THE SEDIMENTATION PROPERTIES OF THE SKIN-SENSITIZING ANTIBODIES OF RAGWEED-SENSITIVE PATIENTS

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One of the important physical properties used to classify antibodies is their sedimentation rate as determined by ultracentrifugation. Until the recent studies of Rockey and Kunkel (1) demonstrating antibodies of intermediate sedimentation rates (8 to 15S), most antibodies have been found to have the sedimentation rates of γ-globulins (7S) or macroglobulins (19S).

The sedimentation properties of the skin-sensitizing antibodies of human atopic allergy have been studied by a number of investigators (1–6). Most work involving sucrose density centrifugation (2, 6) and ordinary preparative sedimentation (3, 4) has indicated that the skin-sensitizing antibodies are to be found in the 7S group of proteins rather than associated with the 19S macroglobulins. In many cases it was impossible to determine whether there were skin-sensitizing antibodies of intermediate sedimentation coefficient. Experiments (7, 5) carried out using the moving partition cell technique of Yphantis and Waugh have suggested that skin-sensitizing antibodies may be found in the 19S or an intermediate (10 to 18S) group.

Because of this difference in experimental results with regard to the sedimentation coefficient of skin-sensitizing antibodies and because in many cases only one or two sera were studied, it seemed worthwhile to reinvestigate the problem. Sera from patients sensitive to ragweed have been investigated by both the Yphantis-Waugh moving partition cell method and by sucrose density gradient centrifugation. The data indicate that the skin-sensitizing antibodies sediment slightly faster (7.4 to 7.9S) than normal human γ-globulin (6.8S).

Materials and Methods

Sera.—Sera from three ragweed-sensitive patients that gave end-point passive transfer reactions at high dilutions (NK and HA at 1/1000 and FM at 1/500) were used. These sera were from patients who had been treated in the past with ragweed extract for hyposensitization.

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Two sera from untreated ragweed-sensitive patients (BS and VF) of slightly lesser passive transfer titer also were used for these studies. Patients were selected on the basis of high sensitivity to ragweed and low risk of hepatitis transfer. All studies were performed on serum from a single bleeding from each donor. The sera were filtered through a 0.22 μ-Millipore filter, tested for sterility, and stored at 4°C. Little, if any, change in skin-sensitizing antibody titer was noted after storage for over a year.

Passive Transfer Assay of Skin-Sensitizing Antibodies.—The passive transfer method of Prausnitz and Küstner (P-K) (8) was used for the assay of skin-sensitizing antibodies as previously described (9). Volunteers with negative histories of allergies and a negative ragweed skin test were selected from the inmates of the District of Columbia Jail. All tests were performed by injecting into the skin of the back in randomly selected sites 0.1 ml of the serum or serum fraction to be tested. Forty-eight hours later the sites were challenged with approximately 0.01 ml of short ragweed extract containing 1000 protein nitrogen units per ml.[1] The titer of skin-sensitizing antibody was obtained by determining the highest dilution of serum or a serum fraction that would produce a definitely positive reaction in 15 minutes after challenge with ragweed extract. All sample dilutions to be compared were tested in the same volunteer at the same time.

Moving Partition Cell Method.—This technique was developed by Yphantis and Waugh (10) and involves the use of an analytical ultracentrifuge cell[2] with a moving partition which separates the cell into top and bottom compartments at the completion of the centrifugation. The depletion of a biological activity in the top compartment is measured, and from this and the speed and duration of centrifugation the sedimentation coefficient of the molecules possessing the biological acitivity may be measured.

The procedure followed in using this method to study skin-sensitizing antibodies was similar to that of Gyenes, Gordon, and Sehon (5). The moving partition cell was sterilized in ethanol and allowed to dry. It was then assembled and the experiment carried out under sterile conditions. The cell was filled with a 1:10 dilution (in 0.15 M sodium chloride) or a 1:3 dilution in phosphate-saline buffer (sodium phosphate 0.05 M, sodium chloride 0.15 M, pH 7.0) of the whole allergic serum. Centrifugation was carried out in a Spinco model E analytical ultracentrifuge. Preliminary runs established the appropriate speed and duration of centrifugation which would effect an optimum change in the P-K titer of the serum in the top compartment. At the end of the run the sample in the top of the cell was removed, and P-K titers were obtained on this material and on a specimen of the uncentrifuged serum.

