CHROMATOGRAPHIC STUDIES OF THE RHEUMATOID FACTOR*

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Since the discovery that sera from patients with rheumatoid arthritis have the capacity to agglutinate sensitized sheep erythrocytes (1), much attention has been directed to the constituents in serum responsible for this phenomenon. This has resulted not only in the development of a number of diagnostic tests, but also in considerable investigation (2) into the nature of the responsible serum constituent. The latter has been referred to as the rheumatoid factor (3).

Ziff and coworkers (4) demonstrated that the agglutinating activity of rheumatoid serum is precipitated in the euglobulin fraction, and Svartz and Schlossmann (5) showed that it precipitated in the "cold globulin" fraction. On zone electrophoresis of the cold globulin precipitate, agglutinating activity was found in the gamma globulin. This has also been demonstrated by paper electrophoresis of precipitated material obtained after dilution of rheumatoid serum with water (6). Ultracentrifugal studies of rheumatoid sera and euglobulin fractions have shown that the factor circulates as a high molecular weight component with a sedimentation coefficient of 22 S (7), and that there is a correlation between the quantity of the 22 S component found and the agglutination titer.

With the development of a suitable chromatographic system for the fractionation of serum proteins, it has been possible to apply the chromatographic method to the investigation of the rheumatoid factor. The cellulose ion exchangers described by Sober and Peterson (8, 9) have therefore been employed by Lospalluto and Ziff (10–12), and by Svartz *et al.* (13), for the fractionation of rheumatoid serum and euglobulin fractions. The present paper describes the isolation of rheumatoid factor using an anion exchanger and further fractionation of the purified material on a cation exchanger.

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Materials and Methods

Sera from patients with rheumatoid arthritis were heated to 56°C. for 30 minutes to inactivate complement, and subsequently were absorbed twice with equal volumes of washed, packed sheep erythrocytes before fractionation. In experiments in which the starting material was a euglobulin fraction, this was prepared by dialysis of the serum at pH 5.8 to 6.0 using M/150 phosphate buffer after dilution with an equal volume of water.

The cellulose ion exchangers diethylaminoethyl cellulose and carboxymethyl cellulose were prepared[1] by the method of Peterson and Sober (8). The elution procedure used was a modification of that described by Sober *et al.* (9). After packing with exchanger which had been previously suspended in 0.01 M phosphate at pH 7, small volumes of 0.15 M NaH_2PO_4 solution were added to adjust the pH to 7. Columns were operated at room temperature using flow rates of 1 to 2 ml. per minute. Stepwise changes in pH and concentration of buffers were employed instead of gradient elution. For anion exchange chromatography, the pH and concentration of eluting phosphate buffers were varied stepwise from pH 8.0 (0.01 M) to pH 4.5 (0.15 and 0.3 M). In cation exchange experiments, the range was pH 5.0 (0.01 M) to pH 7.0 (0.15 M). In experiments in which whole serum was fractionated 1 gm. of exchanger was used for each ml. of serum. When the euglobulin fraction was applied, 30 mg. of protein was applied per gm. of exchanger.

In the earlier experiments, the charge was diluted ten- to fifteenfold with 0.01 M phosphate buffer at pH 7 and then added to the column in small portions. In later experiments, the serum or euglobulin solution was dialyzed against 0.01 M phosphate solution at 4°C. and pH 8.6 for 24 hours before application to the column. It was not possible to equilibrate serum at pH 7 and 0.005 M concentration as described by Sober *et al.* (9) since precipitates of euglobulin containing rheumatoid factor were formed under these conditions. The higher pH used in this work resulted in little or no precipitation of euglobulin and allowed the application of a clear protein solution to the column.

The concentration of protein in each fraction collected was estimated from measurements of ultraviolet absorption at 280 mμ in the Beckman spectrophotometer. Agglutinating activity for sensitized sheep cells was tested by the method of Ziff *et al.* (4). Since, in most experiments, the volumes of the eluates collected were equal to the volume of serum fractionated, each eluate was diluted for testing in the same way as the original serum. Dilutions were made with an isotonic, saline-phosphate buffer at pH 7 (0.075 M NaCl and 0.075 M phosphate).

Precipitation tests with human fraction II[2] were performed on small aliquots of each eluate. 0.1 to 0.2 ml. of eluate was brought to neutrality and isotonicity by addition of 1.05 to 1.15 ml. of the neutral, saline-phosphate buffer. Following this, 0.25 ml. of a 1 per cent solution of human fraction II, which had been previously heated at 63°C. for 10 minutes, was added. The appearance of turbidity or precipitate was considered a positive test. Latex fixation tests were carried out according to the method of Singer and Plotz (14).

Chromatographic eluates containing active constituents were in some instances combined and dialyzed against 0.005 M phosphate at pH 5.8 to 6.0 for 48 hours. The euglobulin precipitate obtained under these conditions contained the bulk of the agglutinating activity.

In some experiments, the starting material for chromatography was a solution of the precipitate (15) formed on addition of heated human fraction II to rheumatoid serum. The precipitate was prepared by adding 0.5 ml. of a 0.5 per cent solution of fraction II to each ml. of a tenfold dilution of serum in isotonic, saline buffer (0.075 M phosphate and 0.075 M NaCl) at pH 7. After the mixture had been allowed to stand for 1 day at 4°C., the precipitate was

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[1] In the later phases of the work, both exchanges were purchased from the Brown Company, New York.

