Protein Allergens of White-Faced Hornet, Yellow Hornet, and Yellow Jacket Venoms†

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ABSTRACT: White-faced hornet, yellow hornet, and yellow jacket venoms have very similar protein compositions; each contains mainly three basic proteins. Two of these proteins have hyaluronidase and phospholipase activities and the third one, designated as antigen 5, is of as yet unidentified biochemical function. These three proteins have molecular weights of about 45 000, 35 000, and 25 000, respectively. The three proteins of white-faced hornet venom have been purified to near homogeneity, while this is the case only for antigen 5 of yellow hornet and yellow jacket venoms. Strong antigenic cross-reaction of the hyaluronidase from these three vespid venoms was observed using specific rabbit anti-venom sera, while weak cross-reactions of phospholipases and of antigen 5s were observed. All three proteins are active as allergens to varying degrees in vespid sensitive individuals. With each vespid venom its antigen 5 seems to be the major allergen. The results help to clarify the commonly observed varying degrees of multiple sensitivity of people to different vespids.

The most common insect sting allergies in the United States are those caused by bees and vespids (cf. Barr, 1971). The vespids include hornets, yellow jackets, and wasps. Such insect hypersensitivity reactions occur in 0.4–0.8% of the population of the United States (Chafee, 1970; Settipane et al., 1972); deaths attributed to this cause approximate 50 per year (Parish, 1965). The immunologic basis of the disease is indicated by the presence of venom specific IgE antibodies in sensitive individuals. These antibodies are demonstrable by direct skin tests on allergic patients with venoms (Loveless & Fackler, 1956; Hunt et al., 1976), by venom-induced histamine release of allergic leukocytes (Sobotka et al., 1974), or by radioimmunoassay of allergic sera with venoms (Light et al., 1977; Sobotka et al., 1978).

The allergens of honeybee venom have been subject to extensive investigations in different laboratories; the protein components of honeybee venom are the major allergens (cf. King et al., 1976). By contrast, the allergens of vespid venoms have been subjected to relatively little study (cf. Shulman, 1968). This difference is related in part to the availability of different venoms, and in part to the lack of detailed biochemical characterization of the vespid venoms. Honeybee venom is a commercial item, while the vespid venoms are not; they have to be specially collected. Nearly all the venom components of honeybee have been characterized biochemically (cf. Habermann, 1972), but those of the vespids have not.

In this paper we will report our chemical and immunological studies of the venom proteins of white-faced hornet (*Vespula maculata*), yellow hornet (*V. arenaria*), and yellow jacket (*V. maculifrons, V. vulgaris,* and *V. germanica*). The venom of yellow jackets was collected from a mixture of the three species, as these species cross-breed and often share the same nests.

Materials and Methods

The venoms of white-faced hornet, yellow hornet, and yellow jacket were collected by Dr. Allen W. Benton of the College of Agriculture, The Pennsylvania State University, as follows: venom sacs, after removal from insects, were extracted by a 5- to 10-s homogenization with 25 mM β-alanine–acetic acid buffer (pH 4.6) containing 0.45% NaCl (about 50 sacs per 5 mL). The extract, following centrifugation and passage through a 0.45-μm Millipore filter, was lyophilized and stored in a freezer until use. Dr. Benton estimated the venom weights by Folin color (Lowry et al., 1951) using bee venom as the standard. In this study, however, all the venom weights given were determined by amino acid analysis following acid hydrolysis. The weights so determined are about one-third to one-half of that determined by Folin color, as indicated in Table I.

Hyaluronic acid was from Nutritional Biochemicals Corp.; Sephadex G-100 and BioRex 70 resin were from Pharmacia and BioRad Laboratories, respectively. Complete Freund's adjuvant was from Difco Laboratories.

Specific rabbit anti-venom sera were prepared using New Zealand red or white rabbits. Each rabbit received six injections of venom on weeks 0, 1, 2, 4, 5, and 7. Each injection, containing 0.2–0.3 mg of venom in 0.2 mL of 0.2 M Tris-HCl buffer (pH 7.95) emulsified with an equal volume of complete Freund's adjuvant, was given in two separate sites subcutaneously behind the neck of the animal. Bleedings were made on weeks 6, 8, and 9. With some of the animals, sera of 6th week bleeding were already useful for immunodiffusion studies.

Hyaluronidase activity was measured by a turbidimetric method (Tolksdorf et al., 1949) with the modification that the assays were made at 25 °C. One unit of activity is defined as that amount of enzyme required to hydrolyze 1 μg of hyaluronic acid per min at 25 °C in 200 μL of a 200 μg/mL solution of substrate in pH 5.3 buffer.

Phospholipase activity was measured by two different methods; one is a titrimetric procedure with the egg yolk as substrate (Shiloah et al., 1973), and one unit of phospholipase activity is defined as that amount of enzyme releasing 1 μmol of acid per min at pH 8 and 25 °C in 3 mL of a 10% (w/v) solution of egg yolk in 0.1 M NaCl. Another method depends on the clearing of egg yolk suspension in agarose gel (Habermann & Hardt, 1972), and purified bee venom phospholipase (King et al., 1976) was used as the standard.

For the determination of protein content of venom, the samples were exhaustively dialyzed in Visking 8/32 tubing against 0.02 N ammonium acetate buffer (pH 4.75). Venom

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TABLE I: Comparative Biochemical Properties of Vespid and Honeybee Venoms.

	white-faced hornet	yellow hornet	yellow jacket	honeybee[e]
hyaluronidase units per mg of venom[a]	370 (range 260-550)	430 (range 330-520)	470 (range 370-530)	370
phospholipase:[b]				
(a) units per mg of venom	22	32	49	62
(b) μg per mg of venom	0.90	1.1	7.0	120
	(range 0.46-2.3)	(range 0.45-2.3)	(range 2.3-8.4)	
% of venom proteins[c] as:				
hyaluronidase	2.2	1.9	2.0	6-24
phospholipase	0.4	0.4	2.3	70
antigen 5	13.2	12.1	15.3	0
% of venom as proteins[d]	22 ± 2	28 ± 3	30 ± 3	>17
ratio of venom weights determined by Folin color & by amino acid anal.[a]	2.0 ± 0.3	3.0 ± 0.6	2.9 ± 0.5	1.0

[a] For each enzyme activity both the mean and the range of different lots for each venom are given. The error of each individual assay is 15% or less. The numbers of different lots of white-faced hornet, yellow hornet, and yellow jacket venoms studied were respectively 6, 2, and 3. [b] Phospholipase activity expressed in units was determined by a titrimetric procedure with egg yolk as substrate, while that expressed in micrograms was made by measuring the clearing of a gel suspension of egg yolk using bee venom phospholipase A_2 as a standard. [c] The amounts of hyaluronidase in vespid venoms were estimated on the assumption that they have the same specific activities as that of honeybee enzyme. The amounts of phospholipase in vespid venoms were estimated by the gel clearing assay using bee venom phospholipase as the standard. The amounts of antigen 5 were estimated from isolation experiments after correcting for a 50% loss in workup. [d] Percent of venom as proteins was determined on amino acid analysis of samples before and after exhaustive dialysis as described in Materials and Methods. Values represent averages of two or more experiments. [e] The values for honeybee venom are from Habermann (1972) and King et al. (1976).

