

CHROMATOGRAPHIC PROPERTIES OF GAMMA GLOBULIN: BEHAVIOR OF SERUM GAMMA MACROGLOBULINS *

By JOSEPH LOSPALLUTO,† JULIO CEGORIANSKY,‡ ARTHUR LEWIS § AND
MORRIS ZIFF

(From the Departments of Medicine (Rheumatic Diseases Group) and Biochemistry, University of Texas Southwestern Medical School, Dallas, Texas)

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The cellulose ion exchangers (1) have proved to be useful chromatographic media for the serum proteins. In the fractionation of normal human serum on the anion exchanger, diethylaminoethyl (DEAE) cellulose, application of buffer gradients of increasing concentration and decreasing pH results in the appearance of components in the effluent fluid in order of increasing electrophoretic mobility (2), indicating that ion exchange is the predominant mechanism involved. In the case of the gamma globulin component, some subfractionation was demonstrated in terms of progressively increasing electrophoretic mobility of column effluents of this fraction. Little attention has been directed, however, to the chromatographic properties of the gamma globulins of high molecular weight, 19S or greater, which are found in large quantities in certain pathological sera, and also in small quantities in normal human sera (3).

In the course of anion exchange fractionation of sera from patients with rheumatoid arthritis, the "rheumatoid factor" (4-6), a gamma globulin constituent of high molecular weight (7), appeared in column effluents far removed from the bulk of the gamma globulin (8). This observation indicated that the chromatographic behavior of certain gamma globulins, and possibly of other serum proteins, on DEAE cellulose is governed not only by ion exchange but also by other factors which depend in part upon molecular size. Our attention was therefore directed to sera known to

contain large amounts of abnormal gamma globulin constituents. The results obtained have been previously presented in abstract form (9).

MATERIALS AND METHODS

DEAE cellulose¹ (1 g for each 30 to 40 mg of protein fractionated) was equilibrated with 0.01 M phosphate buffer at pH 7. The equilibrated exchanger was then poured onto columns (2.5 × 15 cm) equipped with fritted glass filters, and packed under mild air pressure. Before chromatography, sera were diluted with an equal volume of distilled water and dialyzed for two days against 3 to 6 L of 0.01 M phosphate buffer at pH 8.5 to 9. In one instance, the euglobulin precipitate from the serum of a patient with macroglobulinemia was fractionated. This was prepared by 15-fold dilution of 4 ml of the serum with cold water. The precipitate formed was harvested by centrifugation, washed four times with cold water, dissolved in 0.01 M phosphate buffer at pH 8.6 and chromatographed.

Chromatographic fractionation was carried out at room temperature using relatively rapid flow rates of 1 to 2 ml per minute as described previously (8). The protein content of each effluent fraction was determined by measurement of ultraviolet absorption at 280 mμ in the Beckman spectrophotometer. Readings were taken as fractions and were collected in order to follow the course of separation and to determine when a change in the eluting buffer was required. At the end of each chromatographic experiment, samples of each peak were concentrated 10- to 100-fold by ultrafiltration through Mies collodion bags² to a final protein concentration of approximately 1 per cent. Paper electrophoretic analysis was carried out in a Spinco (Durrum) apparatus at pH 8.6 ($r/2 = 0.075$) for 16 hours at 120 v and 4 ma. After staining with bromphenol blue, paper strips were analyzed in a densitometer.³ Ultracentrifugal analyses were carried out in the Spinco Model E analytical ultracentrifuge at 59,780 rpm. The sedimentation constants reported were not corrected for concentration dependence.

Precipitation and elution of the rheumatoid factor were carried out as previously described (8).

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† Fellow of the Helen Hay Whitney Foundation.

‡ Present address: Antirheumatic Center, University of Buenos Aires Medical School, Buenos Aires, Argentina.

§ Participated in preliminary experiments at New York University—Bellevue Medical Center, New York, N. Y.

¹ Brown and Co., 500 Fifth Avenue, New York, N. Y.

² Schleicher and Schuell, Keene, N. H.

³ Analytrol, Spinco, Palo Alto, Calif.

