

The allergic response to stinging insects

IV. Cross-reactions between bee, wasp, and yellow jacket

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The cross-reaction between bee and yellow jacket is not detected by tanned cell hemagglutination, inhibition of hemagglutination, or agar gel double diffusion. Only by immunoelectrophoresis is it possible to demonstrate a common antigen contained only in the body of these insects. Bees and wasps share one common antigen which is not present in their venom sacs. Wasps and yellow jackets have two common antigens, one of which is localized in the body and the other in the venom sacs of both insects. The implications of these results in regard to human hypersensitivity to stinging insects is discussed.

In a previous publication¹ it was reported that patients who have had an allergic reaction to an insect sting may have skin-sensitizing and hemagglutinating antibodies against one or several insects of the order of Hymenoptera. In a subsequent article,² it was also implied that some of the antigens recovered in venom sac extracts may be specific to the venom, whereas other antigens were common to both venom sac and insect body extracts.

We then became interested in investigating the location of the antigens

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shared by the several stinging insects, to discover whether they were localized in the venom sac or in the bodies of the insects.

MATERIALS AND METHODS

Antigen extracts

Extracts of the following were prepared according to our procedure, as described previously¹: (a) bee (*Apis mellifera*), wasp (*Polistes exclamans*), and yellow jacket (*Vespula pennsylvanicus*) whole insect; (b) sacless bee, wasp, and yellow jacket; (c) bee, wasp, and yellow jacket venom sacs.

Rabbit sera

Rabbits were immunized with either whole insect extracts, sacless insect extracts, or venom sac extracts of each type of insect. The immunization procedure is described in a previous publication.²

Hemagglutination

The tanned cell hemagglutination technique was employed as described by Feinberg and his associates³ and modified by Arbesman and his colleagues.⁴

Inhibition of hemagglutination

Inhibition of hemagglutination was performed as described in a previous publication.⁵

Agar gel diffusion

The agar gel double diffusion method was performed according to the principles of Ouchterlony.⁶

Immunoelectrophoresis

Microimmunoelectrophoresis was performed according to the technique described by Scheidegger.⁷

RESULTS

Inhibition of hemagglutination

Optimal dilutions of our various insect extracts for coating of the tanned red blood cells were ascertained as previously described.²

The hemagglutination of cells coated with all types of bee extracts (whole insect, sacless, or venom sac) and agglutinated by their homologous rabbit antisera, was not inhibited by any of the yellow jacket extracts. The same result, namely, no inhibition, was obtained in the attempted inhibition by bee extracts of hemagglutination produced by anti-yellow jacket sera, with the use of cells coated with yellow jacket extracts. A complete absence of common antigenicity (within the limits of these experiments) between bee and yellow jacket, as measured by tanned cell hemagglutination, was evident.

Tables I and II are illustrative of these experiments. In Table I, the inhibition of an anti-bee venom sac rabbit serum was successfully produced by the different extracts of bee, but not by extracts of yellow jacket. In Table II the

Table I. *Inhibition of tanned-cell hemagglutination of cells coated with bee venom sac extract by rabbit serum No. 66,* with use as inhibitors of the bee extracts and yellow jacket extracts indicated at left*

<i>Inhibiting antigen</i>	<i>Inhibition titer†</i>
Bee whole insect	1:80
Bee sacless insect	1:40
Bee venom sac	1:160
Yellow jacket whole insect	0
Yellow jacket sacless insect	0
Yellow jacket venom sac	0

*Anti-bee venom sac rabbit serum diluted 1 to 20,000.

†Expressed as the greatest dilution of the reagent which was able to inhibit the hemagglutination reaction; 0, no inhibition, even by the undiluted reagent.

Table II. *Inhibition of tanned-cell hemagglutination of cells coated with yellow jacket venom sac extract by rabbit serum No. 65,* with use as inhibitors of the bee extracts and yellow jacket extracts indicated at left*

<i>Inhibiting antigen</i>	<i>Inhibition titer†</i>
Bee whole insect	0
Bee sacless insect	0
Bee venom sac	0
Yellow jacket whole insect	1:40
Yellow jacket sacless insect	1:20
Yellow jacket venom sac	1:80

*Anti-yellow jacket venom sac rabbit serum diluted 1 to 2,000.