In order to minimize the variability among volunteers in the testing of these materials, the volunteers were used twice for each titration. One side of the back was used to establish a preliminary P-K titer, and then subsequently a more precise titration was carried out on the other side. The best estimates of the P-K titers from all the volunteers were used for determining the P-K titers for each experiment. The ratio of the reciprocals of the P-K titers in the top compartment and the whole serum was taken as a measure of the depletion of the top fraction in skin-sensitizing antibody activity. This ratio, based on the highest dilutions giving a positive end-point titer, was then used to calculate the sedimentation coefficient (5). The true end-points could have been at a dilution just greater than the last positive reaction or just less than the first negative reaction. On this basis a range was calculated using a combination of the last positive and first negative dilutions in such a way as to give a maximum and minimum value for the sedimentation coefficient. These values are given in parenthesis in Table I.

Sucrose Density Gradient Method.—The method of Martin and Ames (11) has been followed closely in these experiments. Centrifugation was carried out in a Spinco model L ultracentri-

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[1] Obtained from Center Laboratories, Inc., Port Washington, New York.

[2] Obtained from Beckman Instruments, Inc., Spinco Division, Palo Alto, California.

fuge with a SW-39 swinging bucket rotor at 35,000 RPM for varying lengths of time. A sucrose gradient of 5 to 20 per cent in a phosphate-saline buffer (sodium phosphate 0.05 M, sodium chloride 0.15 M, pH 7.0) with a volume of 4.8 ml was used throughout the study. Preliminary studies had shown that reagin activity was unaffected in concentrations of sucrose up to 30 per cent (in the phosphate-saline buffer) for 24 to 48 hours. The sucrose gradients were shown to be linear by refractive index measurements, and storage for 24 hours was shown to have little effect on the gradient. The gradients were generally prepared 1 to 4 hours before use and allowed to cool to 4°C.

The 0.2 ml samples (0.1 ml of serum or a serum fraction and 0.1 ml of the phosphate-saline buffer) were layered on the sucrose gradients with care to avoid mixing. Mineral oil was used to fill the remaining space in the tube. The tubes were spun in a precooled rotor (4°C) which was kept between 0° and 5°C during centrifugation at 35,000 RPM. After completion of the centrifugation, the tubes were tapped from the bottom and generally 24 fractions of 13 drops each were collected from each tube. One ml of the phosphate-saline buffer was added to each tube. Prior to skin testing the fractions were dialyzed against the phosphate-saline buffer to remove the sucrose and then rendered bacteria free by centrifugation at 20,000 RPM for 20 minutes with removal of the supernatant solution for testing. Earlier in the study the fractions were sterilized by filtration through 0.22 μ Millipore filter, but this resulted in considerable loss of skin-sensitizing antibody activity, presumably by adsorption on the filter, and consequently this method had to be abandoned.

All the fractions were tested to determine which ones contained skin-sensitizing antibody activity. In the final tests the active fractions were diluted with an equal volume of phosphate-saline buffer and were again tested in duplicate sites in two or more volunteers. The intensity of the skin reactions was determined by measuring the greatest diameter of the erythema reaction and the diameter perpendicular to it and averaging the diameters from all the volunteers for a given fraction. All the fractions that were compared were tested in the same volunteers at the same time. It should be noted that the antibody activity in the fractions was close to the end-point and further two- or fourfold dilution of the fractions produced negative reactions.

The sedimentation rates of standard proteins of known sedimentation coefficients were determined. Volumes of 0.2 ml of these proteins were layered in 0.2 to 4.0 per cent solutions on the sucrose density gradient and centrifuged as described for the serum samples. The distribution of the protein after the run was determined by measuring the optical density at 280 mμ in a Beckman DU spectrophotometer. The position of the peak protein concentration was determined. The data are presented in Table II.

The sedimentation coefficients of the skin-sensitizing antibodies were calculated by comparison with the sedimentation rates of the standard proteins as described by Martin and Ames (11). These authors had shown a proportional relationship between the rate of sedimentation and the distance of movement through the sucrose gradient. Essentially the same result was obtained using any of our standard proteins to make the calculation.

