

Diagnosis of Hymenoptera hypersensitivity by skin testing with Hymenoptera venoms

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This double-blind study provides evidence that skin testing with dialyzed Hymenoptera venoms is a more accurate, reliable method of diagnosing hypersensitivity to the sting of honeybee, yellow jacket, yellow hornet, white-faced hornet, and wasp than is skin testing with the corresponding whole body diagnostic allergenic extract. Furthermore, the incidence of false-positives was greatly reduced by using the dialyzed Hymenoptera venom (HDV) diagnostic. In this clinical trial, most sensitive individuals had skin test reactions greater than the diluent control at concentrations of 1 µg/ml or below. Levels of venom diagnostic of 100 µg/ml appeared to produce nonspecific local irritation. Skin test with whole body diagnostic allergenic extract did not produce a consistent differentiation between sensitive and nonsensitive individuals. No untoward reactions were seen using the dialyzed Hymenoptera venom (HDV) diagnostic.*

Although anaphylactic reactions to the sting of bees, wasps, hornets, and yellow jackets are the cause of relatively few deaths, estimates have placed the portion of the population which has experienced a systemic reaction after the sting of one of these insects in the range of 0.4% to 0.8%.¹ Whether these abnormally severe responses are the result of toxicity or allergenicity has been the subject of controversy and speculation.

Questioning the type of response (toxic or allergic) induced by Hymenoptera sting has been, in part, due to an inability to distinguish between sensitive and nonsensitive individuals by skin test with currently available diagnostics. Skin sensitivity to suspected antigens, combined with an evaluation of the patient's history, is the usual way in which clinical hypersensitivity is diagnosed. However, skin testing with whole body extracts of bee, wasp, yellow jacket, and hornet has failed to distinguish between sensitive and

nonsensitive persons, or at best has given ambiguous results.^{2, 3-5} The only diagnostic products available for skin testing of patients have been extracts of the individual whole insect body or mixed whole insect bodies.

Recent studies have provided evidence that the systemic reactions experienced by certain individuals to the sting of a hymenopteron are IgE (reagin) antibody-mediated allergic reactions.⁵ Concurrent with these studies, an in vitro method of diagnosing hypersensitivity to Hymenoptera sting was developed. This method utilizes histamine release from the patient's washed leukocytes to the venoms of selected Hymenoptera.⁵ Tests with this method have proved it to be an accurate, reliable way to distinguish patients sensitive to Hymenoptera sting from nonallergic controls. Generally, allergic persons will respond by release of histamine from isolated leukocytes at concentrations of venom equal to or less than 1.0 µg per milliliter.⁵ Normal persons are unresponsive to venom concentrations of 10 µg/ml and higher.

While venom-induced leukocyte histamine release is an accurate in vitro diagnostic technique, the method would be a difficult, if not impossible, tool for the practicing allergist due to the sophisticated and sensitive laboratory techniques and instrumentation required. However, since it has been demonstrated that it is the venom rather than the insect whole body which induces a majority of the hypersensitive reac-

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tions, it has been postulated that the use of venom as a skin test antigen should prove capable of diagnosing insect sting allergy and should correlate better than whole body skin test extracts with the clinical hypersensitive state as determined by history or in vitro histamine release.

The purpose of this study was to test a new type of Hymenoptera diagnostic reagent. A double-blind clinical comparison is made of the accuracy of dialyzed Hymenoptera venom (HDV) diagnostic as compared with whole body insect diagnostic in identifying individuals allergic to honeybee, wasp, white-faced hornet, yellow hornet, and yellow jacket.

MATERIALS AND METHODS

Diagnostics

The whole body diagnostic extracts used were commercially available,* individually lyophilized, whole body extracts of honeybee (*Apis mellifera*), yellow jacket (*Vespula maculifrons*), white-faced hornet (*Vespula maculata*), yellow hornet (*Vespula arenaria*), and wasp (*Polistes sp.*). When reconstituted (phenol saline diluent),* these extracts contained 20,000 PNU/ml. The dialyzed Hymenoptera venoms (HDV) used were prepared as individual, lyophilized powders, each prepared from the venom of a single species of Hymenoptera. Venoms from honeybee, yellow jacket, white-faced hornet, yellow hornet, and wasp were used after dialysis to remove low molecular weight vasoactive materials. The reconstituted strength of the venoms was 0.1 mg protein/ml. Saline (0.9%) containing 0.4% phenol was used as diluent. The lyophilized antigens, both whole body and dialyzed venom, were reconstituted monthly, and fresh dilutions were prepared from the concentrated solution. For histamine release assays, the lyophilized venom was diluted in a TACM* buffer.

