Note about experimental yellow fever (*)

By ARISTIDES MARQUES DA CUNHA, M. D., and JULIO MUNIZ, M. D.

In a previous note, we had given a report about experimental transmission of yellow fever to the *Macacus Rhesus*, which we obtained starting from a case of the disease under treatment at the Hospital of the Oswaldo Cruz Institute.

We collected by this opportunity a few observations about the evolution of the disease in those animals, specially with reference to the fever and the lesions produced in the liver.

After issuing our former note, we had an opportunity to inoculate some *Rhesus* with African strain and were thus enabled to compare the effect of this virus with that of the virus isolated by us in Rio de Janeiro. As a fact, the duration of the illness in *Rhesus* inoculated with the Brazilian strain used by us, would vary from 5 to 11 days.

In a *Macacus cynomolgus* of our series, it lasted for 14 days. Wedo not include in this account the very special case of the *Rhesus n. 20*, which happened to die 36 days after being inoculated and whose liver, examined histo-pathologically, revealed the characteristical lesions caused by yellow fever. We must state, the animal had been inoculated with a mixture of liver and citrated plasma from a *Rhesus* which died from yellow fever, the plasma having been submitted to warming at 55oC. for 30 minutes, with a view to destroy the *virus* that it contained.

Our purpose in making this experiment was to verify the existence in the plasma of anti-bodies, during the terminal stage of the infection.

We are of the opinion that the presence in the serum of protecting substances does not account for the long duration of the illness in *rhesus n. 20*, for, after renewing the experiment in the animal which had received liver and warmed plasma at 55oC, this one died on the 6th day, whereas a witness, infected with liver only, died on the 5th day.

The Brazilian strain, isolated by us, does not determine in every case the death of the animal, and, in such occasions, it is most often uneasy to ascertain whether the animal has been infected or not, which to a certain extent, makes the keeping of the virus in the laboratory rather difficult.

The temperature rise does not always provide accurate indications, for it may be owed to other causes, as we had opportunities to observe. Thus the *Rhesus n. 43*, after having been stung by *Aedes* which had priorly sucked a monkey infected with yellow fever, and having shown the same thermic curve as seen in infected animals, (3rd day 40.5o – 4th day 35.2o) died on the 4th day in hypothermy, showing during the stage prior to collapse, the position generally observed in those animals, during the final period of the disease. This notwithstanding, the anatomo-pathological examination revealed only the presence of pneumonia, with no signs of

(*) Read before the Brazilian Society of Biology, December 26, 1928.

yellow fever lesions in the liver, a result which was confirmed by inoculating this liver to another *Rhesus*. It must be noted that in this case, like in others, the urine appeared yellow, with a big amount of albumin and casts, as observed in the course of yellow fever infection, which is a statement of how little worth are such signs.

Another fact we observed in the animals infected with the *virus* isolated by us, is the highly varying extent of the lesions in the liver. In some animals, there was an extended necrosis with destruction of the greatest part of the hepatic cells; some others would show no necrosis, but only changes in the nuclear structure, a fact being that, in one case (*Rhesus 19*) the anatomo-pathological examination did not reveal anything abnormal in the liver, in spite of this having proved infectant when inoculated to another animal.

The African strain with which we worked, contrarily to that isolated by us, determined the death of all the inoculated animals, in a delay from 4 to 6 days, the same showing lesions in the liver, almost always to a large extent, though we used for the inoculations, much smaller doses than those experimented with Brazilian virus.

From all we stated here above, we must draw the conclusion that though the Brazilian virus isolated by us shows of lesser virulence than the African strain, this is not its most characteristical property, as at times, it is sufficient to cause rapid death in the animal, with extended lesions of the liver, as happens with the African strain. Its variable virulence is the more typical property, this determining the longer or shorter duration of the disease, the not always fatal process, as also the variation of extension observed in the lesions of the liver. This property of the virus we worked on, could be accounted for a weaker adaptableness of this to the organism of the *Rhesus*, so that, through successive passages into this animal, this property might be modified, thus acquiring the virus the same characteristical properties of the African strain. But, remaining within the results of our own series of experiments, we do not feel justified in thinking in this way, because what we reached at was to observe alternating grades of virulence, not a progressive increase of same as was expected.

In view of the occurrence with *Rhesus 19*, which, being killed on the 5th day of the illness, showed no lesions in the liver, in spite of being infected as proved by further inoculations, we tried to verify whether the missing of hepatic lesions was constant at a certain stage of the disease, or only to be observed exceptionally and ascribed to the varying virulence of the virus. Two *Rhesus* inoculated with African strain, were killed, one on the 14th day, 3 days after the fever appeared, the other one on the 3rd day, just after the first sign of thermic rise. The histo-pathological examination showed in both, lesions of the liver, with less extended necrosis in the second animal, in which prevailed nuclear changes (oxychromatic degeneration, chromatolyse). This shows that the missing of hepatic lesions is in no way characteristic of the illness, but a fact which may arise in certain cases, and seems to be dependent on the variable infectiousness of the virus, as we point out here above.

NOTE—The African virus with which we worked was supplied to us by Dr. HENRIQUE ARAGÃO, to whom we present our best thanks.