

THE INCUBATION PERIOD OF YELLOW FEVER IN THE MOSQUITO.

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During the yellow fever epidemic at Orwood and Taylor, Mississippi, in 1898, Carter (1, 2) noted that an interval of from 2 to 3 weeks elapsed between the first infecting cases and the secondary cases arising therefrom. This observation antedates, of course, the demonstrations of mosquito transmission of the disease. In 1900 Reed, Carroll, Agramonte and Lazear (3) in establishing the fact of mosquito transmission discovered that a period of about 12 days after biting was required before the mosquito was capable of inducing the infection in normal individuals. They showed, for example, in one experiment, that several mosquitoes which had fed on yellow fever patients, were non-infective on the 4th and 7th days and infective to the same individual on the 17th day following the original feeding. Guiteras (4) confirmed this finding. Mosquitoes having fed on a yellow fever patient were non-infective to a susceptible person on the 5th and infective on the 20th day after the feeding. Marchoux, Salimbeni and Simond (5) who made numerous such tests concluded that at least 12 days must elapse between the feeding of mosquitoes (*Aedes ægypti*) on yellow fever patients and their ability to transmit the infection to fresh susceptible persons. This period between the initial infecting feeding and the time at which the mosquitoes are capable of inducing infection explains and corresponds to what has been called by Carter "extrinsic incubation."

Now that it has been shown (6) that the virus of yellow fever is transmissible to *Macacus rhesus*, in which species of monkey an experimental disease is produced corresponding to yellow fever in man, it becomes possible to determine more precisely the exact period of this extrinsic incubation. Earlier experiments (6) had shown that whereas

the virus is readily filtrable when in the circulating blood, the filtrates prepared from mosquitoes which had fed on infected animals were ineffective on inoculation. The experiments to follow were designed for the purpose of elucidating the different states in which the virus is supposed to exist in the mosquito during extrinsic incubation and in man and monkey during actual infection. A uniform number of mosquitoes were made to bite normal *Macacus rhesus* monkeys at various intervals after the infectious feed, after which they were ground up and injected into susceptible monkeys.

The temperature in the storage cage in which the mosquitoes were kept during these experiments was registered with a recording thermometer and was found to vary daily between 74° and 90°F.; the coolest time was between 6 and 8 o'clock in the morning and the warmest between 3 and 5 in the afternoon.

Experiment 1.—On January 28, 1928, a normal *rhesus*, No. 50-6, was exposed to Lot 27 of infected *A. ægypti*, eighteen of which fed upon this animal. On February 3 the monkey showed a temperature of 105.8°F., and on that date Lot 35, consisting of 95 normal females, *Aedes ægypti*, were made to feed on the animal.

At intervals of 48 hours, five mosquitoes of this lot were transferred to a suitable cage and a normal monkey exposed until each of these five insects had fed. The mosquitoes were then caught, killed with tobacco smoke, ground up in a mortar with 3.0 cc. of salt solution and the emulsion injected subcutaneously into another normal *rhesus* monkey. This procedure was carried out 2, 4, 6, 8, 10 and 12 days after the original feeding of the mosquitoes on *Rhesus* 50-6. The mosquitoes of this lot died very rapidly so that on February 15, *i.e.*, on the 12th day after the original feeding, only three remained alive, which were used instead of five.

As seen from Table I, the results of this experiment were irregular. None of the mosquitoes which fed on normal monkeys 2, 4, 6, 8 and 10 days after the original infectious feed induced disease, while those tested on the 12th day produced infection. On the other hand, 2, 4 and 8 day mosquitoes, which failed to infect by biting, proved infectious when used for injection into monkeys. 12 day mosquitoes produced infection by both methods of inoculation, while 6 and 10 day insects failed by biting and by injection. All monkeys failing to respond were shown to be susceptible by subsequent inoculation with virulent blood or by biting with known infected mosquitoes.

The length of life of a female *A. ægypti*, under laboratory conditions,

irrespective of whether the insect is infected with yellow fever virus or not, averaged in our experience about 2 months, and about 10 per cent of the insects usually lived over 3 months. Occasionally, however, some batches of mosquitoes became subject to epidemics in which all insects that happened to be in one cage died within a few days, irrespective of their age, food, surrounding temperature, humidity, etc.

TABLE I.
Experiment 1. Mosquitoes, Lot 35, Infected February 3, 1928.

