

The allergic response to stinging insects

III. The specificity of venom sac antigens

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In a previous publication¹ it was implied that, on occasion, patients sensitive to hymenoptera formed antibodies and were allergic not only to venom sac components but also to antigens found in the insect body. Because of controversy as to the value of using whole insect extracts or venom of hymenoptera for hyposensitization of allergic patients, studies were made on the specificity of the antigens of the venom sac compared to the antigens of the whole insect body.

Our study on the ultracentrifugal patterns and the calculated sedimentation coefficients of venom sac extracts and whole insect extracts showed that, indeed, these extracts did differ by these criteria. It was therefore of interest to determine whether antigenic distinctions could also be made.

MATERIALS AND METHODS

Antigen extracts

Extracts of bee, wasp, and yellow jacket whole insect bodies were prepared according to the procedure previously described.² The protein concentration in

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such extracts, by biuret measurement, was generally between 0.4 per cent and 0.6 per cent. Extracts of sacless bee, wasp, and yellow jacket bodies were prepared by the same procedure as the whole insect extracts. The venom sacs had been removed from the insect bodies prior to extraction.² The protein concentration was of the order of 0.4 per cent to 0.6 per cent. Bee, wasp, and yellow jacket venom sacs were collected and extracted as described previously.² The protein concentration was approximately 0.4 per cent.

Rabbit sera

Two rabbits were immunized with yellow jacket whole insect extracts, two with bee, and two with wasp whole insect extracts. One rabbit each was immunized with venom sac extracts of yellow jacket, bee, and wasp. Extracts of sacless bee, wasp, or yellow jacket were injected into one rabbit for each species of insect.

Each rabbit was immunized according to the schedule described in Table I, using an emulsion made from an equal mixture of extract and Freund's adjuvant. After the trial bleeding at week 8, a final bleeding was performed 3 days later. Each rabbit thus received a total of 2 ml. of either venom sac extracts, whole insect extracts, or sacless insect extracts of either bee, wasp, or yellow jacket. The sera were kept at -20°C . until ready for immunological studies.

Hemagglutination

The tanned cell hemagglutination technique was employed as described by Feinberg, Davidson, and Flick,³ and modified by Arbesman and co-workers.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 used as described by Ouchterlony.⁶ The undiluted extracts were always used in the gel diffusion experiments.

Immunoelectrophoresis

Microimmunoelectrophoresis was performed according to the technique described by Scheidegger.⁷ Macroimmunoelectrophoresis, as described by Grabar,⁸ was also used on occasion.

Table I. *Schedule of rabbit immunization*

<i>Week</i>	<i>Amount of emulsion (ml.)</i>	<i>Freund's adjuvant</i>	<i>Site</i>
0	1.0	Complete	Foot pads
1	1.0	Complete	Dorsal skin
4*	1.0	Incomplete	Foot pads
6*	1.0	Incomplete	Foot pads
8*	—	—	—

*Trial bleeding at this time.

RESULTS

Tanned cell hemagglutination

Yellow jacket extracts. The optimal dilution of the stock extract needed to provide a successful coating of antigen on tanned cells was ascertained by attempting several dilutions of each type of extract. The optimal dilution for yellow jacket whole insect extracts and sacless extracts varied from 1:150 to 1:200. For yellow jacket venom sac extracts the optimal dilution was 1:200.

High titers of hemagglutination were obtained with all the rabbit antisera against their homologous antigens. Anti-yellow jacket whole insect rabbit sera gave titers of 51,200 and 128,000; anti-yellow jacket sacless extract serum gave a titer of 51,200, and anti-yellow jacket venom sac serum had a titer of 25,600. This is illustrated in Table II. Anti-venom sac serum had a lower titer with its homologous antigen than anti-whole insect and anti-sacless had against their respective homologous antigens.

By direct hemagglutination, it was shown that anti-yellow jacket whole insect sera reacted well with cells coated with sacless yellow jacket extracts and with cells coated with yellow jacket venom sac extracts, but with a lower titer than with its homologous extract. This is also illustrated in Table II. Essentially similar results were obtained with anti-sacless insect serum, as illustrated in this Table. Finally, anti-venom sac serum also agglutinated cells coated with extracts of yellow jacket, whether or not these extracts contained venom sac components; however, the homologous antigen produced the greatest titer.

