

Fluorescent antibody studies in three patients dying from honey bee sting

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A study was made of the organs and sera of 3 patients, each dying of a single honey bee sting. By means of an immunofluorescent test, antibodies to honey bee antigens were found in the sera, the hearts, and in the adrenal glands. Hemagglutinating antibodies were found in the sera. Some implications of these findings are discussed. The test can be performed with either freshly frozen or formalin-fixed tissues. This method may be of practical value to pathologists and coroners in ascertaining the cause of some cases of sudden death.

A number of persons, perhaps several dozen, die each year in the United States as a result of Hymenoptera stings.^{1, 2} The true incidence of this type of death is difficult to ascertain. However, information obtained from studying the known fatalities showed that most deaths were sudden. They occurred in healthy people. A single sting was sufficient to cause death, and allergic individuals tended to be more vulnerable. These facts suggest that the fatal response may result from anaphylactic shock caused by insect allergy. Therefore, it becomes important to perform postmortem immunologic studies to determine the nature of the reaction responsible for death. In this study, antibodies to honey bee antigens were detected in the sera and organs of three individuals who had died from a single honey bee sting.

MATERIALS AND METHODS

Antigenic extracts

Extracts were prepared according to the procedure of Shulman and associates³ of the following: honey bee (*Apis mellifera*) whole, yellow jacket (*Vespula pennsylvanica*) whole, and wasp (*Polistes* spp.) whole.

Human sera and organs

Blood was removed from the heart of 2 patients (F. H. and J. R.) at the time of autopsy. The sera obtained by centrifuging at 1,500 x g for 15 minutes were frozen quickly and kept frozen until studied. Material for special studies was obtained from 10 organs of each of 2 patients (F. H. and J. S.): adrenal gland, brain, heart, kidney, liver, pancreas, skin, spleen, and thyroid. Only the lung of a third patient (J. R.) was obtained at autopsy. The organs of Patient F. H. were quickly frozen and kept frozen until used. Those of Patients J. R. and J. S. were preserved in formalin in the usual manner.

Rabbit antisera

Rabbits were immunized with whole body extracts of honey bee (*Apis mellifera*), yellow jacket (*Vespula pennsylvanica*), and wasp (*Polistes* spp.) according to the procedure of

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Supported by a grant from the John A. Hartford Foundation, Inc.

Received for publication Sept. 21, 1970.

Langlois and associates.⁴ The rabbit anti-honey bee sera were used for immunofluorescence and all 3 antisera for hemagglutination.

Fluorescent antibody conjugate

Fluorescein-conjugated rabbit anti-honey bee globulin was prepared as follows: The globulin fraction of rabbit antisera was obtained and labeled with fluorescein isothiocyanate according to the procedures of Kendall⁵ and Clark and Shepard,⁶ respectively. The unreacted dye was removed from the fluorescein conjugates by gel filtration,⁷ and the conjugates were further fractionated on a DEAE-cellulose column to obtain the highest staining specificity.⁸ Dilute solutions of conjugates were concentrated, and immunological properties of conjugates were tested.

Hemagglutination

The tanned cell hemagglutination technique was used as described by Feinberg and associates⁹ and modified by Arbesman and associates.¹⁰

Fluorescent antibody test

The indirect fluorescent antibody technique was performed according to the principle of Coons and associates.¹¹ Tissues 6 to 10 microns thick were placed on microscopic slides which previously had been washed with acid alcohol. These sections then were fixed with 95 per cent alcohol for 10 minutes.

Step 1. After adding whole body honey bee extract to the tissues, the slides were placed individually in a moisture chamber and incubated for 30 minutes at 37° C. with constant shaking. The excess whole body honey bee extract was washed away with buffered saline, pH 7.2.

Step 2. Fluorescein-conjugated rabbit anti-honey bee globulin was added to the tissues, and the slides were incubated again as before. The excess labeled antibody was then washed away with buffered saline, pH 7.2, and the slides were examined under a fluorescent microscope.