Ultracentrifuge Characterization of Standard Proteins.—The sedimentation coefficients at infinite dilution, $s^0_{w,20}$, of four proteins were determined for use as standards in the sucrose density gradient procedure. Purified human γ-globulins,[3] rabbit muscle aldolase,[4] bovine catalase,[5] and porcine thyroglobulin[5] were analyzed at different concentrations by velocity ultracentrifugation in a Spinco model E ultracentrifuge. The proteins were dissolved in 0.1 M sodium chlo-

[3] The human γ-globulin preparation was kindly donated by Dr. Arthur Strauss of our laboratory.

[4] Obtained from Nutritional Biochemicals Corp., Cleveland.

[5] Obtained from Mann Research Laboratories, Inc., New York.

ride with the exception of catalase which was dissolved in 0.1 M sodium chloride buffered at pH 8.4 by the addition of 10 per cent by volume borate buffer. The sedimentation coefficients were corrected to standard conditions of 20°C in water ($s_{w,20}$) and the value at infinite dilution ($s^0_{w,20}$) obtained by extrapolation of a plot of $1/s_{w,20}$ *versus* the protein concentration. The values obtained (Table II) are in general agreement with the data obtained by others (12).

Preparation of Macroglobulin Serum Fraction.—Serum was obtained from an individual with no history of allergy or hepatitis. The serum was dialyzed against 0.15 M sodium chloride buffered at pH 8.4 by the addition of 10 per cent by volume borate buffer and then fractionated by gel filtration through sephadex G-200[6] (9). The major constituents of the first peak are macroglobulins and lipoproteins. This material was further fractionated by bringing the solution to a density of 1.21 with the addition of cesium chloride and centrifugation at 39,500 RPM in a Spinco model L ultracentrifuge (No. 40 angle rotor) for 24 hours. The bottom one-third of the tubes containing the α_2M- and β_2M-globulins were removed and dialyzed against the borate saline buffer (pH 8.4).

Starch Block Zone Electrophoresis.—The method was a modification of the procedure of Kunkel and Slater (13) as previously described (9).

Nitrogen Determinations.—Nitrogen analyses of the samples were carried out by digestion with sulfuric acid and hydrogen peroxide followed by the addition of Nessler's solution. The nitrogen content was calculated from the optical density at 440 mμ. When enough material was available, the procedure was carried out using 50 to 200 μg of nitrogen (14). If only small amounts of material were available, a small scale method (15), using 5 to 50 μg of nitrogen, was used with minor modification (16).

EXPERIMENTAL AND RESULTS

Moving Partition Cell Experiments.—In order to test the method, preliminary studies were carried out to estimate the sedimentation coefficients of bovine serum albumin[7] and human γ-globulin.[8] Nitrogen analyses were performed to show the depletion of the protein in the top compartment after centrifugation. The data are presented in Table I and show reasonably close agreement with the expected values.

In the studies on skin-sensitizing antibodies sera from three ragweed-sensitive individuals were used. All three patients had received some hyposensitization treatment for ragweed allergy. One serum (NK), with an especially high P-K titer, was studied more extensively than the other two in order to evaluate the reproducibility of the method. The relative depletion of the skin-sensitizing antibody in the top compartment after centrifugation was measured by end-point P-K titration in duplicate sites in normal volunteers as previously described. The sedimentation coefficient data calculations from these ratios are presented in Table I. The sedimentation coefficients are based on the last positive end-point dilutions while the range, indicated in parenthesis, is based on the combinations of the last positive and first negative end-point dilutions such as to give maximum and minimum values of the sedimentation coefficients, as

[6] Obtained from Pharmacia Fine Chemicals, Inc., Rochester, Minnesota.

[7] Bovine plasma albumin, crystallized obtained from Armour Pharmaceutical Co., Kankakee, Illinois.

[8] Human γ-globulins (fraction II) obtained from Pentex Inc., Kankakee, Illinois.

indicated under Materials and Methods. The sedimentation coefficients of the skin-sensitizing antibody activity vary from 2.9 to 10.2S with most values ranging from 5 to 10S.