Table II. *Hemagglutination titers of rabbit antisera to yellow jacket extracts using cells coated with yellow jacket extracts**

<i>Antiscrum</i>	<i>No.</i>	<i>Cells coated with yellow jackets extracts</i>		
		<i>Whole body</i>	<i>Sacless body</i>	<i>Venom sacs</i>
Whole body	56	128,000	64,000	12,800
	53	51,200	—	—
Sacless body	61	51,200	51,200	6,400
Venom sacs	65	5,120	2,560	25,600

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

This common antigenicity between different types of extracts and antisera to yellow jacket was also demonstrated by inhibition of hemagglutination. Yellow jacket venom sac, whole insect, and sacless insect extracts gave good inhibition of hemagglutination of an anti-yellow jacket whole insect serum. These results are illustrated in Table III. Only a 1:160 dilution of the stock solution of the homologous antigen (whole body extract) was necessary to inhibit this hemagglutination reaction, whereas it required twice as much sacless extract, and four times more venom sac extract to produce the same results. The whole body and sacless extracts provided inhibition of yellow jacket anti-venom sac rabbit serum, but less inhibition than venom sac extract itself. Here again one notes that the homologous antigen (extract of venom sacs) was able to inhibit this reaction with the least concentration of antigen. As was indicated

Table III. *Inhibition of tanned-cell hemagglutination by extracts of yellow jacket using antisera to whole body and venom sac extracts and cells coated with the homologous preparations**

<i>Inhibiting antigen</i>	<i>Serum No. 56: anti-whole body</i>	<i>Serum No. 65: anti-venom sacs</i>
Whole body	160	40
Sacless body	80	20
Venom sacs	40	80

*Each value represents the reciprocal of the greatest dilution of antigen which prevented hemagglutination. The serum was at a 1:2000 dilution.

Table IV. *Inhibition of tanned-cell hemagglutination by extracts of bee using antisera to bee venom sac extract and cell coated with venom sac extract**

<i>Inhibiting antigen</i>	<i>Serum No. 66: anti-bee venom sacs</i>
Whole body	80
Sacless body	40
Venom sacs	160

*Each value represents the reciprocal of the greatest dilution of antigen which prevented hemagglutination. The serum was at a 1:2000 dilution.

Table V. *Inhibition of tanned-cell hemagglutination by extracts of wasp using antisera to wasp venom sac extract and cells coated with venom sac extract**

<i>Inhibiting antigen</i>	<i>Serum No. 68: anti-wasp venom sacs</i>
Whole body	40
Sacless body	10
Venom sacs	80

*Each value represents the reciprocal of the greatest dilution of antigen which prevented hemagglutination. The serum was at a 1:2000 dilution.

in a previous publication,¹ the protein content of these various extracts does not account for this difference in activity of inhibition of hemagglutination.

Bee extracts. The optimal dilution of the stock extract needed to provide adequate coating of antigen on tanned cells was ascertained by attempting several dilutions of each type of extract. The optimal dilution for bee whole insect extract was 1:50, for bee venom sac extracts, 1:100, and for sacless bee extract 1:25. High titers of hemagglutination were obtained with all the rabbit anti-bee extract sera when tested against their homologous antigen. By direct hemagglutination, as with the yellow jacket system, it was demonstrated that anti-bee whole insect sera produced good titers of hemagglutination when the tanned cells were coated with bee venom sac or sacless bee extract. Similar results were obtained whether an anti-bee venom sacs or an anti-sacless bee serum was used. It was also demonstrated by inhibition of hemagglutination that common antigenicity existed between bee venom sac extracts and sacless bee extracts (Table IV). Common antigenicity is demonstrated by the fact

that the extract of the whole bee, and the extract of the whole body without the venom sac, inhibit the hemagglutination reaction of anti-venom sac rabbit serum and its homologous antigen. The homologous extract, however, produces the greater degree of inhibition.

