

STUDIES ON THE ISOLATION AND CHARACTERIZATION OF THE "RHEUMATOID FACTOR"

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Considerable evidence has been accumulated which indicates that a large proportion of the sera from patients with rheumatoid arthritis contains a factor which has the property of agglutinating particulate bodies such as red blood cells or latex particles when they are coated with γ -globulin or antibodies. Some of these reactions have been employed as diagnostic procedures and the factor or group of factors involved has been termed the "rheumatoid factor." A number of observations (1, 2) have demonstrated that the rheumatoid factor is a γ -globulin with the solubility properties of a euglobulin. More recently, ultracentrifuge experiments (3, 4) have indicated that the rheumatoid factor is a high molecular weight γ -globulin that exists in serum and plasma in the form of a complex. This complex can be visualized directly by means of the analytical ultracentrifuge in whole serum and in γ -globulin and euglobulin fractions and has a corrected sedimentation rate (*s* rate) of approximately 22S. Previous studies (4, 5) on the dissociation of the 22S complex by means of acid and urea have demonstrated that it is composed of two major constituents, one a 7S component and the other 19S. The latter was found to be highly active by the serological reactions for rheumatoid factor. The rheumatoid factor thus appeared to belong to the 19S class of γ -globulins which is known to include certain antibodies as well as the Waldenstrom type macroglobulins (6, 7).

Considerable purification of the rheumatoid factor has been achieved by cellulose ion exchange chromatography (8, 9), by elution from pneumococcus-antipneumococcus complexes (10), by elution from sensitized sheep cells (11), by ultrafiltration with membranes of graded porosity (12), and by preparative ultracentrifugation (4, 13), but isolation in sufficient amounts to determine the complete physical and chemical properties of the rheumatoid factor has not been reported. The observations that rheumatoid factor precipitated di-

rectly with γ -globulin (14) which had been aggregated by the procedures of isolation or heating at 63°C. for 10 minutes (3, 13) offered a method of ready concentration from large volumes of serum. Furthermore the direct visualization of rheumatoid factor in the ultracentrifuge offered a means of observing purification particularly from certain unique sera where it represented a substantial portion of the total γ -globulin.

In the present study rheumatoid factor has been obtained in a high state of purity by several different procedures. Investigations utilizing these preparations have demonstrated that rheumatoid factor is a 19S γ -globulin with chemical and immunological properties similar to those of the normal 19S class of γ -globulins.

MATERIALS AND METHODS

Sera from 4 patients with rheumatoid arthritis were employed in the isolation experiments. These were screened from approximately 100 patients with the disease and upon analytical ultracentrifugal examination contained large amounts of 22S complex as well as showing very high titers in the latex fixation and sensitized sheep cell agglutination reactions. Serum P. R. was unique in the group since it contained at least four times as much rheumatoid factor as the others and showed approximately 1,000 mg. per cent of high molecular weight complex. Serum E. P., R. P. and M. S. contained from 50 to 150 mg. per cent of 22S complex which could be visualized by direct ultracentrifugation of whole serum, euglobulin or γ -globulin fractions.

Euglobulin preparations were prepared from these sera by dilution with 12 volumes of deionized distilled water. After washing with distilled water the precipitates were dissolved in 5 per cent NaCl. Aggregated γ -globulin was prepared by heating the γ -globulin (Lederle Fraction II, 10 mg. per ml.) to 63° C. for 10 minutes. Gamma-globulin-rheumatoid factor precipitates were prepared by adding this altered γ -globulin to rheumatoid serum diluted eight times with isotonic saline. The precipitates were washed once with a large volume of saline and then dissolved in glycine-HCl buffer at pH 3 or in 4 to 6 M urea. The minimum volume was utilized which would completely dissolve the precipitates. In certain of the experiments some of the aggregates of γ -globulin were

precipitated by the addition of NaCl to the acid dissolved precipitates. Two-tenths ml. of 14 per cent NaCl was added to each 1 ml. of acid dissolved precipitate and the resulting precipitate discarded.

The euglobulin solution or dissolved γ -globulin-rheumatoid factor precipitates were layered over a sucrose or NaCl density gradient and separated in the ultracentrifuge utilizing a swinging bucket rotor. The details of this method have been published previously (13). Five-tenths to 1.5 ml. protein solution was layered over 4 ml. of sucrose or NaCl. A time of centrifugation was utilized which permitted the 19S class proteins to sediment to the lower third of the tube and leaving the 7S class proteins in the upper third. The γ -globulin aggregates packed as a pellet at the bottom of the tube. The NaCl density gradient was not as efficient as the sucrose but it proved preferable for obtaining rheumatoid factor preparations for carbohydrate analyses as well as for other analytical experiments; this was because of the difficulty of completely removing sucrose by dialysis and the deleterious effect of prolonged dialysis on the isolated preparations. In most experiments iodoacetate or parachloromercuribenzoate were added to the rheumatoid factor preparation during purification to prevent reduction of the disulfide groups in the protein. Final preparations were concentrated by ultrafiltration.