†Expressed as the greatest dilution of the reagent which was able to inhibit the hemagglutination reaction; 0, no inhibition, even by the undiluted reagent.

Table III. *Inhibition of tanned-cell hemagglutination of cells coated with wasp whole insect extract by serum No. 57,* with use as inhibitors of the bee extracts and wasp extracts indicated at left*

<i>Inhibiting antigen</i>	<i>Inhibition titer†</i>
Bee whole insect	1:10
Bee sacless insect	1:10
Bee venom sac	0
Wasp whole insect	1:80
Wasp sacless insect	1:20
Wasp venom sac	1:20

*Anti-wasp whole insect rabbit serum diluted 1 to 40,000.

†Expressed as the greatest dilution of the reagent which was able to inhibit the hemagglutination reaction; 0, no inhibition, even by the undiluted reagent.

inhibition of an anti-yellow jacket venom sac rabbit serum was produced by the different extracts of yellow jacket, but not by bee extracts.

The same results were obtained when either the whole body or sacless extracts of bee and yellow jacket were used as coating antigens against their homologous antisera and inhibited with the various extracts of these two insects. The bee extracts did not inhibit the yellow jacket system, and the yellow jacket extracts did not inhibit the bee system.

Table IV. *Inhibition of tanned-cell hemagglutination of cells coated with yellow jacket whole insect extract by serum No. 56,* with use as inhibitors of the yellow jacket and wasp extracts indicated at left*

<i>Inhibiting antigen</i>	<i>Inhibition titer†</i>
Yellow jacket whole insect	1:160
Yellow jacket sacless insect	1:80
Yellow jacket venom sac	1:40
Wasp whole insect	1:20
Wasp sacless insect	1:10
Wasp venom sac	1:5

*Anti-yellow jacket whole insect rabbit serum.

†Expressed as the greatest dilution of the reagent which was able to inhibit the hemagglutination reaction.

Table V. *Inhibition of tanned-cell hemagglutination of cells coated with yellow jacket venom sac by serum No. 65,* with use as inhibitors of the yellow jacket venom sac or wasp venom sac extracts indicated at left*

<i>Inhibiting antigen</i>	<i>Inhibition titer†</i>
Yellow jacket venom sac	1:80
Wasp venom sac	1:10

*Anti-yellow jacket venom sac rabbit serum.

†Expressed as the greatest dilution of the reagent which was able to inhibit the hemagglutination reaction.

Table VI. *Hemagglutination titers of rabbit antisera using cells coated with extract of wasp whole body**

<i>Antiserum</i>	<i>No.</i>	<i>Hemagglutination titer</i>
Whole bee	60	1:2,000
Whole wasp	57	1:128,000

*Each value represents the greatest dilution which still produced a positive reaction.

Some cross-reactions occurred between bee and wasp. Bee whole insect and sacless bee extracts provided some inhibition of the hemagglutination of wasp whole insect coated cells by an anti-wasp whole insect rabbit serum. Bee venom sac failed to produce inhibition in the same experiment. These results are illustrated in Table III. Furthermore, cross-reaction was not demonstrated between bee venom sac extracts and wasp venom sac extracts by these methods.

The reverse experiment demonstrated that wasp whole body and sacless extracts inhibited the reaction of anti-bee whole body and sacless bee coated cells with their homologous antisera. When bee venom sac extracts were used for coating red cells, all the wasp extracts failed to inhibit the hemagglutination with anti-bee venom sac serum.

A higher degree of cross-reaction occurred between wasp and yellow jacket extracts. In Table IV, one can see that all three types of wasp extracts produced

Table VII. *Hemagglutination titers of rabbit antisera using cells coated with extract of wasp venom sac**

<i>Antiserum</i>	<i>No.</i>	<i>Hemagglutination titer</i>
Yellow jacket venom sac	65	1:320
Wasp venom sac	68	1:2,560

*Each value represents the greatest dilution which still produced a positive reaction.

