On infection of the male "Aedes aegypti" and the possibility of propagation of yellow fever from Stegomyia to Stegomyia without passage through man

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In the course of our experiments with regard to experimental yellow fever, we tried to verify whether it was possible to infect the male Aedes aegypti with the virus. As males do not bite, we employed, to feed them, desfibrinated blood of infected rhesus, to which we added a little honey (5 o/o), to render it more attractive for the mosquito. With such prepared blood, a bit of cotton-wool is humected and placed into the cage where the male Aedes aegypti are kept. These begin soon to suck the blood, though not absorbing exceedingly as female often use to do, for, their digestive tube is of a smaller capacity, and they do not require so much food as the latter. After this first infecting meal, the mosquitoes were fed on honey, until the moment of verifying the infection, which was carried out 14 to 16 days later.

Up to the present time, 5 experiments with Aedes aegypti were effectuated under such conditions, a report of which we give hereunder:

EXPERIMENT 1. M. rhesus N. 232. Inoculated on 24. 12. 28, subcutaneously, with an emulsion in distilled water, of 2 Aedes aegypti, which, 15 days before, had bitten an infected rhesus. This monkey, whose previous temperature was 38°9, remained without a fever for 5 days, showed 40° on the 6th, and 39°9 on the 7th day. On the 8th day, in the morning, it was found lying in its cage with 36° and killed. The autopsy revealed a very marked jaundice, such as seldom observed in rhesus, an extremely yellow liver, large spleen, little changes in kidneys. The histo-pathologic examination did not show, in the liver, the typical changes occurring in yellow fever, but merely fatty degeneration.

Rhesus N. 249 inoculated with 1 c.c. of the blood from rhesus 232, underwent several febrile reactions, for which, however, the virus of yellow fever was not liable, since after this monkey being later inoculated with virulent blood, it had a typical yellow fever and died after 6 days with the characteristic lesions.

EXPERIMENT 2. M. rhesus 412. Was inoculated on the 6th May with an emulsion made of 9 male Aedes aegypti that had sucked on the 22nd and 23rd April 1929, blood from an infected rhesus. This monkey, whose temperature, at the beginning, was 38°9, showed 39° on the 7th; 39°6 on the 8th; 40°6 on the 9th; 40°2 on the 10th May. On the 11th in the morning, temperature had sunk to 35°3, and 35° at night. The monkey was lying in the cage and was killed.
It showed macroscopic changes, typical of yellow fever. The histopathologic examination, for which we are indebted to Dr. MAGARI-NOS TORRES' kindness, revealed fatty degeneration with necrosis in the hepatic cells, and the specific nuclear inclusions.

EXPERIMENT 3. *M. rhesus* 464. Inoculated on the 10. 6. 29 with an emulsion of 7 male *Aedes aegypti* which had sucked on the 28. 5. 29, blood of the infected *rhesus* 408. Temperature 39o. After two days, it had risen to 39o.9, being then the animal bled. Temperature keeps between 39o. and 39o.7 from this date till on the 27th June. On the 1st July, the animal is found dead in its cage. Macroscopic lesions, rather similar to those of yellow fewer, same being confirmed by microscopic examination, which shows, besides, extensive necrosis and fatty degeneration of the liver cells, as also nuclear inclusions (M. TORRES).

EXPERIMENT 4. *M. rhesus* 482. Inoculated on the 27th with an emulsion, in sterile and distilled water of 2 *Aedes aegypti* which had sucked blood from *rhesus* 408 on the 28. 5. 29. Temperature 38o.9. No change in the animal until the 1st of July. On the 2nd, sudden rise of temperature to 40o.7, and on the next morning, it sinks back to 37o.8. Being killed, it shows macroscopically typical lesions, confirmed by histopathologic examination, which reveals, besides, a slight fatty degeneration, infiltration of polymorphonuclear leucocytes and nuclear inclusions. Few liver cells being necrosed (M. TORRES).

The last three positive experiments do not leave place to any doubt about the possibility of infecting male mosquitoes by putting them to suck disfibrinated blood from an infected *rhesus*.

We realized further experiments, with a view to elucidating the question whether male mosquitoes, put together with infected females in the same cage, could get infected, or vice versa.

EXPERIMENT 5. *M. rhesus* 426. This monkey was inoculated with and emulsion of 7 *Stegomyia* which had remained in a glass cage of GÓ-DOY, for 12 days time, together with 10 infected females. Temperature on the 20. 5. 29 (date of the experiment), 39o. Up to the 28th, no noteworthy changes. By that day, the temperature of the animal rose to 40o, this being then bled out. On the 29th, temperature decreases to 39o.6. On the 30th and 31st, it shows 40o, and 39o.9. On the 1st and 2nd, 39o.3 and on the 3rd and 4th, 39o.8 and 40o.2. Henceforth, temperature is getting back to normal; the observation of the animal was given up on the 13. 6. 29, the same having shown, during the last 10 days, 39o to 39o.4, except on the 8th, when it rose to 39o.9.

EXPERIMENT 6. *M. rhesus* 442. The animal was inoculated on the 29. 5. 29 with 0,5 cc. blood from *rhesus* 425, gathered on the 28. 5. 29. Up to the 8. 6. 29, temperature remains about unchanged, never exceeding 39o.7. On the 9th, a sudden thermic rise to 41o, falling on the next morning to 36o.8. The animal is then sacrificed. The macroscopical examination reveals lesions of yellow feve r, which the results of histo-pathologic examination confirm, say: congestion, necrosis and fatty degeneration of liver cells, alterations in HENLE's trabeculae. Nuclear inclusions (M. TORRES).

This result shows to evidence that the males, which had been for twelve days together with ten infected females, were also infected, for,
when injected to a *rhesus*, they determined in it a weakened disease, as was verified from the positive results of the inoculation of blood from *rhesus* 426 to *rhesus* 442.

**EXPERIMENT 7. M. rhesus 483.** This monkey was inoculated with an emulsion of 4 female *Aedes aegypti*, which were left together with male *Aedes* from the 10, 6, 29 to the 27, 6, 29, and were undoubtedly infected (4th experiment). (These male and female *Aedes* had been put on the 10, 6, 29 together into a clean cage and remained there until the 27, 6, 29). The monkey's temperature was 39° on the day of the inoculation; on the 29th, 39°.7; on the 30th, 39°; on the 1st July, 39°.9; on the 2nd and 3rd, 39°; on the 4th 39°.6; on the 5th 40°; on the 6th 40°.9, falling on the 7th to 39°, being then the animal killed. Macroscopical examination of the visera confirmed the true aspect of yellow fever, and the histo-pathologic examination revealed: fatty degeneration, necrosis of the liver cells, alterations in HENLE's trabeculae, nuclear inclusions. (M. TORRES).

In our opinion, these experiments are a support to following facts:

1) it is possible to infect male *Aedes aegypti* with disfibrinated blood from an infected *rhesus*;
2) it is possible to infect male *Aedes aegypti* by putting them together with infected females;
3) it is possible to infect female *Aedes aegypti* by putting them in contact with infected males;

These results seem to us to be the confirmation of a possible infection of mosquitoes, which under normal conditions, is probably not frequent, but which might perhaps account for the explanation of certain cases of yellow fever, which sometimes occur on a place, a long time after the last incidence, without having, during the time elapsed, any intermediate case been observed.