Solid γ -globulin for adsorption of the rheumatoid factor was obtained by heating Fraction II γ -globulin (Lederle) at a concentration of 40 mg. per ml. to 75° C. The resulting precipitate was washed three times with saline and the final packed precipitate suspended in a finely divided state in two volumes of saline. This material was added to rheumatoid serum. In one experiment 2 ml. of the solid suspension was added to 5 ml. rheumatoid serum and allowed to stand overnight. The precipitate was spun off and added to a second 5 ml. of serum. This was carried out with 25 ml. serum before evidence was obtained by precipitin analysis that no more activity was adsorbed by the solid γ -globulin. The precipitate was then washed three times with saline and the rheumatoid factor eluted with glycine-HCl buffer. Following concentration the eluate was layered over the NaCl density gradient and the 19S rheumatoid factor separated in the ultracentrifuge.

Analytical ultracentrifugation was carried out in a Spinco Model E machine utilizing procedures described previously (3). Free solution electrophoresis was done by the method of Longsworth employing a micro cell. Zone electrophoresis experiments were made in a starch supporting medium after first demonstrating that the rheumatoid factor was not adsorbed to the medium in water flow experiments. All electrophoresis experiments were made at pH 8.6, ionic strength 0.1, in barbital buffer.

Carbohydrate assays were carried out by the same procedures previously utilized for analyses of normal 7S and 19S γ -globulin (15). Serological determinations of rheumatoid factor were made by standard procedures (3). Precipitin tests were carried out in capillary tubes

and the interphase precipitate between rheumatoid factor and γ -globulin which was heated at 63° for 10 minutes, visualized. Precipitin curves in studies on the antigenicity of the rheumatoid factor were carried out by procedures described previously utilizing the modified Folin method for analysis of specific precipitates. Five-tenths ml. of antiserum was used and the nitrogen values in Figure 9 were obtained with this volume of serum. Double-diffusion-in-agar analyses were completely comparable to those used in studies on normal 19S γ -globulin (16). Five different types of antisera were employed and material from more than one rabbit was available in each case. Complement was inactivated by heating at 56° C. for 30 minutes. Antiserum 145 was prepared against normal 19S γ -globulin. The antigen contained approximately 85 per cent 19S protein and 10 per cent 7S γ -globulin. The antibodies were primarily against the 19S constituent. Antiserum 137 was prepared against Fraction II γ -globulin which was demonstrated to be free of 19S material, and the antiserum contained no demonstrable antibodies specific for the 19S constituent. Antiserum 154 was prepared against electrophoretically isolated γ -globulin from a patient with rheumatoid arthritis. The antigen in this case contained approximately 60 per cent 7S, 15 per cent 19S, and 25 per cent 22S material. This antiserum reacted strongly with isolated rheumatoid factor even after complete absorption with 7S γ -globulin. Antiserum 169 was prepared against isolated macroglobulin S prepared from the serum of a patient with Waldenstrom's macroglobulinemia. Antiserum 200 was prepared from macroglobulin L of a different case of Waldenstrom's macroglobulinemia. Each of these antisera was used both unabsorbed and absorbed with 7S γ -globulin. The latter was obtained from a Lederle Fraction II preparation and contained no 19S material on analytical ultracentrifugation. The absorption was usually carried out by three separate additions of 7S material starting with equivalence amounts until no further precipitation was evident. The Waldenstrom macroglobulins were prepared by combined euglobulin precipitation and electrophoresis. They consisted of > 95 per cent high molecular weight protein.

RESULTS

1. Isolation from euglobulin and γ -globulin fractions

Experiments published previously indicated that the activity in the various rheumatoid tests was associated with a rapidly sedimenting component that could be directly visualized in the ultracentrifuge in certain high titer sera. Figure 1 shows the analytical ultracentrifuge pattern of whole Serum P. R. along with the γ -globulin and euglobulin fractions. Determination of the sedimentation rate demonstrated that the rapidly sedimenting peak in each case had an *s* rate of approximately

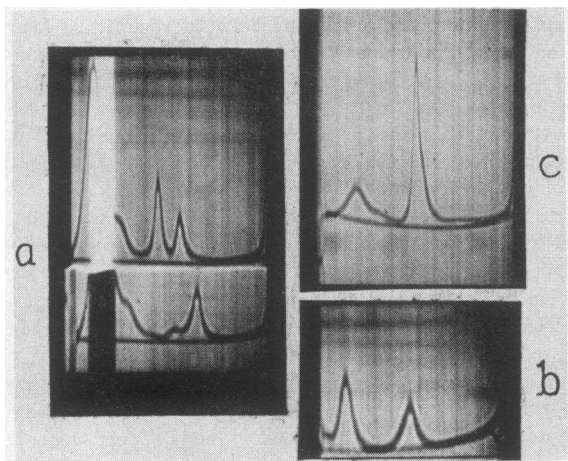


FIG. 1. ULTRACENTRIFUGE PATTERNS OF WHOLE SERUM P. R. (a) AND γ -GLOBULIN (b) AND EUGLOBULIN (c) FRACTIONS

The sedimentation is from left to right. The most rapidly sedimenting peak represents the rheumatoid factor complex. The upper pattern in (a) shows the serum after addition of 19S macroglobulin.