The following controls were run to establish that the observed staining was immunologically specific.

1. Specific inhibition with unconjugated homologous antibody: After Step 1, unconjugated rabbit anti-honey bee globulin was added to the tissues, and the slides were incubated as described. Then conjugated rabbit anti-honey bee globulin was added, as described in Step 2.

2. Elimination of antigen in Step 1: Instead of whole body honey bee extract, an equal volume of saline was added to the tissues.

3. Reaction with heterologous antigen: In Step 1, instead of whole body honey bee extract an equal amount of German roach whole body extract or ragweed extract was added to the tissues.

4. Reaction with normal tissues: In Step 1 normal tissues were used to replace the tissues of honey bee victims. These tissues were obtained from young persons accidentally killed. They had no known history of insect allergy or other allergy.

CASE REPORTS

Case 1

F. H. a 55-year-old healthy man, had no known history of serious illness or insect allergy. About 4:30 P.M. while mowing his lawn barefooted, he was stung by a honey bee on his right foot. A few minutes later, after going indoors, he complained of nausea, chest pain, and numbness and tingling in his right arm. Shortly thereafter he felt as if he was going to vomit and went to the bathroom. Approximately 10 minutes later his wife went to the bathroom and found him dead. The cause of death was reported as coronary insufficiency.

TABLE I. Hemagglutination titers using whole body insect extract in two victims of honey bee sting

Deceased patient	Honey bee	Wasp	Yellow jacket
F. H.	320*	0	40
J. R.	160	0	0

*Each value is the reciprocal of highest dilution of serum which produced a positive reaction.

Case 2

J. R., a 35-year-old previously healthy Caucasian man, died suddenly while painting the outside of his house. Although examination of his skin failed to reveal a puncture site, the possibility of a honey bee sting was suggested because honey bees had been seen around the house at the time of death. The cause of death was reported as "primary" congestion and edema.

Case 3

J. S., a 40-year-old Caucasian man previously stung without alarming reactions, was stung on the scalp by a honey bee. Death followed within 30 minutes. Postmortem examination showed only moderate pulmonary congestion and edema. The cause of death was reported as anaphylaxis.

RESULTS

Hemagglutination

Hemagglutinating antibodies were detected by the tanned cell technique in both of the 2 patients' sera tested. These results are illustrated in Table I. The serum of Patient F. H. showed positive hemagglutination reactions to honey bee and yellow jacket, and that of Patient J. R. showed positive hemagglutination to honey bee only. Controls for this test were inhibition by homologous antigen and reaction with heterologous antigen.

Fluorescent antibody

All 10 organs of the 3 deceased persons exhibited greater autofluorescence than did the organs of the normal control subjects. Two organs, heart and adrenal gland, were significantly stained with fluorescent antibody—an indication of the presence of specific antibody to honey bee antigen in these 2 organs (Figs. 1 and 2). The skin and lung of these 3 subjects also gave positive reactions with the tagged antibody. However, because of the high degree of autofluorescence present in these organs, the exact amount of staining produced by tagged antibody was difficult to judge. No staining was found in control tests (Figs. 3 and 4). No difference has been found between fluorescent staining of quick-frozen and formalin-fixed tissues.

DISCUSSION

The postmortem findings in persons who have died as a result of hypersensitivity to insect stings are usually those of respiratory obstruction or profound shock. Schenken and associates,¹² in their study of autopsy findings in 10 patients who died from bee sting, reported that visceral congestion and pulmonary edema were present in 6, pulmonary emphysema, laryngeal obstruction,