protein or total weights were determined by amino acid analysis of a sample (0.1-0.5 nmol) following hydrolysis in 6 N HCl (50 μL) at 110 °C in sealed and evacuated tubes (<0.1 Torr); the amino acid analyzer used was adapted for the detection of amino acids in the nmole range (Liao et al., 1973). For the determination of amino acid compositions of the purified antigens, the following correction factors were used: 5, 10, and 10%, respectively, for the loss of threonine, serine, and half-cystine after 20-h hydrolysis. Valine, leucine, and isoleucine contents were determined using 72-h hydrolysates. Tryptophan content was determined using samples hydrolyzed in methanesulfonic acid (Liu & Chang, 1971) or in 6 N HCl containing tryptamine (2 mg per mL).

The chromatographed venom fractions were concentrated by ultrafiltration through Visking 8/32 and 23/32 tubings for solutes with molecular weights of greater and less than 30 000, respectively (Berggård, 1961). Concentration by ultrafiltration through Amicon membrane UM-2 was also used for fractions with molecular weights of <30 000.

Disc electrophoresis was carried out in polyacrylamide gel containing pH 4.1 acetate buffer in 6 M urea (Reisfeld et al., 1962) and the gels were stained with Coomassie blue G-250 (Reisner et al., 1975). Sodium dodecyl sulfate ($NaDodSO_4$)[1] gel electrophoresis was carried out as reported (Swank & Kundres, 1971); two of the standard proteins used were obtained from limited pepsin digestion of bovine plasma albumin (King, 1973).

Immunodiffusion was carried out in 1% agarose gel containing 0.1 M Tris-HCl buffer (pH 7.95); wells of 2-4-mm diameter were spaced 10 mm apart from center to center. All samples indicated in the figures were added in two separate equal aliquots about 20 h apart. The gels were dried, washed, and stained 20 h after the second addition of samples (Weeke, 1973).

Immunoelectrophoresis was made in 1% agarose gels (0.08 × 7 × 11 cm) containing 75 mM sodium barbital buffer (pH 8.6). Electrophoresis of the sample (2-10-μL volume) was carried out at 50 V for 2 h. After adding antisera (50 μL) to the trough, immunodiffusion was carried out for about 20 h. The gels were then processed as described for immunodiffusion.

The allergenic activities of the venom fractions were studied by the histamine release method from leukocytes of the appropriate vespid allergic patients (Lichtenstein & Oster, 1964; Siriganian, 1974).

Results

General Biochemical Properties of Vespid Venoms. All the studies described in this paper, with a single exception, were made with extracts of venom sacs rather than with pure venoms. This is because of the technical difficulties to obtain large quantities of vespid venoms. A small sample of white-faced hornet venom was collected on electrical stimulation. When this venom sample was compared with a corresponding sample of venom sac extract by $NaDodSO_4$ gel electrophoresis and disc electrophoresis in acetate-urea buffer, indistinguishable results were observed. Therefore, the findings show that the white-faced hornet venom sac extracts contain mainly venom proteins and that the sac extracts are not heavily contaminated with proteins from other parts of insect body. Similar comparative studies were not made with yellow hornet and yellow jacket venoms and their venom sac extracts. However, it is unlikely that the venom sac extracts of yellow hornet and yellow jacket will have higher levels of contamination than that of white-faced hornet since all three sac extracts were made under identical conditions.

In Table I are given some of the biochemical properties of the venoms of the three vespids: white-faced hornet (WFH), yellow hornet (YH), and yellow jacket (YJ). For purposes of

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; WFH, white-faced hornet; YH, yellow hornet; YJ, yellow jacket.

comparison, some of those properties of honeybee venom are also given. Honeybee venom is known to be rich in phospholipase A_2 and hyaluronidase activities (cf. Habermann, 1972). The three vespid venoms, like honeybee venom, also contain these two enzyme activities. The phospholipase assay procedures used in this work do not establish the specificities of the three vespid venoms as of the A_2 type.

The specific hyaluronidase activities of the vespid venoms are in the range of 260–550 units per mg of venom, while that of honeybee venom is about 370 units per mg of venom. Titrimetric assays using egg yolk as the substrate showed that the specific phospholipase activities of the vespid venoms range from 22 to 49 units per mg of venom. Phospholipase assays of the vespid venoms were also made by measuring the clearing of a gel suspension of egg yolk. With the egg yolk clearing assay, the vespid venoms were found to contain much less phospholipase activity than the bee venom does: 1–7 μg per mg of vespid venom as compared with 120 μg per mg of bee venom. The phospholipase results obtained by the gel clearing assay are not in accord with those obtained by the titrimetric assay where the difference in phospholipase activities of vespid venoms and honeybee venom is much smaller. The reason for this discrepancy in assay results is not known. The data in Table I also indicate that there is wide variation of phospholipase activity in different lots of vespid venoms, as shown with the egg yolk clearing assay, and that there is less variation of hyaluronidase activity.

With honeybee venom, about 77% of its dry weight is accounted for as proteins and peptides, and these two components respectively comprise 17% and 83% of that 77%. Honeybee venom also contains small amounts of biogenic amines in the order of few percent or less (Habermann, 1972). It is likely that the vespid venoms and the honeybee venom are alike in their chemical compositions and that the dry solid weight of vespid venoms is contributed mainly by peptides and proteins as is the case with bee venom. With these assumptions, we estimated the total solid and protein contents of vespid venoms by amino acid analysis before and after exhaustive dialysis of the venom samples. As the vespid venom sac extracts were made initially with nonvolatile buffers, it was not possible to obtain its total solid content on direct weighing.

About 20–30% of the weight of the vespid venoms represents proteins as compared with about 17% for the honeybee venom. Phospholipase is the major protein component of honeybee venom, but this is not the case with the vespid venoms. The results below will show that the major protein component of the three vespid venoms is a basic protein with molecular weight of about 25 000, which will be designated as antigen 5.

The three vespid venoms have very similar protein compositions, as indicated by their chromatograms from a column of Sephadex G-100 (Figures 1a–c). Dialyzed venoms were used in these studies. With each vespid venom there is one main ultraviolet absorbing peak containing the protein antigen 5. With all three vespid venoms, phospholipase is eluted slightly ahead of the main ultraviolet absorbing peak, and the hyaluronidase peak is ahead of the phospholipase peak. There is poor separation of the hyaluronidase and the phospholipase peaks for the two hornet venoms, but there is good separation of these two peaks for the yellow jacket venom. The cuts indicated in Figure 1 were lyophilized to remove the volatile buffer, 0.02 N ammonium acetate (pH 4.75). The recoveries of the fractions in terms of their weight and enzyme activities are in the range of 70–100% and they are given in Table II.