22. The normal 19S component is barely visible in the whole serum pattern and not at all in the euglobulin and γ -globulin fractions. The upper portion of Figure 1a is a duplicate of the lower

pattern except that 19S material from a case of Waldenstrom's macroglobulinemia was added. Similar addition experiments with 19S macroglobulins also showed the higher s rate of the abnormal component in the γ -globulin and euglobulin fractions. The euglobulin fraction of this unusual serum already represented considerable purification of the rheumatoid factor and the activity per mg. of nitrogen was considerably increased. The recovery was approximately 80 per cent. In the other rheumatoid sera, the euglobulin fraction showed considerably larger amounts of contaminating protein and the normal 19S component could be seen as a major peak.

Further purification was achieved by density gradient centrifugation. The euglobulin fraction from Patient P. R. was dissolved in glycine-HCl buffer at pH 4.0 in order to dissociate the 22S complex. It then was layered immediately over a sucrose density gradient. Ultracentrifugation for 16 hours at $110,000 \times G$ permitted the isolation of high molecular weight material at the bottom of the tube free from 7S components which remained in the upper third of the tube. Figure 2b shows an ultracentrifuge pattern of the active

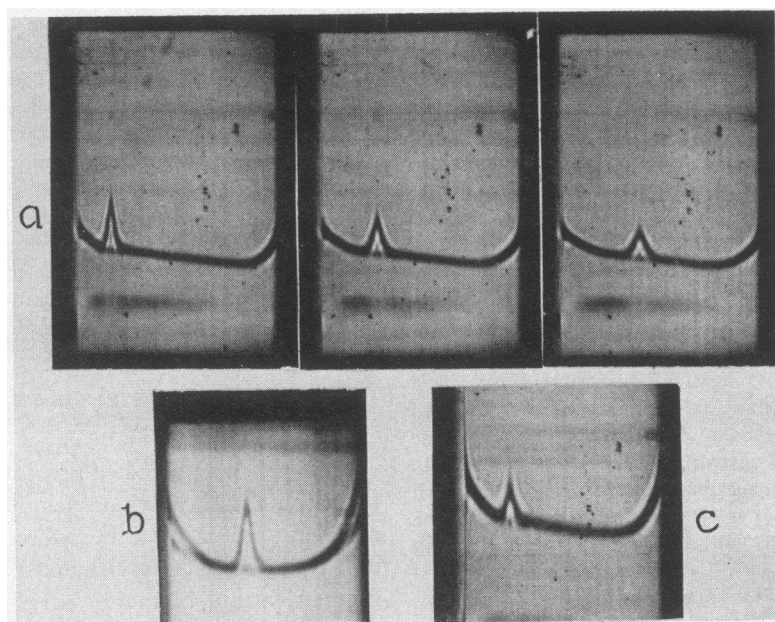


FIG. 2. ULTRACENTRIFUGE PATTERNS OF ISOLATED RHEUMATOID FACTOR PREPARATIONS

The upper patterns (a) are of Preparation II at 12, 20 and 26 minutes at 52,000 rpm. The lower patterns (b) of Preparation I and (c) of Preparation VII are at 28 and 20 minutes, respectively.

material isolated from the lower part of the density gradient tube. It shows a single major peak with an *s* rate of 18.7S. Figure 3 shows the electrophoretic pattern obtained for this preparation by zone electrophoresis in a starch supporting medium. The pattern of the original serum is also shown. The results of activity analyses are indicated and show the marked purification achieved in terms of activity per mg. of protein. The mobility of the activity is similar in the isolated preparation to that in the whole serum with possibly a slight shift to the faster migrating portion of the γ -globulin. Although this preparation appeared quite homogeneous by zone electrophoresis, experiments by moving boundary electrophoresis indicated one peak that was too broad to represent a single component. Table I shows the activity per mg. of this preparation (Preparation I) and other similar preparations. Unfortunately, these tests are only semiquantitative, and the exact activity per mg. is not satisfactorily established. In some experiments the euglobulin or γ -globulin was centrifuged directly without dissociation of the 22S complex with acid. Some dissociation appeared to occur with this procedure alone but it was not always complete and since no detectable loss of ac-

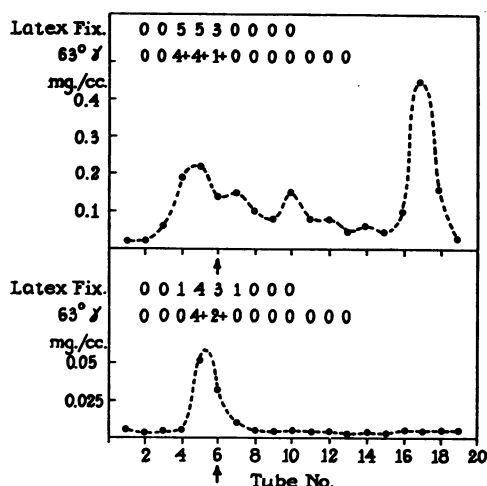


FIG. 3. ZONE ELECTROPHORETIC PATTERN OF SERUM P. R. (UPPER CURVE) AND THE PATTERN OF PREPARATION II ISOLATED FROM THIS SERUM (LOWER CURVE)

The separation was carried on the same starch block and the segments correspond in the two curves. The activity by the latex fixation test is indicated by tube numbers of serial dilutions giving a positive reaction. The precipitin reactivity with heated γ -globulin is also indicated.

