AN UNUSUAL PROTEIN COMPONENT OF HIGH MOLECULAR WEIGHT IN THE SERUM OF CERTAIN PATIENTS WITH RHEUMATOID ARTHRITIS

By E. C. FRANKLIN, M.D., H. R. HOLMAN, M.D., H. J. MÜLLER-EBERHARD, M.D., AND H. G. KUNKEL, M.D.

(From The Rockefeller Institute for Medical Research)

(Received for publication, January 18, 1957)

Normal human serum has long been known to contain a high molecular weight protein component which is demonstrated by direct examination in the ultracentrifuge (1). This material, with a sedimentation constant of approximately 19 S, has been found in recent studies to be composed of two main types of protein, one migrating electrophoretically as an α_2-globulin and the other as a γ-globulin (2). The 19 S γ-globulin is of particular interest because it appears to contain certain antibodies. Evidence has accumulated suggesting that some of the isoagglutinins (1) and Wassermann antibodies (3), the complete Rh agglutinins (4), and certain pathological hemagglutinins (5) fall into this fraction. Chemical and immunologic differences between the 7 S and 19 S γ-globulin indicate that the latter component is not a simple aggregate (6, 7).

Molecules with an *s* rate greater than 19 S are not detectable in significant amounts in fresh normal serum observed directly in the ultracentrifuge. Thus far such proteins have been reported only in the sera of patients with Waldenström's macroglobulinemia where they represent secondary components associated with marked elevation of the 19 S fraction (8).

It is the purpose of this paper to describe a serum protein component with an *s* rate greater than 19 S in the γ-globulin fraction of certain patients with rheumatoid arthritis, and to present preliminary data on its physical and chemical properties. Since sera from patients with rheumatoid arthritis are known to potentiate certain immunologic reactions such as the sensitized sheep cell agglutination, and form a precipitate with γ-globulin, the relation of this component to these serologic reactions was investigated.

Materials and Methods

1. Ultracentrifugation.—Analytical examinations of whole sera and of isolated serum protein fractions were carried out in a Spinco model E ultracentrifuge. Results were quantitated according to methods described by Trautman (9). Although the initial observations were made on electrophoretically prepared γ-globulin fractions, most of the subsequent studies were carried out on whole sera. This was preferable for purposes of quantitation because aggregation and selective loss of the high molecular weight fraction

were sometimes observed during isolation. Physicochemical studies, however, were performed on isolated preparations of γ-globulin.

A refrigerated Spinco model L ultracentrifuge was used to prepare samples rich in high molecular weight components. This was accomplished by 3 to 8 centrifugations of whole sera or γ-globulin fractions at 40,000 R.P.M. The duration of each of these was sufficient to allow all molecules with $s_{20,w}$ greater than 19 S to be packed into a pellet at the bottom

TABLE I

Comparison of the Reaction between Various Preparations of γ-globulin, both Intact and Altered, with Various Control and Rheumatoid Arthritis Sera

γ-globulin	State	Normal	Cirrh.*	Rheum. I	Rheum. II
10 mg./cc.					
Fr. II	Intact	0	0	0	0
Led. C-439	Euglobulin	0	0	2+	3+
	Heated‡	0	0	2+	4+
	Alkali treated	0	1+	2+	4+
Fr. II	Intact	0	0	1+	2+
Cut. Pool "B"	Heated	0	Tr.	2+	4+
Fr. II	Intact	0	0	0	0
Sq. 57	Heated	0	Tr.	2+	4+
Fr. II	Intact	0	0	2+	3+
Sq. 606	Heated	0	0	2+	4+
El. γ-glob.	Intact	0	0	0	0
Normal A.B.	Heated	0	0	2+	3+
El. γ-glob.	Intact	0	0	0	0
Cirrh. C.P.	Heated	0	2+	2+	4+
El. γ-glob.	Intact	0	0	0	0
Pool	Heated	0	Tr.	2+	4+

* Cirrhosis serum with 5 gm. per cent γ-globulin.

‡ Heated = 62–65°C. for 5 to 15 minutes.

of the tube. Depending on the protein concentration, this time varied between 2 and 11 hours. The supernatants were poured off and the pellets resuspended in buffer prior to each centrifugation.

