

Dermal hypersensitivity reactions to imported fire ants

Richard D. deShazo, M.D.,* Craig Griffing, M.D.,* Theodore H. Kwan, M.D.,**
W. A. Banks, Ph.D.,*** and H. F. Dvorak, M.D.** New Orleans, La.,
Boston, Mass., and Gainesville, Fla.

A survey of suburban residents of New Orleans, La., revealed that 58% of the individuals who responded had been stung by imported fire ants (IFA) within the previous year. More than half of the patients stung had dermal reactions that were distinct from the previously reported reactions to IFA in that immediate wheal-and-flare reactions evolved into pruritic, edematous lesions that persisted about the developing pustule for 24 hr or more. Twenty-one volunteers were stung with live IFA, and the course of the reactions was observed. Nine developed persistent reactions after stings. These reactions could be reproduced by the intradermal injection of IFA-whole body extract in only four of these nine subjects. Biopsy specimens of sting reactions at 6 hr demonstrated the reactions to be "late phase reactions" characterized by dense fibrin deposits like those previously noted in dermal reactions to ragweed and insulin. Eosinophils were present in the sting-associated pustules only in individuals who developed late-phase reactions. These data demonstrate that late-phase reactions occur commonly to IFA stings and that this form of insect hypersensitivity may not always be diagnosed by skin testing with whole body extract. (J ALLERGY CLIN IMMUNOL 74:841-7, 1984.)

The IFA, *Solenopsis richteri* Forel and *S. invicta* Buren, were inadvertently introduced from South America into the United States at the port of Mobile, Ala., about 1918 and 1940, respectively.¹ The two species now inhabit 90 to 100 million hectares of land in the southeastern area of the United States. *S. richteri* are restricted to a relatively small area of northwestern Alabama and northeastern Mississippi, whereas *S. invicta* infest all of Florida and Louisiana and parts of North and South Carolina, Georgia, Alabama, Mississippi, Arkansas, and Texas.¹ The latter species has been found also in Puerto Rico.² The ants are present in many habitats and present special

Abbreviations used

WFR: Wheal-and-flare reaction
IFA: Imported fire ant
P-K: Prausnitz-Küstner
WBE: Whole body extract

problems for the farmer because of the damage to farm machinery caused by their mound building. Their vicious stings afflict farmers and urbanites alike. Such stings are always painful and may on occasion cause persistent local reactions or anaphylaxis.^{3, 4, 5}

We report the results of an investigation of dermal reactions to fire ants conducted in suburban New Orleans, La. Our data demonstrate that although life-threatening hypersensitivity reactions appear to be unusual, at least two types of local reactions to fire ant stings occur. One of these types, the typical sting-associated WFR followed by a sterile pustule, has been studied previously.³ The second type noted in our patients, large, persistent local reactions, was found to be common. An extensive histopathologic investigation of these reactions revealed them to be "late-phase reactions," a form of dermal hyper-

From the Herbert H. Harvey Laboratory, Clinical Immunology Section, Tulane University School of Medicine,* New Orleans, La.; the Charles A. Dana Laboratory, Department of Pathology, Beth-Israel Hospital, Harvard Medical School,** Boston, Mass.; and the U.S. Department of Agriculture, Insect Research Laboratory,*** Gainesville, Fla.

Supported by a pilot study grant from the Dean, Tulane University School of Medicine, New Orleans, La.

Received for publication Sept. 15, 1983.

Accepted for publication May 22, 1984.

Reprint requests: Richard D. deShazo, M.D., Professor of Medicine and Pediatrics, Tulane University School of Medicine, 1700 Perdido St., New Orleans, LA 70112.

