

31623

Analysis of Differing Patterns of Cross-Reactivity of Honeybee and Yellow Jacket Venom-Specific IgE: Use of Purified Venom Fractions

John I. Wypych^a, C. John Abeyounis^b, Robert E. Reisman^a

Allergy Research Laboratory, Departments of ^a Medicine and ^b Microbiology, State University of New York at Buffalo, Buffalo General Hospital, Buffalo, N.Y., USA

Abstract. Prior studies of sera from insect sting-allergic patients have analyzed the relationship of coexisting honeybee venom- and yellow jacket venom-specific IgE. Radioallergosorbent (RAST)-inhibition tests with these venoms revealed four different patterns of activity. In this present study, purified fractions prepared from these venoms were used to analyze these varying patterns. The hyaluronidases of yellow jacket venom and honeybee venom showed extensive cross-reaction. The phospholipases from these venoms showed minimal cross-reactivity; antigen 5 was restricted to yellow jacket venom. There was a high molecular weight component in yellow jacket venom with immunologic properties similar to honeybee venom acid phosphatase. Sera from individual patients showed quantitative and qualitative differences in the reactions to the major components of both venoms. The differences in the RAST-inhibition patterns in patients with elevated levels of both honeybee venom- and yellow jacket venom-specific IgE are accounted for by these differences as well as by differences in the cross-reactivity between the individual components.

Introduction

Sera of patients who have had allergic reactions from insect stings may contain IgE antibodies that react with both honeybee and yellow jacket venoms. In radioallergosorbent test (RAST)-inhibition studies using whole venoms [1, 2], the specificity of this IgE activity was examined and four different patterns were found: (1) unique IgE antibody activity with no cross-reaction between serum honeybee venom (HBV)-specific and yellow jacket venom (YJV)-specific IgE; (2) extensive cross-reaction between serum HBV-specific and YJV-specific IgE; (3) reaction directed to a major allergen in YJV and cross-reacting with a minor allergen in HBV, and (4) reaction directed to a major allergen in HBV and cross-reacting with a minor allergen in YJV.

YJV contains three major allergens: phospholipase (PLA); hyaluronidase, and antigen 5 (Ag5) [3]. HBV contains four major allergens: PLA; hyaluronidase; acid phosphatase, and melittin [4-6]. In this report,

we describe the use of purified preparations of these allergens to analyze the various RAST-inhibition patterns with whole venoms. The results suggest that the differing patterns of cross-reactivity observed using whole venoms are due to the specific venom fractions to which the IgE antibody activity is primarily directed.

Materials and Methods

Venom

YJV, *Vespula* species (lot No. 0228508YJ), in bottles containing 24.6 mg, was obtained from Vespa Laboratories, Spring Mills, Pa. HBV was obtained from the Champlain Valley Apiaries, Middlebury, Conn., in 1-gram lots. HBV hyaluronidase and acid phosphatase, in 0.5- and 1.0-mg amounts, were kindly provided by Dr. David Kemeny. The venom and venom fractions were stored at -20°C until use.

Rabbit Antisera

Rabbit antisera to HBV and to mixed species of YJV were prepared as previously described [7].

Human Sera

Sera from 9 individuals containing both HBV- and YJV-specific IgE were used in this study. These sera had been extensively studied by RAST inhibition, and were categorized into 1 of 4 groups, depending upon the results of the RAST inhibition with whole venoms [1, 2].

Group 1. The sera of group-1 patients (BO and SI) showed minimal cross-reactivity between HBV and YJV. There was minimal inhibition of the HBV RAST by YJV and minimal inhibition of the YJV RAST by HBV, suggesting specific antibody activity directed at each venom.

Group 1. The sera of patients in group 2 (GA, FR and MC) showed very extensive cross-reactivity between HBV and YJV. Both venom RASTs were inhibited significantly by both venoms.

Group 3. In tests with sera from patients in group 3 (DE and JA), YJV inhibited both YJV and HBV RAST, but HBV inhibited only the HBV RAST. These studies suggest that the sera reacted with a major allergen in YJV which cross-reacted with a minor allergen in HBV.

