

Comparison of vespid venoms collected by electrostimulation and by venom sac extraction

U. Mueller, M.D.,* R. Reisman, M.D., J. Wypych, Ph.D., W. Elliott, Ph.D.,
R. Steger, B.S., S. Walsh, M.S., and C. Arbesman, M.D. Buffalo, N.Y.

Venoms of three vespid species, yellow jacket, bald-faced hornet, and yellow hornet, obtained by either electrostimulation or venom sac extraction were compared with regard to their enzymatic activity, antigenicity, and allergenicity. Phospholipase A, phospholipase B, and hyaluronidase enzymatic activities were present in all six preparations. The activity of venom sac extracts lay in the range found in different batches of venoms obtained by electrostimulation for each species. Analysis of sera from vespid-sensitive patients in the radioallergosorbent test (RAST) with discs coupled with either venom sac extracts or venoms obtained by electrostimulation showed a good correlation of the results within all three species ($r = 0.95$). In RAST inhibition the potency of venom sac extracts and venom obtained by electrostimulation was similar for each species. Analysis of rabbit antisera to the six preparations revealed similar patterns in immunoelectrophoresis and identity reactions between the major antigens within each species. Tissue protein contamination was detected in all venom sac extracts but not in venoms obtained by electrostimulation. (J ALLERGY CLIN IMMUNOL 68:254, 1981.)

In recent years clinical and immunologic studies have shown that Hymenoptera venom preparations are reliable for the diagnosis and treatment of stinging insect allergy.¹⁻³ These studies have also suggested that extracts prepared by crushing whole insect bodies (whole body extracts), which have been used for many years, are ineffective, probably lacking sufficient venom content.⁴⁻⁶ Honeybee venom can be

collected in large amounts by the method of electrostimulation as described by Benton et al.⁷ The commercially available honeybee venom is collected in this manner. Problems have been encountered in adapting this method to the collection of vespid venoms (hornet, yellow jacket, wasp).^{8, 9} As a result, the commercially available vespid venoms are collected by extraction of venom sacs. Venoms collected by this technique contain tissue protein components.^{10, 11} Venom proteins in venom sac extracts may be decreased in relative concentrations by the admixture of extraneous protein, and the preparation therefore may be less potent when expressed in activity per milligram of protein.

As part of our research in the field of stinging insect allergy, the electric shock method for the collection of venom has been modified for the collection of vespid venoms.⁸ In this report, vespid venoms obtained by the two methods, electrostimulation and venom sac extraction, are compared with regard to their enzymatic activity, antigenicity, and allergenicity.

From the Departments of Medicine and Biochemistry, State University of New York at Buffalo, and the Allergy Research Laboratory of the Buffalo General Hospital, Buffalo, N.Y.

This study was supported in part by the U.S.P.H.S. Research Grant, R01-AI14501 from the National Institutes of Health, Allergy and Clinical Immunology Branch, Bethesda, Md., and a grant from the *Swiss Foundation for Medical and Biologic Fellowships of the Swiss Academy of Medical Sciences.

Received for publication Feb. 26, 1981.

Accepted for publication July 1, 1981.

Reprint requests to: Robert E. Reisman, M.D., 50 High St., Buffalo, NY 14203.

Abbreviations used

RAST:	Radioallergosorbent test
SDS:	Sodium dodecyl sulfate
PLA:	Phospholipase A
PLB:	Phospholipase B
s:	Venom sac extract
v:	Venoms obtained by electrostimulation
b:	Whole insect bodies
sb:	Sacless bodies

MATERIALS AND METHODS**Antigens**

s Preparations. Venom sac extracts from yellow jacket (*Vespa maculifrons* [Y]), bald-faced hornet (*Vespa maculata* [M]), and yellow hornet (*Vespa arenaria* [A]) prepared by Vespa Laboratories were obtained from Dr. Daniel Mullally, Head of the Collaborative Products Section, IAIDP-NIAID, Bethesda, Md.

These venom sac extracts were prepared by Vespa Laboratories in the following way: Either the entire nest containing insects or insects that had been trapped outside the nest were treated with CO₂ anesthesia and frozen at -20° C. For venom collection the insects were allowed to thaw, and venom sacs were dissected and placed in a vial containing 0.025M β -alanine acetic acid buffer, pH 4.6, in 0.54% saline. In this solution the venom sacs were crushed in a tissue homogenizer for 10 sec and centrifuged, and the supernatant was filtered through Millipore 0.8 μ m and stored at 4° C. The crushed sacs were extracted overnight in the same buffer, centrifuged, filtered, and combined with the original supernatant. The combined material was filtered through Millipore 0.45 μ m, assayed for protein by the Lowry method, and lyophilized.