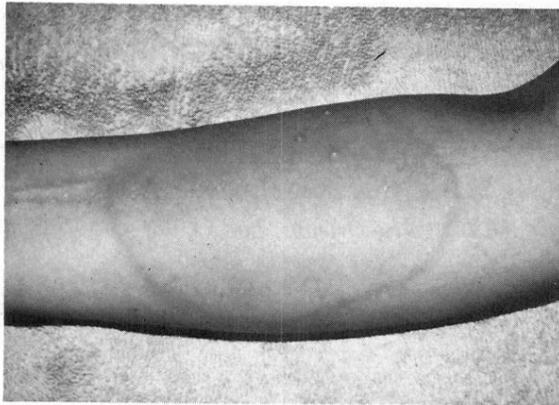


FIG. 1. Persistent dermal reaction to fire ant stings. This individual sustained three stings from a single fire ant on her arm 6 hr previously. She developed this large pruritic, painful, erythematous, edematous dermal reaction that peaked in size at 24 hr.

sensitivity previously reported to occur by both IgE-dependent and IgE-independent mechanisms.⁶

MATERIAL AND METHODS

Residential survey

Fifty consecutive homes on two randomly selected streets in a densely populated, middle-class residential area of a New Orleans suburb were visited. Land in this area was uncultivated until development began 12 yr before the survey. Respondents were then asked to fill out a questionnaire that included information on the numbers and ages of individuals in the household and the frequency of fire ant stings within the previous year. Types of local reactions were described. Respondents were asked if individuals who were stung had the previously described local reaction characterized by intense itching for 1 to 2 hr followed by the presence of a small pustule or had larger, persistent, itchy, red, swollen reactions lasting at least 24 hr that we had noted in many of our patients. Residents were also asked if symptoms associated with systemic allergic reactions, including generalized itching, urticaria, wheezing, laryngeal edema, or vasomotor collapse, had occurred.

Patterns of dermal reactivity to fire ant stings

Twenty-one individuals with histories of previous fire ant stings volunteered to participate in this phase of the study. Twelve had histories of fire ant stings of the sting-pustule type. These individuals were termed "nonreactors" for purposes of the study. Nine individuals had histories of persistent local reactions to fire ant stings and were termed "reactors." Individuals from these two groups were stung in the Tulane University Medical Center Clinic with live fire ants entomologically identified by one of us (W. A. B.) to be *S. invicta*. These ants were collected from mounds in the area of the survey. Reactions were observed during 48 hr and measured at 1, 6, 12, and 24 hr after fire ant sting. The

relative size of the reactions, expressed in square millimeters, was calculated by multiplying the two largest perpendicular diameters of palpable induration as previously described.⁷

Skin testing with fire ant extract

Volunteers from the two groups were also skin tested with commercial fire ant extract (IFA-WBE, *S. invicta*, Center Laboratories, Port Washington, N. Y., lot 12909). Intradermal skin test titrations were performed by injecting increasing concentrations (expressed as reciprocals of dilutions ranging from 1×10^{-5} to 2×10^{-2} of the original 20,000 PNU/ml extract) of extract in 0.02 ml volumes. These sites were observed during 24 hr, and the size of reactions was recorded. Passive transfer studies with the use of treated and untreated serum from two patients with persistent local reactions were performed on a volunteer who had not experienced previous IFA-sting reactions. Transfer of a WFR to Bermuda grass established the volunteer as an adequate recipient.⁷

Histologic studies

Skin biopsy specimens. Four biopsy specimens of sting sites were taken from the skin of three individuals who were demonstrated to be "nonreactors" by IFA sting. Two biopsy specimens were taken of lesions 30 min after sting, one biopsy specimen was taken at 6 hr after sting, and one biopsy specimen was taken at 24 hr after sting. Five biopsy specimens were taken from the skin of three "reactors"; one at 30 min, one at 1 hr, and three at 6 hr.

The four millimeter-punch biopsy specimens were obtained by use of 1% lidocaine (Xylocaine; Astra, Worcester, Mass.) field anesthesia and were processed for 1 μ m Giemsa-stained sections as previously described.⁷ Biopsy specimens were fixed in paraformaldehyde-glutaraldehyde for 5 hr at room temperature and transferred to 0.1M sodium cacodylate buffer (pH 7.4). Tissues were postfixed in osmium tetroxide, dehydrated, and embedded in Epon. These sections were stained with Giemsa's reagent and examined by light microscopy. A biopsy specimen of a 6-hour reaction from a "reactor" was also studied by use of previously described immunofluorescent techniques to detect the presence of immunoglobulin, complement, and fibrin-fibrinogen.⁷

Criteria used for classification of dermal reactions

We have classified human late-in-time dermal reactions as late-phase reactions, Arthus-type reactions, or delayed hypersensitivity reactions by use of criteria discussed in detail in a previous publication.⁸

