

The allergic response to stinging insects

VI. Fractionation of whole body and venom sac extracts of wasp

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Extracts of whole body and of venom sac of wasps (Polistes exclamans) were fractionated by DEAE-cellulose chromatography. Nine fractions were separated from whole body, and eight from venom sac preparations. Fractions 6 of venom sac and 8 of whole body were the active components in direct skin testing. The antigenic heterogeneity of each of the fractionated products was examined by means of appropriate rabbit antisera to wasp extracts. All nine of the whole body fractions gave precipitation, as did four of the eight venom sac fractions. They variously gave between one and three lines of precipitation. In addition to this, fractions 6 and 7 of whole body showed, by testing with rabbit antiserum, common reactions, respectively, with bee and yellow jacket, and were presumed to be the components of wasp that were responsible for cross-sensitizations in patients. A final discussion, comparing the findings for bee, wasp, and yellow jacket, is given in the third paper of this series.⁶

Studies on three types of stinging insects revealed complicated patterns of antigen distribution.¹⁻⁴ Several antigenic constituents were found to be present in both the whole body and venom sac extracts of bee, wasp, and yellow jacket. It was also shown that allergenic components were distributed in the venom sac and in the body of each insect. In order to elucidate more clearly the actual distribution and nature of each of these components, it was necessary to apply methods of fractionation to these extracts and to examine the isolated fractions. Such procedures have already been described for bee,⁵ and will be for yellow jacket.⁶ This report deals with such methods of analysis, as applied to the wasp.

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This investigation was supported in part by Research Grants AI-01303 from the National Institute of Allergy and Infectious Diseases and AM-04195 from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, Bethesda, Md.

Received for publication July 19, 1965.

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**Recipient of Research Career Award K6-AI-1377 from the United States Public Health Service.

MATERIALS AND METHODS

Anion exchange chromatography and insect extracts

Venom sac and whole body extracts of wasp (*Polistes exclamans*) were prepared as previously described,* and were examined chromatographically as described in the previous paper.⁵

Test methods

The methods of skin testing, gel diffusion, and production of rabbit antisera were also all the same as described in the previous paper.⁵

RESULTS

Chromatographic fractionation

The fractionation of wasp whole body extract by column chromatography revealed an elution pattern of nine peaks, obtained at various pH levels: five peaks were eluted at pH 7.5, two at 6.5, and one each at 6.0 and 5.0. The fractionation of wasp venom sac extract showed a total of eight peaks: four peaks at pH 7.5, and one each at 6.5, 6.0, 5.5, and 5.0. Nearly as many fractions were obtained from wasp venom sac as from wasp whole body, but the pattern of elution was markedly different; a very high peak of venom sac, peak 4, was eluted late with buffer I, pH 7.5; this peak did not occur in the fractionation of wasp whole body extract. Only one peak, peak 5, was eluted at pH 6.5, in wasp venom sac fractionation, and it was small when compared to peaks 6 and 7 of wasp whole body extract, which were also eluted at pH 6.5. One peak was eluted at pH 5.5 with venom sac and none with whole body.

As with the fractionation of bee extracts,⁵ each peak was considered as a fraction and the tubes were appropriately pooled together. There was no activity as judged by either clinical or immunological methods in the intervals between the peaks.

Skin reactivity in humans

Table I shows the results of skin testing of patients with fractions of venom sac and whole body of wasp extracts, as well as with unfractionated insect extracts. The protein concentration of each extract and fraction was estimated by ultraviolet light absorption (optical density), by means of criteria and assumptions previously discussed.¹ Within reasonable limits, the concentrations of all of these fractions were essentially the same. Five patients were tested, all of whom had positive skin tests to wasp, bee, and yellow jacket whole body extracts. Examination of venom sac fractions showed that fractions 2, 3, 7, and 8 of wasp venom sac were devoid of allergenic activity. Fraction 4 was positive in three patients but at very low titers. Fraction 1 was positive in four patients, and at somewhat higher titers in three of them. Only fractions 5 and 6 were positive in all five patients, and fraction 6 gave higher titers; in fact, it gave higher titers than any other venom sac fractions. Accordingly, this fraction can be considered as the major allergenic component, and perhaps the only one.

*Insects were obtained from Greer Drug and Chemical Corp., Lenoir, N. C. They contained no larvae or pupae; wasps lacking venom sacs were not studied.

