

REACTIONS TO MOSQUITO BITES^{1, 2}ANNE HUDSON,³ J. A. McKIEL,⁴ A. S. WEST⁵ AND T. K. R. BOURNS⁶

The clinical reaction to mosquito bites is characteristic; a localized erythematous region around the puncture site, heat, pruritus and possibly some degree of pain. The severity of the reaction varies from person to person and the time elapsing between the bite and the appearance of a skin reaction may vary from a few minutes to 48 hours.

Many theories have been put forward to explain the nature of reactions to insect bites. These have included attempts to attribute the wheal and erythema to mechanical damage, irritation of the skin caused by a specific toxin secreted by the insect. Experiments have shown that, while these factors may contribute to the cause, they are not responsible for the type of reaction produced; this appears to be clearly allergic in nature.

From the evidence available, it is apparent that a characteristic sequence of events takes place in all subjects exposed to mosquito bites over a period of time. Evidence for this sequence has seldom been presented by a single subject. It has been compounded from observations on young children and persons with no known history of mosquito bites, from persons known to have been exposed intermittently to a moderate number of mosquito

bites, and from persons who have been exposed intensively for long periods of time.

A series of experiments which gave a clear indication that some sequence occurred was carried out by Mellanby in 1946. He selected a group of students who were not known to have had previous exposure to the bites of *Aedes aegypti*. When the experiment was begun, the first (delayed) reactions to the bites appeared 24 hours after they were received. During the subsequent months more bites were received and immediate reactions occurred in addition to the delayed response. Further exposure produced a reduction in the latent period between immediate and delayed reactions until finally the latter disappeared completely. Mellanby suggested that continued exposure would result in no reaction at all.

Heilesen, in 1949, experimented with 10 Danish children aged from 6 months to 3 years. No reaction was given to the first bites received. Four of the children were exposed to repeated bites, and all gradually developed delayed reactions.

These observations, together with many others led McKiel (1955) to extend Mellanby's classifications of reactions from 4 to 5 stages:

Stage	Immediate Reaction	Delayed Reaction
I	—	—
II	—	+
III	+	+
IV	+	—
V	—	—

In the work to be described we have been concerned with confirmation of the allergic nature of reactions to insect bites and with the establishment of a quantitative estimation of the time and number of

¹ Contribution from the Department of Biology, Queen's University, Kingston, Ontario, Canada.

² These studies have been supported by the Defence Research Board of Canada and the United States Public Health Service.

³ Research Associate.

⁴ Formerly, Research Officer, Defence Research Kingston Laboratory; present address, Laboratory of Hygiene, Dept. National Health and Welfare, Ottawa.

⁵ Professor of Zoology.

⁶ Formerly Research Associate; present address Dept. Zoology, University of Western Ontario, London.

bites necessary to sensitize laboratory rabbits. At the present time attempts are being made to demonstrate circulating antibodies to "bite substance" and to obtain mosquito salivary fluid for analysis.

Man is unreliable as a subject for investigations on the bite reaction, because it is often difficult to obtain an accurate account of past exposure to insects. A laboratory animal with a known history, and one which gives a clear response, is more useful. Rabbits have proven to be more satisfactory than rats, mice or guinea pigs.

Our first attempts to sensitize rabbits were made by exposure to mass-biting by mosquitoes.

1. Clean rabbits were tested with 2 or 3 bites to detect any existing sensitivity.

2. The rabbits were exposed to large numbers of mosquitoes (approximately 5,000) for two hours on successive days for five days.

3. A resting period of 5 to 7 days was allowed for the development of sensitivity.

4. Challenge bites by two mosquitoes were given to detect sensitivity.

As a result of these tests, it was found that when sensitivity developed a wheal appeared within 10-15 minutes and reached a maximum usually within one hour. However, the proportion of rabbits which would become sensitized by this method was unpredictable, and there was no means of determining the course of development of sensitivity in individuals. In some cases it was believed that an animal may have become sensitized and then temporarily desensitized by exposure to large numbers of insects. Other methods were attempted to determine the relation between time of exposure and the number of bites received.

PAIRED BITES ON SUCCESSIVE DAYS

1. Rabbits were given a preliminary test for sensitivity as above.

2. Two bites per day were given daily and the sites observed at hourly periods between one and 24 hours.

Results have shown clearly that the minimum period required for the appear-

MEAN WHEEL DIAMETER SHOWN BY 9 RABBITS SENSITIZED TO *A. AEGYPTI* BY TWO BITES PER DAY.

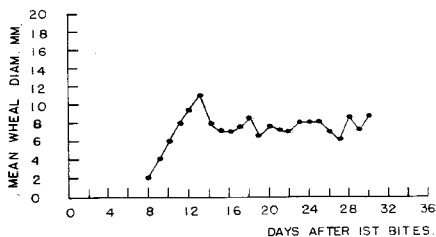


FIG. 1.

ance of small wheals was between 7 and 9 days. Between 10 and 14 days a maximum wheal size was reached, followed on successive days by fluctuating wheal sizes intermediate between the minimum and maximum. The latter condition persisted for 30 days (duration of the experiment). The maximum wheal size was frequently not reached until 12 hours after the bites were given, but this was a highly individual effect.

In Figure 1 the mean wheal diameter given by 9 rabbits during the experimental period is shown. Figure 2 shows the mean wheal diameter, the range and standard error at the 9th, 13th, 15th and 25th days. Since the standard errors between the 9th and 13th days do not overlap, it is

MEAN WHEEL DIAMETER, STANDARD ERROR AND RANGE OF WHEEL DIAM. SHOWN BY 9 RABBITS SENSITIZED TO *A. AEGYPTI* BY 2 BITES A DAY.