TABLE I
Patients with hyperglobulinemia

Patient	Diagnosis	Total serum protein	Serum albumin	Serum globulin	Serum gamma globulin
		g %	g %	g %	g %
M. S.	Liver disease	17	2.55	14.45	10.71
J. C.	Primary amyloidosis	8.2	2.59	5.65	3.36
M. K.	Multiple myeloma	17	2.55	14.45	10.71
C. R.	Multiple myeloma	8.6	3.0	5.60	5.56*
B. B.	Macroglobulinemia	11.2	3.57	7.64	5.75
S. K.	Macroglobulinemia	10.9	2.08	8.80	7.35

* Represents per cent beta globulin; gamma globulin content was 0.05 g per cent.

Rabbit antisera against normal human 7S and 19S gamma globulins (10) were used in capillary precipitation tests. The rabbit antiserum to 19S gamma globulin had been absorbed with human 7S gamma globulin prior to use. Both antisera were kindly provided by Dr. Edward C. Franklin of the Rockefeller Institute.

The sera⁴ investigated were obtained from one normal individual and from patients with the following: lymphosarcoma, 1; liver disease [an atypical case of the syndrome described by Bearn, Kunkel and Slater (11)], 1; primary amyloidosis, 1; multiple myeloma, 2; and macroglobulinemia (Waldenström syndrome), 2. Pertinent laboratory data on the patients enumerated are given in Table I.

RESULTS

Three types of chromatographic pattern were obtained with the sera investigated. In one type, represented by a normal serum and sera of patients with ordinary hyperglobulinemia, substantially all of the gamma globulin was found in the first chromatographic peak eluted at pH 7. A second type, represented by a serum from a patient with beta myeloma, was characterized by elevation of an intermediate peak eluted at pH 6. In the third type, which was obtained with sera from patients with macroglobulinemia, the major peak, composed mainly of macroglobulins, was eluted at pH 5 at the tail end of the chromatograms.

The first type of pattern is illustrated by sera obtained from patients with primary amyloidosis (Figure 1) and multiple myeloma (Figure 2). In both instances, the serum gamma globulin con-

centration was high (Table I) and was eluted almost completely in the pH 7 peak. Little or no gamma globulin was found in subsequent chromatographic fractions (Figures 1 and 2, Table II). The same gamma globulin distribution was observed with a normal serum⁵ and with the sera of one patient with lymphosarcoma and another with liver disease of the type described by Bearn, Kunkel and Slater (11). In all of these, the major portion of the gamma globulin was eluted in the pH 7 peak and was entirely of the 7S variety. The small amount of gamma globulin found

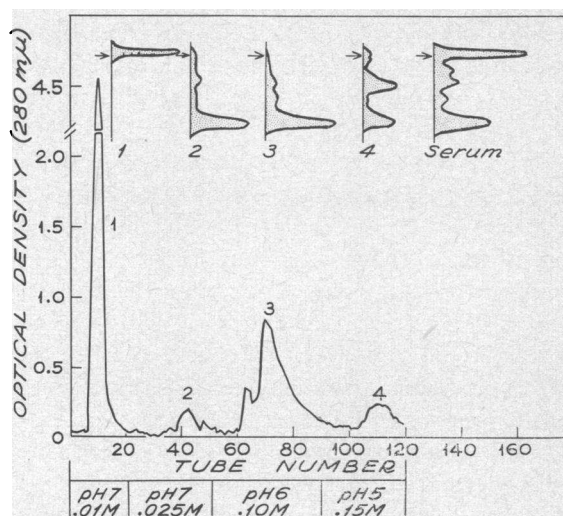


FIG. 1. CHROMATOGRAPHIC PATTERN OF SERUM FROM A PATIENT (J.C.) WITH PRIMARY AMYLOIDOSIS, AND PAPER ELECTROPHORETIC PATTERNS OF CHROMATOGRAPHIC FRACTIONS. Three ml serum on 6 g DEAE cellulose; 5 ml fractions.

⁴ The sera of the patients with multiple myeloma (M.K.), lymphosarcoma (V.H.), and one with macroglobulinemia (S.K.) were kindly provided by Dr. Elliott Osserman of Columbia University. The other macroglobulinemia serum (B.B.) was obtained through the courtesy of Dr. Gerald Weissman of the Mt. Sinai Hospital, N. Y.