[2] Obtained through the courtesy of Dr. J. N. Ashworth of the American Red Cross.

harvested by centrifugation and washed four times with buffered saline. The washed precipitate, obtained usually from 25 to 75 ml. of serum, was then redissolved in 2 to 5 ml. of 4 M urea solution as described by Franklin *et al.* (7) and applied to a diethylaminoethyl cellulose column. The elution method was substantially the same as the one used with serum except that urea was added to the initial eluting buffer (pH 7.0, 0.025 M) in a concentration of 4 moles per liter. The urea containing buffer was used in the initial phases of the chromatogram to insure separation of the components before recombination could take place. It was then replaced by a buffer of the same pH and concentration but containing no urea when significant amounts of the first component, usually 7 S gamma globulin, had appeared in the column effluents.

Protein estimations on pooled, concentrated fractions were made by a modification of the biuret method of Mehl (16). The reagent used contained 10 mM of $CuSO_4$ and 50 mM of glycerol per liter dissolved in 0.75 M K_3PO_4. For each determination, 2.5 ml. of protein solution containing 0.1 to 2 mg. N was added to 2.5 ml. of reagent and allowed to stand for 45 minutes before reading at 550 mμ in the Beckman spectrophotometer. A standard curve was constructed using a solution of human fraction II previously analyzed by the Kjeldahl method. Hexose determinations were made with the anthrone reagent (17).

Electrophoresis was performed on paper strips using the hanging strip method in a Durrum type cell at pH 8.6 ($\Gamma/2 = 0.075$ M) for 16 hours at 120 volts and 4 milliamperes. Papers were stained with bromphenol blue and scanned with a transmission densitometer (Spinco). Ultracentrifugal analyses were carried out through the courtesy of Dr. H. G. Kunkel of The Rockefeller Institute in the Spinco model E ultracentrifuge. Agar double diffusion tests were carried out by the method of Preer (18).

RESULTS

Anion Exchange Chromatography of Serum and Euglobulin.—The diagram shown in Fig. 1 was obtained with a 25 ml. pool of rheumatoid serum from two individuals fractionated on a 5 x 45 cm. column containing 20 gm. of diethylaminoethyl cellulose. While the first peak contained most of the gamma globulin, as previously shown (9), positive tests for the rheumatoid factor, both by precipitation (insert graph) and agglutination methods (shaded area), were obtained only in the last peak.

Since the rheumatoid factor precipitates mainly in the euglobulin fraction, a sample of euglobulin was fractionated in the same manner (Fig. 2). The separation of the rheumatoid factor (shaded area) from the main gamma globulin component (first peak) is clearly evident. Moving boundary electrophoresis of the active chromatographic component at pH 8.6 ($\Gamma/2 = 0.1$) revealed the presence of material consisting of over 70 per cent gamma globulin.

Cation Exchange Chromatography of Serum and Chromatographic Fractions on Carboxymethyl Cellulose.—Chromatography of a rheumatoid serum on carboxymethyl cellulose is illustrated in Fig. 3. Tests for rheumatoid factor indicated that both agglutinating activity and the capacity to precipitate with heated fraction II were associated, as previously demonstrated (19), only with the major gamma globulin peak eluted at pH 6.5 (0.075 M). The second gamma globulin peak which appeared at pH 7.0 (0.15 M) showed no agglutinating activity but did precipitate with heated fraction II.

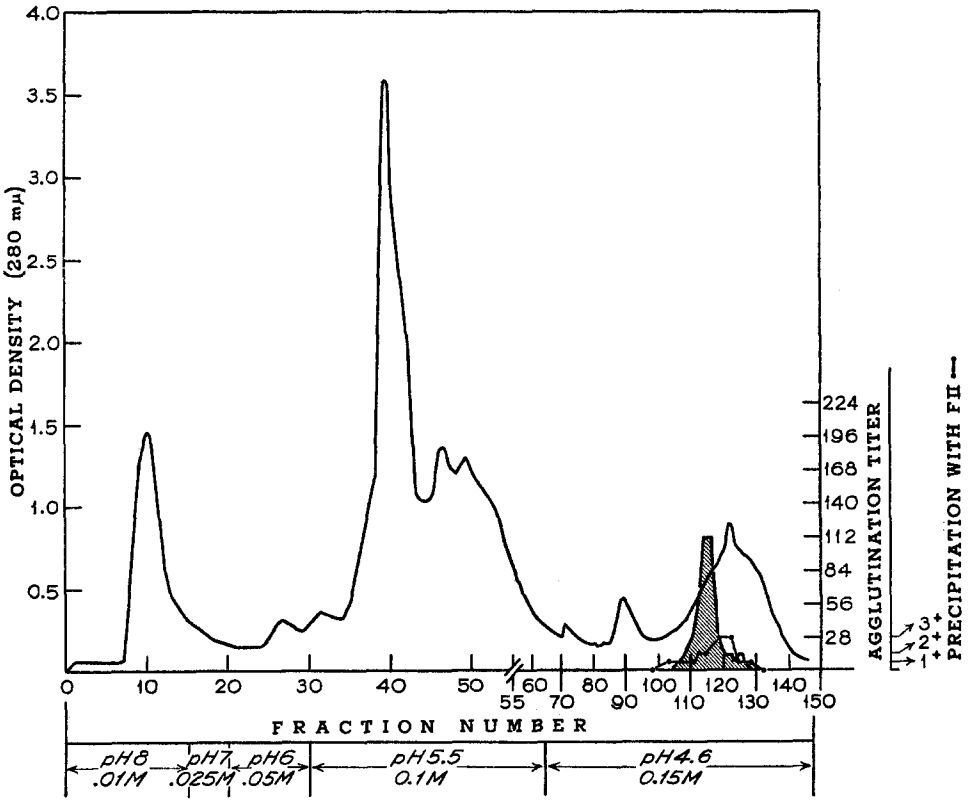

FIG. 1. Anion exchange chromatogram of pooled rheumatoid serum (25 ml.). Shaded area indicates sensitized sheep cell agglutination titer. Inset graph (—O—O) indicates precipitation with fraction II.