Late-phase reactions are biphasic in that they are comprised of a WFR that occurs within 30 min and fades away to be followed shortly thereafter by a second reaction at the same site. The second phase peaks in size 6 or more hr after challenge. Both phases of this reaction are characterized histopathologically by mast cell degranulation and edema. The second phase is accompanied also by a mild and incon-

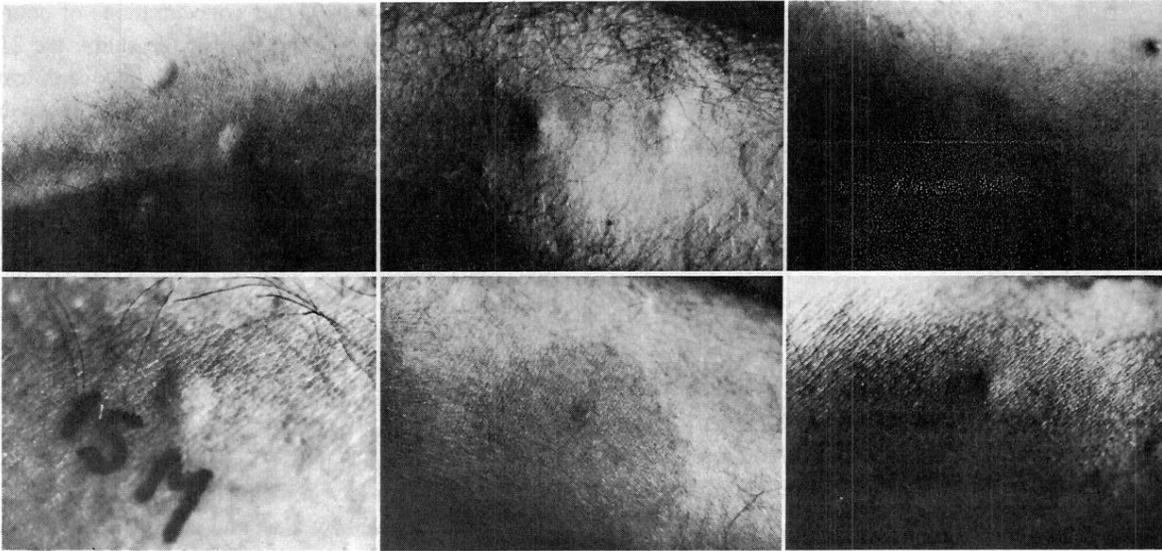


FIG. 2. Time course of dermal reactions to fire ant stings in "nonreactors" and "reactors." In nonreactors (*top panels*) WFRs prominent by 20 min (*left*) resolved within 2 hr. Developing pustules were present at 6 hr (*middle*) with a small area of surrounding edema. By 24 hr (*right*) the characteristic pustules and one excoriated pustule were present. In this panel three pustules, two of which are excoriated (*center of panel*), are present. Similar immediate reactions occurred in reactors (*lower panel, left*), but by 6 hr an intensely pruritic area of erythema and edema surrounded the sting (*center*). The developing pustule was almost always excoriated, and for that reason, 24-hour lesions (*right*) usually had no pustule. That lesion, consisting of a central papule with surrounding edema, peaked in size by 24 hr.

sistent, perivascular, cellular infiltrate composed of a mixture of lymphocytes, monocytes, neutrophils, eosinophils, and basophils. Dermal vessels are intact and lack immunoglobulin deposits.

Arthus reactions are characterized by damage and thrombosis of small blood vessels accompanied by immunoglobulin deposits and a predominantly neutrophilic infiltrate in the walls of small blood vessels. This infiltrate extends into the surrounding dermis. Fibrin deposits are present in the intervascular dermis as well as in thrombi.

Histologically, delayed hypersensitivity reactions have infiltrates of lymphocytes. Small numbers of granulocytes, monocytes, and macrophages are often noted as well. Fibrin is present in the same intervascular distribution as that found in late-phase reactions. At late intervals (i.e., 24 hr), initial late-phase reactions provoked by antigen may take on the characteristics of delayed hypersensitivity reactions, perhaps reflecting the evolution of an independent response to the same antigen challenge.