$NaDodSO_4$ gel electrophoresis and disc electrophoresis in acetate–urea buffer were carried out with the venom fractions

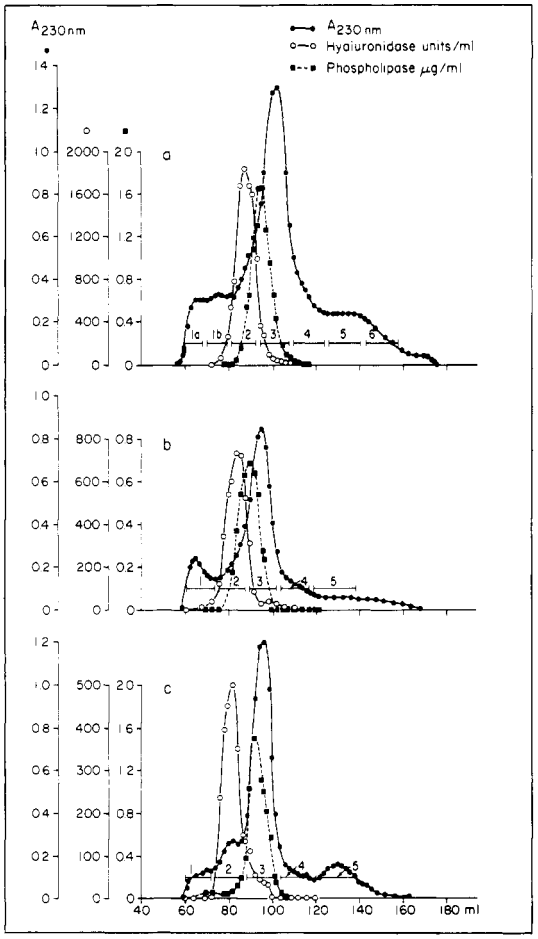

FIGURE 1: Chromatography of vespid venoms on a column (200 × 0.9 cm) of Sephadex G-100. (a) Twenty-eight milligrams of white-faced hornet venom or 5.6 mg of venom proteins; (b) 14 mg of yellow hornet venom or 3.5 mg of venom proteins; (c) 11 mg of yellow jacket venom or 3.6 mg of venom proteins. Column was eluted with 0.02 N ammonium acetate buffer (pH 4.75) at room temperature and at a flow rate of about 10 mL per h.

obtained from Sephadex G-100 chromatography (Figures 2a–f). The following generalizations may be made for all three vespid venoms: the major ultraviolet-absorbing fraction for each venom contains mainly one major and one minor basic protein of molecular weights of about 25 000 and 35 000, respectively. The fraction preceding the main ultraviolet-absorbing fraction contains, in addition to the basic protein of molecular weight of about 35 000, a basic protein of molecular weight of about 45 000. The fraction following the main ultraviolet-absorbing fraction contains proteins of molecular weight of less than 10 000. For reasons which are not clear, the venom component with molecular weight of 45 000 gave variable staining with Coomassie blue dye when the whole venom was used for $NaDodSO_4$ gel electrophoresis. This variability was not seen when partially purified fractions were used.

Immunodiffusions of the venom fractions from Sephadex G-100 were carried out (Figures 3a–d). At least three antigenic components were detected with each venom.

Purification of White-Faced Hornet Venom Proteins. The hyaluronidase- and the phospholipase-rich fractions of white-faced hornet venom, fractions 2 and 3 of Figure 1a, were separately rechromatographed on Sephadex G-100 to reduce the level of their cross-contamination (results not shown). They were then separated on a column of Bio-Rex 70 ion-exchange resin using a pH 6.6 phosphate buffer with a linear sodium

TABLE II: Recovery of Venom Fractions from G-100 Chromatogram.

fraction	WFH venom (Figure 1a) weight (mg)	hyaluronidase (units)	phospholipase (units; μg)[a]	YH venom (Figure 1b) weight (mg)	hyaluronidase (units)	phospholipase (μg)[a]	YJ venom (Figure 1c) wt (mg)	hyaluronidase (units)	phospholipase (units; μg)[a]
1	0.39			0.24			0.42		
2	0.67	16 000	230; 11	0.41	5800	2.4	0.24	3600	140; 8
3	1.58	1 400	220; 12	0.86	900	4.7	1.12	480	420; 120
4	0.93			0.34			0.36		
5	0.98						0.45		
6	0.59								
total	5.14	17 400	450; 23	1.85	6700	7.1	2.59	4080	560; 128
dialyzed venom	5.6	19 800	340; 22	3.5	8500	8	3.6	5800	590; 140
recovery (%)	92	90	130; 105	53	79	89	72	70	94; 91

[a] Phospholipase activity was measured by a titrimetric assay (units) and by a gel clearing assay (μg).

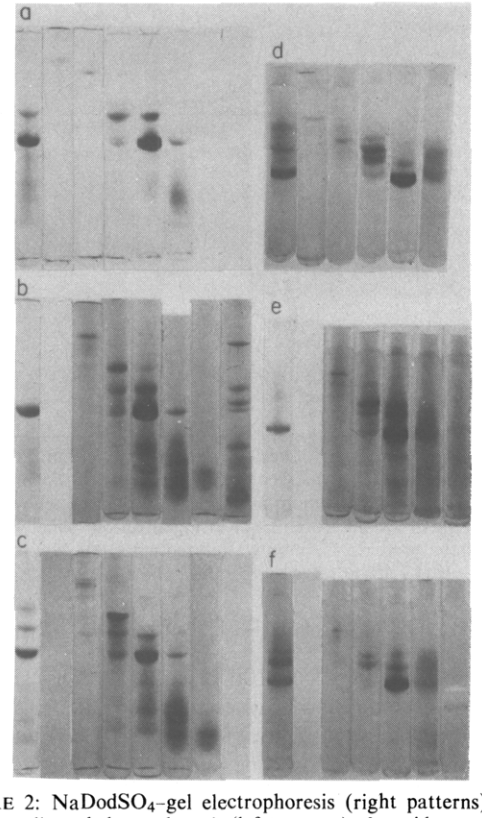

FIGURE 2: $NaDodSO_4$-gel electrophoresis (right patterns) and acetate-urea disc gel electrophoresis (left patterns) of vespid venom fractions. From left to right: (a and d) Dialyzed white-faced hornet venom (16 μg), fractions 1a, 1b, 2, 3, and 4 of Figure 1a (78 μg of venom protein equivalent). (b and e) Dialyzed yellow hornet venom (27 μg), fractions 1, 2, 3, 4, and 5 of Figure 1b (52 μg or venom protein equivalent). In b there is an additional gel on the far right containing fraction 4 (10 μg of venom protein equivalent) together with a mixture of four standard proteins, bovine plasma albumin, its two pepsin fragments and ribonuclease with molecular weights of 66 000, 35 000, 29 000, and 13 600, respectively. (c and f) Dialyzed yellow jacket venom (26 μg), fractions 1, 2, 3, 4, and 5 of Figure 1c (60 μg of venom protein equivalent).