Lyophilized samples were provided for this study. Individual vials were reconstituted in distilled water to contain 3 to 5 mg protein/ml. The protein content was rechecked by the Biuret method. The solutions were aliquotted and re-lyophilized for further procedures.

v Preparations. The electrostimulation method used for the collection of vespid venoms is a modification of the method originally described by Benton et al.⁷ and has been described elsewhere in detail.⁸

The collection apparatus was placed over the main entrance of the hive and other entrances were obstructed. In general, 30 to 40 volts were used for yellow jacket and yellow hornets, and 50 volts were required for the bald-faced hornet. Each nest was milked for 5 to 10 min. Once milking was completed, the voltage was turned off, the hive was allowed to calm, and the collection apparatus was removed. The glass plate and the polyethylene sheet on which the venoms are collected were placed in a labeled plastic bag and stored at 4° C until further processing, which took place within 24 hr. The polyethylene sheet was then cut into small pieces, placed in a large beaker together with the

TABLE I. Enzymatic activities of venoms obtained by v and s procedures from yellow jacket, bald-faced hornet, and yellow hornet

	PLA*	PLB*	Hyaluronidase†
Yellow jacket			
v Mean‡	72.1	22.8	4.9
Range‡	25-125	18-27.5	2.4-7.4
s	43	35	2.5
Bald-faced hornet			
v Mean	25.4	12.4	6.6
Range	16.3-35.7	6.4-20.4	4.8-8.4
s	15.5	5.1	5.6
Yellow hornet			
v Mean	57.2	7.2	10.1
Range	26-80	5.4-8.4	8.6-12.4
s	45.4	5.7	12

*Data expressed as μ g fatty acid released/min/mg protein.

†Data expressed as mg hyaluronic acid digested/10 min/mg protein.

‡Mean values and range obtained in two to four different batches of v.

venom scraped off the glass plate, and mixed thoroughly with two portions of double-distilled water for two 15-min periods. The two portions were pooled, centrifuged, and lyophilized. The venoms were then reconstituted in borate-buffered saline, pH 8.2, filtered through Millipore 1.2 μ m, assayed for protein by the Biuret method, and re-lyophilized in appropriate aliquots.

Venoms from three yellow jacket species (Y), *V. vulgaris*, *V. maculifrons* and *V. germanica*, and from two hornet species, *V. arenaria* (A) and *V. maculata* (M), were collected by this method. Venoms from the three closely related Y species¹² were pooled.

Insect body extracts. Saline extracts were prepared from b or sb (the rear end of the abdomen containing the venom apparatus was cut off) by rapid extraction methods previously described.⁶ Extracts were filtered through Millipore 1.2 μ m, assayed for protein by the Biuret method, aliquotted, and lyophilized.

Immunodiffusion studies

Rabbit immunization. Rabbits were immunized with 1 ml of an emulsion of equal parts of each allergen preparation in saline and complete Freund's adjuvant. Two to three rabbits were used for each venom or body preparation. Each rabbit received five to six injections at 2- to 3-wk intervals. Trial bleedings were performed 1 to 2 wk after the second to sixth injection. Two-hundred micrograms of yellow jacket venom protein were used per injection. Higher doses (500 μ g protein/injection) were needed to obtain peak antibody responses with hornet venom preparations and with body extracts. Antisera with peak antibody responses from two to three rabbits for each venom preparation were pooled for immunodiffusion studies.

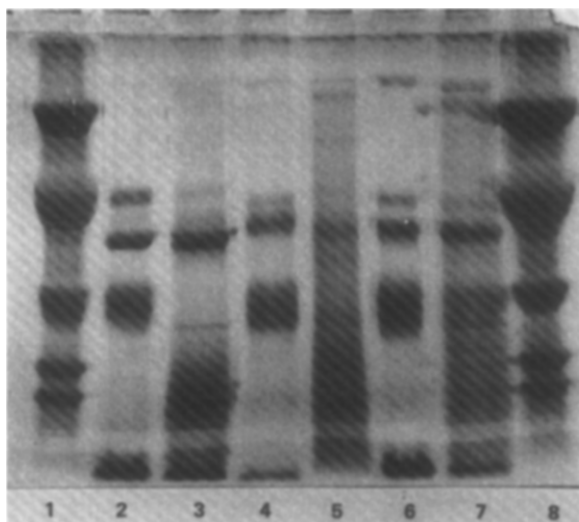


FIG. 1. SDS-electrophoresis. Columns 1 and 8 are markers (from the top): bovine serum albumin, mol.wt. 66,000; ovalbumin, 45,000; trypsinogen, 24,000; β -lactoglobulin, 18,400; and lysozyme 14,300. Column 1, Yv; 3, Ys; 4, Mv; 5, Ms; 6, Av; 7, As. For description, see text.