Cellular content of pustules

Pustule fluid from three "reactors" and two nonreactors was aspirated by syringe from 24-hour-old fire ant sting sites.

These fluids were filtered onto a 5.0 μm filter (Gelman Scientific Co., Ann Arbor, Mich.) that was stained with Papanicolaou's stain. Two hundred cells were counted for the differential count.

Statistical methods

Linear regression analysis was used to analyze skin test-titration curves.⁹

RESULTS

Survey results

Completed questionnaires were returned from 31 of the 50 homes visited. Of the 113 people occupying these homes, 65 (58%) of the individuals had had one or more fire ant stings within the previous year. Fifty-six percent reported reactions that were different from the previously described WFR with subsequent pustule in that they were large, persistent, itchy, red, and edematous and lasted for 24 hr. A photograph of one such reaction, which occurred after three stings from a single fire ant, is demonstrated in Fig. 1. No respondent had a history compatible with a systemic allergic reaction. Analysis of the incidence of stings by age demonstrated that both adults and children across the entire age range were stung.

Patterns of dermal reactivity to fire ant stings

WFRs were prominent by 20 min and resolved within 2 hr in "nonreactors" (Fig. 2, *upper panels*). Local pruritus was most intense during that time peri-

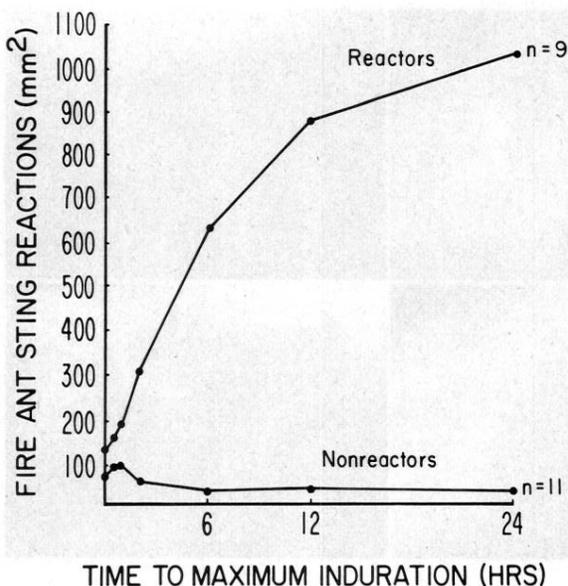


FIG. 3. Clinical course of local reactions to live fire ant stings in nine reactors and 11 nonreactors observed during 24 hr. Peak induration occurred by 24 hr and resolved rapidly over the following 24 hr.

od. By 6 hr, a developing pustule was present with a small area of surrounding edema, although significant quantities of fluid could not be aspirated until much later. By 24 hr the characteristic pustule previously associated with imported fire ant stings³ was present.

Similar immediate WFRs occurred in "reactors" (Fig. 2, lower panels). In contrast to the course in "nonreactors," reactions were biphasic with immediate (20 to 30 min) and "late" (developing after 1 to 2 hr) components. The biphasic nature of these reactions was most discernible with stings inducing smaller late reactions. The area of local edema at the sting site developed into a well-defined, intensely pruritic area of erythema and induration that was predominant by 6 hr and grew in size over the 24 hr. Large reactions were frequently painful if they were touched. These late reactions peaked in size by 24 hr (Fig. 3) and were uniformly smaller in size by 48 hr. The size of the WFR at 20 min was directly correlated to the size of the induration of the reaction at 6 hr (data not shown), i.e., the larger the reaction at 20 min, the larger the reaction at 6 hr.

No differences in appearance or time course of the pustular lesions were noted in "reactors" and "nonreactors"; pustules occurring in "reactors" appeared to be distinct from the edematous reaction occurring around them.

Skin testing with fire ant WBE

Preliminary skin test titrations revealed that all individuals tested, including those never stung by fire

ants, had WFRs with higher concentrations of commercial IFA-WBE. In this part of the study, the 21 volunteers previously stung by live IFA had skin test titrations with commercial IFA-WBE (Fig. 4). Skin test sites were observed during 24 hr. Of the nine individuals found to be "reactors" by live fire ant stings, only four had similar reactions to skin testing with IFA-WBE. The sizes of WFR at 20 min in these four individuals were uniformly larger across the range of extract concentrations than either those of 12 individuals with isolated WFR to IFA or the five individuals with late reactions to insect sting but not IFA-WBE ($p \leq 0.05$). The titration curves in the latter two groups were not significantly different ($p \geq 0.05$). No pustules were produced by skin testing with WBE in any individual tested.