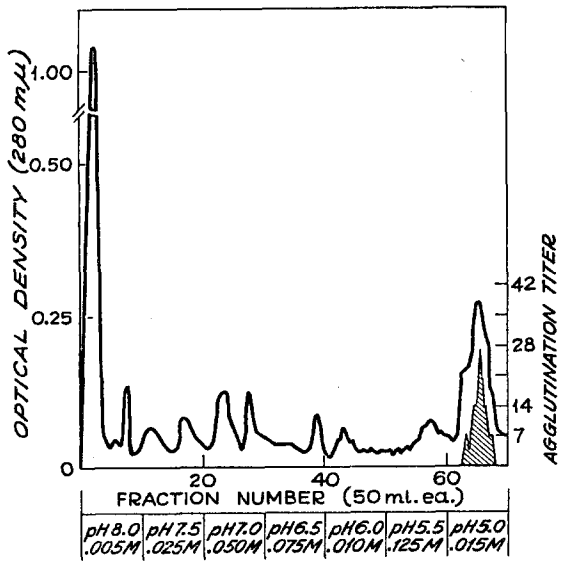

FIG. 2. Anion exchange chromatography of euglobulin from 100 ml. pooled rheumatoid serum. Titer euglobulin = 112. Shaded area indicates sensitized sheep cell agglutination titer.

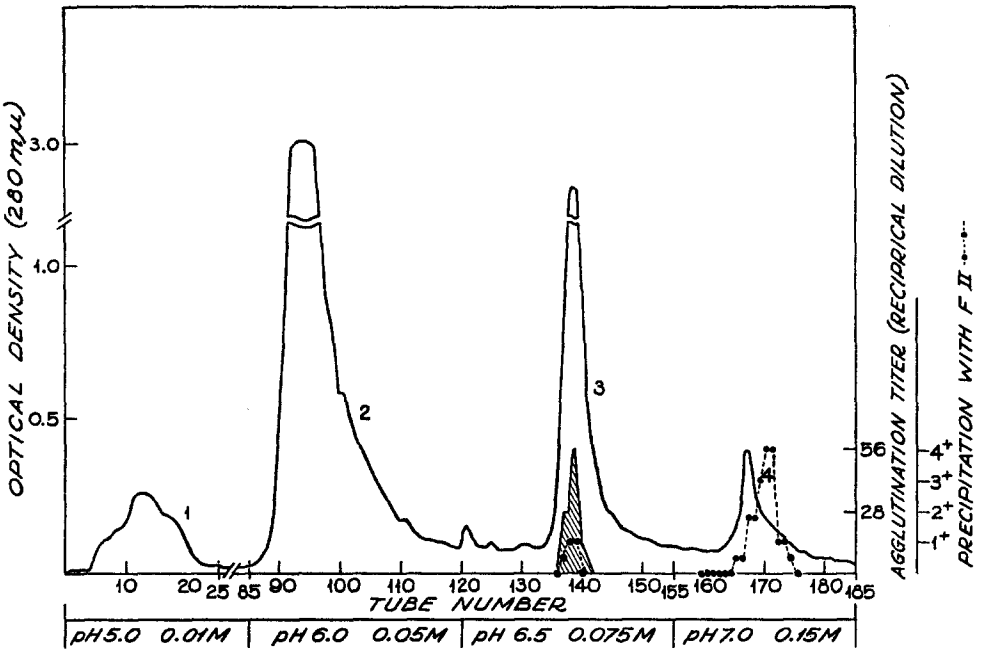

FIG. 3. Chromatographic pattern of rheumatoid serum (G. I. 10 ml.) on carboxymethyl cellulose (10 gm.). Shaded area shows sensitized sheep cell agglutinating activity. Interrupted curve shows precipitation with heated fraction II.

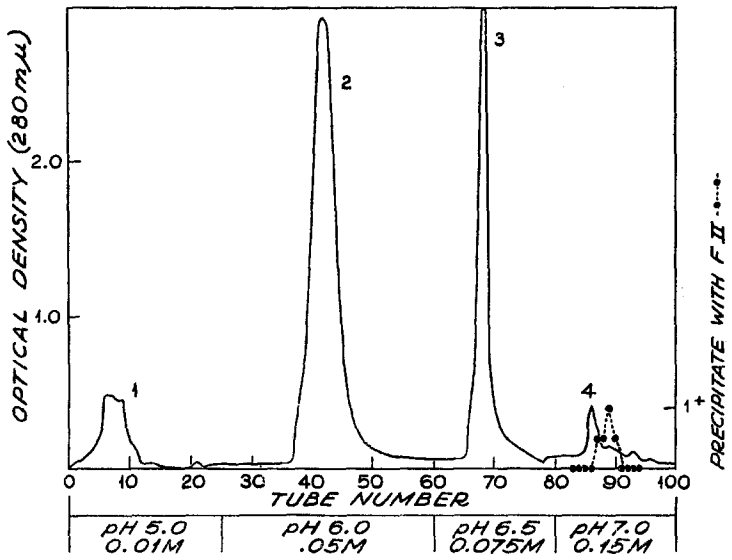

FIG. 4. Cation exchange chromatography of normal serum (G. F., 5 ml. on 6 gm. carboxymethyl cellulose). Ten ml. fractions were collected. Interrupted curve indicates precipitation with heated fraction II.

