

HYPERSENSITIVE REACTIONS IN MAN TO SPECIFIC MOSQUITO BITES*

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Reactions in man to mosquito bites have been studied by a number of workers.¹⁻¹⁰ This reaction, observed after repeated exposure to mosquito bites, differs according to the degree of sensitization and, likely, the degree of susceptibility. The earliest stage of sensitivity is characterized by a delayed type of hypersensitivity reaction that does not appear until some hours after the initial bite; the skin around the puncture swells, reddens, and may itch for several days thereafter. The development of an immediate response together with the delayed reaction represents an intermediate stage of sensitization. A later stage of sensitization is characterized by the immediate reaction, which occurs within an hour following the bite, and consists of a soft, whitish wheal surrounded by a reddened area with pruritus usually less intense than that of the delayed. The disappearance of the delayed response, leaving only an immediate reaction, takes place at a later stage of sensitization. Still further exposure to the insect bites may eventually result in a state of desensitization with no immediate or delayed reaction appearing.

It is generally agreed that the immediate and delayed reactions to bites of mosquitoes are of an allergic nature.⁷ The allergens concerned in the process of sensitization and desensitization have not, it is believed, been isolated and chemically identified, although preliminary observations have given some indication of the characteristics of the skin-reactive components.^{1, 8, 11} Because of this, it is difficult to predict precisely the relations of sensitizing antigens in the salivary fluids of closely related species. Nevertheless, it is hoped that the additional evidence on the specificity of the skin reaction to mosquito bites presented here will facilitate the understanding of variations in the hypersensitive behavior of man to insect bites.

A unique opportunity to test human beings was exploited at the University of Illinois, where

a number of graduate students and faculty members in the Department of Zoology had been engaged in studies of several different species of culicine mosquito of the genera *Anopheles* and *Aedes*. In some instances the investigators have provided blood-meals for as long as 4 years and have passed through various stages of sensitization and desensitization to mosquito bites. The observations on the reactions of these subjects to the bites of homologous (the sensitizing species or species used in their experimental work) and heterologous insects, along with the reactions of subjects with no known history of deliberate exposure to mosquito bites, form the basis for this report.

MATERIALS AND METHODS

Mosquitoes. One strain of *Aedes aegypti* and 8 anopheline species were used, the latter including *A. albimanus*, *A. atroparvus*, *A. aztecus*, *A. punctipennis*, *A. pseudopunctipennis*, *A. quadrimaculatus*, *A. freeborni*, and *A. "southern occidentalis"* (originally collected from the Irvine Ranch, Orange County, California, and believed closely related to *A. freeborni*). All of the species were identified by morphologic and salivary chromosomal studies and kept under laboratory colonization in accordance with the methods of Klassen *et al.*¹²

Human beings. Sixteen volunteers, both male and female, varying in age from 20 to 48 years, were involved in the investigations; these subjects provided a diversity of previous exposures. Bites were received by placing the bared inner forearm against a cage or a series of tubes containing mosquitoes. Each person was exposed to the experimental bites of at least two species on the first day, followed by an interval of 24 hours, and a further exposure to an additional one or two species. When subjects were exposed to five species, a third exposure to biting was provided after an interval of another 24 hours. At least five, and more often, twenty or more bites, by any one species were received on one arm, and the other arm was likewise exposed to a second

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TABLE 1
Summary of prior exposure of human subjects to mosquito bites