In addition, density gradient centrifugation was carried out in a swinging bucket rotor containing a sucrose gradient in isotonic saline. The gradient was formed by mild stirring and diffusion of three sucrose layers with concentrations up to 25 per cent at the bottom, and 0.9 per cent saline at the top. Serum (0.2 ml.) diluted with 1 volume of saline was layered above and centrifuged for 5 hours at 110,000 *g*. The distribution of γ-globulin in the gradient was determined by quantitative immunologic assay using rabbit antiserum against 7 S γ-globulin free of 19 S material, and against 19 S γ-globulin. The latter was prepared by absorbing antisera to whole γ-globulin with 7 S γ-globulin (7).

2. Electrophoresis.—Zone electrophoresis was carried out according to methods previously described, using starch and polyvinyl as supporting media and barbital buffer pH 8.6, Γ/2 0.05–0.1 (10, 11). The blocks were divided into ½ inch segments and eluted by displacement filtration. Protein concentration was determined by the modified Folin tyrosine method. Eluates were subsequently used for ultracentrifugal and biological studies.

3. Biological Tests.—A direct reaction between rheumatoid arthritis serum and Fr. II γ-globulin was described by Epstein and associates (12). Observations with numerous preparations of γ-globulin in the present study indicated that fresh γ-globulin preparations isolated under optimal conditions either reacted very weakly or failed to give a direct reaction with formation of a precipitate. Table I shows the results obtained with seven different preparations of γ-globulin. One preparation, a Squibb Fraction II sample supplied in 1951 and the oldest and least soluble of the group reacted strongly in the unaltered state. Another relatively fresh preparation obtained from Cutter Laboratories also gave a distinct reaction. The other γ-globulin fractions, including two isolated from single sera and one isolated from pooled sera by starch zone electrophoresis did not show capillary tube precipitation in 30 minutes. However, each preparation, following heating at 62–65°C. for 5 to 15 minutes gave a precipitate either immediately or within one-half hour with the two rheumatoid sera used in this experiment. Active preparations were also obtained from the euglobulin fraction prepared by dialysis against distilled water, as well as by treatment of γ-globulin with alkali (pH 12.5). The activity could be further concentrated from all the preparations by short periods of ultracentrifugation. The active material sedimented rapidly and was clearly partially aggregated γ globulin. No activity remained in the supernate at the position of 7 S material which contained the bulk of the γ globulin. In the remainder of this study, the γ-globulin precipitation test was performed using heated Lederle Fr. II γ-globulin. The altered γ-globulin preparations were layered over sera in capillary tubes. They were read at 2 minutes and 30 minutes by observing the precipitate at the interphase. The titers of highly active sera were determined by layering different dilutions over γ-globulin solutions of increased density, thus allowing a sharp interphase to be formed.

Sensitized sheep cell agglutination reactions were carried out with absorbed sera and protein fractions by the procedure of Heller and associates (13). The sensitization was carried out at $\frac{1}{10}$ the basic agglutination titer and sheep serum was omitted.

RESULTS

1. Presence of a High Molecular Weight Component in the Whole Serum and the γ-globulin Fraction of Certain Patients with Rheumatoid Arthritis

31 sera from patients with rheumatoid arthritis, one with associated psoriasis, and 37 sera from normal control subjects and patients with the diseases listed in Table II were examined in the analytical ultracentrifuge. All sera were diluted with an equal volume of normal saline and with few exceptions ultracentrifugal examination was carried out within 1 week after being drawn. In all the sera examined a peak previously shown to have an $s_{20,w}$ = 19 S separated in 12 to 24 minutes at 52,640 R.P.M. This can be observed in the pattern of the normal serum shown in Fig. 1. In 14 of the sera from patients with rheumatoid arthritis this peak divided into 2 parts, one of which corresponded to the normal 19 S component and one of which sedimented more rapidly. Fig. 1 shows the appearance of this second rapidly sedimenting peak at 8 minute intervals in the serum of one patient with rheumatoid arthritis.

Fig. 2 illustrates the presence of this material in three additional sera with measurable amounts of this unusual component.