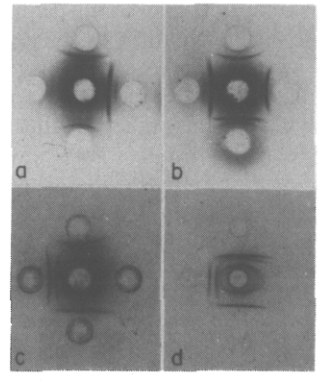

FIGURE 3: Immunodiffusion of vespid venoms and their fractions. The amounts of venoms and fractions are given in venom protein equivalents. The central wells are filled with specific anti-venom sera, and the peripheral wells are filled with test samples. Sample designations of peripheral wells starting from top of each pattern and continuing in counterclockwise direction are as follows: (a) white-faced hornet venom (32 μg), fractions 1a, 1b, and 2 of Figure 1a (120 μg); anti-white-faced hornet venom sera (40 μL). (b) Purified hyaluronidase fraction (2 μg) of Figure 4a, fractions 2 and 3 of Figure 1a (120 μg), and purified phospholipase fraction of Figure 4b (0.2 μg); anti-white-faced hornet venom sera (40 μL). (c) Yellow hornet venon (12 μg), fractions 2, 3, and 4 of Figure 1b (84 μg); anti-yellow hornet venom sera (40 μL). (d) Yellow jacket venom (18 μg), fractions 2, 3, and 4 of Figure 1c (18 μg); anti-yellow jacket venom sera (20 μL).

chloride gradient, as shown in Figures 4a and 4b.

Multiple chromatographic forms of hyaluronidase were observed in Figure 4a. The main hyaluronidase fraction indicated in Figure 4a contained about 47% of the enzyme units applied to the column. Variable recoveries of hyaluronidase activities were encountered on concentration of the fraction by ultrafiltration. This loss of enzyme activity was prevented on addition of human plasma albumin to the fraction prior to its concentration. Albumin probably prevents the loss of hyaluronidase by saturation of nonspecific binding sites of the vessels used. The hyaluronidase fraction, recovered in the absence of added albumin, is homogeneous on acetate disc and $NaDodSO_4$ gel electrophoreses (results not shown), immunodiffusion (Figure 3b), and immunoelectrophoresis at pH 8.6 (Figure 5, 1a). $NaDodSO_4$ gel electrophoresis indicates its molecular weight to be about 45 000.

In Figure 4b, the small ultraviolet-absorbing peak 1 eluted near the beginning of the chromatogram contains the phospholipase activity, while the two large peaks 2 and 3 near the middle of the chromatogram contain antigen 5. There was a significant loss of phospholipase activity in this purification step, as only about 45% of the enzyme applied to the column was recovered in the chromatogram. The main phospholipase cut 1 of Figure 4b contains about 30% of the enzyme applied to the column, and only 30% of the enzyme was recovered on concentration. Addition of human plasma albumin to the fraction prior to its ultrafiltration did not improve the recovery of phospholipase, but it did improve the stability of the concentrated solution. These observations suggest that phospholipase is being inactivated by nonspecific adsorption to vessels as well as by some other mechanism(s). Acetate disc and $NaDodSO_4$ gel electrophoreses of the recovered phospholipase

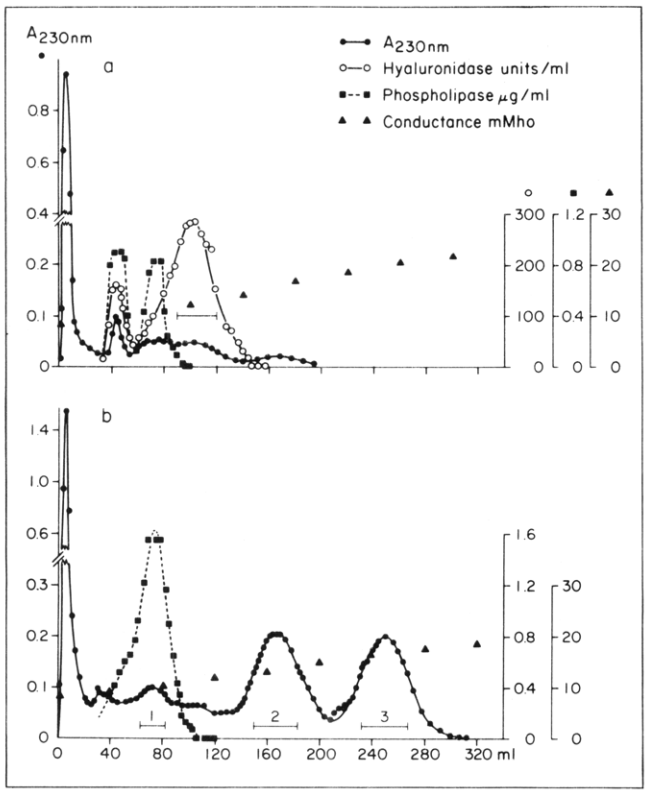

FIGURE 4: Chromatography of white-faced hornet venom fractions on a column (20 × 0.6 cm) of Bio-Rex 70. Patterns a and b were obtained, respectively, with fractions 2 and 3 of Figure 1c, as isolated from 16 mg of venom proteins. The column was eluted at a flow rate of 60 mL per h with a linear NaCl gradient of 50 mM per 100 mL in 0.1 M sodium phosphate (pH 6.6).

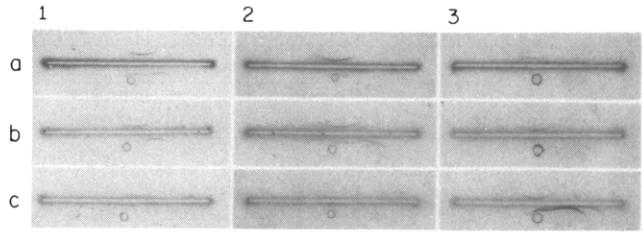

FIGURE 5: Immunoelectrophoresis of vespid venom fractions. The antigens tested are as follows: (1a–c) Upper and lower wells filled with purified hyaluronidase (0.5 μg) and phospholipase (0.4 μg) of white-faced hornet venom (Figure 4), respectively. (2a–c) Upper and lower wells filled with the hyaluronidase-rich fraction 2 and the phospholipase-rich fraction 3 of yellow hornet venom (Figure 1b), respectively. The antisera troughs are filled as follows: (1a, 2a, and 3a) anti-white-faced hornet venom sera; (1b, 2b, and 3b) anti-yellow hornet venom sera; (1c, 2c, and 3c) anti-yellow jacket venom sera.