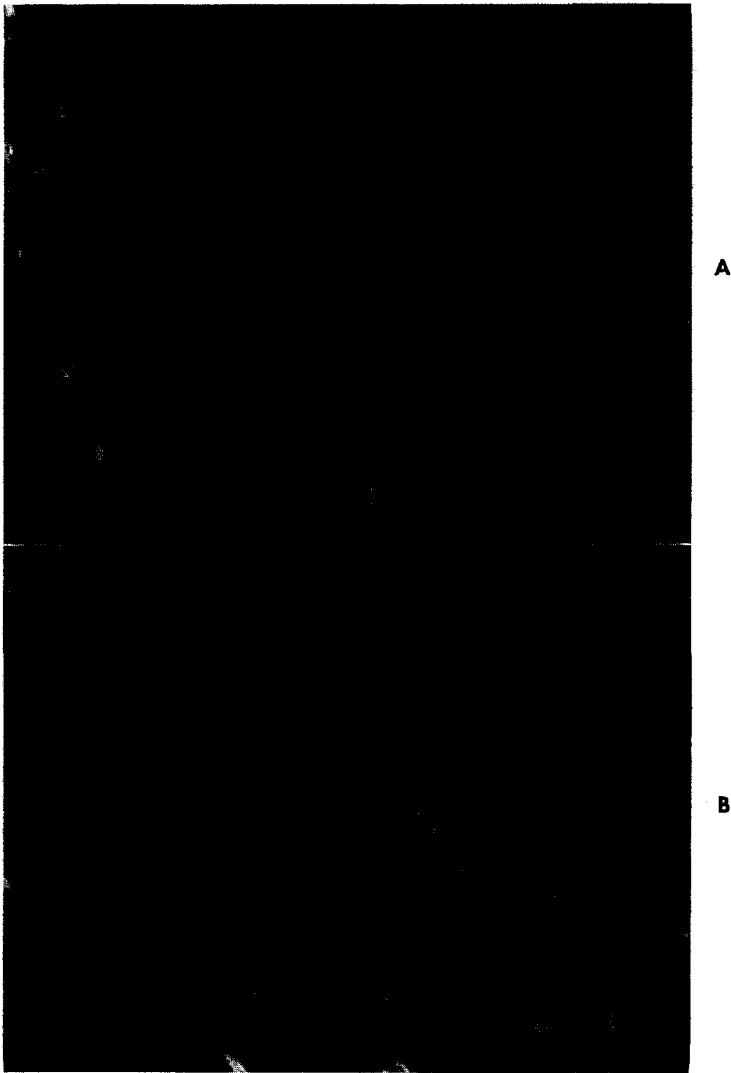


FIG. 1. Indirect fluorescent antibody procedure. Tissues treated first with whole body honey bee extract and then with fluorescein-conjugated rabbit anti-honey bee globulin. **A**, F. H. heart, and **B**, J. S. heart. The lightest areas are those emitting the yellow-green light of fluorescein and indicate sites of antibody present in the tissue sections. The darker areas indicate the less intense autofluorescence of other tissue elements. All photomicrographs of the frozen tissue sections were taken under a fluorescent microscope ($\times 220$).

or edema in 4, and cerebral edema in 4. In Hobson's¹³ patient, visceral, hepatic, and renal congestion were marked. Williams¹⁴ noted that the major autopsy finding in 2 patients dying from wasp sting was visceral congestion. However, O'Connor and associates¹⁵ stated that 13 of the 42 insect sting deaths studied were caused by some type of heart failure. Recently, Barnard,¹⁶ studying postmortem findings in 50 patients who died of insect sting, divided the major