Double-diffusion gel precipitation. Immunodiffusion studies were conducted in disposable plastic dishes as described previously.¹² For initial titration of trial bleedings, 25 μ l of serial twofold dilutions of antisera in 3% human serum albumin were arranged peripherally around the center well containing 25 μ l of the corresponding allergen preparation at a concentration of 100 μ g/ml. The highest dilution at which at least one precipitation line was formed was designated as the titer of the trial serum. Titers of $>1/32$ were obtained for all antisera. Subsequently, antigen titration studies were done and indicated that 400 μ g/ml was the optimal antigen concentration for resolution of precipitation lines.

Immunoelectrophoresis. Plates of 8 by 10 cm were coated with 12 ml of 1% agarose in a Tris-barbital buffer, pH 8.3 (Tris, 0.08M; barbital, 0.024M; NaN_3 , 0.003M). Seventy-five micrograms of antigen protein were applied to each well. Electrophoresis was run at 10 V/cm for 50 min in Tris-barbital buffer. Troughs were filled with 150 μ l of the respective antisera. Immunodiffusion was carried out for 24 hr at room temperature in a moist chamber. The plates were then washed, dried, and stained with Coomassie Brilliant Blue, 0.2%.

RAST

Specific IgE antibodies to v and s preparations of the three vespids were measured as described previously, with some minor modifications.¹³ Preliminary experiments revealed that s preparations are poor coupling antigens for the RAST, probably because of competitive inhibition by the alanine added during the extraction procedure. Therefore both v and s preparations were dialyzed before coupling. For dialysis, 8 to 50 mg of each venom preparation were dissolved in distilled water to a concentration of 1 to 3 mg protein/ml and dialyzed in Spectra/Por 3 dialysis bags

(Spectrum Medical Industries, Inc., Los Angeles), with a molecular cutoff of 3500 over 3 days against distilled water. The dialysate was centrifuged, and the supernatant was assayed for protein and was lyophilized. The procedure resulted in a protein recovery of 20% to 37% for v and s. One milligram of dialyzed Yv or Ys, 2 mg of dialyzed v and s of the hornets, and 10 mg of sb were used to couple 200 cellulose discs. Results were expressed in percent of the net binding of a serum pool from ragweed-sensitive patients to ragweed discs as described previously.¹⁴ A value of $\geq 10\%$ was regarded as significant. The background activity bound when normal human serum was assayed was 0.7% for ragweed discs and varied between 0.7% (As, Ms, and Mv) and 1.5% (Yv) of the total activity added. The maximal binding went up to 15%.

Sera from 31 patients from western New York state with systemic allergic reactions after vespid stings were obtained before starting immunotherapy. Sera from another 16 patients on immunotherapy with either Yv or Ys were collected before starting immunotherapy and after reaching the maintenance dose of 50 to 100 μ g protein. Serum of a patient with high IgE antibodies to Ysb⁹ was used as a reference for the estimation of IgE antibodies to Ysb.

RAST inhibition

This method was performed as described previously.⁶ One hundred microliters of serial threefold dilutions of the venom preparations starting at 625 μ g protein/ml were used for the absorption of 50 μ l of serum from two patients with high IgE-antibody levels to all three vespid venom preparations. Discs were coupled with dialyzed v preparations of the three vespids. All tests were done in duplicate. The concentration for 50% inhibition of binding was determined on the basis of a sigmoid curve calculated by a microcomputer program. Experiments were repeated if the r value of the calculated curve was <0.9 .

Enzymatic activities

PLA and PLB activities of the venom preparations were measured by titration of fatty acids.¹⁵ The substrate, either 5 ml of egg yolk homogenate (PLA) or 3 mg of lysophosphatidylcholine (PLB), was incubated with the venom in a final volume of 10 ml, containing 1.3×10^{-3} M Na desoxycholate, 0.5% bovine serum albumin, and 3 mM CaCl_2 . Enzyme activity at 40° C was followed by automatic titration of free fatty acids at pH 8, with 0.01N NaOH. One unit of activity releases 1 μ M of fatty acid/min/mg protein.

Hyaluronidase activity was assayed by the turbidimetric method of Tolksdorf, as described in the Worthington Manual.¹⁶ The amount of hyaluronic acid digested was determined from a hyaluronic acid standard curve. Activity is expressed as mg hyaluronic acid digested/10 min/mg protein. Two to four replicates were done for each assay. The error of each individual enzyme assay was less than 25%.

