On the infection of M. RHESUS by contact of the uninjured skin and conjunctiva with the excreta of infected mosquitoes. (')

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Continuing with our projected experiments on the infection of M. rhesus by excreta of infected mosquitoes, we have considered it advisable to test whether the dilution of that material, obtained by working according to the technique described in our previous paper (?), could also infect the monkey if put in contact with the skin or in the conjunctiva, without injuring the epithelium.

For this purpose we have chosen 2 monkeys and verified, with the aid of a binocular lens, that the parts intended for the experiments were quite intact.

The first rhesus, no. 427, was tested by putting a few drops of the infecting dilution in one of the conjunctival sacs; in the other one, rhesus no. 428, put also some drops of the same fluid in contact with the skin of the inguinal region, where they remained for about half an hour. The dilution was prepared with excreta of 9 mosquitoes which had bitten rhesus no. 373 on 18th April and fed, at the moment of the experiment, on a guinea-pig.

Both experiments began on May 21, 1929.


On the two following days and up to 28, the temperature did not exceed 39°.8. On the 29th, however, the temperature had risen to 40°.3; the monkey was then bled and its blood injected into rhesus no. 443. On the 30th the temperature fell to 40°.1 and to 39° on the 31st. From this date forward it showed slight fluctuations near 39°. On June 5, the temperature had fallen to 37°.8 when the monkey was killed.

The autopsy showed the viscera as in yellow fever cases; therefore, the histo-pathological examination of the liver, made by Dr. TORRES revealed: the outlines of the liver cells preserved; fatty infiltration around the portal veins; neither intra-nuclear bodies, nor necrosis of the liver cells.

EXPERIMENT 2. M. rhesus no. 443. Inoculated on May 29 with 0.5 cc. of blood taken from rhesus no. 427. Temperature: 39°.2.

June 2, its temperature was 39°.9, on the following day 40°.7 in the morning and 41° on the afternoon. On June 4, the temperature had fallen to 37°. At 3 p. m., the temperature was 35° when the monkey was killed.

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(4) ARAGÃO, Dr. H. de BEAUREPAIRE e COSTA LIMA, Dr. A. da—Sobre a transmissão do virus da febre amarela pelas fezes de mosquitos infectados. Red before the Sociedade Brasileira de Biologia, May 29, 1929. Published in Brasil-Médico, 1929, XLIII, 25, June 15, 669-671 and also in Suplemento das Memorias of the Instituto Oswaldo Cruz, 8, June 22, 1929, 101-108, 2 figs.
The histo-pathological examination of the liver revealed: acidophile intra-nuclear bodies, extensive necrosis and fatty degeneration of the liver cells. Foci of infiltration by polymorphonucleated leucocytes.

EXPERIMENT 3. *M. rhesus* 428, whose skin was in contact for nearly half an hour with the dilution of excreta, was set at liberty in its cage.

On the following days and until the 27th May it showed fluctuations of temperature either somewhat above or a little below 39⁰. On the 28th the temperature had risen to 40.3, falling the next day to 40. The monkey was then bled and the blood used for inoculating *rhesus* no. 445. On the 30th, in the morning, it was found dead in the cage.

The viscera showed the typical aspect observed in yellow fever and the stomach was almost filled with black material.

The histo-pathological examination of the liver showed disarrangement of the columns of hepatic cells, with extensive necrosis and fatty degeneration of the hepatic cells; intranuclear bodies. Congestion,

EXPERIMENT 4. *M. rhesus* no. 445. Injected on May 29, with 0.5 of blood taken from the *rhesus* no. 428. Temperature 39.3. On the following day 38.6; on the 31st: 39.7. On June 1, the temperature had risen to 40.5. On the next day, the third day after the monkey had been inoculated, we found it dead in the cage. It was autopsied too late.

The histo-pathological examination revealed: nuclear inclusions not abundant. The liver cells showed parenchymatous degeneration. Dr. TORRES could not perfectly observe the necrosis of those cells.

Though the experiments above described conclusively demonstrate that an attack of yellow fever may be induced in the healthy *rhesus* by simple contact of infected feces with conjunctiva or uninjured skin, we have considered it advisable to repeat the experiment relative to the last mentioned manner of transmission.

For this purpose we used 2 monkeys: no. 449, for being infected, through the intact skin, by yellow fever virus contained in excreta and no. 448, for being infected by the bite of the mosquitoes that had expelled such excreta.

EXPERIMENT 5. *M. rhesus* no. 449. Received on the afternoon of June 3, on the skin of the throat, some drops of a dilution of excreta, expelled by 9 of the mosquitoes used in the control experiment no. 7.

On the morning of the following day, as it was possible that the monkey had scratched itself, we examined again the contaminated region of the skin, having verified its integrity.

Temperature: 39.2. On the first days following contamination the record of temperature showed no rise above 39.3, till June 7 when the temperature was 40.1. The monkey was then bled. On the next day temperature was 40, and 39.5 on the 9th. This temperature was maintained for the two subsequent days. On the 12th and 13th a new rise of temperature to 40.2. By that occasion the monkey was bled again. From that date until the 18th the temperature varied from 39 to 40, this temperature being again observed on the 19th and 20th.

On June 21 (temperature 39.7), the monkey was given a subcutaneous injection of 0.5 cc. of blood obtained from infected *rhesus* no. 476.
On the two following days the temperature did not exceed 39.5. On the 24th again the temperature rose to 40.3 and on the 25th to 40.9. On the 26th, at noon, it sunk to 38.3. On the 27th the monkey was found dead in its cage.

The histo-pathological examination of the liver revealed: "extensive fatty degeneration and slight necrosis of liver cells. Many hepatic cells show intranuclear bodies" (TORRES).

The question here naturally arises: the monkey was already infected when it was reinoculated with infected blood on the 21st June, or it became infected by that blood? It was solved by the subsequent experiment.

EXPERIMENT 6. M. rhesus no. 467, on June 13th, injected subcutaneously with 1 cc. of blood, taken, on the 7th, 12th and 13th, from rhesus no. 449. Temperature 39.0.

On June 14, 39.4. On the 15th, 40.0; this temperature was maintained for the 7 subsequent days at about the same degree or with small fluctuations until June 23rd. On this day, the temperature had fallen to 39.8. On the next day it sunk to 38.6, and the monkey died during the night.

The histo-pathological examination of the liver showed: "extensive necrosis and fatty degeneration of the liver cells; intranuclear bodies; congestion" (TORRES).

EXPERIMENT 7. M. rhesus no. 448. Was bitten on June 3 by 12 mosquitoes (the excreta of 9 of these mosquitoes were used in experiment 5) that had 28 days previously bitten infected rhesus no. 405. Temperature 39.0.

The first rise of temperature, which was somewhat fluctuant in character, did not reach its height until June 7th or 8th, when the temperature was 39.9. From that date until 21st the temperature, always somewhat fluctuant, did not exceed 39.9. On the 21st (temperature 39.2) the monkey was inoculated subcutaneously with 0.5 cc. of blood of the rhesus 476, without showing any signs of infection.

The result of this experiment shows the occurrence of a mild case of yellow fever produced by the bite of 12 mosquitoes that have fed on an infected monkey 28 days previously.

In the light of these results we feel convinced that excreta of infected mosquitoes, when simply put in contact with the uninjured skin, are sufficient to produce cases of yellow fever.