

The Journal of Allergy

VOL. 23

NOVEMBER, 1952

No. 6

Original Articles

IMMUNO-SEROLOGIC STUDIES OF THE IMMEDIATE WHEEL-TYPE ALLERGIES

A PRELIMINARY REPORT

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DURING the past year we have continued our studies to determine, if possible, the location of the skin sensitizing antibody in certain fractions of ragweed-reacting sera. Newell¹ has shown that this antibody was present in the electrophoretically separated gamma globulin. Campbell and associates² have reported skin-sensitizing activity in other serum fractions. In the studies³ we reported last year the indications were that the antibody could not be accounted for in its entirety in the gamma globulin fraction and our further studies confirm this view.

Generally, the isolation of individual electrophoretic main components from sera can be applied only to the fast and slow moving end components, namely, albumin and gamma globulin. Mixtures of end components as albumin and alpha₁ globulin or gamma and beta globulin may be obtained in bulk if electrophoretic separation is carried out in macro scale. Such mixtures may then be further separated by subsequent re-electrophoresis. In the study of some sera we have combined chemical fractionation with electrophoretic separation and analysis in order to obtain further data.

In our current work we have preferred to express the end point of reactivities, as obtained by passive transfer test, not in terms of dilution but rather in terms of the actual amount of the protein component injected into the skin site. These amounts were calculated on the basis of colorimetric or refractometric evaluations of the sera and their fractions. From these data and the electrophoretic analysis the amount of each component present in each site was then calculated. Details of experimental procedures will be given in a future report.

From The Institute of Allergy, The Roosevelt Hospital, New York.

Presented at the Eighth Annual Meeting of The American Academy of Allergy, Chicago, Ill., February 18-20, 1952.

Aided in part by a grant from the United States Public Health Service.

Received for publication February 14, 1952.

TABLE I. ELECTROPHORETIC AND IMMUNOLOGICAL DATA ON RBR. SERUM AND CERTAIN DERIVED FRACTIONS

	PER CENT COMPOSITION*					MICROGRAMS INJECTED AT END-POINT REACTIVITY					REACTION AT END-POINT	PER CENT OF REACTION DUE TO GAMMA GLOBULIN
	ALBUMIN	ALPHA ₁	ALPHA ₂	BETA	GAMMA	TOTAL PROTEIN	ALBUMIN	ALPHA ₁	ALPHA ₂	BETA	GAMMA	
A Serum	52.2	7.0	10.7	15.4	14.7	9.55	5.0	0.7	1.0	1.5	1.4	13
B Fr. I	86.7	9.8	3.5	0	0	1535	1331	150	54	0	0	-
C Fr. I-F	+++						265					-
D Fr. I-S		+	+++									-
E Fr. III	0	0	9.1	21.1	69.8	9.0	0	0	0.8	1.9	6.3	+
F Fr. III-F	0	0	+++	+	+		0	0	0	90		57
G Fr. III-S					+++						11	100
Column	1	2	3	4	5	6	7	8	9	10	11	13

*Fraction distribution in relative concentrations where electrophoretic analyses performed. Qualitative estimates expressed for other fractions as follows: necessarily present: +++; likely, though not necessarily present: +. Fractions I and III (B and E, respectively) represent the ascending and descending endcomponents obtained from Rbr. serum on electrophoresis in macro scale in phosphate buffer pH 7.8, ionic strength 0.1. Fr. I-F (C) is the separated ascending, Fr. I-S (D) is the separated descending endcomponent of Fr. I, obtained on re-electrophoresis in barbiturate buffer. Likewise Fr. III-F (F) and Fr. III-S (G) are the separated ascending and descending endcomponents obtained on re-electrophoresis of Fr. III in barbiturate buffer. According to the method of preparation Fr. I-F and Fr. III-S can be considered as highly pure albumin and gamma globulin, respectively.

Sites were prepared by the injection of 0.1 ml. of ragweed-reacting serum or its fraction. The sites were tested in the usual manner. Pertinent data as obtained with one of several sera studied (Rbr) are summarized in Table I.

ALBUMIN AND ALPHA₁ GLOBULIN

First let us consider the question of the albumin and alpha₁ globulin fractions. In the total serum, passive transfer tests yielded an end-point reaction with the Rbr. serum in a skin site containing 5 micrograms of albumin (A, column 7). In the electrophoretically separated fraction I a site containing 1,331 micrograms albumin (B, column 7) yielded no reaction and with Fr. I-F in a site containing 265 micrograms of albumin (C, column 7) there was also no reaction; thus it seems proper to conclude that albumin as such does not carry any skin-sensitizing antibody. In both of these tests, let it be noted, detectable beta and gamma globulin were absent.

Now as to the alpha₁ globulin; an end-point reaction was obtained with the total serum when alpha₁ was present in the amount of 0.7 microgram in the test site (A, column 8). However, in a test with fraction I, when 150 micrograms of alpha₁ were present in the skin site, there was no reaction (B, column 8); hence it would seem that alpha₁ does not contain skin-sensitizing antibody.

