On transmission of the virus of yellow fever by excreta of infected mosquitoes

By Dr. H. de BEAUREPAIRE ARAGÃO and Dr. A. da COSTA LIMA.

As soon as appeared the first cases of yellow fever in Rio de Janeiro, it came to our mind to verify how the infection would result in individuals bitten by infected mosquitoes, whether exclusively by the secretion of the salivary glands of these insects, that passes along the proboscis when biting, or by the droplets of clear fluid which are always ejected through the anus, immediately after they withdraw the proboscis.

After some preliminary experiments, which have shown us the most efficient technique to be applied to, we undertook others, which we here detailly expose, and which lead us to the conclusion that defecations of infected mosquitoes, when inoculated subcutaneously, are sufficient to infect the Macacus rhesus.

The mosquitoes we used in these experiments (Aedes (Stegomyia) aegypti) bred at the laboratory, were taken off from the common cages, transferred to glass containers of GODOVY (fig. 1) and then kept supplied with honey.

In order to infect them, we applied the lower end of these containers to the skin of a monkey previously inoculated with the yellow fever virus, at the first day of fever. The infected mosquitoes were kept in these same containers until they had become infectant. Whenever we intended to perform some experiment, we removed the necessary specimens to small glass tubes (fig. 2), one of the openings of which was covered with a piece of flannel, the other one plugged with cotton-wool. The flannel shows the advantage of being rather easily passed through by the proboscis of the mosquito and not being soaked with any droplet of excreta. Each of these tubes containing a single infected mosquito, remained for one or two days in a rather not too moist chamber, and, during that time, in order to force them to bite readily on occasion of the experiment, they were not fed. We chose then two rhesus, one to be bitten by the mosquitoes exclusively through the pores of the flannel of the tubes; the other one to be inoculated with the dilution of excreta of the same mosquitoes. As soon as these had filled themselves with blood of the first rhesus, we removed the tubes from the skin of the monkey and placed them horizontally upon a stand of any kind. The mosquitoes, with their abdomen made heavy with the blood they have imbibed freely, almost do not move and after a few seconds lie on the wall of the tube. The excretion of droplets of fluid, which often begins while the insect is sucking blood, becomes more abundant when the mosquitoes are at rest. They then eject droplets, which, not seldom becoming confluent, form a small drop perfectly visible with bare

(1) Read before the Sociedade Brasileira de Biologia, May 29, 1929.
eye, and which may be easily marked with a point of ink outside the glass. We then removed the mosquitoes from the tubes, after having lightly etherized them, and gathered the material to be inoculated. For this purpose, we took a syringe containing a little sterilized water, and pressing on the piston, we allowed a small quantity of water to fall on the points where the dejections were sticking. With the tip of the needle, we made the dilution, and aspirated this again into the syringe. This operation was repeated with all the tubes, so as to gather into the syringe some material of the dejections of all mosquitoes which had bitten the first rhesus. We inoculated then this dilution, subcutaneously, into the other monkey, making also in its skin some scarifications on which we dropped a little of such material.

Here are summarized our observations:

EXPERIMENT 1—M. rhesus no. 245. Temperature at the outset 38°.8. Bitten on the 7th January 1929 by mosquitoes which, on the 17th December 1928 had fed on the infected rhesus no. 226, on the first day of fever. These mosquitoes have been, since that date, fed on honey, as we use to do with infected mosquitoes.

This animal did not show a very marked febrile reaction, the maximum having been 39°.6 on the 5th day after having been bitten. From this date, temperature went on decreasing. On the 8th day, at night, temperature had fallen to 37°.2 and the monkey was lying in the cage. He was then killed. Autopsy and histopathologic examination did not reveal any lesion of yellow fever.

EXPERIMENT 2—M. rhesus no. 246. Temperature at the outset: 38°.5. Inoculated also on the 7th January, with a dilution of faeces of mosquitoes which had fed on M. rhesus no. 245.

On the third day after the inoculation, the fever had risen to 40° and on the 4th day, to 40°.1. Hence temperature sunk forthwith, a series of small fluctuations being observed in the meantime. On the 22nd, viz. 15 days after inoculation, temperature rose again to 40°.5 oscillating, however, during the three succeeding days, between 39°.6 and 39°.4. Finally, on the 26th, it had fallen to 36°.2 the monkey looking then very weak.

By killing it on the same day, we verified that the viscera showed the characteristic appearance observed in cases of yellow fever. Nevertheless, the histopathological examination of the liver did not reveal the presence of the typical lesions of yellow fever.

EXPERIMENT 3—M. rhesus no. 400. Initial temperature: 38°.8. Bitten on the 30th April by 6 mosquitoes which had fed on the infected rhesus no. 369, on the first day of fever.

On the 5th of May, say, five days after having been bitten, it showed in the morning 40°. On the following day, temperature had fallen to 39°, rising however again, in the evening, to 40°.1. On the 7th, in the morning 40°.2 and in the evening 40°.3. On the 8th: morning 38°.8. At night, temperature had fallen to 37°.8 being then the animal killed. Macroscopically, the viscera and histopathologically, the liver, showed the presence of the typical lesions of yellow fever.
EXPERIMENT 4—*M. rhesus* no. 401. Initial temperature: 39°3. Inoculated on the 30th of April with excreta of mosquitoes that had bitten the *rhesus* no. 400.

Two days after the inoculation, temperature had risen to 40°. From then, it oscillated between 39°7 and 39°5.