SDS electrophoresis

SDS-electrophoresis was performed according to the discontinuous gel method of Laemmli¹⁷ as described previously.¹⁴ Resolving gel contained a gradient from 10% to

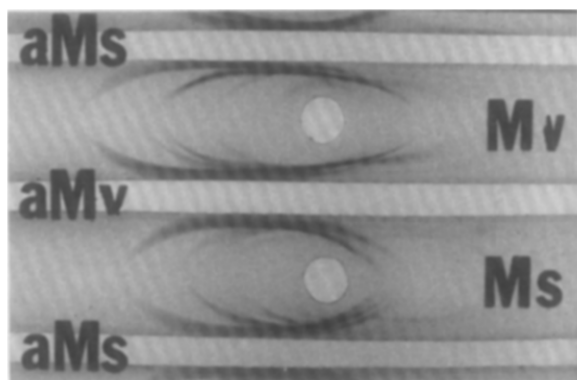


FIG. 2. Immunoelectrophoresis of bald-faced hornet venom obtained by electrostimulation (*Mv*) or venom sac extraction (*Ms*) against the corresponding antisera (*aMv*, *aMs*). The anode is to the right. Some faint additional lines are observed near the application well when *Ms* is assayed against *aMs*.

20% cyanogum. One hundred micrograms of protein of each venom preparation were applied to the preformed wells. Gels were stained in Coomassie Brilliant Blue R250, 0.25%.

RESULTS

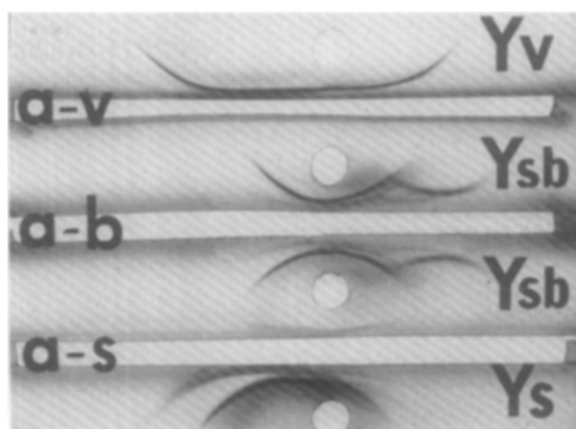
Biochemical analysis

Enzymatic activities of PLA, PLB, and hyaluronidase are given in Table I. The batch-to-batch variability for the three enzymes as found in *v* preparations was quite considerable in all three species. The enzyme activities of *s* preparations lay usually within the range of values observed for different *v* batches of the same species but tended to be lower than the mean value obtained from different *v* batches.

SDS-electrophoresis (Fig. 1) showed generally similar patterns in the high molecular weight (mol.wt.) region, with a strong band at about mol.wt. 35,000, one somewhat fainter at about or slightly above 45,000, and a faint one at greater than 66,000, which were in similar positions in the corresponding *v* and *s* preparations. The band at 45,000 was definitely weaker in *s* than in *v* preparations. A significant difference was found in the low molecular weight region where all *v* preparations showed a broad band at about 25,000, which was absent or markedly weaker (yellow hornet) in *s*. The *s* preparations, however, showed a heavy band at about 15,000, which was only very faint in *v*. All *s* preparations contained faint bands between these major bands, which were not present in *v* preparations.

Rabbit antisera

In immunoelectrophoresis, three to nine components were detected in each of the venom preparations with the corresponding antisera. The precipitation pat-



FIGS. 3A to 3C. Immunoelectrophoresis of *v* and *s* preparations as well as sb against corresponding antisera. *a-b* is antiserum to the corresponding body extract. The anode is to the right. Although the precipitation patterns of *v*-/anti-*v* and *s*-/anti-*s* appear similar, only *a-s* reacted with sb.

FIG. 3A. Immunoelectrophoresis of yellow jacket preparations.

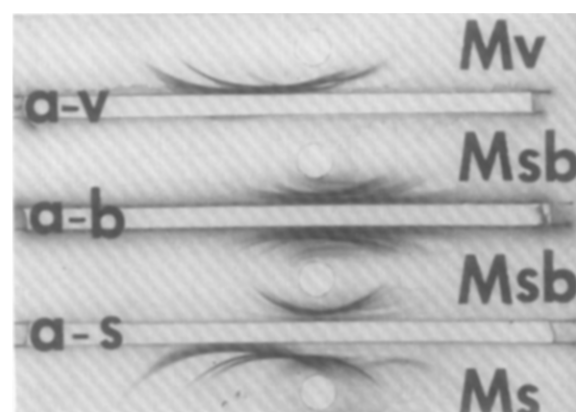


FIG. 3B. Immunoelectrophoresis of bald-faced hornet preparations.

