

Allergens in Hymenoptera venoms

IV. Comparison of venom and venom sac extracts

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Honeybee venom sac extract is compared with pure venom. All five known allergens of venom are present in venom sac extract. Enzyme analyses indicate that the sac extracts contain 11% to 16% venom. At least 10 additional components, several of which are proteins, are present in venom sac extract. Radioallergosorbent test (RAST) studies of yellow jacket venom and venom sac extract yielded a correlation of $r = 0.94$, with only some weakly reactive sera positive to only one preparation. A few sera were substantially more reactive with venom sac extract. Venom sac extracts appear to be suitable for in vitro diagnostic use, but the extraneous proteins and peptides may make them less suitable than pure venoms for use in immunotherapy.

Recent studies¹⁻⁴ indicate that pure insect venoms are superior to whole body extracts for diagnosis and therapy of stinging insect allergy. Honeybee venom is relatively easy to obtain in a pure state by electrical stimulation,⁵ but it is more difficult to obtain venoms from bumblebees and vespid wasps. Some investigators have prepared venoms by removal from isolated venom sacs with a micro syringe. Although it is still time consuming to prepare, one alternative is the use of venom sac extract. In this study, pure venom is compared to venom sac extract by biochemical, physicochemical, and immunologic methods. Bee venom was used for the biochemical and physicochemical studies, since its protein components are relatively well characterized.^{4, 6-10} Yellow jacket venom and venom sacs were compared by radioallergosorbent test (RAST) in order to compare allergen contents for diagnostic purposes.

MATERIALS AND METHODS

Venom and venom sacs

Bee venom was obtained by electrical stimulation and stored either as an air-dried preparation at -20°C or as a liquid at 4°C . Bee venom was purchased from Champlain Valley Apiaries, Middlebury, Vt. Yellow jacket venom obtained by electrical stimulation was purchased from Davis Insect Venoms, Oshawa, Ontario. A further sample, purchased from Dr. A. Benton, was supplied by Pharmacia

Laboratories. Venom sacs were dissected from freshly killed insects and quick frozen in dry ice. They were supplied by Davis Insect Venoms.

Venom sac extracts

One hundred bee, wasp, or yellow jacket sacs were crushed with a glass rod in 1 ml of water. After standing 10 min, the suspension was centrifuged and the supernatant decanted and freeze-dried. For bumblebee, 10 sacs were extracted per milliliter. One hundred bee venom sacs yielded about 35 mg of freeze-dried extract.

Separation and purified allergens

The methods of separating venom⁴ and isolating the various components^{4, 8, 9} have been previously described. The method for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been previously described.⁴

Enzyme assays

The assays for acid phosphatase,¹⁰ hyaluronidase,¹⁰ and phospholipase⁴ have been described.

Human reaginic sera

Sera were obtained from individuals clinically allergic to yellow jackets and from bee-allergic individuals from one week to one year after the last reaction. All patients had not received any immunotherapy to stinging insects.

Antisera

Antisera were prepared in rabbits and guinea pigs.^{4, 9}

RAST

The method used for RAST has been described in detail.^{4, 9, 11, 12} Yellow jacket venom was coupled with the use of 5 mg/100 discs (0.22 gm). Venom sac extract was coupled with 20 mg/200 discs. All tests were run in duplicate and each experiment repeated. Specific binding was

From the Department of Pathology, Creighton University School of Medicine.

Received for publication Nov. 12, 1976.

Accepted for publication Feb. 1, 1977.