Killed on the 8th and autopsied, though showing a temperature of 39°5. At the same time, we extracted blood from the heart and inoculated it into *rhesus* no. 415 for the next experiment.

The viscera looked out with normal appearance and also the histopathological examination resulted negative.

EXPERIMENT 5—*M. rhesus* no. 415. Temperature at the beginning: 38°8. Inoculated on the 8th May, intraperitoneally, with blood extracted from the heart of *rhesus* no. 401.

On the 13th attained 40°5 and that high temperature was maintained, between 40°6 and 40°3 on the following day.

On the 15th, in the morning, it showed 38°5 and at 19.40 o’clock, when killed, 36°8.

Autopsy revealed typical lesions of yellow fever, this verification being later on confirmed after histo-pathological examen of the liver. There were acidophile nuclear inclusions, scattered necrosis of the liver cells, fatty degeneration, infiltration and congestion.

EXPERIMENT 6—*M. rhesus* no. 417. Initial temperature: 38°8. Bitten on the 10th of May by 5 mosquitoes which had fed, on the 1st April, on the infected *rhesus* no. 373, on the first day of fever.

On the 15th, at night, temperature was 39°9. By the following days, it kept between 40° and 40°5. On the 17th, the monkey was bled, and the blood inoculated into the *rhesus* no. 422. On the 18th, temperature rose to 40°8 and was maintained for the two subsequent days at 39°. On the 21st in the morning, it had sunk to 38°5. On the 22nd on the morning, the monkey was found dead in its cage.

The liver, spleen, and kidneys showed macroscopically the characteristic appearance as observed in cases of yellow fever. There was also an hemorrhage in the stomach.

Histopathological examination of the liver revealed: numerous acidophile nuclear inclusions, fatty degeneration and slight necrosis of the liver cells.

EXPERIMENT 7—*M. rhesus* no. 418. Initial temperature: 39°1. Inoculated on the 10th May with excreta of 4 mosquitoes which had bitten *rhesus* 417.

On the 14th in the morning, the fever had attained 40°, and maintained itself at this degree until the 16th. By that day, some blood was taken off and inoculated into *rhesus* 423. On the 17th, the temperature sunk to 39°2 in the morning and 38°7 at night. On the 18th, a new rise to 40°3 and on the 19th, to 41°3, falling the next day to 37°7, until at night. On the 21st, the monkey was found dead in its cage.

The viscera showed typical lesions of yellow fever and the greatest part of the material contained in the stomach had turned black by altered blood. That verification was confirmed by the histo-pathological examination
of the liver, which revealed: acidophile nuclear inclusions, slight scattered necrosis of the liver cells, fatty degeneration, infiltration and congestion.

**EXPERIMENT 8—** *M. rhesus* no. 422. Initial temperature: 39°.4. Inoculated on the 17th May intra-peritoneally, with blood taken from *rhesus* no. 417.

It showed on the 20th, in the morning, 40°.1 and at night, 40°.7. On the 11st, temperature sunk to 39°6. On the 22nd, in the morning, 35°6 and at night, 33°, being then the animal killed.

The viscera showed macroscopic lesions of yellow fever.

Histopathological examination revealed: acidophile nuclear inclusions, extensive necrosis of hepatic cells, fatty degeneration, congestion and infiltration by polymorphonucleated leucocytes.

**EXPERIMENT 9—** *M. rhesus* no. 423. Initial temperature: 38°.9. Inoculated on the 17th May, intra-peritoneally, with blood taken from *rhesus* no. 418.

The highest temperature observed in this monkey, 40°.2, was attained on the 24th May. On the 25th, it lowered considerably, and at night, the animal was found dead in its cage.

It showed small foci of peritonitis, congested organs, and its blood invaded by a Gram-positive coco-bacillus. The histopathological examination revealed, like the former cases, nuclear acidophile inclusions, an intensive necrosis of liver cells, fatty degeneration and congestion.

From all experiments carried on, we may draw the conclusion that the dejections of mosquitoes, whose bites are already infectant, are equally infectant.

The same as what happens with bites of infected mosquitoes, which not always cause the *rhesus* to die, even when this is bitten by several insects, faeces may produce mild or severe infection, although without fatal results, the animal recovering from the infection. We need only see what happened in the case of the monkeys of our experiments 2 and 5.

We feel convinced that the monkeys of the experiments 1 and 2, considering the results obtained later on, and though were negative the histopathological examination of the liver of both, had contracted the disease.

From the above mentioned experiments remains demonstrated that it will be advisable to gather some blood of the monkey in observation, as soon as it shows a serious rise in temperature, inoculating this blood into another *rhesus*, in order to obtain a confirmation that the former was really infected, specially in such cases when it recovers from the infection or dies afterwards in consequence of any other cause (2).

Rio de Janeiro, May 29, 1929.

**ADDITIONAL NOTE**—We have lately verified that the dilution of excreta of infected mosquitoes, when put in contact with the skin or with the ocular conjunctive, proved also infective to the *M. rhesus*. In this way we made a repeated trial that will be further described in a separate article.

(2) The histo-pathological examinations have been performed by Dr. MAGARINOS TORRES, to whom we should like to express our heartiest thanks.
Fig. 1—Gaiola de vidro de GODOY.
Fig. 1—Glass container of GODOY.

Fig. 2—Tubo de vidro usado nas nossas experiencias (metade do tamanho natural).
Fig. 2—Glass tube used in our experiments (half the natural size).