A WFR followed by a second edematous reaction prominent at 6 hr was transferred by the P-K technique by use of one of two reactor sera. Neither wheal and flare nor late reactions could be induced with the other sera by use of concentrations of IFA-WBE not evoking nonspecific reactions.

Histologic studies

Skin biopsy specimens. Both reactor and nonreactor biopsy specimens exhibited a similar sequence of histologic changes resulting in the clinically observed pustule common to both (Fig. 5, A to C). Numerous cytoplasmic vacuoles were present at 30 min within keratinocytes, and clefts and vacuolar spaces were noted around vessels. The clinically apparent edema correlated with the histologic finding of separated collagen bundles in the dermis. Neutrophils and rare basophils were observed in and around some of the superficial and middermal venules. Vacuolar changes in keratinocytes and spaces around vessels remained similar to those described above at 1 hr, but neutrophils were more numerous in and around vessel walls. A dermoepidermal split consistent with necrobiosis had formed above the area of collagen alteration at 6 hr. Below this area neutrophils filled the dermis, forming bandlike cords that fanned out into the lower dermis in an anastomosing trabecular network. These cords, which did not maintain a perivenular relationship, also contained eosinophils and numerous necrotic cells.

"Reactors" could be distinguished by the deposition of fibrin with trapping of edema fluid in the reticular dermis circumferential to the central pustule (Fig. 6, A and B). The zone of edema extended for distances up to several centimeters from the central pustule as judged by gross examination. Fibrin deposition, noted as fibrin strands on Giemsa-stained 1 μ m sections, was observed at 30 min but was most prominent at 6 hr. Associated lymphocytic, granulocytic,

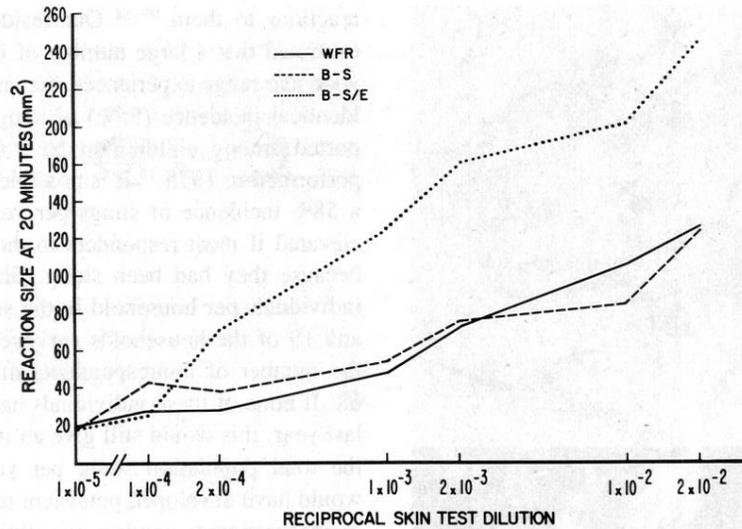


FIG. 4. Results of intradermal skin test titration with increasing concentrations of commercial IFA-WBE in "reactors" and "nonreactors." Wheal size at 20 min is plotted as a function of increasing concentrations of extract. The mean titration curve of five reactors to ant sting who failed to develop biphasic reactions to IFA-WBE (*B-S*) was similar to that of 12 nonreactors who had isolated WFRs. The mean titration curve of four reactors who developed biphasic reactions to both stings and extract (*B-S/E*) was different in that this group had uniformly larger reactions at dilute, nonirritant concentrations of extract.

