

PARTITION OF THE SKIN-SENSITIZING ANTIBODY AMONG ALCOHOL-FRACTIONATED PLASMA PROTEINS

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IN PREVIOUS studies allergic (skin-sensitizing) antibodies¹ have been reported to be among the gamma globulins of serum proteins separated by classical electrophoretic techniques. Recently, however, Campbell and associates,² have reported the concentration of these antibodies in the alpha and beta globulin component of serum proteins separated by the newer electrophoretic-convection procedure. The present paper describes our studies on the reagin content of the plasma proteins of four patients with ragweed hay fever fractionated by Cohn's alcohol precipitation method.³

METHODS

Plasma Fractionations.—Ten ml. of fasting venous blood was obtained by venepuncture and immediately mixed with 1.75 ml. of a solution of 2.2 Gm. per cent dextrose, 2.2 Gm. per cent sodium citrate, and 0.8 Gm. per cent citric acid. The blood was centrifuged at 2,000 r.p.m. for 15 to 20 min. to obtain a clear plasma. Five ml. of this plasma was then fractionated by Cohn's alcohol precipitation technique (Method 10) as modified by Lever and associates.⁴ Fractions I, II, and III were precipitated at -5° C. in an ethanol concentration of 19 per cent, pH 5.8 to 5.9, and ionic strength of 0.04 M. The supernatant and washing of this precipitate were combined and Fraction IV+V precipitated in 0.01 M. zinc acetate. The I+II + III precipitate was twice extracted for Fraction II with a 14 per cent alcoholic solution at pH 5.5, ionic strength of 0.005 M. and glycine concentration of 40 Gm./L. These extractions were combined and Fraction II was precipitated in 22 per cent ethanol and 0.071 M. disodium tartrate.⁴

The IV+V precipitate was taken up in 5 ml. distilled water as a cloudy solution and equilibrated with a cationic resin (Dowex 50) to remove the zinc ion, after which a clear solution was obtained. The criterion for sufficient ion exchange was failure of a pink color to develop when 0.25 ml. of the protein solution was mixed with 0.05 ml. of 0.001 M. dithizone in CHCl_3 . The protein solution was then adjusted to a pH of 7.4 and ionic strength of 0.16 M. by adding 4 ml. to a test tube containing the dried crystals from 0.4 ml. of a solution of 1.5 M. NaCl buffered with 0.1 M. phosphate.

The I+III precipitate was taken up in 5 ml. 0.15 M. NaCl buffered with phosphate at pH 7.4. Fibrinogen was allowed to clot out overnight at 4° C., the residual fraction thereafter being referred to as IIId.[†] All fractions were

Supported in part by a research grant from the Asthma Fund, Harvard Medical School, and by a U.S.P.H.S. grant.

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Received for publication, June 16, 1952.

[†]The symbol IIId is here used to differentiate this fraction from Cohn's Fraction III, in which the fibrinogen is removed by further treatment with alcohol, rather than simple clotting.

sterilized by Seitz filtration and stored at -10° to -20° C. until used. Fractions were considered "undiluted" when present in the above states, where their protein concentrations were approximately equal to those which they had had in the original unfractionated plasma.

Fraction IV + V is shown electrophoretically to contain the albumin, α_1 globulin, and about half the α_2 globulin. Fraction IIId has in addition to the beta globulins some α_2 globulin and 10 to 20 per cent of the gamma globulins. Fraction II consists only of gamma globulins.

Skin Testing.—Nonreactive, nonragweed-sensitive patients from the allergy clinic were chosen as recipients. Skin sites were prepared with five-fold dilutions of the original plasma and fractions in 0.1 ml. amounts. The sites were carefully marked. Forty-eight hours later the prepared skin sites were each tested with 0.01 ml. of 1:1000 ragweed extract in Coca's fluid.⁵ The wheal and flare response was recorded directly by tracing over it on matte-acetate paper.*

Protein Determinations.—All protein concentrations were estimated by the Biuret reaction as modified for quantitative analysis.³

TABLE I. PARTITION OF RAGWEED REAGIN AMONG PLASMA PROTEIN FRACTIONS

	WHOLE PLASMA	FRACTION IV + V	FRACTION IIId	FRACTION II
T. S.	625*	125	625	5
A. R.	Not tested	5	25	1
B. R.	25	1	25	Negative
W. O.	25	5	25	1

*Reciprocal of highest dilution showing definite positive passive transfer reactivity.

RESULTS

Table I shows the results of skin testing in all four patients and in Fig. 1 is presented a reproduction of the tracings made on the plasma of T.S. It is clear that the skin-sensitizing antibody of ragweed allergy is concentrated in Fraction IIId. Table II presents the relative proportions of proteins in these fractions as referred to the original plasma.

TABLE II. RELATIVE PROTEIN CONTENTS OF FRACTIONS AND ORIGINAL PLASMA

	PLASMA	IV + V	IIId	II
T. S.	100*	67	21	12
A. R.	100	65	18	17
B. R.	100	70	17	13
W. O.	100	62	23	15

*Expressed as per cent of original plasma.