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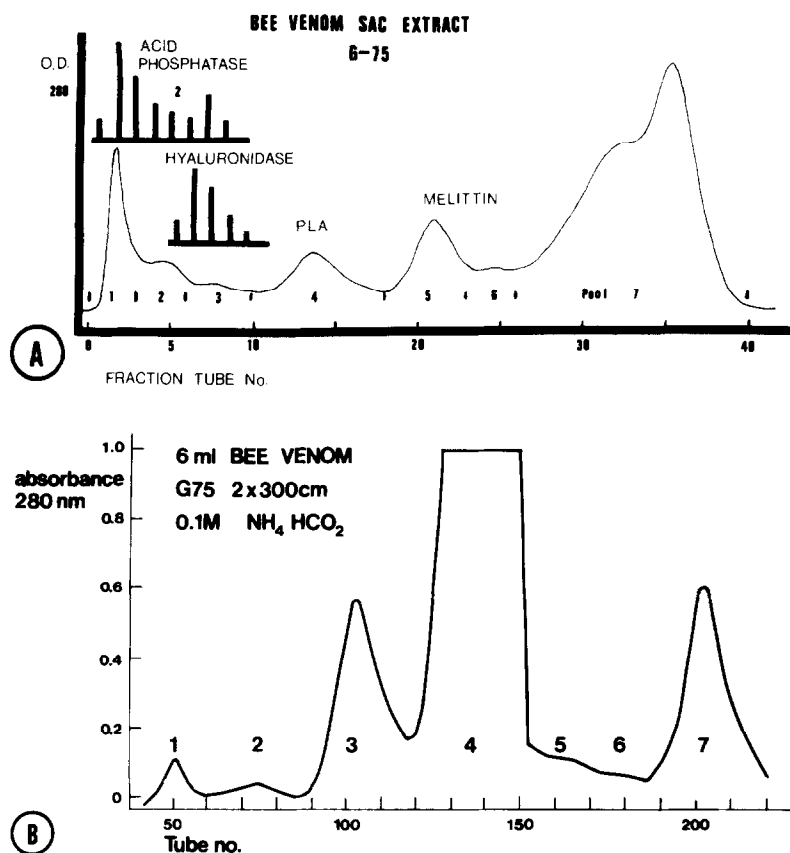


FIG. 1. A, Sephadex G-75 separation profile of 35 mg of bee venom sac extract (100 sacs). The activities of acid phosphatase and hyaluronidase are shown by the bar graphs; other tubes with no results shown were negative. The peak and pool numbers are indicated below the curve and above the axis. The 1.6×100 cm column was run at 14 ml/hr with the use of 0.1 M ammonium formate, pH 4.5, as eluant. B, Sephadex G-75 profile of bee venom. (From Hoffman, D. R., and Shipman, W. H.: *J. ALLERGY CLIN. IMMUNOL.* **58**:551, 1976.)

reproducible to $\pm 10\%$ or $\pm 0.4\%$ of total counts added, whichever was greater.

RESULTS

The gel filtration profile of honeybee venom sac extract on Sephadex G-75 is shown in Fig. 1. The G-75 profile of bee venom is illustrated by Hoffman and Shipman⁴ and in Fig. 1. Several differences are evident. In the venom sac extract the first (exclusion) peak is much larger, as is peak 2. Peak 5 is much less in the venom sac extract and the low molecular weight peaks (pool 7) are much larger. All of the peaks present in venom appear in the venom sac extract.

The enzyme, acid phosphatase, or allergen B (9, 10) is found in the exclusion peak from the G-75 separation of bee venom. In the venom sac extract acid phosphatase was found in the exclusion peak and peak 3. Peak 3 corresponds to about 50,000 d or the molecular weight of allergen B monomer.⁹ Allergen B was detectable in the exclusion peak from venom sac extract by immunodiffusion with the use of guinea pig antiserum. The specific activity of acid phos-

phatase in venom sac extract was 11% of that of pure venom.

Hyaluronidase was found in the leading side of peak 3 in the venom sac extract. The specific activity of hyaluronidase in the venom sac extract was 16% of that in venom. Phospholipase was found in peak 4 of the venom sac extract and melittin in peaks 5 and 6. Since the venom sac extract is less concentrated than venom, melittin appears to have a smaller molecular weight because most of it is no longer in the aggregated form.⁸

Allergen C was found in peak 1 of bee venom sac extract by immunodiffusion with guinea pig and rabbit antisera. The enzyme contents of bee venom sac extract and other Hymenoptera venom sac extracts relative to bee venom are given in Table I. Honeybee venom sac extract appears to be 11% to 16% venom based on this data.