Fractionation of normal serum was also carried out on the cation exchanger in order to determine whether similar active chromatographic constituents were present. The result (Fig. 4) clearly indicated the presence of a precipitating, non-agglutinating component (peak 4). No agglutinating activity for sensitized sheep cells was observed in any peak. The same results were obtained on fractionation of a second normal serum. In both instances the precipitating activity appeared to be lower than that found with the corresponding peak from a rheumatoid serum (Fig. 3); i.e., 1+ as compared with 4+. The

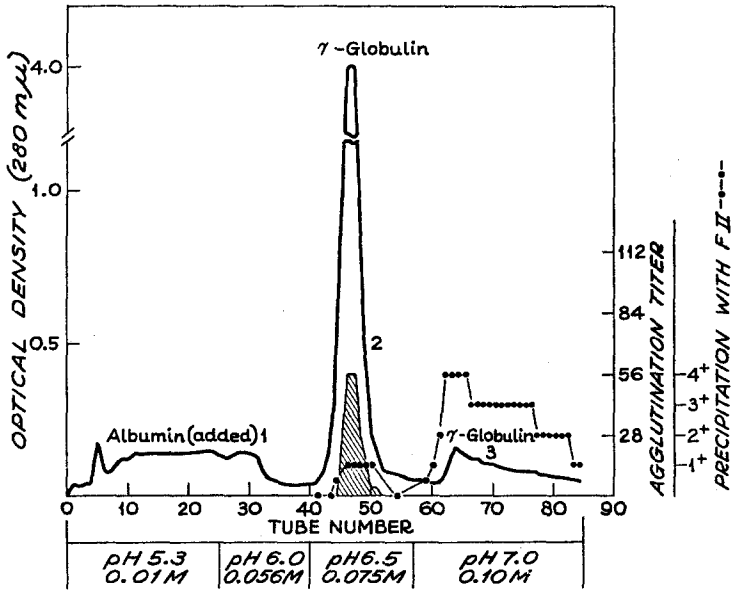

FIG. 5. Cation exchange chromatography of active material obtained by anion exchange chromatography of rheumatoid serum (Fig. 1).

corresponding peaks from two other normal sera showed no precipitating activity.

The active effluents obtained by anion exchange chromatography of whole rheumatoid serum (Fig. 1) were pooled and fractionated further on carboxymethyl cellulose. The combined active fractions (indicated by the shaded are to which crystalline bovine serum albumin had been added were dialyzed against 0.01 phosphate solution (pH 5.0) for 1 day. The small amount of euglobulin precipitate formed dissolved in 2 ml. of 0.15 M phosphate buffer (pH 5.0). Small volumes of this solution were diluted tenfold with the albumin containing supernatant and the diluted solutions successively applied to a column of carboxymethyl cellulose.

In the chromatographic pattern obtained, (Fig. 5), the protein appearing in the early effluents consisted mainly of the albumin added. The second peak

contained both albumin and gamma globulin and the third gamma globulin alone. Activities were demonstrated in both the second and third peaks. The protein of the second peak agglutinated sensitized sheep erythrocytes and precipitated with heated fraction II, while that of the third showed only the precipitation reaction.

The precipitating and non-agglutinating fractions of peak 3 were pooled, dialyzed against water and lyophilized. The dried protein was then dissolved in 6 ml. of saline-phosphate buffer (0.075 M NaCl, and 0.075 M phosphate at pH 8.0). An aliquot of this solution containing 0.061 mg. of protein was titrated against unheated fraction II in a total volume of 1.5 ml. by

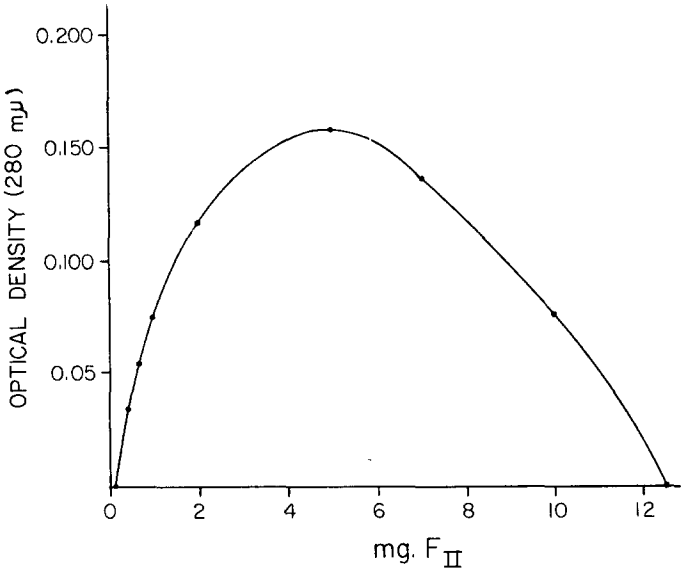

FIG. 6. Precipitation curve of factor II obtained from rheumatoid serum (peak 3, Fig. 6); 0.061 mg. protein, peak 3; final volume = 1.5 ml.

the method described above. The precipitates formed after two days at 4°C. were washed three times with the cold saline buffer, then redissolved in 0.05 M NaOH and the ultraviolet absorption of each solution was read at 280 mμ. The curve obtained (Fig. 6) is similar to one type obtained by Epstein *et al.* (15) with whole rheumatoid serum.