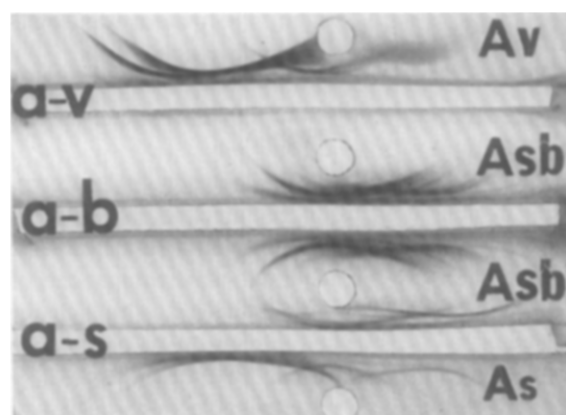
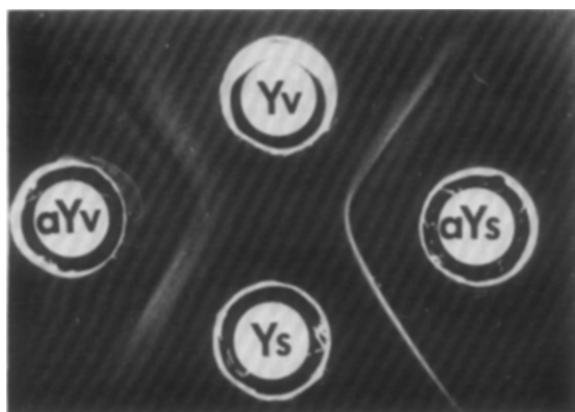


FIG. 3C. Immunoelectrophoresis of yellow hornet preparations.



FIGS. 4A to 4C. Double-diffusion gel precipitation analysis of v and s preparations with the corresponding antisera (aY, aM, aA). Identity reactions are shown for the major antigens in v and s for all three vespid species.

FIG. 4A. Precipitation analysis of yellow jacket preparations.

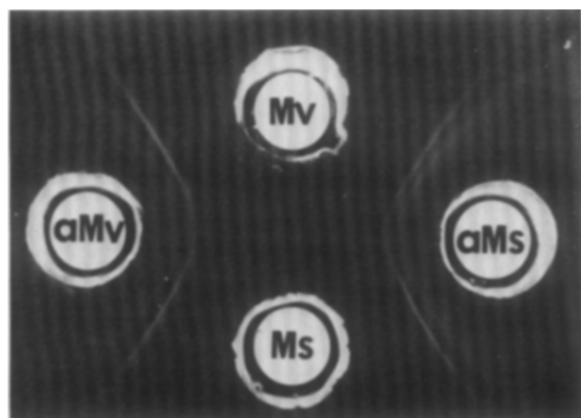


FIG. 4B. Precipitation analysis of bald-faced hornet preparations.

terns obtained with v and s preparations of the hornets were similar (Figs. 2, 3B, and 3C). Faint additional lines near the application well or on the anodic side were found in the s system. The pattern was somewhat different in v and s of Y extracts (Fig. 3A), where v was obtained from *V. maculifrons*, *V. germanica*, and *V. vulgaris* and pooled, whereas s was obtained from *V. maculifrons* only.

In Figs. 4A to 4C v and s preparations are compared in double-diffusion gel precipitation with the respective antisera to s or v. Identity reactions were observed between the major antigenic components of v and s of each of the three species. Although the antiserum to Ys appeared slightly more potent than that to Yv, anti-Av was more potent than anti-As.

Figs. 3A to 3C show immunoelectrophoresis patterns of venom preparations from Y, M, and A, as well as the corresponding sb extracts. Antigenic com-

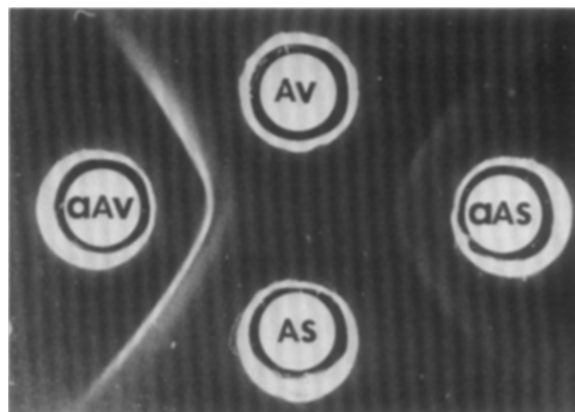


FIG. 4C. Precipitation analysis of yellow hornet preparations.

