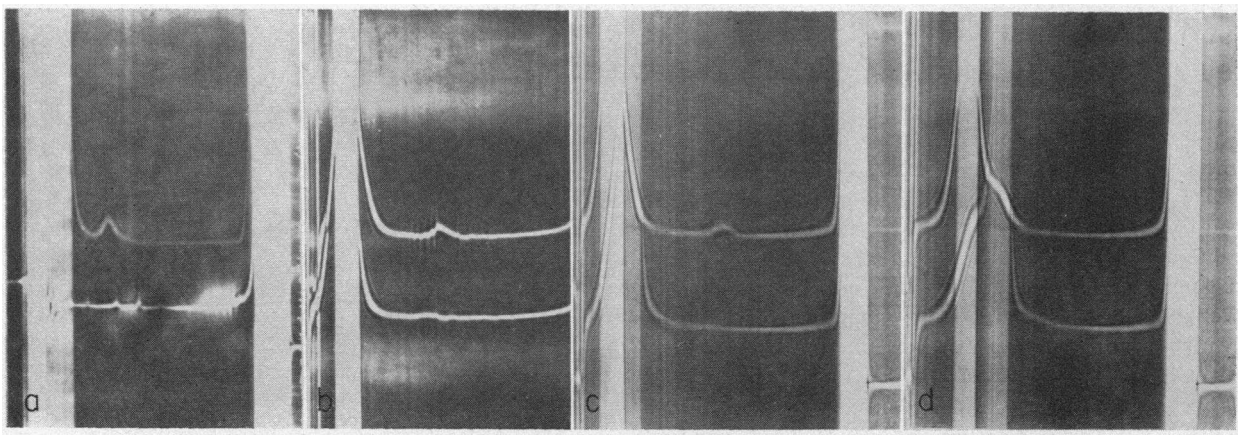

FIGURE 1 Analytical ultracentrifuge studies of serum from patients with myeloma and hyperviscosity syndrome. (*a*) Normal human serum (top) and Pur serum (bottom) both undiluted at 8 min after reaching 59,780 rpm at 37°C. Note the absence of high molecular weight polymers, even the normal 19S peak in serum Pur. The small deflections are concentration artifacts. Sedimentation is to the right. (*b*) Normal human serum (top) and serum Ca (bottom) undiluted, 8 min after reaching speed. (*c*) Normal human serum (top) and serum Ca (bottom) diluted 1:4, 16 min after reaching speed. (*d*) Normal human serum (top) and serum Ca (bottom) diluted 1:4, 32 min after reaching speed. Again note absence of polymers in serum Ca.

weight polymers. The results obtained with serum Pur and Ca are shown in Fig. 1.

The physicochemical properties of the isolated γG1 myeloma proteins are shown in Table II. During the isolation procedures, the γG3 protein of patient Hu consistently disintegrated into its Fab fraction and peptides, precluding further characterization. By ultracentrifugal analysis at 20°C, the sedimentation coefficients of the three γG1 proteins were in the range of 6.30–6.40. In plots of the sedimentation constants against concentration (Fig. 2), no marked concentration dependency was demonstrable.

Studies to determine the radius of each protein and hence its diffusion constant were carried out by gel filtration through a calibrated Sephadex G-200 column (see Methods). The elution volume of each protein was determined. Protein Pur eluted 20 ml before, protein Cl eluted 6 ml after, and protein Ca eluted with normal human IgG. The effective radius of each protein was calculated to be 58.2, 49.9, and 52.2 A, respectively. By use of diffusion coefficients derived from these values and sedimentation coefficients obtained above, we calculated molecular weights. These ranged from 144,400 for protein Cl, and 158,000 for protein Ca to 163,000 for protein Pur. The molecular weight for protein Pur was also determined by equilibrium ultracentrifugation and by this method it was 161,700. Thus the proteins which were obtained from these patients with hyperviscosity

TABLE II

Physicochemical Characteristics of Three IgG Myeloma Proteins

Protein	Sedimentation coefficient, $s°_{20,w}$	Diffusion constant, $D°_{20,w}$	Intrinsic viscosity	Frictional ratio, f/fo	Radius	Hexose content	Mol wt
			cc/g		*A*	%	
Pooled IgG (Cohn fraction II)	6.70	4.1	5.5	1.48	52.2	1.1	158,000*
Ca IgG	6.38	4.1	5.5	1.48	52.2	0.8	158,000*
Pur IgG	6.30	3.68	10.1	1.63	58.2	0.8	163,800* 161,700‡
Cl IgG	6.40	4.26	3.7	1.43	49.9	0.6	144,400*

Partial specific volume ($\bar{V}$) for protein Pur was 0.743. Values of 0.740 were assumed for the other proteins.

* Molecular weight by sedimentation and diffusion.