Group 4. The sera of group 4 (LE and VI) showed the opposite pattern in RAST inhibition of that found for group 3. The HBV RAST was inhibited by HBV only, and the YJV RAST was inhibited by both HBV and YJV. These studies suggest that the sera reacted with a major allergen in HBV which cross-reacted with a minor allergen in YJV.

Enzyme Assays

PLA and hyaluronidase assays were performed as described previously [8, 9]. Acid phosphatase activity was determined by using the Sigma Acid Phosphatase Kit. Melittin was detected using the hemolytic test developed by Habermann and Zeuner [10].

Immunodiffusion

Venom fractions were compared in radial immunodiffusion using 50 × 5 mm Falcon plates filled with 3 ml of 1% Agarose melted in 0.1 M borate-buffered saline (pH 8.0). Six wells, 5 mm in diameter, were equally spaced from a similar central well. Well capacity was about 25 µl.

YJV Ag 5 was defined by a fraction which was potent in the RAST assay, gave a single line in immunodiffusion distinct from the hyaluronidase and PLA lines, and had no detectable enzyme activity.

Radioimmunoassays

The RAST was performed as previously described [11]. Discs were coupled with either 5 µg of dialyzed YJV, 100 µg of HBV, or 400 ng of venom fractions. The results were calculated as percent bound of total activity added, and adjusted if dilution was necessary, assuming linearity.

For RAST inhibition, 50 µl of serum (diluted in normal human serum, if necessary) was added to 50 µl bovine serum albumin (BSA) diluent, as in the RAST, or to BSA diluent containing a known amount of inhibiting antigen. Tubes were incubated at 4–6°C for 20 h. The next day, an appropriately coupled disc was added to each tube, followed by incubation overnight at 31°C. The remainder of the test was done as previously described.

Fractionation of HBV

HBV fractionation was carried out using technology previously described, including negative-pressure dialysis concentration [12],

tandem-column gel filtration [13], PC-affinity column [3], and the SP-Sephadex-120 chromatography [14]. Briefly, after negative-pressure dialysis of HBV, 50-mg amounts of protein (pH 4.75), in 0.5 M ammonium acetate buffer were passed through tandem columns of ACA-44, ACA-54 and P-60 acrylamide gels. The leading peaks of several such fractionations were pooled, concentrated and analyzed, and were found to be rich in hyaluronidase and acid phosphatase. After dialysis against a pH-9.0 Tris-HCl buffer and passage through the PC-affinity column, the effluent was found to contain only hyaluronidase activity and showed one line in immunodiffusion. Acid phosphatase was retained by the PC column (under the initial wash conditions) but was eluted with Tris-buffer containing 0.5 M NaCl. Immunodiffusion tests showed one line which did not cross-react with hyaluronidase or PLA. While the starting material had elevated acid phosphatase activity, this protein in the first eluate had reduced acid phosphatase activity, suggestive of denaturation.

The PLA-rich fractions were obtained by elution with 0.1 N acetic acid (pH 4.75). The PLA-rich peaks (later peaks) from several gel-filtration columns were pooled and passed through the ACA-54 gel filtration column. The PLA-rich peak was dialyzed against 0.01 M Tris-citrate (pH 8.0) and passed through an SP-Sephadex-120 column. Highly purified PLA was eluted, using an 0.002-M (pH 4.5), acetate buffer containing 0.3 M NaCl. The leading fraction contained PLA activity only and showed one line in immunodiffusion.

Melittin peaks from gel filtration which occurred after the PLA peak were pooled, concentrated, and passed through a P-60 gel column. This fraction contained no enzyme activity, but had very high hemolytic activity, and did not cross-react with any of the other fractions.

Fractionation of YJV

YJV was fractionated following several of the procedures developed by King et al. [3], using 24–50 mg of venom protein at a time.