Table VIII. *Hemagglutination titers of rabbit antisera using cells coated with extract of wasp whole body**

<i>Antiserum</i>	<i>No.</i>	<i>Hemagglutination titer</i>
Whole yellow jacket	56	1:3,200
Whole wasp	57	1:128,000

*Each value represents the greatest dilution which still produced a positive reaction.

some inhibition of yellow jacket whole insect extract coated cells agglutinated by an anti-yellow jacket whole insect rabbit serum. Other experiments showed that the reverse was also true. All three types of yellow jacket extract produced some inhibition of wasp whole body extract coated cells agglutinated by an anti-wasp whole body rabbit serum.

Common antigenicity between yellow jacket venom sac extract and wasp venom sac extract was demonstrated. These results are shown in the experiment described in Table V. As illustrated, wasp venom sac extract produced some degree of inhibition of an anti-yellow jacket venom sac rabbit serum.

Direct hemagglutination

By means of a direct tanned cell hemagglutination technique, these findings on inhibition were corroborated by the following experiments:

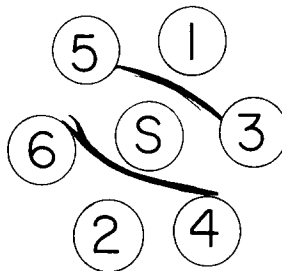
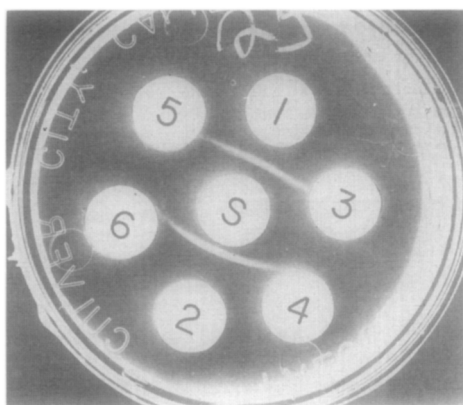
As illustrated in Table VI, an anti-bee whole body rabbit serum partly agglutinated cells coated with wasp whole body extract, but to a degree much lower than the homologous antiwasp serum.

An anti-yellow jacket whole body rabbit serum agglutinated cells coated with wasp whole body extract, but with a titer less than the homologous one (Table VII).

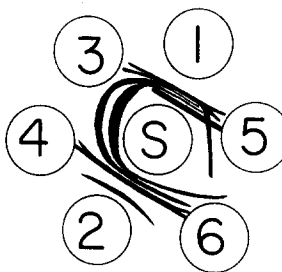
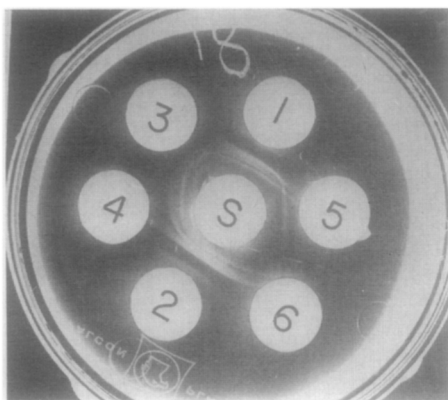
Table VIII illustrates the comparative hemagglutination of cells coated with wasp venom sac extract by an anti-yellow jacket venom sac rabbit serum, and by the homologous anti-wasp venom sac serum. Some slight degree of cross-reaction is visible.

Gel diffusion

Agar gel double diffusion experiments gave results which correlated very well with the results obtained by tanned-cell hemagglutination. Fig. 1 illustrates

**Fig. 1**

Gel diffusion precipitation. Well *S*, Anti-bee venom sac rabbit serum. Peripheral wells: 1, Bee venom sac extract. 2, Bee venom sac extract. 3, Yellow jacket whole insect extract. 4, Wasp whole insect extract. 5, Yellow jacket venom sac extract. 6, Wasp venom sac extract.