Procedures

The study was a double-blind design, although the whole body extracts were necessarily distinct from the HDV venoms due to the differences in measurement of potency and appearance when reconstituted. However, within the whole body extracts, or venoms, a coding system was used such that honeybee was not distinct from wasp, hornet, etc.

All subjects enrolled in the study were volunteers, educated to the intent of the study, who gave informed consent prior to participation. The subjects were separable into three categories as discussed in the following paragraphs.

Hymenoptera-sensitive subjects. Each of these 11 subjects had experienced symptoms suggestive of systemic anaphylaxis within sixty minutes of an insect sting. The sting occurred no longer than eighteen months prior to enrollment of the subject into the study. To qualify as having anaphylactic symptoms, the individual must have experi-

enced one or more of the following: generalized urticaria or angioneurotic edema, respiratory difficulty due to laryngeal edema or asthma, cardiovascular collapse, generalized pruritis, or generalized erythema. In addition to having anaphylactic symptoms, subjects to be considered sensitive also had to demonstrate histamine release to venoms.

"Normal" nonsensitive subjects. Each of these 10 subjects had experienced a hymenopteron sting within eighteen months of being enrolled in the study, with reactions suggestive neither of systemic hypersensitivity nor excessive local reaction.

"Never-stung" subjects. The 10 persons in this group did not recall being stung at any time by any of the Hymenoptera species.

Skin testing of sensitive patients began initially with scratch tests; then intradermal skin tests were done if no positive reaction was elicited to scratch tests at 10^{-1} μ g/ml. The intradermal tests were begun with concentrations of 1×10^{-3} to 1×10^{-1} μ g/ml (HDV) or protein nitrogen units (PNU)/ml (whole body diagnostic), and each successive test was increased by 10-fold until a positive reaction was elicited or maximum concentration of 100 μ g/ml HDV, 1,000 PNU/ml whole body extract, was reached. Nonsensitive and "never-stung" subjects were not scratch-tested (based on results with the "sensitive" subjects) but were progressed through 10-fold intradermal tests until a positive reaction or the maximum concentration (100 μ g/ml or 1,000 PNU/ml) was reached.

During each battery of tests, histamine (0.1 mg/ml) was used as a positive control and phenol saline diluent as a negative control. A 1+ positive reaction to the diagnostic antigen was any reaction consisting of a wheal greater than control but not more than 5 mm in diameter, with erythema unequivocally greater than that produced by the negative control at 15 min. A 2+ positive reaction was considered a wheal of 5 mm to 15 mm in diameter with associated erythema.

On the same day or prior to the in vivo skin testing, in vitro histamine release was done independently on samples of washed, isolated blood leukocytes of each of the Hymenoptera-sensitive subjects and several of the nonsensitive or "never-stung" subjects. The histamine release results were utilized to confirm the subjects' sensitivity and to identify the specific insect venom(s) (honeybee, wasp, white-faced or yellow hornet, or yellow jacket) to which the subject was allergic.

RESULTS

Eleven subjects reporting reactions indicative of hypersensitivity were enlisted into the study. Each of the subjects reported a systemic reaction immediately following an insect sting. The insect was identified in 6 cases and was unknown in 5 cases. Table I describes the reaction for each sensitive subject and indicates the insect responsible, if known. In addition, the results of histamine release and skin testing are given for the venom of the responsible insect or the venom giving the maximum histamine release. For

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*TACM-THAM—buffered saline containing human serum albumin (HSA), calcium, and magnesium.⁵

