

On the contamination of hæmolymp in mosquitoes infected by the yellow fever virus

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

The observations of all the authors who studied yellow-fever transmission seem to indicate that after the virus has been taken into the mosquito's intestinal tract, a certain number of days must elapse before the insect is able to transmit it by its bites to man or to *M. rhesus*.

This period might well be explained as representing the time required for the germ to pass from the mosquito stomach to the coelomic cavity and hence, carried by means of the hæmolymp, to the salivary glands.

It follows from this hypothesis that the virus should be found in the coelomic fluid or, in other words, that this fluid should also be capable of infecting a monkey.

This hypothesis therefore was recently opposed by a very interesting experiment of HINDLE (1). He inoculated two monkeys, one with the legs containing hæmolymp of two mosquitoes infected sixty four days previously, and the other with the rest of the body. This monkey died of yellow fever four-and-a-half days later, whilst the first was not infected. 'The results of this experiment indicate that the virus may be present in the organs of infected mosquitoes without necessarily invading the coelomic fluid' (HINDLE).

Nevertheless, the experimental criterium adopted by HINDLE for reaching such a conclusion does not hold good after the results we obtained with mosquito's excreta. It might have failed if the experiment had given a contrary result and induced him to conclude to the infectious nature of the hæmolymp. We believe that the legs of the 2 mosquitoes used by HINDLE were not contaminated by excreta at the moment of the experiments.

We may draw this conclusion after having repeated his experiment but with legs of mosquitoes contaminated by excreta a few moments before the inoculation.

EXPERIMENT 1. *M. rhesus* no. 429. This monkey received, on May 21, subcutaneously a few cc. of an emulsion of legs of infected mosquitoes in sterilized water. We used 10 mosquitoes, 6 contaminated on *rhesus* no. 373, 33 days previously and 4 that had been fed on infected *rhesus* no. 405, 15 days before. All these mosquitoes had expelled many droplets of excreta on the wall of the tubes and walked freely on these contaminated material.

After having etherized them, we take off their legs and prepared an emulsion.

(1) HINDLE, E. An experimental study of yellow fever. Trans. Roy. Soc. Trop. Med. Hyg., 1929, XXII, 5, 417.

Temperature of inoculated *rhesus*: 38°7.

On the first days the temperature was normal. On the 29th, 8 days after the inoculation, it rose in the afternoon to 40°. The monkey then was bled and its blood injected into *rhesus* no. 444. On the 2 following days the temperature fell to 39° and to 39°5. On June 1, a new rise to 40°, falling the next day to 39°7. On June 4 we found the monkey dead in its cage. The autopsy and the histo-pathological examination of the liver showed typical lesions of yellow fever. The result of this last examination was given by TORRES in the following terms: "Necrosis of the liver cells so extensive that it was almost impossible to observe normal ones. Disarrangement of the columns of hepatic cells. Congestion. Nuclear inclusions".

EXPERIMENT 2. *M. rhesus* no. 444. Inoculated on May 29th with 0.5 cc. of blood withdrawn from *rhesus* no. 429. Temp. 39°. On the 3 following days after the inoculation the temperature remained normal. On June 2 rose to 39°8. On June 3, 40°2, on June 4, 39°8 and on June 5, 39°7.

On June 6, at 11 a. m., when the temperature was very low, the monkey was killed. The autopsy and the histo-pathological examination revealed the presence of typical lesions of yellow fever. The result of this last examination was the following: "Nuclear inclusions in hepatic cells. Necrosis. Congestion" (TORRES).

In order to verify if the hæmolymph of infected mosquitoes contains the yellow fever virus or is free from it we considered better to withdraw that fluid directly from the pericardial chamber, where it flows more abundant, principally inside the dorsal vessel, by adopting the following technique. After having fed on blood, the infected mosquito is etherized and its wings and legs cut off. We stick it then firmly, ventral side downwards, to the glueing face of an adhesive tape, mounted on a piece of cork plate, serving as operating table. The puncture of the pericardial chamber is by its nature a microscopic operation and it is indispensable for its performance to use a binocular microscope giving a magnification of about 60 diameters (occ. 4, object. a₃—ZEISS).

The region to be punctured, the membranous space between the 4th and 5thth tergites (figs. 1 and 2), must be sterilized by cauterizing it with the point of a heated glass stylet. For the puncture we use glass tubes, drawn out a few moments before the operation so as to form a micropipette (fig. 3). With careful handling of this micropipette it is not difficult to reach to the pericardial chamber, what is ascertained by the rising of hyaline fluid in the pipette, immediately after the perforation of the intersclerital membrane. If by an intempestive handling of the pipette the stomach is perforated, instead of a clear fluid, we get blood, easily distinguished by its color. The end of the micropipette, filled with hæmolymph, is introduced under lens control in a cut of the skin of the *rhesus* made by a Gillette blade. We thus make an intradermal inoculation of the fluid, the monkey being inoculated by several micropipettes introduced in various cuts.

EXPERIMENT 3. *M. rhesus* no. 501. Temperature 39°1. Inoculated on July 20 with the hæmolymph of 8 mosquitoes previously fed on blood of 3 infected monkeys. On the 4 following days the temperature remained nor-

mal. On July 25 it had risen to 40°3. On the next day, 40°7. On July 27, 37° in the morning. The monkey died at noon of this day. The histo-pathological examination of the liver made by Dr. TORRES showed typical lesions of yellow fever.

We also tried to prove the infective action of the mosquitoes used in this experiment by inoculating the *rhesus* no. 502 with an emulsion of their bodies. This monkey showed the rise of fever on July 24 and 25, the temperature falling the next day to 38°9. On the 27th we found it dead. The histo-pathological examination of the liver also revealed typical lesions of yellow fever.