DISCUSSION

Fraction IIId consists primarily of the beta globulins, while the bulk of the alpha globulins are found with albumin in Fraction IV + V. Our results, therefore, are in accord with those of Campbell, who demonstrated the reagin content of sera separated by the electrophoresis-convection technique to lie principally with the alpha and beta globulins and with the more recent

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work of Cooke and associates⁶ who have found passive transfer activity to lie predominantly among the "mid-portion" serum proteins separated by the classical Tiselius electrophoreses.

By an earlier fractionation scheme (Method 6 of Cohn) Enders,⁷ and others found that most of the classical antibodies of normal serum lie among the gamma globulins in Fraction II. The somatic agglutinin of the typhoid organism which was in Fraction III was a notable exception to this. The present report would indicate that antiragweed antibody will constitute a second exception, if we can assume that the Fraction II's obtained by the earlier and later fractionation schemes are essentially the same. In addition,

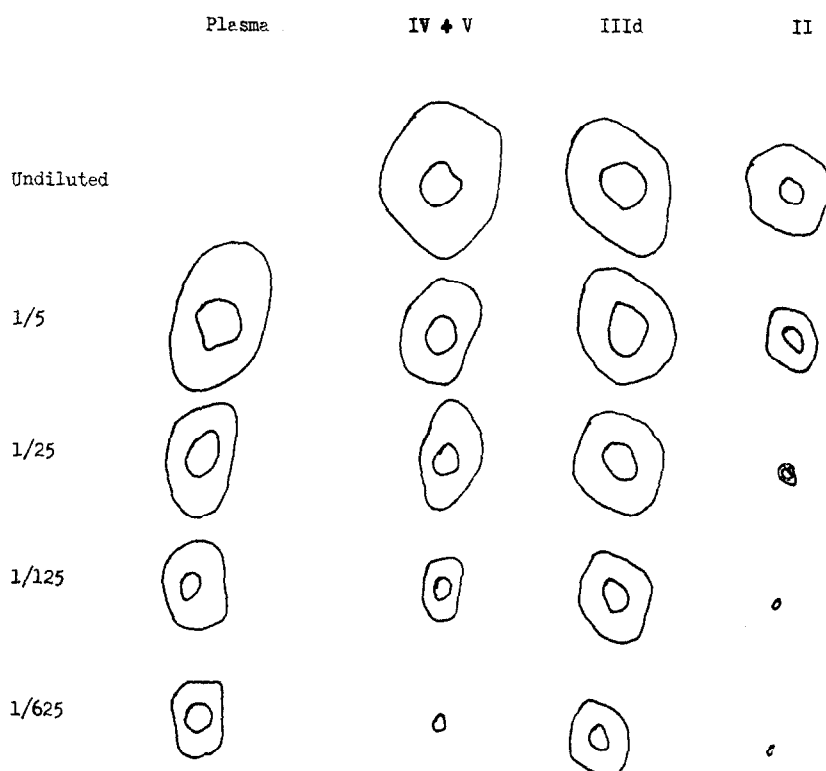


Fig. 1.—Skin sites sensitized with 0.1 ml. and tested 48 hours later with 0.1 ml. 1/1000 ragweed extract in Coca's fluid. Plasma from patient T. S. with ragweed hay fever. (Reduced to $\frac{2}{3}$ actual size).

the presence of considerable passive transfer activity in Fraction IV+V, which is essentially free of gamma globulins, is further evidence that anti-ragweed antibodies are not gamma globulins.

SUMMARY

The skin-sensitizing antibody of ragweed hay fever has been shown to be concentrated among the proteins of Fraction IIIId, as separated by alcohol precipitation in the cold.

REFERENCES

1. Newell, J. M., Sterling, A., Oxman, M. F., Burden, S. S., and Krejci, L. E.: Electrophoretic Separation of Antibody From Human Allergic Serum, *J. ALLERGY* **10**: 513, 1938-39.
2. Campbell, D., Cann, J. R., Friedman, T. B., and Brown, R. A.: Reaginic Serum Fractions Obtained by the Electrophoresis Convection Method, *J. ALLERGY* **21**: 519, 1950.
3. Cohn, E. J., and others: A System for the Separation of the Components of Human Blood, *J. Am. Chem. Soc.* **72**: 465, 1950.
4. Lever, W. F., and others: Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. XL. Quantitative Separation and Determination of the Protein Components in Small Amounts of Normal Human Plasma, *J. Clin. Invest.* **30**: 99, 1951.
5. Vaughan, W. T., and Black, J. H.: *Practice of Allergy*, C. V. Mosby Co., St. Louis, 1948, p. 261.
6. Cooke, R. A., Sherman, W. B., Menzel, A. E. O., Chapin, H. B., Howell, C. M., Scott, R. B., Myers, P. A., and Downing, L. M.: ACTH and Cortisone in Allergic Diseases, *J. ALLERGY* **22**: 211, 1951.
7. Enders, J. F.: Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. X. The Concentration of Certain Antibodies in Globulin Fractions Derived From Human Blood Plasma, *J. Clin. Invest.* **23**: 510, 1944.