**Fig. 2**

Gel diffusion precipitation. Well *S*, Anti-bee whole insect rabbit serum. Peripheral wells: 1, Bee whole insect extract. 2, Bee whole insect extract. 3, Bee venom sac extract. 4, Bee venom sac extract. 5, Wasp whole insect extract. 6, Yellow jacket whole insect extract.

the reaction between an anti-bee venom sac rabbit serum in the center well and extracts of bee, wasp, and yellow jacket. One can see that the anti-bee venom sac serum produced two lines of precipitate with bee venom sac extract in wells 1 and 2; no precipitation occurred between the anti-bee venom sac serum and yellow jacket whole insect or venom sac extracts, nor with wasp whole insect or wasp venom sac extracts. In Fig. 2, an anti-bee whole insect serum in the center well produced five lines of precipitation with its homologous antigen in wells 1 and 2, two lines of precipitation with bee venom sac extract in wells 3 and 4, one line with wasp whole body extract in well 5, and one line (as indicated by a slight deviation of a line from well 2) with yellow jacket whole body in well 6. The line of precipitation of wasp whole body extract was con-

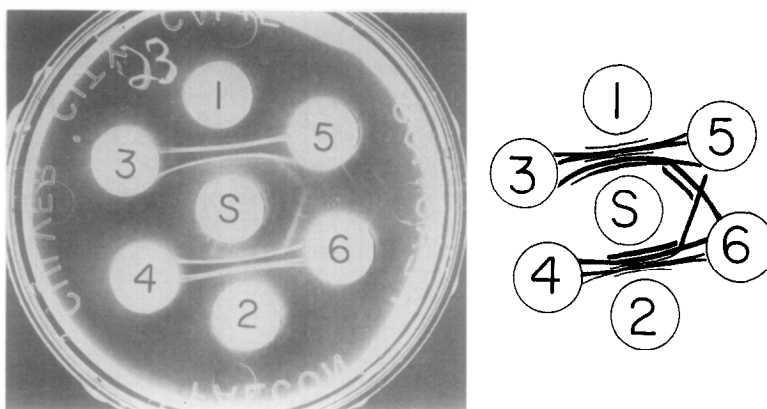


Fig. 3

Gel diffusion precipitation. Well *S*, Antisacless wasp extract. Peripheral wells: 1, Sacless wasp extract. 2, Sacless wasp extract. 3, Bee venom sac extract. 4, Yellow jacket venom sac extract. 5, Yellow jacket whole insect extract. 6, Bee whole insect extract.

tinuous with one of the bee system lines (hence, a reaction of identity), and different from the two lines of the same system which were in common with the lines of bee venom sac extract. This result supported what had been found by hemagglutination, and, along with the results of the previous experiment (Fig. 1), indicated that the common antigen between bee and wasp is localized in the body of the insect, but not in the venom sac.

Fig. 3 illustrates an anti-wasp sacless insect rabbit serum in the center well which gave six lines of precipitation with its homologous antigen in wells 1 and 2, none with bee venom sac extract in well 3, none with yellow jacket venom sac extract in well 4, two lines with yellow jacket whole body extract in well 5, and one line with bee whole body extract in well 6. The two lines of precipitation obtained with yellow jacket whole body extract and the line obtained with bee whole body extract do not cross-react with each other, and they cross-reacted with different lines of the wasp system. These results demonstrated that bee and yellow jacket have no major common antigen, and that the antigens in wasp whole body extracts, which are common on one hand with bee whole extracts and on the other hand with yellow jacket extracts, are totally different from one another.

Immunoelectrophoresis

The relationship between the venom sacs of each of our insects was investigated by immunoelectrophoresis. In a previous publication² it was found that this method revealed more antigens than did the agar double diffusion technique.

In Fig. 4, the anti-yellow jacket venom sac serum gave one line of precipitation with yellow jacket venom sac extract in the center well, one line with wasp venom sac extract in the lower well, and none with bee venom sac extract in the upper well.

