

Comparison of the venom immunogenicity of various species of yellow jackets (genus *Vespula*)

K. Wicher, D.M.Sc.,* R. E. Reisman, M.D., J. Wypych, Ph.D., W. Elliott, Ph.D., R. Steger, R. S. Mathews, Ph.D., and C. E. Arbesman, M.D.
Buffalo, N. Y., and Athens, Ga.

Venoms from various yellow jacket species were examined by two-dimensional thin-layer chromatography (TDTLC), double-diffusion gel precipitation (DDGP) using rabbit antisera, and the radioallergosorbent test (RAST). Comparison of representative venoms by the TDTLC showed that the venoms of V. vulgaris and V. maculifrons have a larger number of Ninhydrin (triketohydrindene hydrate)-positive substances than the venom of V. squamosa. The results of the DDGP confirmed the differences; venoms of V. vulgaris, V. maculifrons, V. flavopilosa, and V. germanica have one or more major components with immunogenic identity. The venom of V. squamosa has a species-specific major component and some minor components immunologically identical to the other venoms examined. Sera from 21 patients with a history of anaphylaxis following yellow jacket stings were examined by the RAST. Using the venoms of V. maculifrons, V. vulgaris, V. flavopilosa, and V. germanica as coupling antigens, most sera reacted similarly. The sera did not react with V. squamosa. These results suggest that the major component in venom obtained from the four yellow jacket species has immunogenic identity. Venom of V. squamosa differs from the remaining venoms. As a practical corollary, with the exception of venom from V. squamosa, common sensitivity appears to exist among the yellow jacket venoms examined.

Ground-nesting members of the genus *Vespula* (yellow jackets) are the most common cause of an allergic reaction from an insect sting. In North America, approximately 12 species are recognized.¹ They vary in geographic distribution; some are distributed transcontinentally (e.g., *Vespula vulgaris*) and others are restricted to certain regions (e.g., *Vespula maculifrons* is found in the eastern part of North America, and *Vespula pennsylvanica*, with a few ex-

ceptions, is found in the western region of North America). No information is available concerning the species specificity of the venoms of the members of the genus *Vespula*. From a practical point of view, it is important to know whether there is common allergenicity or immunogenicity among the venoms of these different yellow jacket species. Does sensitization to venom of one species imply sensitization to venom of all species? Would specific immunotherapy with venom from one species provide immunity to venom of all species?

These studies were further prompted by the clinical observation of individuals who failed to show demonstrable serum venom-specific IgE when examined shortly after an insect sting reaction, usually attributed to a yellow jacket.

MATERIALS AND METHODS

Venoms

The venoms from *V. vulgaris*, *V. maculifrons*, *V. germanica*, and *V. flavopilosa* were collected by means of an electric grid² from species present in New York state (Buf-

From the Departments of Medicine, Microbiology, and Biochemistry, State University of New York at Buffalo, the Allergy Research Laboratory of Buffalo General Hospital, Buffalo, and the Department of Entomology, University of Georgia, Athens.

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

*Present address: New York State Department of Health, Division of Laboratory and Research, Albany, N. Y.

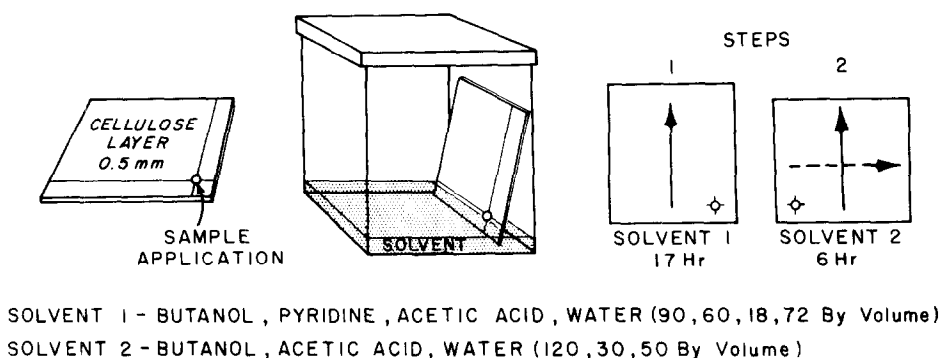


FIG. 1. Diagram of two-dimensional thin-layer chromatography procedure.