⁵ Following submission of the manuscript of this paper, it was reported by Fahey and Horbett (12) that gamma globulin from normal individuals may be fractionated on DEAE cellulose into light and heavy components.

TABLE II
Ultracentrifugal analysis of chromatographic fractions containing gamma globulin, normal serum, and hyperglobulinemia sera

Patient	Diagnosis	Serum	Elution		Effluent volume	Sedimentation constant of gamma globulins S_{20W}	% Total protein of peak
			pH	Molarity			
K. S.	Normal subject	25 ml	7	0.01	400 ml	6.6*	100
V. H.	Lymphosarcoma	2	7	0.01	100	6.6	100
M. S.	Liver disease	2	7	0.01	80	6.2	100
J. C.	Primary amyloidosis	3	7	0.01	80	7.2	100
M. K.	Multiple myeloma, γ type	2	5	0.15	100	17	4
C. R.	Multiple myeloma, β type	2	7	0.01	60	6.2	100
			6†	0.10	140	6.3	62
						9.1	11
						12	4
						14	3
			5†	0.15	100	6.2	12
						8.6	55
						10	23
						12	8
						14	2

* An 18S gamma globulin component was noted in the fraction eluted at pH 5 (0.15M) but was too small to be measured.

† The group of components eluted at this pH had the electrophoretic mobility of beta globulin.

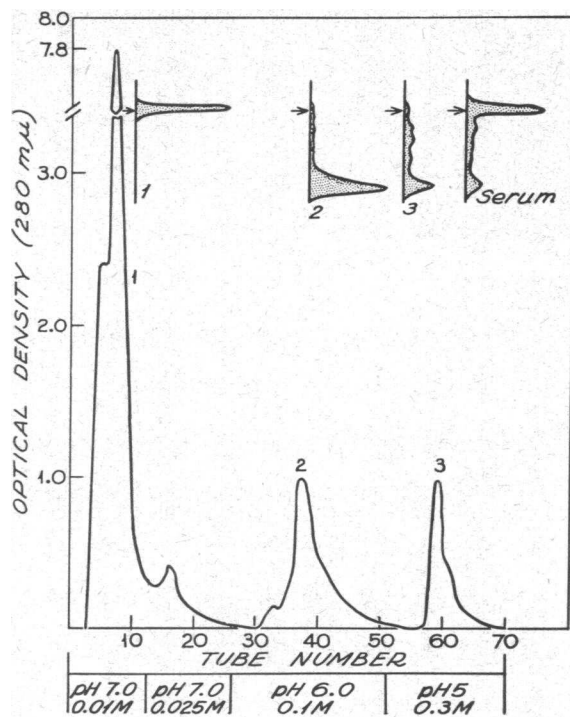


FIG. 2. CHROMATOGRAPHIC PATTERN OF MULTIPLE MYELOMA SERUM (M.K.) OF GAMMA TYPE, AND PAPER ELECTROPHORETIC PATTERNS OF CHROMATOGRAPHIC FRACTIONS. Two ml serum on 4 g DEAE cellulose; 5 ml fractions.

in two instances in the pH 5 peak was of the 19S type.

The second type of chromatographic pattern was obtained with the serum of a patient with beta multiple myeloma (Figure 3). The distinguishing feature of this pattern is the great elevation in the pH 6 peak. In this instance, the pH 7 (0.01) peak was relatively small and con-

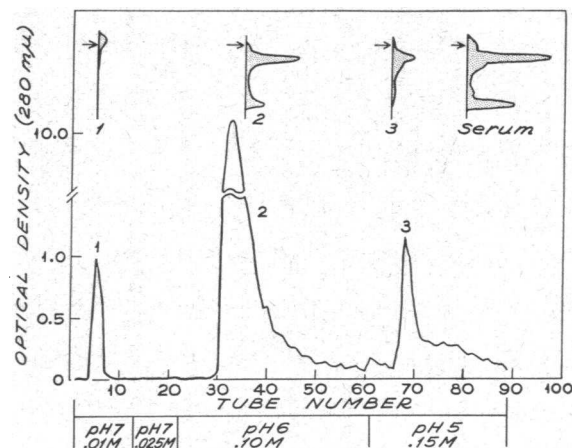


FIG. 3. CHROMATOGRAPHIC PATTERN OF MULTIPLE MYELOMA SERUM (C.R.) OF BETA TYPE, AND PAPER ELECTROPHORETIC PATTERNS OF CHROMATOGRAPHIC FRACTIONS. Two ml serum on 6 g DEAE cellulose; 5 ml fractions.