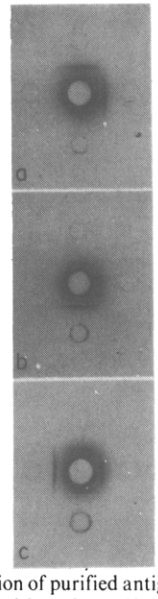

FIGURE 6: Immunodiffusion of purified antigen 5s of vespid venoms. The peripheral wells are filled with antigens (0.4–0.7 μg) as follows: starting from the top in counterclockwise direction, white-faced hornet venom antigen 5, yellow jacket venom antigen 5, yellow hornet venom antigen 5, and a repeat of white-faced hornet venom antigen 5. The central wells (40 μL) in patterns a, b, and c are filled with anti-white-faced hornet venom sera, anti-yellow hornet venom sera, and anti-yellow jacket venom sera, respectively.

gave only one band with molecular weight of about 35 000 (results not shown). Immunodiffusion of the phospholipase fraction showed one strong precipitin band and one faint band (Figure 3b). Immunoelectrophoresis at pH 8.6 showed that the main precipitin band is of two electrophoretic forms with identical antigenic specificity (Figure 5, 1a).

The two chromatographic forms of antigen 5, cuts 2 and 3 of Figure 4b, are homogeneous and indistinguishable from each other on acetate disc and $NaDodSO_4$ gel electrophoresis (results not shown); both forms gave only one band with molecular weight of about 25 000. On immunoelectrophoresis at pH 8.6, both forms of antigen 5 gave identical long precipitin arcs (results not shown). The two forms of antigen 5 are also indistinguishable on amino acid analysis and immunodiffusion and only the results for one of these two forms are shown (Table II and Figure 6). The combined amount of these two forms of antigen 5 in Figure 4b represents 46% of the weight of fraction 3 (Figure 1a) applied to the Bio-Rex 70 column, and the amount represents a yield of 2.9 mg per 100 mg of venom. The actual recovered yield is about half that amount owing to loss during ultrafiltration. Lyophilization of solutions of antigen 5 containing high salt concentration was found to cause denaturation.

Antigen 5 was found not to contain detectable levels of the following enzymic activities: choline esterase (Augustinsson, 1971); elastase, lysozyme, and phosphatase (Decker, 1977); protease (Rinderknecht et al., 1968); fibrinogenlytic and fibrinolytic activities (Ouyang & Huang, 1976); ribonuclease (Wang & Moore, 1977); deoxyribonuclease (Liao, 1974); phospholipase; and hyaluronidase.

Purification of Yellow Hornet Venom. Fractions 1–4 of Figure 1b, obtained from Sephadex G-100 chromatography of dialyzed yellow hornet venom, were combined and chromatographed on an ion-exchange column of Bio-Rex 70. As is the case with white-faced hornet venom, the two main ultraviolet-absorbing peaks, 1 and 2, eluted last in the chromatogram (Figure 7a) contain antigen 5, and the two small ultraviolet-absorbing peaks eluted near the breakthrough volume contain hyaluronidase and phospholipase, but these two enzymes were not separated. Better than 90% of hyaluronidase units applied to the column were found in the chromatogram, but only about 40% of phospholipase applied were found.

The ion-exchange chromatographic conditions used for yellow hornet venom differ from those used for white-faced hornet venom by the presence of 1% glycerin in the eluting buffer. The purpose of adding glycerin was that it might stabilize the phospholipase activity, but this objective was not realized. In two other separate experiments, where only fraction 2 of Figure 1b or the dialyzed venom was used, complete loss of phospholipase activity was observed, although the recovery of hyaluronidase activity was about 90% (results not shown).

TABLE III: Amino Acid Compositions of Antigen 5s from Three Vespid Venoms.[a,b]

amino acid	WFH av	WFH nearest integer	YH av	YH nearest integer	YJ av	YJ nearest integer
Lys	19.5 ± 0.5	20	20.9 ± 0.4	21	22.9 ± 0.7	23
His	5.1 ± 0.1	5	7.9 ± 0.7	8	6.2 ± 0.6	6
Arg	8.2 ± 0.3	8	6.3 ± 0.2	6	6.0 ± 0.9	6
Asp	31.0 ± 1.4	31	28.0 ± 0.4	28	25.7 ± 1.2	26
Thr	13.3 ± 0.6	13	13.1 ± 0.5	13	10.1 ± 0.5	10
Ser	9.1 ± 0.7	9	8.9 ± 0.2	9	12.5 ± 21	13
Glu	22.9 ± 1.2	23	22.0 ± 0.7	22	26.6 ± 1.8	27
Pro	9.1 ± 0.4	9	10.2 ± 0.4	10	8.6 ± 1.0	9
Gly	18.6 ± 0.6	19	20.0 ± 0.6	20	19.3 ± 1.3	19
Ala	10.6 ± 0.4	11	12.9 ± 0.8	13	13.1 ± 0.6	13
½-Cystine	9.4 ± 0.9	9	9.2 ± 1.0	9	5.5	6
Val	11.4 ± 0.9	11	13.9 ± 1.5	14	13.6 ± 0.3	14
Met	3.4 ± 0.3	3	4.7 ± 0.5	5	4.0	4
Ile	12.0	12	8.0 ± 0.2	8	7.1 ± 0.2	7
Leu	10.0	10	9.6	10	12.7 ± 0.6	13
Tyr	9.7 ± 0.7	10	10.3 ± 0.3	10	12.1 ± 0.1	12
Phe	7.2 ± 0.4	7	5.1 ± 0.4	5	4.7 ● 0.3	5
Trp	4.7 ± 0.8	5	4.9 ± 0.4	5	5.4 ± 1.2	5

[a] Calculated on the assumption that the number of Lys, Arg, Asp, Glu, Gly, and Ala residues is respectively 20, 8, 31, 23, 19, and 11 for WFH antigen 5; 21, 6, 28, 22, 20, and 13 for YH antigen 5; 23, 6, 25, 26, 19, and 13 for YJ antigen 5. [b] Averages of duplicate analyses of at least two different preparations.

