

Leukocyte histamine release in Hymenoptera-allergic patients

Correlation with skin test reactivity and changes following hyposensitization therapy

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We have studied 46 Hymenoptera-allergic patients and 11 nonallergic controls by grading the severity of their sting reaction, determining their skin test reactivity, and performing human leukocyte histamine release with a commercial mixed stinging insect extract. A significant correlation was found between increasing severity of sting reaction and increasing skin test reactivity ($p < 0.001$). In 41/46 allergic patients from 15 to 100 per cent release of total cellular histamine occurred, whereas in the 11 nonallergic controls no greater than 10 per cent release of total cellular histamine was observed. Skin test reactivity correlated significantly with cell sensitivity in the allergic patients ($p < 0.001$). Following hyposensitization therapy, cell sensitivity generally did not change, but significant increases in antigen-neutralizing capacity (A.N.C.) of allergic serum did occur in 11/15 Hymenoptera-allergic patients.

At the present time, there are conflicting opinions in the literature concerning the correlation of skin test reactivity and the severity of reactions to Hymenoptera stings.¹⁻³ In addition, a significant number of "false positive" skin tests have been reported in nonallergic subjects, as well as occasional "false negative" skin tests in Hymenoptera-allergic patients who have experienced severe sting reactions.^{1, 2, 4} The diagnosis of Hymenoptera allergy is based primarily on the history, and skin testing is used primarily to determine which antigen dilution to select in initiating hyposensitization therapy. Once hyposensitization therapy is begun, there is no consensus in the literature, or in *in vitro* testing that allows the clinician to decide when therapy may be safely discontinued. The present practice is to continue injections indefinitely because severe reactions to stings have occurred within a year of discontinuing injection therapy.⁵

We have studied 46 Hymenoptera-allergic patients by grading the severity of their sting reaction, by serial dilution skin testing, and by leukocyte histamine release with Hymenoptera antigen. In 15 of these 46 patients, cell sensitivity

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TABLE I. Classification of Hymenoptera sting reactions and the percentage of 46 allergic patients with each grade of reaction

Grade 1 (19%)	Large local contiguous reaction greater than 15 cm. in diameter
Grade 2 (43%)	Generalized pruritus and urticaria; may or may not have mild dyspnea
Grade 3 (17%)	Any of the above plus at least 2 of the following: Generalized angioedema; mild wheezing; nausea and vomiting; dizziness
Grade 4 (21%)	Any of the above plus at least one of the following: Severe dyspnea or asthma; dysphagia with tongue swelling; hoarseness with laryngeal edema; hypotension or shock; syncope

before and after hyposensitization therapy, as well as antigen-neutralizing capacity (A.N.C.) of serum, have been determined. The objectives of this study were: (1) to determine if skin test reactivity correlated with the severity of sting reaction in Hymenoptera-allergic patients, (2) to determine if leukocyte histamine release could better separate Hymenoptera-allergic patients from nonallergic controls than is possible by skin testing, (3) to determine if leukocyte histamine release correlated with skin test reactivity in allergic patients, and (4) to determine the effect of hyposensitization therapy on leukocyte histamine release and A.N.C. of serum.

MATERIALS AND METHODS

Selection of patients

The 46 Hymenoptera-allergic patients, each having been referred to one of the authors, had exhibited a spectrum of sting reactions graded 1 through 4, as shown in Table I. Their ages ranged from 4 to 55 years. The 20 healthy nonallergic control subjects had all been stung by Hymenoptera in the distant past and had experienced only minimal local swelling confined to the sting bite. Their ages ranged from 20 to 40 years.

Skin testing

Hollister-Stier glycerosaline mixed stinging insect extract containing equal portions of bee, wasp, yellow jacket, and hornet was used for intracutaneous skin testing as well as for leukocyte histamine release. A sufficient quantity of this extract was obtained in concentrated form with the same lot number to complete the present study and prevent differences in extract potency. This concentrated extract contained, in a 1:10 w/v dilution, 10,000 protein nitrogen units (PNU) per milliliter, and fresh serial dilutions were made from it every 2 weeks for skin testing. Patients who had experienced severe sting reactions were first tested by the scratch method with a 1:1,000,000 w/v dilution and, if negative, serial intracutaneous testing was performed starting with the 1:1,000,000 dilution. The skin test reactivity of a subject was the highest dilution of antigen that elicited at least a 5 × 5 mm. wheal with surrounding erythema when read at 20 minutes. Negative diluent controls and positive histamine controls (0.1 µg per milliliter histamine phosphate) were included in the skin testing of each subject. All but 4 of the 46 patients were skin tested 1 to 3 months after their sting reaction to avoid a refractory period and to avoid the decrease in skin reactivity that may occur with passage of time following a sting.⁶ The remaining 4 patients were skin tested 8 to 12 months after their sting reactions. The 20 nonallergic subjects were skin tested 2 to 20 years from the time of their stings. All sympathomimetic and antihistamine medications were excluded 48 hours prior to skin testing.