Wasp extracts. The proper dilution of the stock extract for each type of wasp extract was studied as with the yellow jacket and bee systems. The optimal dilution for wasp whole insect extracts was 1:50 to 1:100, 1:00 for sacless wasp extracts, and 1:50 for wasp venom sac extracts. Direct hemagglutination tests and inhibition tests also demonstrated a common antigenicity between the insect body and its venom sac extract. A typical result of inhibition of hemagglutination is illustrated in Table V. Similar results and hence interpretations are obtained, as with the other two insects studied.

Gel diffusion and immunoelectrophoresis

Gel diffusion. By the double diffusion gel method, four to five lines of precipitation, representing (at least) four to five antibody-antigen systems, were obtained when a whole body extract of either bee, wasp, or yellow jacket was precipitated by its corresponding antiserum. These results corresponded well to those reported by Foubert and Stier.⁹ Hardly any difference was found in the number of lines or in the shape of the precipitation bands when whole body extracts were compared with sacless insect extracts. In Fig. 1, wasp whole body extract and sacless wasp extract were precipitated by an anti-wasp whole body rabbit serum. The number of lines of precipitation is the same in the case of sacless wasp extract, although the line nearest the antigen well is smaller. Similar results were obtained with bee and yellow jacket.

A smaller number of lines of precipitation was obtained when venom sac extracts were analyzed by their homologous antiserum. A typical example is shown in Fig. 2 where bee venom sac extract was precipitated by its homologous antiserum. All the peripheral wells contain bee venom sac extract, but they are located at increasing distances from the center well which contained the serum.

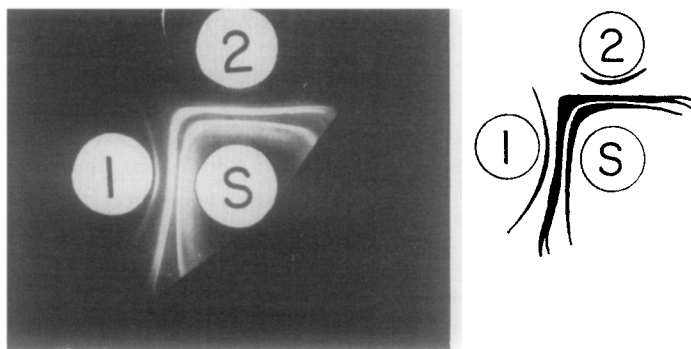
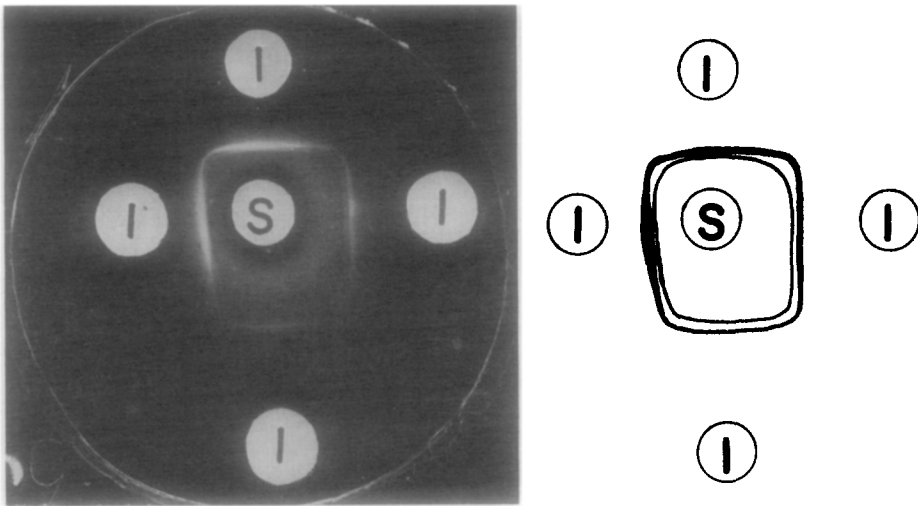
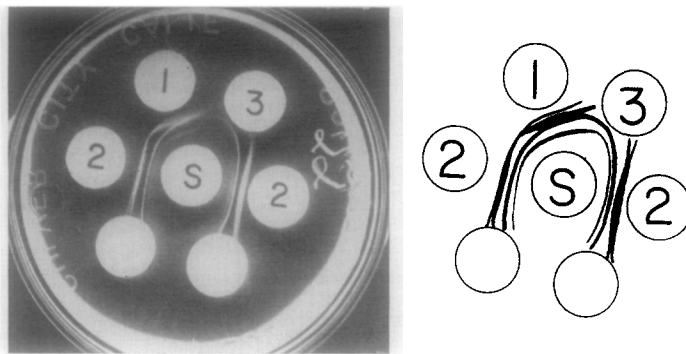