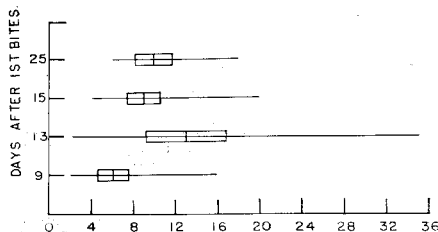


FIG. 2.

concluded that there is a significant difference in the wheel diameters obtained on these days. Between the 13th and 15th and 13th and 25th days the standard errors do overlap and it is concluded that there is no significant difference between wheel sizes produced after the maximum has been attained.

PAIRED BITES ON ALTERNATE DAYS

The second method decreased the number of bites; two bites per day were given on alternate days. The degree of sensitivity produced was less, although some small reactions were obtained, and it was shown that less than 12 bites over a period of 12 days were required to produce some sensitivity.

The development of the reaction as shown by these experiments supports the theory of its allergic nature.

SPECIFICITY

Attempts have been made to determine the specificity of the reaction to mosquito bites. Rabbits sensitized to *A. aegypti* by various methods were tested with other species of *Aedes*, some *Culex* and some *Anopheles*; challenge bites with the sensitizing species were given prior to each test, and control bites with the sensitizing species were included in all tests. The overall results were inconclusive; examples are shown in Table 1.

Rabbits 11-52, listed in the table, were selected as examples because they gave a good response to challenge bites, and were positive to control bites. They were thought to be near the period of peak sensitivity although this cannot be accurately stated since the methods of sensitization varied and the heterologous tests were performed some days after the last

TABLE 1.—Specificity of Mosquito Bite Reactions in Rabbits

Rabbit No.	Method of sensitization	Result of challenge bites	Results of bites with heterologous species		Control bites with sensitizing species
			Positive	Negative	
11	With <i>Aedes aegypti</i> Mass biting	+	<i>Aedes trichurus</i> <i>A. stimulans</i>	—	+
15	2 bites per day	+	<i>A. trichurus</i> <i>A. punctor</i> <i>A. excrucians</i>	—	+
16	"	+	<i>A. trichurus</i> <i>A. stimulans</i>	—	+
65	"	+	<i>A. stimulans</i> <i>A. excrucians</i> <i>A. vexans</i>	<i>Anopheles occidentalis</i>	+
75	2 bites per day on alternate days	+	<i>A. vexans</i> <i>A. excrucians</i>	<i>A. occidentalis</i>	+
52	With <i>Aedes canadensis</i> 2 bites per day	+	<i>A. punctor</i> <i>A. stimulans</i>	<i>Mansonia perturbans</i>	+
53	"	±	<i>Anopheles occidentalis</i> <i>Aedes stimulans</i>	<i>Anopheles quadrimaculatus</i> <i>A. punctipennis</i> <i>Aedes aegypti</i>	+

sensitizing bite. Rabbit 53 gave a poor response to challenge bites but was positive to the control. This rabbit had been observed to show a peak sensitivity on the 16th day of the sensitization period and was tested on the 24th consecutive day.

It appears that the degree of sensitivity to heterologous species may be determined by the time during the sensitization period at which the test is made; those rabbits which were at, or close to, the maximum reaction stage gave a greater indication of specificity than those which were tested later during the period of sensitization. This would show agreement with the serological hypothesis that specificity may be lost with increasing duration of sensitization.

ANALYSES OF WHOLE MOSQUITO EXTRACT

Up to the present time most attempts to determine the antigenic factors in mosquito bites have involved the use of whole mosquito extract. A saline extract has been used to sensitize rabbits and has been subjected to certain analytical procedures (McKiel, 1955). Chromatographic fractionation of whole extracts has revealed that the active principle is comprised of at least four components, eluates of which have produced positive reactions in individuals giving strong immediate reactions to mosquito bites.

Calculations of the amount of active material required to produce a bite reaction gave a value of 0.0013 μg of chromatographically purified material. This estimated quantity is probably greater than the amount actually injected by the mosquito.

The use of whole extract obviously complicates any investigation such as this, and we are therefore at the present time devising a method for extracting salivary fluid from the mosquito.

SEROLOGICAL EXPERIMENTS

One of the most perplexing aspects of the reactions to mosquito bites is our in-

ability to demonstrate circulating antibodies in bite-sensitive serum. Precipitin ring tests will give positive results on sera obtained from injected animals; however, this reaction does not involve only the 'bite substance.' It is possible that the amount of antibody present in bite sensitive animals is extremely small and therefore requires a much more sensitive test. Some experiments have been made using the tanned-cell technique of Boyden (1951); further tests have been delayed until the antigenic factors can be obtained either from saliva or from purification from salivary glands.

Attempts have been made to obtain passive transfer of sensitivity (McKiel, 1955 and in the present work); these have been unsuccessful.

SUMMARY

1. Evidence is presented for the occurrence of a definite sequence of events during sensitization of human subjects to mosquito bites.

2. Experiments with different methods of sensitizing rabbits have given information about the relation of time and the number of bites required to produce sensitivity. The results have supported the theory of the allergic nature of the reaction.

3. Chromatographic fractionations of extracts of whole mosquitoes have revealed that the active principle has at least four components.

References

BOYDEN, S. V. 1951. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent haemagglutination by anti-protein sera. *J. Expt. Med.* 93:107-120.

HEILESEN, B. 1949. Studies on mosquito bites. *Acta Allergologica*, 2:245-267.

McKIEL, J. A. 1955. Reactions to mosquito bites. Studies of causation and remedial measures. (Unpublished Ph.D. Thesis, Queen's University, Kingston, Ontario.)

MELLANBY, K. 1946. Man's reaction to mosquito bites. *Nature, Lond.*, 158:554.