Date of experiments	No. of days after original feeding	No. of monkey	Mode of transmission	Results
Feb. 5	2	51-1	5 mosquitoes fed	No reaction
" 5	2	51-2	Same mosquitoes macerated and injected	Feb. 17. Died. Postmortem findings typical
" 7	4	51-3	5 mosquitoes fed	No reaction
" 7	4	51-4	Same mosquitoes macerated and injected	Feb. 13. Died. Postmortem findings typical
" 9	6	51-5	5 mosquitoes fed	No reaction
" 9	6	51-6	Same mosquitoes macerated and injected	No reaction
" 11	8	51-7	5 mosquitoes fed	No reaction
" 11	8	51-8	Same mosquitoes macerated and injected	Feb. 17. Died. Postmortem findings typical
" 13	10	51-9	5 mosquitoes fed	No reaction
" 13	10	52-0	Same mosquitoes macerated and injected	No reaction
" 15	12	52-1	3 mosquitoes fed	Feb. 26. Died. Postmortem findings typical
" 15	12	52-2	Same mosquitoes macerated and injected	Feb. 24. Died. Postmortem findings typical

According to Howard, Dyar and Knab (7), these epidemics have been studied by numerous workers and have been attributed mostly to fungi and occasionally to bacteria which are supposed to invade the digestive tract and the body cavities. Having no other explanation, it seems to us that the high mortality in the mosquitoes of Lot 35 was due to one of these parasitic infections. If this was the case, it seems also possible that the yellow fever virus might in the 6 and 10 day mosquitoes have been destroyed by the invading organisms. This

hypothesis seems to be supported by the results obtained in subsequent experiments.

Experiment 2.—*Rhesus* 51-8, which in the above experiment on February 11 had been inoculated subcutaneously with the emulsion of five macerated mosquitoes, continued to show normal temperature until February 15, when the temperature suddenly rose to 105.3°F. The monkey was immediately exposed to a lot of

TABLE II.
Experiment 2. Mosquitoes, Lot 36, Infected February 15, 1928.

Date of experiments	No. of days after original feeding	No. of monkey	Mode of transmission	Results
Feb. 18	3	54-5	10 mosquitoes fed	No reaction
" 18	3	54-6	Same mosquitoes macerated and injected	Feb. 27. Died. Postmortem findings typical
" 20	5	54-7	10 mosquitoes fed	No reaction
" 20	5	54-8	Same mosquitoes macerated and injected	Feb. 27. Died. Postmortem findings typical
" 22	7	54-9	10 mosquitoes fed	No reaction
" 22	7	55-0	Same mosquitoes macerated and injected	Mar. 1. Died. Postmortem findings typical
" 24	9	55-1	10 mosquitoes fed	Mar 2. Died. Postmortem findings typical
" 24	9	55-2	Same mosquitoes macerated and injected	Feb. 29. Died. Postmortem findings typical
" 26	11	55-3	10 mosquitoes fed	Mar. 2. Died. Postmortem findings typical
" 26	11	55-4	Same mosquitoes macerated and injected	Mar. 6. Died. Postmortem findings typical
" 28	13	55-5	10 mosquitoes fed	Mar. 5-9. Fever. Recovered
" 28	13	55-6	Same mosquitoes macerated and injected	Mar. 8. Died. Postmortem findings typical

normal *A. ægypti*, of which 130 became engorged. These were labelled "Lot 36" and were used in the next experiment.

Beginning on the 3rd day after feeding on *Rhesus* 51-8, and thereafter on every other day, ten mosquitoes were transferred to a suitable cage and fed on a normal monkey. After the monkey was removed, the engorged mosquitoes were caught, killed with tobacco smoke, ground up in a mortar with 3.0 cc. of salt solution and the emulsion injected subcutaneously into another normal monkey. This procedure was carried out on the 3rd, 5th, 7th, 9th, 11th and 13th days from the date when the mosquitoes had originally fed on the infected monkey. No mortality

occurred among the mosquitoes of this lot to interfere with the completion of the experiment.