Table II shows the results of quantitative determinations of the unusual higher molecular weight component in various sera. In one patient with rheu-

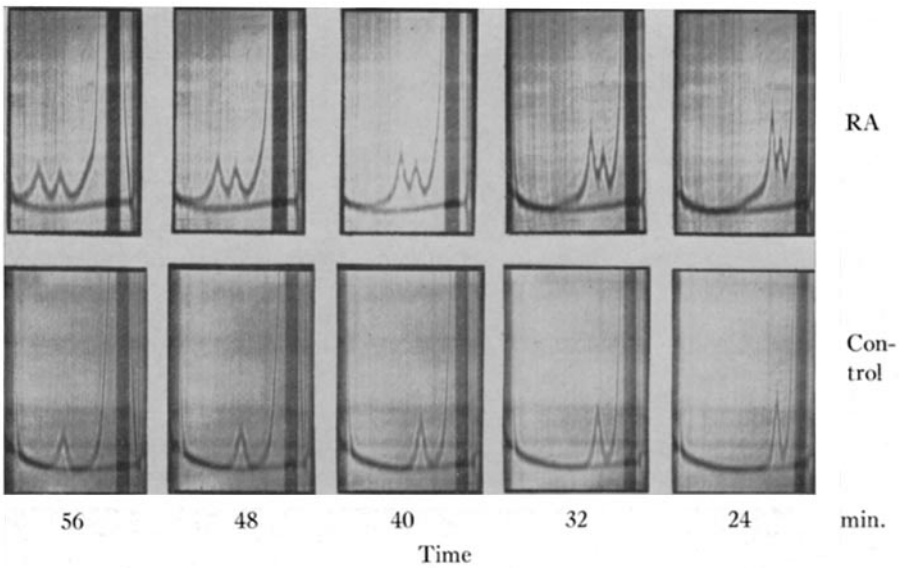

FIG. 1. Ultracentrifugal patterns at 8 minute intervals of a normal serum and a serum from a patient with rheumatoid arthritis. A second component sedimenting more rapidly than the normal 19 S component is visible in the rheumatoid arthritis serum.

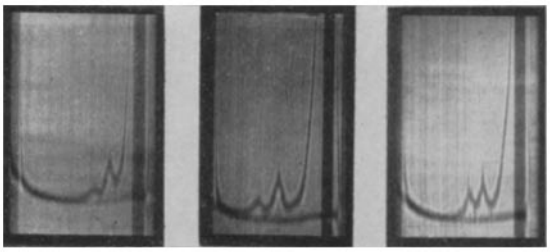

FIG. 2. Representative ultracentrifugal patterns of three sera from patients with rheumatoid arthritis containing smaller amounts of the high molecular weight component than the serum of Fig. 1.

matoid arthritis the concentration of this material was 340 mg. per cent (Fig. 1) which is greater than that of the 19 S component which normally makes up about 5 per cent of the serum proteins (2). In six others the concentration varied between 75 and 175 mg. per cent while in seven of the patients with

rheumatoid arthritis it was present in a concentration of less than 75 mg. per cent. This was too small to be accurately quantitated and was noted only as a distinct asymmetry preceding the 19 S material.

In the control group, none of the sera, with the exception of one serum from a patient with infectious mononucleosis with a heterophile titer of 3584, contained detectable amounts of material sedimenting more rapidly than the 19

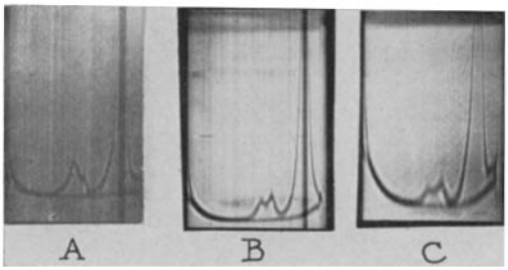

FIG. 3. Ultracentrifugal patterns of the γ-globulin fractions electrophoretically prepared from the sera of 3 patients with rheumatoid arthritis showing the normal 19 S and the unusual high molecular weight component in different relative concentrations.

TABLE II

Results of Analyses for the 22 S Component in the Sera of Patients with Rheumatoid Arthritis and Control Sera

Mg. Per Cent 22 S	Diagnosis															
	Normal	Rheumatoid arthritis	Psoriasis rheumatoid arthritis	Lupus erythematosus	Cirrhosis	Hepatitis	Rheumatic Fever	Sarcoid	Infectious mononucleosis	Nephrosis	Hemochromatosis	Wilson's disease	Scleroderma	Muscular dystrophy	Bronchiectasis	Tuberculosis	Osteoarthritis
None	6	16	1	7	5	4	2	2	2	1	1	1	1	1	1	1	1
Less than 75		7							1								
75–125		3															
125–175		3															
>300		1															

S component. In the latter serum the heavier material was not distinguishable as a peak but as a small, widely dispersed elevation above the base line. The concentration of the normal 19 S component was also unusually high.

