

Hymenoptera sensitivity

I. Conjunctival testing in insect allergy

*Bertram Kahn, Captain, MC, USA, and Howard J. Schwartz,
Captain, MC, USA,* Fort Sam Houston, Texas*

Conjunctival testing to a whole body mixture of bee, wasp, hornet, and yellow jacket in Coca's solution was carried out in an attempt to develop a simple clinical test which would be useful in verifying a history suggestive of stinging insect allergy. Approximately 60 per cent of 52 insect-sensitive patients undergoing hypo-sensitization therapy demonstrated positive reactions; whereas only 8 per cent of control subjects reacted similarly. This suggests that false positive conjunctival reactivity to Hymenoptera is unlikely, but that clinically significant numbers of patients will be falsely negative. Further implications are discussed.

Medical reports of deaths from insect stings date back to the early part of the nineteenth century, but not until 1914 was the anaphylactic nature of these reactions first recognized.¹ Much work has been done since that time with regard to therapeutic and immunologic considerations. Diagnostic modalities, however, are still in their infancy. Although hyposensitization has been reported to be an excellent therapeutic tool in Hymenoptera-sensitive individuals, skin testing of these patients with specific insect extracts has shown a wide variety of reactions and has even been negative in several patients who have sustained severe reactions from stings.²⁻⁶ In fact, as pointed out by one of the authors in a previous paper, the unreliability of skin testing has been so marked that the medical history alone appears to be the main criterion for a diagnosis of hypersensitivity to stinging insects as a prelude to hyposensitization treatment.⁷

This is most unfortunate for two reasons. First, many patients will present

From the Allergy Clinic, Brooke General Hospital, Fort Sam Houston, Texas.

Received for publication July 25, 1966.

*Present address: Department of Medicine, Massachusetts General Hospital, Boston, Mass.

themselves as possibly having been stung by one of the Hymenoptera group and yet are uncertain of the identity of the insect which attacked them. Second, Hymenoptera hyposensitization of a nonallergic individual may be hazardous, as will be discussed later. In view of these problems, it was decided to examine the possibility of using conjunctival testing for the verification of Hymenoptera sensitivity and to compare this method with direct skin testing in both insect-sensitive and nonsensitive individuals.

TECHNIQUE

Conjunctival and intracutaneous tests were performed in three groups of individuals: (1) Patients with a history of hypersensitivity to stinging insects who were currently receiving perennial aqueous mixed insect hyposensitization, no less frequently than one injection every 3 weeks, at this clinic (Brooke General Hospital). All treated patients used in this study had been undergoing hyposensitization for intervals of time ranging from at least 3 months to 3 years. (2) Atopic patients under treatment for allergic rhinitis or bronchial asthma but without a history of Hymenoptera sensitivity. (3) Nonatopic healthy adults without a personal or family history of asthma, hay fever, or insect sensitivity.

The first group was further divided into those with systemic and those with local reactions, based on clinical history. Those with systemic reactions included patients who, following a Hymenoptera sting, developed one of the following: urticaria, angioneurotic edema, asthma, hypotension, or collapse with unconsciousness. In addition, many experienced dizziness, thickened speech, nausea, a sense of constriction in the chest, and other vague symptoms of a generalized reaction. The local reactors included patients who developed immediate and progressively severe edema involving at least one half of the entire extremity that was stung, but who did not develop any of the systemic problems described above. All testing was carried out with a whole body mixture of bee, wasp, yellow jacket, and hornet in Coca's solution.*

All three groups of patients were given a conjunctival test with a 1:10 concentration of the Hymenoptera whole body mixture. A 1 c.c. tuberculin syringe, without a needle, was used to place one drop of antigen into the lower conjunctival sac of one eye and one drop of control Coca's solution, similarly, in the other eye. Reactions^{8, 9} were read after 5 and 10 minute intervals by each of the investigators, independently, and recorded as follows: 1+, congestion of the sclera and lower conjunctiva with slight redness and swelling of the caruncle; 2+, more diffuse and intense scleral redness; 3+, extremely diffuse and intense scleral redness with marked involvement of the upper palpebral conjunctiva; and 4+, chemosis of conjunctiva and caruncle.

At the same time all three groups of patients were skin tested with 0.01 to 0.02 c.c. of a 1:100 dilution of the whole body Hymenoptera mixture and with 0.01 to 0.02 c.c. of Coca's solution as a control. The tests were performed intracutaneously on the lateral surface of the arm at a site which had not previously

*Materials kindly furnished by Hollister-Stier Laboratories.

been used for any recent allergy or other type injection.^{10, 11} Reactions were read at 15 minutes only, delayed reactions not being investigated or recorded. The immediate reactions were graded according to wheal size as follows: -, 0 to 4 mm. (negative); 1+, 5 to 8 mm.; 2+, 9 to 12 mm.; 3+, 13 to 16 mm.; and 4+, > 16 mm. Flare formation and pseudopods, if any, were not considered in the measurement of the wheal but were recorded separately.