Subject	Sex	Age (years)	Prior exposure to various species*	Comments
1	M	28	Irregular exposure to Aeg; deliberate exposure to Ppp, Azt, Quad, and Alb	Several months elapsed since deliberate exposure to Azt and Quad
2	M	28	Deliberate exposure to Aeg and Alb	Regular exposure to Aeg for several years and to Alb for a few months preceding test
3	M	27	Irregular exposure to Aeg; deliberate exposure to Alb	Exposure to Alb for at least 1 year, but more infrequently during the year preceding test
4	M	46	Irregular exposure to Aeg; deliberate exposure to Alb, Ppp, and Azt	Exposure for several years to many anopheline species
5	M	28	Irregular exposure to Aeg, Alb; deliberate exposure to Pp	
6	M	23	Irregular exposure to Aeg, Alb; deliberate exposure to Pp	
7	F	21	Irregular exposure to Aeg, Alb; deliberate exposure to Quad, Pp	
8	F	23	Irregular exposure to Alb; deliberate exposure to Aeg, Quad	Regular exposure to Aeg and Quad occurred 2 years preceding test
9	F	22	Irregular exposure to Aeg, Alb	
10	F	22	No deliberate or known irregular exposure	
11	M	28	No deliberate or known irregular exposure	Slight tendency to allergies
12	F	24	No deliberate or known irregular exposure	
13	M	23	No deliberate or known irregular exposure	Moderately allergic
14	F	25	No deliberate or known irregular exposure	Strongly allergic
15	F	20	No deliberate or known irregular exposure	
16	M	48	No deliberate or known irregular exposure	

* Ppp = *Anopheles pseudopunctipennis*, Azt = *Anopheles aztecus*, Quad = *Anopheles quadrimaculatus*, Alb = *Anopheles albimanus*, Aeg = *Aedes aegypti*, Pp = *Anopheles punctipennis*.

species. Similar techniques had been used by the research personnel to provide blood-meals during mosquito-rearing procedures.

Measurement of skin reactions. Immediate reactions were observed during the hour following the bite. The diameter of the wheal was measured with vernier calipers, and the degree of surrounding flare was recorded. Subjects were asked to report any stinging or itching sensations during or directly following the bite.

Delayed reactions were first observed about 18 to 24 hours after the bite. Measurements were

made of the diameter of the papular area and the surrounding erythema, if present. Subjects were questioned as to the degree of pruritus, and its presence was recorded. The persistence of delayed reactions on subsequent days was also recorded.

RESULTS

The full details of the skin-reaction measurements and the extent of flare, stinging, or pruritus, erythema and duration of reaction, and a detailed history of each of the human subjects are contained in a Thesis submitted in partial

TABLE 2
Summary of delayed (D) and immediate (I) hypersensitive reactions of human subjects with no known history of deliberate exposure to mosquito bites*

Subject	Mosquito species to which the subjects were exposed											
	<i>Aedes aegypti</i>		<i>Anopheles pseudopunctipennis</i>		<i>Anopheles albimanus</i>		<i>Anopheles atroparvus</i>		<i>Anopheles freeborni</i>		<i>Anopheles "southern occidentalis"</i>	
	D	I	D	I	D	I	D	I	D	I	D	I
10	++	0	++	0	++		++	0				
11	+	0	+	0	+		+	0				
12	+	0			+				+	0	+	0
13	++	0			+				++	0	++	0
14	+++	+++			+++				+++	+++		
15	++	0			++						++	0
16	0	0			0	±					0	0

* Reaction symbols: 0 = negative, ± = doubtful, + = slight, ++ = moderate, +++ = strong, ++++ = intense.

Absence of symbol indicates that subject was not exposed to that species.

fulfillment of the requirements of the degree of Master of Science in Zoology in the Graduate College of the University of Illinois.¹³

An attempt is made here to summarize this information. The 16 subjects are arbitrarily placed in three categories: 1) nonsensitized, 2) sensitized, and 3) desensitized according to their respective reactions to specific mosquito bites and considering their prior exposures. Pertinent facts concerning the history of each subject are summarized in Table 1.

Nonsensitized. Seven of the 16 subjects with no known history of deliberate or prolonged exposure to mosquito bites constitute the category of nonsensitized persons. The results of the experimental exposure of each of these volunteers to the bites of *A. aegypti* and to two or three anopheline species appear in Table 2. Six subjects showed slight to strong delayed type of hypersensitivity responses. The seventh person (subject 16) reacted with only a doubtful immediate type of response. In six instances the nonsensitized subjects showed little difference in the type or extent of their reaction to the various mosquito species. The observations did not suggest any apparent effect resulting from the varying sequences of species to which the subjects were exposed. In one instance (subject 14) a strong immediate reaction occurred when this person was bitten by *A. aegypti* and *A. freeborni*, but no immediate reaction followed the bite of *A.*

albimanus; a strong delayed reaction was observed to all three species. Apart from this single observation of a definite difference in the immediate response to different species, no evidence for selective or specific reactivity was detected in the nonsensitized subjects when exposed to the several species of mosquito.