In vitro tests

Human leukocyte histamine release as described by Lichtenstein and Osler⁷ and modified by May and associates⁸ for small volumes of blood was performed in the 46 Hymenoptera-

TABLE II. Skin test reactivity to mixed stinging insect antigen in Hymenoptera-allergic patients and nonallergic controls*

Antigen dilutions (w/v)	Patients	Controls
1:1,000,000	• • • • •	
1:100,000	• • • • •	
1:10,000	• • • • •	
	• • • • •	
	• • • • •	
1:1,000	• • • • •	• • •
	• • • • •	
1:100	• •	• • • • •
		• • • • •
		• • • • •
		• •
Totals	46	20

*Each dot represents the highest dilution of antigen causing a positive skin test in a given subject.

allergic patients and in 11 of the control subjects. Histamine was specifically determined by condensation with o-phthalaldehyde and measured fluorometrically. Calculations of per cent histamine release were as follows: Reaction tube histamine (containing leukocytes, 10 per cent normal human serum, antigen, and Tris ACM buffer) minus control tube histamine (containing leukocytes, 10 per cent normal human serum, and Tris ACM buffer) minus antigen blank tube (containing antigen and Tris ACM buffer) equals *net reaction tube histamine*. The total histamine tube (containing lysed leukocytes and Tris ACM buffer) minus Tris ACM buffer equals *net total histamine*: $\frac{\text{Net reaction tube histamine}}{\text{Net total histamine}} \times 100 = \text{per cent histamine release}$

Inclusion of the antigen blank tube was crucial in the calculations because it was found that the antigenic extract contained free histamine and, therefore, exhibited fluorescence. Because of this contributory fluorescence only high dilutions of antigen were used in the determination of histamine release curves. In our hands, leukocyte histamine release was highly reproducible by replicate determinations. The per cent leukocyte histamine release was determined 15 times in Patient M. A. at an antigen concentration of 2 μg protein nitrogen (PN) per milliliter. The mean per cent histamine release was 72 per cent $\pm$ 5.5 per cent S.D. Internal histamine standards were included in each experiment, and their recovery was greater than 90 per cent.

A.N.C. of allergic serum refers to the capacity of allergic serum in the presence of antigen to inhibit leukocyte histamine release.⁹ May's method⁸ was used and involves preincubating several concentrations of antigen in separate tubes with either equal volumes of normal human serum or the patient's serum at 37° C. for 30 to 60 minutes. Equal aliquots of the same patient's leukocyte suspension are then added to all of these tubes and incubated for an additional 45 minutes at 37° C., during which time histamine release occurs. The final concentration of either allergic or normal human serum in the reaction tubes is 10 per cent. If the patient has "blocking" or neutralizing antibody, less histamine release will occur in the tubes preincubated with the patient's allergic serum compared to the tubes containing the same amounts of antigen preincubated with normal human serum. The A.N.C. of an individual allergic patient's serum is obtained by:

$$\text{A.N.C.} = \frac{G_{50} \text{ AHS}^9}{G_{50} \text{ NHS}}$$

G_{50} AHS = the antigen concentration required for 50% histamine release when preincubated with allergic human serum.

TABLE III. Correlation of skin test reactivity with severity of sting reaction in 42 Hymenoptera-allergic patients

Severity of sting reaction	1:100 1:1,000	1:10,000	1:100,000 1:1,000,000
Grade 1	● ● ● ● ● 43%	● ● 14%	0%
Grade 2	● ● ● ● ● 50%	● ● ● ● ● 72%	● 7%
Grade 3	● 7%	● ● 14%	● ● ● ● ● 29%
Grade 4			● ● ● ● ● 64%
Total	0% 14	0% 14	14

G_{50} NHS = the antigen concentration required for 50%
histamine release when preincubated with
normal human serum.