It was decided beforehand that if any disagreement in interpretation occurred between the investigators, the result would be discarded. However, no such disagreement occurred. Patients with active conjunctival congestion or unequal conjunctival vascularity were excluded from the study. Of the entire group, only one control subject demonstrated a positive conjunctival test to Coca's solution. In this subject, a similar reaction to antigen instillation occurred; the latter was, therefore, regarded as a negative test in the final analysis.

RESULTS

The hyposensitized patients were divided into two groups consisting of 43 with systemic and 9 with local reactions. The results of their skin and eye tests are shown in Table I along with the results of the atopic and nonatopic control patients. The skin test reactions were further analyzed as shown in Table II. Ninety-five per cent of the patients with systemic reactions and 89 per cent of those with local reactions were positive to Hymenoptera intradermal testing. Six with systemic reactions were already positive at a 1:1,000 dilution of the mixed stinging insect antigen and, therefore, were not exposed to the more potent 1:100 concentration. Interestingly, 71 per cent of both atopic and nonatopic controls also showed positive intradermal reactivity. However, in none of these latter two groups were the reactions greater than 2+, whereas both former groups had a total of 3 patients with 3+ or greater reactivity. Notwithstanding, a remarkably high percentage of nonsensitive patients did appear to be skin test sensitive.

This was far different from the reactions produced by eye testing as shown in Table II. Here, neither control group showed any greater positive reactivity than 8 per cent; whereas the local and systemic reactors were positive in 56 and 60 per cent, respectively. That these results were statistically valid was shown by the corrected chi square method, p (eye testing) = 0.001, whereas p (skin testing) > 0.10 < 0.05.

DISCUSSION

The results of the skin test studies confirm previously reported failure to distinguish significantly between the clinically Hymenoptera-sensitive patients and the atopic and nonatopic controls; for although the former did show an 89 to 95 per cent positive response, the latter's response was also quite high, 71 per cent. On the other hand, as seen from above, the results of the eye test studies showed a significant difference between the clinically sensitive patients and the atopic and nonatopic controls. It was thus felt that the eye test might be more helpful than the skin test in distinguishing those individuals

Table I

Patient group	No. tested	Skin test results										Eye test results					
		1:1000			1:100					Control		1:10			Control		
		1+	2+	3+	-	1+	2+	3+	4+	+	-	-	1+	2+	+	-	-
Hyposensitized systemic reaction	43	4	1	1	2	24	10	0	1	0	43	17	19	7	0	43	
Hyposensitized local reaction	9			1	1	6	1			0	9	4	5		0	9	
Atopic control	14				4	8	2			0	14	12	2*		1*	13	
Nonatopic control	14				4	9	1			0	14	13	1		0	14	

*One of the two atopic control patients with a 1+ reaction to the 1:10 eye test also had a 1+ reaction to the control eye test. Thus in the final analysis in Table II his conjunctival test is considered negative. This was the only instance in which any of the reported patients showed a positive conjunctival or intradermal test to the control solution.

Table II. Comparison of eye and skin test results

Group	Skin*			Eye†		
	Total tested	Total +	% +	Total tested	Total +	% +
Systemic reaction	43	41	95	43	26	60
Local reaction	9	8	89	9	5	56
Atopic control	14	10	71	14	1	7
Nonatopic control	14	10	71	13	1	8

*With the use of the corrected chi square method $p > 0.10 < 0.05$.

†With the use of the corrected chi square method $p = 0.001$.

who are truly Hymenoptera-sensitive from those who are not. However, although the eye test results in far fewer false positive reactions than does skin testing, in the present study there were approximately 40 per cent false negative reactions among the allergic patients. Notwithstanding, 7 patients with systemic reactions were the only ones who showed a greater conjunctival response than 1+. This is compatible with the work of other investigators who have shown the clinical importance of conjunctival testing as opposed to direct skin testing in patients with serum sickness,¹²⁻¹⁴ and even as a prognosticator of constitutional reactivity in hay fever patients undergoing hyposensitization therapy.¹⁵

The results of the skin test studies were not surprising in view of a previous investigation of one of the authors⁷ as well as an earlier study done by Bernton and Brown.¹⁶ The latter showed that almost 40 per cent of "normal" persons possess positive skin reactivity on testing with honeybee extract. Mueller found that patients with similar levels of skin sensitivity exhibited

varying degrees of clinical reactivity and vice versa.⁵ Unfortunately, in all the studies referred to above, as well as in our own, passive transfer was not done to confirm the reaginic nature of the positive skin tests. This will have to be done to rule out the possibility of Hymenoptera extract nonspecific whealing ability and to help determine whether inhalation of insect material is playing a role in the process of sensitization, as suggested by other investigators.¹⁷⁻²⁰

Interestingly, if it can be shown that injections of Hymenoptera stimulate the formation of skin-sensitizing antibodies in nonatopic as well as atopic non-insect-sensitive individuals, then an immunological relationship between it and such a parasite as *Ascaris* will have been established. Several workers have previously demonstrated that injections of *Ascaris* and *Trichina* can induce the formation of reagins in nonatopic individuals.²¹⁻²⁴ Studies of E. H. Walzer and M. Walzer²⁵ have already demonstrated the induction of skin-sensitizing antibodies against Hymenoptera in nonatopic dogs by means of repeated subcutaneous or intracutaneous injections. The possible association of these induced reagins with future clinical reactivity remains to be demonstrated. If this is shown to occur, then hyposensitization would be theoretically hazardous if given to "normal" patients because of diagnostic error.