Seven of the sera with measurable amounts of the unusual high molecular weight fraction were separated by starch zone electrophoresis. In the 5 with the largest amounts, the same component was present in the γ-globulin fraction (Fig. 3) while in two others with initially low concentration it was no

longer demonstrable. In the one γ-globulin fraction (Fig. 3 A) from the serum shown in Fig. 1, the normal 19 S component is barely visible because of the high concentration of the unusual component. Examination of the β and α_1-globulin and albumin fractions of one, and the α_2-globulin fractions of two sera with high concentrations of the high molecular weight material showed none of the abnormal component, although 19 S material which is normally present in the α_2-globulin (2) was observed. Ultracentrifugal analysis of the joint fluid and its γ-globulin fraction from the patient containing a serum level of 340 mg. per cent revealed the high molecular weight component in each case, but at a concentration somewhat lower than in the serum.

2. Physical and Chemical Characteristics of the High Molecular Weight Component

The γ-globulin from the serum containing the largest amount of this component was separated by starch zone electrophoresis into 7 fractions of different mobilities. Each of these was then examined in the analytical ultracentrifuge. Fig. 4 shows the high molecular weight protein distributed throughout most of the γ-globulin fraction. Its electrophoretic mobility was, however, somewhat faster than that of the peak of the γ-globulin.

Although evidence of heterogeneity in the ultracentrifuge was obtained in certain instances, an attempt was made to determine an approximate sedimentation coefficient of the unusual protein.

Since in the whole γ-globulin fractions examined the high molecular weight component never made up more than 20 per cent of a three component system, it was difficult to calculate its sedimentation coefficient at infinite dilution by analysis of its s *vs.* c dependence. Therefore, 9 fractions of γ-globulin from 4 different sera were examined at different protein concentrations and the s rates of the normal 19 S and the unusual high molecular weight components were determined. From the observed values, the s rate of a component at infinite dilution can be calculated by applying a correction for the concentration of itself and all slower sedimenting material as previously described (2). The kj of 7 S γ-globulin was 0.007 ml./mg., that of the 19 S and heavier material was assumed to be 0.017 ml./mg. (2). The observed sedimentation coefficients in barbital buffer for the unusual rapidly sedimenting component varied between 15.9 and 18.3 S. Application of the corrections for concentration differences and addition of 10 per cent to convert from barbital buffer to distilled water resulted in a calculated $s^0_{20,w}$ of 21.2 and reduced the range of variation to 0.8 S (20.9 to 21.7 S). This suggested that the same molecular species was present in each of the 4 sera examined. The s rate of the normal 19 S component calculated at the same time was somewhat low (17.2 to 18.5 S) compared to previously determined values of 18.5 S (2), and suggested that the value 21.2 for the unusual component might be low also. Since this could be due to the large excess of 7 S material present, one preparation of γ-globulin was repeatedly centrifuged in 5 per cent NaCl to decrease the concentration of 7 S material and to increase that of the heavier molecules. Examination of this preparation which contained approximately 25 per cent 7 S material at two dilutions and application of the same corrections resulted in approximate $s^0_{20,w}$ of 18.2 and 22.7 S for the normal and abnormal components, respectively. For purposes of convenience the unusual high

molecular weight material will be referred to subsequently as the 22 S component. However, in view of the broadness and asymmetry of the observed peak in the ultracentrifuge it appears quite likely that this component is composed of more than one molecular species, and that the values obtained represent either the sedimentation coefficient of the major constituent or the average of all of them. Accurate determinations must await the isolation of the substance in pure form.

