

Passive Transfer between Experimental Animals of Hypersensitivity to *Aedes aegypti* Bites¹

John R. Allen²

Department of Biology, Queen's University, Kingston, Ontario, Canada.

(Submitted for publication, 7 June 1965)

ALLEN, J. R. Passive transfer between experimental animals of hypersensitivity to *Aedes aegypti* bites. *Experimental Parasitology* 19, 132-137. Attempts were made to demonstrate passive transfer between experimental animals of immediate-type hypersensitivity to *Aedes aegypti* bites. Seven out of eight guinea pig recipients of sera from donors sensitized by oral secretion and adjuvant showed immediate-type skin reactions when tested by *A. aegypti* bite. No passive transfer was demonstrated with sera from donors sensitized by *A. aegypti* bites, nor was transfer successful when rabbits were used as recipients. Spleen cells from donors sensitized by oral secretion and adjuvant were used in experiments to demonstrate passive transfer of delayed-type hypersensitivity to *A. aegypti* bite. Successful transfers were obtained with guinea pig recipients. The cells were apparently capable of transferring both immediate- and delayed-type hypersensitivities. No success was achieved with rabbit recipients.

INTRODUCTION

Evidence supporting the thesis that skin reactions to mosquito bites represent allergic phenomena was reviewed by McKiel and West (1961). A few reports of successful passive transfer between human subjects of hypersensitivity to mosquito bites are in the literature. Hecht (1929) reported one successful attempt at passive transfer of the immediate-type (wheal and erythema) hypersensitivity to bites of *Anopheles maculipennis* Meig. Brown *et al.* (1938) demonstrated passive transfer of immediate-type hypersensitivity to *Aedes aegypti* bite. In this case, serum from an individual exhibiting "skin necrosis" after *A. aegypti* bite was used in the

Prausnitz-Keustner procedure to transfer the hypersensitive state to two recipients. In the literature, there appears to be no report of any successful passive transfer of the delayed-type hypersensitivity to mosquito bites. Also, there are no reports of passive transfer between experimental animals of immediate- or delayed-type hypersensitivities to mosquito bites.

Work described here was designed to demonstrate passive transfers of both types of hypersensitivity between experimental animals.

MATERIALS AND METHODS

Oral secretion was collected from *A. aegypti* (L.) by the method of Allen and West (1962). Briefly, the method of collection involved inducing mosquitoes to probe a membrane which contained distilled water; as they probed, mosquitoes injected salivary components through the membrane, and the materials collected in this way were concentrated by freeze-drying. The freeze-dried oral

¹ This work was supported by research grant AI-01155 from the National Institutes of Health, United States Public Health Service and by research grant 6801-19 from the Defence Research Board of Canada to Professor A. S. West. It formed part of a Ph.D. thesis submitted by the author at Queen's University.

² Present address: College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

secretion was stored under vacuum in sealed flasks. Each flask contained a very small amount of material. The mean dry weight of oral secretion in 72 such flasks, was 0.433 mg per flask. However, since the fraction of active material in oral secretion was unknown and possibly inconstant, the doses of oral secretion given in these essentially qualitative experiments were expressed in "flask units."

Rabbits and guinea pigs were used as experimental animals. Rabbits were all-white males weighing 3-4 kg; guinea pigs were all-white males weighing 400-450 gm.

Guinea pigs and rabbits were subjected to injections of oral secretion with Freund's complete adjuvant. Oral secretion was dissolved in sterile physiological saline, buffered at pH 7, prepared as described by Evans (1922). Eight flasks of oral secretion were dissolved in each milliliter of saline and the solution was emulsified with an equal volume of Freund's complete adjuvant (Difco; 0638) before being injected. Injections containing 1.5 ml of oral secretion solution were given to guinea pigs; half the dose was given subcutaneously, bilaterally in the neck region, just anterior to each scapula, and half the dose was given intramuscularly bilaterally in the hind leg muscles. Rabbits received injections containing 3.5 ml of oral secretion solution, given in similar sites to those used in guinea pigs.

As previously shown (Allen and West, 1966), such procedures can sensitize experimental animals to *A. aegypti* bites. Sensitized animals may be obtained which show delayed-type reactions only, delayed- and immediate-type reactions, or immediate-type reactions only, corresponding to stages II, III, or IV of reactivity, as described in man by Mellanby (1946).

