HOST TEMPERATURE AND THE TRANSMISSION OF ARBOVIRUSES BY MOSQUITOES

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ABSTRACT. The viremic period in arbovirus infections of primates is frequently accompanied by a rise in body temperature. It has been suggested that this response of the vertebrate host may be advantageous to the parasite, since the raised temperature may enhance the attractiveness of the host to feeding mosquitoes. The viremia is usually transient, lasting for 2–4 days only, and when followed by life-long immunity, it is the only period in the whole life-span of the primate during which virus is available for transmisson.

This hypothesis is now supported by the results of laboratory tests, in which mosquitoes were allowed the choice of alternative artificial warm sources, one of them 1–3° C warmer than the other. Provided that both sources were present simultaneously the mosquitoes selected the warmer of the two in significantly greater numbers.

INTRODUCTION

Whereas the parasites of malaria and filariasis appear in the peripheral blood stream in their transmissible form at regular intervals (according to their respective circadian rhythms) and are thus available to feeding mosquitoes night after night (Hawking et al. 1968, Gillett 1968, 1971), arboviruses, on the other hand, tend to be available for transmission during a single viremic episode in the whole life-span of the vertebrate host. In primates the typical viremia lasts for 2 to 4 days and is followed by life-long immunity. Since, however, the feeding activity of the mosquitoes, on which transfer and, therefore, survival of the virus depends, is governed largely by their own circadian rhythm, the actual period during which virus can escape from the vertebrate host may be very restricted indeed.

In yellow fever among African monkeys the virus is transmitted by the arboreal mosquito *Aedes (Stegomyia) africanus* Theobald, whose feeding activity occurs during the single hour after sunset (Haddow et al. 1947). Moreover, there is no similar feeding peak at dawn; and, as Lumsden (1952) has subsequently shown, the actual feeding period each evening may be as short as 20 min. This means that although the virus may be continuously present in the peripheral blood for 48–96 hr and at a sufficiently high titre for take-up for about half of this period, the rigid regime of the mosquitoes may lop the effective period down to a total of a mere 40–60 min in the whole life of the vertebrate.

The viremic period in primates is typically accompanied by a rise in body temperature of 0.5 to 3° C, the highest temperature recorded by us being 41.1° C (Gillett et al. 1950). Now the warm air surrounding or rising from the host's body is known to be one of the short range attractants to feeding mosquitoes (Howlett 1910, Marchand 1918, Peterson and Brown 1951, Wright 1968); and Gillett (1974) has suggested that the higher body temperature of the viremic vertebrate may be advantageous to the virus in that more mosquitoes may be attracted to it than to its aviremic neighbors. We have now tested this hypothesis using artificial warm sources in place of living primates.

MATERIALS AND METHODS

THE MOSQUITOES. Female pupae of *Aedes (Stegomyia) aegypti* (L.) were taken from the Brunel colony (a strain of mixed origin, coming mainly from the London School of Hygiene and Tropical Medicine but with an admixture from the Wellcome Laboratories, Berkhampstead). The pupae were washed twice in tap water and placed on moist absorbent tissue on the floor of the test chambers. The adult mosquitoes, which emerged from the pupae already inside the test chambers, remained unfed, except for the water which they sucked up from the moist tissue.

THE TEST CHAMBERS. The containers, in which each test was made were of three types. They consisted of acrylic (perspex) cylinders 10 cm. in diameter and 10 cm. in length (12 cm. in length in type 3). Types 1 and 2 stood vertically and allowed, respectively, for a single or for two warm tubes to be placed at the top of the cylinder; type 3 stood horizontally and allowed for two tubes to be placed simultaneously, one at either end. Types 1 and 2 were placed on 10 sheets of folded tissue (Kleenex medical wipes) on the floor of a white enamel rectangular photographic dish; the tissue was kept constantly moist by the addition of water to the photographic dish both before emergence of the adults from the pupae and afterwards. In type 3 it was necessary to place the tissue inside the horizontal cylinder and to keep it moist by irrigation through a Pasteur pipette.

THE ARTIFICIAL WARM SOURCE. Flexible polyethylene (polythene) tubing, 1 cm. outside diameter, circulating warm water from a water bath of constant temperature was used as the warm source. The tubing was clipped into a groove at the top or at the ends of the test chambers (Figure 1). The temperature measured at the site of the test chambers, after an initial warm-up period of 10 min. was ca. 0.5° C lower than that of the water bath.

PROCEDURE. When the adult mosquitoes, already inside the test chambers, were not less than 2 days old, and usually between 5 and 6 days old, the tubing carrying the flow of warm water was placed in the groove or grooves at the top or at the ends of the test chambers, and the maximum number of mosquitoes probing simultaneously within a period of 2 min was recorded. Before each experiment the tubing was washed in alcohol and all manipulations were made with the experimenter wearing surgical rubber gloves. Precautions were also taken against breathing over or near the test chambers.