TABLE I
Serological activity of eight different preparations of rheumatoid factor homogeneous in the ultracentrifuge

Preparations	Latex fixation	Sensitized sheep cell agglutination	Sensitized sheep cell agglutination + sheep serum
<i>1 mg./ml. conc.</i>			
I P. R. euglob.*	1/10,240	1/640	1/5,120
II P. R. 63° prec.*	1/20,480	1/1,280	1/5,120
III R. P. euglob.†	1/10,240	1/5,120	1/5,120
IV R. P. 63° prec.*	1/10,240	1/5,120	1/10,240
V R. P. Solid eluate*	1/10,240	1/5,120	1/5,120
VI E. P. 63° prec.‡	1/10,240	1/320	1/5,120
VII M. S. 63° prec.*	1/5,120	1/640	1/5,120
VIII E. L. 63° prec.*	1/10,240	1/2,560	1/10,240

* Precipitate dissolved in acid.

† No acid employed in this isolation.

‡ Precipitate dissolved in urea.

tivity was observed following acid dissociation, the euglobulin was usually dissolved in acid buffer prior to zone centrifugation. All of the titers by the latex test in Table I are after 1.5 hours incubation. Higher titers were obtained following overnight standing.

Isolation procedures of this type from euglobulin and γ -globulin fractions cannot be considered a method for the complete isolation of the rheumatoid factor because of contamination with normal 19S γ -globulin. The latter has similar properties and is carried along. However Serum P. R. contained such a uniquely large amount of rheumatoid factor as indicated by the ultracentrifuge patterns of the euglobulin and γ -globulin fractions, that contamination by normal 19S γ -globulin must have been less than 10 per cent. This is not true of the preparations from the sera of Patients R. P. and E. P. where the contamination was significant although not sufficient to be reflected in the titers of the serial dilution tests shown in Table I.

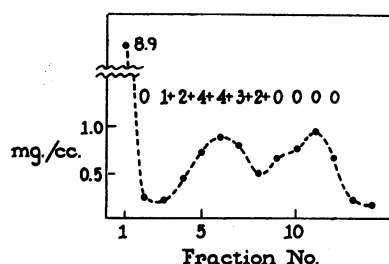


FIG. 4. CURVE OBTAINED BY PROTEIN ANALYSES ON FRACTIONS OF DISSOLVED SPECIFIC PRECIPITATES, SEPARATED BY DENSITY GRADIENT ULTRACENTRIFUGATION

Fraction 1 is from the bottom of the centrifuge tube; Fraction 14 is from the top. The peak in Fractions 4 through 7 represents the 19S rheumatoid factor. The activity in the precipitin reaction is indicated above the curve.

II. Isolation from specific precipitates

In order to eliminate normal 19S γ -globulin from isolated fractions of rheumatoid factor the specific property of this material to precipitate with aggregated γ -globulin was utilized for complete purification. The rheumatoid factor was precipitated from whole serum and euglobulin or γ -globulin fractions. The precipitates were dissolved in glycine-HCl buffer, layered over a density gradient, and separated in the ultracentrifuge. Figure 4 illustrates the pattern obtained by analyses of fractions taken from the density gradient tube at the end of the experiment. Three main peaks are visible. One, which comes from the pellicle at the bottom of the tube, represents primarily aggregated γ -globulin. The second peak which was the active fraction represented 19S rheumatoid factor and the third peak in the upper portion of the tube consisted of material with an s rate between 6 and 12 (usually primarily approximately 7S). The latter was inactive in the various serological reactions and originated primarily from the lower molecular weight portion of the soluble 22S complex from the original serum (4, 5). Combination of the fractions from the 19S peak (Tubes 4 through 7 in the experiment shown in Figure 4) furnished highly purified preparations of rheumatoid factor. Figures 2a and 2c illustrate ultracentrifuge patterns of two of these preparations. The activity per mg. of some of these preparations is shown in Table I. This method made it possible to isolate rheumatoid factor from relatively weakly positive sera. Prepara-

tion VII was obtained from Serum M. S. which had only approximately one-tenth the activity of Serum P. R. Recovery of activity was difficult to determine from activity analyses but by indirect calculation from the amount of 22S component observed in the original serum a value close to 80 per cent could be obtained. In some experiments the dissolved precipitates were partially fractionated prior to centrifugation by precipitation of the aggregated γ -globulin from the acid solution by raising the ionic strength with NaCl. Urea (4 to 6 M) was also used to dissolve the specific precipitates prior to centrifugation (Preparation VI) and results similar to those with acid were obtained. With both the urea and acid experiments, ultracentrifugation was begun within a few minutes after dissolving the precipitates and the rheumatoid factor then sedimented out of these solutions into the density gradient. As a result it was exposed to urea or acid only for a relatively short time.