In order to estimate the number of nonvenom protein components in venom sac extract, the seven G-75 fractions were denatured with dithiothreitol-SDS and electrophoresed in 7.5% acrylamide gels in SDS. A

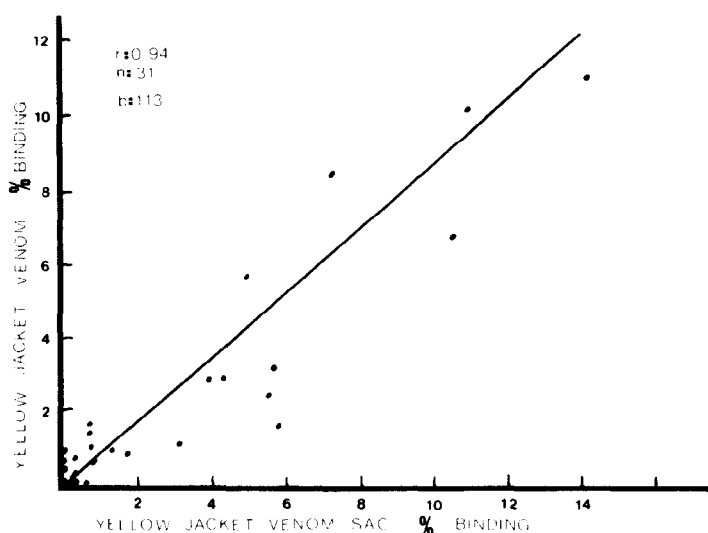


FIG. 2. Correlation of RAST results with the use of 31 sera with yellow jacket venom discs and yellow jacket venom sac extract discs. Five control and bee-sensitive sera appear at the origin. The 11 sera giving greater than 2% binding and 5 of the weakly positive sera were from yellow jacket-sensitive individuals. The remaining sera were from honeybee-sensitive individuals.

total of about 10 to 12 bands not present in bee venom were visible, including major components of about 60,000 and 3,000 d. At least 8 of these substances were larger than 3,000 d.

RAST studies were performed with 18 sera from yellow jacket-sensitive individuals, 10 from honeybee-sensitive individuals, and 3 control sera. Each serum was tested with yellow jacket venom sac extracts discs and two preparations of yellow jacket venom discs. Both venom preparations were similar. The correlation of RAST with venom sac extract discs and with venom discs is shown in Fig. 2. The slope of the linear regression line was 1.13 and the correlation coefficient, $r = 0.94$. All sera with greater than 1% binding above background were positive to both preparations. Three sera of 0.5% to 1.0% binding were positive to only one of the two preparations. Two sera showed substantially greater binding to venom sac extract than to venom.

In order to study stability of venoms, liquid bee venom from the 1971 crop stored at 4° C, liquid venom from 1975, and air-dried venom from 1976 all obtained from the same source were compared. The G-75 profiles and enzyme activities were all similar with a variation of $\pm 20\%$. Batches of venom from the same year show a similar amount of variation. The 1971 venom was darker in color and Sephadex G-25 profiles of the low molecular weight peak from the G-75 separation was quite different from that of fresh venom. The higher molecular weight components of bee venom appear to be quite stable if stored dry at 0° C or as a 40% solution at 4° C.

TABLE I. Venom content of venom sac extracts expressed as bee venom

Extract (1 mg/ml)	Acid phosphatase (mg/ml)	Hyaluronidase (mg/ml)
Bee venom	1.00	1.00
Bee venom sac	0.11	0.16
Bumblebee venom sac	0.42	0.45
Yellow jacket venom sac	0.06	0.21
Paper wasp venom sac	0.14	0.22

DISCUSSION

This study indicated that an aqueous extract of honeybee venom sacs contains all of the 5 known allergens of honeybee venom. With the use of enzyme activities to measure venom content, from 11% to 16% of the venom sac extract appears to be venom. The remainder is made up of at least 10 other components, many apparently protein, not found in venom.