TABLE I. Reaction to Hymenoptera sting

Hypersensitive subjects			Max. % histamine release/ venom conc.		Minimum conc. eliciting a positive skin test		
Patient No.	Sting reaction	Responsible insect	%	$\mu\text{g/ml}$	Whole body (PNU/ml)	Venom ($\mu\text{g/ml}$)	Venom source*
009	Swelling of entire arm, generalized pruritus and erythema, swollen eyelids and lips	?	75	0.1	100	10.0	Honeybee
010	Generalized pruritus and urticaria, facial swelling	Yellow jacket	100	0.1	>1,000	0.1	Yellow jacket
007	Generalized pruritus and urticaria, difficulty in breathing	Yellow jacket	71	1.0	1,000	1.0	Yellow jacket
005	Generalized erythema, difficulty in breathing, possible shock	Yellow hornet	66	1.0	1,000	10.0	Yellow hornet
003	Generalized pruritus, erythema and urticaria, nausea, dizziness	Honeybee (multiple)	30	0.01	1,000	1.0	Honeybee
002	Generalized pruritus, periorbital swelling	Yellow jacket	30	1.0	1,000	0.1	Yellow jacket
008	Generalized pruritus, erythema and urticaria, difficulty in breathing, swelling of throat	?	48	1.0	100	1.0	Yellow jacket
012	Generalized pruritus, erythema, urticaria	?	94	0.01	>1,000	0.1	Honeybee
036	Generalized pruritus and urticaria	?	44	1.0	10	0.1	Yellow jacket
025	Generalized pruritus and urticaria, cardiovascular collapse	?	81	1.0	>1,000	10.0	Yellow jacket
023	Generalized pruritus and urticaria, swollen eyelids, lips, and tongue	Yellow jacket	73	0.01	100	0.1	Yellow jacket

*Hymenoptera-dialyzed venom showing maximum % histamine release or the venom from the responsible source, if known.

comparison, the skin test results obtained using whole body extracts are listed in Table I. With the exception of Patient 003, who received multiple stings, each of the subjects in both the sensitive and nonsensitive groups reported that his reaction was induced by a single insect sting.

The 10 nonsensitive subjects all reported minimal reactions to their insect stings varying from slight itching to local swelling and erythema. In all ten cases whole body extracts gave positive reactions only at 1,000 PNU/ml or greater, and the venoms gave positive skin tests at 100 $\mu\text{g/ml}$ or greater in 8 subjects. One control subject gave a positive skin reaction to yellow hornet venom at 1 $\mu\text{g/ml}$ and released 29% of his leukocyte histamine on challenge with 1 $\mu\text{g/ml}$ yellow hornet venom. The other such patient gave a positive skin reaction to 10 $\mu\text{g/ml}$ yellow jacket venom, but was not tested for histamine release.

For the purposes of statistical analyses, the subjects in the "sensitive" group were recategorized as sensitive or nonsensitive by histamine release to each of the 5 insects tested according to the criteria such that:

histamine release of 30% or greater to venom concentrations of 1 $\mu\text{g/ml}$ or less was considered positive and diagnostic of hypersensitivity to the insect tested; histamine release of 15% to 29% was classed as a nondiagnostic sensitivity; and histamine release of less than 15% to a venom concentration of 1 $\mu\text{g/ml}$ was considered diagnostic of lack of hypersensitivity or the "normal" state. The same criteria were applied to subjects in the nonsensitive and "never-stung" groups who were assessed by histamine release.

Each of the subjects who had experienced a severe reaction (sensitive group) released greater than, or equal to, 30% of their leukocyte histamine following challenge with at least one of the Hymenoptera venoms tested. Several of these individuals were sensitive to several venoms or showed nondiagnostic (15% to 29%) sensitivities to other insect venoms. Two of the 4 nonsensitive (by history) individuals had nondiagnostic leukocyte sensitivities as demonstrated by histamine release of 15% to 29%. Three of the 7 individuals who had never been stung, when tested for histamine release, responded to venom, 2 released

TABLE II. Correlation between venom histamine release and skin test reactivity—whole body or venom—in Hymenoptera and control patients