The fractions containing agglutinating activity (peak 2) were combined and concentrated by lyophilization. Sufficient material was available in this case for study in the ultracentrifuge. Of the two components shown in the ultracentrifugal curve (Fig. 7), the first, with sedimentation coefficient of approximately 4 S is albumin and the second, a 19 S component is gamma globulin as indicated by electrophoretic analysis. Agglutination of sensitized sheep cells with this fraction was noted only at a concentration of 10 μg. per ml. whereas fractions of the 19 S proteins prepared by ultracentrifugation (20) showed agglutination at concentrations of 1 μg. per ml. or less. It was apparent from these results that the specific activity of this preparation was too low for the amount of 19 S gamma globulin present, and that preliminary fractionation

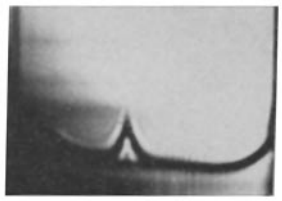

FIG. 7. Ultracentrifugal pattern of protein from peak 2, Fig. 5 taken at 28 minutes, 52,000 R.P.M.

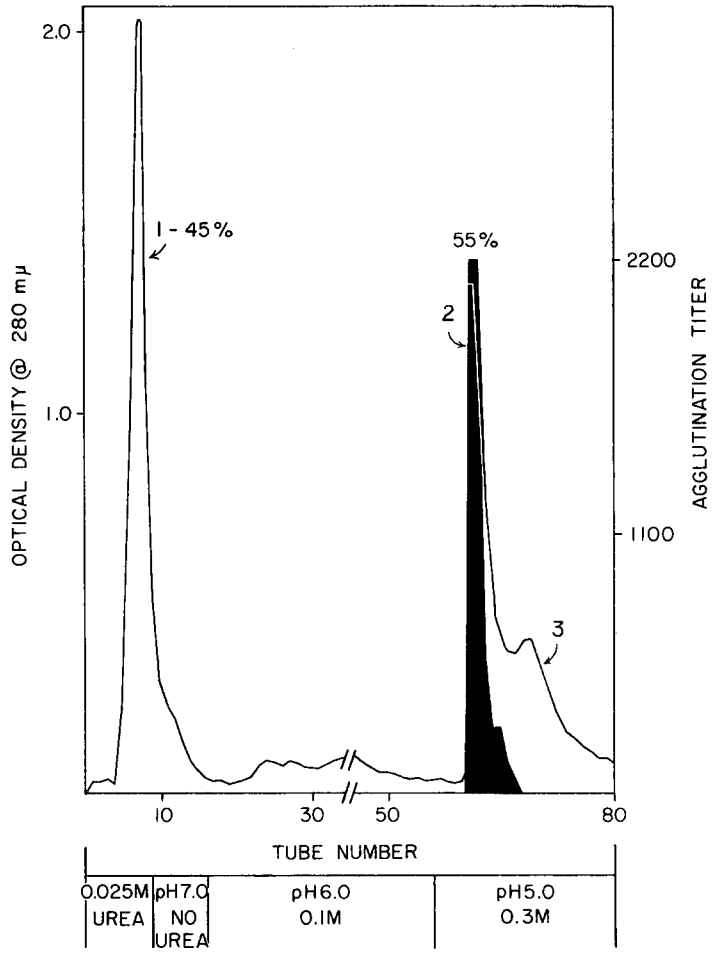

FIG. 8. Anion exchange chromatography of the urea dissolved fraction II precipitate from rheumatoid serum; shaded area indicates sensitized sheep cell agglutination titer in reciprocal dilution.

would be required to separate the rheumatoid factor macroglobulin from the inactive macroglobulin which is present in most sera (21).

Fractionation of Fraction II Precipitated Rheumatoid Factor on Diethylaminoethyl and Carboxymethyl Cellulose.—

Preliminary separation was achieved by precipitation of rheumatoid factor with human fraction II. This precipitate was prepared from a number of individual rheumatoid sera, redissolved in 4 M urea, applied to the cellulose anion exchanger, and fractionated as described above.

The elution diagram shown in Fig. 8 was obtained with the precipitate produced by the addition of 125 ml. of 0.5 per cent, heated fraction II solution to 250 ml. of a tenfold dilution of rheumatoid serum (G.C.) in saline. After washing four times with cold, buffered saline, the

TABLE I

Activity of Fractions Obtained by Anion Exchange Chromatography of Urea Dissolved Fraction II Precipitate from Rheumatoid Serum (Fig. 8)

Test	Peak 1	Peak 2		Peak 3	
		Reciprocal dilution	Concentration at highest dilution	Reciprocal dilution	Concentration at highest dilution
			μg./ml.		μg./ml.
Sensitized sheep cell agglutination	0	2,250	0.4	0	—
Latex agglutination	0	50,000	0.02	800	0.3
Precipitation with heated fraction II	0	+++	—	+	—
Addition of rheumatoid serum	++	—	—	—	—
Addition of normal serum	0	—	—	—	—

precipitate was redissolved in 3 ml. of 4 M urea, and applied to the diethylaminoethyl cellulose column.

Three major chromatographic components are seen. The first comprised approximately 45 per cent, and the second two partially resolved peaks approximately 55 per cent of the total protein recovered. The results of tests performed on eluates of each peak at the point of maximum protein concentration are shown in Table I. It is clear that peak 2 contains highly active rheumatoid factor. Agglutination of sensitized sheep cells was observed at a concentration of 0.4 μg. per ml. and agglutination of latex particles at a concentration of 0.02 μg. per ml. Peak 3 precipitated with fraction II and agglutinated latex particles at a concentration of 0.3 μg. per ml., but did not agglutinate sensitized sheep cells. Peak 1 was devoid of all activity. When the latter was tested for its ability to replace human fraction II as a precipitant of rheumatoid factor, however, precipitates were obtained, although these

were smaller than obtained with heated fraction II. No precipitation was observed when peak 1 was added to normal serum.

Precipitates were also obtained on addition of the fractions of peak 1 to those of peak 2, demonstrating the recombination of the constituents of the original precipitate.

