Notes about Yellow Fever.

By Drs. A. MARQUES DA CUNHA and JULIO MUNIZ.

The occurrence of a few cases of yellow fever in Rio de Janeiro, gave us an opportunity to effect some researches about this disease. The object of the present note is to report upon the first results of these researches.

We shall divide our work into two parts. In the first we shall relate our attempts with a view to isolating the "Leptospira icteroides" from yellow fever patients, which attempts have led to absolutely negative results. In the second part, we shall deal with the transmission of the disease to experimental animals, represented, in the present case, by the Macacus rhesus, whose receptivity for Yellow Fever has been demonstrated in Africa by Stokes, Bauer and Hudson. We made use also once, in our experiments, of the Macacus cynomolgus which alike proved sensible, according to Dr. B. Aragão's verification.

RESEARCH OF THE "LEPTOSPIRA ICTEROIDES".

We undertook searching the Leptospira icteroides not only for considering this micro organism as a possible factor of the disease, but also under the hypothesis, expressed by Schuffner the Leptospira might represent a germ of secondary invasion. For this reason, our research were effected, not merely during the period in which blood is likely to infect, but also during advanced stages of the illness in which the presence in the blood of micro organisms of secondary invasion should appear as more likely to exist.

We used, for our attempts, blood extracted during various stages of the illness, since the first day up to the agonial period. The blood was extracted and placed into tubes containing a physiologic citrated 20% solution. Very often, the sowing was effected immediately after extracting the blood. Other ones were made after a few hours cooling on icebox at the temperature of 5 to 10°. We sowed, at times plasma only, at other times total blood. Of each sample of blood we never used to sow less than six tubes of culture, each of these with quantities varying from one drop to 2 and 3 ccm. The medium we always used was a medium of NOGUCHI for Leptospira, having sometimes sowed, besides the medium referred to, variants of this medium, such as NOGUCHI's for Leptospira with a coating of paraffin, the same medium with a fragment of fresh blood organ. We also sowed tubes with RINGER and ascite's lique and also tubes with rabbit serum, diluated in saline solution in the proportion of 1/4, 1/5 and 1/30.

The cultures were kept at the room temperature and observed for a period of not less than a month. All the cultures, which during this delay, would present any cloudiness, were carefully examined in dark field. Under these conditions, we examined bloods of 13 patients, some of which, more
than once, were at different stages of the illness. The results, as to trying
to cultivate the *Leptospira icteroides*, remained always negative.

With the blood of some of these patients, we effected intra peritoneal
inoculations in Guinea pigs. With the blood, kidney and liver of those Gui-
nea pigs, we made passages to another series of Guinea pigs, which were
used to provide material for a third passage. With the blood of the ani-
mals of these three lots, we made sowings in NOGUCHI’s medium for
*Leptospira*. The organs (liver, kidney and adrenals) were examined in
dark field after impregnation.

We also inoculated Guinea pigs with viscera (liver and kidney) ob-
tained from autopsies of a few cases of yellow fever as well as with urine
extracted from the corpse. All these examinations were of no profit to
make the presence of *Leptospira* obvious.

**TRANSMISSION OF THE YELLOW FEVER TO THE *MACACUS RHESUS*.

The first attempt we made to obtain the infection of *Macacus rhesus*
with blood of yellow fever patients was effected only after some times had
elapsed since the illness appeared amongst us, for, at the beginning of
our researches, we could not dispose of such animals. It was only more
than one month after the first case broke out, that we were able to rea-
lize our first attempt in this way. We inoculated then, by via intraperito-
neal, a *rhesus* with the blood of a benign case of about 24 hours duration
The patient was H. M., admitted at the Hospital annexed to the Oswaldo
Cruz Institute. This animal showed on the 6th day after the inoculation, a
high temperature (40°) which dropped on the following day.

A few days later we observed a new thermic rise, after which the
temperature grew normal again, and the animal did not show any more
troubles, till now.

Later we inoculated another *rhesus* with the blood of the patient
F. W. also admitted at the Hospital of the Oswaldo Cruz Institute. This
patient showed also a mild form of the disease. The blood was extracted
about 48 hours after appearing the first symptoms, and injected per via
intraperitoneal to the *rhesus* monkey n. 6. The temperature of the animal,
taken every day at evening (diagram n. 1) went on without change,
oscillating between 38.1 and 39.1 until on the 10th day, it grew to 39.7.
The temperature taken on the next morning (10 A. M.) was 39.6. The hours
later, the temperature had fallen to 38°. The animal was then killed
and autopsied. The urine extracted in the necropsy proved to be
yellow containing albumin and cylinders. The histological examination of
the liver, revealed lesions, notwithstanding of not a large extent similar to
those described by STOKES, BAUER and HUDSON: necrosis, fatty dege-
neration, and polymuclear infiltration,

With the liver of that monkey preserved in icebox for 4 days, another
*rhesus* n. 8 was inoculated whose thermic curve is shown in graphic
n. 2. On the 8th day after the inoculation, the temperature, after having
shown feeble rises of 39.5 and 39.3 in the course of the two days before,
was in the morning 37.6 (8.40 A. M.). At 13 o’clocks the temperature had
dropped to 35.2 being then the animal sacrificed and autopsied.
The histopathologic examination of the liver did not reveal the typical lesions as described by STOKES, BAUER and HUDSON, for there was no necrosis of the hepatic cells, or if any, it was so scarce as not to be seen in examining several sections. There was, beside the fatty degeneration and polymorphonuclear infiltration, certain alterations in the nuclear structure, a detailed study of which will be made by Dr. MAGARINOS TORRES.