Histamine release							
Whole bodies				Venom			
<i>Honeybee (A)</i>							
	(++)	(±)	(-)		(++)	(±)	(-)
Skin test	≥30%	15%-30%	<15%	Skin test	≥30%	15%-30%	<15%
≤10 (++)			3	≤1 (++)	2		
100 (±)	2	1	3	10 (±)	1		1
≥1,000 (-)	1		12	≥100 (-)		1	17
	$X^2 = 6.16$ $H_0:H_1 = 1:5\frac{3}{4}$				$X^2 = 17.77$ $H_0:H_1 = 1:1,925$		
<i>Wasp (B)</i>							
	(++)	(±)	(-)		(++)	(±)	(-)
Skin test	≥30%	15%-30%	<15%	Skin test	≥30%	15%-30%	<15%
≤10 (++)			4	≤1 (++)			
100 (±)		1	4	10 (±)			2
≥1,000 (-)		1	12	≥100 (-)		2	18
	$X^2 = 1.69$ $H_0:H_1 = 1\frac{1}{2}:1$				$X^2 = 0.22$ $H_0:H_1 = 3\frac{1}{4}:1$		
<i>Yellow jacket (C)</i>							
	(++)	(±)	(-)		(++)	(±)	(-)
Skin test	≥30%	15%-30%	<15%	Skin test	≥30%	15%-30%	<15%
≤10 (++)	1	1	2	≤1 (++)	8		
100 (±)	4		3	10 (±)	1	2	2
≥1,000 (-)	4	1	6	≥100 (-)			9
	$X^2 = 2.62$ $H_0:H_1 = 1:1$				$X^2 = 26.44$ $H_0:H_1 = 1:147,300$		
<i>Yellow hornet (D)</i>							
	(++)	(±)	(-)		(++)	(±)	(-)
Skin test	≥30%	15%-30%	<15%	Skin test	≥30%	15%-30%	<15%
≤10 (++)	1	2	1	≤1 (++)	3	3	
100 (±)	1		3	10 (±)	1	1	2
≥1,000 (-)	2	4	8	≥100 (-)		2	10
	$X^2 = 3.44$ $H_0:H_1 = 1:1\frac{1}{2}$				$X^2 = 12.37$ $H_0:H_1 = 1:130$		
<i>White-faced hornet (E)</i>							
	(++)	(±)	(-)		(++)	(±)	(-)
Skin test	≥30%	15%-30%	<15%	Skin test	≥30%	15%-30%	<15%
≤10 (++)	1		4	≤1 (++)	1		1
100 (±)		1	3	10 (±)	1	1	3
≥1,000 (-)	1	1	11	≥100 (-)		1	14
	$X^2 = 2.93$ $H_0:H_1 = 1:1\frac{1}{4}$				$X^2 = 7.41$ $H_0:H_1 = 1:11$		

histamine in the nondiagnostic range, and 1 released 30% of his cellular histamine.

To determine the accuracy of diagnosis by (1) skin testing with Hymenoptera whole body extracts or (2) skin testing with Hymenoptera venoms, the categorization of subjects determined by histamine release

(sensitive, nondiagnostic, and nonsensitive) was used as a standard to be compared with the results obtained with each method of skin testing. The whole body skin test results were grouped as: (1) positive at 10 PNU/ml or less—generally considered indicative of specific hypersensitivity; (2) positive at 100 PNU/

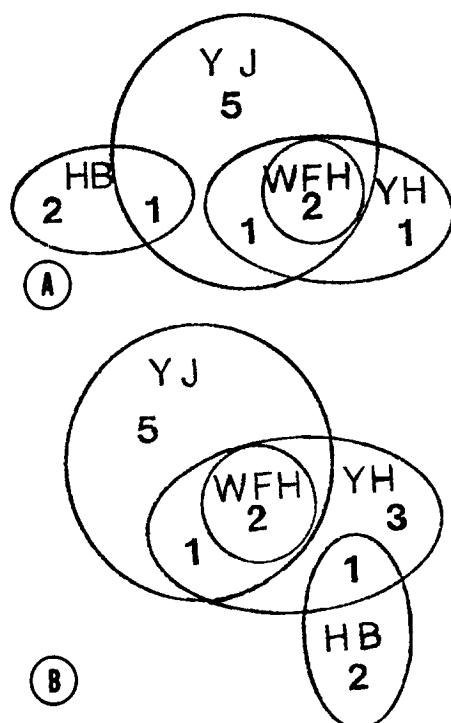


FIG. 1. A, Multiple sensitivities as determined by histamine release. B, Multiple sensitivities as determined by skin testing. YJ, Yellow jacket; HB, honeybee; WFH, white-faced hornet; YH, yellow hornet.