Each experiment consisted of a number of runs, each of which was made up of a series of tests, in any one of which the number of mosquitoes was restricted in order to ensure accurate counting of the insects probing at the warm tube or tubes. In each alternate test the conditions were reversed: if the insects were presented with the cooler tube before being presented with the warmer tube first in one test, they were presented with the warmer tube first in the next (Experiment 1);

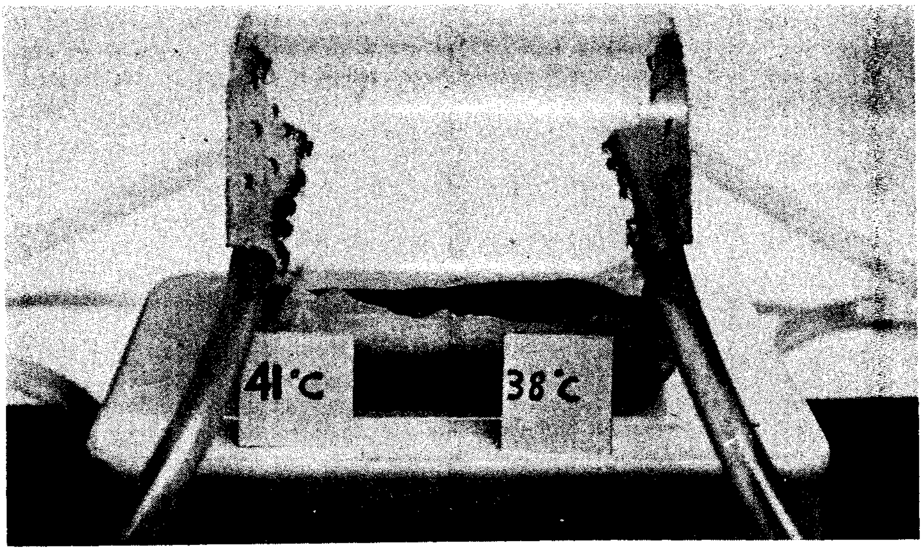

Fig. 1. Type 3 test chamber showing arrangement for testing response of mosquitoes to two warm tubes at different temperatures.

if the warmer tube was placed on the left of the test chamber in one test, it was placed on the right in the next.

The room in which the experiments were conducted was maintained at a mean temperature of 24° C and RH 55–70%.

RESULTS

Experiment 1 using type 1 test-chamber was planned to find out whether the mosquitoes would show any preference for one warm source or the other when these were 3° C apart and presented separately, one after the other. The results are set out in Table 1, and show clearly that the mosquitoes failed to discriminate between the two; 531/1048 (51%) of the probing mosquitoes chose the warmer tube —X^2 $(n=1)=0.187$ $p>0.50$.

Experiments 2–4 using types 2 and 3 test-chambers were planned to find out whether the mosquitoes would show any preference for one warm source or the other when these were presented simultaneously with temperature differences of 1°, 2° and 3° C. The two warm sources were 12 cm. apart except in Runs 1 and 2 of Experiment 4 in which type 2 chambers were used; these allowed for only 6 cm. between the two warm tubes.

The results are set out in Tables 2–4

Table 1. Attraction of mosquitoes (*Ae. aegypti*) to two artificial warm sources, presented sequentially—one 3° C warmer than the other.

Run No.	No. of tests per run	No. of mosquitoes per test	Maximum number of mosquitoes probing during 2 min. exposure at 37° C	(%)	at 40° C	(%)	X^2 (n=1)	p
1	12	15	121	(48)	129	(52)	0.256	>0.50
2	7	20	122	(52)	112	(48)	0.427	>0.50
3	10	20	167	(49)	170	(51)	0.027	>0.80
4	10	16	107	(47)	120	(53)	0.745	>0.30

X^2 $(n=3) = 1.454$ $p > 0.50$.

Table 2. Attraction of mosquitoes (*Ae. aegypti*) to two artificial warm sources, presented simultaneously —one 1° C warmer than the other.

Run No.	No. of tests per run	No. of mosquitoes per test	Maximum number of mosquitoes probing during 2 min. exposure				X^2 (n=1)	p
			at 39° C	(%)	at 40° C	(%)		
1	16	50	47	(47)	54	(53)	0.485	>0.30
2	10	47	130	(45)	158	(55)	2.722	>0.05
3	10	47	102	(41)	145	(59)	7.486	<0.01

X^2 (n=2) = 10.694 $p < 0.01$.