The liver of this animal kept in icebox for 6 days, and injected to *rhesus* n. 14, gave way to an infection with thamic curve, as usually found in these animals, which graphic n. 3 shows. This animal presented on the 6th day, a sudden drop of temperature, after which it was sacrificed. The autopsy revealed signs as generally observed in experimental yellow fever, viz. in the histopathologic examination the presence, though on a very small scale of the typical STOKES, BAUER and HUDSON lesions, necrosis, fatty degeneration and polymorphonuclear infiltration could be made patent.

Later on, other animals were inoculated totaling to this date, 8 successive passages of virus. In these passages, we generally used liver stuff, finely triturated with saline solution, filtrated on wire cloth and injected subcutaneously. We used twice blood per via intraperitoneal, once being blood extracted after autopsy and inoculated to a *cynomolge* which happened to die 14 days after the inoculation. The second time, we used blood extracted alive, from the animal in the febrile stage two days before being sacrificed. The blood proceeded from the *rhesus* n. 14, whose temperature, was by the time, 40.5°. The inoculated animal, *rhesus* n. 16, had an infection with sudden drop of temperature on the 6th day. In view of such results, we are of opinion that, for passing the virus, either liver or blood extracted alive from the animal in the febrile stage has to be preferred.

We should like to call now attention to some facts observed by us in the course of our researches.

The first refers to fever. In some of the animals, the maximum temperature observed in the course of the disease, was not a high one (39.7 in *rhesus* n. 6, 39.5 in *rhesus* n. 8 and 39.3 in *rhesus* n. 16). Should we adopt the criterium of STOKES, BAUER and HUDSON who only consider a fever, a temperature of 40° and upwards, then these animals would not have shown fever whatever. If however, we compare these temperatures with those observed formerly in the same animal, we shall see there has been an obvious thamic rise. What we just affirmed goes out clearly examining the temperature graphics of *rhesus* n. 16, which in spite of not showing a little elevated maximum temperature, 39.3, points out, on the former temperatures of 37.6 and 37.8 a plain increase of 1.5°. Accordingly, we think verifying temperature rises in comparison with former temperature observed in the same animal, but not in relation with a determined unvarying limit for all the animals, constitutes a better criterium to know whether or not there was a fever in the course of the infection.

Another fact of higher importance refers to the observed lesions. Thus, in *rhesus* n. 8, inoculated with liver from *rhesus* n. 6, which showed typical lesions, even if to a small extent, there were in the liver, none of
the lesions as described by STOKES, BAUER an HUDSON in Africa, while there was no necrosis of the hepatic cells. In this way, from the lesions observed in the liver, and according to what was established on this subject by the Commission above referred to, it was not possible, after histopathologic examination of the liver, to make a diagnosis of yellow fever. The liver of this infected monkey, inoculated to *rhesus* n. 14 gave way to an infection with all the characteristics of the experimental disease, including the histopathologic lesions of the liver, thus demonstrating that *rhesus* n. 8 was in a fact infected by yellow fever.

This fact occurred anew later on, in another animal, *rhesus* n. 19, in a still more noteworthy manner. In that case, almost all the lesions generally found and described up to now, as characteristics of experimental yellow fever would miss completely, hardly existing periportal fatty infiltration. Nor even could be found there the alteration of the nuclear structure observed in *rhesus* n. 8, meantime, the liver of that animal against any expectation, reproduced, when inoculated to *rhesus* n. 22, an infection with all the characters of experimental yellow fever, including the liver lesions, necrosis of hepatic cells, fatty degeneration, polynuclear infiltration, etc. We must point out that the *rhesus* n. 14 was sacrificed on the 5th day posterior to the inoculation, after having shown temperature rise on the 2nd and 4th days. The temperature was, by the time the animal was killed, 38.8 i., e., more or less normal, the animal having undergone no hypothermic period, as observed in other cases.

Thus, though we acknowledge the importance of the liver lesions in characterizing experimental yellow fever, we may affirm that the missing of same does not elude the possibility of infection of the animal. The two above stated cases, controlled in our series of inoculations furnished a formal proof thereof.
N. 1—Rhesus 6

N. 2—Rhesus 8

N. 3—Rhesus 14

N. 4—Cynomolgus 15

N. 5—Rhesus 16

N. 6—Rhesus 17
N. 7—Rhesus 19

N. 8—Rhesus 22