

REMOVAL OF A SKIN-REACTIVE COMPONENT IN RAGWEED POLLEN EXTRACT BY HUMAN SERUM PROTEIN PRESENT AS AN IMMUNOLOGIC PRECIPITATE

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IN THIS article the allergenic activity of a fraction of ragweed pollen is shown to be removable by means of a precipitate made by mixing rabbit anti-human-gamma-globulin serum and serum from a patient or subject who had received a series of injections of ragweed pollen extract. The procedure depends on the capacity of antibody to combine with antigen when the antibody itself is present as an insoluble immunologic precipitate. It has been shown by others that rabbit antiequine serum will precipitate soluble equine tetanal antitoxin and that rabbit antiequine serum does not bring about dissociation of diphtheric toxin-antitoxin complexes,^{1, 2} nor does precipitated antitoxin lose its antitoxic activity.³ More recently it was shown that a precipitate made with rabbit antiequine serum and equine antiovalbumin serum would combine with ovalbumin⁴ but that in similar experiments equine antihemocyanin serum, presumably because of stearic hindrance, failed to combine with hemocyanin.⁵

MATERIALS AND METHODS

Rabbit antiserum for human gamma globulin was prepared essentially as described elsewhere.⁶ Immunologic precipitates were made in most instances by mixing twenty parts of rabbit antiserum and one part of human serum (see below). After standing overnight in an icebox, the precipitates were washed by suspension and centrifugation in three exchanges of chilled saline. The precipitate was then suspended in a finely divided state in a solution of allergenic extract. After thirty minutes, the precipitate was removed by centrifugation and the allergenic activity of the supernatant was assayed in the skin of a suitable subject. A reduction in allergenic activity of the supernatant, as compared with an unabsorbed aliquot of the solution of allergenic extract from which the supernatant was prepared, was considered to be evidence that the allergen had been removed by the precipitate.

The human sera were obtained from unselected normal subjects and from three normal subjects and two ragweed pollen-sensitive patients who had received a series of injections of ragweed pollen extract. All sera obtained from the injected normal subjects and patients had neutralizing activity as determined by titration in passively sensitized skin sites in normal subjects.⁷

Crude allergenic extract was prepared from defatted ragweed pollen extracted with distilled water in a proportion of 1 to 10 by weight. Fraction I⁸

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was prepared essentially as described elsewhere.⁹ A precipitate was obtained from crude pollen extract at pH 4 by the addition of ammonium sulfate to saturation. The precipitate was redissolved and again precipitated four successive times. Fraction I was then obtained by precipitation and reprecipitation with ammonium sulfate at 50 per cent saturation, following which the ammonium sulfate was removed by dialysis. The resulting solution was not entirely free of Fraction II as judged by the persistence of some skin reactivity after heating for one hour at 70° C. One unit of Fraction I is defined as 0.00001 mg. of nitrogen precipitable with phosphotungstic acid.

RESULTS

Among twenty-one experiments designed to detect removal of allergenic activity by precipitated serum from normal subjects or from patients who had received a series of injections of crude ragweed pollen extract, evidence for removal was obtained in eighteen. Such evidence was not consistently obtained when the supernatants were made with dilutions of crude pollen extract tested in the skin of patients with ragweed hay fever. However, if such tests were made in the skin of normal persons passively sensitized with serum from pollen-sensitive patients or if Fraction I was substituted for crude pollen extract, the precipitates obtained from 0.1 to 0.5 ml. of human serum consistently removed or markedly diminished the skin reactivity of solutions containing 150 to 1,000 Noon units of crude pollen extract or 2 to 200 units of Fraction I in a volume of 1 ml. Five similar tests done with serum from normal subjects who had received no injections of ragweed pollen extract gave no evidence of removal of allergenic activity, and in a sixth subject equivocal evidence for such removal was obtained (see below).

Within the quantitative limits of the procedures involved, the correlation between the results obtained with the precipitates made with the human sera and the titers obtained by the neutralization tests made in skin with mixtures of human serum and ragweed pollen extract,⁷ indicated that the neutralizing or blocking antibody was involved. However, the design of the experiments was not such as to yield results sufficiently quantitative to warrant the conclusion that this antibody alone was involved, a point which will require further study.

An experiment is described in some detail below and in Figs. 1 and 2. In this experiment the supernatants were tested in triplicate in order to minimize error which might be introduced by the variability of reactions obtained with the skin test procedure. It was shown that equivalent and fivefold concentrations of Fraction I, in terms of unabsorbed extract added, exhibited no evidence of allergenic activity and that a concentration twenty times greater gave skin reactions distinctly less intense than those obtained with unabsorbed Fraction I or Fraction I exposed to a resuspended precipitate prepared from serum from a normal person who had received no injections of ragweed pollen extract (Fig. 1). When the concentration of Fraction I, in terms of unabsorbed extract added, was 100 times greater, however, intense skin reactions were again obtained. In this experiment, the concentration of Fraction I, in

which the precipitate was dispersed and subsequently removed, was 1,000 times that required to produce an intense reaction in the skin of the test subject. These results indicated, therefore, that more than 90 per cent of the allergenic activity of the extract had been removed by the precipitate.

