

SEDIMENTATION STUDIES OF SKIN-SENSITIZING ANTIBODY

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THE skin-sensitizing antibody of human atopic allergy does not readily cross the placental barrier; however, the "blocking" antibody induced by hyposensitization passes the placenta freely.¹ Campbell and associates² have demonstrated that Rh saline agglutinating antibody, which also does not cross the placenta readily, is associated with a more rapidly sedimenting fraction of the serum globulins than the Rh incomplete or "blocking" antibody which does pass the placenta. In view of the analogous relationship between the atopic allergy antibody system and the Rh antibody system with regard to placental transfer of antibody, studies were initiated in this laboratory to determine whether skin-sensitizing antibody, like the Rh saline-agglutinating antibody, is also associated with the more rapidly sedimenting globulin fraction.

MATERIALS

Serums were obtained, from twelve allergic persons, which produced strongly positive transfer skin test reactions to various test allergens including grass, weed, epidermal, and mold extracts. The group of patients from whom the serums were obtained included both adults and children suffering from major allergic diseases, such as asthma, hay fever, perennial allergic rhinitis, eczema, urticaria, and various combinations of these. Details of age, sex, diagnosis, treatment history, and test sensitivity are given in Table I. There were approximately equal numbers of serums from treated and untreated persons.

All serums were stored in a refrigerator at 4° C. and processed within six weeks of the time obtained. In the one case in which both pre- and posttreatment sera were obtained from the same patient, the pretreatment serum was stored in the Deep Freeze at -20° C. for six months.

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Skin tests were performed intradermally with 0.02 ml. of 1:500 dilutions of commercial test allergens.*

METHODS

Volumes of 6 ml. of each serum were diluted with an equal volume of 1 per cent saline containing 10 per cent borate buffer (pH 8.4, $\mu = 0.125$) and centrifuged for ten to twelve hours at 40,000 r.p.m. in a Spinco Model L preparative centrifuge. The supernatant was decanted, and the remaining gelatinous pellet of heavy materials was resuspended in the borate saline diluent. Both supernatant and resuspended pellet were recentrifuged through four more cycles at 40,000 r.p.m. for ten to twelve hours. At each cycle any further pellet material

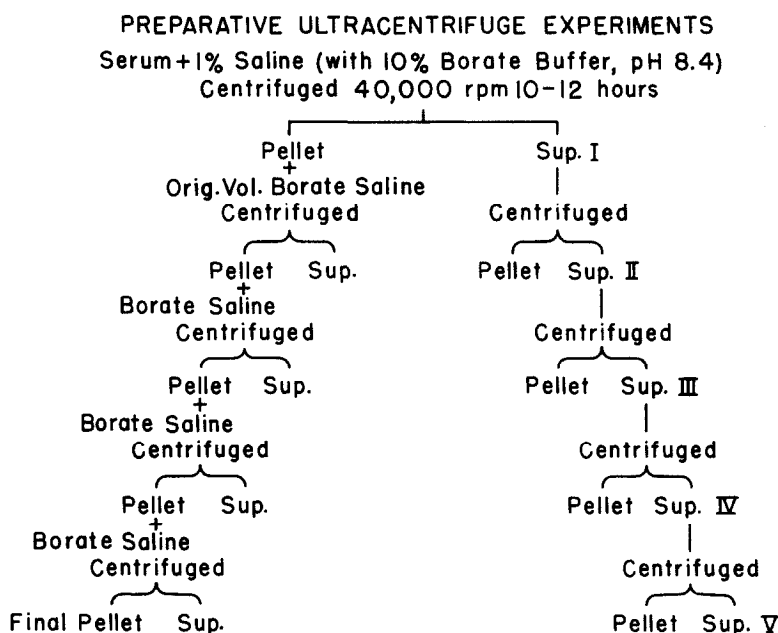


Fig. 1.—Method of serum fractionation by means of preparative ultracentrifugation.