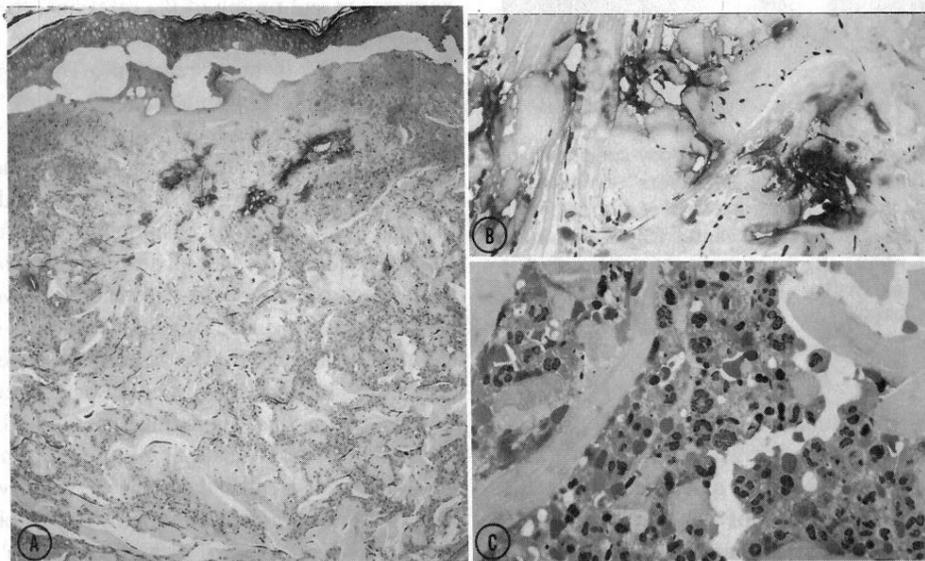


FIG. 5. Photomicrographs of 1 μ m Giemsa-stained biopsy specimens of fire ant bites. "Reactors" and "nonreactors" exhibited a similar sequence of histologic changes eventuating in pustule formation. These changes are described in detail in the text. At 6 hr the following changes were observed. **A**, Formation of a subepidermal cleft and changes consistent with necrobiosis. Extensive dermal infiltrates are also present (see *C*). (Giemsa stained; magnification $\times 40$.) **B**, Collagen alteration consistent with necrobiosis. (Giemsa stained; magnification $\times 960$.) **C**, Dermal infiltrates composed of neutrophils and eosinophils (prominent cytoplasmic granules) and many necrotic cells. (Giemsa stained; magnification $\times 960$.)

and histiocytic infiltrates were sparse or absent. Immunofluorescence studies of a reactor biopsy specimen at 6 hr indicated that fibrin was present in large amounts throughout the reticular dermis. Although

antifibrinogen antibodies react with fibrinogen, fibrin, and with certain of their breakdown products, the material identified in these sections could be identified confidently as fibrin because of its fibrillar appear-

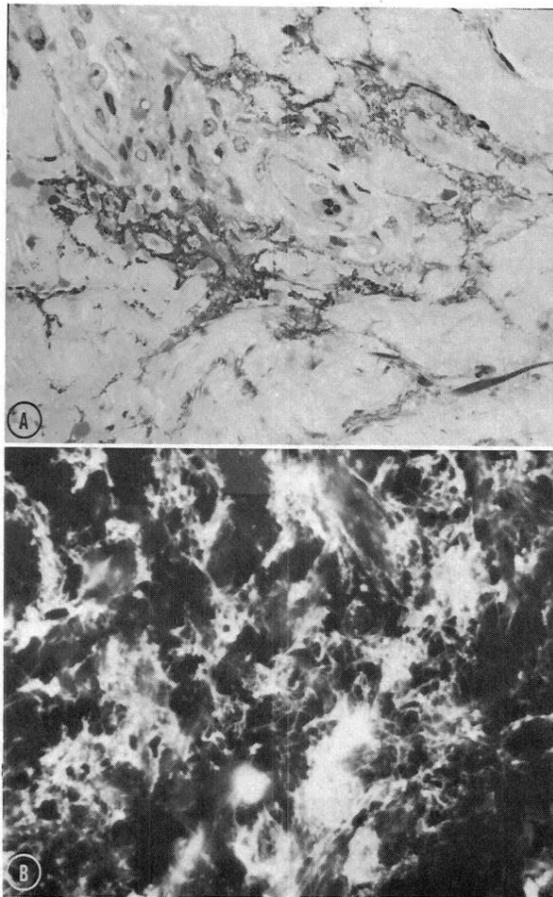


FIG. 6. Photomicrographs of 6-hour biopsy specimens of "reactor" fire ant sting sites. **A**, "Reactors" exhibited fibrin deposits in the dermis. Here swirls of compacted fibrin are present around a central vessel. (Giemsa stained; magnification $\times 960$.) **B**, Immunofluorescence studies demonstrated extensive, intense staining for fibrin (magnification $\times 160$).