Fig. 1

Gel diffusion precipitation. Well *S*, anti-wasp whole insect rabbit serum. Peripheral wells: *1*, wasp whole insect extract; *2*, wasp sacless extract.

**Fig. 2**

Gel diffusion precipitation. Well *S*, anti-bee venom sac rabbit serum. Peripheral well: *I*, bee venom sac extract.

**Fig. 3**

Gel diffusion precipitation. Well *S*, anti-wasp sacless extract rabbit serum. Peripheral wells: *1*, wasp whole insect extract; *2*, wasp sacless extract; and *3*, wasp venom sac extract.

One can see two lines of precipitation. Two lines of precipitation were also obtained with yellow jacket venom sac extract and its antiserum. Wasp venom sac extract produced two to three lines of precipitation, depending on the extract studied.

We were then interested in finding out if there was any difference between venom sac antigens and the antigens contained in the insect body. Our purpose was to see if all the antigens present in the venom sac extract were also present in the insect body.

In Fig. 3 an anti-wasp sacless insect serum was reacted against wasp whole insect, wasp venom sac, and wasp sacless insect extract. One can see that four

lines occurred with the whole insect extract and with the sacless insect extract, all showing complete identity. Only two lines appeared with the wasp venom sac extract, of which one is very faint. It appeared then that wasp venom sac extract contained two of the four antigens of sacless wasp extract, as demonstrated by the Ouchterlony method.

In Fig. 4 an anti-wasp venom sac serum was reacted against all three types of wasp extracts. Three bands of precipitation occurred with wasp whole insect and wasp venom sac extract. One line was very faint and broad, nearer to the antigen well; a second line was very sharp and clear, and the third line, the

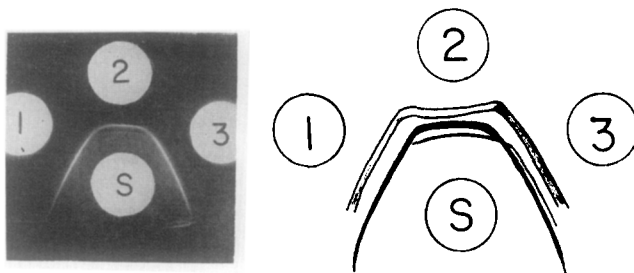


Fig. 4

Gel diffusion precipitation. Well *S*, anti-wasp venom sac rabbit serum. Peripheral wells: 1, wasp sacless extract; 2, wasp venom sac extract; and 3, wasp whole insect extract.

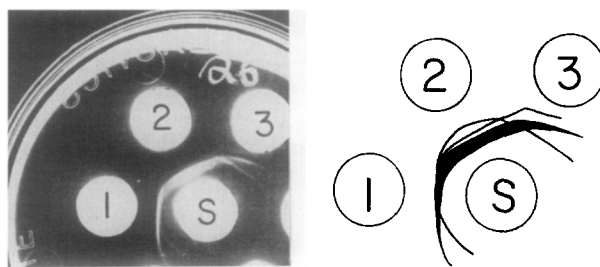


Fig. 5

Gel diffusion precipitation. Well *S*, anti-wasp whole insect rabbit serum. Peripheral wells: 1, wasp sacless extract; 2, wasp whole insect extract; and 3, wasp venom sac extract.