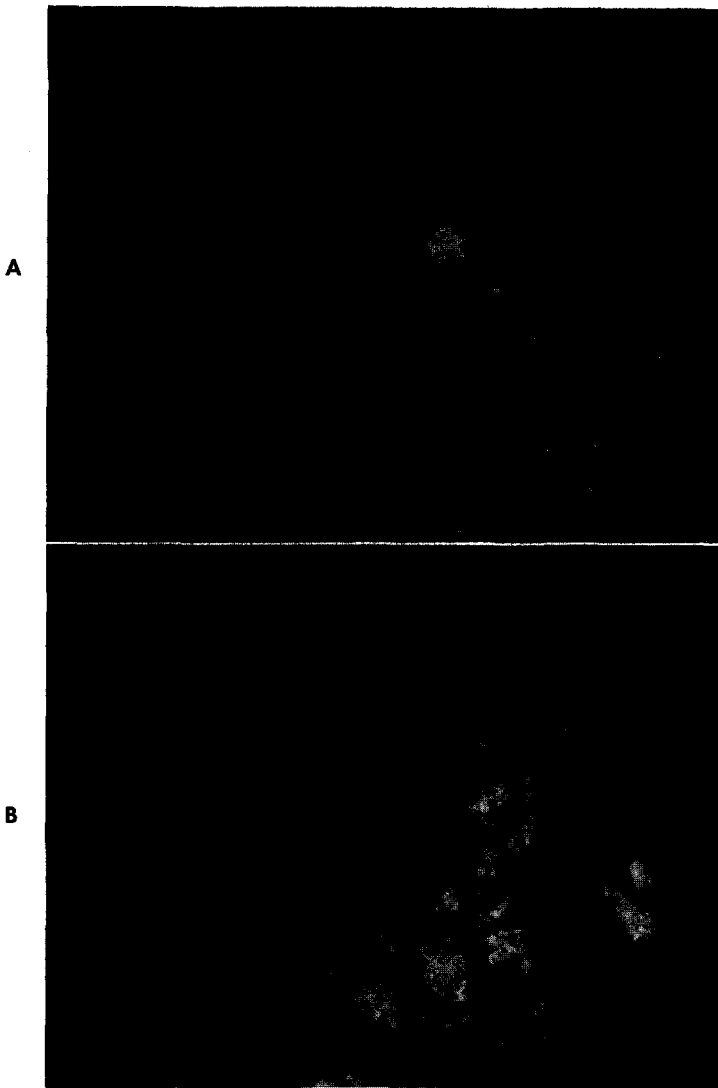


FIG. 2. Indirect fluorescent antibody procedure. Tissues treated first with whole body honey bee extract and then with fluorescein-conjugated rabbit anti-honey bee globulin. **A**, F. H. adrenal gland, and **B**, J. S. adrenal gland. The lightest areas are those emitting the yellow-green light of fluorescein and indicate sites of antibody present in the tissue sections. The darker areas indicate the less intense autofluorescence of other tissue elements. All photomicrographs of the frozen tissue sections were taken under a fluorescent microscope ($\times 220$).

pathologic reactions into 4 categories: the respiratory tract, the vascular system, the nervous system, and anaphylaxis. The 3 patients described in this paper were reported as having had coronary insufficiency, "primary" congestion with edema, and anaphylaxis, respectively. Until now, there has been no explanation as to why the primary site of a fatal reaction may occur in