TABLE III. Frequency of false-positive and false-negative skin tests

	False-positive				False-negative			
	Venom		Whole body		Venom		Whole body	
	No.	%	No.	%	No.	%	No.	%
Honeybee	0	—	3	13.6	0	—	1	4.5
Wasp	0	—	4	18	0	—	0	—
Yellow jacket	0	0	2	9	0	—	4	18
Yellow hornet	0	—	1	4.5	0	—	2	9
White-faced hornet	1	4.5	4	18	0	—	1	4.5

ml—equivocal with regard to specific hypersensitivity; and (3) positive only at 1,000 PNU/ml or greater—a nonspecific, presumably irritative reaction. Skin test responses to Hymenoptera venoms were grouped in a similar manner, although reactions at or above 100 μ g/ml were considered to be nonspecific, reactions at 1 μ g/ml or less indicated hypersensitivity, and reactions at 10 μ g/ml were considered equivocal.

The results of these correlations are shown in the

chi-squares, Table II. A chi-square value and odds of the primary hypothesis (H_0) of no relationship against the alternative hypothesis (H_1) of some relationship ($H_0:H_1$), as described by Jeffreys,⁶ were calculated. Both values are shown for each portion of the table.

In the case of yellow jacket (Table II, C), yellow hornet (Table II, D), and white-faced hornet (Table II, E), skin testing with *whole body extracts* did not identify sensitive patients (those who gave $\geq 30\%$ histamine release to the specific insect venom). In these cases the odds in favor of a correlation were negligible; i.e., the odds of a correlation between the whole body skin tests and histamine release tests were 1:1.25. For the same insects, skin testing with the corresponding HDV diagnostic resulted in substantial evidence in favor of a relationship between sensitivity as defined by histamine release and sensitivity as indicated from the results of skin testing. Odds in favor of a relationship were 1:11 in the case of white-faced hornet, 1:130 for yellow hornet, and 1:147,300 for yellow jacket.

A statistical evaluation of the results of honeybee sensitivity as diagnosed by whole body skin testing and histamine release gave moderate evidence for a relationship between the results obtained by the two methods ($0.10 > p > 0.05$, odds 1:5.4 in favor of a relationship). However, it must be noted that of the three patients giving strongly positive histamine release (30% or greater), none showed strongly positive whole body skin tests. Skin tests with the honeybee HDV resulted in decisive evidence in favor of a correlation between the two methods, odds of 1:1,925.

No highly wasp-sensitive individual was identified by the venom histamine release method (Table II, B). Two persons with nondiagnostic sensitivity had negative ($>100 \mu$ g/ml) HDV skin tests. The same negative result was obtained using the whole body extracts. However, in the case of the whole body skin tests, 4 patients who were nonsensitive by histamine release gave strongly positive skin tests. If these results are considered, 18% false-positives occurred using wasp whole body extract.

False-positive results were consistently more frequent for other whole body skin tests as compared to HDV skin tests. A summary of the data obtained with regard to false-positive (strongly positive skin test but negative histamine release) and false-negative reactions (negative skin test with histamine release $>30\%$) is shown in Table III. These results assume that the allergen to which the untreated patients are sensitive is venom.

Table IV summarizes skin test results and compares them to sensitivity as determined by histamine release (22 patients $\times$ 5 tests/patient = 110 tests).

TABLE IV. Results of 110 venom, 110 whole body, and 110 histamine release tests on 22 sensitive, nonsensitive, or "never-stung" individuals

Histamine release (to venom)							
<i>Whole body extracts (5)</i>				<i>HDV venoms (5)</i>			
Skin test	≥30%	15%-29%	<15%	Skin test	≥30%	15%-29%	<15%
≤10	3	3	14	≤1	14	3	1
100	7	3	16	10	4	4	10
≥1,000	8	7	49	≥100	0	6	68
Total, 110				Total, 110			
$\chi^2 = 3.2$				$\chi^2 = 72.6$			
$H_0:H_1 = 1\frac{3}{4}:1$				$H_0:H_1 = 1.7 \times 10^{14}$			