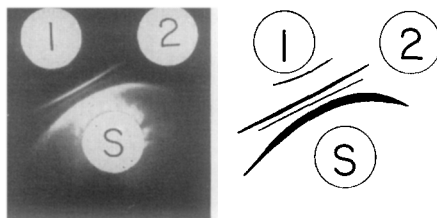
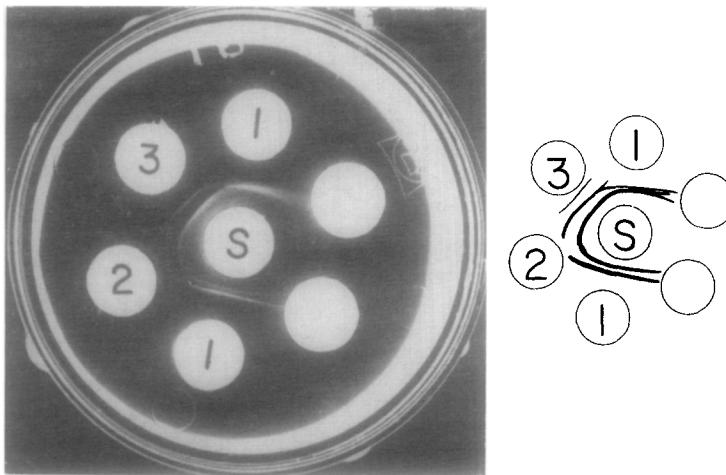
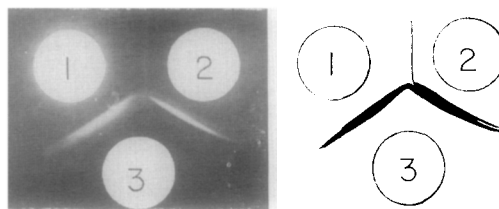


Fig. 6

Gel diffusion precipitation. Well *S*, anti-bee whole insect rabbit serum. Peripheral wells: 1, bee whole insect extract; and 2, bee venom sac extract.

**Fig. 7**

Gel diffusion precipitation. Well *S*, anti-bee venom sac rabbit serum. Peripheral wells: *1*, bee venom sac extract; *2*, bee sacless extract; and *3*, bee whole insect extract.

**Fig. 8**

Gel diffusion precipitation. Well *1*, anti-bee venom sac rabbit serum absorbed with sacless bee extract. Well *2*, nonabsorbed anti-bee venom sac rabbit serum. Well *3*, bee venom sac extract.

nearest to the antiserum well as formed by wasp venom sac extract, became the medial line on the wasp whole insect side and was absent on the sacless insect extract side.

Fig. 5 shows the same results but in a different spatial arrangement. Wasp venom sac and wasp whole insect extracts gave three lines with an anti-venom sac serum. The innermost line on the wasp venom side became the outermost line on the wasp whole insect side, indicating that the antigen producing it is more concentrated in the wasp venom sac extract. Sacless wasp extract again produced only two lines with the anti-wasp venom serum.

Bee extracts were also investigated for the same purpose. In Fig. 6, anti-bee whole insect serum produced four lines with its homologous antigens, and one line with bee venom sac extract.

In Fig. 7, anti-bee venom sac serum gave two lines (possibly a third line) with bee venom sac and bee whole insect extract, whereas sacless bee extract produced one line. Similar results were obtained with the yellow jacket system.

Absorption studies were performed. We were able to demonstrate that sacless extracts of each of the three types of insects studied were incapable of

removing all the antibodies from its homologous insect anti-venom sac serum. Such an experiment is illustrated in Fig. 8. The center well contains bee venom sac extract, the upper right well contains anti-bee venom rabbit serum non-absorbed, and the upper left well contains anti-bee venom sac serum absorbed with an excess of sacless bee extract. Two lines appeared between bee venom sac extract and anti-bee venom sac serum on the right. Only one line appeared between the antigen well in the center and the absorbed bee venom sac serum absorbed with sacless extract on the left. One vertical line appeared between both upper antisera wells due to an excess of sacless bee extract in the upper left well; this line corresponded to the line which had been removed between the center and the upper left well, constituting further proof of one common antigen between the venom sac extract and sacless insect extract.