Sucrose Density Gradient Experiments.—The preliminary sucrose density gradient experiments with the standard proteins yielded values for the sedimentation coefficients that are close to those obtained by velocity ultracentrif-

TABLE I

Sedimentation Data from Moving Partition Cell Experiments

Experiment	Material	Speed	Time	Sedimentation rate	No. of P-K recipients
		RPM	*min.*	*S*	
Standard proteins*					
1	Bovine serum albumin (1 per cent)	59,780	82	3.7	
2	Bovine serum albumin (1 per cent)	59,780	72	4.3	
3	Human γ-globulin (1 per cent)	59,780	37	7.4	
Allergic sera‡					
4	NK (1:10)	59,780	46	6.1 (6.0–6.3)§	3
5	NK (1:10)	59,780	28	9.8 (8.1–10.3)	1
6	NK (1:10)	50,740	29	5.0 (2.6–5.5)	3
7	NK (1:10)	50,740	29	6.8 (6.0–10.8)	1
8	NK (1:10)	50,740	29	7.2 (5.7–8.1)	1
9	NK (1:10)	50,740	29	8.5 (8.1–9.1)	1
10	NK (1:10)	50,740	29	10.2 (8.1–10.8)	1
11	HA (1:3)	50,740	35	5.7 (3.5–6.5)	4
12	FM (1:3)	50,740	35	2.9 (1.3–5.7)	6

* Protein concentrations determined by nitrogen analysis.

‡ Skin-sensitizing antibody determined by passive transfer (P-K) test.

§ Range based on combinations of endpoint P-K titers as described in text.

ugation as shown in Table II. Any of the proteins could be used as the reference protein without any appreciable change in the result.

The aldolase was found to have a faster sedimenting component when observed in the analytical ultracentrifuge. When studied by the sucrose density gradient method, this fast component was seen as a small faster sedimenting peak or shoulder on the leading edge of the main component. The thyroglobulin preparation had some material which sedimented more slowly than the main component; however, the γ-globulin and catalase showed only a single peak by either method. In all cases the movement of the main protein peak was measured.

Five sera, three from treated allergic patients and two from untreated patients, were studied by sucrose density gradient centrifugation. Of the sera studied, only two (NK and HA) were of sufficiently high titer that accurate skin test assays could be carried out to determine the fraction containing the most skin-sensitizing antibody activity. In the three remaining sera (FM, VF, and BS) the titers were lower, and it was possible to narrow the region of peak activity down only to three or four fractions. The sedimentation coefficients calculated from the movement of the peaks of the skin-sensitizing antibody activity relative to the human γ-globulin standard protein are given in Table III. In those cases where it was possible to determine accurately the peak of activity, a sedimentation rate of 7.4 to 7.9S was obtained with an average value of 7.7S. When the lower titer sera were studied by this method ranges from 6.8 to 9.4S were obtained, and the average of the mid-points of the ranges was 8.3S.

TABLE II

Sedimentation Data on Standard Proteins

Protein	Sedimentation coefficient* $s^0_{w,20}$	Sedimentation coefficient by sucrose density gradient‡
Human γ-globulin	6.8	—
Aldolase (rabbit muscle)	7.8	7.6
Catalase (bovine)	11.9	11.9
Thyroglobulin (porcine)	18.6	18.7

* Sedimentation velocity data extrapolated to infinite dilution.

‡ Sedimentation coefficients calculated assuming the human γ-globulin was 6.8 S.

The values obtained by the sucrose density gradient method assume that the partial specific volumes ($\bar{v}$) of skin-sensitizing antibodies are similar to the value of 0.74 reported for the γ-globulin (12) since γ-globulin was the reference protein used in its calculation. The error introduced by a small difference between the assumed and actual $\bar{v}$ is small, especially for slowly sedimenting materials. If we make use of the data of Martin and Ames (11) and assume that $\bar{v}$ for the skin-sensitizing antibodies is 0.80, the corresponding sedimentation coefficient would be 8.2S instead of 7.7S. If we assume that $\bar{v}$ is 0.50, the sedimentation coefficient would be 7.1S. It is very unlikely that the correct $\bar{v}$ is beyond the range of 0.70 to 0.75. This would correspond to a corrected range of 7.5 to 7.8S for the average sedimentation coefficient of the skin-sensitizing antibodies.

The effect of protein concentration on sedimentation through the sucrose gradient was evaluated by centrifuging samples of γ-globulins of varying concentration ranging from 0.2 to 4.0 per cent. As the concentration of the γ-

globulins increased to about 1 per cent, the shape of the curves changed from symmetrical to slightly skewed with the long tail on the side of the leading edge (Fig. 1). Since the γ-globulin solution was free of aggregates as demonstrated by analytical ultracentrifugation, it seemed likely that the skewing was due to slight mixing at the sample-gradient interface. Over the range of concentrations studied, there was no change in the rate of sedimentation of the protein peak. The slight skewing present with the higher concentrations of protein, therefore, did not change the calculated sedimentation coefficient.