The results indicate the degree of correlation between whole body of HDV skin test and histamine release results, without respect to individual insects.* Both by chi-square and the calculation of odds statistical technique, negligible evidence was found to support a relationship between whole body skin testing and histamine release. On the other hand, the data obtained show a decisive correlation between HDV skin tests and venom-induced histamine release in the diagnosis of sensitivity to the various Hymenoptera ($H_0:H_1 = 1.7 \times 10^{14}$). Additionally, if cases in which subjects were found to be sensitive by histamine release but were negative ($\geq 1,000$ whole body, ≥ 100 venom) by skin test are considered false-negatives and cases in which negative histamine release tests were coupled with strongly positive skin tests are regarded as false-positives, then there were 17.7% overall false-positive reactions obtained using whole body extract in contrast to 0.9% for the HDV diagnostic. Seven percent overall false-negative results occurred using the whole body extracts, whereas none were obtained using HDV.

We found that approximately one third of the hypersensitive people had multiple sensitivities. The relationship of these multiple sensitivities is shown in Fig. 1. *A* groups the subjects by histamine release, and *B* groups them by HDV skin test results. These results indicate that cross-reactions between venom can occur, but further work is needed to define the specificity and patterns of Hymenoptera sensitivities.

DISCUSSION

To be a useful diagnostic, a skin test reagent must necessarily contain the allergen(s) thought to induce the allergic response in question. Furthermore, the diag-

nostic must contain the antigen in sufficient quantity that allergic manifestations ranging from "exquisitely sensitive" to borderline can be clearly identified. However, neither the antigen itself nor extraneous materials within the diagnostic should induce toxic or nonspecific reactions in "normals." Since evidence now suggests that a high percentage of patients having anaphylaxis to Hymenoptera sting react to venom antigens,⁵ it has been suggested that the whole body extracts currently in use contain large amounts of irrelevant antigens and unpredictable amounts of active material.⁷ On this basis, a logical extension of *in vitro* diagnostic studies seemed to be the development of an antigenically relevant, venom diagnostic.

The results of this double-blind study provide evidence that skin testing with dialyzed Hymenoptera venoms is a more accurate, reliable method of diagnosing hypersensitivity to the sting of honeybee, yellow jacket, yellow hornet, and white-faced hornet than is skin testing with the corresponding whole body diagnostic. Of primary importance in this regard were our data showing that the occurrence of false-positive reactions using the HDV diagnostic were negligible. Results with the wasp diagnostic were inconclusive due to a lack of adequate numbers of patients having wasp sensitivity, and they therefore showed the poorest correlation.

The decision to use the histamine release categories of <15% and $\geq 30\%$ to designate, respectively, nonsensitive and sensitive individuals came from observation of the data as subjects were tested. It was unknown at the time the study began how results obtained using the dialyzed and lyophilized commercial diagnostic products would correspond to those obtained using laboratory-prepared venom reagents. Using the leukocytes of 5 patients, Hymenoptera venoms (HDV) were compared by histamine release to nondialyzed, TACM*-diluted samples of venom.

*Certain assumptions are necessary to statistically pool and analyze the data as in Table IV. These assumptions are: (1) any variation among the subjects is accidental; (2) any variation among the 5 Hymenoptera venoms is accidental; (3) the variation among subjects reacting to one Hymenoptera venom or to another is accidental; and (4) the unit of measure is sites.

*THAM-buffered saline containing HSA, calcium, and magnesium.⁵

Only with honeybee venom were similar dose-response curves obtained. These results suggest that the methods or reagents used during preparation of the dialyzed and lyophilized material may have resulted in loss of antigenic activity.