Sensitized. Subjects are considered here as persons sensitized to a particular mosquito species when they had had a more or less frequent prior exposure and had exhibited both an immediate and delayed response or an immediate response alone to that species. It is evident from Table 3 that there were extensive cross-reactions among sensitized persons; this was especially marked, as would be expected, in the case of those with a history of exposure to species of *Aedes* and *Anopheles* (Table 1). For example, subjects 1, 2, 3, 4, 5, 8, and 9 had had varying degrees of experience with a number of anopheline species in addition to *A. aegypti* and appeared to react similarly to the species of both genera tested even when the previous history indicated a more regular and longer exposure to species of one of the genera.

There were, however, some indications of a degree of specificity in some of the responses to bites that were measured. Subject 9 was sensitized to the bites of *A. aegypti* and *A. albimanus* and responded with strong to intense immediate and delayed reactions accompanied by erythema

TABLE 3
Summary of delayed (D) and immediate (I) hypersensitive reactions* of human subjects apparently sensitized and/or desensitized to the bites of certain species of mosquito

Subject	Mosquito species† to which subjects appeared:		Mosquito species with which subjects were challenged experimentally								
	Sensitized	Desensitized	Aeg	Alb	Atp	Azt	Pp	Ppp	Quad	So	Fbi
1	Aeg	Ppp, Azt, Alb	D + I +	0 0		0 0		0 0	+ 0		
2	Aeg, Alb		D + I +	+++ +++	+ +++			+++ +++			
3	Aeg	Alb	D +++ I +++	0				0		++	++
4	Aeg, Alb, Azt	Ppp	D + I +	+ ++		+ +++		0 0		++	++
5	Aeg, Pp		D + I +			+ +					0
6		Aeg, Pp	D 0 I 0			+ +	0 0			0 0	+ 0 0 0 0
7		Quad, Alb, Pp, Aeg	D 0 I 0	0 0					0 0		
8	Aeg, Quad, Alb		D ± I +++	+++ +++		+++ +++		+++ +++	0 +		
9	Aeg, Alb		D +++ I +++	+++ +++		+++ ++		+++ +++	+++ +++		

* See Table 2 for interpretation of reaction symbols.

† Aeg = *Aedes aegypti*, Ppp = *Anopheles pseudopunctipennis*, Azt = *A. aztecus*, Alb = *A. albimanus*, Pp = *A. punctipennis*, Quad = *A. quadrimaculatus*, So = *A. "southern occidentalis"*, Fbi = *A. freeborni*, Atp = *A. atroparvus*.

and pruritus for up to 4 and 5 days. When this person was exposed to *A. aztecus*, the wheals and flares of the immediate response were considerably smaller than that observed with *A. aegypti* or *A. albimanus*, and no pruritus was reported during either the immediate or delayed type of hypersensitivity observation periods. Likewise, subject 3, apparently sensitized to *A. aegypti*, reacted to a lesser degree to *A. "southern occidentalis"* and to *A. freeborni*, but the observation that this person also appeared desensitized to another anopheline, *A. albimanus*, may be a complicating factor here and is discussed later.