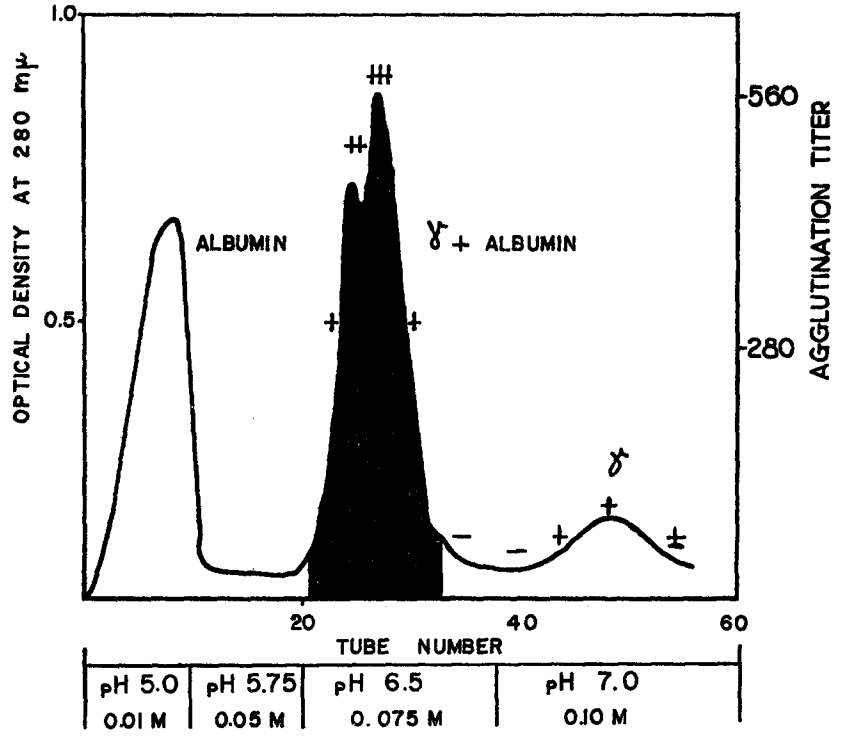

FIG. 9. Cation following anion exchange chromatography of peak 2, Fig. 8; shaded area represents sheep cell agglutination in reciprocal dilution (+ signs indicate precipitation with Fraction II).

The fact that the eluates comprising peak 3 had no sensitized sheep cell agglutinating activity but did agglutinate latex particles and precipitate with heated fraction II suggested that at least in some rheumatoid sera, the precipitate obtained with fraction II consists of a minimum of two components derived from the serum. Further evidence for this was obtained from cation exchange chromatography. The pool of active fractions (30 ml.) from peak 2 (Fig. 8), to which 20 mg. of bovine serum albumin had been added to stabilize activity, was dialyzed at pH 5.0 (0.01 M) for 24 hours at 4°C. The equilibrated solution was then chromatographed on a column containing 4 gm. of carboxy-

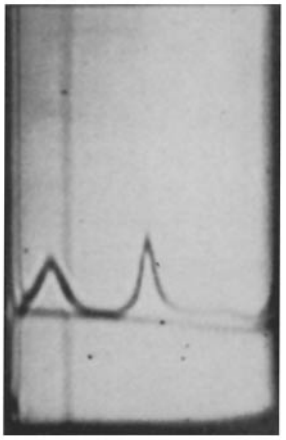

FIG. 10. Ultracentrifugal pattern of agglutinating peak of Fig. 9 at 32 minutes, at 52,000 R.P.M.

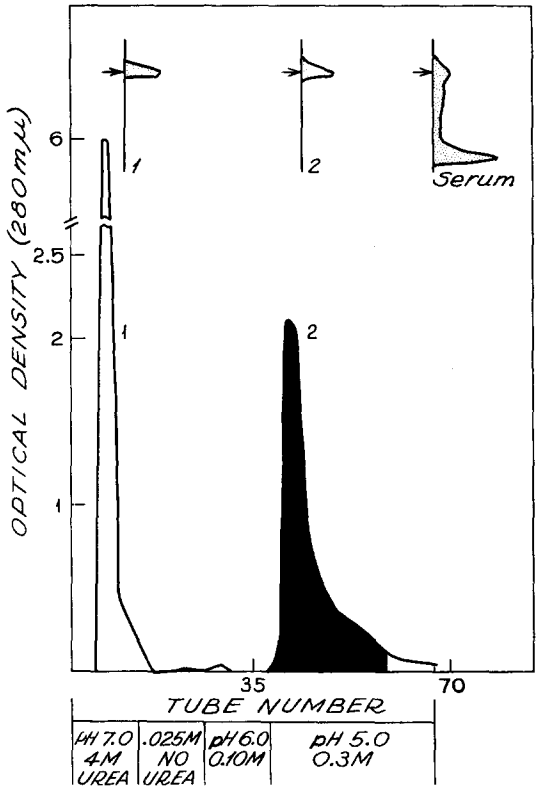

FIG. 11. Anion exchange chromatography of urea dissolved fraction II precipitate from 75 ml. of a rheumatoid serum; shaded area indicates sheep cell agglutination activity.

methyl cellulose as described above. The first peak (Fig. 9) consisted of albumin on electrophoretic analysis. The second peak, containing gamma globulin plus albumin, gave positive tests for agglutination and precipitation. The protein of the third peak, consisting entirely of gamma globulin, gave positive precipitation tests, as indicated by the plus signs, but did not agglutinate sensitized sheep cells. It should be pointed out that chromatography of peak 2 (Fig. 8) on carboxymethyl cellulose was attended by considerable loss of protein.