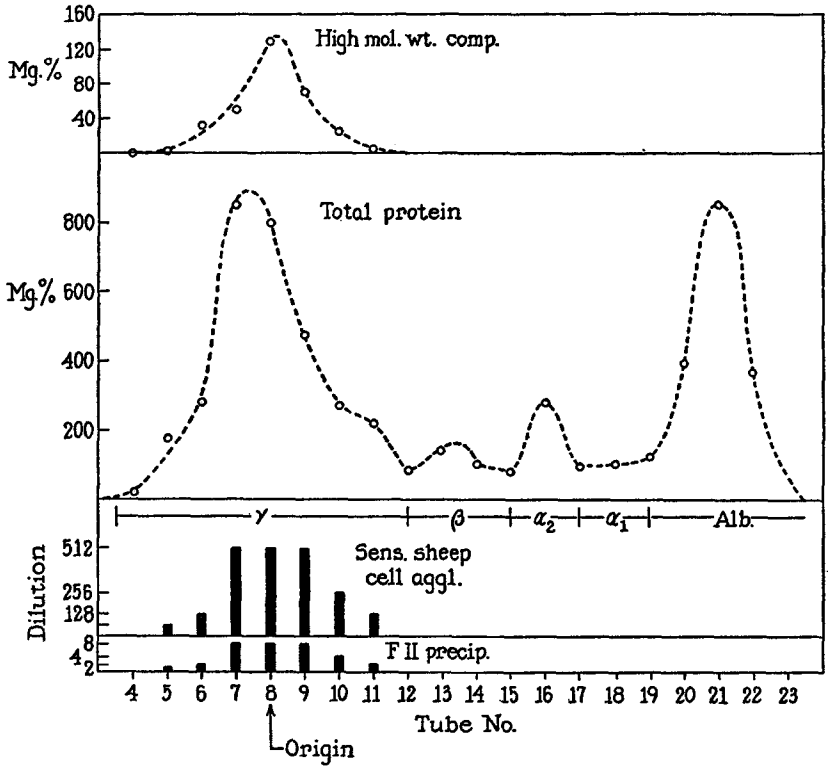

FIG. 4. Electrophoretic distribution of the serum proteins of a patient with rheumatoid arthritis compared to the unusual high molecular weight component (above) and the activity in the sensitized sheep cell agglutination and γ-globulin precipitation tests (below).

To characterize further this component, attempts were made to dissociate it with 4 to 6 molar urea, acid buffer pH 3, and saline in concentrations up to 15 per cent. Urea in concentrations between 4 to 5 molar resulted in disappearance of this component and a corresponding increase in the 19 and 7 S material. Numerous observations of this type which will be reported in detail separately (14) indicated that the high molecular weight protein was a complex consisting of approximately 50 per cent 19 S material and 50 per cent of another protein probably of the 7 S type and that it was dissociated into its component parts in urea. The 19 S material released on dissociation sedimented exactly as the

normal 19 S and showed a protein and carbohydrate content similar to that previously described for 19 S γ-globulin (6, 14). Because of its apparent heterogeneity and broad electrophoretic distribution it is quite likely that the 22 S component is composed of several complexes with different molar ratios of 7 and 19 S γ-globulin. Assuming the molecular weight of the 7 S material to be 160,000 and that of the 19 S γ-globulin to be 900,000 the approximate average molar ratio of 7 to 19 S material in this complex would be of the order of 5 to 1. Although the component was most readily dissociated in urea, it could also be broken down with acid buffers to a 19 S fraction and another of lower molecular weight.

TABLE III

The Relationship of the Amount of the 22 S Component to Activity in the γ-globulin Precipitation and Sheep Cell Agglutination Tests

Amount of 22 S material	γ-globulin precipitation					Sensitized sheep cell agglutination						
	Dilution					Dilution						
	Neg.	0–25	50	100	>150	<32	64	128	256	512	1024	2048
None	6	8	2			1	2		1	1		
<75 mg. per cent		3	4					1	2	2		
75–125 mg. per cent			2	1				1			2	
125–175 mg. per cent			1	1	1						2	1
>300 mg. per cent					1					1		

3. Biological Properties

Table III shows the relationship of the concentration of 22 S material to the γ-globulin precipitation and the sensitized sheep cell agglutination tests. Some correlation was observed particularly between the intensity of the precipitation reaction and the amount of 22 S material. Ultracentrifugal analysis was comparatively insensitive and biologic activity was found to be present in the highly active sera at dilutions where 22 S material was no longer detectable by ultracentrifugal examination. It is, therefore, not surprising that many of the sera with lower titers did not show 22 S material on direct examination. However, occasional discrepancies were noted, particularly with the sheep cell agglutination test in which the titer failed to correlate well with the concentration of 22 S material. The most striking exception was noted in the serum containing 340 mg. per cent of 22 S material. Although it contained the greatest amount of unusual component and reacted most strongly in the γ-globulin precipitation test, it was less positive in the sensitized sheep cell agglutination

reaction than sera containing smaller amounts of this component. However, with both reactions all the sera that were most positive showed measurable amounts of 22 S component. The latex fixation test (18) was also carried out on a number of these sera. The titer was highest in sera showing the most 22 S component.

