Report upon some researches on Yellow Fever.

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(Chief Service)

The cases of yellow fever, which lately occurred in Rio de Janeiro offered us an opportunity for a series of experimental researches on the subject, the result of which are the reason of the present work.

Before relating them, we avail ourselves of this opportunity to tender here with our best thanks to Drs. CLEMENTINO FRAGA, ROCHA LIMA, FREDERICO SOPER, NELSON DAVIS, JOHANNES BAUER, BARROS BARRETO, SINVAL LINS, BRUNO PESTANA, RAUL DI PRIMO, AMADEU FIALHO, ARRUDA BOTELHO, CARLOS DRUMMOND, as also to Drs. CARLOS CHAGAS, ALCIDES GODOY and to all our colleagues, companions of Manguinhos, for the valuable help lent to us, in various manners, for these investigations.

The subjects we were more immediately interested in, were the following:

I. Transmission of the yellow fever to monkeys.
II. Researching the virus in the blood and organs of persons who died from yellow fever.
III. Research of the *Leptospira icteroides* in the patient, in the corpse, and in infected monkeys.
IV. Identity of the African and American yellow fever.
V. Serological diagnostics of yellow fever.
VI. Vaccination against yellow fever.

TRANSMISSION OF YELLOW FEVER TO MONKEYS.

The discovery, by STOKES, BAUER and HUDSON, that yellow fever in Africa was transmissible to the *Macacus rhesus*, opened a wide field to experimental researches on the subject, in which up to the present, only the experiments tried in man had been undoubtedly successful. The yellow fever cases in Rio de Janeiro, offered an opportunity for us to also make attempts to transmit the American disease to a few kinds of monkeys, making use, up to now, to that object, of specimens of the following species: *Macacus rhesus*, *Macacus cynomolgus*, *Macacus speciosus* all of these, of Asiatic origin, and of the common Brazilian species *Pseudocebus azarae*.

The three first species proved sensible, for it was possible to cause in same, the yellow fever infection, as well by inoculating human blood, as by the biting or inoculations of mosquitoes experimentally infected through patients, as also by inoculating into animals of these kinds, blood and organs of monkeys, infected at the laboratory.
Most of our observations were made in *Macacus rhesus*, of which we could dispose of a rather fair lot.

The inoculations of human material in *Macacus rhesus* and *cynomolgus* were effected with material extracted from 21 patients, some of whom with severe, other with slight forms of the illness, there being 16 with 10 to 72 hours of contracting the disease and 5 with a duration of more than three days. These bloods were inoculated to 26 *rhesus* and 1 *cynomolgus*.

Sometimes, owing to our disposing at the beginning of but few animals, we were compelled to inoculate the blood of two patients to a single animal, nevertheless, since we had *rhesus* in higher quantity, it was possible to inject each blood to each monkey, and several times, the blood of a same patient, at different days of the illness, could be injected to several monkeys.

The quantities of injected blood oscillated between 5 and 10 c.c., seldom more. At times, the blood was citrated, in other cases it was not and in the latter, what was injected was the serum, sometimes rather sanguinolent, owing to the presence in it of many red globules.

The mortal injections, obtained in monkeys inoculated with the blood of patients with less than a 72 hours sickness, amounted to 4, having proved negative all those effected with the blood of individuals after more than 72 hours of the illness.

Of the monkeys which did not die consecutively to injections of blood from patients with less than 72 hours, a certain number would show more or less typical febrile reactions, of which obviously a certain number were a mild infection, while those monkeys, when inoculated, later on, with virulent liver showed no reaction, proving thus to be immunized. Other monkeys, however, under the same conditions as the previous ones, when injected with the same virus, died consecutively to the injection, appearing not to have had an own specific infection, nor was the immunity they perhaps acquired in consequence of the injection of the patient's blood, not sufficient to protect them against the infection produced by injecting the virulent stuff.