falo and surrounding areas). Venoms of *V. maculifrons* and *V. squamosa* collected by the same method in Georgia were provided by one of the authors (R. S. M.), who also identified the insect species. Pharmacia Inc. (Piscataway, N. J.) kindly provided venom collected from various species of yellow jackets by multiple punctures of venom sac followed by immersion in a mildly acid beta alanine buffer solution. This reagent will be referred to here as *Vespula* species mixture (*Vespula* sp-m). Venoms from individual species were dissolved in borate-buffered 0.9% saline at pH 9.0, filtered through a 0.23- μ Millipore filter, and the protein determined by the biuret method. All venoms were stored at a protein concentration of about 2 mg/ml. All samples were aliquoted and stored at -65° C until used.

Two-dimensional thin-layer chromatography (TDTLC)

Representative venoms were examined by TDTLC (Fig. 1), using the method described previously.³ In brief, the venom sample in a concentration of 1 mg/0.1 ml was applied on the lower right corner of a glass plate (20 $\times$ 20 cm) precoated with a layer of cellulose (MN 300-50, E. Merck, Darmstadt). The plate was then placed in a tank containing solvent No. 1 (butanol-pyridine-acetic acid-water, 90:60:18:72) for 17 hr. As the solvent moved up the plate, the separation of the sample took place. When the solvent front approached the upper edge of the plate, the plate was air dried and placed in solvent No. 2 (butanol-acetic acid-water, 120:30:50) for 6 hr in such a position as to allow the horizontally-spread substance to move vertically. When the solvent front reached the proper height, the plate was removed, dried, exposed to Ninhydrin (triketohydrindene hydrate; Pierce Chemical Co., Rockford, Ill.), which stains free alpha amino groups of amino acids and other amine-containing substances yellow or purple, and the spots were marked.

Immunization of rabbits

Eight- to ten-pound New Zealand white rabbits were injected intradermally in multiple sites with venom incorporated in Freund's complete adjuvant. All animals received three injections, each containing 100 μ g/ml, at about 3-wk

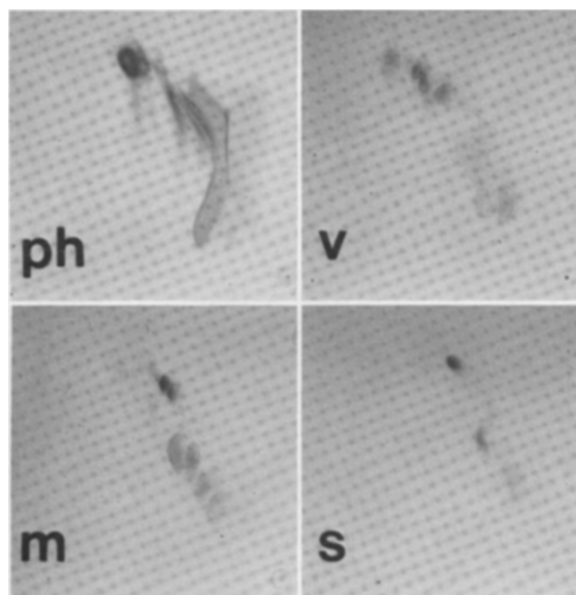


FIG. 2. Results of two-dimensional thin-layer chromatography. Glass plates have been stained with Ninhydrin. Venoms: ph, Pharmacia mixed species (referred to in text as *Vespula* sp-m); v, *V. vulgaris*; m, *V. maculifrons*; s, *V. squamosa*.

intervals; 10 to 14 days after the last immunization, the animals were bled under general anesthesia.