Briefly, venom at a concentration of 5 mg/ml in 0.05 M ammonium acetate buffer (pH 4.75) containing phenylmethylsulfonylfluoride, a protease inhibitor, was thoroughly dialyzed against the starting buffer Tris-HCl 0.05 M (pH 8.0). The dialyzed venom (after addition of CaCl₂ to a final concentration of 0.05 M) was passed through a PC-affinity column. Effluent of the column contained both hyaluronidase and Ag 5. An initial eluate using Tris-HCl (pH 8.0) and 0.5 M NaCl, contained a unique protein which had no enzyme activity, showed one line in immunodiffusion, and was different from Ag 5. A second eluate obtained after passage of 0.1 M acetic acid (pH 4.75) was highly enriched in PLA and showed one line in immunodiffusion.

The effluent from the PC-affinity column which contained hyaluronidase and Ag 5, was concentrated against 0.50 M ammonium acetate buffer (pH 4.75) and passed through a CM-25 chromatography column. Effluent from this column contained Ag 5, had no enzyme activity and showed one line in immunodiffusion. Eluate following a NaCl gradient in the acetate buffer contained a large amount of hyaluronidase activity. After concentration and dialysis, the hyaluronidase-rich fraction was put on a heparin-sepharose column and equilibrated with the same ammonium acetate buffer. Elution with increasing amounts of NaCl led to 3 peaks high in hyaluronidase activity, the last two of which had very high enzyme activity, and showed only single lines in immunodiffusion.

Table 1. Characteristics of venom fractions

	Enzyme activity				Immunodiffusion number of lines reacting with rabbit antisera
	PLA μM fatty acids/ mg protein/min	hyaluronidase turbidity reduction units	acid phosphatase units activity/ mg protein	melittin hemolytic activity %	
Bee Venom					
Whole venom	440	6.3	76.3	65	3
Isolated fractions					
PLA ₂	3,896	0	0	0	1
Hyaluronidase	0	188	0	0	1
Acid phosphatase	0	0	1,938	0	1
Melittin	0	0	0	100	0
Yellow Jacket Venom					
Whole venom	774	8.25			3-4
Isolated fractions					
PLA	1,353	0			1
Hyaluronidase	0	225			1
Ag 5	0	0			1

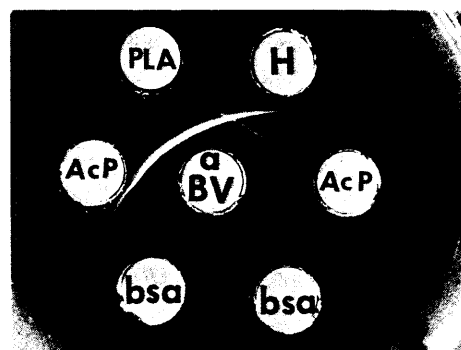


Fig. 1. Gel diffusion experiment showing the reaction of a rabbit anti-HBV serum (aBV) with phospholipase (PLA), hyaluronidase (H), and acid phosphatase (AcP) fractions of HBV, and diluent control (bsa). Single precipitin lines of nonidentity are present with each of the isolated fractions.

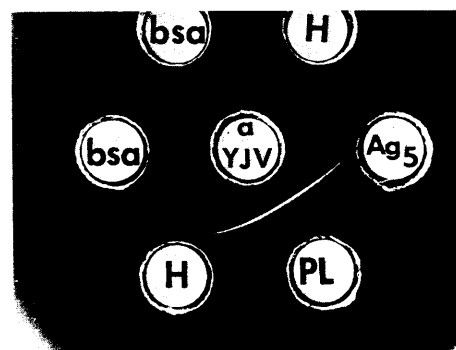


Fig. 2. Gel diffusion experiment showing the reaction of rabbit anti-YJV serum (aYJV) with phospholipase (PL), hyaluronidase (H), and antigen 5 (Ag5) fractions of YJV, and diluent control (bsa). Single precipitin lines of nonidentity are present with each of the isolated fractions.