TABLE III

Sedimentation Data from Sucrose Density Gradient Experiments

Sera	Duration of centrifuge run	Migration of peak activity	Sedimentation rate
	hrs.	*cm*	*S*
From treated patients			
NK	13	1.72	7.6
NK	21	2.58	7.6
NK	21	2.48	7.4
NK	21	2.67	7.9
HA	13	1.72	7.6
HA	21	2.67	7.9
FM	13	1.72–2.10*	7.6–9.4
FM	21	2.48–2.86	7.4–8.5
From untreated patients			
VF	13	1.72–2.10	7.6–9.4
BS	13	1.53–2.10	6.8–9.4

* The range of P-K activity is given where the titers were too low to permit the determination of the peak activity.

In order to determine whether the curve showing the distribution of skin-sensitizing antibody activity (Fig. 1) represents antibody with more than one sedimentation rate, samples were separately pooled from the leading and trailing portions of the curves from eight, 21-hour, sucrose density gradient runs with one serum (NK). The fractions with the maximum activity were discarded. The two pooled fractions were concentrated and recentrifuged for 21 hours in similar sucrose density gradients. The curves of skin-sensitizing antibody activity from both the leading and trailing sides showed a distribution and sedimentation rate similar to that of the original curve indicating that the activity represents predominantly a population with one sedimentation rate.

A single serum (NK) was fractionated by starch block electrophoresis and the distribution of the skin-sensitizing antibodies determined by P-K test. The antibody activity was found in the fast γ-globulin to slow β-globulin region as

has been previously described (21, 9). The active region was divided into three parts, and materials from the γ-globulin and β-globulin ends, excluding the intermediate materials, were studied by the sucrose density gradient method.

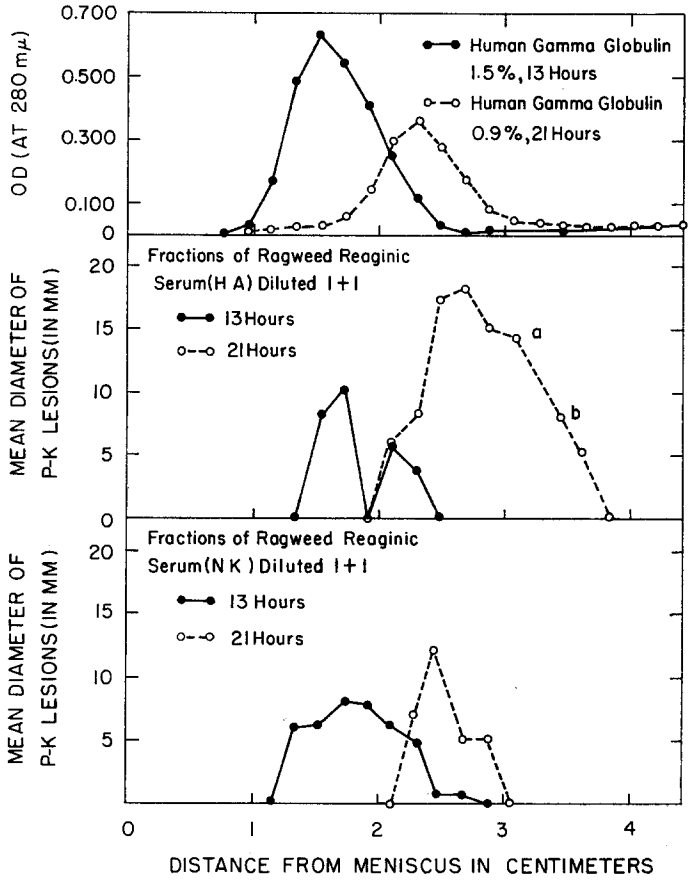

FIG. 1. Curves showing the distribution of activity by sucrose density gradient centrifugation. ● and ○, distribution after centrifugation for 13 and 21 hours respectively. For an explanation of points *a* and *b* see text.

No differences in the sedimentation properties of the skin-sensitizing antibodies from fractions of different electrophoretic mobility were found.

In an attempt to demonstrate complex formation of skin-sensitizing antibody and macroglobulin as suggested by Augustin and Hayward (3), equal parts of allergic serum (NK) and a 5.5 per cent solution of α_2M- and β_2M-globulins (approximately 70 per cent α_2M-globulins) as prepared in Materials and Methods were mixed giving a serum to macroglobulin weight ratio of 1.23.