Double-diffusion gel precipitation (DDGP)

Sera from trial and final bleedings were examined by DDGP, using small Petri dishes containing 3 ml of 1% agarose in borate-buffered saline. The wells, 5 mm in diameter, containing 25 μ g of reagents, were 7 mm apart (edge to edge). The plates were incubated for 3 days in a moisture chamber at room temperature.

Radioallergosorbent test (RAST)

Sera from 21 patients with a clinical history of anaphylaxis following yellow jacket stings were examined by the RAST using cyanogen bromide-activated paper disks

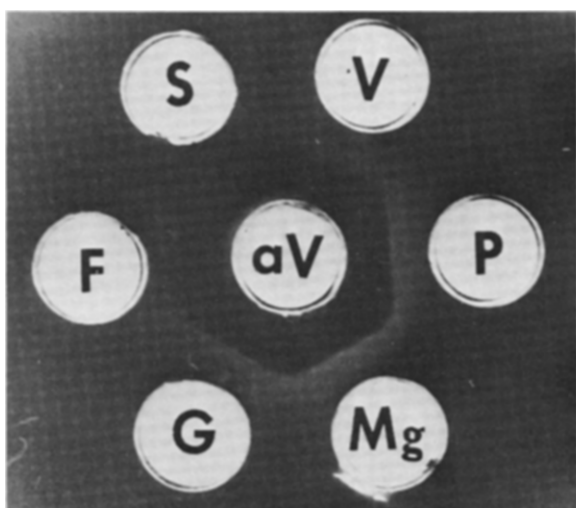


FIG. 3A. Results of double-diffusion gel-precipitation experiments. Venoms: S, *V. squamosa*; F, *V. flavopilosa*; G, *V. germanica*; V, *V. vulgaris*; M, *V. maculifrons* (Mg, Georgia; MI, New York); P, Pharmacia mixed species (referred to in text as *Vespula* sp-m). Reaction of venoms with rabbit antisera to *V. vulgaris* (aV) is shown.

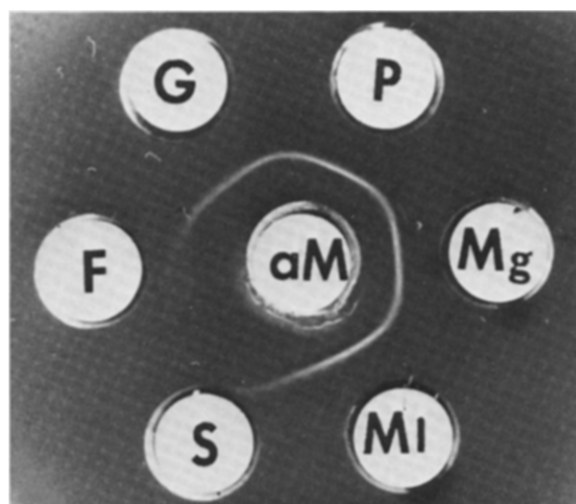


FIG. 3C. See legend for Fig. 3A. Reaction of venoms with rabbit antisera to *V. maculifrons* (aM) is shown.



FIG. 3B. See legend for Fig. 3A. Reaction of venoms with rabbit antisera to *V. squamosa* (aS) is shown.

coupled with the following venoms: *V. maculifrons* collected in New York state and Georgia, *V. vulgaris*, *V. flavopilosa*, *V. germanica*, and *V. squamosa*. For coupling, all venoms were used in concentration of 1 mg/200 disks. The results were expressed as percentage of positive standard.⁴ Levels above 10% were considered significant.