TABLE I. DIAGNOSIS AND TREATMENT STATUS OF THE SERUM DONORS

PATIENT	SEX	AGE (YEARS)	DIAGNOSIS	TEST ALLERGEN	HYPOSENSI- TIZATION (YEARS)
D. N.	M	22	Allergic rhinitis	<i>Distichlis spicata</i>	None
N. N.	M	23	Asthma	<i>Olea europaea</i>	None
N. P.	M	24	Hay fever	<i>Lolium perenne</i>	None
M. C.	F	15	Asthma and rhinitis	Mixed feathers	3
J. R.	F	10	Asthma and eczema	<i>Artemisia californica</i>	1.5
T. V.	M	15	Asthma	Cattle hair	2
J. S.	M	9	Asthma and rhinitis	Altenaria	2
J. B.	M	5	Asthma	<i>Bromus rigidus</i>	0.3
M. S.	M	13	Asthma	<i>Cynodon dactylon</i>	2.5
A. W.	M	30	Hay fever	<i>Lolium multiflorum</i>	None
A. L.	M	38	Asthma and urticaria	Rabbit hair	None
P. J.	M	32	Asthma	<i>Franseria acanthicarpa</i>	None and 0.5*

*Pre- and posttreatment serums obtained.

*Hollister-Stier Laboratories, Inc., Los Angeles, California.

formed from the supernatants was discarded and the suspending fluid from the pellet materials was likewise discarded. A schematic representation of the fractionation procedure is presented in Fig. 1.

Four serums (Table III), diluted with an equal volume of borate saline, were fractionated by means of a Waugh moving-partition cell³ in a Spinco Model E analytic ultracentrifuge. The cell was filled with 0.55 to 0.65 ml. of the diluted serum. The runs were carried out at 52,640 r.p.m. at a rotor temperature of 23 to 25° C. The deceleration schedule was started approximately thirty minutes after gaining full speed, when the S19 peak had just reached the initial position of the moving partition. During the course of slow deceleration, the S19 peak moved completely into the bottom compartment. The rotor decelerated from 52,640 to 37,000 r.p.m. on the slow brake, from

TABLE II. PASSIVE TRANSFER TEST DATA ON PREPARATIVE CENTRIFUGE FRACTIONS

PATIENT	SERUM (DILUTED 1:1)	FINAL SUPERNATANT (SUP. V)	FINAL PELLET
D. N.	3+	1+	0*
N. N.	3+	2+	1+*
N. P.	3+	1+	0*
M. C.	4+, 3+	4+, 3+	4+, 3+
J. R.	4+, 3+	3+, 2+	2+, 2+
T. V.	4+, 4+	3+, 2+	3+, 2+
J. S.	4+, 3+	4+, 4+	4+, 3+
J. B.	4+, 4+	3+, 3+	3+, ±
M. S.	4+, 3+	3+, 2+	4+, 3+
A. W.	4+, 4+	4+, 4+	4+, 4+
A. L.	4+, 3+	4+, 1+	4+, 3+
P. J. pretreatment	4+, 3+	3+, 2+	3+, 3+
P. J. posttreatment	4+	4+, 4+	2+, 0

Each score represents an individual test on a separate donor.

*Final pellet diluted to original volume; therefore, protein concentration is 0.1 per cent instead of 0.6 per cent.

TABLE III. PASSIVE TRANSFER TEST DATA ON PARTITION-CELL FRACTIONS

PATIENT	TOP 1	TOP 2	BOTTOM 2	BOTTOM 1*
A. W.	4+, 3+	2+, 2+	3+, 1+	4+, 3+
A. L.	3+, 2+	2+, 2+	1+, 1+	3+, 1+
P. J. pretreatment	4+, 3+	3+, 3+	2+, 2+	3+, 2+
P. J. posttreatment	4+, 2+	2+, ±	3+, ±	4+, 2+

*The fraction "bottom 1" is the only one that contains demonstrable S19 material.