Results

Characteristics of Venom Fractions

The characteristics of the venom fractions are shown in table 1. Four fractions were isolated from HBV. Three of these fractions contained PLA, hyaluronidase, or acid phosphatase activity; none had hemolytic activity. Each fraction gave a single precipitation line of nonidentity with the other two frac-

tions in immunodiffusion against anti-whole HBV rabbit sera (fig. 1). A fourth fraction, which apparently was melittin, had hemolytic activity but no enzyme activity.

Three fractions from YJV were isolated. Each gave a single precipitation line of nonidentity in immunodiffusion against rabbit antisera (fig. 2). The PLA and hyaluronidase fractions contained no other enzymatic activity. The Ag 5 fraction had no enzyme activity.

Table 2. Direct RAST results with whole venom and venom fractions

	Serum dilution	Bee venom					Yellow jacket venom			
		whole venom	fractions				whole venom	fractions		
			AcP	Hyal	PLA	Mel		Hyal	A5	PLA
Group 1										
BO	1:1	12.9	14.8	14.0	4.0	0	9.1	7.2	2.9	0
SI	1:1	10.6	15.2	18.2	4.0	0	10.8	5.2	7.9	1
Group 2										
FR	1:2	12.1	22.6	23.4	16.2	1	16.6	39.1	2.1	5.0
GA	1:2	21.0	27.2	25.4	26.2	1	27.2	33.8	7.6	3.0
MC	1:4	43.9	151.6	173.4	83.7	2	36.9	187.2	11.5	10.7
Group 3										
DE	1:2	8.6	12.8	16.9	8.8	1	19.0	11.7	11.4	6.2
JA	1:1	4.6	6.8	7.3	5.8	0	11.7	8.6	1.4	3.7
Group 4										
LE	1:3	35.3	91.1	78.8	89.4	12.7	10.1	30.5	0.5	2.4
VI	1:1	10.7	17.2	18.6	14.0	0	14.8	21.4	0.1	4.5

Results expressed as percent net binding. AcP = Acid phosphatase; Hyal = hyaluronidase; Mel = melittin.

Direct RAST Results

The RAST results are shown in table 2. The data are expressed as net percent binding of total radiolabeled anti-IgE. As described above, the patients' sera are divided into four groups, based upon the initial RAST-inhibition studies with HBV and YJV, as both the coupling and inhibiting antigens.

Group 1: No Cross-Inhibition of RAST. The two sera of patients in group 1 showed reactions with HBV fraction acid phosphatase, hyaluronidase and PLA, and YJV fraction hyaluronidase and Ag 5. The HBV PLA titers were low and there was no reaction with YJV PLA.

Group 2: Extensive Cross-Inhibition of RAST. The three sera in group 2 showed fairly strong reactions with HBV fraction acid phosphatase, hyaluronidase and PLA, and no reaction with melittin. Strong reactions were also found with YJV hyaluronidase, and mild-to-moderate reactions with YJV Ag 5 and PLA. The high-titered hyaluronidase-specific IgE characterized this group.

Group 3: YJV as the Major Antigen. The two sera from patients in group 3 reacted with HBV PLA, hyaluronidase and acid phosphatase fractions and with all three YJV fractions. Patient JA had low titers of Ag 5-specific IgE.

Group 4: HBV as the Major Allergen. The sera from the 2 patients in group 4 showed strong reactions with HBV fraction acid phosphatase, hyaluronidase, and PLA. Patient LE also reacted with melittin. Both patients reacted fairly strongly with YJV hyaluronidase, and minimally with PLA and Ag 5.

Inhibition of Hyaluronidase RAST

The cross-reactivity of HBV and YJV hyaluronidase in RAST inhibition is shown in table 3. The HBV hyaluronidase obtained from Dr. D. Kemeny was used as the inhibiting antigen in this experiment. In general, there was cross-reactivity between the two hyaluronidases with homologous and heterologous inhibition of the YJV and HBV hyaluronidase RAST. Patients SI and LE showed no inhibition of the HBV hyaluronidase RAST by small quantities of YJV hyaluronidase. Unfortunately, larger quantities of hyaluronidase were not available to complete the analysis.