RESULTS

Two-dimensional thin-layer chromatography

The comparison of four yellow jacket venoms (Fig. 2) indicated that the *Vespula* sp-m (Pharmacia) had



FIG. 3D. See legend for Fig. 3A. Reaction of venoms with rabbit antisera to Pharmacia mixed species venom (aP) is shown.

the largest number of components when compared with the venoms of *V. vulgaris*, *V. maculifrons*, and *V. squamosa*. The Pharmacia product definitely contains beta alanine and appears to contain insect body protein and probably a protein used as a stabilizer, in addition to the venom components. The conclusion concerning body protein present in the Pharmacia venom was based upon the finding of similar spots in the chromatogram of yellow jacket sacless body extracts and the failure of any of the yellow jacket venoms collected by electrical stimulation to show this pattern. *V. vulgaris* and *V. maculifrons* venoms had a comparable number of spots, but *V. squamosa* venom

TABLE I. Comparison of different yellow jacket venom RASTs of sera from patients with histories of yellow jacket sting anaphylaxis

Patient serum	Coupling yellow jacket species venom					
	<i>V. maculifrons</i>		<i>V. vulgaris</i>	<i>V. flavopilosa</i>	<i>V. germanica</i>	<i>V. squamosa</i>
S. A.	135*	128†	117	93	106	1
B. D.	254	251	209	93	211	2
B. F.	73	82	80	33	42	1
H. J.	38	48	19	30	2	<1
N. D.	26	36	15	19	ND	
A. W.	24	32	34	18	5	
S. M.	24	18	14	13	13	
P. S.	22	13	18	18	15	<1
Y. S.	17	21	20	25	ND	<1
DeG. M.	17	20	14	9	ND	11
R. L.	15	37	19	11	13	<1
P. M.	12	19	13	13	12	
G. J.	11	13	8	5	ND	
D. P.	7	2	1	5		
C. M.	5	9	<1	2		
P. F.	5	<1	4	9		
M. T.	4	<1	1	4		
B. M.	2	5	6	12	ND	
S. D.	<1	2	5	<1	<1	
M. S.	<1	<1	2	3	ND	
M. P.	<1	6	5	12	ND	

RAST results are expressed as percentage of a positive control serum. Levels above 10% are considered significant.

*Local product (New York).

†From Georgia.

differed substantially by having fewer components as well as less intense spots. These results obtained by TDTLC with *V. squamosa* were done in replicate and were reproducible.

Double-diffusion gel precipitation

All antisera used were titrated against homologous antigens (100 µg/ml). The titers varied from 1:16 to 1:32. For the DDGP experiments, the antisera were used undiluted and all antigens were in a concentration of 100 µg/ml.

Representative results of the DDGP experiments are shown in Fig. 3. Rabbit antiserum to venom of *V. vulgaris* (Fig. 3A) reacted with venom of *V. vulgaris*, *Vespula* sp-m, *V. maculifrons* (Georgia), *V. germanica*, and *V. flavopilosa*, with precipitation lines of identity indicating that all these venoms, in the experimental conditions used, have a major component of immunogenic identity. It is not certain whether the hazy precipitation lines closer to the venom wells indicate a reaction with a second component of the venoms. Since the concentration of all venoms was similar (100 µg/ml), it may be assumed that the venom of

V. flavopilosa contains less of the major component than the other venoms. No visible reaction was obtained with the venom of *V. squamosa*. When the antiserum to venom of *V. squamosa* was used (Fig. 3B), a strong precipitation line, not having common or related immunogenic properties with the venom of *V. vulgaris* or *Vespula* sp-m, was identified. However two weak precipitation lines, demonstrating identity with the other venoms, were observed, suggesting that the venom of *V. squamosa*, in addition to a species-specific substance, has components immunogenically identical to those of other venoms used for examination. Antiserum to the venom of *V. maculifrons* (Georgia) (Fig. 3C) reacted with all other venoms used except *V. squamosa*. The precipitation line of common identity seems to confirm the results obtained with antiserum to *V. vulgaris* (Fig. 3A). The pattern of precipitation reaction using the antiserum to *Vespula* sp-m (Fig. 3D) indicated that this Pharmacia product contains a mixture of venoms from different species. A precipitation line of immunogenic identity was observed between the Pharmacia product and the other venoms used. Because the venom of *V. fla-*

vopilosa has a lower concentration of the major component (Figs. 3A and 3C), this precipitation line does not appear as a clear-cut line of identity with adjacent venoms. A precipitation line of immunogenic identity located closer to the center well, between the *Vespula* sp-m, *V. vulgaris*, *V. maculifrons*, and *V. flavopilosa*, was also observed. There were some other precipitation lines: a very faint reaction with the *Vespula* sp-m, *V. vulgaris*, and *V. maculifrons*, and a strong precipitation line reacting only with the venom of *V. squamosa*. The precipitation reaction with antiserum to *Vespula* sp-m suggests that this product contains major and minor components of all venoms examined.