37,000 to 26,000 r.p.m. on the medium brake, and from 26,000 to 6,000 r.p.m. on the rapid brake. After the rotor had reached a speed of 6,000 r.p.m., the final deceleration was carried out on slow brake. The rotor chamber was opened when the speed had been reduced to 1,000 r.p.m., and as soon as the rotor stopped it was immediately removed with utmost gentleness and tipped so as to stabilize the concentration gradient in the partition cell. A syringe and blunt needle were used to remove the fractions from the top and bottom compartments of the cell. In this manner the sera were separated into "top 1" and "bottom 1" fractions. Six such runs were carried out for each serum, and the corresponding "top 1" and "bottom 1" fractions were pooled. A sample of the pooled "top 1" fraction of each serum was examined for the presence of the S19 peak in a regular centrifuge cell at 52,640 r.p.m. in the

Spinco analytic ultracentrifuge. The remaining portions of the "top 1" fractions of each of the four sera were rerun in the Waugh partition cell following the identical time schedule used in the first fractionation. The fractions obtained from the "top 1" fraction by this method were designated "top 2" and "bottom 2."

Sedimentation patterns of the various supernatant and final pellet fractions of the same four sera used for the partition-cell experiments were obtained in the Spinco analytic ultracentrifuge at 52,640 r.p.m. and a rotor temperature of 23 to 25° C. The supernatants were run in the ultracentrifuge undiluted at a protein concentration of 2.5 to 3.5 per cent.

Passive transfer tests were performed with the various fractions obtained by these procedures. Except in the cases of three serums (D. N., N. N., and N. P.), these tests were performed in duplicate with two separate donor subjects for each serum and fractions thereof. The final pellet materials for testing were prepared by resuspension of one pellet, derived from 12 ml. of diluted serum, in 2 ml. of borate saline, giving a protein concentration of approximately 0.6 per cent. Three exceptions to this are indicated in Table II. In the case of these three sera (D. N., N. N., and N. P.) the pellets were diluted to the original volume of diluted serum, so that if all the activity were concentrated in the pellet it could be more easily compared with the skin-sensitizing activity of the diluted serum.

RESULTS

The analytic ultracentrifuge experiments performed with the first supernatants (Sup. I) from the preparative ultracentrifuge fractionations (Fig. 1) were almost completely devoid of the S19 components. The second supernatants (Sup. II) and all succeeding supernatants were absolutely free (within the resolving capabilities of the method) of the S19 components (Fig. 2). Analytical ultracentrifuge studies of the final pellet solution (Fig. 2) showed a high concentration of the S19 components, a smaller S6-7 peak, and a still smaller concentration of much more rapidly sedimenting material (S40-50). The analytical ultracentrifuge experiments carried out with the pooled "top 1" solutions showed absolutely no evidence of any of the S19 components.

Free boundary electrophoresis experiments carried out with the pellet solutions (0.6 to 1.0 per cent protein in barbital buffer, pH 8.6, $\mu = 0.1$) showed two peaks with mobilities -1.7×10^{-5} and -4×10^{-5} cm.² volt⁻¹ sec.⁻¹. These peaks correspond to fast gamma globulin and alpha₂ globulin components. The alpha₂ globulin peak constitutes about 55 per cent of the protein present.

Results of the passive transfer skin tests are tabulated in Tables II and III. It is quite evident that skin-sensitizing activity has been neither significantly removed nor significantly concentrated in any of the fractions prepared by either method of fractionation. Control experiments have demonstrated that the borate saline used as a diluent does not influence the passive transfer skin tests.

DISCUSSION

During the preliminary stages of this study, Sehon⁴ reported data clearly identifying "blocking" antibody with the S7 globulins. He also drew the inference from his work, using the Waugh partition-cell method and the ragweed allergen system, that skin-sensitizing antibody was associated with the S19 globulins, thus supporting the analogy with the Rh antibody system.