Passive sensitization of nonallergic leukocytes was performed by the method of Levy and Osler.¹⁰ A Hymenoptera-allergic donor was chosen whose serum PK titer was 1:100. This allergic serum was then preincubated at 37° C. with the nonallergic donor leukocytes plus EDTA, 4×10^{-3} M, and heparin, 10 μ g per milliliter for 90 minutes. The allergic serum was then removed by washing twice with buffer, and the sensitized normal donor leukocytes were then incubated with mixed stinging insect antigen for determination of leukocyte histamine release as described previously. This experiment was repeated with a portion of the same Hymenoptera-allergic serum heat-inactivated at 56° C. for 2 hours.

RESULTS

Table II shows the skin test reactivity to serial w/v antigen dilutions of mixed stinging insect extract in the 46 patients and the 20 controls. It is noteworthy that 17 nonallergic subjects reacted to a 1:100 dilution of insect extract and 3 reacted to a 1:1,000 antigen dilution. In addition 2/46 allergic patients reacted only to the 1:100 antigen dilution and 12/46 patients reacted only to the 1:1,000 antigen dilution. Thus if one skin tests with these lower dilutions of antigen one may see overlap of the patients and nonallergic controls. Forty-two of the 46 Hymenoptera-allergic patients who had been skin tested within 3 months of their sting reaction were divided into 3 equal groups of 14 on the basis of their skin test reactivity. Table III relates their skin test reactivity to the severity of sting reaction. The percentages refer to the proportion of patients in each of the three skin test groups experiencing a given grade of sting reaction. The correlation between increasing skin test reactivity and increasing severity of sting reaction is highly significant ($p < 0.001$ by chi square).

Fig. 1 is an example of the striking differences we repeatedly observed in leukocyte histamine release between a typical allergic patient and a nonallergic control. The cell sensitivity (antigen concentration causing 50 per cent histamine release) of Patient D. R. was 0.03 μ g PN per milliliter. Not only were higher percentages of histamine release obtained in the allergic patients but also this release occurred at low antigen concentrations (0.01 – 0.1 μ g PN per milliliter).

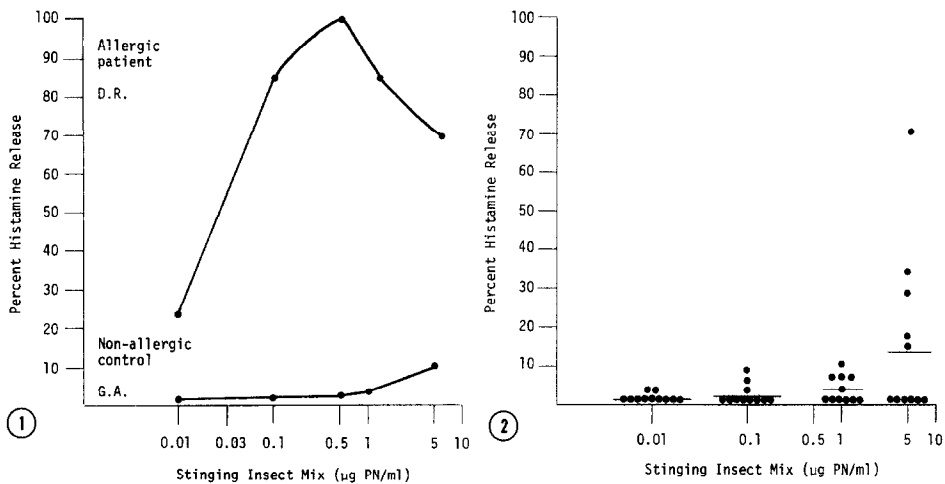


FIG. 1. Comparison of leukocyte histamine release between a Hymenoptera-allergic patient and a nonallergic control.

FIG. 2. Scattergram of per cent leukocyte histamine release in 11 nonallergic subjects at several antigen concentrations.

Fig. 2 is a scattergram of the per cent leukocyte histamine release that occurred in the 11 nonallergic subjects at several antigen concentrations. The points of each individual subject could be connected to form 11 histamine release curves as in Fig. 1. At the high antigen concentration of 5 μ g per milliliter some nonspecific histamine release occurred. Therefore, this antigen concentration was not included in subsequent studies. To further confirm that we were measuring an allergic release of histamine from leukocytes, rather than a nonspecific toxic release by the antigen mixture, we attempted to passively sensitize the leukocytes of 6 nonallergic subjects with serum from a Hymenoptera-allergic patient. The leukocytes of 2 nonallergic subjects were successfully passively sensitized. In Fig. 3 are shown the leukocyte histamine release curves of a nonallergic leukocyte donor before (closed circles) and after (open circles) passive sensitization with Hymenoptera-allergic serum. As shown, heat-inactivated allergic serum failed to passively sensitize the nonallergic donor leukocytes.