Radioallergosorbent test

In general terms, the twenty one sera reacted very similarly with the venoms of *V. maculifrons*, *V. vulgaris*, and *V. flavopilosa* (Table I). Sera of three patients (S. A., B. D., and B. F.) had less reaction with *V. flavopilosa* venom. There were several minor differences with the borderline sera (DeG. M., M. B., and M. P.). Two of the nine sera (H. J., and A. W.), reacted with these three venoms but not with the venom of *V. germanica*. There was no major discrepancy in the reactions between the disks coupled with the venoms of *V. maculifrons* collected locally and those collected in Georgia. The disks coupled with venom from *V. squamosa* failed to react with any of the sera except for that of DeG. M., confirming the differences found in the DDGP experiments.

DISCUSSION

The venoms selected for examination were those most readily available in the Northeastern and Southern United States. As mentioned, there are at least 12 recognized species of the genus *Vespula*. It is not known whether the results presented in these experiments can be extended to other species.

Judging from the different pattern of the TDTLC, it seems that this method may find practical application in the analysis of venoms from various insects. Only a very few of the components of yellow jacket venom have been identified at the present time using this technique. However, some biologic amines, i.e., nor-epinephrine, serotonin, and dopamine, have been localized.

There is a definite discrepancy between the results obtained by TDTLC and DDGP. The TDTLC showed that the venom consisted of a variable number of components, whereas the number of precipitation lines with DDGP was rather limited. Perhaps the rabbit recognizes only a limited number of venom components. Many of the Ninhydrin spots shown in

the TDTLC are biogenic amines and low-molecular weight peptides that might not be strong antigens and thus would require higher concentrations and/or longer immunization periods to elucidate antibody production.

The antisera produced in rabbits cannot be considered hyperimmune sera. Despite this, the DDGP experiments provided a tentative answer to the question of whether the venoms of different species of the genus *Vespula* vary. The venoms of *V. maculifrons* (regardless of whether collected in New York or Georgia), *V. vulgaris*, *V. germanica*, and, possibly, *V. flavopilosa* have immunogenically identical major component(s) not detected in the venom of *V. squamosa*. *V. squamosa* seems to have another major component, probably species specific (Fig. 3B). From the DDGP experiments using antiserum to *Vespula* sp-m, one may conclude that the venoms used for examination may contain more than one immunogenically identical substance (Fig. 3D).

The antisera to the various yellow jacket venoms when examined by DDGP against bee and bald-faced hornet venoms (not presented in the results), did not react with bee venom, but did react with one precipitation line against bald-faced hornet venom, confirming limited immunogenic cross-reactivity between the yellow jacket and the hornet reported previously from this laboratory.⁵

The results of the RAST measuring specific IgE were similar to the results of the DDGP examination. Only one sera reacted, in low titer, with the venom of *V. squamosa*, whereas with few exceptions the sera of the 21 patients responded similarly with venoms of *V. maculifrons*, *V. vulgaris*, *V. flavopilosa*, and, to some extent, *V. germanica*.

The range of RAST titers varied considerably. Some of the sera in this group were selected because these individuals had clinical anaphylaxis following yellow jacket stings and yet failed to have specific IgE antibodies. In no instance did the use of another type of yellow jacket venom for coupling detect the presence of specific IgE. Thus, the RAST results would suggest cross-allergenicity among the various yellow jacket venoms examined. In addition, it would appear that specific immunotherapy with one of these major yellow jacket venoms should be adequate to provide protection against some venom of other yellow jacket species. This latter conclusion must await specific analysis of serum venom-specific IgG antibodies.

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