We observed once that the blood of a patient, (severe and mortal case), when injected on two successive days, caused the death of the animal with the sample extracted 36 hours after the illness had begun, whilst the one we extracted from the patient after only 10 hours, provoked in the animal only a few small atypical febrile oscillations. This monkey, however showed to be immune against a further injection of virus, thus, demonstrating, it had had a slight infection, insufficient, notwithstanding, to bring death as a result.

The mosquitoes which sucked the patient as well after 10 as after 36 hours of the illness, infected themselves and proved capable to transmit the disease to the *Macacus rhesus*.

Of the 4 cases in which the blood inoculation produced an infection fatal to the monkey, three proceeded from patients with a very mild attack of yellow fever and one from a severe and mortal case, which seems to demonstrate that slight cases are more favourable to the infections of monkeys than serious ones. In most of the cases, the blood injections were effected on the same day of the gathering, but there is no inconvenience
in keeping the blood in the ice-box at 8 centigrades, for we had two positive inoculations, using blood kept for 48 hours at this temperature and one with blood kept under the same conditions, during 9 days. Those same bloods, which were positive with 48 hours cooling, gave negative results when inoculated after 14 days cooling at 0°, which gives us way to suppose that the cool storage of the virus further then 10 days would not be safe, nor temperatures from zero could be particularly favourable to the object, for we did not observe a better preservation of the virus in such temperature.

The results of the inoculations of blood from patients to the Macacus rhesus, show that those monkeys are not so sensible as was expected, and that on a great number of animals, only but few happen to die, and other, either get a slight infection or show nothing at all, or even some ones do not acquire a firm immunity, capable to make them resist to a further inoculation of virus.

The activity of the virus, isolated from the animal does not appear to be immediately a very strong one; after 4 passages, we did not obtain more than 0,2 c.c. of blood as a minimum mortal dosis, but surely, with successive inoculations in rhesus, virus will at last be got at, capable of killing at a dosis of 0,0001 milligram, as occurs with those isolated in Africa.

The illness in the rhesus infected by inoculating patient's blood, or blood and organs of another rhesus, or that obtained by the bite of Aedes aegypt or by inoculating emulsions of those insects, develops within a period varying from 5 to 15 days, this latter being the longest time observed by us. The illness transcurs, amongst ourselves, in a way absolutely similar to that observed in animals infected with virus isolated in Africa by the American Commission.

In the cases of quick evolution, the temperature sometimes rises since the first days; other times there are, at first, some alternatives of rising of falling, more or less accentuated, of the temperature, and, on the 4th or 5th day the thermic curve rises suddenly from 1 to 1,5 grade over the primitive temperature of the animal, on the following day, it begins to fall suddenly, the animal entering then into accentuated hypothermy, at last dying in collapse. The annexed diagrams (figs. 6, 7 and 8) give a clear idea of the thermic curves in such cases.

Generally, during the period when the temperature rises suddenly, the animal loses appetite and begins to show less liveliness. As soon as temperature begins to drop quickly under the normal, the monkey gets sad, keeps confined in a corner of its cage, allowing to be caught easily; very weakened, it supports its body on the forelimbs, a little disjoint one from the other, its head hanging forwards in a very characteristic position. Finally the animal falls all its length in the cage and does not take a long time to die (figs. 1 to 3).

Not always, however, a sudden accentuated drop of temperature is a certain premonition that the animal is to die, since it can take back temperature, after a sudden fall of about 3 grades under the normal.

When the illness lasts much, there occur, as a rule, two or more thermal risings, followed by periods of nearly normal or under normal
temperature, before the *rhesus* dies (figs. 4 & 5). There are besides cases in which the illness evolves for days subsequent to the inoculation of virulent blood, without serious thermic alterations; the curve remains almost inaltered and only rises during the two days before the animal dies (fig. 9).

Besides the infections with human blood, it has been possible to get the infection of the *Macacus rhesus* through the sting of infected mosquitoes which had sucked a patient 17 to 30 days before, being in this last case sufficient one mosquito to cause the monkey a mortal infection.