Fig. 4 summarizes the maximum percentage of leukocyte histamine release in the 46 patients and 11 controls. Each closed circle represents one patient and depicts both the maximum per cent histamine release and the antigen concentration at which this occurred. Only 5/46 (11 per cent) of allergic patients were indistinguishable from the normal controls (represented by open circles), and none of the controls released a significant percentage of histamine.

The 46 patients were arbitrarily divided into three groups on the basis of 50 per cent leukocyte histamine release. Group 1 consisted of the 16 patients whose "relatively insensitive cells" failed to release 50 per cent of their total cellular histamine at any antigen concentration. The remaining 30 patients, all of whom released at least 50 per cent of their total cellular histamine, were divided into two equal groups of 15 and designated as having "sensitive cells" or

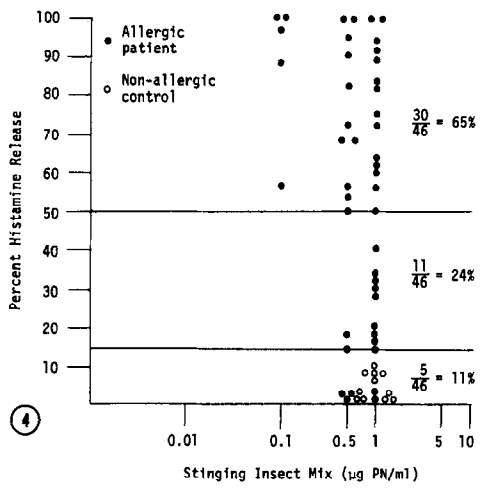
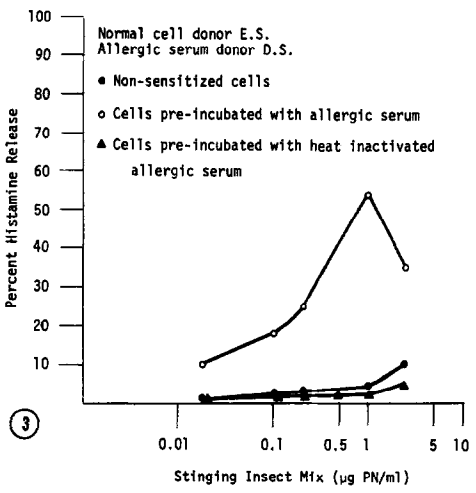


FIG. 3. Leukocyte histamine release of nonallergic donor cells before and after passive sensitization with Hymenoptera-allergic serum. Passive sensitization does not occur with heat-inactivated Hymenoptera-allergic serum (triangles).

FIG. 4. The antigen concentrations eliciting maximum leukocyte histamine release in 46 Hymenoptera-allergic patients and 11 nonallergic controls.

TABLE IV. Correlation of skin test reactivity with 50% leukocyte histamine release in 46 Hymenoptera-allergic patients

Skin test reactivity	Patients with "relatively insensitive" cells ($< 50\%$ release at all antigen concentrations)	Patients with "sensitive" cells (50% release at 1-0.2 μg PN)	Patients with "highly sensitive" cells (50% release at $< 0.2 \mu\text{g}$ PN)
1:100	● ● ● ● ●	● ● ● ● ●	●
1:1,000	● ● ● ● ● ● ● ● ● ● 87%	33%	6%
1:10,000	● ● 13%	● ● ● ● ● ● ● ● ● ● 67%	● ● ● ● ● 27%
1:100,000			● ● ● ● ● ● ● ● ● ●
1:1,000,000	0%	0%	67%
Total	16	15	15

"highly sensitive cells" depending on the antigen concentration that elicited 50 per cent leukocyte histamine release (i.e., cell sensitivity). The 15 patients with "highly sensitive cells" released 50 per cent of their total histamine at antigen concentrations of less than 0.2 μg PN per milliliter. These three patient groups of varying cell sensitivity were correlated with their corresponding skin test reactivity (Table IV). The percentages represent the proportion of patients in each of the three groups demonstrating the skin test reactivity to each of the antigen dilutions as shown. There is a significant correlation of increasing skin test reactivity with increasing cell sensitivity as measured by 50 per cent leukocyte histamine release ($p < 0.001$ by chi square).

