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POSSIBILITY OF HEREDITARY TRANSMISSION OF YELLOW FEVER VIRUS BY AEDES AEGYPTI (LINN.)

By CORNELIUS B. PHILIP

(*From the West African Yellow Fever Commission, International Health Division of the Rockefeller Foundation, Lagos, Nigeria*)

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Hereditary passage of yellow fever virus through mosquito eggs was first investigated by Marchoux and Simond (1906) who reported successful transmission to a human being through the first generation offspring of an infective mosquito. Rosenau and Goldberger failed to confirm this work; in fact none of thirteen susceptible human beings, bitten by mosquitoes reared from eggs of infected female stegomyia, showed any signs of yellow fever. Stokes, Bauer and Hudson (1928) also obtained negative results when they allowed 79 mosquitoes, reared from the eggs of infective females, to bite a normal *rhesus* monkey. Hindle (1929) has pointed out the importance of further experimentation, especially as he has shown by inoculation of various parts of infected mosquitoes that the virus is not confined to the salivary glands. It was felt by the West African Yellow Fever Commission that information on this important point was still inadequate, and at the suggestion of Dr. Henry Beeuwkes, the problem was again taken up.

EXPERIMENTAL

Two methods were employed, direct inoculation into test animals of an emulsion of eggs laid by infective mosquitoes, and transmission tests with adults reared from such eggs. Since it has been shown by injection (Bauer and Hudson, 1928) that yellow fever virus is present in infectious form in adult mosquitoes at all times subsequent to their infecting blood-meal, it seems logical to assume that, if virus is present in the ova, it could be detected by similar injection procedures. This seems especially likely when large numbers of ova are employed as in the experiments here reported.

A group of 172 normal females of *Aedes aegypti* was fed on an infected monkey during the initial period of fever (Asibi strain of virus). These mosquitoes were subsequently shown to be infective, since all normal monkeys bitten by them died of typical yellow fever. The mosquitoes were then segregated and 65 males added to obtain fertile eggs. At desired intervals ova were collected on strips of continuously wet blotting paper. The ova were never allowed to dry. All were used within two to three days and the majority within twenty-four hours after oviposition. Batches of eggs were removed after the first or infecting blood-meal, and after the second, fourth and fifth testing (normal) meal, that is 14, 41 and 54 days after the first feeding. Of the first two batches of ova, one half were employed for direct injection into test animals, and the other half placed in water to obtain adults for subsequent feeding tests. As the original lot of mosquitoes laid an insufficient number of eggs after the fourth blood-meal, they were given a fifth meal for the rearing of adults to be used in additional biting experiments.

It is known that in certain diseases (Rocky Mountain spotted fever particularly), there is an "activation" of the etiologic agent following a blood-meal of the maturing arthropod host. For that reason, more than one feeding of blood was allowed for each lot of mosquitoes, both parent and offspring. After the first feeding of each batch there was allowed a period of time at least as long as the incubation period (8 days or more). This gave a check on the relationship of both time and number of blood-meals of parents and progeny to the problem in hand.

Experiment I

A. *Eggs.* Approximately 400 eggs, laid soon after the first infecting feeding, were washed off of the blotting paper into 50 c.c. normal saline and gently centrifuged. Three c.c. of the supernatant fluid were drawn off and injected into normal *M. rhesus* C; the remainder of the liquid was discarded. The animal registered normal temperatures for 15 days. It was then found susceptible by the injection of known virulent blood, and, during the febrile attack which resulted, was killed as a source of virus for other experiments.

After centrifugalizing, the ova were drained, ground in a sterile mortar with neutralized glass powder, diluted with 5 c.c. normal saline, and 2.5 c.c. of the resulting mixture injected into each of two normal monkeys, A and B. No reaction followed for a period of 15 days and both died of yellow fever when tested later for susceptibility.

B. *Adults.* The second half of the eggs were immersed while still wet and the adult females that emerged, designated as Lot 185, were used in the following three tests:

1. A normal *rhesus* (D), was bitten by 254 insects of this hatching. No apparent reaction occurred over a period of 19 days, nor was any noted when it was tested for susceptibility with known infectious material. In the light of the subsequent experiments, it seems probable that this animal was insusceptible to yellow fever rather than immunized by the bites of the mosquitoes.

2. Fourteen days after they had been fed on Monkey D, 110 specimens of the same lot were allowed to bite another normal monkey, (E). This animal continued to show normal temperatures for a period of 17 days. It was then inoculated with proved infectious blood, but although a sharp febrile reaction resulted, the monkey recovered.

3. Forty-two days after the first feeding, the 96 insects remaining alive were stupefied with tobacco smoke and macerated in 4 c.c. of normal saline. The suspension was then injected subcutaneously into normal *M. rhesus* F. No reaction was noted during a period of 17 days in this animal which when later exposed to the bites of an infective lot of mosquitoes, died of typical yellow fever.

Experiment II

A. *Eggs.* The parent lot of mosquitoes was given a second blood-meal on a normal animal 14 days after their infecting blood-meal. Approximately 400 ova were collected and treated exactly as in Experiment I. Injection of supernatant fluid after concentration in the centrifuge was omitted in this and in the following experiments. Two normal monkeys, G and H, were injected with 2.5 c.c. each of the egg emulsion, as before. The animals remained normal for 15 days and then proved susceptible to inoculation of virulent blood, developing fatal yellow fever.

B. *Adults.* After removal of enough for use in section A of this experiment the remainder of the eggs of the second laying by the original mosquito lot was immediately immersed for hatching without drying. The adult females that emerged were labelled Lot 187 and employed in the two following tests:

1. A normal monkey (I) was bitten by 350 insects of this lot 38 days after the original infecting meal of the parent lot. After a period of 22 days, the animal still remained normal. It died with characteristic lesions following inoculation of virulent blood.