We also succeeded twice in obtaining the infection of a *rhesus* by inoculating emulsions on mosquitoes, experimentally infected. In the first case, we got at this result, inoculating an emulsion of 10 *Aedes aegypti* which, 5 days ago, had sucked a patient. This experiment shows that the virus yellow fever exists in the mosquito, suitable for infection, prior to this being able to transmit it, by stinging, that is to say, before it has virus in the salivary glands, which confirms the results previously obtained in Africa by BAUER and HUDSON.

Not all the *Macacus rhesus* inoculated with the virus of another *rhesus* infected before, show a typical illness and happen to die. Lots of them escape, after having shown high thermic alterations and very yellow urines, charged with albumin, as in the rule in the advanced stages of the illness in *rhesus*. This fact should perhaps be ascribed to the still not strong enough virulence of the virus or may-be, to another still unknown cause.

As we had already an opportunity to say, besides the *Macacus rhesus*, we also tested the sensibility to the yellow fever virus, of the *Macacus cynomolagus*, *Macacus speciosus* an the *Pseudocebus azarae*.

The first one, the *Macacus cynomolgus*, was infected by us, inoculating it with blood of two patients with a slight yellow fever, and the animal happened to die after 8 days with a typical form of the disease, a characteristic curve and macro and microscopic anatomopathological lesions, quite identical to those of the *Macacus rhesus*. A second *cynomolgus*, inoculated with 0,2 c.c. of blood from an infected *rhesus* died in the same way as the former, 6 days after the inoculation, with characteristic lesions and thermic curve (Fig. 8).

In both cases, we dealt with adult and very developed animals. We have a feeling that the *cynomolgus* is a monkey as sensible as the *rhesus* to the inoculation of human or animal virus, and owing to its big size, offers an advantage for certain experiments, as it is able, not only to provide a large quantity of blood but also of organs, which is of a great importance in the case of preparation of anti-yellow fever vaccine.

Of the kind *Macacus speciosus* we only had a specimen 3 months old. This monkey was inoculated with an emulsion of the liver from a *rhesus* which had died from yellow fever. It had a long lasting form of the disease, with a few thermic rises (fig. 2) and happened to die, after 12 days inoculation, showing characteristic but less acknowledged lesions than the two other already experimented.

As regards the *Pseudocebus azarae*, we had opportunity to inoculate only 2 specimens; one with the blood of a patient with 72 hours illness; there was no reaction; another one, injected with virus of a *rhesus*, had a
fever during several days, but, having been sacrificed still during the febrile stage, it did not reveal anything suspect, according to the anatomopathological examination of its viscera.

Besides those kind of monkeys, we did note use any other in the experiments effected till now.

The anatomopathological lesions in monkeys sensible to yellow fever infection, the *M. rhesus*, the *M. cynomolgus* and the *M. speciosus*, are in a general way the same and wholly identical to these observed in monkeys infected with African virus, and most similar to the lesions in man, and like these absolutely characteristic and constant.

These lesions are, as a rule, the following:

Slightly accentuated jaundice, in few cases only to be noted by a slightly yellowish colour of the skin and the mucosae and by the shade of the aorta. Hemorrhages in the stomach and intestines may be observed, specially in cases when the illness is a longlasting one; they miss or seldom appear, and then, in acute forms, seem to be limited to the stomach. Slight petechiae can be observed in the pleura, in the pericardium and the endocardium. Heart generally pale and flaccid. Liver of a shammy colour in many cases, in other ones yellowish in the whole of the organ, or greyish with more or less extensive yellowish spots, lobules visible and vessels injected. The organ is more or less friable, its colour being in relation to the fat it contains.

Spleen slightly enlarged in volume, with dark red, sometimes rather blueish shade. The area of the section is neat, pulp consistent, and glomeruli visible.

Kidneys a little increased, yellowish grey, of lighter colour than the normal ones, with a reddish grey section area on the cortical and central coats distinct. The capsule appears clearly. The bladder contains sometimes an intense yellow coloured urine, rich in albumin carrying hyaline and granulated casts.