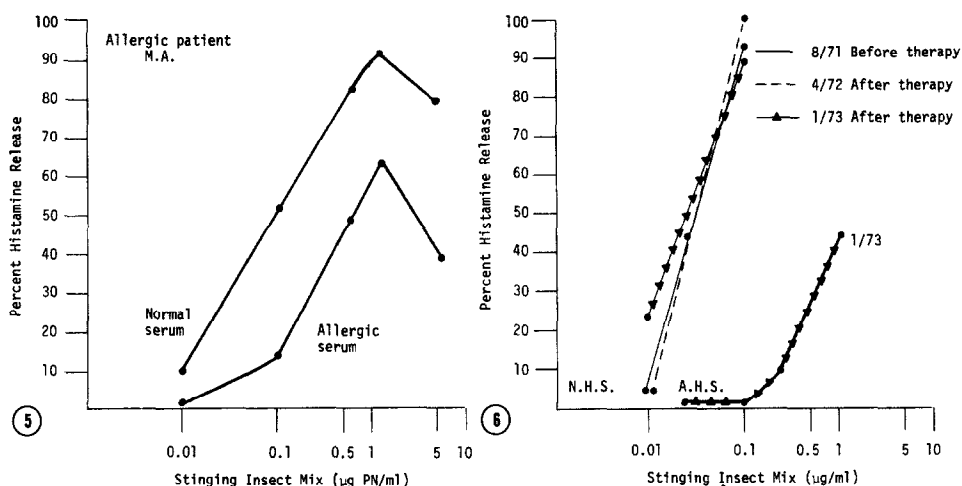


FIG. 5. A.N.C. of the allergic serum of Patient M. A. $\text{A.N.C.} = \frac{\text{G50 AHS}}{\text{G50 NHS}} = 6.8$.

FIG. 6. Serial determinations of cell sensitivity in Patient M. G., before and after hyposensitization therapy. N.H.S. = normal human serum used to determine cell sensitivity. A.H.S. = allergic human serum used to determine A.N.C. On 1/73 A.N.C. = 50.

By this method of leukocyte histamine release with stinging insect antigen we considered a 30 per cent release of total cellular histamine as significant since it had not been observed in the 11 nonallergic controls at the antigen dilutions used. If 30 per cent is a significant amount of histamine release, it might be expected to represent the *in vitro* analogue of our threshold positive skin test, which was a 5×5 mm. wheal with erythema. Therefore, we compared the sensitivity of skin testing with the sensitivity of 30 per cent leukocyte histamine release. We determined in each of 44 patients the antigen dilution w/v that caused the first positive skin test and the antigen dilution w/v that caused 30 per cent leukocyte histamine release. The two patients reacting on skin test to a 1:100 antigen dilution were excluded since they did not differ from the majority of the normals in this respect. In the individual patient, if a positive skin test occurred at a higher antigen dilution than that antigen dilution that elicited 30 per cent leukocyte histamine release, then skin testing was considered the more sensitive test. Conversely, if 30 per cent leukocyte histamine release occurred at a higher antigen dilution than that antigen dilution eliciting a positive skin test, then 30 per cent leukocyte histamine release was considered the more sensitive test. It was found that 30 per cent leukocyte histamine was less sensitive than skin testing in 11/44 patients, equally sensitive in 12/44 patients, and more sensitive in 21/44 patients.

A typical determination of A.N.C. of allergic serum is shown in Fig. 5 for Patient M. A. whose A.N.C. is 6.8. This means that 6.8 times as much antigen is required for 50 per cent leukocyte histamine release if antigen is preincubated in allergic rather than in normal serum. The A.N.C. of 5 consecutive patients determined prior to hyposensitization therapy ranged from 0 to 2.0, and was within the error of our technique. Therefore, we did not observe significant

TABLE V. A.N.C. of serum from 15 Hymenoptera-allergic patients after hyposensitization therapy

Patient	A.N.C.*	Months on injection therapy	Average maintenance dose in milliliters†	Restung
E. U.	0	30	0.5	+
D. Sh.	2.5	6	0.5	
D. R.	2.5	6	3.0	
R. Z.	2.5	18	0.5	
E. P.	4.0	12	0.5	
G. D.	4.0	6	0.2	
V. V.	5.0	30	0.5	+
C. L.	5.0	12	0.5	
D. S.	6.0	30	0.3	
M. A.	6.8	24	0.5	
M. R.	7.0	6	0.5	
E. M.	10.0	36	0.3	+
C. P.	14.0	24	0.5	+
M. G.	50.0	30	1.0	+
M. E.‡	No release	18	0.25	

*A.N.C. of less than 2.0 is insignificant and within the error of our technique.