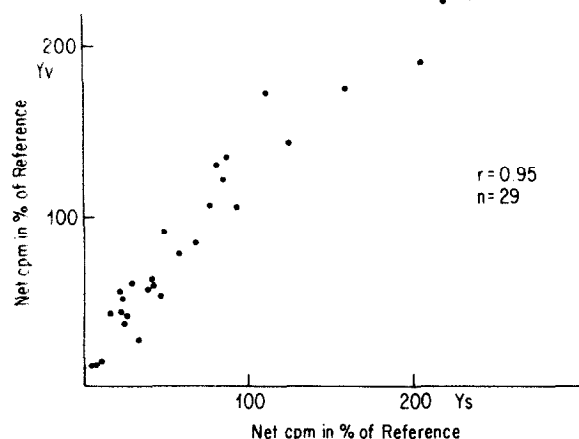
ponents of the venom preparations lay largely on the cathodic side, and those of the sb extracts near the application well or on the anodic side. Antisera to the three v preparations did not react at all with sb, whereas several precipitation lines were observed between sb and the corresponding antisera to s. The reaction between sb and anti-s prominent in M and A but was weak in Y.

Human IgE antibodies

Sera from 31 patients with systemic reactions after vespid stings were investigated by RAST using discs coupled with either v or s of all three species. Figs. 5A to 5C show the results obtained with v- and the corresponding s-coupled discs. A good correlation (Spearman's $r = 0.95$) was found for all three species. The values obtained with v discs tended to be somewhat higher than those obtained with s discs, especially for Y and M.

The different preparations were further tested for their potency in RAST inhibition. Fig. 6A shows studies on Y preparations. With serum 1, v was somewhat more potent than the three batches of s, but with serum 2, the opposite was true. Fig. 6B shows studies on M preparations. Here, v was somewhat more potent in patient 2, and s more potent in patient 1. Finally, Fig. 6C shows studies on A preparations. With both sera, v was more potent than s. The concentrations of v and corresponding s for 50% inhibition of binding lay within 1 log for all three species. The batch-to-batch range, as tested in Ys and in Av, was small.

Sixteen patients on immunotherapy, eight on Yv, and eight on Ys were investigated for the presence of IgE antibodies against Ysb. Significant levels were found in only one patient (12.9% of the reference) before starting immunotherapy, but they decreased to background levels during immunotherapy with Ys.



FIGS. 5A to 5C. Specific IgE antibodies to v and s preparations of three vespid species. A good correlation (Spearman's rank $r = 0.95$) was observed for all species.

FIG. 5A. Specific IgE antibodies to yellow jacket venoms.

DISCUSSION

The problems involved in the collection procedure of vespid venoms by either venom sac extraction or electrostimulation have been addressed in detail elsewhere.^{8, 9} This article compares venoms from three vespid species, obtained by the two methods with regard to their antigenic and allergenic potency and to their possible contamination with tissue protein components.

Analysis of enzymatic activities of PLA, PLB, and hyaluronidase revealed considerable batch-to-batch variability of all three enzymes in the v of the three vespid species, confirming results previously reported by other authors for s.^{18, 19} In this study, enzymatic activities detected in s were usually in the range of those found in v but tended to be somewhat lower than the mean value found for v. The much larger difference in enzymatic activity of the honeybee v and s described by Hoffman¹⁰ may be a result of the different venom sac extraction procedure used by this author or may be species-dependent.

Analysis of molecular composition of v by SDS-electrophoresis shows three major bands at about 45,000, 35,000, and 25,000 mol.wt., as previously described by King et al.¹⁹ and identified by these authors as hyaluronidase, PLA, and antigen 5. An additional faint band with a molecular weight greater than 66,000 was also observed, most prominently in A. Although bands at greater than 66,000, at 45,000, and at 35,000 were also present in all s preparations, the band at 25,000 was virtually absent or faint in s. A heavy band, however, was present at about 15,000 mol.wt. in all s preparations. A possible explanation for this discrepancy between v and s is that antigen 5

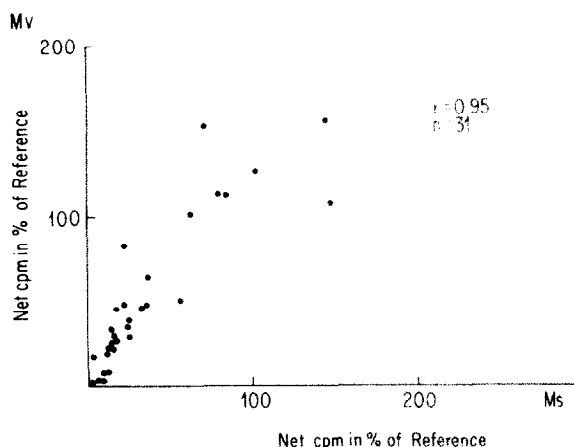


FIG. 5B. Specific IgE antibodies to bald-faced hornet venoms.