In Fig. 5, anti-wasp venom sac rabbit serum gave six lines with wasp venom

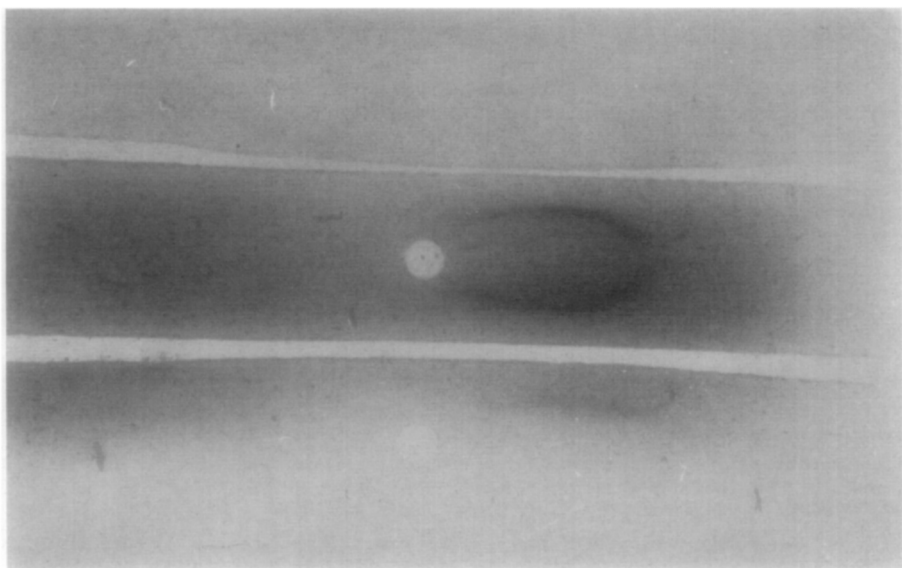


Fig. 4

Immunoelectrophoresis. Upper well: Bee venom sac extract. Center well: Yellow jacket venom sac extract. Lower well: Wasp venom sac extract. Troughs: Anti-yellow jacket venom sac rabbit serum.

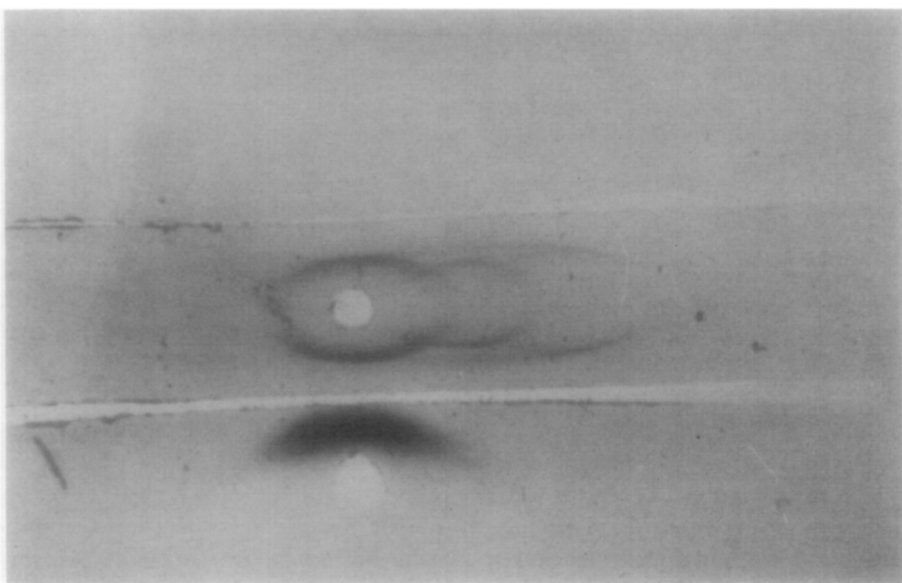


Fig. 5

Immunoelectrophoresis. Upper well: Bee venom sac extract. Center well: Wasp venom sac extract. Lower well: Yellow jacket venom sac extract. Troughs: Anti-wasp venom sac rabbit serum.