†All doses expressed in equivalent terms of a 1:100 w/v extract dilution.

‡No release of histamine occurred when antigen was preincubated with serum from Patient M. E.

pretreatment A.N.C. of serum from Hymenoptera-allergic patients. In Table V are listed A.N.C. values for the sera of 15 patients together with the average dose and the duration of hyposensitization therapy in each patient. There does not appear to be a correlation between A.N.C. and either the dose or duration of injection therapy. However, 5 of these patients have been restung and experienced only a large local or no reaction, indicating that the injection therapy has been effective. Patient M. E. (Table V) is remarkable in that no release of histamine occurred at any antigen concentration when preincubated in his serum.

Cell sensitivity (antigen concentration causing 50 per cent histamine release) before and after hyposensitization therapy is shown in a representative patient (M. G.) in Fig. 6. There was no change in leukocyte sensitivity in this patient after 1.5 years of injection therapy with an average dose of 1.0 c.c. of a 1:100 w/v dilution of extract, yet an A.N.C. of 50 was demonstrable. Table VI lists the cell sensitivities before and after hyposensitization therapy in 15 patients along with the average dose and duration of therapy in each patient. Changes in cell sensitivity are represented by the ratio of:

$$\frac{\text{Cell sensitivity after hyposensitization}}{\text{Cell sensitivity before hyposensitization}}$$

Ratios from 0.5 to 2.0 were considered insignificant since these were within the error of our technique and, therefore, represented no net change in cell sensitivity. Ratios less than 0.5 represent an increase in cell sensitivity, and ratios greater than 2.0 represent a decrease in cell sensitivity. As can be seen in Table VI, 4 patients developed an increase in cell sensitivity after hyposensitization therapy and in only one patient, E. U., was a decrease in cell sensitivity noted. The majority of patients, 10/15, showed no net change in cell

TABLE VI. Changes in cell sensitivity of 15 Hymenoptera-allergic patients after hyposensitization therapy

Patient	Cell sensitivity change*	Months on injection therapy	Average maintenance dose in milliliter†	Restung
C. P.	0.04	24	0.5	+
D. Sh.	0.10	6	0.5	
C. L.	0.14	12	0.5	
M. R.	0.14	6	0.5	
E. P.	1.0	12	0.5	
G. D.	1.0	6	0.2	
M. G.	1.0	30	1.0	+
M. A.	1.0	24	0.5	
V. V.	1.0	30	0.5	
R. Z.	1.0	18	0.5	+
D. R.	1.0	6	3.0	
D. S.	1.0	30	0.3	
E. M.	1.0	36	0.3	+
M. E.	1.0	18	0.25	
E. U.	11.5	30	0.5	

*Cell sensitivity change represents ratio of: $\frac{\text{cell sensitivity after hyposensitization}}{\text{cell sensitivity before hyposensitization}}$

†All doses expressed in equivalent terms of a 1:100 w/v extract dilution.

sensitivity. No correlation seems to exist between the dose or duration of injection therapy and changes in cell sensitivity.

DISCUSSION

Although Mueller¹ has noted that skin test reactivity to Hymenoptera antigen usually parallels clinical sensitivity, exceptions do occur, with some patients showing negative skin tests despite histories of severe reaction to stings. Schwartz² and Barnard³ found no correlation between the levels of clinical and dermal sensitivity in their studies of Hymenoptera-allergic patients. Schwartz also noted no significant difference between the skin test reactivity of allergic patients and nonallergic controls.² Bernton and Brown⁴ found that 37.5 per cent of 200 normal subjects had a positive skin test to a 60 PNU per milliliter concentration of honey bee extract. In the present study, we found an excellent correlation between severity of sting reaction and skin test reactivity with serial dilutions of antigen in 42 patients examined. Our results are perhaps explained by our practice of not skin testing patients during the refractory period following a sting, which may last up to 4 weeks, but testing patients before several months have elapsed following the sting, after which time skin reactivity may decrease.⁶ In addition, fresh dilutions of extracts were made up every 2 weeks because of loss of allergenic extract potency with time, which can occur at high dilutions.¹¹⁻¹³ Furthermore, it should be emphasized that our results were obtained with a specific commercial mixed stinging insect antigen of the same Lot number and thus, depending on the potency and composition of this antigen, these results may not apply to other Lots of the same extract or to other commercial extracts.