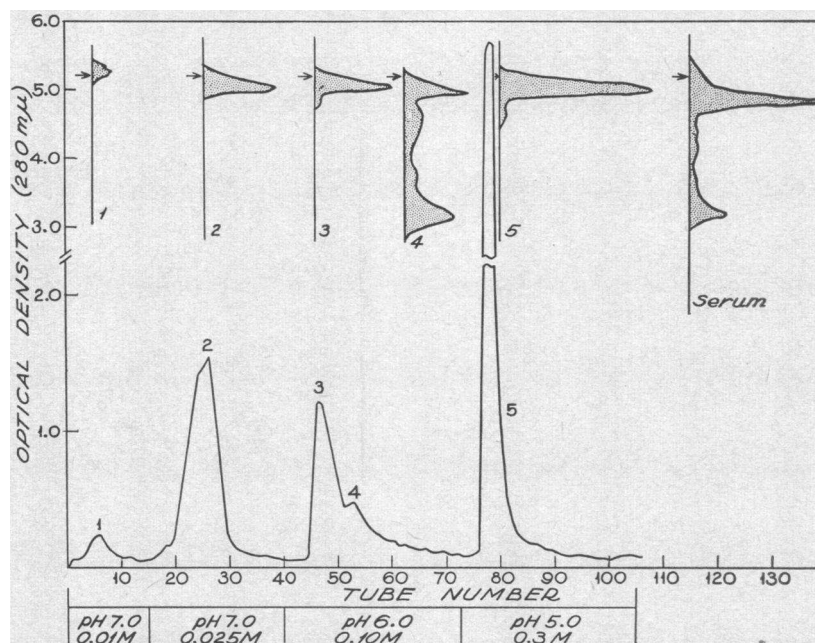


FIG. 4. CHROMATOGRAPHIC PATTERN OF SERUM FROM A PATIENT (B.B.) WITH MACROGLOBULINEMIA, AND PAPER ELECTROPHORETIC PATTERNS OF CHROMATOGRAPHIC FRACTIONS. Two ml serum on 5 g DEAE cellulose; 4 ml fractions.

sisted entirely of 7S gamma globulin. On ultracentrifugal analysis, the pH 6 peak contained five components, one of which was albumin and the rest beta globulins by paper electrophoresis. The

sedimentation constants ranged between 6.3 and 14S, the 6.3S being the major component (Table II). The pH 5 peak consisted almost entirely of beta globulins with sedimentation constants rang-

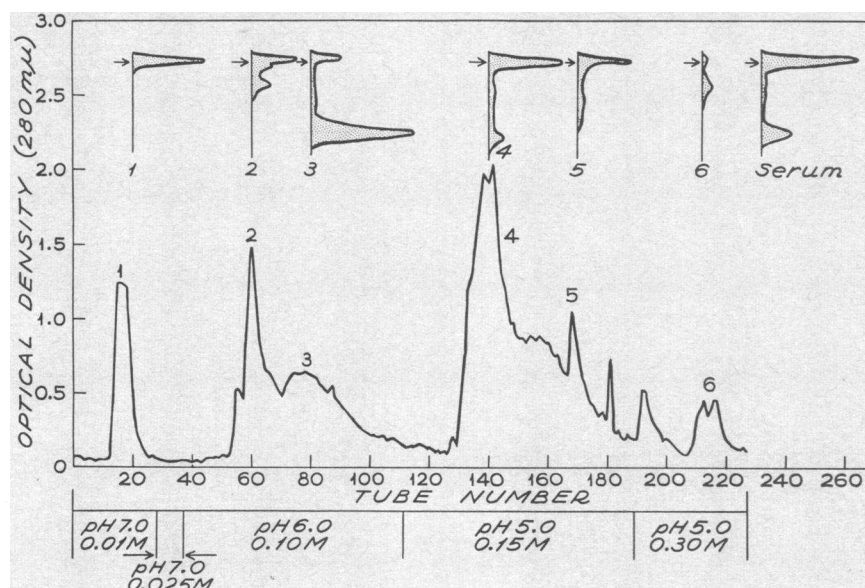


FIG. 5. CHROMATOGRAPHIC PATTERN OF SERUM FROM A PATIENT (S.K.) WITH MACROGLOBULINEMIA, AND PAPER ELECTROPHORETIC PATTERNS OF CHROMATOGRAPHIC FRACTIONS. Four ml serum on 10 g DEAE cellulose; 4 ml fractions.