Inhibition of PLA RAST

The results of RAST-inhibition studies with HBV and YJV PLAs are shown in table 4. In general, there was minimal cross-reactivity between these two phospholipases. In group 2, serum MC showed little inhibition of the YJV PLA-RAST by YJV PLA, presum-

Table 3. Cross-reactivity of hyaluronidase from honeybee venom and yellow jacket venom in RAST inhibition

	Serum dilution	Hyaluronidase disc	% Inhibition with		
			bee venom hyaluronidase 15 µg	yellow jacket venom hyaluronidase 1.5 µg	15 µg
Group 1					
SI	1:6	HBV	79	3	
	1:6	YJV	55	65	
Group 2					
FR	1:2	HBV	85		84
GA	1:2	HBV	50		50
MC	1:50	HBV	79	41	
	1:50	YJV	83	68	
Group 3					
JA	1:4	HBV	56	33	
	1:4	YJV	70	57	
Group 4					
LE	1:20	HBV	96	0	
	1:20	YJV	91	64	

Table 4. Cross-reactivity of PLA from honeybee venom and yellow jacket venom in RAST inhibition

	Serum dilution	PLA disc	% Inhibition with	
			bee venom PLA 3 µg	yellow jacket venom PLA 3 µg
Group 1				
BO	1:1	HBV	23	0
SI	1:1	HBV	50	14
Group 2				
FR	1:1	HBV	59	16
	1:2	YJV	10	45
GA	1:4	HBV	42	15
	1:4	YJV	10	100
MC	1:10	HBV	45	17
	1:10	YJV	19	18
Group 3				
DE	1:1	HBV	70	41
	1:2	YJV	38	87
JA	1:1	HBV	29	15
	1:2	YJV	1	91
Group 4				
LE	1:10	HBV	82	13
VI	1:3	HBV	65	63
	1:3	YJV	45	68

ably due to the presence of large amounts of IgG antibody to PLA. In group 3, serum DE showed some cross-reactivity between the PLAs (about 40% inhibition). Serum VI in group 4, appears to be an exception, with comparable inhibition of the HBV and YJV PLA RAST by PLA from both HBV and YJV.

Inhibition of Ag 5 RAST

Ag 5 is specific for vespid venoms and is not present in HBV. The possibility of a cross-reacting antigen was examined by inhibition experiments using Ag 5-coupled discs and HBV, YJV, and Ag 5 as inhibiting antigens. The results are shown in table 5. There was good inhibition of the Ag 5 RAST by YJV and the homologous fraction, and little or no inhibition with HBV.

Inhibition of Acid Phosphatase RAST

Acid phosphatase is specific for HBV and is not thought to be present in vespid venoms. The possibility of a cross-reacting antigen was examined by inhibition experiments using acid phosphatase-coupled discs and YJV or HBV acid phosphatase (D. Kemeny), as inhibiting antigens. The results are shown in table 6. Only serum MC showed significant inhibition of the acid phosphatase RAST by YJV (48%). With the other three sera, there was minimal inhibition.

Discussion

Patients who have had allergic reactions following stings often have positive skin test reactions and/or serum IgE antibodies reacting with both YJV and HBV. Current recommendations are to give venom immunotherapy with all venoms to which there is detectable IgE antibody activity. Our previous studies using RAST inhibition have shown that sera from allergic patients which contain both HBV- and YJV-specific IgE demonstrate four patterns of cross-reactivity. The clinical application of these results suggests that some patients would not require immunotherapy with both venoms for adequate protection against subsequent stings. The purpose of the present studies was to further investigate the explanation for these different RAST-inhibition patterns using purified venom fractions.

The fractions obtained from HBV and YJV appeared to be quite pure. The four fractions obtained from HBV had single enzyme or hemolytic activity (melittin). In immunodiffusion, melittin was not detected. The other fractions had individual lines of nonidentity. The three isolated major fractions of YJV appeared to be purified with single enzyme activity (except for Ag 5) and single precipitin lines of nonidentity in immunodiffusion with rabbit anti-YJV serum.