III. Isolation by adsorption on solid γ -globulin

Previous observations (13) indicated that solid γ -globulin prepared by heat precipitation under controlled conditions adsorbed the rheumatoid factor which then could be eluted by means of acid buffers or urea. Analyses of these eluates indicated that they consisted of from 50 to 70 per cent 19S material. Removal of the eluting solvent by dialysis gave highly active saline solutions but relatively poor recovery because of considerable precipitation. Direct ultracentrifugation in the density gradient of concentrated eluates in acid avoided precipitation and resulted in good recovery of 19S rheumatoid factor in highly purified form. One preparation, the activity of which is listed in Table I, consisted of 96 per cent of one component by ultracentrifuge analysis. This method had advantages over the dissolved precipitate procedure because the absence of the dissolved aggregates of γ -globulin facilitated the purification in the ultracentrifuge. The major limitation was in the fact that some preparations of solid γ -globulin removed only slight activity from the original serum while others removed all activity. Sera from different patients also varied in respect to the removal of activity.

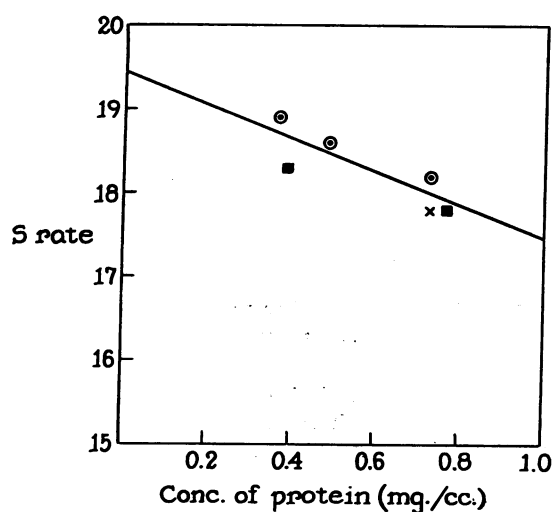


FIG. 5. THE s VS. c PLOT FOR SEVERAL PREPARATIONS OF ISOLATED RHEUMATOID FACTOR SHOWING THE SEDIMENTATION RATE AT INFINITE DILUTION

By the method of least squares, $y = -1.97x + 19.44$. The different symbols represent different preparations.

IV. Physical and chemical properties

Figure 5 shows a plot of the s vs. c relationship for several preparations of isolated rheumatoid factor examined in the ultracentrifuge. The extrapolated line obtained by the method of least squares shows after correction for the buffer solvent that $s^{\circ}_{20,w} = 19.4S$.

Five samples of isolated rheumatoid factor prepared from acid dissolved precipitates were studied by free solution electrophoresis. All of these preparations were found to be heterogeneous. In two instances a single peak was observed but this was broader than would have been expected for a single component (Figure 6a). Reversible boundary spreading experiments confirmed this heterogeneity. In three instances a second peak could be seen on the faster side of the main peak.

This is illustrated in Figure 6b. Both peaks were broader than expected for single components. Ultracentrifugal analysis of these same preparations demonstrated that the second peak also represented 19S protein and was not due to contaminants. In one experiment the second peak made up 18 per cent of the total protein by electrophoresis but by ultracentrifugal analysis there was less than 5 per cent of proteins other than those in the 19S peak. In addition, strong evidence was obtained that the faster migrating material was rheumatoid factor in that it again precipitated with aggregated γ -globulin when this was added to the isolated preparation. In several experiments all detectable protein in the isolated fraction reprecipitated on the addition of aggregated γ -globulin. The mobility of the major peak ranged from 1.1 to 1.5×10^{-5} in these experiments.

Table II shows the results of nitrogen and carbohydrate analyses on three different prepara-

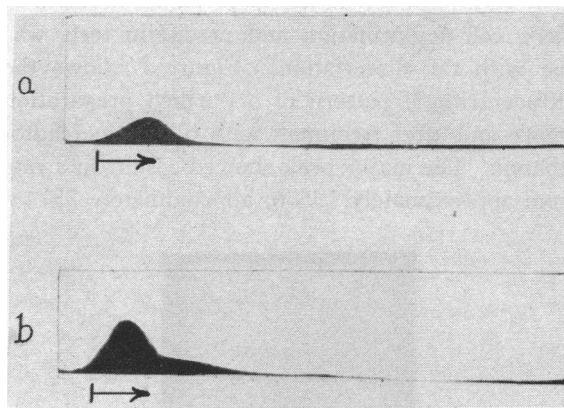


FIG. 6. FREE SOLUTION ELECTROPHORESIS PATTERNS OBTAINED ON TWO PREPARATIONS OF ISOLATED RHEUMATOID FACTOR (UPPER, PREPARATION II; LOWER, PREPARATION VIII)

Photographs taken at approximately two hours at field strength of approximately 6 volts per cm.