TABLE III

Precipitation tests on chromatographic fractions using rabbit antiserum to 7S and 19S gamma globulin

Effluent fraction		Waldenström syndrome (Figure 4)		Rheumatoid factor, F II precipitate	
pH	Molarity	Anti-7S	Anti-19S	Anti-7S	Anti-19S
7	0.01	+++	0	+++	0
7	0.025	++	+		
6	0.10	+	+		
5	0.30	+++	++	+++	+++

ing from 6.2S to 14S. The major ultracentrifugal component, comprising 55 per cent of the total protein of this peak, had an S rate of 8.6S (Table II).

Chromatograms of the third type, demonstrated by the sera of patients with macroglobulinemia (Figures 4 and 5), are characterized by the occurrence of gamma globulins in each of the peaks and the presence of a large pH 5 peak composed mainly of gamma globulin. Ultracentrifugation of the whole serum of Patient B. B., of which 53 per cent of the total protein was gamma globulin (Table I), indicated the presence of four components, one of which had a sedimentation constant

of 4S and three sedimentation constants of 8, 17, and 25S, respectively. The 17S component was the major component. Unlike those of the normal, multiple myeloma, and other hyperglobulinemic sera studied above, all chromatographic peaks, as mentioned, contained large amounts of protein with the electrophoretic mobility of gamma globulin. The protein of the pH 7 (0.01 M) peak was entirely gamma globulin, but too small in concentration to examine in the ultracentrifuge. It gave no reaction with an antiserum to 19S gamma globulin (Table III), but did react with an antiserum to 7S gamma globulin. The pH 7 (0.025 M) and pH 6 peaks consisted mainly of a 17S macroglobulin with small amounts of 8S and approximately 26S material. The pH 5 peak consisted mainly of an 18S component and was free of light gamma globulin.

A second serum from a patient with macroglobulinemia (S.K., Figure 5) contained 67 per cent gamma globulin. On ultracentrifugation, there were five components, one of which had a sedimentation constant of 4.7S, the others having constants of 6.7, 17, 25, and 31S, respectively. The 17S component was the major gamma globu-

TABLE IV

Ultracentrifugal analysis of chromatographic fractions containing gamma globulin, macroglobulinemia sera

Patient	Serum	Elution		Effluent volume	Sedimentation constant of gamma globulins S _{20W}	% Total protein* of peak
		pH	Molarity			
B. B.	ml 2	7	0.01	40	8	17
		7	0.025	60	17	71
					27	12
		6	0.10	80	7.7	8
					18	81
					28	11
		5	0.3	64	18	60
					27	9
		7	0.01	100	6.3	100
		6	0.10	128	17	58
S. K.	4				24	7
		6	0.10	248	16	16
					25	5
		5	0.15	360	18	47
					24	21
					31	9
		5	0.15	70	18	92
					25	8
		5	0.15	130	18	70
					25	24
S. K. (Euglobulin fraction)	4†				31	6

* In those instances where the total is not 100 per cent, the difference represents non-gamma globulin components.

† The euglobulin fraction was prepared from this volume of serum.

lin component, making up 40.5 per cent of the total protein of the serum. The pH 7 (0.01 M) peak consisted entirely of gamma globulin with a sedimentation coefficient of 6.3S. The serum macroglobulins with sedimentation constants of approximately 18, 25, and 31S were found distributed over the pH 6 and pH 5 peaks (Table IV). Other serum proteins were also eluted in the pH 6 and pH 5 peaks; beta globulin (4.9S) and albumin (3.8S) were eluted in the pH 6 peak, and alpha globulins (3.6S) chiefly in the pH 5 peak.