Desensitized. Five persons showed evidence of being desensitized to the bites of one or more species of mosquito by their lack of reactivity to these species following a previous history of exposure to them. Their responses are briefly summarized in Table 3. Subject 6 and possibly subject 7 appeared desensitized to the bites of *A. aegypti* as well as to one or more anopheline species, and they failed to react even to species not previously encountered in deliberate exposures. Conversely, subjects 1, 3, and 4 were desensitized to the bites of anopheline mosquitoes, and yet all demonstrated slight to strong immediate and delayed responses to the bites of *A. aegypti*. Subject 3, who was apparently desensitized to the bites of *A. albimanus*, showed slight delayed and moderate immediate reactions to *A. freeborni* and *A. "southern occidentalis"*. Similarly, another subject (4), who was desensitized to the bites of *A. pseudopunctipennis*, reacted with a slight delayed and a moderate to strong immediate response to the challenge with *A. albimanus* and *A. aztecus*. Some evidence for a common antigenic component in the secretions associated with mosquito bites is suggested by the observations on subject 6 who, when exposed to *A. "southern occidentalis"* and *A. freeborni*, appeared to be desensitized in the same way in which he was desensitized to *A. punctipennis*. In addition, the close phylogenetic relation that is recognized between *"southern occidentalis"* and *A. freeborni* is also indicated in the response to bites of these species. Subjects 3 and 6, who received bites from the two species, reacted equally (in the case of subject 6, not at all) to both of them.

DISCUSSION

The inaccuracy of data concerning the past exposure of human beings to the bites of a variety

of species of mosquito makes it difficult to draw definitive conclusions from results obtained during a study of this nature. However, certain of the observations are of particular interest and warrant further discussion.

In general, during this investigation it was noted that subjects with no history of deliberate exposure to mosquito bites exhibited little immediate reaction (with the exception of subject 14); whereas subjects with deliberate or prolonged exposures displayed varying degrees of both immediate and delayed responses or an apparent loss of all reactivity. These observations are in agreement with the pattern of development of skin reactions in man after repeated exposure to bites originally described by Mellanby³ and later extended by Hudson *et al.*⁶

No significant differences in reactions to bites were observed between the males and females in this study, but such differences may have been masked because of their varied past exposures.

With regard to the persons categorized as nonsensitized, it appears that the differences occurring in the degree of response to the bites are more significant between individual subjects than among the reactions of any one subject. The concept of individual susceptibility to mosquito bites is not new.^{3, 6, 10} It is not surprising that subject 14, with a strong case history of allergies, was much more highly reactive than other persons in this category. The delayed response of the nonsensitized persons to various anopheline and *A. aegypti* bites was relatively nonspecific. The characteristic of a less precise specificity has been attributed to delayed hypersensitivity reactions by researchers concerned with more defined protein systems.¹⁴ Subject 16 appears to be nonreactive in comparison with the other nonsensitized subjects; this state may be related to the age of this person. A decrease in the reaction to bites with increasing age has been reported earlier.⁶

There is some suggestion of specificity in the reactions to bites in the sensitized persons, but cross-reactions are also evident. As McKiel and West¹⁰ have indicated, the degree of specificity varies with the degree of sensitization, and in the present investigation the state of reactivity is very likely quite different among these subjects with varied durations of prior exposures. Another factor influencing the cross-reactivity in this study is the history of mixed exposures for irregular periods of time. It is difficult to assess the extent of nonspecificity in a person who has had

experience of unequal extent with the anopheline species and with *A. aegypti*.

Cross-reactivity in addition to a degree of specificity are both evident when the desensitized state is considered; this apparent conflicting observation is probably also influenced by the varied prior exposure to the two genera, *Aedes* and *Anopheles*. Subjects 1, 3, and 4 reacted to bites of *A. aegypti* although they appeared desensitized to the bites of the anopheline species, an observation suggesting specificity at the generic level. Gordon and Crewe³ similarly reported that a person partially desensitized to the bites of *A. aegypti* reacted strongly to the bites of *Culex molestus* Forskal. Subjects 6 and 7, however, exhibited negative or doubtful reactions to *A. aegypti* as well. This may be the result of prolonged prior exposure to *A. aegypti* rather than cross-reactivity after regular exposure to *Anopheles* or, what is even more likely, both factors may contribute. Rockwell and Johnson,⁶ working with mosquito extracts, also reported nonspecificity, for persons desensitized to *A. aegypti* extract, to the delayed reaction after *C. pipiens* bites. The distinction between desensitization leading to a loss of the delayed reaction as opposed to the immediate reaction has been suggested in the past.^{4, 5, 9, 10} McKiel and West^{9, 10} have indicated the relative ease with which subjects can be desensitized with respect to the delayed reaction in contrast to the immediate reaction, which is apparently more difficult to affect. Mellanby² reported that only persons who had been exposed to mosquito bites for several years displayed a loss of the immediate reaction. It is of significance, then, that desensitization with a disappearance of both immediate and delayed responses has been observed here in persons having a history of previous deliberate exposure, but certainly not for many years. Subjects 6 and 7, for example, had not been deliberately or irregularly exposed to mosquito bites up to a year or less preceding the test challenge.