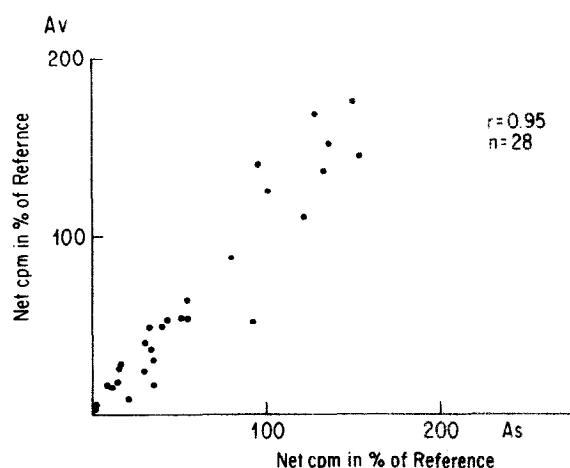
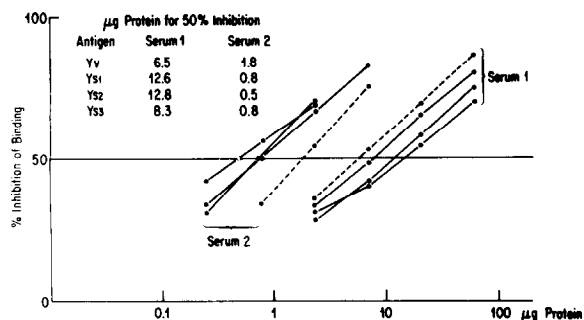


FIG. 5C. Specific IgE antibodies to yellow hornet venoms.

was split into subunits during the work-up procedure of s preparations. The assumption of the presence of different proteins, one in v only and the other in s, seems less convincing if one considers the similarity of immunoelectrophoresis patterns, identity reactions between major components in immunodiffusion, and the close correlation of RAST results. Several minor bands that are present only in s preparations may represent tissue protein components.^{10, 14}

Analysis of sera from patients with vespid sting hypersensitivity by RAST showed a very good correlation of specific IgE antibodies to v and the corresponding s for all three species studied. Values obtained with v-coupled discs tended to be somewhat higher than those obtained with s-coupled discs, suggesting a slightly higher potency of dialyzed v preparations. In RAST inhibition experiments, undialyzed v and s of Y and M were of similar potency, whereas



FIGS. 6A to 6C. RAST inhibition experiments with v (dashed lines) or s (solid lines) preparations. Sera from two patients with high IgE antibody levels to all three vespid species were investigated. Discs were coupled with dialyzed v preparations of the three vespids.

FIG. 6A. RAST for yellow jacket venoms.

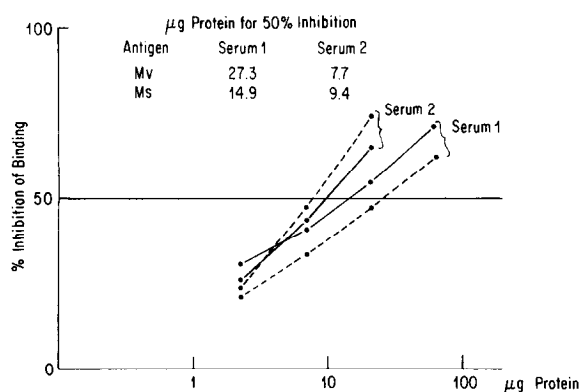


FIG. 6B. RAST for bald-faced hornet venoms.

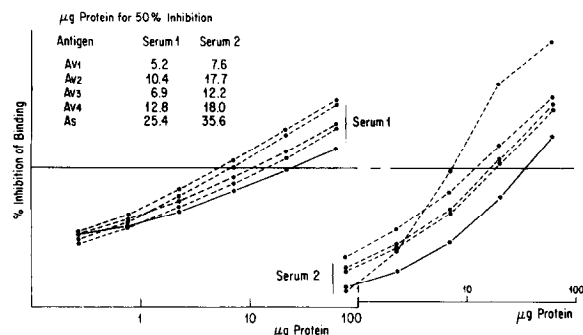


FIG. 6C. RAST for yellow hornet venoms.

Av was somewhat more potent than As in both sera studied. The concentrations for 50% inhibition by v or the corresponding s lay within a narrow range for all three species, indicating comparable allergenic potency of v and s.

Immunodiffusion studies with rabbit antisera showed identity reactions between the major antigens in v and s within each species. Immunoelectrophoresis patterns were similar in v and s preparations of the two

hornets but were somewhat different in Y. This is probably because pooled Yv was obtained from three different yellow jacket species and Ys from only one. It appears that the major antigens in the different Y species have somewhat different electrophoretic motility, resulting in elongated precipitation lines in immunoelectrophoresis of the pooled Yv. Immunodiffusion, however, shows identity reactions between the major antigens of Yv and Ys.