Table 1. Skin tests with wasp venom sac and wasp whole body fractions, and with whole insect extracts

	Optical density	Patient				
		Pal.	Hya.	Ful.	Gre.	Bea.
<i>Wasp venom sac fractions</i>						
Fract. 1	0.10	10	10 ³	nr	10 ³	10 ²
Fract. 2	.22	nr	nr	nr	nr	nr
Fract. 3	.08	nr	nr	nr	nr	nr
Fract. 4	.15	10	10	10	nr	nr
Fract. 5	.11	10	10	10 ²	10	10
Fract. 6	.14	10 ³	10 ²	10 ³	10 ⁵	10 ³
Fract. 7	.05	nr	nr	nr	nr	nr
Fract. 8	.03	nr	nr	nr	nr	nr
<i>Wasp whole body fractions</i>						
Fract. 1	0.09	10	10 ²	10	10 ³	10 ²
Fract. 2	.13	nr	10	10	10	10 ²
Fract. 3	.31	10 ²	10	10	10	10
Fract. 4	.10	10	10	10	nr	nr
Fract. 5	.12	nr	10	10	nr	nr
Fract. 6	.42	10	10 ²	10 ³	10 ²	10 ²
Fract. 7	.26	10 ²	10 ²	10 ³	10	10
Fract. 8	.21	10 ⁴	10 ³	10 ²	10 ⁵	10 ⁴
Fract. 9	.04	nr	nr	nr	10	nr
<i>Whole insect extracts</i>						
Bee		10 ⁴	10 ³	10 ⁴	10 ²	10 ³
Wasp		10 ³	10 ⁴	10 ⁵	10 ²	10 ⁴
Yellow jacket		10 ²	10 ³	10 ²	10 ²	10 ³

The optical density readings are essentially proportional to the protein concentrations of the samples.

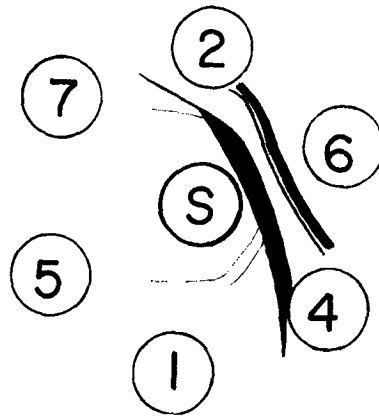
Each skin-test value represents the reciprocal of the greatest dilution still producing a positive wheal and flare reaction.

nr = no reaction.

Examination of whole body products showed that most of the whole body fractions were positive by skin testing. Distinctions could still be made, nonetheless. Fractions 2, 3, 4, 5, and 9 were either negative or gave low titers. In contrast, fractions 1, 6, and 7 gave positive skin tests in all five patients with moderate titers. But the most potent activity was found for fraction 8 which gave positive skin tests in all five patients and at high titers.

Gel diffusion

Fig. 1 illustrates that only wasp venom sac fractions 1, 2, 4, and 6 gave lines of precipitation with a rabbit anti-wasp venom sac serum. Fraction 6 gave three lines; the outer two were specific to fraction 6 and the inner one cross-reacted with the lines formed by fractions 2 and 4. Fraction 2 had two lines of precipitation merging with the inner line of fraction 6. The same phenomenon occurred with fraction 4. Fraction 1 had one line which showed a reaction of complete identity with the inner line of fraction 4. Thus, fractions 1, 2, 4, and 6 shared common antigens, and fraction 6 showed two additional antigens that were specific for this fraction. One of these antigens would seem to be common to the body components, since fraction 6 of venom sac cross-reacted with frac-

**Fig. 1**

Gel diffusion precipitation. Center well, anti-wasp venom sac rabbit serum No. 68. Peripheral wells, fractions of wasp venom sac extract, as numbered.

tion 8 of whole body when they were precipitated by an anti-wasp whole body serum. This reaction is illustrated in Fig. 2. Fraction 6 gave only one line with the anti-wasp whole body serum and it had a reaction of complete identity with fraction 8 of whole body.

All wasp whole body fractions gave precipitating lines with an anti-wasp whole body rabbit serum. In Fig. 3, fraction 1 gave one broad line, fraction 2 gave two lines merging with the fraction 1 broad line. Fractions 3 and 4 also gave two lines. From this figure, one can see that fractions 1 through 4 accounted for the fastest diffusing antigens (those nearer the antiserum well) of whole body extract and that they are closely related. Fraction 5 showed one line of precipitation near the serum well; the apparent line formed near the reservoir of fraction 5 is due to the proximity of fraction 6 and the reservoir of wasp whole body extract. Fraction 6 had three lines of precipitation; two of the inner lines had reactions of identity with two lines of whole body, which themselves were cross-reacting with the two lines formed by fraction 7. The outer line formed by fraction 6 was the only line of all the fractions present to react with the outermost line formed by whole body extract. This line probably represents the antigen of fraction 6 that reacted with an anti-bee rabbit serum, as described below. Fraction 8 had one line of precipitation.

It is interesting to note that fractions 6 and 7 of whole body extract were fractions containing antigens common to other species of stinging insects. This is illustrated in Fig. 4 where fraction 6 was precipitated by an anti-bee whole body rabbit serum. No other fraction had that property. Fig. 5 illustrates the reaction between fraction 7 of wasp and anti-yellow jacket whole body serum. No other fraction than fraction 7 reacted with an anti-yellow jacket extract rabbit serum. The fact that fractions 6 and 7 of wasp whole body extract, both eluted at pH 6.5, cross-reacted with different stinging insects may explain why they elicited positive skin tests in patients sensitive to bee, wasp, or yellow jacket.