Immunoelectrophoresis. Several components were revealed in our extracts by the immunoelectrophoresis technique. In Fig. 9, bee whole insect extract, venom sac extract, and sacless insect extract were submitted to immunoelectrophoresis and precipitated by an anti-bee whole insect rabbit serum. Nine lines of precipitation occurred with the whole insect extract in the center well, one band with venom sac extract in the upper well, and seven with the sacless extract in the lower well. Similar results were obtained with yellow jacket extracts. In Fig. 10, a sacless insect extract and a whole insect extract of wasp gave twelve to thirteen arcs of precipitation against an anti-wasp whole insect extract serum, whereas wasp venom extract against the same antiserum produced five arcs. In Fig. 11, the Osserman method¹⁰ of combined immunoelectrophoresis and gel diffusion was used to study the relation between wasp venom antigens and sacless wasp extract. Wasp venom extract was electrophoresed in the upper and lower well, and wasp anti-venom sac serum was placed in the lower trough. In the upper trough, however, was placed a sacless wasp extract—an antigen preparation, and one which could be expected to contain only a few antigens for this antiserum. These antigens would be expected to diffuse straight across toward the other trough, giving lines of precipitation which would run parallel to the

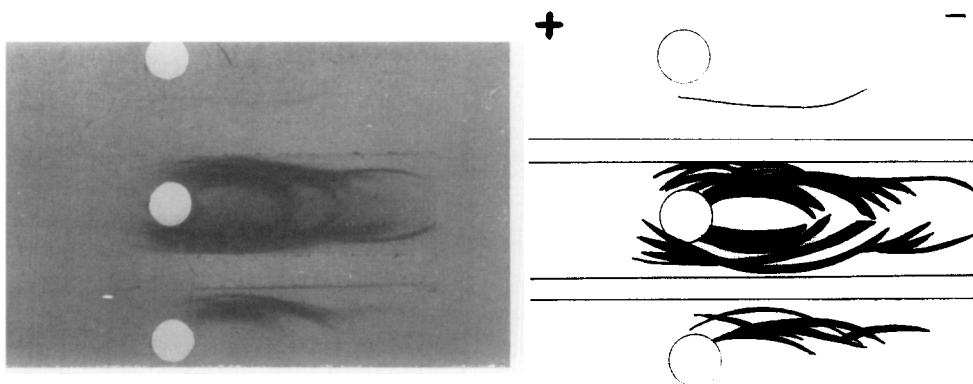
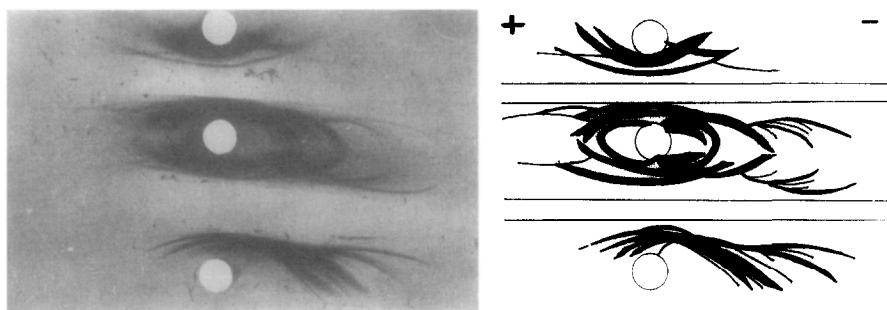
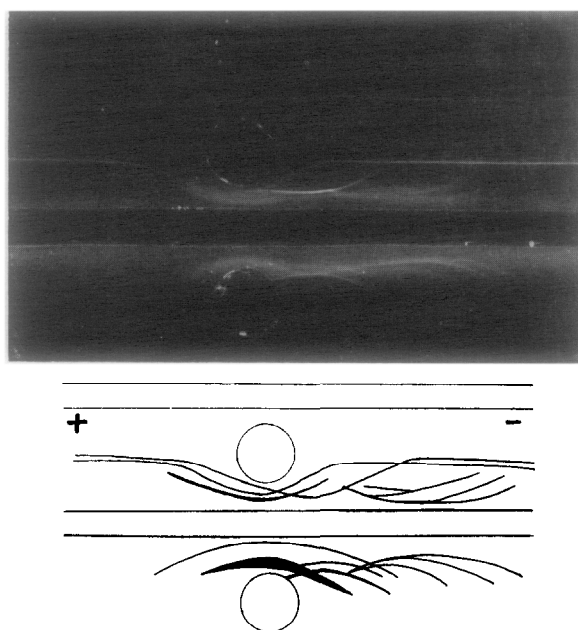


Fig. 9

Immunoelectrophoresis. Upper well: bee venom sac extract. Center well: bee whole insect extract. Lower well: bee sacless extract. Troughs: anti-bee whole insect rabbit serum.