The results are summarized in Table II. This experiment shows that the virus is definitely infectious during the entire period of the extrinsic incubation. Typical yellow fever was produced in monkeys by injecting the substance of the mosquitoes at any time after the insects had ingested the virus from an infected animal. Although then present in the mosquitoes in an infectious form, the virus was not, however, transmitted by bite until from the 9th day on. As seen from this table, all the animals which developed infection, either as a result of the injection of mosquito emulsion or through bite of the infected insects, died with the exception of one, *Rhesus* 55-5, which had been bitten by the 13 day mosquitoes. 5 days after the bite this monkey developed high temperature which continued for another 5 days. Final recovery ensued and upon subsequent inoculation with a large dose of virulent blood, the animal again ran a febrile period but did not succumb. The three monkeys which were bitten by 3, 5 and 7 day mosquitoes and did not respond, were found as susceptible as normal animals to later inoculation with virulent blood.

In order to confirm these findings, one more experiment was carried out, with the same technique and the same number of mosquitoes, but over the time intervals at which Experiment 1 was performed.

Experiment 3.—*Rhesus* 51-3, which in Experiment 1 had been bitten by 4 day mosquitoes without response, was on February 20 exposed to two infected mosquitoes, of which one only fed. On the afternoon of February 23, the animal's temperature was 106.0°F.; it was then exposed to a lot of normal *A. ægypti* and 102 of these became engorged. These, labelled "Lot 42," were used in the following experiment.

Beginning on the 4th day after feeding on *Rhesus* 51-3, and thereafter on every other day, ten of these mosquitoes were placed in a suitable cage and made to feed on a normal monkey. After the monkey was removed, the engorged mosquitoes were caught, killed with tobacco smoke and ground up in a mortar with 3.0 cc. of salt solution. The emulsion was injected subcutaneously into another normal monkey.

The results are given in Table III. All monkeys which had been inoculated with macerated mosquito emulsions at various intervals after the insects had their infectious feed, died of typical yellow fever infection. Those, on the other hand, which had been bitten by the

same mosquitoes at the same intervals, gave no response with the exception of one monkey, No. 56-5, on which 12 day mosquitoes were allowed to feed, and which died of typical yellow fever. Monkeys 55-7, 55-9 and 56-1 were later either inoculated with virulent blood or exposed to mosquitoes which were known to be infected and died of yellow fever, thus proving their susceptibility. However, Monkey 56-3, which was bitten by 10 day mosquitoes and showed no reaction,

TABLE III.

Experiment 3. Mosquitoes, Lot 42, Infected February 23, 1928.

Date of experiments	No. of days after original feeding	No. of monkey	Mode of transmission	Results
Feb. 27	4	55-7	10 mosquitoes fed	No reaction
" 27	4	55-8	Same mosquitoes macerated and injected	Mar. 10. Died. Postmortem findings typical
" 29	6	55-9	10 mosquitoes fed	No reaction
" 29	6	56-0	Same mosquitoes macerated and injected	Mar. 8. Died. Postmortem findings typical
Mar. 2	8	56-1	10 mosquitoes fed	No reaction
" 2	8	56-2	Same mosquitoes macerated and injected	Mar. 11. Died. Postmortem findings typical
" 4	10	56-3	10 mosquitoes fed	No reaction; later proved immune
" 4	10	56-4	Same mosquitoes macerated and injected	Mar. 16. Died. Postmortem findings typical
" 6	12	56-5	10 mosquitoes fed	Mar. 16. Died. Postmortem findings typical
" 6	12	56-6	Same mosquitoes macerated and injected	Mar. 12. Died. Postmortem findings typical

proved refractory when given 2 weeks later a large dose of virulent blood. It is impossible to determine whether the last animal was naturally immune or had acquired immunity as the result of the mosquito bite.

SUMMARY.

1. The yellow fever virus was found in infectious form in *Aedes ægypti* throughout the entire period of the extrinsic incubation, as demonstrated by the injection of the bodies of mosquitoes into normal

rhesus monkeys at daily intervals after the insects had fed on an infected animal.

2. The virus was transmitted through the bite of the mosquitoes, in one experiment on and after the 9th day, and in two experiments on the 12th day after the initial infecting feed.

3. The pathologic changes produced by the injection of the infected mosquitoes into normal monkeys during the extrinsic incubation were in every respect those of typical experimental yellow fever.

4. The monkeys withstand easily the subcutaneous injection of the mosquito emulsion. No acute inflammatory reaction was observed at the site of injection in any of the seventeen animals inoculated with this material in these three experiments.

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