In direct RAST analysis, there were varying degrees of reactions with these fractions. Sera from a majority of patients showed very definite reactions with PLA, hyaluronidase, and acid phosphatase fractions from HBV as well as with the YJV reactions Ag 5, hyaluronidase, and PLA. Serum from only 1 patient reacted with melittin. There were differences in the degree of reactions with each of these fractions and this may account for the RAST-inhibition patterns obtained with whole venom. The presence of IgG antibody to whole venoms may certainly influence the RAST results. The limited quantities of the individual venom fractions prevented an indepth analysis of the specific IgG antibody activity with these fractions.

The results of these RAST-inhibition fractions were applied to the categorization of patients into four groups based upon RAST inhibition with whole venoms. The two sera in group 1 contained specific antibodies directed to each of the two venoms and showed reactions with acid phosphatase, PLA, and hyaluronidase fractions from HBV, and Ag 5 and hy-

Table 5. Inhibition of yellow jacket venom-antigen 5 RAST with yellow jacket venom and antigen 5

	Serum dilution	% Inhibition with		
		antigen 5 15 µg	whole yellow jacket venom 15 µg	honeybee venom 15 µg
Group 1 SE	1:3	68	91	0
Group 2 GA	1:2	95	98	10
Group 3 DE	1:2	98	79	0

Table 6. RAST inhibition of acid phosphatase-coupled discs by yellow jacket venom and honeybee venom acid phosphatase

	Serum dilution	% Inhibition with	
		whole yellow jacket venom 100 µg	honeybee venom acid phosphatase 15 µg
Group 1 SI	1:6	4	74
Group 2 MC	1:50	48	82
Group 3 JA	1:4	8	71
Group 4 LE	1:20	22	75

aluronidase fractions from YJV. There was little antibody reactivity with YJV PLA. In further studies, acid phosphatase gave fairly marked inhibition in the HBV RAST. We conclude that with these two sera, the HBV RAST is primarily driven by reactions with acid phosphatase and, perhaps, PLA, and that the YJV RAST is primarily driven by reactions with Ag 5.

The sera in group 2 showed extensive cross-reaction with comparable inhibition given by HBV and YJV of the HBV and YJV RASTs. Patients in this

group had large amounts of IgE antibody directed at hyaluronidase. This appeared to be the major reason for the rather extensive cross-reactivity. There were also fairly substantial reactions with acid phosphatase and PLA from HBV. The patients in this group had IgG antibody to PLA and it is possible that this affected the total RAST.

Sera from patients in group 3 reacted primarily with a major allergen in YJV. The sera reacted with Ag 5 and PLA from YJV, and had lower titers to hyaluronidase than the sera in group 2. However, these patients also had antibodies to HBV PLA. It is possible that IgG antibody to HBV influenced the results of these RAST-inhibition studies.

For sera of the patients in group 4, HBV appeared to be the major allergen. There were large amounts of antibody reacting with PLA and acid phosphatase from HBV. There was very little antibody reacting with Ag 5 and PLA from YJV. IgE antibody to YJV hyaluronidase was probably responsible for driving the YJV RAST and was easily inhibitable by the HBV hyaluronidase.

The overall results of the study indicate that there are quantitative and qualitative differences in the reactions of sera from individual patients to the major components of HBV and YJV. The differences in the RAST-inhibition patterns in patients with elevated levels of both HBV- and YJV-specific IgE are accounted for by these differences as well as by the differences in cross-reactivity between the individual components. There is extensive cross-reactivity between the hyaluronidases from HBV and YJV, but minimal cross-reactivity between the PLAs. Ag5 is restricted to YJV. The RAST-inhibition studies, shown in table 6, suggest the possible presence of a component in YJV with immunologic properties similar to HBV acid phosphatase.