This mixture was allowed to stand at 37°C for 3 hours and at 4°C for 3 days, after which the sedimentation rate of the skin-sensitizing antibody was determined by the sucrose density gradient method. There was no measurable change in the sedimentation coefficient of skin-sensitizing antibodies under these conditions.

DISCUSSION

The sucrose density gradient method has provided the best data that we have been able to obtain about the sedimentation properties of skin-sensitizing antibodies. Using the two high titer sera, six determinations were carried out giving an average value of 7.7S (and a range of 7.4 to 7.9S) for the movement of the peak of the skin-sensitizing antibody activity (Table III). These data and the results from the density gradient studies with our standard proteins are plotted

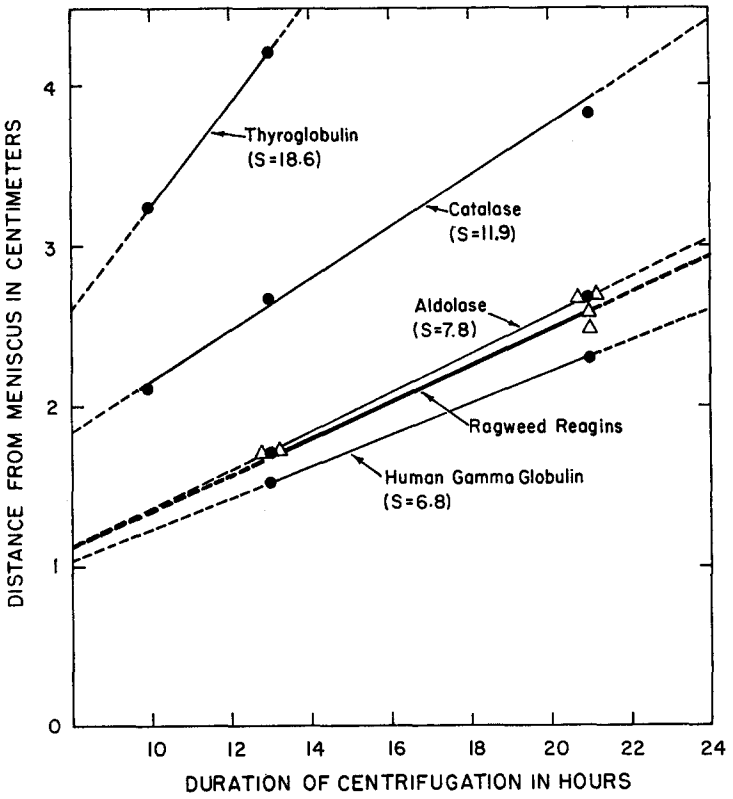

FIG. 2 Rate of movement of proteins in the sucrose density gradient. ●, average distance that the peak concentration of standard proteins moved for each duration of centrifugation. △, distance that the peak skin-sensitizing antibody activity moved in separate determinations of sera HA and NK.

in Fig. 2. The sedimentation rate of the skin-sensitizing antibodies appears to be very similar to that of aldolase and significantly faster than the human γ-globulin. The reproducibility of the method makes it seem unlikely that the major skin-sensitizing components in these sera are to be found among the macroglobulins or even in the 10 to 11S range.

In Fig. 1 we have plotted the distribution of skin-sensitizing antibody activity for single runs of both our high titer sera after 13 and 21 hours of centrifugation. For comparison similar curves were included for human γ-globulin. The biphasic character of the curve for the 13 hour run with serum HA is considered to be an artifact since this is the only run that showed such a distribution. It can be seen that the peak of the skin-sensitizing antibody activity moves slightly faster than the human γ-globulin. The slightly asymmetric shapes of the curves, showing the distribution of skin-sensitizing antibody activity, suggest the possibility that lower concentrations of more rapidly sedimenting materials with skin-sensitizing antibody activity may be present. If, for example, the points *a* and *b* in Fig. 1 marked the peaks of these more rapidly sedimenting components, they would have sedimentation coefficients of 9.1 and 10.2S respectively. A more likely explanation for the skewed shape of these curves is that it is due to the same kind of interfacial mixing that was observed with the standard γ-globulin solutions.