It was found that all 20 nonallergic subjects reacted to a 1:100 dilution of the extract. We have attributed this to free histamine, which has been described in

Hymenoptera venom¹⁴⁻¹⁶ and which we have measured in the extract by the fluorometric assay of Shore, Burkhalter, and Cohn,¹⁷ which is specific for histamine. This nonallergic, histamine-induced wheal-and-flare response to low dilutions of Hymenoptera extract may explain some of the "false positive" skin tests in normals. Our observations, however, would tend to confirm Mueller's that an occasional patient demonstrates a disproportion between skin test reactivity and severity of sting reaction since we noted patients reacting on skin testing to 1:100 or 1:1,000 antigen dilutions who had experienced Grade 2 or 3 sting reactions. In these patients either an exquisite nonimmunologic sensitivity to the toxins contained in Hymenoptera venom or intravascular entry of venom may have explained their more severe clinical reactions.

Histamine release from washed human leukocytes with Hymenoptera antigen adequately separated allergic patients from nonallergic patients with the antigen dilutions used. Mixed stinging insect venom contains mellitin, phospholipase A, phospholipase B, histamine, lecithin, and mast cell degranulating peptide,¹⁴⁻¹⁶ which are active against cell membranes and may release histamine from basophils by a toxic or lytic mechanism. The use of pure venoms rather than whole body extracts for leukocyte histamine release does not circumvent this problem. However, in the dilutions of antigen used, no significant release of histamine occurred with nonallergic leukocytes. Furthermore, the passive sensitization of nonallergic leukocytes demonstrates that a heat-labile homocytotropic factor (presumably IgE antibody) in Hymenoptera-allergic serum sensitizes normal leukocytes for the allergic release of histamine, ruling out a nonspecific histamine release effect of insect antigen. Only 5 of 46 Hymenoptera-allergic patients overlapped the nonallergic controls by comparison of maximum leukocyte histamine release, giving an incidence of 11 per cent "false negative" with this *in vitro* test. However, if one looks at the overlap on skin testing between the allergic and nonallergic groups, it can be seen that 2 patients and 17 normals reacted to a 1:100 w/v antigen dilution and 12 patients and 3 normals reacted to a 1:1,000 w/v antigen dilution. Overall there seems to be more overlap of allergic and nonallergic groups by skin testing than by leukocyte histamine release. Thus, in our patients, leukocyte histamine release separated 90 per cent of the Hymenoptera-allergic patients from the nonallergic controls and was somewhat better in this respect than skin testing.

When the sensitivity of 30 per cent release of total cellular histamine was compared with the sensitivity of skin testing in 44 patients, leukocyte histamine release was found to be more sensitive in 48 per cent of the patients. Therefore, it appears that leukocyte histamine release is somewhat more specific and more sensitive than skin testing with Hymenoptera extract. This slight advantage in specificity and sensitivity that leukocyte histamine release provides, compared with skin testing, is probably negated by the fact that the former requires 5 hours to perform in a laboratory by trained technicians, whereas the latter is a rapid, easily performed office procedure. It is noteworthy that skin test reactivity in the Hymenoptera-allergic patients was highly correlated with leukocyte histamine release. In the 20 nonallergic subjects who were skin tested there was overlap with the allergic patients only at low dilutions (1:100, 1:1,000) of the

Hymenoptera extract. Therefore, we feel that skin testing with higher dilutions of fresh insect extract (1:10,000, 1:100,000) does separate allergic from non-allergic subjects. The excellent correlation of skin test reactivity with 50 per cent leukocyte histamine release allows one to predict that a subject reacting on skin testing to a 1:10,000 extract dilution usually will release 50 per cent of his total cellular histamine. Furthermore, the close correlation between skin test reactivity and severity of sting reaction suggests that those subjects reacting to a 1:100,000 or 1:1,000,000 antigen dilution might well experience a Grade 3 or 4 reaction if stung. Thus, we do not feel that leukocyte histamine release replaces the usefulness of skin testing, but rather that it complements skin testing and reaffirms that skin testing has diagnostic specificity and correlates with clinical sensitivity if properly performed and interpreted. The complementary roles of skin testing and leukocyte histamine release were seen in 2 allergic patients who reacted on skin testing to a 1:100 antigen dilution, yet released at least 30 per cent of their total cellular histamine; and in 2 patients who failed to release more than 10 per cent of their total histamine, yet reacted on skin testing to a 1:10,000 antigen dilution.