‡ Molecular weight by equilibrium ultracentrifugation.

syndrome were not significantly larger than the usual values obtained in study of pooled human IgG.

The results of intrinsic viscosity determinations at 25°C are shown in Fig. 3 and Table II. The value of 5.5 cc/g for pooled normal IgG (Cohn fraction II) is in good agreement with the data of Miller and Metzger (13). An identical value was obtained for myeloma protein Ca. Myeloma protein Pur had a markedly increased value of 10.1 cc/g, comparable to the intrinsic viscosity of gamma macroglobulin (13). In contrast, the abnormal protein of patient Cl gave a value of only 3.7 cc/g, identical with the value reported for human serum albumin (2).

The frictional ratio of each protein was calculated. Values of 1.48 were determined for normal IgG and protein Ca while values of protein Pur and Cl were 1.63 and 1.43, respectively.

The relative viscosity of high concentration solutions of gamma globulin was investigated. Solutions of aggregrate free human IgG ranging from 148 mg/ml to 80 mg/ml were studied. The results are presented in Fig. 4. It can be seen that high concentrations give relative viscosity values within the range seen in patients with hyperviscosity syndrome.

Carbohydrate, because of its hydrophilic properties, may influence the configuration of protein molecules (2). The total hexose content of the three IgG proteins, however, did not exceed 1% (Table II).

DISCUSSION

Relative serum viscosity, the ratio between the descent time of a solvent (usually distilled water or saline) and the descent time of the test serum as determined in a viscometer, is a simple but useful measurement in patients with dysproteinemias. The absolute level and the rate of change provide information on the progress of the disease in both treated and untreated patients.

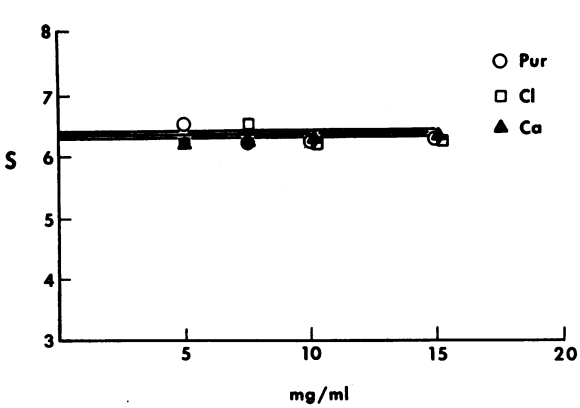

FIGURE 2 Plot of sedimentation constants (S) against concentration of isolated myeloma proteins from patients Ca, Pur, and Cl.

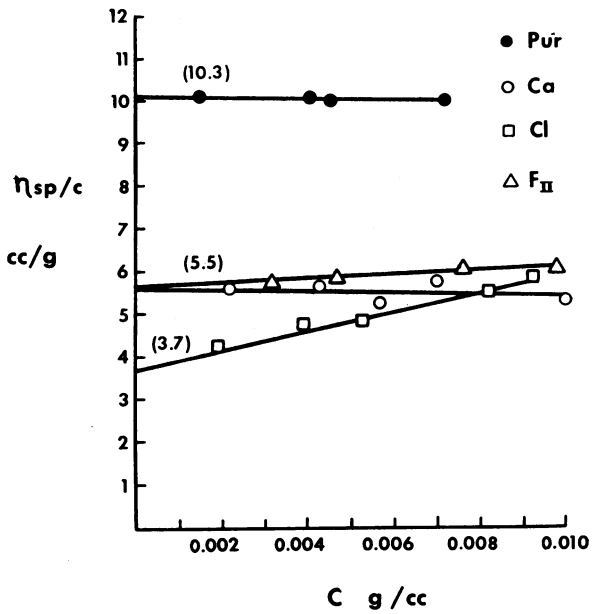

FIGURE 3 Plot of specific viscosity divided by concentration ($\eta sp/C$) against concentration (C) to obtain the intrinsic viscosities of myeloma proteins Ca, Pur, and Cl and normal IgG at 25°C.

Normal sera average approximately 1.5 relative units (14). In patients with increased immunoglobulin levels, relative serum viscosity usually is slightly increased. In IgG myeloma it ranges from 2 to 4, and in IgA myeloma the values may be higher, particularly if high molecular weight polymers are present. In patients with Waldenström's macroglobulinemia, relative serum viscosity ranges from 2 to 40. The hyperviscosity syndrome usually does not occur if relative serum viscosity is less than 6; in general, hemorrhagic and (or) visual symptoms first appear when the values are in the range of

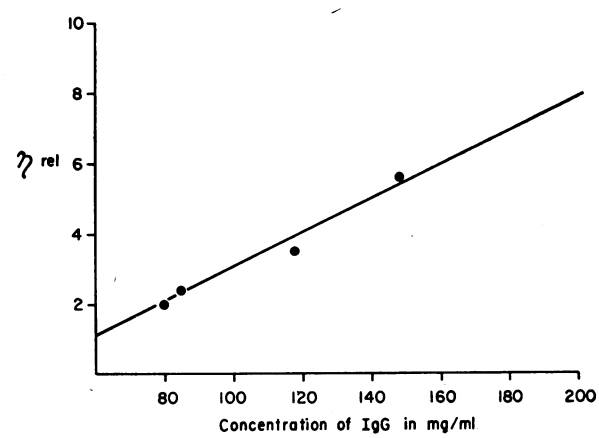

FIGURE 4 Plot of relative viscosity values obtained with various concentrations of normal human IgG.