Unfortunately, neither testing with individual fractions nor RAST-inhibition studies provide a practical solution to the problem of appropriate treatment for those individuals who have both serum HBV-specific and serum YJV-specific IgE. It is too expensive to obtain individual fractions in large quantities. The RAST-inhibition test is primarily a laboratory research tool. However, with knowledge of these patterns of cross-reactivity, accompanied by the clinical details, the physician is often able to make an educated decision regarding the appropriate therapy.

References

- 1 Reisman, R.E.; Müller, U.R.; Wypych, J.I.; Lazell, M.I.: Studies of coexisting honeybee and vespid venom sensitivity. *J. Allergy clin. Immunol.* 73: 246-252 (1984).
- 2 Reisman, R.E.; Wypych, J.I.; Lazell, M.I.: Further studies of patients with both honeybee and yellow jacket venom-specific IgE. *Int. Archs Allergy appl. Immun.* 82: 190-194 (1987).
- 3 King, T.P.; Sobotka, A.K.; Alagon, A.; Kochoumian, L.; Lichtenstein, L.M.: Protein allergens of white-faced hornet, yellow hornet and yellow jacket venom. *Biochemistry* 17: 5165-5174 (1978).
- 4 Sobotka, A.K.; Franklin, R.M.; Adkinson, N.F., Jr.; Valentine, M.; Baer, H.; Lichtenstein, L.M.: Allergy to insect stings. II. Phospholipase A: the major allergen in honeybee venom. *J. Allergy clin. Immunol.* 57: 29-40 (1976).
- 5 Kemeny, D.M.; Harris, M.D.; Youlten, J.F.; Mackenzie-Mills, M.; Lessof, H.H.: Antibodies to purified bee venom proteins and peptides. *J. Allergy clin. Immunol.* 71: 505-514 (1983).
- 6 Hoffman, D.R.: Allergens in bee venoms. III. Identification of allergen B of bee venom as acid phosphatase. *J. Allergy clin. Immunol.* 57: 354-366 (1977).
- 7 Reisman, R.E.; Mueller, U.; Wypych, J.; Elliott, W.; Arbesman, C.E.: Comparison of the allergenicity and antigenicity of yellow jacket and hornet venoms. *J. Allergy clin. Immunol.* 69: 268-274 (1982).
- 8 Nair, B.C.; Nair, C.; Denne, S.; Wypych, J.; Arbesman, C.E.; Elliott, W.B.: Immunologic comparison of phospholipase A present in *Hymenoptera* insect venoms. *J. Allergy clin. Immunol.* 58: 101-109 (1976).
- 9 Tolksdorf, S.; McCready, M.B.; McCulloch D.P.; Schwenk, E.: *Worthington manual*, p. 35 (Worthington Biochemical, Freehold 1968).
- 10 Habermann, E.; Zeuner, G.: Comparative studies of native and synthetic melittins. *Arch. Pharmacol.* 270: 1-9 (1971).
- 11 Light, W.C.; Reisman, R.E.; Rosario, N.A.; Arbesman, C.E.: Comparison of the allergenic properties of bee venom and whole bee body extract. *Clin. Allergy* 6: 293-300 (1976).
- 12 Kemeny, D.M.; Banks, B.E.C.; Lawrence, A.J.; Pearce, F.L.; Vernon, C.A.: The purification of hyaluronidase from the venom of the honeybee (*Apis mellifera*). *Biochem. Int.* 2: 45-51 (1981).
- 13 Littler, S.J.; Wypych, J.I.; Noble, R.E.; Abeyounis, C.J.; Reisman, R.E.: Allergenic components of bald-faced hornet (*V. maculata*) venom. *J. Allergy clin. Immunol.* 76: 1-8 (1985).
- 14 Walsh, S.G.: Biochemical studies of phospholipase A₂ from bee venom; Diss., State University of New York at Buffalo (1981).

Received: July 29, 1988

Correspondence to: Robert E. Reisman
Allergy Research Laboratory
State University of New York at Buffalo
Buffalo General Hospital
100 High Street
Buffalo, NY 14203 (USA)