Since the main peak of skin-sensitizing antibody activity, with an average sedimentation cofficient of 7.7S, may represent the summation of activities of separate components such as monomeres and dimeres of a 7S unit, it seemed necessary to attempt separation of active components with different sedimentation coefficients. Centrifugation for greater lengths of time did not resolve additional peaks of activity. Finally, samples from the leading and trailing portions of the antibody activity curves were recentrifuged in sucrose density gradients and produced distributions of antibody activity essentially identical with the original sample. This indicates that the skin-sensitizing antibodies in this serum appear to be homogeneous with regard to sedimentation rate.

The three lower titer sera, which included the two sera from untreated patients, gave ranges from 6.8 to 9.4S. The average of the mid-points of the ranges was 8.3S. This is greater than the average of the peak activities (7.7S) as determined with the higher titer sera and is in accord with the slightly skewed shape of the antibody activity curve. There is no evidence of any differences between the sedimentation properties of the skin-sensitizing antibodies of treated and untreated patients.

The data obtained by the moving partition cell method are quite consistent with the more accurate data obtained by the sucrose density gradient method. The difficulty in obtaining accurate end-point P-K titrations, even with the use of great care, has prevented us from obtaining more accurate values by this method. The range of values from 2.9 to 10.2S is indicative of this problem.

The data of Augustin and Hayward (3) and our previous studies (4) both involving preparative ultracentrifugation without the use of density gradients are quite consistent with our present data. Also the sucrose density gradient studies of Stanworth (2) and Barnett *et al.* (6) would agree that the sedimentation coefficients of skin-sensitizing antibodies are close to 7S. The movement of our peak of skin-sensitizing antibody activity is slightly slower than the 8 to 11S reported by Rockey and Kunkel (1). Our data do not seem consistent with the experiments of Gyenes, Gordon, and Sehon (5). Their values of 12, 14, and 22S for the sedimentation coefficients of skin-sensitizing antibodies in sera from ragweed-sensitive patients as determined by P-K testing with the moving partition cell method are much higher than our results.

Considering the work from a variety of other laboratories as well as our own, it seems quite probable that the sedimentation coefficients of most skin-sensitizing antibodies are slightly greater than human γ-globulins (6.8S) in the range of 7.4 to 7.9S. This work provides additional support for the concept of Rockey and Kunkel (1) that there is a class of antibodies of intermediate sedimentation coefficient between the 7S γ-globulins and the 19S macroglobulins. It seems very unlikely that the major portion of skin-sensitizing antibodies is to be found to sediment in the macroglobulin range (15 to 20S).

The suggestion of Augustin and Hayward (3) concerning complex formation between skin-sensitizing antibodies and macroglobulins could not be confirmed in our experiments. It is possible, however, that a complex is formed which subsequently becomes disassociated in the sucrose gradient.

The data suggesting that skin-sensitizing antibodies sediment just slightly faster than human γ-globulins are consistent with sephadex G-200 gel filtration studies previously reported (9). Other studies have indicated that skin-sensitizing antibodies are to be found among the β_2A globulins (17, 9, 22). Ultracentrifuge studies of normal human β_2A globulins (18) and β_2A myeloma globulins (19, 20) have shown that the major portion of the proteins has an S value close to 7 with lesser amounts of more rapidly sedimenting materials. Thus the sedimentation experiments provide data quite consistent with the association of skin-sensitizing antibodies with the β_2A-globulin fraction.

SUMMARY

The sedimentation coefficients of the skin-sensitizing antibodies to ragweed were evaluated by the moving partition cell method and the sucrose density gradient method. The most reliable results were obtained by sucrose density gradient ultracentrifugation which showed that the major portion of skin-sensitizing antibodies to ragweed sediment with an average value of 7.7S (7.4 to 7.9S). This is about one S unit faster than γ-globulins (6.8S). The data from the moving partition cell method are in agreement with these results. Our

studies failed to demonstrate heterogeneity of the skin-sensitizing antibodies with regard to sedimentation rate.

We are indebted to Dr. Halla Brown for furnishing the allergic sera and to Dr. Vernon Knight and Dr. Howard C. Goodman for their advice and help. The cooperation of Mr. Donald Clemmer, Director of the District of Columbia Department of Corrections, and the inmate volunteers of the District of Columbia Jail have been invaluable.

Addendum.—After submitting this article, a study by Dr. A. I. Terr and Dr. J. D. Bentz was published in *Proc. Soc. Exp. Biol. and Med.*, 1964, **115,** 721, which reported data that are in general agreement with this study.

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