ance. No significant fluorescent staining for IgG, IgA, IgM, IgE, C3, or fibronectin was observed.⁷

Pustule contents. Adequate quantities of pustule fluid for differential counts of cellular constituents were available by 24 hr after sting. Three blister fluids from "reactors" and two from "nonreactors" were studied. Light microscopic examination revealed amorphous, apparently necrotic, cellular debris and leukocytes. No bacteria were noted. Neutrophils averaged 75%, lymphocytes 12%, and eosinophils 13% in "reactors." Neutrophils averaged 94%, lymphocytes 6%, and no eosinophils were detected in "nonreactors."

Discussion

Few attempts have been made to analyze either the incidence of fire ant stings or the incidence of allergic

reactions to them.¹⁰⁻¹² Our residential survey demonstrated that a large number of individuals across a wide age range experienced fire ant stings. An almost identical incidence (55%) of stings per year was reported among children in New Orleans in a study performed in 1975.¹³ It is possible that our finding of a 58% incidence of stings per year could be falsely elevated if most responders to the survey responded because they had been stung. Since the number of individuals per household in the survey averaged 3.6 and 19 of the households surveyed did not respond, the number of nonrespondents may be estimated at 68. If none of these individuals had been stung in the last year, this would still give an incidence of 35% of the total population stung per year of which 28% would have developed persistent reactions. Such high incidence figures confirm our clinical impression that reactions to fire ant stings are a common problem.

Our investigation has determined that the "reactors" in this study experienced dermal reactions previously classified as "late-phase reactions," late-in-time dermal responses that occur as a result of mast cell degranulation by immunologic or nonimmunologic means.^{7, 14, 15} This was substantiated by the appearance and timing of the reactions, their histopathology, and the direct relationship of the size of the WFR to the 6-hour reaction. The presence of eosinophils in the 24-hour blister fluids of "reactors" but not in blister fluids of "nonreactors" appears to reflect the release of mediators of immediate hypersensitivity in late-phase reactions and the subsequent recruitment of eosinophils into the lesions. The dependence of late-phase reactions induced by IFA stings on homocytotropic antibody was demonstrated in one instance by passive transfer techniques by use of IFA-WBE. The failure to transfer the reaction in the second instance could have occurred for a variety of reasons, including the possibility that in some cases these reactions may occur on a nonimmunologic basis, like those that develop when compound 48/80, a nonspecific mast cell degranulator, is injected into the skin. The high incidence of late-in-time reactions in the survey population supports the notion that such nonimmunologic reactions may in fact occur. It is also possible that immunologic reactions of types other than those documented here may occur also. Further studies, by use of IFA-venom and RAST techniques are required to clarify this matter.

Previously reported studies with ragweed antigen have suggested that late-phase reactions can be blocked by pretreatment with oral corticosteroids or antihistamines, especially combinations of H1 and H2 blockers.¹⁶ Whether administration of these agents at the time of envenomation or shortly thereafter could

decrease the severity of IFA sting-induced late-phase reactions is presently unclear. However, this possibility is presently being investigated.

Another finding of note was that local hypersensitivity reactions to fire ant stings were not necessarily reproduced by intradermal injection of IFA-WBE. WBE skin test titrations of individuals who were "reactors" to fire ant stings but not to IFA-WBE were similar to those of "nonreactors." Thus, the putative antigens or agents (such as piperidine phosphate) responsible for the dermal responses noted in "reactors" may be absent or of low concentration in commercially available IFA-WBE. Data on the utility of skin testing with IFA-WBE for diagnosis of both systemic and local fire ant reactions have been conflicting and may relate to differences in the extracts used for testing.^{5, 17-19} Our data demonstrate that certain individuals with late-phase reactions after IFA sting could be unreactive to IFA-WBE (at concentrations not causing irritant reactions) and thus be judged to have "negative skin tests," a confusing diagnostic result.