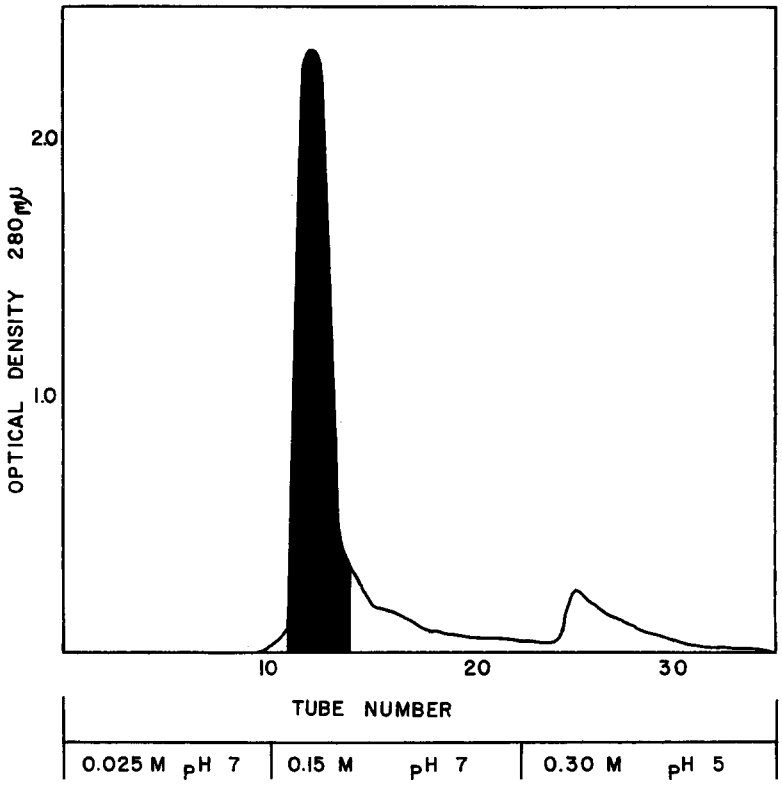

FIG. 12. Anion exchange chromatography of rheumatoid factor, peak 2, Fig. 11; shaded area indicates sensitized sheep cell agglutinating activity.

Ultracentrifugation of the active material of peak 2 from the cation exchanger (Fig. 9) indicated the presence of two major components (Fig. 10), the first with a sedimentation coefficient of 4 S and the second of 19 S. Electrophoresis of the preparation demonstrated the presence of albumin which had not separated completely from the gamma globulin. The absence of significant amounts of contaminants other than the added albumin both by electrophoretic and ultracentrifugal analysis indicated that the active component was composed entirely of 19 S gamma globulin.

The demonstration of precipitating and non-agglutinating protein in peak 3 (Fig. 9) is in agreement with the results obtained by direct chromatography on rheumatoid and normal serum on carboxymethyl cellulose (Fig. 3 and 4).

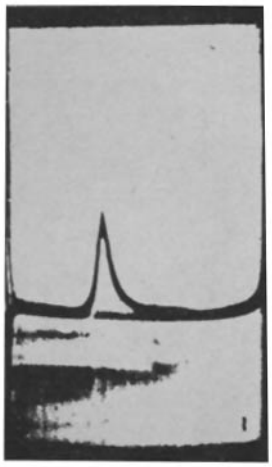

FIG. 13. Ultracentrifugal pattern of active material (Fig. 12) taken at 24 minutes; 52,000 R.P.M.

TABLE II

Activities of Rheumatoid Factor Obtained after Two Successive Anion Exchange Fractionations of Urea Dissolved Precipitate from Rheumatoid Serum

Test	Concentration at End-point
	μg. protein/ml.
Sensitized sheep cell agglutination	0.1–0.5
Latex particle agglutination	0.02
Precipitation with fraction II	10

Preparation of Rheumatoid Factor by Anion Exchange Chromatography.—

In an experiment designed to prepare larger amounts of factor, 375 ml. of a 0.5 per cent solution of heated fraction II was added to 75 ml. of rheumatoid serum and the final volume was brought to 1125 ml. with isotonic saline-phosphate buffer of pH 8.0. The precipitate that formed after two days at 4°C. was washed 4 times with cold saline buffer, then redissolved in 5 ml. of 4 M urea and fractionated in the manner previously described except that no albumin was added.

The chromatogram (Fig. 11) showed two peaks, each with the electrophoretic mobility of gamma globulin. The first peak consisted entirely of 7 S gamma globulin as shown by agar double diffusion tests (18). The second peak, containing the rheumatoid factor activity, was rechromatographed on diethyl-

aminoethyl cellulose using a modified elution technique as shown in Fig. 12. The product consisted of approximately 95 per cent of 19 S gamma globulin, (Fig. 13) with small amounts of heavier sedimenting material. There was no evidence of contaminating 7 S gamma globulin on examination by agar double diffusion tests using rabbit antisera to 19 S and 7 S gamma globulin[3] as well as by ultracentrifugation of the active material. The results of tests performed on this preparation are shown in Table II. In addition the hexose content was determined on two different preparations of this type and values of 5.2 and 5.3 per cent respectively were obtained.

DISCUSSION

It has been pointed out that the serum from some patients with rheumatoid arthritis contains a high molecular weight component with a sedimentation coefficient of 22 S (7). Analysis of this unusual component has shown that it consists of approximately equal quantities of 7 S and 19 S gamma globulins and that the 19 S constituent alone gives the reactions of the rheumatoid factor. The chromatographic method used in this work has made possible the isolation of the active constituent through the separation of 7 S and 19 S gamma globulin on the basis of the anomalous behavior of the larger molecular variety on diethylaminoethyl cellulose (22).