Passive Transfer of Immediate-Type Hypersensitivity

Serum samples were taken from the experimental animals before sensitization and at various stages during the sensitization course.

Serum samples were also taken from animals sensitized by *A. aegypti* bites.

Various volumes of the different sera were injected into recipient animals which had no known previous contact with mosquitoes. Injections were given intravenously, intraperitoneally or subcutaneously. Twenty-four hours later, recipient animals each received a test bite from *A. aegypti*.

The immediate-type skin reactions in experimental animals differ from the wheal and erythema reactions of man. McKiel (1959) noted that the wheal, in rabbits and guinea pigs, does not reach a maximum until approximately an hour after challenge with allergen, and erythema is usually lacking.

In these experiments, the skin sites for *A. aegypti* test bites were on the dorsolateral region of the shoulder or flank. And, as described by Allen and West (1966), the criterion for a positive immediate-type reaction was a wheal of more than 3.5 mm occurring 45 minutes after test bite, while the criterion for a positive delayed-type reaction was a reaction of at least 4 mm in diameter measured 24 hours after test bite. A statistical analysis had shown a very low probability ($p \ll 0.001$) that the diameters chosen represented too low values.

Passive Transfer of Delayed-Type Hypersensitivity

Animals sensitized by *A. aegypti* bites show no macroscopically visible delayed-type reactions to bites (McKiel, 1959). In these experiments, rabbits and guinea pigs were sensitized, as before, by injections of oral secretion and Freund's complete adjuvant. Both control, unsensitized animals and sensitized animals showing delayed- or delayed- and immediate-type reactions in response to *A. aegypti* bites were used as donors. Spleens were removed from donors under ether anesthesia. The organs were placed in a cold Tyrode solution made up as described by Boughton and Schild (1962). Cells were separated and suspended in fresh Tyrode solution, then

centrifuged at approximately $2.5 \times 10^3 \times g$ for 5 minutes. The supernatant was decanted off and fresh Tyrode solution added. This washing procedure was repeated twice more. All manipulations of organs and cells were carried out at 5°C. The final suspension of cells was injected slowly, intraperitoneally into a recipient animal. Approximately 10 ml of cell suspension were injected in each case, and an estimate of the number of cells injected was made on an aliquot of the suspension, using a hemocytometer. Recipient animals were tested by an *A. aegypti* bite 48 hours after the transfer of cells. Control transfers were made with cells from unsensitized animals.

In one experiment, the effect of heating cells at 60°C for 15 minutes was investigated. Cells were obtained from the spleens of 4

sensitized donors; they were mixed and washed and the suspension was divided into two approximately equal portions. One portion was injected into a recipient, and the other portion was held in a water bath at 60°C for 15 minutes before being injected into a second recipient guinea pig.

RESULTS AND DISCUSSION

Passive Transfer of Immediate-Type Hypersensitivity

From the results shown in Table I, it appears that successful passive transfers of immediate-type hypersensitivity to *A. aegypti* bites were achieved, with guinea pigs as recipients. These recipients, with the exception of guinea pig H, showed immediate-type hypersensitivity after receiving serum from rabbit or guinea pig donors sensitized by oral

TABLE I
Passive Transfer of Immediate-Type Hypersensitivity to Aedes aegypti Bites by Means of Sera

Serum donor	Donor's reaction to <i>A. aegypti</i>		Serum injection	Recipient	Recipient's reaction to <i>A. aegypti</i>	
	Imm. ^a	Del. ^a			Imm.	Del.
Rabbit U ^b	—	—	2 ml S/C ^c	G. pig A	—	—
Rabbit OS 1	+	—	1.5 ml I/V	G. pig B	+	—
Rabbit OS 1	+	—	2 ml S/C	G. pig C	+	—
Rabbit OS 1	+	—	2 ml S/C	G. pig D	+	—
Rabbit OS 2	—	+	2 ml I/V	G. pig E	—	—
Rabbit OS 3	—	+	2 ml S/C	G. pig F	—	—
Rabbit OS 4	—	+	2 ml I/P	G. pig G	—	—
Rabbit OS 5	+	+	1.3 ml I/V	G. pig H	—	—
Rabbit OS 6	+	+	2 ml S/C	G. pig I	+	—
Rabbit OS 7	+	+	2 ml S/C	G. pig J	+	—
Rabbit B 1	+	—	2 ml S/C	G. pig K	—	—
Rabbit B 2	+	—	2 ml S/C	G. pig L	—	—
Rabbit OS 1	+	—	6 ml I/V	Rabbit A	—	—
Rabbit OS 7	+	+	8 ml I/V	Rabbit B	—	—
G. pig OS 1	+	—	1 ml S/C	G. pig M	+	—
G. pig OS 2	+	—	2 ml S/C	G. pig N	+	—
G. pig B 1	+	—	2 ml S/C	G. pig O	—	—
G. pig B 1	+	—	2 ml S/C	G. pig P	—	—