Table 3. Attraction of mosquitoes (*Ae. aegypti*) to two artificial warm sources, presented simultaneously —one 2° C warmer than the other.

Run No.	No. of tests per run	No. of mosquitoes per test	Maximum number of mosquitoes probing during 2 min. exposure				X^2 (n=1)	p
			at 38° C	(%)	at 40° C	(%)		
1	12	47	130	(41)	188	(59)	10.579	<0.01
			at 39° C	(%)	at 41° C	(%)		
2	10	47	136	(46)	159	(54)	1.793	>0.10
3	10	47	104	(43)	143	(57)	6.158	<0.02

X^2 (n=2) = 18.530 $p < 0.001$.

Table 4. Attraction of mosquitoes (*Ae. aegypti*) to two artificial warm sources, presented simultaneously —one 3° C warmer than the other.

Run No.	No. of tests per run	No. of mosquitoes per test	Maximum number of mosquitoes probing during 2 min. exposure				X^2 (n=1)	p
			at 38° C	(%)	at 41° C	(%)		
1	41	48	325	(45)	395	(55)	6.806	<0.01
2	44	27	175	(42)	241	(58)	10.471	<0.01
3	13	80	247	(39)	381	(61)	28.592	<0.001

X^2 (n=2) = 45.870 $p < 0.001$.

and show a consistent preference for the warmer tube:

at 1° C difference 357/636 (56%) of the probing mosquitoes chose the warmer tube—X^2 (n=1)=9.566, $p<0.01$.
at 2° C difference 490/860 (57%) of the probing mosquitoes chose the warmer tube—X^2 (n=1)=16.744, $p<0.001$.
at 3° C difference 1017/1764 (58%) of the probing mosquitoes chose the warmer tube—X^2 (n=1)=41.327, $p<0.001$.
These results suggest a slight trend towards increased discrimination with increasing difference of temperature, but there is, in fact, no significant difference between the results at 1° C difference and at 3° C (X^2 (n=1)=0.442 $p>0.50$).

DISCUSSION

Anyone who has conducted transmission trials with live viruses and live primates will know how difficult it may be to judge when the viremia has reached a sufficient titre to risk feeding the mosquitoes. If the trial is made too soon the titre of virus circulating may be too low to infect a significant proportion of the insects; if it is made too late the level of neutralizing antibodies may already have started to rise. These difficulties are particularly acute when one is dealing with inapparent infections, as is often the case with yellow fever virus in African primates.

Fortunately, even in so-called inapparent infections, the peak of viremia is often accompanied by a short lasting rise in temperature. Indeed, it is routine procedure in such trials for the experimenter to read the temperature of the primate in question twice a day from the time of infection onwards (or from a few days beforehand) and more frequently as the expected incubation period draws to a close. In previous transmission trials made by one of us (J.D.G.) on yellow fever, Ntaya, Zika and chikungunya viruses in primates, this procedure was always followed and the characteristic rise in temperature, often only slight, was frequently the only clue that optimum conditions had been reached for the trial, although with some species of primates temperature changes have not always proved reliable (Hughes 1943).

This was the background that led to the suggestion that, despite the maxim that a 'good' parasite produces minimum disturbance to its host, it may in fact be advantageous for the virus to bring about a rise in temperature in the vertebrate host. Indeed, with the critical period of virus availability so severely reduced by the transient nature of the single viremic episode and compounded by the highly restricted feeding periods of the invertebrate hosts, it was argued, *a priori,* that some other factor must intervene to tip the balance in favor of transfer if the parasite is to survive (Gillett 1974).

In our experiments using 2 warm sources simultaneously, each within the range of normal and febrile primates respectively, the warmer source was selected in significantly greater numbers; the results in each of Tables 2–4 show ratios of 1.28, 1.32 and 1.36 in favor of the warmer source. Indeed in all 9 separate runs the results are in the expected direction, even when the difference is not significant.

Recent work by Edman and Webber (1975), investigating the significance of host size, showed that cockerels attracted more mosquitoes when in direct competition with rabbits. The results of our experiments suggest that temperature may have been the key factor in these results: the normal body temperatures of rabbit and cockerel are 38.4° and 40.5° C respectively.

Temperature, however, is only one of several stimuli that serve to attract mosquitoes to the host. But a rise in temperature, even of modest proportions, is liable to increase body odor and skin moisture, both factors known also to influence feeding mosquitoes (Smart and Brown 1956, Platt et al. 1957, Brouwer 1960). In nature, then, the febrile reaction accompanying the viremic phase may be much more powerful than our experiments indicate, using temperature in isolation. The critical experiment would be to make the test using normal and viremic primates.

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