GAMMA GLOBULIN

Let us now consider the question of the gamma globulin fraction and its immunologic property of skin sensitization. In the original unseparated serum, a positive end-point reaction was obtained in a skin site containing 1.4 micrograms of gamma globulin (A, column 11). Referring now to fraction III which contained in addition to the predominant gamma globulin also beta and alpha₂ globulin, an end-point reaction was obtained in the site containing 6.3 micrograms of gamma globulin (E, column 11) and in fraction III-S the end-point reaction was obtained in the site which contained 11 micrograms of gamma globulin (G, column 11). Since it appears that 11 micrograms of gamma globulin alone account for the endpoint reaction in fraction III-S, one may calculate that the 1.4 micrograms of the gamma globulin in the original serum account only for 13 per cent of the reactivity of the serum. One must of course understand that all protein components included in the gamma globulin do not necessarily contain sensitizing antibody, but also in many other experiments that we have done, reactive gamma globulin has been separated from most of the sera but has accounted for only from 13 per cent to 25 per cent of the total serum reactivity, never 100 per cent; however, there have been serum fractions in which no detectable gamma globulin was present and reactivity was nevertheless obtained. In other words, gamma globulin is not essential for the transfer of sensitizing antibody and further there have been two instances in which purified gamma globulin did not transfer any sensitization, although the serum from which it was derived was reactive.

ALPHA₂ AND BETA GLOBULIN COMPONENTS

Tests with the whole serum yielded an end-point passive transfer reaction in the site containing 1.5 micrograms of beta globulin (A, column 10) and one

TABLE II. THE INDIVIDUALITY OF THE REACTIONS OF SERA FROM TYPICAL RAGWEED HAY FEVER PATIENTS

PASSIVE TRANSFER SITES*		REACTIONS OF SITES TESTED WITH THE FOLLOWING EXTRACTS†							
SERUM	MIXED WITH	LOW RAGWEED	AMARANTH	CHENOPOD	COCKLEBUR	IVA	MUGWORT	SAGE	RUSSIAN THISTLE
Hre. Hre.	Saline L.R. 1000 U.	+++ 0	++ 0	++ 0	++ 0	+++ 0	+++ 0		++ 0
Aar. Aar.	Saline L.R. 1000 U.	++ 0	++ +	++ +++	++ +	+++ +	++ +		++ +
Wilk. Wilk.	Saline L.R. 1000 U.	+++ ++	++ 0	++ 0	++ ±	++ +	+++ ++	+++ ++	
Elaz. Elaz.	Saline L.R. 1000 U.	+++ 0	++ 0	± 0	++ 0	+++ 0	0 0	0 0	

*The various sera, Hre., Aar., etc., were mixed in equal parts with saline or low ragweed (1000 protein nitrogen units per c.c.), and 1/10 c.c. of each such mixture was injected into the skin of normal test subjects to create sites for later testing.

†Forty-eight hours after sites were made, they were tested by the injection of 1/40 c.c. of each of the various extracts into various sites as shown.

Note: If one compares both the passive transfer test (serum and saline) and the neutralization test (serum and ragweed), it will be observed that no two of these sample sera behave exactly alike.

(Reprinted from Cooke, R. A.: J. ALLERGY 15: 212, 1944.)

microgram of α_2 globulin (A, column 9); since only 13 per cent of the total reactivity is accounted for in the gamma globulin it may be reasoned that the balance of reactivity lies in the α_2 or in beta globulin or both. We have at the present time no positive direct evidence in this respect, since these components have not been separated in purified form, but there is indirect evidence that the α_2 globulin has not contributed to the reactivity of this Rbr. serum since with fraction I in a site containing 54 micrograms of α_2 globulin no reaction was obtained (B, column 9). Observations on other sera, however, do not rule out entirely a possible presence of reactivity in α_2 globulin of certain sera.

In the many samples of separated components that we have tested, beta globulin has been present in most of those that showed passive transfer capacity but we did have instances of reactivity in the entire absence of detectable beta or α_2 globulin.

As yet, we have not been able to account for the totality of serum reactivity in any instance, where sera were likewise fractionated electrophoretically and where the reactivities of the isolated fractions were evaluated quantitatively in the manner outlined. As the results so far obtained do not permit a general unqualified interpretation, we shall continue our studies on this point. On the other hand, about 75 per cent of the activity of a sensitizing plasma was accounted for by the reactivities of the plasma fractions obtained according to Cohn's Method 10.^{5, 6*} Continued electrophoretic-chemical studies using this method are in progress.

In reviewing the sum total of our studies, one significant fact seems to emerge and that is the variation that has been found in sera from different ragweed-sensitive patients as to the location of the sensitizing antibody in the α_2 , beta and gamma globulin fractions. This is in conformity with the previous biological studies which indicate what was described as a "finger-print individuality" of the ragweed sensitizing sera,⁴ since the antibodies of such sera when studied in detail with regard to their reactivities and neutralizations show definite though sometimes minor biologic variations (Table II).

SUMMARY

1. The skin-sensitizing antibody for ragweed has not thus far been found to exist in the albumin or α_1 components of serum. It appears to exist in gamma globulin, but with variations.

2. It appears to exist in the beta and possibly in the α_2 globulin or in the interzones to these components.

3. Neither gamma globulin nor beta globulin nor α_2 is essential to skin reactivity since reactions have been found in the absence of each of these components in electrophoretically detectable amounts.

4. The differences observed in different sera suggest a variation in the distribution of the sensitizing antibody through the globulin area and in this way agrees with the biological variations that ragweed sensitive sera have been shown to possess.

*These latter experiments were carried out jointly with Miss Ella M. Russ, B.S., of the Department of Medicine of the New York Hospital-Cornell Medical Center, New York.

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