TABLE II
Carbohydrate content of several preparations of isolated rheumatoid factor *

Protein	N	Hexose	Hexosamine	Sialic acid	Total carbohydrate	Hexosamine/Hexose
	%	%	%	%	%	
Norm. γ -glob. (7S)	15.64	1.22	1.14	0.22	2.58	0.94
Norm. γ -glob. (19S)	14.47	5.20	2.9	1.70	9.80	0.55
Rh. f. Prep. II	14.54	5.40	2.9	1.80	10.10	0.55
Rh. f. Prep. IV		5.3	2.0	1.70		
Rh. f. Prep. V		5.1				

* Previous results for normal 7S and 19S γ -globulin are also indicated.

tions of isolated rheumatoid factor. Preparation II was analyzed in considerable detail and the figures illustrated were obtained from duplicate assay. The experimental error of these methods has been described previously (15). Hexosamine and sialic acid values on Preparation IV were the results of single determinations. Insufficient material was available to check the one low hexosamine figure. Numerous other studies on the hexose content of different preparations gave similar results. The carbohydrate content of the rheumatoid factor was very close to that previously reported for the normal 19S fraction of γ -globulin (15) which is shown in Table II for purposes of comparison.

Mercaptoethanol and other sulfhydryl reagents were found to dissociate the rheumatoid factor in a manner similar to that reported for Waldenstrom macroglobulins (17) and the cold agglutinins (7). Some of these results have been published previously (4). Activity in the latex fixation, sensitized sheep cell agglutination and precipitin tests was lost with the dissociation. Figure 7 shows the ultracentrifugal pattern of a purified preparation before and after treatment with 0.1 M mercaptoethanol. The major peak showed a shift in s rate from approximately 19S to approximately 7S.

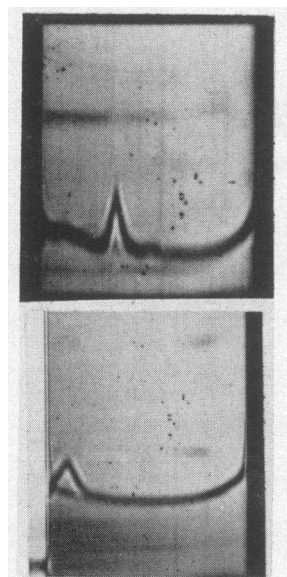


FIG. 7. ULTRACENTRIFUGE PATTERNS OF ISOLATED RHEUMATOID FACTOR BEFORE (UPPER) AND AFTER (LOWER) DISSOCIATION WITH MERCAPTOETHANOL. Patterns at 28 minutes in each case.

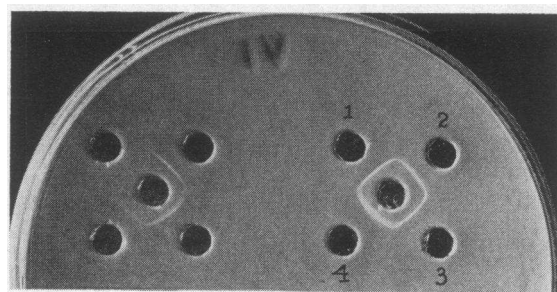


FIG. 8. OUCHTERLONY PLATES SHOWING REACTION OF VARIOUS ANTISERA WITH ISOLATED RHEUMATOID FACTOR

The antisera used in both experiments were the same except that in the left series each antiserum was absorbed with 7S γ -globulin. The rheumatoid factor is in the middle well in both instances. The antisera beginning at the upper left and proceeding clockwise are as follows in both instances: 1) As. 137 against 7S γ -globulin; 2) As. 145 against normal 19S γ -globulin; 3) As. 154 against rheumatoid factor γ -globulin; 4) As. 169 against an isolated Waldenstrom macroglobulin.

V. Immunological studies

Various modifications of the Ouchterlony procedure indicated a close immunological relationship between the isolated rheumatoid factor, normal 19S γ -globulin and isolated macroglobulins prepared from cases of Waldenstrom's macroglobulinemia. Figure 8 illustrates one of these experiments. Preparation IV of isolated rheumatoid factor was placed in the center well and reacted with four different antisera, both unabsorbed and after absorption with 7S γ -globulin. A single major line which fuses is visible with the unabsorbed antisera. Absorption removed the line with antiserum 137 which contained primarily antibodies to 7S γ -globulin. The absorbed antisera reacted with the rheumatoid factor in a single line and little difference in reactivity could be detected between the antisera against normal 19S, rheumatoid factor or macroglobulin. Control experiments with isolated normal 19S γ -globulin gave almost identical results with these same antisera.