Initial experiments indicated that the rheumatoid factor was separated from the major portion of the gamma globulin during anion exchange chromatography of whole serum (Fig. 1). With the knowledge (7) that the factor was a macroglobulin, it appeared that its unusual chromatographic behavior was due to its large size. It was then concluded that the isolation of the purified factor would require preliminary separation from the other 19 S proteins, and this was done by precipitation with fraction II. Dissociation of this complex with 4 M urea and subsequent chromatography led to the separation of the constituents of the precipitate. The 19 S constituent did indeed have the properties of very highly purified rheumatoid factor (Table II).

In a number of experiments, the chromatographic peak representing purified rheumatoid factor appeared to be resolvable into two components on the anion exchanger (Fig. 8). Of these, one agglutinated sensitized sheep cells and latex particles and precipitated with heated fraction II. The second, although it agglutinated latex particles and precipitated with heated fraction II, did not agglutinate sensitized sheep cells (Table I). The former will be referred to as factor I and the latter as factor II.

The existence of two distinct factors was also indicated by fractionation with the cation exchanger, carboxymethyl cellulose. Chromatograms of whole rheumatoid serum on carboxymethyl cellulose yielded two peaks, only one

[3] Antisera to 7 S and 19 S gamma globulins were kindly provided by Dr. E. C. Franklin, of The Rockefeller Institute.

of which agglutinated sensitized sheep cells although both gave precipitation with fraction II. When active material from anion exchange experiments was chromatographed on the cation exchanger (Figs. 5 and 9), two factors were again demonstrated, only one of which agglutinated sensitized sheep cells. This was true of both the fractions obtained from chromatography of whole serum and the urea dissociated fraction II precipitate.

The precipitation curve between factor II and unheated fraction II (Fig. 6) resembles the usual precipitin curve. However the data are insufficient to determine whether this type of curve is more characteristic of factor II than of factor I, since an inflection point of the type shown is not often obtained with whole rheumatoid serum (23).

The results obtained from cation exchange chromatography of two of four normal sera are of particular interest with regard to factor II. Although the peak corresponding to the one containing factor I in the case of rheumatoid serum demonstrated no activity of any kind, the peak corresponding to factor II showed small but definite precipitating activity in the case of the two positive sera.

It would appear from the results obtained that rheumatoid sera contain at least two factors. These differ, as far as the present data go, in that factor I reacts with sheep cells sensitized with rabbit gamma globulin while factor II does not. This difference could be explained in two ways. One possibility is that factor I can react non-specifically both with human and rabbit gamma globulins, whereas factor II can react only with human gamma globulin. A second possibility is that factor I, since it reacts with antibody sensitized sheep cells, has complement-like activity for antigen-antibody systems in addition to being able to react directly with fraction II.

The demonstration of factor II in normal serum provides a possible explanation for recent observations that sera from patients with sarcoidosis, kala-azar (24), and syphilis (23), agglutinate latex particles and precipitate with fraction II but do not agglutinate sensitized sheep cells. These findings suggest the presence in these sera of a component with the properties of factor II. It is quite possible from the observations reported here, therefore, that these so called false positive reactions are due to an increased concentration of a normally occurring serum constituent.

Factor I, as described here, has been demonstrated to be a 19 S gamma globulin. Although insufficient material was available for ultracentrifugation of factor II, it is quite likely, also, to be a 19 S gamma globulin on the basis of its chromatographic behavior on diethylaminoethyl cellulose and because the active component of the urea dissociated precipitate, which contains both factors, has been shown to contain only 19 S gamma globulin. It has been demonstrated that the circulating, agglutinating and precipitating factors in rheumatoid (7) and various abnormal sera (24) are of the 22 S variety. This

indicates that both 19 S factors form complexes with 7 S gamma globulin in the circulation.

The agglutinating activity against sensitized sheep cells and precipitating activity of purified factor I are similar in magnitude to those found by other investigators (20). The hexose content as determined by the anthrone reaction is in good agreement with the value found by Müller-Eberhard *et al.* (25) for normal gamma globulin.

The use of 4 M urea as a solvent for protein precipitates may be open to objection because of the denaturing effects of this substance. The effect of urea on rheumatoid factor activity, however, appears to be minor since Franklin *et al.* (7) have shown that this factor, when isolated from fraction II precipitates, had the same specific activity when the latter were dissolved in 4 M urea or glycine at pH 3.0. Also, the activity of factor I is in rough agreement with the specific activity of the circulating 22 S component as calculated from the data of Franklin *et al.* (7).

SUMMARY

Serum and serum fractions from patients with rheumatoid arthritis have been subjected to anion exchange chromatography on diethylaminoethyl cellulose. The rheumatoid factor activity was clearly separated from the bulk of the gamma globulin by virtue of the anomalous behavior of macroglobulins on this column.

Purification of rheumatoid factor by precipitation with fraction II, resolution in 4 M urea, and two successive fractionations on an anion exchanger yielded a highly active preparation consisting of approximately 95 per cent 19 S gamma globulin with no detectable amount of 7 S contaminant.

Evidence was obtained for the existence of two factors by cation exchange chromatography (carboxymethyl cellulose) of rheumatoid serum and of macroglobulin previously separated by anion exchange chromatography. One factor (factor I) agglutinated sensitized sheep cells and latex particles and also precipitated with human fraction II. The second (factor II) agglutinated latex particles and precipitated with fraction II but did not agglutinate sensitized sheep cells.

A substance with the properties of factor II was demonstrated in two of four normal sera by cation exchange chromatography. It was suggested that the agglutination of latex particles and precipitation with human fraction II, observed in the case of certain abnormal sera, may be due to elevated concentrations of factor II.

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