To test further the role of the high molecular weight component in the serologic reactions, attempts were made to obtain preparations relatively enriched in 22 S material. Repeated preparative ultracentrifugation of 2 sera yielded preparations containing approximately 15 times as high a concentration of heavy molecules as the sera from which they were prepared with a simultaneous 4- to 15-fold decrease in the level of 7 S γ-globulin. One of these showed a 16-fold and the other one a 32-fold increase in the titer of the γ-globulin precipitation test. Similar results were obtained with the electrophoretically isolated γ-globulin preparation from 1 serum. These studies were limited somewhat because in the process of preparation, aggregation of molecules took place resulting in a broad range of high molecular weight components.

TABLE IV

Distribution of Rheumatoid Arthritis Factors, γ-Globulin and Total Protein in Fractions of Serum Separated by Density Gradient Zone Centrifugation

Fr. No.	Total protein	Immunol. γ-glob.	Sheep cell agglutin. dilution	γ-glob. precip. react.
	mg./cc.	*mg./cc.*		
1 top	.88	0.05	0	0
2	5.20	0.5	0	0
3	5.52	2.2	0	0
4	2.48	0.9	0	0
5	0.88	0.1	1/64	+
6	0.56	0.08	1/256	+++
7 bottom	0.24	0.05	1/64	+

Further direct evidence that the activities in the precipitation test and sheep cell agglutination test were contained in a high molecular weight fraction was obtained by centrifuging whole serum in a density gradient. Table IV shows the results obtained from the serum of a patient with rheumatoid arthritis. In this case, as well as in 3 others tested, both the activity in the sheep cell agglutination and the γ-globulin precipitation test were associated with proteins sedimenting more rapidly than the bulk of 7 S γ-globulin. The latter was detected at various levels of the density gradient tube by means of quantitative immunological assay. No activity by either test was found in the upper fractions which contained more than 90 per cent of the 7 S γ-globulin. Activity was completely localized near the bottom of the tubes at a point at which less than 10 per cent of the total protein was found and at which 19 S γ-globulin as determined by specific antiserum to this fraction was localized. This was also

true of activity measured by the latex fixation test. These experiments, although indicating an association of biologic activities with high molecular weight proteins, did not differentiate the 19 and 22 S types.

The role of the 22 S component in the γ-globulin precipitation and sheep cell agglutination tests was further demonstrated by repeatedly absorbing positive sera or γ-globulin fractions with heated or otherwise altered γ-globulin. Following multiple absorptions there was almost complete loss of activity in both tests in the supernatant solutions and on ultracentrifugal examination

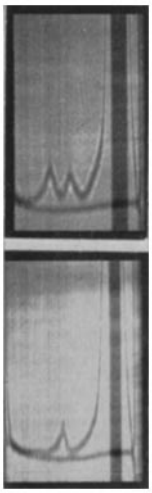

FIG. 5. Comparison of the ultracentrifugal patterns of the serum of a patient with rheumatoid arthritis (above) and the same serum (below) following 5 absorptions with altered γ-globulin.

there was a diminution or complete disappearance of the 22 S component (Fig. 5). The small amount of inhomogeneous heavier material noted in this figure following absorption was introduced at least in part with the γ-globulin preparation used in the procedure.

The electrophoretic mobilities of these biologic activities was determined by testing electrophoretically separated protein fractions. In the γ-globulin precipitation test, fractions from six sera were tested. Two of these were also studied in the sensitized sheep cell agglutination reaction. The electrophoretic distribution varied somewhat in different sera. Fig. 4 illustrates the results with the serum of Fig. 1. In this case as well as in 4 others tested with γ-globulin and in the two tested with sensitized sheep red cells, the activity was found throughout the whole γ-globulin region with a peak somewhat faster than γ-globulin. In one other, the peak of activity corresponded with that of

γ-globulin. In none of the sera tested was activity detected in the β, α_2, α_1-globulins or albumin. Fig. 4 shows that in one serum in which the distribution of activity as well as the 22 S component were determined both migrated somewhat faster than the γ-globulin peak, although it was not possible to determine if their distribution was absolutely identical.