When experiments were carried out with parallel wells and isolated rheumatoid factor compared directly with normal 19S γ -globulin, single lines were observed with the different antisera and the line for normal 19S protein fused with that for rheumatoid factor without spur formation. In these experiments both the normal 19S line and the rheumatoid factor line showed curvature to-

ward the antigen well indicating, as demonstrated by Korngold and Van Leeuwen (18), that the observed reaction was due to a high molecular weight component.

Figure 9 illustrates precipitin curves of these same absorbed antisera with isolated rheumatoid factor as well as with isolated Waldenstrom macroglobulins and normal 7S γ -globulin. Figure 9a shows the curves obtained with rheumatoid factor, macroglobulin L and macroglobulin B with antiserum to normal 19S γ -globulin. The rheumatoid factor reacts slightly more strongly. A similar relationship is seen in Figure 9b where antiserum to rheumatoid factor was employed. Figure 9c shows that rheumatoid factor reacts more strongly

with antiserum to macroglobulin S than do heterologous macroglobulins B and L. However, homologous macroglobulin L reacts most strongly with antimacroglobulin L (Figure 9d). It appeared clear that rheumatoid factor reacted strongly per unit of nitrogen as compared with isolated Waldenstrom macroglobulins against macroglobulin antisera absorbed with 7S γ -globulin. In the course of these experiments, three different antisera to rheumatoid factor γ -globulin were studied. None of these showed any reaction with rheumatoid factor following absorption with normal γ -globulin rich in 19S components.

DISCUSSION

The use of sera containing very high titers in the serological tests and uniquely large amounts of rheumatoid factor as demonstrated directly in the ultracentrifuge made it comparatively easy to isolate this factor in a highly purified state. Serum P. R. which was used extensively in this study contained approximately 10 mg. per ml. of rheumatoid factor complex and the γ -globulin fraction consisted of approximately 40 per cent of this material. The high concentration of rheumatoid factor in this serum made isolation possible directly from euglobulin and γ -globulin fractions with only slight contamination with normal 19S class proteins. With other sera this contamination proved substantial and the specific property of precipitation with aggregated γ -globulin had to be utilized for satisfactory purification. The methods used for isolation all utilized preparative ultracentrifugation for final purification and since the high molecular weight property was somewhat unique the isolated fractions consisted of close to 100 per cent rheumatoid factor. This was indicated both by the homogeneity of the analytical ultracentrifuge patterns and by the reactivity and utilization of all of the protein in the precipitin tests with aggregated γ -globulin. Despite this evidence of biological homogeneity, free solution electrophoresis experiments indicated considerable heterogeneity and it was clear that a group of different proteins was involved. The heterogeneity was similar to that described for γ -globulin itself (19). This was to be expected since activity curves on whole serum separated by zone electrophoresis indicated that the activity peak was too

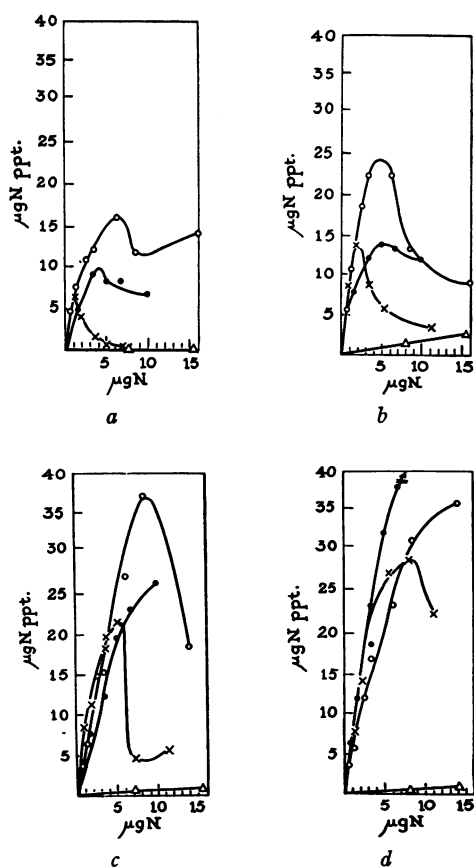


FIG. 9. PRECIPITIN CURVES OF ISOLATED RHEUMATOID FACTOR (○—○), MACROGLOBULIN L (●—●), MACROGLOBULIN B (×—×) AND 7S γ -GLOBULIN (Δ — Δ) WITH VARIOUS ANTISERA ABSORBED WITH 7S γ -GLOBULIN

- Antiserum 145 against normal 19S γ -globulin.
- Antiserum 154 against rheumatoid factor γ -globulin.
- Antiserum 169 against macroglobulin S.
- Antiserum 200 against macroglobulin L.

broad to represent a single protein species. In addition, it is known that in serological tests employing human γ -globulin the latter reacts with more rheumatoid factor protein than does rabbit γ -globulin in tests where it is the reactive substance (20, 21). The picture resembles that known for the usual mixtures of species specific and cross-reacting antibodies. Recently, Lospalluto, Lewis and Ziff have succeeded in separating at least two of these factors by ion exchange chromatography (8). In the present study, absorption experiments were also carried out with antigen-antibody complexes by the method of Vaughan (20) and with finely dispersed solid rabbit γ -globulin. Only part of the isolated rheumatoid factor was absorbed leaving material that reacted strongly with human γ -globulin. In addition, one macroglobulin preparation was isolated from a sarcoid serum that showed no activity in the sensitized sheep cell test but high titers by the tests employing human γ -globulin. The accumulated evidence points to the existence of multiple, closely related macroglobulins which have the common property of reacting with human γ -globulin.

