About the time required by the infected Stegomyia to expell virulent excreta (*)

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Having shown the infectious nature of the excreta of mosquitoes already infective by its bites, it remained to inquire whether such action would also be observed by using excreta of mosquitoes, that had bitten a yellow fever monkey, before attaining the 9th day after contamination, viz. the period considered by the authors as a minimum for the bites to become infective.

Accordingly we have undertaken some experiments in order to obtain data regarding the infection before that period. Though, by these experiments, we are not yet able to inform exactly in which day after the contamination the mosquito faeces become infective, they so far prove that the yellow fever virus may be present in the excreta at least 5 days after the mosquito has fed on an infected monkey. This we observed in two experiments and also verified in another case the infection produced by excreta of mosquitoes that had 7 days previously bitten an infected monkey. We give now brief notes of these experiments and of others performed in order to confirm the results we obtained.

EXPERIMENT 1. M. rhesus no. 434. Temperature 39°. Received on May 25 a subcutaneous injection of a dilution of excreta of 6 mosquitoes that had been fed 5 days previously on blood of the infected rhesus no. 422, when the temperature of which had risen above 40°. In order to use fresh excreta at the time of the experiment the mosquitoes were then fed on a guinea-pig.

On the 4 following days the temperature remained normal. On May 30 it had risen to 39°.9, falling the next day to 38°.8. The monkey was then bled and its blood inoculated, on May 31, in rhesus no. 447. From that date forward the temperature has fluctuated, either a little above or a little bellow 39°. On June 12 we injected the monkey subcutaneously with 0.5 cc. of blood taken from infected rhesus 464; the temperature of that monkey was then 39°.9. Six days latter we observed a more marked rise of fever (40°), the temperature falling to normal on the following days. On June 29 we reinoculated the monkey with 1 cc. of blood taken from infected rhesus no. 480. The first rise of temperature was observed on July 4 (40°.1). On the next day the temperature was 40°.9; on 6th—40°.5; on 7th—40°. On 8th, at 11 a. m., it was killed.

The histo-pathological examination of the liver revealed: "Extensive fatty degeneration and necrosis of liver cells. Nuclear inclusions" (TORRES).

The reported experiment does not prove that the monkey was infected before the inoculations of blood. We believe, however, that there is

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some reason to suppose the occurrence of a mild infection after the inoculation of excreta. In fact, the result which we have obtained in the next experiment seems to confirm that suspicion.

EXPERIMENT 2. *M. rhesus* no. 447. Temperature 38°.7. Injected subcutaneously, on May 31st, with 0.5 cc. of blood withdrawn from *rhesus* no. 434. This monkey showed a rise of temperature only on June 5 (39°.8). From this day until the 12th there were slight fluctuations between 39° and 39°.5. On June 13 the temperature rose again to 39°.9; on the 14th to 40°.8. From this day until June 21 we observed always temperatures over 39°: 15th: 39°.9; 16th: 40°.5; 17th: 39°.9; 18th: 40°.2; 19th: 39°.9; 20th: 40°.; 21st: 39°.4. On this last day the monkey was inoculated subcutaneously with 0.5 cc. of blood of the infected monkey 476, without showing any reaction.

The case seems to be also of a mild character, though somewhat more severe than that of *rhesus* no. 434.

We also tried to prove the infective action of the mosquitoes used in experiment 1 by inoculating, on June 8, *rhesus* no. 457 with an emulsion of the bodies of 3 of the surviving insects, 19 days after they had been contaminated.

EXPERIMENT 3. *M. rhesus* no. 457. Inoculated, as above described, on June 8. This monkey did not show any marked rise of temperature. Therefore, on June 14, viz. on the sixth day after inoculation, the temperature dropped to 37°.8 early in the morning, but on the afternoon it had risen to 38°.5. The subsequent course was passed without marked accidents, although it had occurred a slight rise of temperature (39°.7) on the 21st. On the 24th the monkey was injected subcutaneously with 5 cc. of blood withdrawn from the infected monkey 476. On the 28th the temperature rose to 40°.2 and on the next day had sunk to 33°.7. The monkey was then killed. The histo-pathological examination of the liver revealed: "Fatty infiltration. Congestion. The fatty degeneration and the necrosis of liver cells are slight. Specific nuclear inclusions" (TORRES).

Besides the mosquitoes used in experiment 1 we have kept 5 specimens, also fed, at the same time, on infected monkey 422. These specimens were killed on June 15, viz. 26 days they had been contaminated and triturated in sterilized water. A few cc. of the emulsion thus prepared were injected subcutaneously into *rhesus* 473.