In blinded tests using leukocytes from subjects who had identified the insects, the histamine release was $\geq 30\%$ when the offending insect venom was tested. From analysis of the data obtained using all four venoms in each patient studied, it appeared that there was an equivocal range of borderline venom sensitivities. Patients who exhibited sensitivity to multiple venoms, as well as several responding to a single insect venom, released 15% to 29% of their leukocyte histamine when challenged with the highest dose (1 $\mu\text{g}/\text{ml}$) of venom tested. Rather than forcing the reactions of these subjects into sensitive or nonsensitive categories, their responses were designated as equivocal. While the quantity of allergen required for 50% histamine release has often been used as a convenient way to assess the relative cell sensitivities of a population of patients, other investigators have used the 30% point if the dose-response curves are linear at this level.⁸ It is possible that for many of the subjects who reacted with a maximum histamine release of greater than or equal to 30% but less than 50%, an increase to 50% histamine release might have been attained if the assay had been titrated one concentration higher (10 $\mu\text{g}/\text{ml}$). In all cases, "sensitivity" was ultimately based on histamine results, not on the history alone.

In skin test diagnosis, it is not the absolute concentration of antigen required to give a positive skin test that is of primary importance, but rather the gap between the quantity to which most sensitive patients react and "normals" begin to react. In this clinic trial, levels of venom diagnostic of 100 $\mu\text{g}/\text{ml}$ appeared to produce nonspecific local irritation in many patients and controls. Most sensitive individuals had positive skin test reactions at concentrations of 1 $\mu\text{g}/\text{ml}$ or less. At 10 $\mu\text{g}/\text{ml}$ equivocal responses occurred in several patients, and several control patients were also positive. In contrast, whole body diagnostic skin tests did not produce a consistent differentiation between sensitive and nonsensitive individuals. Using whole body extracts, there were 14 false-positive skin test reactions and 8 false-negative skin test reactions. The dialyzed venom only gave one false-positive skin reaction.

The purpose of enlisting subjects into this study on the basis of "sensitive," "nonsensitive," or "never-stung," as determined by patient history, was to obtain a variety of subjects—some expected to give positive results to certain insects and some expected

to be negative to all insects. We thought that individuals who had never been stung would be negative to all skin tests and that individuals stung with only minor local reactions would probably also be negative. However, at least 1 sensitive and up to 5 equivocal subjects (based on histamine release) were found in these control groups. In the nonsensitive group it is conceivable that some of the individuals are potentially sensitive; i.e., they have IgE antibody to venom, but sufficient levels of blocking antibody protect them from generalized or severe reactions when they are stung. Positive histamine release that occurred in "never-stung" individuals is more difficult to explain but may simply reflect a forgetfulness about previous stings on the part of the subject.

No untoward reactions were seen using the HDV diagnostics. Positive responses in this group of subjects were limited to immediate local wheal-and-flare reactions following intradermal injections using concentrations of 0.1 to 10 $\mu\text{g}/\text{ml}$.

Until the questions of multiple sensitivities and/or cross-reactivity are resolved, the diagnostic use of individual venoms seems to be necessary. While some studies report the existence of proteins with similar characteristics in the various venoms,⁹ other investigators have found the antigen(s) to be distinct.¹⁰ In this study the only consistent multiple reactivity was found in the two white-faced hornet-sensitive people who were a subset of both the yellow hornet- and the yellow jacket-sensitive groups.

The importance of venom proteins, as opposed to those contained in whole body extracts, as the antigenic factors in a majority of Hymenoptera-hypersensitive individuals is supported by data from several laboratories. Reisman and co-workers,¹¹ using Prausnitz-Küstner (P-K) tests and radioallergen sorbent test (RAST) inhibition studies, have obtained results suggesting that venom antigen(s) are probably more important than whole body antigens. Additionally, whole body extract skin tests, when compared to venom RAST results, were found to incorrectly select the large number of false-positive reactions. In a separate study, 2 patients (1 honeybee-sensitive and 1 vespid-sensitive), who failed to be protected after appropriate therapy with whole body extracts, have been treated with the insect venom corresponding to their sensitivity.^{7, 12} Venom treatment resulted in an increase in their IgG-blocking antibody titers, and in both cases a subsequent sting caused only local irritation.

The results of this study, then, suggest that more accurate diagnosis of Hymenoptera hypersensitivity can be made possible by using a venom diagnostic in preference to a whole body diagnostic. The lower

incidences of false-positive reactions that occur using venom diagnostics will not only make meaningful diagnosis of this disease possible but will also reduce the number of patients who may be currently treated unnecessarily. Further clinical experience with Hymenoptera venoms will be needed to define their stability, potency, and range of clinical significance.

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