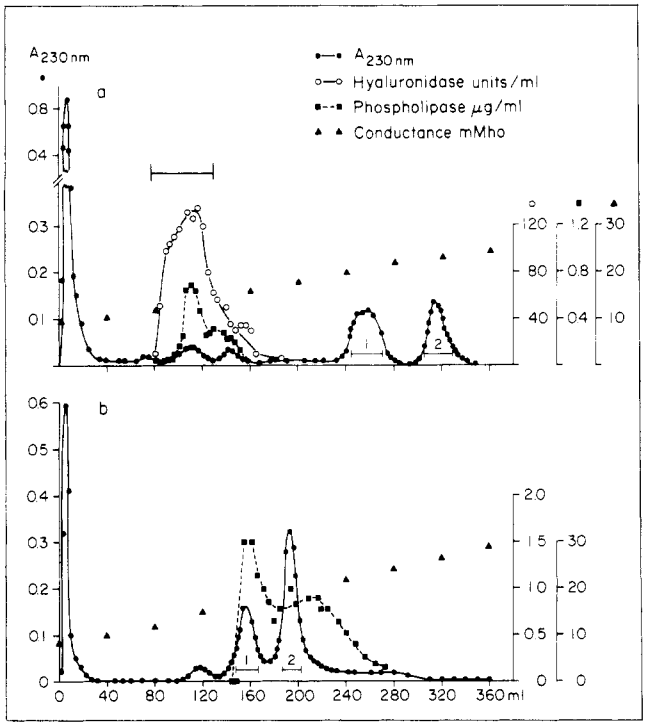

FIGURE 7: (a) Chromatography of yellow hornet venom on Bio-Rex 70. The combined fractions 1 through 4 of Figure 1b, from 4.2 mg of venom proteins, were used. (b) Chromatography of the antigen 5 and phospholipase-rich fraction of yellow jacket venom on Bio-Rex 70. Fraction 3 of Figure 1c, from 4.6 mg of venom proteins, was used. The chromatographic conditions used are the same as those in Figure 4 with the modification that the buffer contains 1% glycerin.

On concentration of the indicated fractions of Figure 7a by ultrafiltration through appropriate membranes, the recoveries of hyaluronidase, phospholipase, and antigen 5 were about 90%, 15%, and 50%, respectively. The poor recovery of antigen 5 is due to incomplete retention of the antigen by the ultrafiltration membrane, but the poor recovery of phospholipase is due to its instability. $NaDodSO_4$ gel electrophoresis of the recovered phospholipase- and hyaluronidase-containing fractions showed the presence of two main bands of equal intensity corresponding to molecular weights of about 45 000 and 35 000 and one faint band of molecular weight of about 25 000 (results not shown). The two antigen 5 fractions are homogeneous and indistinguishable on acetate disc and $NaDodSO_4$ gel electrophoresis, immunodiffusion, and amino acid analysis, and their molecular weights were estimated to be about 25 000. Only the results of immunodiffusion and amino acid analysis of one of these two forms of antigen 5 are shown in Figure 6 and Table III.

Purification of Yellow Jacket Venom Protein. Fractions 3 of Figure 1c, the antigen-5- and phospholipase-rich fraction of yellow jacket venom, was rechromatographed on Sephadex G-100 to remove the contaminating hyaluronidase, then it was separated on a column of Bio-Rex 70 under the same chromatographic conditions as those for yellow hornet venom. All the phospholipase activity applied to the column was found as a broad zone in the middle of the chromatogram (Figure 7b). In this same region of the chromatogram were also present two ultraviolet-absorbing peaks which were both later shown to contain antigen 5. On concentration of these two indicated peaks, 30–50% of the expected absorbance units were recovered, but no phospholipase activity was recovered. The same result was obtained with two other separate experiments. The cause for the observed inactivation of phospholipase is not known. The two recovered fractions both gave single bands of identical mobilities on acetate disc and $NaDodSO_4$ gel electrophoresis, and their molecular weights were estimated to be about 25 000. They were also indistinguishable by immunodiffusion and by amino acid analysis; only the results for one of them are given in Table III and Figure 6.

Under the chromatographic conditions used in Figure 7, hyaluronidase was eluted in the same region as that for antigen 5 and phospholipase. This was shown on direct separation of yellow jacket venom on Bio-Rex 70.

Immunological Specificity of Vespid Venom Proteins. Immunoelectrophoresis at pH 8.6 was used to examine the immunological cross-reactions of the phospholipase- and the hyaluronidase-rich fractions of these three vespid venoms.

TABLE IV: Summary of Antigenic Cross-Reaction of the Venom Proteins of White-Faced and Yellow Hornet and Yellow Jackets with Specific Rabbit Antisera.[a]

	venom specific antisera		
	WFH	YH	YJ
hyaluronidase,			
WFH	+	+	+
YH	+	+	−
YJ	+	+	+
phospholipase,			
WFH	+	+	−
YH	−	+	−
YJ	−	−	+
antigen 5,			
WFH	+	+	+
YH	−	+	+
YJ	−	−	+

[a] Identical cross-reactions of hyaluronidases and phospholipases were obtained with three different sets of specific antisera. The indicated cross-reaction of antigen 5 was obtained with one of the three sets of antisera tested and negative results were obtained with the other two sera.

Purified hyaluronidase of WFH venom gave a single precipitin arc with anti-WFH venom, anti-YH venom, or anti-YJ venom sera (Figure 5, 1a–c); the line with anti-WFH venom or with anti-YH venom sera is clearly visible, but that with anti-YJ venom is barely visible. Purified phospholipase of WFH venom gave two coalescing precipitin arcs with anti-WFH venom or with anti-YH venom sera, and it did not give a precipitin arc with anti-YJ venom (Figure 5, 1a–c).

The hyaluronidase-rich fraction 2 of YH venom from Figure 1b showed a single precipitin arc with anti-YH venom or anti-WFH venom sera, but not with anti-YJ venom sera (Figure 5, 2a–c). The phospholipase-rich fraction 3 of YH venom from Figure 1b gave two coalescing precipitin arcs migrating toward the cathode and one faint precipitin arc migrating toward the anode with anti-YH venom sera; only the anodal component was detected with anti-WFH venom sera, and none was found with anti-YJ venom sera (Figure 5, 2a–c). By analogy to WFH venom, the cathodal migrating precipitin arc is assumed to be the phospholipase of YH venom.

The hyaluronidase-rich fraction 2 of YJ venom from Figure 1c gave a single precipitin arc with all three anti-venom sera (Figure 5, 3a–c). The phospholipase-rich fraction 3 of YJ venom from Figure 1c gave two sets of two coalescing precipitin arcs with anti-YJ venom sera (Figure 5, 3a–c). The faint set closest to the antisera trough represents the antigen 5 component and this is indicated by the immunodiffusion experiment in Figure 3c. The strongly staining set represents phospholipase. Neither set of precipitin arcs was detected with anti-WFH venom or anti-YH venom sera. The immunoelectrophoresis experiments described in Figure 5 (1–3) were repeated with specific antisera from another set of rabbits. Results similar to those of Figure 5 were seen.

The immunological cross-reaction of the antigen 5s from vespid venoms was studied with immunodiffusion instead of immunoelectrophoresis. This is because the precipitin lines of all three vespid antigen 5s lie too close to the antisera trough for easy visualization. These results of immunodiffusion are shown in Figure 6. Antigen 5 of WFH venom formed a precipitin line with anti-WFH venom sera as well as with anti-YH and anti-YJ venom sera. Antigen 5 of YH venom formed a precipitin line with anti-YH and anti-YJ venom sera, but not with anti-WFH sera. Antigen 5 of YJ venom only formed a precipitin line with anti-YJ venom sera, and not with the other two anti-venom sera.