2. Two hundred and fifty *A. aegypti* of the same lot fed on a second normal *rhesus* (J) 14 days after the first test. No reaction occurred during the following 3 weeks and, when tested for susceptibility with infectious blood, this animal succumbed to yellow fever.

Experiment III

A. *Eggs.* Ova were again collected after the fourth blood-meal of the parent lot of aedes on a normal monkey, 41 days from the time of their initial feeding. About 400 eggs were washed, concentrated and ground in a sterile mortar without the use of abrasive material. The mixture was then diluted to 5 c.c. with normal saline and divided for injection into two normal animals (K and L) as in the previous experiments. No reaction was noted during the succeeding 17 days and both monkeys died following inoculation of yellow fever virus.

B. *Adults.* As already explained a fifth blood-meal for the parent lot of mosquitoes was necessary to obtain eggs for the last experiment. This blood-meal occurred 54 days from the time of the original infecting feeding. The ova

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collected were immediately immersed for hatching. The adult females obtained were designated as Lot 204 and employed in the two following tests:

1. Normal *M. rhesus* M was bitten by 44 specimens of this lot. It showed no reaction during a period of 24 days and was not susceptible when tested with

TABLE I

Results of Attempts to Transmit Yellow Fever Virus to Normal M. rhesus through First Generation A. aegypti from Infective Parents

Experiment No.	*Rhesus*	Date	Exposure	Results	Susceptibility test
I	A	Jan. 28	2.5 c.c. saline emulsion of 200 eggs	No reaction for 15 days	Died, yellow fever
Eggs and adults	B	do	do	do	do
obtained after	C	do	3.0 c.c. saline used for washing above eggs	do	High fever, killed for virus
1st blood-meal of parent lot, Jan. 24	D	Mar. 2	Bitten by 254 reared females, Lot 185	No reaction for 19 days	Insusceptible
	E	Mar. 16	Bitten by 110 of above lot	No reaction for 17 days	Fever, recovered
	F	Apr. 13	Injected with 96 of above lot	do	Died, yellow fever
II	G	Feb. 12	2.5 c.c. saline emulsion of 200 eggs	No reaction for 15 days	Died, yellow fever
Eggs and adults	H	do	do	do	do
obtained after	I	Mar. 11	Bitten by 350 reared females, Lot 186	No reaction for 22 days	do
2nd blood-meal of parent lot, Feb. 7	J	Mar. 25	Bitten by 250 of same lot	No reaction for 21 days	do
III	K	Mar. 14	2.5 c.c. saline emulsion of 200 eggs	No reaction for 19 days	Died, yellow fever
Eggs and adults	L	do	do	do	do
obtained after	M	Apr. 22	Bitten by 44 reared females, Lot 204	No reaction for 24 days	Insusceptible
4th and 5th blood-meals of parent lot, Mar. 5 and 18	N	May 1	Injected with 41 of same lot	No reaction for 15 days	Died, yellow fever

known infectious blood. This was the second insusceptible animal encountered during these experiments.

2. Nine days from the time of the first blood-meal 41 insects in Lot 204 remained alive. These were stupefied with tobacco smoke, macerated in 3 c.c.

normal saline in a sterile mortar and injected into a normal *rhesus* (N) which showed no reaction during the following 15 days. It developed fatal yellow fever when later tested for susceptibility. This test was made 63 days after the original infecting blood-meal of the parent lot of mosquitoes.

In section A of Experiments I and II, neutral glass powder was used as an abrasive aid in pulverizing the chorion of the eggs. In sections B of Experiment I, and in A and B of Experiment III, the eggs and adults were ground up by friction alone. At no time were the ova used in the above experiments allowed to dry. All injections were made subcutaneously and carried out immediately following maceration thus eliminating possible deleterious effects from standing in suspension. It therefore seems unlikely that any explanation other than absence of the virus can account for negative results following the various injections.

These results are in agreement with those of Stokes, Bauer and Hudson (1928). The data are summarized in Table I.

SUMMARY AND CONCLUSIONS

Attempts to obtain passage of yellow fever virus from one generation to the next in *A. aegypti* were unsuccessful. Subcutaneous injections at varying intervals of a saline emulsion of 200 eggs laid by an infective lot of mosquitoes produced no reaction in six normal *M. rhesus* monkeys. Negative results were also obtained in five biting and two injection experiments with progeny of the same infective lot of mosquitoes in which seven normal monkeys were used. The eggs consisted of batches laid after the first, second and fourth blood-meals of the original lot; the latter feeding occurred 41 days after the initial infecting meal. The imaginal offspring represented rearings following the first, second and fifth blood-meals of the parent lot. The last feeding occurred 54 days after the first.

It is concluded that under the conditions of the experiments here reported hereditary transmission of yellow fever by *A. aegypti* is improbable. Variations in age and in number of blood-meals of parent and offspring mosquitoes had no effect in achieving passage of the virus from one stage of the insect to another.

REFERENCES

Bauer, J. H., and Hudson, N. P., 1928. The Incubation Period of Yellow Fever in the Mosquito. *J. Exp. Med.*, **48,** 148.

Rosenau, M. J., and Goldberger, J., 1906. Yellow Fever Institute Bulletin No. 15, Washington, Government Printing Office.

Hindle, E., 1929. An Experimental Study of Yellow Fever. *Trans. Royal Soc. Trop. Med.*, **22,** 405.

Marchoux, E., and Simond, P.-L., 1906. Études sur la fièvre jaune. *Ann. de l'Inst. Pasteur*, **20,** 104.

Stokes, A., Bauer, J. H., and Hudson, N. P., 1928. Experimental Transmission of Yellow Fever to Laboratory Animals. *Am. J. Trop. Med.*, **8,** 103.