DISCUSSION

The sera of many patients with rheumatoid arthritis potentiate certain agglutination reactions such as those of sensitized sheep cells (15), some types of bacteria (16), certain human red cells and their antisera (17), and latex particles (18) or red cells coated with Fr. II γ-globulin (13). This property has been attributed to the presence of an agglutination-activating factor or factors. It has been further shown that this substance takes part in certain precipitating antigen-antibody reactions and is thereby removed from the sera (19), and that it can react with Fr. II γ-globulin to give a precipitate (12).

The present observations indicate that a precipitate forms in the presence of altered γ-globulin and that the serum factor reacting with the altered γ-globulin is probably related to a high molecular weight protein which is not present in detectable amounts in normal sera. The latter, with a sedimentation coefficient of approximately 22 S, can be observed directly by ultracentrifugal examination of strongly positive sera and γ-globulin fractions prepared from them. Evidence was obtained indicating that the factor responsible for the sheep cell agglutination reaction was also a high molecular weight γ-globulin although a direct relationship to the 22 S component was not completely established. The other reactions of rheumatoid sera mentioned above with the exception of the latex fixation test were not specifically studied. However, it seems possible that they too are related to the 22 S material.

The exact incidence of the 22 S component in sera of patients with rheumatoid arthritis is difficult to determine from this study. Not all of the patients utilized were actually observed and some selection of cases occurred, particularly in regard to the sheep cell agglutination reaction. An approximate estimate of the frequency would place the figure at somewhere in the region of $\frac{1}{3}$ of the cases. This figure includes a number of instances in which instead of a well defined component only an asymmetry preceding the 19 S peak was seen. Such small asymmetries in the ultracentrifugal patterns would not have been considered significant in the absence of the clearly defined peaks noted in the same location in other instances. It would appear that the ultracentrifuge is a rather insensitive tool and that it shows the unusual high molecular weight component only in those sera which contain relatively large amounts, and which give very positive γ-globulin precipitation and sensitized sheep cell agglutination tests. This is in line with the known higher incidence of positive results for these reactions in patients with rheumatoid arthritis (20).

Because of the insensitivity of ultracentrifugation it is also not possible to exclude the presence in normal sera of components sedimenting more rapidly than the 19 S material. Higher molecular weight peaks appear in normal sera that have been enriched by repeated preparative ultracentrifugation (2). Although these appear to be produced at least in part by aggregation during these procedures, it is possible that small amounts may be present normally. These components are probably different from the 22 S component described here in that they usually sediment more rapidly (approximately 28 S), and fail to react with altered γ-globulin or sensitized sheep cells even when present in easily detectable amounts following concentration.

The unusual high molecular weight component may not be a single distinct chemical entity but rather a group of proteins or protein aggregates. Its electrophoretic distribution is greater than would be expected for a homogeneous protein. Similarly the peak noted in the ultracentrifuge is broad. Preliminary studies have indicated that urea dissociates the high molecular weight material into 2 components with sedimentation coefficient of 19 S, and approximately 7 S. These observations favor the possibility that the 22 S component is a complex of 19 S material and some lower molecular weight protein and that it exists in a soluble state in the sera of certain patients with rheumatoid arthritis. It then precipitates readily under various conditions, particularly upon the addition of small amounts of slightly altered γ-globulin.

The dissociation of the high molecular weight protein component to give 19 S material suggests a relationship to normal 19 S γ-globulin. Since the latter probably contains a number of antibodies (3 to 5), it is possible that this 19 S material from the rheumatoid arthritis sera is also an antibody which interacts with a second protein. Whether the second protein is antigen or a component of complement or non-specific 7 S γ-globulin remains obscure. The possibility that the 22 S component might represent a soluble antigen-antibody complex appears worthy of further investigation.

SUMMARY

In the sera of a number of patients with rheumatoid arthritis an unusual, high molecular weight protein component could be detected by direct ultracentrifuge analysis of whole serum. This material sedimented more rapidly than the normal 19 S component and reached concentrations up to 340 mg. per cent. Similar components were not observed in a limited control series.

The high molecular weight material was present in the γ-globulin fraction of serum and joint fluid. It had an $s_{20,w}$ of approximately 22 S and could be dissociated into 2 fractions, one of which had a sedimentation coefficient of approximately 19 S.