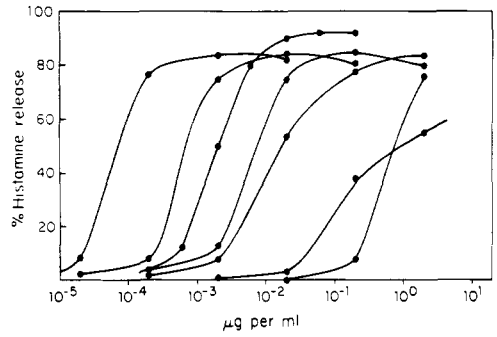

FIGURE 8: Histamine release curves from leukocytes of a yellow jacket venom allergic patient (donor 1, Table VII) on challenge with yellow jacket venom or its fractions. From left to right: fraction 2 of Figure 7, fraction 3 of Figure 1c, dialyzed YJ venom proteins, and fractions 4, 2, 1, and 5 of Figure 1c, respectively. A brief description of the composition of these fractions is given in Table VII.

These immunodiffusion experiments were repeated with specific anti-venom sera from two other sets of rabbits. No cross-reaction was detected with these two sets of antisera, as had been found for the first set shown in Figure 6, as each antigen 5 only formed a precipitin line with its specific antisera. These immunodiffusion studies of the three vespid antigen 5s with the three sets of specific rabbit anti-vespid venom sera were reproducible on repeat experiments. Therefore the observed difference with the three sets of sera is probably due to variability in immune response of the individual rabbits. The precipitin lines of the three antigen 5s studied are specific, as no lines were obtained with rabbit antisera of unrelated specificity.

The above results on the antigenic cross-reactions of the proteins of different vespid venoms are summarized in Table IV. In addition, none of the vespid venom fractions studied in this work was found to react with rabbit anti-honeybee venom sera.

Allergenic Activity of Vespid Venom Proteins in Man. The different vespid venom fractions from Figures 1, 4, and 7 were tested for their allergenic activities by the histamine release assay with leukocytes from appropriate vespid sensitive donors. Each set of vespid venom fractions was tested using leukocytes from 5–10 donors. The results are summarized in Tables V, VI, and VII. In these tables the concentrations of dialyzed vespid venom required to give 50% histamine release are given in parentheses; for all others only the ratios of concentration of the dialyzed venom and the fraction required for 50% histamine release are given. As a representative example one complete set of data for the fractions of YJ venom, which is summarized in Table VII for donor number 1, is given in Figure 8.

The results in Tables V–VII show that for all three vespid venoms the allergenically most active fractions are the ones containing antigen 5, hyaluronidase, and phospholipase. The antigen 5 rich fractions for WFH, YH, and YJ venoms are, respectively, 3.2, 11, and 3.3 times (geometric mean of 5–10 donors) more active than the venom proteins, and in each case they represent 25–31% of the venom proteins. Therefore the data suggest that the clinically important allergens are contained in these fractions. When the purified antigen 5s from WFH, YH, and YJ venoms were tested, they were found to be, respectively, 19, 12, and 56 times more active than the crude venom proteins. The large increases in the allergenic activities of the purified antigen 5s, as compared with those of the an-

TABLE V: Histamine Release Assays of White-Faced Hornet Venom Proteins with Sensitive Human Leukocytes.

HR act. for donor[a]	dialyzed venom	fraction								
		1a, Fig 1a proteins with MW[c] >50 000 3[b]	1b, Fig 1b proteins with MW <50 000 4[b]	2, Fig 1b hyal & phosph rich 12[b]	3, Fig 1b phosph & Ag 5 rich 28[b]	4, Fig 1b proteins with MW <25 000 17[b]	5, Fig 1b large polypeptides 18[b]	Fig 4a hyal -	3, Fig 4b Ag 5 -	1, Fig 4b phosph -
1	(7.7×10^{-2})	0.13	1.3	3.1	0.19	0.08	<0.001	77	0.33	
2	(9.9×10^{-2})	<0.001		2.5	>9.9	9.9	0.10	<0.001	99	99
3	$(1.1 \times 10^{-2}$	<0.001	<0.001	0.37	3.7	0.39	0.006	0.55	6.1	16
4	(2.2×10^{-3})	0.07	0.01	0.55	5.5	0.73	0.017	1.1	12	67
5	(1.4×10^{-2})	<0.001	<0.001	2.8	9.3	1.4	<0.001	1.8	9.3	35
	geometric mean:	0.006	0.011	1.3	3.2	0.79	0.006	0.61	19	44

[a] The concentrations in μg per mL of the dialyzed venom required for 50% histamine release (HR) from sensitive leukocytes are given in parentheses. For all other samples, their activities are expressed as the ratios of concentration of dialyzed venom and the fraction required for 50% HR. [b] Percent of venom proteins calculated from the data of Table II. [c] MW, molecular weight.

tigen 5 rich fractions, are unexpected since antigen 5 is the major protein component (greater than 46%) in these fractions for all three venoms as evidenced by the ion-exchange chromatographic data of Figures 4 and 7.

The purified hyaluronidase and phospholipase from WFH venom were found to be 0.61 and 44 times as active as the WFH venom proteins. These values should be taken as approximate estimates because their concentrations were calculated from their enzymic activities on the assumption that these two enzymes from WFH venom have the same specific activities as those from honeybee venom. The concentrations for the dialyzed venom proteins and the purified antigen 5 were all calculated from the results of amino acid analysis. This was not possible for these two purified enzymes because of their limited amounts.

Discussion

The above results show that the venoms of white-faced hornet, yellow hornet, and yellow jacket are alike in their compositions. About 25% and 75% of the venom weight represent respectively proteins and peptides, as determined by amino acid analysis before and after exhaustive dialysis of the samples. We did not study the peptides of these three vespid venoms. One of the peptides of yellow jacket venom is known to be a bradykinin analogue (Yoshida et al., 1976); no similar bradykinin analogue was found in white-faced hornet venom (Geller et al., 1976).

The three vespid venoms have very similar protein compositions as evidenced by the gel electrophoretic and chromatographic data. Three proteins were characterized from each of the three vespid venoms. They are a hyaluronidase, a phospholipase and a protein designated as antigen 5 of unknown biochemical function (Table I). These three proteins have molecular weights of about 45 000, 35 000, and 25 000, respectively. These three proteins of white-faced hornet venom have been purified to near homogeneity, while this is the case only for antigen 5 of yellow hornet and yellow jacket venoms. The protein compositions of vespid venoms are distinct from that of honeybee venom. Vespid venoms contain antigen 5, which is absent in honeybee venom. Phospholipase is the major protein component of honeybee venom, but it is a minor component for the vespid venoms.