Studies with sb extracts showed that antisera to s reacted clearly with the corresponding sb in all three species, while antisera to v did not. Commercial vespid venom preparations, therefore, do contain tissue protein components in an amount sufficient to induce an immune response in rabbits. The apparently weaker reactivity of antiserum to Ys than that of antiserum to Ms or As with sb could indicate a lower degree of tissue protein contamination of Ys. Alternatively, it may be caused by the lower dose of Ys needed to obtain peak antibody responses to the major antigens in rabbits.

To investigate a possible clinical relevance of this tissue protein contamination of commercial preparations, sera of patients on treatment with either Ys or Yv were examined for the presence of specific IgE antibodies to Ysb before immunotherapy and after reaching maintenance dose. No increase in these IgE antibodies was induced by either treatment in any of the 16 patients studied. A single patient with initially elevated IgE to sb even showed a decrease to normal after reaching maintenance dose of Ys. These observations, although not excluding the possibility of a sensitization to tissue components introduced by immunotherapy with commercial s preparations, do indicate that this is probably a rare event.

In conclusion, both v and s preparations of the three vespids studied were shown to be potent allergens and appear suitable for the diagnosis and immunotherapy of hypersensitivity to vespid stings. The v preparations have a potential advantage of being free of contaminating tissue protein.

We thank Dr. A. Green of Buffalo for providing sera of patients under immunotherapy with commercial Yvs (Pharmacia).

REFERENCES

1. Lichtenstein LM, Valentine MD, Sobotka AK: Insect allergy: the state of the art. *J ALLERGY CLIN IMMUNOL* 64:5, 1979.
2. Reisman RE: Stinging insect allergy. *J ALLERGY CLIN IMMUNOL* 64:3, 1979.
3. Yunginger JW: The sting - revisited. *J ALLERGY CLIN IMMUNOL* 64:1, 1979.
4. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM: A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 229:157, 1978.

5. Mueller U, Thurnheer U, Patrizzi R, Spiess J, Hoigne R: Immunotherapy in bee sting hypersensitivity: bee venom versus whole body extract. *Allergy* **34**:369, 1979.
6. Wypych JJ, Reisman RE, Elliott WB, Steger RJ, Arbesman CE: Immunologic and biochemical evaluation of the potency of whole insect body extracts. *J ALLERGY CLIN IMMUNOL* **63**:267, 1979.
7. Benton AW, Morse RA, Stewart JD: Venom collection from honey bees. *Science* **142**:228, 1963.
8. Eskridge EM, Elliott WB, Elliott AH, Eskridge PB, Doerr JC, Schneller N, Reisman RE: Adaptation of the electrical stimulation procedure for the collection of vespid venoms. *Toxicon* (in press, 1981).
9. Simon RP, Benton AW: A method for mass collection of wasp venoms. *Ann Entomol Soc Am* **62**:277, 1969.
10. Hoffman DR: Allergens in Hymenoptera venoms. IV. Comparison of venom and venom sac extracts. *J ALLERGY CLIN IMMUNOL* **59**:367, 1977.
11. Reisman RE, Wypych J, Lazell M, Arbesman CE: Sensitization to nonvenom components in a nonvenom preparation. *J ALLERGY CLIN IMMUNOL* **64**:281, 1979.
12. Wicher K, Reisman RE, Wypych J, Elliott W, Steger R, Mathews RS, Arbesman CE: Comparison of the immunogenicity of venoms of various species of yellow jackets (genus *Vespula*). *J ALLERGY CLIN IMMUNOL* **66**:244, 1980.
13. Light WC, Reisman RE, Rosario NH, Arbesman CE: Comparison of the allergenic properties of bee venom and whole bee body extract. *Clin Allergy* **6**:293, 1976.
14. Mueller U, Elliott W, Reisman R, Ishay J, Walsh S, Steger R, Wypych J, Arbesman C: Comparison of biochemical and immunologic properties of venoms from four hornet species. *J ALLERGY CLIN IMMUNOL* **67**:290, 1981.
15. Nair BC, Nair C, Denne S, Wypych J, Arbesman CE, Elliott WB: Immunologic comparison of phospholipase A present in Hymenoptera insect venoms. *J ALLERGY CLIN IMMUNOL* **58**:101, 1976.
16. Talksdrorf S, McCready MB, McCullagh DR, Schwenk K: in *Worthington Manual*. Freehold, New Jersey, 1968. Worthington Biochemical Corp. (Distributor), p. 35.
17. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680, 1970.
18. Richman PG, Baer H: A convenient plate assay for the quantitation of hyaluronidase in Hymenoptera venoms. *Anal Biochem* (in press, 1981).
19. King TP, Sobotka AK, Alagon A, Kochoomian L, Lichtenstein LM: Protein allergens of white faced hornet, yellow hornet and yellow jacket venoms. *Biochemistry* **17**:5165, 1978.