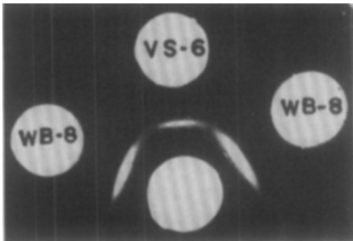


Fig. 2
Gel diffusion precipitation. Center well, anti-wasp whole body rabbit serum No. 57. Well *WB-8*, wasp whole body fraction 8. Well *VS-6*, wasp venom sac fraction 6.

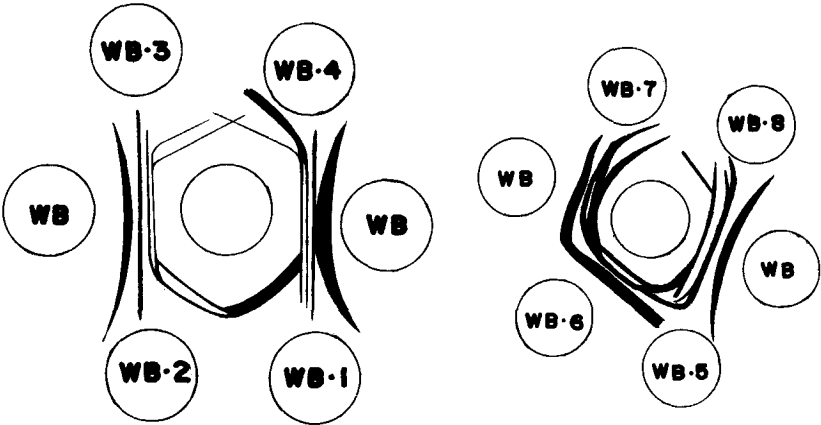


Fig. 3
Gel diffusion precipitation. Center wells, anti-wasp whole body rabbit serum No. 57. Peripheral wells, well *WB*, wasp whole body extract; wells *WB-1* to *WB-4* and *WB-5* to *WB-8*, fractions of corresponding number, of wasp whole body extract.

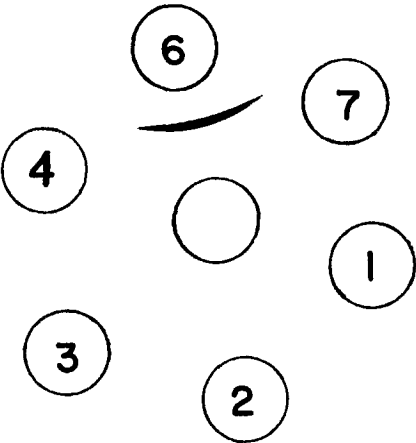
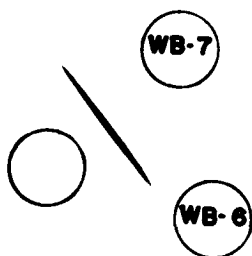


Fig. 4
Gel diffusion precipitation. Center well, anti-bee whole body rabbit serum No. 59. Peripheral wells, fractions of wasp whole body extract, as numbered.

**Fig. 5**

Gel diffusion precipitation. Center well, anti-yellow jacket body rabbit serum No. 56. Well *WB-6*, wasp whole body fraction 6. Well *WB-7*, wasp whole body fraction 7.

DISCUSSION

Well-defined fractions were obtained from wasp whole body and wasp venom sac extracts by column chromatography on DEAE-cellulose. Of the nine fractions obtained from wasp whole body extracts, fraction 8 was the most active as judged by skin tests in sensitive patients. The other fractions were either negative or gave only weak reactions, except for fractions 6 and 7, but these were less potent than fraction 8. The close antigenic relationship of fractions 1, 2, 3, and 4 of wasp whole body could explain their allergenic activity by the presence of a common antigenic determinant as shown by gel diffusion studies. The same phenomenon can explain the activity of fractions 1, 2, and 4 of wasp venom sac, since gel diffusion studies showed a common antigen between those fractions and fraction 6 of wasp venom sac.

The fact that fraction 6 of wasp whole body was precipitated by an anti-bee serum and that fraction 7 was precipitated by an anti-yellow jacket serum may explain their capacity to produce wheal and flare in patients sensitive to wasp, bee, and yellow jacket. Thus, patients who become sensitive to the antigens of fractions 6 and 7 after an encounter with a wasp could also become allergic to bee and yellow jacket, aside from becoming sensitive to wasp. Fraction 6 of wasp venom sac shared a common antigen with fraction 8 of wasp whole body extract, confirming the results of previous studies^{3, 4} showing that the same venom sac antigenic material is distributed throughout the insect body. The other two antigens of fraction 6 of wasp venom sac were specific to venom sac extracts, again confirming previous results.^{3, 4}

The cross-reacting antigens of wasp whole body extracts with anti-bee and anti-yellow jacket rabbit serum were contained in two different wasp whole body fractions, fractions 6 and 7. This result confirmed previous studies^{3, 4} that wasp extracts shared a common antigen with bee and yellow jacket, but that bee and yellow jacket did not share an antigen with each other. This could be so only if the wasp antigen cross-reacting with an anti-bee rabbit serum was different from the wasp antigen cross-reacting with an anti-yellow jacket rabbit serum.

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