SUPERNATANT OR SOLUTION INJECTED			REACTIONS OBTAINED FOLLOWING THE INTRACUTANEOUS INJECTION IN TRIPLICATE OF 0.02 mL. OF SOLUTION OR SUPERNATANT INDICATED		
OBTAINED FROM	DILUTION	FRACTION I IN TERMS OF UNABSORBED EXTRACT ADDED U./mL.	← 5 cm. →		
Saline "absorbed" with Precipitate from LOM Serum	UNDILUTED	0			
Fraction I 100 u./mL.	1:1000	0.1			
Fraction I - 100 u./mL., Absorbed with Precipitated: Normal Serum LOM Serum	Normal Serum	1:1000			
		1:1000			
		1:200			
		1:50			
		1:10			
Saline Solution Used in Preparing Supernatants & Dilutions					

Fig. 1.—The inner area (shaded) represents the area and configuration of the wheal, the outer (unshaded) area that of the erythema. Tracings were made by overlaying the test sites with cellophane. All readings were made exactly twelve minutes after injection of the solution to be tested.

The precipitate used in this experiment represented 0.4 ml. of Lom serum. This serum was active in sensitizing normal skin in a dilution of 1:100, required the addition of 750 u of Fraction I per milliliter of serum to prevent sensitization of normal skin, and had a neutralizing capacity (?) lying between 300 and 1,000 Noon units of crude pollen extract.

The same supernatants were also tested in sites in the skin of a normal subject sensitized passively with serum obtained from a patient with ragweed hay fever (Fig. 2). The level of reactivity of the sites was so low as to require the use of undiluted supernatant, and a titration was therefore not attempted. No evidence of allergenic activity was evident in the supernatant obtained after absorption with precipitated serum from the treated patient. The precipitate obtained from serum from a normal subject gave equivocal evidence of absorption as judged by the slightly less intense reactions obtained with this supernatant as compared with the unabsorbed allergenic solution. This was the only

instance in which a precipitate made with serum from a normal subject, who had received no injections of pollen extract, appeared to remove allergenic activity, and its significance is not clear at this time.

A single preliminary experiment along similar lines was carried out with insulin labeled with I^{131} and serum from a diabetic patient highly resistant to insulin. It was shown by paper electrophoresis, using a technique which has

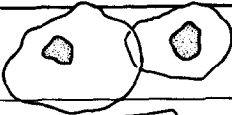
SOLUTION OR SUPERNATANT MADE WITH :		REACTIONS OBTAINED FOLLOWING THE INTRACUTANEOUS INJECTION OF 0.02 mL. OF SOLUTION OR SUPERNATANT INDICATED ← 5 cm. →
FRACTION I IN TERMS OF UNABSORBED EXTRACT ADDED U./mL.	PRECIPITATED SERUM	
100	0	
25	0	
10	0	
0	LOM	
100	Normal	
100	LOM	
SALINE SOLUTION USED IN PREPARING SUPERNATANTS & DILUTIONS		

Fig. 2.—For explanation, see legend to Fig. 1.

been described elsewhere,¹⁰ that this serum had the capacity to bind labeled insulin. Absorption of insulin by the precipitate made with the patient's serum was evident, whereas this was not seen when a precipitate made with serum from a normal subject was used as a control. The experiment was complicated by absorption of insulin onto glass surfaces and this interfered with a quantitative estimate of the amount of binding with the precipitate. Although the use of a radioactive tracer is not applicable to such complex mixtures as pollen extracts, it is felt that the precision presumably obtainable with a radioactive tracer, when a single antigen is used, warrants further studies of binding by antibody present as an immunologic precipitate by such means.

DISCUSSION

The results indicate that at least one skin-reactive component in ragweed pollen extract, Fraction I, combines with and may be removed by precipitated antibody derived from the serum of normal persons and patients after they had received a series of injections of crude ragweed pollen extract. Although combination of antibody with antigen under these circumstances has been recognized for many years, we are not aware of attempts to apply this principle to the detection of antibody.

The procedure, though having the disadvantage common to all methods which entail bio-assay (skin testing), has certain advantages in studies designed to detect and perhaps quantitate antibody for allergens (skin-reactive antigens). It appears to be well suited for detection of such allergens, when these are present in immunologically complex mixtures. Since the antibody is present as a virtually insoluble precipitate, there is little likelihood of adding substances to the solution to be injected which would produce nonspecific reactions. Furthermore, tests can be made in the skin of those who are already sensitive and therefore preparation of sites by first injecting serum containing skin-sensitizing antibody is unnecessary.

The nature of the antibody upon which the results depended is in doubt. Since the sera giving positive results were obtained from both normal persons and patients who had received injections of ragweed pollen extract, it is probable that the "neutralizing" or "blocking" antibody was involved, rather than the skin-sensitizing antibody. The failure to obtain positive results in some of the early experiments in which crude extract was used instead of Fraction I suggests that either Fraction II fails to combine with antibody under the circumstances of the experiment or that the persons selected failed to develop antibody for this fraction.

SUMMARY AND CONCLUSIONS

Evidence is presented indicating that combination occurs between a component of ragweed pollen extract and human antibody for this component when the antibody is present as a specific immunologic precipitate. The human antibody involved is presumably "blocking" or "neutralizing" in nature, rather than "skin-sensitizing." The procedure would appear to offer a means of detecting antibody for allergenically active substances in antigenically complex mixtures. Further study will be required in order to determine its suitability for quantitative studies.

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