Thus, in the case of the macroglobulinemia sera, which contained little low molecular weight gamma globulin, the abnormal, heavy components were distributed over a number of chromatographic peaks with the bulk of the gamma globulin appearing in the pH 5 peak, the last to be eluted. The first peak, eluted at pH 7.0, which contained 7S gamma globulin, was relatively small in size. This is in contrast to the results obtained with the serum of normal individuals, patients with rheumatoid arthritis (8), and the other hyperglobulinemic states described above.

A clear demonstration of the chromatographic behavior of the macroglobulins was obtained on chromatography of the euglobulin fraction from the serum of Patient S.K. The pattern obtained showed only two major components, both of which appeared in the pH 5 peak and consisted entirely of gamma globulin. On ultracentrifugal analysis (Table IV), one fraction contained two components with sedimentation constants of 18 and 25S, while the other contained three components with constants of 18, 25, and 32S. The relative amounts of these components in the second fraction, however, were different from those in the first fraction, a higher concentration of heavier components appearing in the second fraction.

A definitive separation of gamma globulins of differing size was obtained with sera of patients with rheumatoid arthritis. The presence in such sera of the rheumatoid factor, a 19S gamma globulin (7), offered a unique opportunity to study this phenomenon. The factor was first precipitated from the serum by addition of human Fraction II and the precipitate washed and dissolved in 4 M urea. The urea solution was then applied to the DEAE cellulose column and the pattern

obtained (8) showed a distinct separation into two major peaks, eluted at pH 7 (0.01 M) and pH 5 (0.3 M), respectively, each having the electrophoretic mobility of gamma globulin. On ultracentrifugal analysis, the first peak had a sedimentation constant of 7S and the second consisted almost entirely of 19S gamma globulin with no detectable 7S protein (8). No contamination of the 7S with 19S gamma globulin was detectable by immunochemical testing (Table III). Rheumatoid factor activities including precipitation with Fraction II (13), agglutination of sensitized sheep cells (14), and agglutination of latex particles (15, 16) were found only with the second or 19S peak.

Immunological tests. Capillary precipitin tests performed on the chromatographic fractions obtained with the serum from a patient with macroglobulinemia (Figure 4) and from the precipitate between rheumatoid factor and Fraction II (8) are given in Table III. The rabbit antisera used (10) were: 1) an antiserum to 7S gamma globulin, and 2) an antiserum to 19S gamma globulin which, after absorption with 7S gamma globulin, reacts only with the macroglobulin. All of the fractions shown in Table III gave positive precipitin tests with the 7S antiserum as might be expected, since all contained gamma globulin. The presence of macroglobulins, however, is clearly demonstrated only in the pH 7 (0.025 M), pH 6, and pH 5 peaks of Figure 4 and the pH 5 peak of the rheumatoid factor-Fraction II precipitate by the positive precipitin reactions obtained with the antiserum to 19S gamma globulin.

DISCUSSION

The gamma globulin of normal human serum consists of at least four ultracentrifugally distinct components with sedimentation coefficients of 7, 19, 28, and 44S, respectively (3). The first two may be detected in unconcentrated serum and the latter two only after considerable enrichment. The experiments reported in this paper, a development of the chromatographic technique of Sober, Gutter, Wyckoff and Peterson (2), provide a basis for the separation of the 7S from the heavier gamma globulins. In the cases of normal sera; multiple myeloma sera; the sera from patients with liver disease, lymphosarcoma, and primary amyloidosis; and the precipitate between

Fraction II and the rheumatoid factor, it was possible to separate 7 and 19S gamma globulins in a single chromatographic step. The first peak obtained in each instance consisted entirely of 7S gamma globulin as shown both by ultracentrifugation and immunological tests. Beyond this point, little or no gamma globulin was eluted except for the small amount, presumably macroglobulin, present at the tail end of the normal serum chromatogram.

Although the two multiple myeloma sera studied contained considerable amounts of myeloma protein, these were eluted early in the chromatographic procedure. The protein of the gamma globulin type present in the serum of M.K. (Figure 2) was eluted in the pH 7 peak, while the myeloma protein of the beta globulin variety in the serum of C.R. (Figure 3) was eluted mainly in the pH 6 peak, which has usually contained the beta globulins. These results are consistent with those reported by Fahey, McCoy and Goulian (17), and with the observations that myeloma proteins are of the low molecular weight variety (18). In contrast, the patterns obtained with the two macroglobulin-rich sera (Figures 4 and 5) show a series of peaks of which those eluted last tend to be large and contain a large amount of gamma globulin. In this respect, the patterns differ markedly from those obtained with normal and ordinary hyperglobulinemic sera (Figures 1 and 2).