^a Imm = immediate type reaction (45 minutes after bite). Del = delayed-type reaction (24 hours after bite).

^b U = unsensitized; OS = sensitized by oral secretion and adjuvant; B = sensitized by *A. aegypti* bites; I/V = intravenous; I/P = intraperitoneal.

^c S/C = subcutaneous.

secretion with adjuvant, when the donor showed immediate-type reactions to *A. aegypti* bite.

No transfer of hypersensitivity was demonstrable with sera from donors sensitized by *A. aegypti* bites. Several other similar attempts, in which sera from bite-sensitized donors were used, have also been unsuccessful (Dubin *et al.*, 1948; McKiel, 1955).

No transfer of hypersensitivity was evident in the two experiments in which rabbits were used as recipients. It is possible that insufficient volumes of serum were transferred in these experiments; Gell and Hinde (1954), for example, used volumes of 50-70 ml of plasma in their successful passive transfers of the "immediate component" of Arthus reactions between rabbits.

The passive transfers of immediate-type hypersensitivity that were demonstrated may well not be analogous to the Prausnitz-Keustner transfers demonstrable in man. The positive results shown might be interpreted as being examples of passive cutaneous anaphylaxis, a phenomenon which may be demonstrated in guinea pig recipients but not in rabbits (Ovary, 1958). Nonetheless, results in Table I show that sensitized animals may produce transferrable antibodies which react with antigens injected by the feeding mosquito. They also provide the first direct evidence of involvement of antibodies in experimental animals' skin reactions to mosquito bites.

Passive Transfer of Delayed-Type Hypersensitivity

Table II shows the results of experiments involving the transfer of spleen cells. Passive transfer of the delayed-type hypersensitivity to guinea pig recipients was apparently achieved with spleen cells from guinea pigs sensitized by oral secretion with adjuvant. Cells from donors which also exhibited immediate-type reactions to *A. aegypti* bite apparently transferred both the delayed- and

immediate-type hypersensitivities to guinea pig recipients.

No successful passive transfers of hypersensitivity between rabbits was demonstrable. Possibly insufficient numbers of cells were transferred.

When spleen cells were taken from 4 donors (guinea pigs 10-13) and pooled, one aliquot of these cells injected into recipient guinea pig F apparently produced transfer of hypersensitivity, whereas the second (heat-treated) aliquot of these cells produced no demonstrable transfer of hypersensitivity to guinea pig G.

In this type of passive transfer experiment, it is possible that active sensitization by transferred allergen may occur. Turk (1961) has shown this to happen when cells were transferred from tuberculin-sensitized animals to normal recipients. Turk concluded that a transient delayed hypersensitivity could be transferred by intact leucocytes, spleen cells or lymph node cells, and that this could be followed in some cases by active sensitization which may persist far beyond the time when donor cells would have been destroyed by homograft reaction. Such active sensitization was presumed to be brought about by small amounts of allergen transferred with the cells.

In the experiments reported here, it seems unlikely that positive reactions obtained in recipients test-bitten 2 days after cell transfer could have been the results of active sensitization. Allen and West (1966) showed that no positive delayed-type reactions were obtained in guinea pigs sensitized by oral secretion plus adjuvant until at least 7 days had elapsed after the injection. Also, from Table II, it may be seen that heat treatment of spleen cells for 15 minutes at 60°C appeared to destroy their ability to transfer the hypersensitivity. Such heat treatment did not destroy the sensitizing properties of oral secretion (Allen and West, 1966). It seems reasonable to conclude that at least a tran-

TABLE II
Passive Transfer of Hypersensitivity to Aedes aegypti Bites by Means of Spleen Cells^a