Immunodiffusion studies with the purified or partially purified venom proteins and specific anti-venom sera showed that there is partial antigenic cross-reaction of the venom proteins from the three vespids studied (Table IV). The most extensive cross-reaction was obtained with hyaluronidases of the three venoms all of which cross-react, while phospholipases show less cross-reaction. Phospholipases of WFH and YH venoms showed cross-reaction, but they did not cross-react with that of YJ venom. Nair et al. (1976) reported a similar result on the cross-reactions of vespid venom phospholipase by inhibition studies of venom phospholipase activity with homologous or heterologous antisera. Antigen 5s of the three vespid venoms showed some cross-reactivity, but this result was obtained with only one of the three sets of antisera tested. The similar amino acid compositions, molecular sizes, and charges of antigen 5s of the three vespid venoms (Table III and Figure 2) suggest their homologies at the amino acid sequence level; therefore, it is not surprising that they might also exhibit immunological cross-reactions. The results taken together indicate that greater antigenic cross-reactions occur between the two hornet venom proteins than between the hornet and the yellow jacket venom proteins. Vespid venom proteins do not cross-react with those of honeybee venom.

The allergenic activity tests show that for most patients

TABLE VI: Histamine Release Assays of Yellow Hornet Venom Proteins with Sensitive Human Leukocytes.

HR act. for donor[a]	dialyzed venom 100[b]	fraction 1, Fig 1b proteins with MW[c] >50 000 7[b]	2, Fig 1b hyal & phosph rich 12[b]	3, Fig 1b phosp & Ag 5 rich 25[b]	4, Fig 1b proteins with MW <25 000 10[b]	2, Fig 7a antigen 5 -
1	(7.5×10^{-2})	0.08	1.4	8.3	1.3	4.2
2	(5.3×10^{-1})	6.6	130	15	5.0	7.6
3a	(8.0×10^{-1})		8.9	130	16	8.0
3b	(6.0×10^{-2})		0.3	3.0	6.0	1.0
4	(5.5×10^{-3})	0.11	0.55	120	28	5500
5a	(5.5×10^{-3})	0.03	0.06	11	1.8	22
5b	(8.0×10^{-3})	0.05	1.1	8.0	2.0	8.0
6	(6.0×10^{-2})	0.06	10	0.4		1.5
	geometric mean:	0.13	2.5	11	4.8	12

[a-c] See corresponding footnotes in Table V.

TABLE VII: Histamine Release Assays of Yellow Jacket Venom Proteins with Sensitive Human Leukocytes.

HR act. for donor[a]	dialyzed venom 100[b]	fraction 1, Fig 1c proteins with MW[c] >50 000 12[b]	2, Fig 1c hyal rich 6.7[b]	3, Fig 1c phosph & Ag 5 rich 31[b]	4, Fig 1c proteins with MW <25 000 10[b]	5, Fig 1c large polypeptides 13[b]	2, Fig 7b Ag 5 -
1	(2.1×10^{-3})	0.002	0.12	3.0	0.26	0.003	26
2	(1.4×10^{-3})	0.016	0.20	0.2	0.2	0.001	14
3	(3.7×10^{-3})	0.11	1.5	11	1.9	0.01	67
4	(2.1×10^{-3})	<0.001	0.038	2.1	0.23	<0.001	21
5	(9×10^{-3})		0.9	20	3.6	0.05	1300
6	(1.4×10^{-3})	<.001	0.004	1.9	0.02	<0.001	18
7a	(3.5×10^{-3})		0.18	2.7			23
7b	(1.3×10^{-2})		0.19	4.6			43
8	(2.5×10^{-2})		0.19	13			25
9	(6.0×10^{-2})		0.30	2.0			24
10	(6.0×10^{-3})		0.09	3.3			46
	geometric mean:	0.005	0.12	3.3	0.34	0.003	56

[a-c] See corresponding footnotes in Table V.

antigen 5 is an important allergen from the three vespid venoms studied (Tables V–VII). The relative allergenic activities of antigen 5, hyaluronidase, and phospholipase of white-faced hornet venom (Table V) showed that there are varying responses of individuals to these three proteins. The situation is similar to those in ragweed pollen and bee venom allergies (King, 1976) when susceptible individuals show varying sensitivities to the different antigens present in pollen or venom. The present findings may help to explain the observation that the majority of vespid sensitive individuals show multiple sensitivity to different vespid venoms (Kern et al., 1976; Zelenick et al., 1977). That is to say that a person who is sensitized to only one vespid on natural exposure can show varying degrees of reactivity to the other vespids. This phenomenon appears to be a consequence of multiple allergens in each vespid venom which are immunologically cross-reactive and to which individuals develop differential sensitivity.

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Temporal Relationship of Translation and Glycosylation of Immunoglobulin Heavy and Light Chains†

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ABSTRACT: The initial glycosylation of MPC 11 γ_{2b} heavy chains occurs quantitatively in vivo when the nascent heavy chains reach a size of approximately 38 000 daltons. Nonglycosylated, completed MPC 11 heavy chains cannot be glycosylated in these cells. Other classes of mouse heavy chains (i.e., μ, α, and γ_1) also appear to be glycosylated as nascent chains; nonglycosylated, completed heavy chains cannot be glycosylated by the cell in any of these cases. In contrast, variant MPC 11 cells synthesizing a heavy chain with a carboxy-terminal deletion appear to glycosylate some heavy chains prior to chain completion and some heavy chains after chain completion and release from the polysomes. Similar to the variant MPC 11 cells, MOPC 46B cells (which synthesize a κ light chain containing an oligosaccharide attached to an asparagine located 28 residues from the amino terminus) glycosylate the majority of light chains prior to chain completion but also some light chains after chain completion and release from the polysomes. In addition, it appears that, although completed MOPC 46B light chains can be glycosylated if they are present in a monomeric form, they cannot be glycosylated if they are present in a covalent dimeric form.

In recent years, a substantial amount of work has been done to characterize the addition of core oligosaccharides to protein. This initial glycosylation event involves the transfer of a large-molecular-weight oligosaccharide containing *N*-acetylglucosamine, mannose, and glucose as a unit from a lipid intermediate to an asparagine acceptor residue on the polypeptide (Waechter et al., 1973; Hsu et al., 1974; Waechter and Lennarz, 1977). Recent evidence has suggested that soon after transfer there is processing of the oligosaccharide (Robbins et al., 1977; Tabas et al., 1978). Previously, we have shown that the transfer of the oligosaccharide can occur while the nascent immunoglobulin heavy chain is still bound to the ribosome, i.e., very soon after the acceptor residue is synthesized and inserted into the lumen of the endoplasmic reticulum (Bergman and Kuehl, 1977). Using various experimental systems and procedures, several laboratories have obtained results which led them to the same conclusion (Kiely et al., 1976; Rothman and Lodish, 1977; Sefton, 1977; Bielinska and Boime, 1978). However, other studies have suggested that glycosylation may occur on completed chains after their release from the ribosome (Shubert, 1970; Buxbaum and Scharff, 1973; Eagon et al.,

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