The ultracentrifugal analysis of the chromatographic fractions of the sera from patients with macroglobulinemia is of particular interest. In general, the pH 5 peak, the last to be eluted, contains high molecular weight gamma globulin but no gamma globulin of the 6S to 8S variety. Substantially all of the latter protein is found in the early peaks and usually in the pH 7 (0.01 M) peak, the first to be eluted. Analysis of the individual peaks from the macroglobulin-rich sera showed that the gamma globulin fraction of all of the peaks which contained macroglobulin consisted mainly of a 15 to 18S component with smaller amounts of heavier components.

The elution of mixtures of macroglobulins with a similar distribution of S rates in separate chromatographic peaks was observed in some experiments. Particularly striking is the comparison of the pH 7 (0.025 M) and the pH 6

(0.1 M) peaks of Patient B.B. (Figure 4, Table IV), which shows that the only significant difference in composition of the two peaks is the presence of a larger percentage of 7S gamma globulin in the pH 7 peak. On the basis of previously reported observations on the chromatography of human serum albumin (19), it is suggested that the proteins eluted in the pH 7 peak may represent macroglobulins present in excess of the binding capacity of the column at the buffer concentration used to elute this peak.

The mechanism responsible for the separation of the macroglobulins from 7S gamma globulins on DEAE cellulose is not clear. The tendency of the heavy proteins to appear in chromatographic eluates of higher salt concentration than those in which 7S gamma globulin is eluted, even when the pH of the eluting buffer is kept constant (8), may be dependent on a number of factors. Evidence against the possibility that solubility plays an important part is the observation that the chromatographic patterns of the macroglobulinemic sera were similar despite the fact that one serum (S.K.) gave a heavy euglobulin precipitate on dilution with water, while the other (B.B.) gave none.

The increased affinity of the high molecular weight gamma globulins for DEAE cellulose may be based upon at least two factors which differentiate these proteins from the 7S gamma globulins. One is their higher carbohydrate content (20), and the other their greater total charge. Since the 19S gamma globulins consist of six or more 7S molecules, the total charge on the macroglobulin would be greater than that of its individual 7S constituents. It is also possible that the high molecular weight gamma globulins have greater net charge, but because of differences in the shapes of the molecules and resultant differences in resistance to flow, the electrophoretic mobility is similar in magnitude to that of 7S gamma globulin. These properties could be responsible for both increased electrostatic and molecular binding. It is noteworthy that in experiments using the cation exchanger, carboxymethyl cellulose, 19S gamma globulin was bound so firmly that it could be eluted completely only after elution with 0.1 M sodium hydroxide. This suggests that non-ionic forces play an important role in the binding of the macroglobulins.

While it has been possible to separate 7S and 19S gamma globulins effectively by the technique described, little can be said about the separation from each other of the minor gamma globulin constituents of larger molecular size. Though fractionation of the 17S, 25S, and 30S components was achieved (Table IV), complete separation of these components from one another was not effected. It should also be mentioned that the difference in chromatographic behavior observed with gamma globulins of different size may eventually be demonstrable among the other serum proteins which are heterogeneous on ultracentrifugal analysis; i.e., the 5S and 12S beta globulins and 4S and 19S alpha-2 globulins (3).

SUMMARY

1. Gamma globulin has been separated on DEAE cellulose into components which differ in molecular size.

2. When sera from patients with liver disease, lymphosarcoma, primary amyloidosis, multiple myeloma, and macroglobulinemia were studied, the appearance of the chromatographic pattern of the serum on DEAE cellulose in conjunction with paper electrophoresis made it possible to detect the presence of increased amounts of macroglobulins. It has been possible in this way to differentiate between the chromatographic patterns of diseases with ordinary hypergammaglobulinemia (including multiple myeloma) and macroglobulinemia.