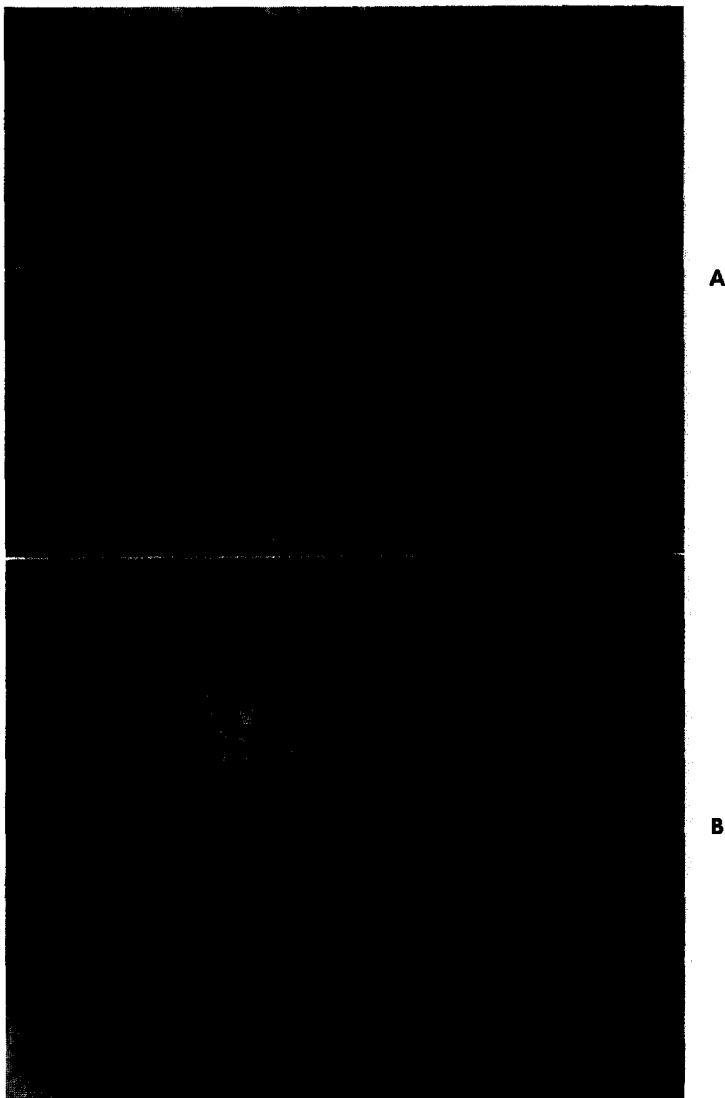


FIG. 3. Indirect fluorescent antibody procedure. Examples of controls. Controls were obtained by elimination of antigen in Step 1. **A**, F. H. heart, and **B**, J. S. heart. No staining was found in control tests. All photomicrographs of the frozen tissue sections were taken under a fluorescent microscope ($\times 220$).

one system or in another. The effect of various components of venom, the route and amount of challenging antigen, the degree of hypersensitivity of the victim, and other mechanisms have been suggested.

McCormick¹⁷ alone has reported the feasibility of detecting antibodies to Hymenoptera antigens in postmortem immunologic studies. By using both semimicro precipitin tube tests and Ouchterlony agar diffusion plates, McCormick found that the serum from a wasp sting victim reacted strongly to wasp extract and slightly to honey bee extract—an indication of the presence of spe-