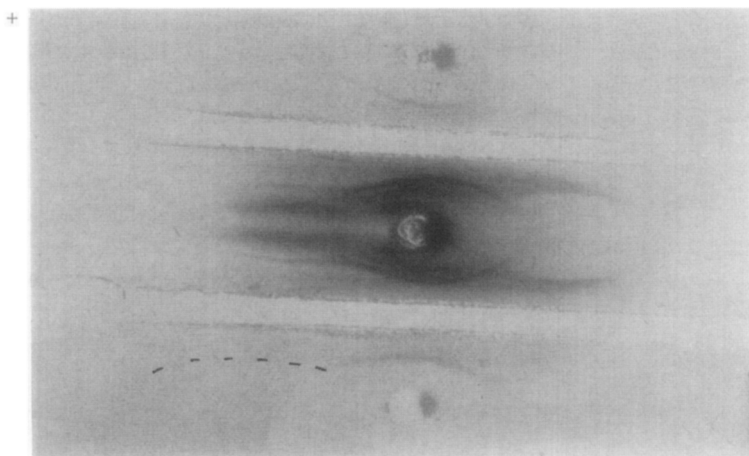


Fig. 6

Immunoelectrophoresis. Upper well: Yellow jacket whole insect extract. Center well: Bee whole insect extract. Lower well: Wasp whole insect extract. Troughs: Anti-bee whole insect rabbit serum. The inked line depicts a very faint line of precipitation which could not be reproduced photographically.

sac extract in the center well, none with bee venom sac extract in the upper well, and one broad line with yellow jacket venom sac extract in the lower well.

An anti-bee venom sac rabbit serum failed to detect any common antigen with yellow jacket or with wasp venom sac extract by immunoelectrophoresis. An anti-bee whole insect rabbit serum, in contrast, gave two lines of precipitation with wasp whole insect extract in the lower well (Fig. 6), but also gave one line of precipitation with yellow jacket whole insect, thus confirming the suggestive evidence in Fig. 2. A common antigen was also demonstrated when an anti-yellow jacket whole insect serum was used against a bee whole insect extract submitted to electrophoresis in agar gel.

DISCUSSION

Foubert and Stier⁹ reported that bee, wasp, yellow hornet, black hornet, and yellow jacket contained from four to six antigenic fractions as studied by agar gel double diffusion. We demonstrated by the same technique approximately the same number of antigenic fractions in bee, wasp, and yellow jacket, as were reported by these authors. Our results were published previously.² In the same publication we clearly demonstrated that by another immunological method, immunoelectrophoresis, a greater number of antigens could be detected—12 to 13 for wasp, 9 for bee, and 9 to 10 for yellow jacket. Foubert and Stier⁹ also demonstrated that two antigens were common between bee and yellow jacket. We were unable to show any antigenic relation between bee and yellow jacket by hemagglutination or inhibition of hemagglutination, although agar gel double diffusion and immunoelectrophoresis did demonstrate one common antigen between bee and yellow jacket. This apparently minor component was distributed in the whole body of both insects, but it was not present in their

venom sacs. Some degree of cross-reaction occurred between bee and wasp, as measured by the immunological methods employed in this study. Here again, the common antigen was present in the body of the insect, and it was not found in the venom sacs.

The greatest degree of cross-reaction occurred between wasp extracts and anti-yellow jacket sera, and conversely, between yellow jacket extracts and anti-wasp sera. Two antigens were common to yellow jacket and wasp. Similar results were published by Foubert and Stier.⁸ Furthermore, our study demonstrated that one of the common antigens was localized in the venom sac of wasp and yellow jacket (Fig. 5) (Table V and Table VII).

Loveless¹⁰ has also reported a common antigenicity between wasp and yellow jacket. She has shown that patients immunized by progressive stings with yellow jacket will be protected not only from subsequent yellow jacket stings, but also from wasp stings. The reverse was also true. Injections of emulsions of wasp venom or stings by wasps were able to protect not only against reactions from wasp stings, but also from stings by yellow jackets.

It is then possible that an individual stung by a wasp can be sensitized to yellow jacket by means of the common antigen existing in their venom sacs. As for bee, it seems probable that an individual must become sensitized to the insect body antigens in order to have antibodies which cross-react with yellow jacket and wasp. We have demonstrated such cross-reacting antibodies in a previous publication¹¹ by tanned cell hemagglutination and passive transfer studies. The manner in which a patient becomes sensitized to these insect body antigens is unknown, although inhalation of insect desquamations has been implied by some authors.¹² Another possibility to consider is that the stinger is also injected, and it probably contains some body proteins different from pure venom.

Although definite differences were shown between venom sac extracts and whole body extracts, this may all be a quantitative rather than a qualitative difference, despite the fact that the protein concentration of each antigen was essentially the same. Obviously, there is some contaminate of body proteins in our venom sac extracts. In subsequent publications we will present data on pure bee venom and its relationship to the other types of bee extract as well as with the extracts of wasp and yellow jacket.

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