Yellow jacket venom sac extract gives a good correlation ($r = 0.94$) with yellow jacket venom in RAST, although some sera that give weak reactions are positive with only one or the other. A few sera react more strongly with venom sac extract.

With the use of hyaluronidase, acid phosphatase, and phospholipase assays to determine activity, it was found that concentrated venoms, solutions, and air-dried venoms are stable at 4° C for years and freeze-dried venom sac extracts are stable for at least several months at -20° C. Aqueous venom sac extracts may

contain proteases from damaged cells and therefore be less stable.

It appears that fresh venom sac extracts contain all of the venom allergens in native forms. Venom content can be determined by enzyme assays. The sac extracts are useful for diagnosis with the use of in vitro procedures such as RAST. Use in skin-testing would require evaluation of nonspecific skin reactivity and of the stability of dilute aqueous extracts. Since the venom sac extracts contain a large amount of nonvenom proteins and peptides, the use of pure venom rather than venom sac extract is preferable in immunotherapy.

Mr. Charles Mraz, Mr. Calvin Davis, and Dr. Saiyiid Rizvi made generous contributions of insect venoms. Expert technical assistance was rendered by Mrs. Glendar Haskin and Mrs. Camillia Ferguson. The manuscript was typed by Ms. Rita Christensen.

REFERENCES

1. Lichtenstein, L. M., Valentine, M. D., and Sobotka, A. K.: A case for venom treatment in anaphylactic sensitivity to Hymenoptera sting, *N. Engl. J. Med.* **190**:1223, 1974.
2. Reisman, R. E., Light, W. C., Okazaki, T., Ilea, V., and Arbesman, C. E.: Stinging insect allergy. IV. Comparison of bee venom and whole body extract in human insect hypersensitivity, *J. ALLERGY CLIN. IMMUNOL.* **55**:79, 1975.
3. Sobotka, A. K., Valentine, M. D., Benton, A. W., and Lichtenstein, L. M.: Allergy to insect stings. I. Diagnosis of IgE-mediated Hymenoptera sensitivity by venom induced histamine release, *J. ALLERGY CLIN. IMMUNOL.* **53**:170, 1974.
4. Hoffman, D. R., and Shipman, W. H.: Allergens in bee venom. I. Separation and identification of the major allergens, *J. ALLERGY CLIN. IMMUNOL.* **58**:551, 1976.
5. Benton, A. W., Morse, R. A., and Stuart, J. D.: Venom collection from honeybees, *Science* **142**:228, 1963.
6. Shepherd, G. W., Elliott, W. B., and Arbesman, C. E.: Fractionation of bee venom. I. Preparation and characterization of four antigenic components, *Prep. Biochem.* **4**:71, 1974.
7. Habermann, E.: Bee and wasp venoms, *Science* **177**:314, 1972.
8. King, T. P., Sobotka, A. K., Kochoumian, L., and Lichtenstein, L. M.: Allergens of honeybee venom, *Arch. Biochem. Biophys.* **172**:661, 1976.
9. Hoffman, D. R., Shipman, W. H., and Babin, D.: Allergens in bee venom. II. Two new high molecular weight allergenic specificities, *J. ALLERGY CLIN. IMMUNOL.* **59**:147, 1977.
10. Hoffman, D. R.: Allergens in bee venom, III. Identification of allergen B of bee venom as an acid phosphatase, *J. ALLERGY CLIN. IMMUNOL.* **59**: , 1977.
11. Hoffman, D. R., and Haddad, Z. H.: Diagnosis of multiple inhalant allergies in children by radioimmunoassay, *Pediatrics* **54**:151, 1974.
12. Hoffman, D. R., and Haddad, Z. H.: Diagnosis of IgE-mediated hypersensitivity reactions to food antigens by radioimmunoassay, *J. ALLERGY CLIN. IMMUNOL.* **54**:165, 1974.