**Fig. 10**

Immunoelectrophoresis. Upper well: wasp venom sac extract. Center well: wasp whole insect extract. Lower well: wasp sacless extract. Troughs: anti-wasp whole insect rabbit serum.

**Fig. 11**

Immunoelectrophoresis. Wells: wasp venom sac extracts. Upper trough: wasp sacless insect extract. Lower trough: anti-wasp venom sac rabbit serum.

troughs and straight in shape, except where each would merge in a reaction of identity with some particular arc of precipitation resulting from the corresponding antigen in the mixture from the electrophoresis well. A total of seven arcs of precipitation occurred, of which two showed merger with the two straight lines. This showed that these two components of venom sac extract were also present in sacless body extract.

Bee venom sac extract and yellow jacket venom sac extract showed only two arcs of precipitation by immunoelectrophoresis, of which one was common to the insect body components.

DISCUSSION

High titers of antibodies were easily produced by rabbits immunized with different types of hymenoptera extracts. These antibodies were readily detected by hemagglutination and gel diffusion reactions.

A limited degree of common antigenicity between venom sac extracts and sacless insect extracts was demonstrated by tanned cell hemagglutination and inhibition of hemagglutination. Sacless insect extract-coated cells were agglutinated by anti-venom sac sera and vice versa, venom sac extract-coated cells were agglutinated by anti-sacless insect rabbit sera. Furthermore, sacless insect extracts were capable of producing, to a limited degree, inhibition of anti-venom sac extract hemagglutination. These results were obtained with all three types of hymenoptera studied.

By the gel diffusion technique we were able to demonstrate the presence of one or two common antigens between venom sac extract and sacless insect extract. Absorption studies demonstrated clearly, however, that venom sac extracts also contained specific antigens that were not distributed in the sacless extract.

The immunoelectrophoretic experiments demonstrated the complex nature of whole insect extracts, as well as of sacless extracts. It provided us with another tool to study the specificity of venom sac antigens.

As many as seven antigens were demonstrated in wasp venom sac extracts by this method, compared with 12 or 13 components for sacless insect extracts. Of the seven components of wasp venom sac extracts, two were seen to be common to the wasp insect body. For bee and for yellow jacket, one out of two venom sac components was common to the insect body.

We are well aware that some contamination of our venom sac extract by body components is possible because of our method of extraction, and further studies are in progress on pure venom collected by special electrical devices.^{11, 12} The demonstration of antigens specific to venom sac extracts, and of antigens common to venom sac extract and sacless insect extracts, lends support to our hypothesis¹ that certain human beings may form antibodies against venom components only, whereas others may produce antibodies against both types of antigens, the specific and the common. Furthermore, as will be demonstrated in a subsequent publication, the antigens common to yellow jacket, wasp, and bee are located mostly in the body of the insects. Patients sensitive to different types of hymenoptera, as demonstrated by passive transfer studies, hemagglutination, and inhibition of hemagglutination,¹ probably formed antibodies against body antigens, which are the ones common to several types of stinging insects.

It must be remembered that the studies presented in this paper deal only with rabbit antisera; the antigen-antibody system which produces anaphylactoid reactions in humans might quite possibly differ in some respects.

SUMMARY

Common antigens were demonstrated in venom sac and sacless insect extracts of hymenoptera by hemmagglutination, gel diffusion, and immunoelectrophoresis. The insects studied were bee, wasp, and yellow jacket. Antigens specific to venom sac extracts of hymenoptera were also demonstrated by the same methods. There

is at least one, and possibly two, antigens specific for the venom sac, for each of these insects. The complex antigenic nature of hymenoptera extracts was illustrated by immunoelectrophoresis. These results are discussed in the light of previous studies on human sera of patients sensitive to hymenoptera.

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