Leukocyte histamine release, therefore, is most useful in those patients with a vague history of a Hymenoptera sting reaction, those patients experiencing systemic reactions upon being stung several times on one occasion, and those patients who experience a large local sting reaction and in addition react on skin testing to only low dilutions of extract. In these cases we have elected to hyposensitize the patient who releases a significant percentage of total cellular histamine (30 per cent or more) but not to hyposensitize the patient who fails to release significant histamine. In the Hymenoptera-allergic patient who reacts on skin testing to a 1:10,000 or higher antigen dilution, we feel that leukocyte histamine release adds little to patient management as hyposensitization therapy is indicated. In addition, in any patient who gives a clear history of a significant sting reaction (Grade 2, 3, or 4), we recommend hyposensitization therapy. We feel that the clinical history and serial dilution skin testing properly performed should guide the physician in the management of Hymenoptera-allergic patients and that leukocyte histamine release is useful for patient management mainly in the specific instances noted previously.

The question of when to discontinue hyposensitization is of paramount importance to physicians treating Hymenoptera-allergic patients. Since significant decreases and even loss of cell sensitivity have been described in hay fever patients treated with ragweed extract,¹⁸⁻²² we explored this phenomenon in Hymenoptera-allergic patients. At this time, with the amounts of antigenic extract administered, we have observed a significant decrease in cell sensitivity in only one of the 15 patients studied before and after hyposensitization therapy. This patient, E. U., is one of 5 followed for over 30 months and has been on 0.5 ml. of a 1:100 w/v dilution of stinging insect mixture. Furthermore, 4 of the 15 patients actually increased their cell sensitivity after hyposensitization therapy. It may be that what is usually considered adequate treatment is not sufficient to significantly decrease cell sensitivity since the average injection dose of these 15 patients was 0.5 ml. of a 1:100 w/v dilution given every 4 weeks.

Since 5 patients have been restung without any unusually severe reaction, one has to consider other factors besides decreased cell sensitivity as being important in protection. The previous studies of changes in leukocyte histamine release following injection therapy have dealt with hay fever patients allergic to inhalant allergens that cross mucosal barriers and elicit local nasal allergic manifestations. The present study deals with anaphylaxis whereby a foreign substance when injected parenterally produces from mild to severe generalized allergic symptoms. Therefore, previously described immunologic and cellular changes of leukocyte histamine release following hyposensitization therapy of pollen-allergic patients may not apply to patients allergic to injections of foreign material.

The present study has shown significant increases in A.N.C. of serum in 11/15 patients following hyposensitization therapy. Patient E. U., who demonstrated no significant A.N.C., was the only one of the 15 patients who demonstrated a significant decrease in cell sensitivity, which suggests that both A.N.C. and cell sensitivity changes may be important factors contributing to protection from stings. A larger study of cell sensitivity and A.N.C. changes following hyposensitization is presently in progress to determine which is the more important in most patients. More determinations of A.N.C. of serum from allergic patients before hyposensitization need to be performed to determine the significance of A.N.C. levels of patients following hyposensitization. Furthermore, perhaps A.N.C. of serum should be determined without the preincubation of serum with antigen before adding the leukocytes, as has been suggested by May and associates.²³ By future prospective studies a specific level of A.N.C. of serum, a decrease in cell sensitivity, or a combination of these may be found that would ensure protection if patients are restung. This would involve serial determinations of cell sensitivity and of A.N.C. of allergic patients while on hyposensitization therapy and particularly following any natural stings experienced by these patients. If A.N.C. of serum is followed after hyposensitization therapy is discontinued and a fall in level with time is observed, an optimal time interval between "booster" injections might be found that may differ from the arbitrary 3 to 4 months now recommended.⁶ Determination of A.N.C. of serum as measured by inhibition of leukocyte histamine release provides an isolated in vitro system to study the interaction of sensitized leukocytes, antigen, and antigen-specific "blocking" or "neutralizing" antibody in the individual Hymenoptera-allergic patient. Hopefully by further study of this in vitro system, the guesswork of when to stop hyposensitization therapy and how frequently to give "booster" injections will be removed from the management of these patients.

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