The authors would like to express gratitude to Colonel Glen K. Arney, MC, USA, for review of the manuscript and Major Elinor Pearson, ANC, USA, for her assistance in the statistical analysis.

REFERENCES

1. Samter, M., Alexander, H. L., Talmadge, D., Rose, B., Sherman, W., Vaughan, J.: Immunologic Diseases, Boston, 1965, Little, Brown & Company, p. 684.
2. American Academy of Allergy: Report of Insect Committee for 1961, *J. ALLERGY* 33: 468, 1962.
3. American Academy of Allergy: Insect Allergy Committee, March 1963 Report, *J. ALLERGY* 35: 181, 1964.
4. Frazier, C. A.: Allergic Reactions to Insect Stings: A Review of 180 Cases, *South. M. J.* 57: 1023, 1964.
5. Mueller, H. L.: Further Experiences With Severe Allergic Reactions to Insect Stings, *New England J. Med.* 261: 374, 1959.
6. Stier, R. A., and Stier, R. F. E.: Results of Desensitization in Allergy to Insect Stings, *Gen. Practice* 19: 103, 1959.
7. Schwartz, H. J.: Skin Sensitivity in Insect Allergy, *J. A. M. A.* 194: 703, 1965.
8. Vaughan, W. T., and Black, J. H.: Practice of Allergy, ed. 2, Saint Louis, 1948, The C. V. Mosby Company, p. 205.
9. Sherman, H.: Personal Communication, 1966.
10. Bowman, K. L., and Walzer, M.: A Study of the Retractoriness to Whealing in Normal (Nonatopic) Skin, *J. ALLERGY* 3: 503, 1932. Abst.
11. Lowell, F. C., and Williams, M.: The Effect of Subcutaneous Injections of Allergenic Extract on the Local Reactivity to Intramuscularly Injected Extract, *J. ALLERGY* 34: 35, 1963.
12. Spicer, S.: Ophthalmic Test for Horse Serum Sensitivity Contrasted With the Intradermal, *J. Immunol.* 15: 335, 1928.
13. Abram, L. E.: An Evaluation of Conjunctival Testing in Extrinsic Respiratory Allergy, *J. ALLERGY* 20: 66, 1949.
14. Kojis, F. G.: Serum Sickness and Anaphylaxis, *Am. J. Dis. Child.* 64: 93, 1942.
15. Sherman, H., and Baron, B.: Studies in Hypersensitiveness of the Mucous Membrane. V. Comparative Studies of Skin and Ophthalmic Reactions in Hay Fever Patients Presenting Constitutional Reactions, *J. ALLERGY* 15: 163, 1944.
16. Bernton, H. S., and Brown, H.: Studies on the Hymenoptera. I. Skin Reactions of Normal Persons to Honeybee (*Apis Mellifera*) Extract, *J. ALLERGY* 36: 315, 1965.
17. MacLaren, W. R., Eisenberg, B. C., Frank, D. E., and Kessler, J.: Reactions to Insect Allergens. The Incidence of Response to Testing Among Allergic and Nonallergic Persons, *California Med.* 93: 224, 1960.

18. Perlman, F.: Insects as Inhalant Allergens, *J. ALLERGY* **29**: 302, 1958.
19. Wiseman, R. D., Woodin, W. G., Mueller, H. C., and Myers, M. A.: Insect Allergy as a Possible Cause of Inhalant Sensitivity, *J. ALLERGY* **30**: 191, 1959.
20. Hellreich, E.: Evaluation of Skin Tests With Insect Extracts in Various Allergic Diseases, *Ann. Allergy* **20**: 805, 1962.
21. Brunner, M.: Active Sensitization in Human Beings, *J. ALLERGY* **5**: 257, 1934.
22. Baron, B., and Brunner, M.: Active Sensitization in Human Beings With Trichina Antigen, *J. ALLERGY* **13**: 459, 1942.
23. Coca, A. F., Walzer, M., and Thommen, A. A.: Asthma and Hay Fever in Theory and Practice, Springfield, Ill., 1931, Charles C Thomas, Publisher, p. 205.
24. Davidson, A. G., Baron, B., and Walzer, M.: Factors Influencing Reagin Formation in Experimental Human Sensitization to *Ascaris Lumbricoides* Antigen, *J. ALLERGY* **18**: 359, 1947.
25. Walzer, E. H., and Walzer, M.: Studies in Experimental Hymenoptera Sensitivity in the Dog, Presentation at the American Academy of Allergy Convention, Feb. 12, 1964.