This monkey was previously bitten by the same mosquitoes.

EXPERIMENT 4. *M. rhesus* no. 473. Inoculated, on June 15, as above described. The first rise of temperature was observed on June 19 (40°.5) and it continued high on the two following days. On the 22nd, early in the morning, we found the monkey dead in its cage. The histo-pathological examination of the liver revealed: "Necrosis and fatty degeneration of liver cells. Nuclear inclusions. Congestion" (TORRES).

The blood of *rhesus* 473, withdrawn on June 19 and inoculated on June 21 into *rhesus* 455, reproduced the yellow fever lesions in this monkey.

EXPERIMENT 5. *M. rhesus* no. 455. Inoculated, on June 21, as above described. The first rise of temperature (40°.1) was observed on June 27; on the 28th—39°.9; on the 29th—35°.8, in the morning, when the monkey was killed. The histo-pathological lesions of the liver observed by Dr.
TORRES were: "Necrosis and fatty degeneration of liver cells. Specific nuclear inclusions".

If by an examination of the foregoing cases we cannot draw a sure conclusion to answer to the question here discussed, therefore, by recording them, we desire to invite special attention on the facts reported in experiment 2, which we consider as indicating the monkey was infected by the blood taken from the animal that was injected with excreta of mosquitoes contaminated 5 days previously. However, our following experiment was undoubtedly conclusive on this point of view.

EXPERIMENT 6. *M. rhesus* n°. 479. Received on June 25 subcutaneously a dilution of excreta of two mosquitoes that had been contaminated on *rhesus* n°. 476, 5 days previously. Temperature 38°.9. On June 29th: 40°. From this day until July 3 the temperature was high, near 40°.5. On the 4th July, it dropped to 38°.7. On the following day (5th) the monkey was found dead in the cage. The histo-pathological examination of the liver showed: "Congestion. Fatty degeneration and necrosis of liver cells. Specific nuclear inclusions" (TORRES).

EXPERIMENT 7. *M. rhesus* n°. 489. Inoculated on July 1 with 1 cc. of blood withdrawn from *rhesus* n°. 479 on the 29th and 30th June. Temperature 39°.3. This monkey did not show any rise of temperature, but on July 6, as the temperature had fallen to 35°, it was killed. The histo-pathological examination of the liver revealed: "Congestion. Fatty degeneration and numerous liver cells with nuclear inclusions" (TORRES).

We have tried also to infect *M. rhesus* by using mosquitoes that had been contaminated by feeding on infected monkey 7 days previously.

The possibility of that infection seems to have been determined by the following experiment.

EXPERIMENT 8. *M. rhesus* n°. 456. Was inoculated subcutaneously on June 8 with a dilution of excreta of 2 mosquitoes that had been fed on infected *rhesus* n°. 445, on June 1. The temperature record of this monkey was not of a typical case of yellow fever. From June 10 the temperature was high, almost without any marked change. On June 23 it was 40°. On the next day, on the afternoon, it was 39°.5. We then inoculated 0.5 cc. of blood withdrawn from infected *rhesus* n°. 476. On the next morning (June 25), as the monkey looked very weak, we killed it. The histo-pathological examination of the liver revealed: "Extensive necrosis and fatty degeneration of liver cells; nuclear inclusions very abundant. Congestion. Agglomeration of polymorpho-nucleated leucocytes" (TORRES).

Certainly these lesions were produced before the inoculation of the infective blood.

The 2 mosquitoes used in the foregoing experiment were kept and 13 days later killed and triturated in sterilized water. This emulsion was injected subcutaneously into *rhesus* n°. 469. This monkey was also bitten by the same mosquitoes.

EXPERIMENT 9. *M. rhesus* n°. 469. On June 14 was bitten and received 2 cc. of the emulsion of the 2 mosquitoes used in experiment 8. The height of the febrile paroxym was reached on the 18th, when the temperature was 41°.1. The monkey was killed in the 20th, in the morning, when the temperature was very low. The histo-pathological examination of
the liver showed: "Intense necrosis and fatty degeneration of liver cells. Specific nuclear inclusions" (TORRES).

As we have, for the first time, recorded cases in which yellow fever in *M. rhesus* followed the inoculation of infective excreta of mosquitoes contaminated 7 or even 5 days previously, and considering our previous verification of infection produced by contact of infective excreta with the uninjured skin, we believe that the observations here reported ought to call renewed attention not only to the mode of infection in general, but specially to the result that may follow the contamination of the skin by excreta of mosquitoes more recently infected.