We would like to thank Drs. Brian Butcher and Sam Lehrer for their helpful suggestions and review of the manuscript and Dr. Nina Dhurandhar for performing the analysis of blister-fluid contents. Mr. Al Dufour provided assistance with the photography, and Ms. Gail White and Ms. Jessi Weiss prepared the manuscript. Ms. Cathy Rolston, R. N., assisted with the fire ant stings and biopsies.

REFERENCES

1. Lofgren CS, Adams CT: Economic aspects of the imported fire ant in the United States. In Breed MD, Michner CA, Evans HE, editors: *Biology of social insects*. Boulder, Colo., 1982, Westview Press, Inc, p 420
2. Buren WF: Red imported fire ant now in Puerto Rico. *Fla Entomol* 65:188, 1982
3. Caro MR, Derbes VJ, Jung R: Skin responses to the sting of the imported fire ant (*Solanopsis saevissima*). *AMA Arch Derm* 75:475, 1957
4. Lockey RF: Systemic reactions to stinging ants. *J ALLERGY CLIN IMMUNOL* 54:132, 1974
5. James FK, Pence HL, Driggers DP, Jacobs RL, Horton DE: Imported fire ant hypersensitivity. Studies of human reactions to fire ant venom. *J ALLERGY CLIN IMMUNOL* 58:110, 1976
6. deShazo RD, Levinson AI, Dvorak HF: Late-phase reactions: paradigm or epiphenomena? *Ann Allergy* 51:166, 1983
7. deShazo RD, Levinson AI, Dvorak HF, Davis RW: The late-phase skin reaction: evidence for activation of the coagulation system in an IgE-dependent reaction in man. *J Immunol* 122:692, 1979
8. deShazo RD, Boehm TM, Kumar D, Galloway JA, Dvorak HF: Dermal hypersensitivity reactions to insulin: correlations of three patterns to their histopathology. *J ALLERGY CLIN IMMUNOL* 69:229, 1982
9. Daniel WW: *In Biostatistics: a foundation for analysis in the health sciences*. New York, 1978, John Wiley & Sons, Inc, p 254
10. Adams CT, Lofgren CS: Red imported fire ants (Hymenoptera:Formicidae): frequency of sting attacks on residents of Sumter County, Georgia. *J Med Entomol* 19:378, 1982
11. Adams CT, Lofgren CS: Incidence of stings or bites of the red imported fire ant (Hymenoptera:Formicidae) and other arthropods among patients at Ft. Stewart, Georgia. *J Med Entomol* 19:366, 1982
12. Yeager W: Frequency of fire ant stinging in Lowndes County, Georgia. *J Med Assoc Ga* 67:101, 1978
13. Clemer DI, Serfling RE: The imported fire ant: dimensions of the urban problem. *South Med J* 68:1133, 1975
14. Dolovitch J, Hargreave FE, Chalmers R, Shier KJ, Gaudie J, Bienstock J: Late cutaneous allergic responses in isolated IgE-dependent reactions. *J ALLERGY CLIN IMMUNOL* 52:38, 1973
15. Solley GO, Gleich GJ, Jordan RE, Schroeter AL: The late phase of the immediate wheal-and-flare skin reaction. Its dependence upon IgE antibodies. *J Clin Invest* 58:408, 1976
16. Smith JA, Mansfield LE, deShazo RD, Nelson HS: An evaluation of the pharmacological inhibition of the immediate and late cutaneous reaction to allergen. *J ALLERGY CLIN IMMUNOL* 65:118, 1980
17. Paull BR, Coghlan TH, Vinson SB: Fire ant venom hypersensitivity. I. Comparison of fire ant venom and whole body extract in the diagnosis of fire ant allergy. *J ALLERGY CLIN IMMUNOL* 71:448, 1983
18. Strom GB, Boswell RN, Jacobs RL: Imported fire and sting sensitivity: correlation in vivo between venom and whole body extract skin testing. *J ALLERGY CLIN IMMUNOL* 65:202, 1980 (abst)
19. Rhodes RB, Schafer WL, Schmid WH, Wubbena PF, Dozier RM, Townes AW, Wittig HJ: Hypersensitivity to the imported fire ant. A report of 49 cases. *J ALLERGY CLIN IMMUNOL* 56:84, 1975