8–10. The level at which symptoms appear, however, varies considerably from patient to patient, although it is apparently constant for the individual patient.

Serum or plasma viscosity is determined by the physical attributes of the constituent proteins, including total concentration and the size, shape, and flexibility of the molecules (15). In recent years hydrodynamic studies have provided information on the configuration of the IgG molecule. It appears to be a globular but slightly extended molecule, devoid the alpha helix and probably in the beta configuration (16, 17). Noelken, Nelson, Buckley, and Tanford (16) postulated a flexible Y-shaped molecule with the Fc piece forming the stem of the Y and the Fab fragments forming the arms. Data obtained by electron microscopy (18, 19) have confirmed the general shape of the IgG molecule and indicate that the Fab arms have a wide range of flexibility.

Of the physical properties of proteins that determine serum viscosity, only molecular size has been adequately documented in the devlopment of the hyperviscosity syndrome. In the two cases of IgG myeloma described by Smith, Kochwa, and Wasserman (1) the presence of high molecular weight IgG polymers in the serum created a hyperviscous state identical with that seen in macroglobulinemia. Subsequent physicochemical studies on the myeloma protein of one of the two patients showed a sedimentation constant of 13.2S, a molecular weight of about 808,000, and an intrinsic viscosity of 9.5 cc/g (20). In two subsequent reports of the hyperviscosity syndrome in IgG myeloma, aggregation of the 7S myeloma globulin could not be demonstrated (21, 22).

The data obtained in the present study indicate that abnormalities in IgG concentration and in molecular shape may also result in the hyperviscosity syndrome. The myeloma protein of patient Ca had normal sedimentation and diffusion constants, hence a molecular weight similar to normal IgG. Further, the intrinsic viscosity, effective radius, and frictional ratio were the same as normal human IgG. The protein, however, was present in the patients' serum high concentrations (17 g/100 ml). Normal IgG, when present in such concentrations and when added to the value of normal human serum (1.4–1.8), is clearly in the range of patients with hyperviscosity syndrome. Patient Ca had a value exceeding that obtained with similar concentrations of normal IgG. This could be due to interaction between the myeloma protein and other serum proteins.. If this occurred it did not result in visible aggregate formation as this would have been seen in the analytical ultracentrifuge studies. It appears reasonable that high concentrations of myeloma proteins or of normal IgG can lead to the hyperviscosity syndrome. There may be additional factors or subtle configuration changes which cannot be detected by the current methodology.

In the case of protein Pur, the IgG protein also had a normal sedimentation and molecular weight, but the intrinsic viscosity and effective radius and frictional ratio in the absence of high molecular weight polymers indicate a markedly asymmetrical molecule. Inherent polypeptide configuration or changes in shape due to the water of hydration might account for the asymmetry of the molecule, although neither possibility can be evaluated from the present data.

Somer (15), in his extensive review of serum viscosity in dysproteinemic states, noted a linear relationship between protein concentration and relative serum viscosity in such patients; this finding indicated that a high concentration of protein alone can result in the hyperviscosity syndrome. The linear correlations, however, were obtained only after sera with extremely high viscosities had been excluded from the data. The hyperviscosity of the excluded sera probably was attributable to asymmetry of the protein molecules, as in the case of patient Pur. The intrinsic viscosity established for the myeloma protein of patient Cl, who had no clinical manifestation of hyperviscosity, strengthens this hypothesis. This protein had a sedimentation rate of 6.4S, an effective radius of 49.9, and a molecular weight of 144,400. The assumption that this protein is a compact molecule would explain why this patient, despite the high protein content, showed only a slight increase in relative viscosity.

The presence of γG1 and γG3 proteins, as well as kappa and lambda light chains, indicates that the hyperviscous properties are not limited to a single subclass of IgG.

There appears to be then at least three causes for the hyperviscosity syndrome in patients with IgG multiple myeloma. They are: (*a*) the presence of aggregates of the myeloma IgG, which is readily recognized in the analytical ultracentrifuge even upon dilution of the serum (*b*) the presence of large quantities of IgG protein (this form may require additional undefined factors), and (*c*) the molecular shape of the myeloma protein.

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