Evidence for a direct relationship between the 22 S component and the γ-globulin precipitation test was obtained. The latter reaction was found to occur

in the presence of altered, aggregated γ globulin. Absorption of serum with altered γ globulin removed the 22 S component. There also appeared to be a connection with the sheep cell agglutination reaction and the latex fixation test. The 22 S fraction was always observed in sera giving the most positive tests. Procedures of density gradient and repeated preparative ultracentrifugation demonstrated that each of these reactions was caused by a high molecular weight fraction.

The relationship between the unusual protein complex and various 19 S γ-globulins and 19 S antibodies is discussed.

The authors are very much indebted to Dr. William H. Kammerer, Dr. Richard H. Freyberg, and Dr. I. J. Estrin for their aid in obtaining some of the serum specimens employed in this study.

BIBLIOGRAPHY

1. Pedersen, K. O., Ultracentrifugal Studies on Serum and Serum Fractions, Uppsala, Almqvist and Wiksells, 1945.
2. Wallenius, G., Trautman, R., Kunkel, H. G., and Franklin, E. C., Ultracentrifugal studies of major non-lipide electrophoretic components of normal human serum, *J. Biol. Chem.*, 1957, **225,** in press.
3. Davis, B. D., Moore, D. H., Kabat, E. A., and Harris, A., Electrophoretic ultracentrifugal and immunological studies on Wassermann antibody, *J. Immunol.*, 1945, **50,** 1.
4. Campbell, D. H., Sturgeon, P., and Vinograd, J. R., Separation of complete and incomplete Rh antibodies by centrifugation, *Science*, 1955, **122,** 1091.
5. Gordon, R. S., Jr., The preparation and properties of cold hemagglutinin, *J. Immunol.*, 1953, **71,** 220.
6. Müller-Eberhard, H. J., Kunkel, H. G., and Franklin, E. C., Two types of γ-globulin differing in carbohydrate content, *Proc. Soc. Exp. Biol. and Med.*, 1956, **93,** 146.
7. Franklin, E. C., and Kunkel, H. G., Immunologic differences between the 19 S and 7 S components of normal human γ-globulin, *J. Immunol.*, 1957, **78,** 11.
8. Waldenström, J., Abnormal proteins in myeloma *in* Advances in Internal Medicine, (W. Dock and I. Snapper, editors), Chicago Year Book Publishers, Inc., 1952, **5,** 398.
9. Trautman, R., Operating and comparating procedures facilitating schlieren pattern analysis in analytical ultracentrifugation, *J. Physic. Chem.*, 1956, **60,** 1211.
10. Kunkel, H. G., Zone electrophoresis *in* Methods of Biochemical Analysis, (D. Glick, editor), New York, Interscience Publishers, Inc., 1954, **1,** 141.
11. Müller-Eberhard, H. J., and Kunkel, H. G., Carbohydrate of γ-globulin and myeloma proteins, *J. Exp. Med.*, 1956, **104,** 253.
12. 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. and Med.*, 1956, **91,** 235.

13. Heller, G., Kolodny, M. H., Lepow, I. H., Jacobson, A. S., Rivera, M. E., and Marks, G. H., The hemagglutination test for rheumatoid arthritis with characterization of the rheumatoid agglutinating factors by analysis of serum fractions prepared by ethanol fractionation, *J. Immunol.*, 1955, **74,** 340.
14. Müller-Eberhard, H. J., Franklin, E. C., and Kunkel, H. G., data to be published.
15. Rose, H. M., Ragan, C., Pearce, E., and Lipman, M. O., Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis, *Proc. Soc. Exp. Biol. and Med.*, 1949, **68,** 1.
16. Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., The etiology of rheumatoid arthritis, *Am. J. Med. Sc.*, 1931, **181,** 12.
17. Waller, M. V., and Vaughan, J. H., Use of anti-Rh sera for demonstrating agglutination activating factors in rheumatoid arthritis. *Proc. Soc. Exp. Biol. and Med.*, 1956, **92,** 198.
18. Singer, J. M., and Plotz, C. M., The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis, *Am. J. Med.*, 1956, **21,** 888.
19. Vaughan, J. H., Behavior of the rheumatoid arthritis agglutinating factor with immune precipitates, *J. Immunol.*, 1956, **77,** 181.
20. Epstein, W., and Ragan, C., Serologic reactions in rheumatoid arthritis, *Am. J. Med.*, 1956, **20,** 487.