Suggestive evidence that the rheumatoid factor contains considerable carbohydrate was obtained by a number of workers prior to its isolation (20, 22, 23). The present observations indicate approximately 10 per cent carbohydrate made up of hexose, hexosamine and sialic acid. The analytical values are very close to those previously obtained for normal 19S γ -globulin and for certain macroglobulins from the sera of patients with Waldenstrom's macroglobulinemia.

The immunological results with the isolated rheumatoid factor also indicate a close relationship to normal 19S γ -globulins and pathological macroglobulins. Antisera to crude rheumatoid factor preparations did not show antibodies specific for this type of protein and these antisera could be absorbed completely by normal proteins. The possibility must be considered that rheumatoid factor exists in normal serum and is a component of normal 19S γ -globulin, thus accounting for some cross-reactions between different antisera. However, quantitative studies indicated that the close immunological relationship between rheumatoid factor, normal 19S γ -globulin and pathological macroglobulins could not be accounted for by small

amounts of rheumatoid factor in the latter preparations. All the evidence pointed to the fact that these 19S proteins with different biological properties behaved very similarly as antigens. Future work may indicate fine differences between rheumatoid factor and normal 19S proteins as has already been demonstrated between different Waldenstrom macroglobulins (24). Difficulties in the complete isolation of adequate amounts of normal 19S proteins hampered final resolution of this question. In addition, the secondary reaction of rheumatoid factor with soluble and insoluble antigen-antibody complexes (13, 20) obscured certain of the quantitative interpretations particularly in regions of antigen excess for rheumatoid factor.

Considerable evidence has accumulated indicating that the 19S class of γ -globulins in normal and certain pathological sera are made up of a group of different antibodies. Certain of the isoagglutinins, the saline agglutinating rh antibodies, certain of the Wasserman antibodies, heterophile antibodies, cold agglutinins, certain of the typhoid agglutinins and many other antibodies have been localized in this fraction (6, 7, 25, 26). The Waldenstrom macroglobulins appear to be exceptions and seem to bear a relationship to the 19S class of γ -globulins similar to that of the myeloma proteins to the major 7S class of γ -globulins. Since the rheumatoid factor, on the basis of the ultracentrifugal data, the carbohydrate content, the ready dissociation with mercaptoethanol and the immunological results, is closely related to the 19S class of γ -globulins, the possibility is raised that it represents an antibody. Final proof of such a hypothesis must await clear elucidation of the antigen involved. Certain evidence suggests that ordinary γ -globulin of the 7S variety is itself the antigen. No other protein has yet been found that will react with the rheumatoid factor in a similar fashion. This reaction is observed in the soluble complex in rheumatoid arthritis sera, in the precipitin reaction with aggregated γ -globulin, in the specific adsorption on solid γ -globulin, and in the agglutination of red cells coated with incomplete antibodies. In the latter reaction the rheumatoid factor acts in a manner analogous to Coombs sera or rabbit antibody to γ -globulin and sera containing rheumatoid factor actually have been utilized for this purpose (27).

SUMMARY

Highly active purified preparations of rheumatoid factor were obtained from selected sera showing high titers in the latex fixation and sensitized sheep cell agglutination reaction. Procedures of density gradient ultracentrifugation were utilized for isolation from euglobulin fractions, from dissolved specific precipitates produced by the addition of aggregated γ -globulin to rheumatoid serum and from eluates of solid γ -globulin to which rheumatoid factor had been adsorbed.

The isolated preparations were homogeneous in the analytical ultracentrifuge and showed a corrected sedimentation rate of approximately 19S. Dissociation into smaller subunits was produced readily with mercaptoethanol with simultaneous loss of activity. Free solution electrophoresis showed the preparations to be primarily γ -globulin with evidence of heterogeneity. The accumulated evidence indicated that the rheumatoid factor consists of multiple, closely related macroglobulins.

Carbohydrate analyses showed a total content of approximately 10 per cent consisting of hexose and hexosamine sugars and sialic acid. The results were very similar to those previously obtained for normal 19S γ -globulin. Immunological studies also indicated a close relationship to this normal fraction.

The question of whether the rheumatoid factor represents an antibody is discussed. The close relationship to the broad 19S class of γ -globulins which are known to contain a variety of antibodies is an additional point in favor of this hypothesis although it remains to be proven.

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