Cell donor	Donor's reaction to <i>A. aegypti</i>		Cells transferred	Recipient	Recipient's reaction to <i>A. aegypti</i>	
	Imm.	Del.			Imm.	Del.
G. pig						
1	—	—	2×10^9 I/P	G. pig A	—	—
2	—	—				
3	—	+	2×10^9 I/P	G. pig B	—	+
4	—	+				
5	—	+	2×10^9 I/P	G. pig C	+	+
6	+	+				
7	+	+	3×10^9 I/P	G. pig D	+	+
8	+	+				
9	+	+	1×10^9	G. pig E	+	+
Rabbit						
1	—	+	2×10^9 I/V	Rabbit A	—	—
2	+	+	4×10^9 I/P	Rabbit B	—	—
3	+	+				
4	+	+	3×10^9 I/P	Rabbit C	—	—
5	+	+				
G. pig						
10	+	+	2×10^9 , I/P	G. pig F	+	+
11	+	+				
12	—	+	2×10^9 heat- ^b treated cells, I/P	G. pig G	—	—
13	+	+				

^a See Table I footnotes for explanation of abbreviations.

^b Heat-treatment of cells: 60°C for 15 minutes before injection into recipient (G. pig G).

sient passive transfer of delayed-type hypersensitivity was obtained in these experiments. No such demonstration has previously been reported.

It is of interest that the transfer of cells from donors showing immediate- and delayed-type reactions caused recipient guinea pigs to show both types of reaction in response to test bite. The appearance of circulating antibodies as well as delayed-type reactions in recipients of washed cells has been noted by Chase (1951) and Harris *et al.* (1954). The experimental results obtained by us also give an indication that the transferred cells were responsible for the production of circulating

antibody (and immediate-type reactions) in recipients.

REFERENCES

- ALLEN, J. R., AND WEST, A. S. 1962. Collection of oral secretion from mosquitoes. *Mosquito News* **22**, 157-159.
- ALLEN, J. R., AND WEST, A. S. 1966. Some properties of oral secretion from *Aedes aegypti*. *Experimental Parasitology* **19**, 124-131.
- BOUGHTON, B., AND SCHILD, H. O. 1962. Transfer of delayed hypersensitivity by lymph node cells in testis autosensitization. *Immunology* **5**, 522-532.
- BROWN, A., GRIFFITHS, T. H. D., ERWIN, S., AND DYRENFORTH, L. Y. 1938. Arthus phenomenon from mosquito bites. *Southern Medical Journal* **31**, 590-595.

- CHASE, M. W. 1951. Development of antibody following transfer of cells taken from lymph nodes of sensitized or immunized animals. *Federation Proceedings* 10, 404-405.
- DUBIN, I. N., REESE, J. D., AND SEAMANS, L. A. 1948. Attempt to produce protection against mosquitoes by active immunization. *Journal of Immunology* 58, 293-297.
- EVANS, A. C. 1922. A buffered physiologic salt solution. *Journal of Infectious Diseases* 30, 95-98.
- GELL, P. G. H., AND HINDE, I. T. 1954. Observations on the histology of the Arthus reaction and its relation to other known types of skin hypersensitivity. *International Archives of Allergy and Applied Immunology* 5, 23-46.
- HARRIS, S., HARRIS, T. N., AND FARBER, M. B. 1954. Studies on the transfer of lymph node cells. I. The appearance of antibody in recipients of cells from donor rabbits injected with antigen. *Journal of Immunology* 72, 148-160.
- HECHT, O. 1929. Die Hautreaktionen auf Insektenstiche als allergische Erscheinungen. *Archiv für Schiffsund-Tropenhygiene* 33, 364-366.
- OVARY, Z. 1958. Immediate reactions in the skin of experimental animals provoked by antibody-antigen interaction. *Progress in Allergy* 5, 459-508.
- McKIEL, J. A. 1955. Reactions to mosquito bites, studies of causation and remedial measures. Ph.D. thesis, Queen's University, Kingston, Ontario.
- McKIEL, J. A. 1959. Sensitization to mosquito bites. *Canadian Journal of Zoology* 37, 341-351.
- McKIEL, J. A., AND WEST, A. S. 1961. Nature and causation of insect-bite reactions. *Pediatric Clinics of North America* 8, 795-816.
- MELLANBY, K. 1946. Man's reactions to mosquito bites. *Nature* 158, 554.
- TURK, J. L. 1961. Active sensitization to tuberculin with cells from tuberculin-sensitized guinea pigs. *Nature* 189, 889-890.