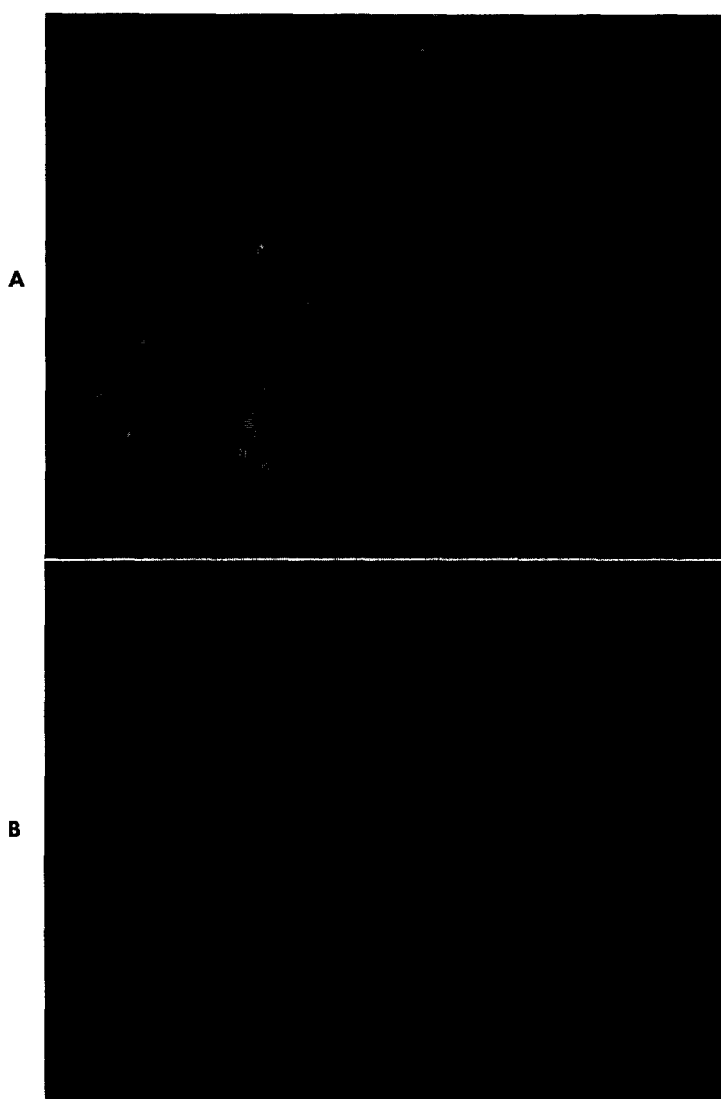


FIG. 4. Indirect fluorescent antibody procedure. Examples of controls. Controls were obtained by elimination of antigen in Step 1. **A**, F. H. adrenal gland, and **B**, J. S. adrenal gland. No staining was found in control tests. All photomicrographs of the frozen tissue sections were taken under a fluorescent microscope [$\times 220$].

cific antibodies in the serum. In this study, we have demonstrated the presence of hemagglutinating antibodies in the sera of honey bee sting victims. Specific antibodies to Hymenoptera antigens also have been found in the sera of living patients allergic to Hymenoptera.^{18, 19}

The role of hemagglutinating antibodies in anaphylaxis is not entirely understood. Beekeepers having multiple bee stings and developing protection have been found to have positive hemagglutination tests to whole body bee

extract and to bee venom. In penicillin hypersensitivity, the hemagglutination test for IgM antibodies to penicillin has been found positive in individuals receiving penicillin without any reaction. However, the majority of patients showing positive hemagglutinating tests for IgG antibodies experienced anaphylaxis to penicillin.^{20, 21} In this postmortem study, a positive hemagglutinating antibody titer to whole body honey bee extract was found in the sera of two honey bee victims. These facts suggest that the victims have responded immunologically to previous stings, but the relationship of the antibodies producing the hemagglutination reactions and the manifestations of clinical anaphylaxis is uncertain.

Some patients, shortly after a sting, have died of myocardial insufficiency documented by electrocardiographic abnormalities.^{22, 23} The usual explanation has been that hypotension, a component of anaphylactic shock, has caused anoxia of the cardiac muscle or the formation of a thrombus in a coronary artery, either of which results in myocardial infarction. These patients may die as a result of heart failure soon after a sting or days, or even weeks, later when they have become ambulatory and normotensive. Our finding of antibodies in the heart suggests that coronary insufficiency and myocardial infarction may not necessarily be secondary to anaphylactic shock but that the heart in these cases may be primarily involved as a shock organ.

Anaphylaxis has long been considered the cause of rapid death occurring after a sting. The adrenal gland is notably involved both in anaphylaxis and in nonimmunologic shock (tuberculosis of the adrenal, sepsis, etc.).²⁴ The fact that antibodies were found in significant quantity in this organ may mean that, in cases of anaphylaxis resulting from a sting, the adrenal is the primary shock organ. Thus, our findings of antibodies in the heart and in the adrenal gland may explain 2 clinically recognized mechanisms of death in Hymenoptera victims.

From time to time, coroners are confronted with cases of unexplained death. No clues may be apparent from the available history. The site of a sting, and even of several stings, may be unrecognized at autopsy. Therefore, the finding of antibodies in the sera or organs by immunofluorescence may be of practical value to pathologists and coroners in explaining some cases of sudden death.

We thank Drs. Richard Whelton, Monroe Coleman, and Joaquin Dorantes for contributing specimens of human tissues and Dr. Frank Jannotta for his many contributions to the project.

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