3. The chromatographic method employed offered a means of preparing 7S gamma globulin free of heavier molecular species. In the case of the rheumatoid factor, the 19S gamma globulin has been prepared free of the 7S variety.

REFERENCES

- Peterson, E. A., and Sober, H. A. Chromatography of proteins. I. Cellulose ion-exchange adsorbents. *J. Amer. chem. Soc.* 1956, **78**, 751.
- Sober, H. A., Gutter, F. J., Wyckoff, M. M., and Peterson, E. A. Chromatography of proteins. II. Fractionation of serum protein on anion-exchange cellulose. *J. Amer. chem. Soc.* 1956, **78**, 756.
- Wallenius, G., Trautman, R., Kunkel, H. G., and Franklin, E. Ultracentrifugal studies of major non-lipide electrophoretic components of normal human serum. *J. biol. Chem.* 1957, **225**, 253.
- Conference on serological reactions of rheumatoid arthritis: Summary of First Conference (Jan. 23, 1957), R. W. Lamont-Havers, Ed. New York, Arthritis and Rheumatism Foundation, 1958.
- Ziff, M. The agglutination reaction in rheumatoid arthritis. *J. chron. Dis.* 1957, **5**, 644.
- Vaughan, J. H. Serum responses in rheumatoid arthritis. *Amer. J. Med.* 1959, **26**, 596.
- Franklin, E. C., Holman, H. R., Müller-Eberhard, H. J., and Kunkel, H. G. An unusual protein component of high molecular weight in the serum of certain patients with rheumatoid arthritis. *J. exp. Med.* 1957, **105**, 425.
- Lospalluto, J., and Ziff, M. Chromatographic studies of the rheumatoid factor. *J. exp. Med.* 1959, **110**, 169.
- Ziff, M., and Lospalluto, J. Unusual chromatographic behavior of serum macroglobulins. *J. clin. Invest.* 1959, **38**, 1057.
- Franklin, E., and Kunkel, H. G. Immunologic differences between the 19 S and 7 S components of normal human γ -globulin. *J. Immunol.* 1957, **78**, 11.
- Bearn, A. G., Kunkel, H. G., and Slater, R. J. The problem of chronic liver disease in young women. *Amer. J. Med.* 1956, **21**, 3.
- Fahey, J. L., and Horbett, A. P. Human gamma globulin fractionation on anion exchange cellulose columns. *J. biol. Chem.* 1959, **234**, 2645.
- Epstein, W., Johnson, A., and Ragan, C. Observations on a precipitin reaction between serum of patients with rheumatoid arthritis and a preparation (Cohn Fraction II) of human gamma globulin. *Proc. Soc. exp. Biol. (N. Y.)* 1956, **91**, 235.
- Ziff, M., Brown, P., Lospalluto, J., Badin, J., and McEwen, C. Agglutination and inhibition by serum globulin in the sensitized sheep cell agglutination reaction in rheumatoid arthritis. *Amer. J. Med.* 1956, **20**, 500.
- Singer, J. M., and Plotz, C. M. The latex fixation test: I. Application to the serologic diagnosis of rheumatoid arthritis. *Amer. J. Med.* 1956, **21**, 888.
- Singer, J. M., and Plotz, C. M. The latex fixation test: II. Results in rheumatoid arthritis. *Amer. J. Med.* 1956, **21**, 893.
- Fahey, J. L., McCoy, P. F., and Goulian, M. Chromatography of serum proteins in normal and pathologic sera; the distribution of protein-bound carbohydrate and cholesterol, siderophilin, thyroxine-binding protein, B₁₂ binding proteins, alkaline and acid phosphatases, radioiodinated albumin and myeloma proteins. *J. clin. Invest.* 1958, **37**, 272.
- Putnam, F. W. Abnormal human serum globulins. II. Physical constants and amino end groups. *J. biol. Chem.* 1958, **233**, 1448.
- Boman, H. G., and Westlund, L. E. Protein chromatography on an anion-exchange resin. *Arch. Biochem.* 1956, **64**, 217.
- Müller-Eberhard, H. J., Kunkel, H. G., and Franklin, E. C. Two types of γ -globulin differing in carbohydrate content. *Proc. Soc. exp. Biol. (N. Y.)* 1956, **93**, 146.