Our data, however, do not support this hypothesis. All our supernatants and "top" fractions containing no detectable S19 peaks show high concentrations of skin-sensitizing activity as compared with whole serum. One cycle of preparative centrifugation was sufficient to reduce the concentration of the S19 components to an almost imperceptible level, and even after five such

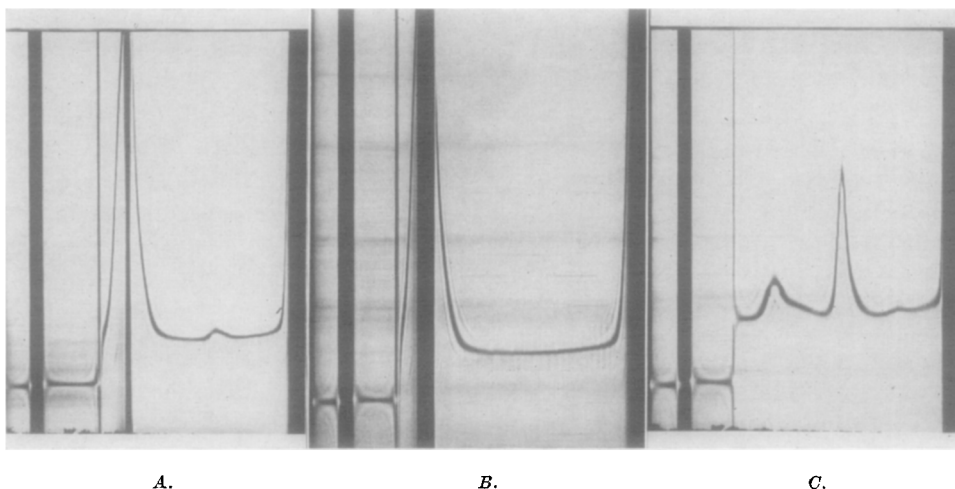


Fig. 2.—Sedimentation patterns at 52,640 r.p.m. of a serum sample (A. W.) and two of the derived fractions, all in borate saline. A, Serum 2 per cent protein concentration thirty minutes after gaining full speed. B, Second supernatant (Sup. II), 3 per cent protein concentration, thirty-two minutes after gaining full speed, showing the absence of the S19 components. C, Final pellet solution, 0.6 per cent protein concentration, twenty-four minutes after gaining full speed.

successive centrifugations the last supernatant showed skin-sensitizing antibody activity. The final pellet solution contained a high concentration of the S19 material relative to the concentration in serum; however, the pellet solution was no more active than the serum. It is possible that the skin-sensitizing antibody is concentrated in the pellet but is partially inactivated during the process of pellet formation, but this does not seem likely. The pellet solutions containing augmented amounts of S19 material showed almost equal skin-sensitizing activity with both whole serum and the S19-depleted fractions.

Our Waugh partition-cell data are entirely consistent with the data obtained by the preparative ultracentrifuge method. The fractions depleted of S19 material ("top 1," "top 2," and "bottom 2") showed as much antibody activity as fraction "bottom 1" that contained the S19 components. The advantage of the use of the partition cell is that the separations are carried out under direct visual control and under well-defined convection-free conditions.

More recent experiments from Sehon's laboratory⁵ have shown that there is no correlation between the concentration of the S6-7 components and the skin-sensitizing antibody activity. It is to be noted that in the methods described, by which our fractions have been obtained, a separation of materials of intermediate sedimentation coefficient selectively into one or another of these fractions was not obtained. The skin-sensitizing antibody may be present in low concentration, even if it is of high activity, in allergic serums: therefore, it is possible that the antibody may be of intermediate sedimentation coefficient and may be divided in approximately equal amounts between the "heavy" and "light" fractions prepared by our methods. It is also possible that the antibody activity could be associated with antibodies of different sedimentation coefficients. If the skin-sensitizing activity were associated with molecules of high molecular weight but low density, this might account for both the lack of placental passage of the antibody and the lack of concentration of the activity in the pellets.

At the inception of this study it was hoped that the skin-sensitizing antibodies might prove to be molecules of the S19 group, thus providing a convenient characteristic for their concentration and purification. The results obtained are thus further disappointing, as they leave the characterization and in vitro manipulation of these antibodies as elusive as they have been for the past decades.

SUMMARY

Serums from allergic patients have been fractionated by preparative and analytic ultracentrifugation into fractions devoid of the S19 components and fractions containing S19 components. Skin-sensitizing activity has been found to be associated with both kinds of fractions. The skin-sensitizing activity could not be correlated with the S19 globulin fraction alone.

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