Differences in reaction to the bites of different species of *Aedes* and *Anopheles* have been recognized.^{2, 4, 5} Mellanby² even reported that one man may simultaneously give only a delayed reaction to *Anopheles*, but both an immediate and a delayed reaction to *Aedes*. This was observed here in subject 1, who showed a delayed reaction to *A. quadrimaculatus*, and at the same time an immediate and a delayed reaction to *A. aegypti*. Subject 5 also showed only an immediate response to

the bites of two anopheline species and both immediate and delayed responses to the bites of another anopheline and of *Aedes*. Mellanby² suggested that the two reactions, delayed and immediate, are quite distinct and are probably caused by different antigens in the saliva of the mosquito. Rockwell and Johnson⁶ have also proposed the presence of multiple allergens in the saliva to account for some of the differences in the reactions of human beings to bites of *Anopheles* and *Aedes*.

Finally, it seems that skin reactions to mosquito bites are not completely specific but may show some signs of specificity. Further, the disparity in the results of various workers claiming a high degree of specificity or, on the other hand, cross-reactivity, seems to be greatly influenced by the selection of subjects with different prior exposures and hypersensitive tendencies.

In view of the undesirability of using the human subject for extensive experimentation with mosquito antigens, further investigations on hypersensitive responses were undertaken using the guinea pig as an experimental animal. The results of these studies will form the basis of a later report.

SUMMARY

Sixteen persons providing a diversity of previous exposures were challenged by bite with *Aedes aegypti* and with at least two species of anopheline mosquito. Immediate and delayed skin reactions were recorded. The subjects fell conveniently into three categories: 1) nonsensitized, 2) sensitized, and 3) desensitized according to their respective reactions to specific mosquito bites and their history of exposure. Persons with no prior deliberate exposure to particular mosquito bites exhibited a delayed but little immediate response, whereas those with deliberate or prolonged exposures displayed varying degrees of both immediate and delayed responses or loss of reactivity. Six of the seven nonsensitized persons showed little difference in the extent of their delayed reactions to various species. One highly allergic person provided a good example of the role of individual susceptibilities in the skin response to mosquito bites. Another subject appeared to represent a case of nonreactivity due to age. Instances of both cross-reactivity and specificity between the two genera and among the anopheline species were noted in the immediate and the delayed response of sensitized persons

and in the loss of these responses in desensitized persons. The observation of the disappearance of both the immediate and the delayed response in those deliberately exposed to mosquito bites for only a relatively short period of time (less than a year) is of significance in view of the report by others that a desensitized state leading to the loss of the immediate reaction requires an exposure of many years.

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ERRATUM

In the article, "Immunoelectrophoretic analysis of several strains of *Entamoeba histolytica*," by Iris M. Krupp, which appeared in the November 1966 issue of the *Journal* (15: 849-854), the three figures were misplaced. The proper order for the figures and their legends is as follows:

Figure 1 is the two-column diagram on page 852: "Diagrammatic representation of IEP pattern of several strains of amebae reacted with homologous and heterologous antisera (anode on the left)."

Figure 2 is the one-column diagram on page 850: "Precipitin arcs formed in the double diffusion test when anti-*E.h.* 200 serum was reacted with five strains of *E. histolytica*."

Figure 3 is the one-column diagram on page 852: "Double diffusion test illustrating the common antigenic components between *E.h.* 200 and *E.h.* DKB when both antigens were reacted with anti-*E.h.* 200 serum."