The microscopic examination of the organs reveals slight hemorrhages in the lung, heart, stomach and intestine in a few cases. In the liver, intense fatty degeneration, a more or less strong necrosis of cells with acidophilous alterations of the protoplasma and pycnosis of the nucleus, and caryorrhexis. Not seldom, the cells lose their normal connections, other appear with an aspect of amebas. The nuclei do not colour well and seem to be altered in shape. The necrosis of the cells occupies generally the medium zone of the lobule all along the HENLE's layers, but may extend further and spare only the coat of cells surrounding the portal space. Slight leucocytarian infiltrations in the liver are frequently observed.

In the spleen, an intense hyperplasia of the follicles and increase of the phagocytarian endothelial cells in the periphery of the glomerule is to be observed. In the kidneys the tunefaction troubles lesions of acute nephritis, fatty degeneration and necrosis of cells. Hyaline and granulous casts, sometimes those of calcium. In the liver and kidneys, the lesions are the classical as described by Prof. ROCHA LIMA for yellow fever in man.

The fatty degeneration is to be observed, on a more or less large scale, in several organs, but in the brain, hardly a congestion is observed, without nothing the presence of fat in the nervous cells according to what
was controlled by Prof. JAKOB and Drs. AMADEU FIALHO and E. VILLELA.

The nuclear alterations of the liver cells are being the object of very interesting studies on the part our colleague Dr. MAGARINOS TORRES.

The above stated lesions, as well the macro as the microscopic ones, may be of varying intensity, according to the case, and do not miss in animals which die from the infection.

When however, the animals are sacrificed in the beginning of the fever period, or other are with low post-thermic temperature fall, but have been inoculated with a still little active virus, and in which probably the illness would be likely to evolve towards curing, the classic lesions of the liver and kidneys, may miss or be very rare.

Every time however, an animal is sacrificed, when in the period of hyper or hypothermy, but whose serum is already yellow owing to biliary elements contained in it, and whenever there is albumin in the urine, then there is no lack of lesions of liver necrosis nor of the nephritis besides fatty degeneration, etc.

The anatomo-pathologic lesions of the monkeys infected with yellow fever in Brazil, show no difference with those obtained with the African virus.

II

RESEARCH OF THE VIRUS IN BLOOD AND ORGANS FROM PERSONS WHO DIED FROM YELLOW FEVER.

We had an opportunity of prospecting the virulence of the blood and the organs of yellow fever patients to the Macacus rhesus with material from 7 autopsies, effected 1 to 4 hours post mortem, in persons who had died on the 4th and 5th day of the illness. The monkeys were inoculated, half an hour to two hours, after the autopsy, having been controlled, by this opportunity, by means of culture, the complete lack of bacteria of secondary association within the organs, which were gathered with all possible aseptic care, the fragments of which were repeatedly washed in sterile physiologic water, before wakening use of them. One part of the organ, after having been squashed in a mortar with sterile sand, was diluted with 4 parts of saline solution, filtrated through gauze and the emulsion thus obtained served then for injections to the monkeys, subcutaneously, at a dose of 2 to 5 cubic centimeters.

The inoculations were made with blood, liver, kidneys, spleen, bone marrow and brain. The liver was injected isolatedly to 3 Macacus rhesus, as well as the blood and kidney to two other of these monkeys. Four rhesus were inoculated with mixtures, of various organs. None of the 9 inoculated animals happened to die from yellow fever, and of those which experienced febrile reactions, two which were sacrificed, would show nothing from which might be supposed they were injected with yellow fever virus.

Anyhow, as far one may conclude from these results, it seems that the virus of yellow fever does not exist either in the blood or in the organs of yellow fever patients, which confirms its total disappearing in the human organism after the third day of the illness.
RESEARCH OF THE *LEPTOSPIRA ICTEROIDES*.

In 15 cases of yellow fever, the researches intended to discover the *Leptospira icteroides* through microscopic verification, isolating in culture and through inoculation to animals of the most varied material, blood, urine of patients at different stages of the illness, as well as material gathered in organs of corpses, liver and kidney, besides blood and urine, all provided negative results. The examinations in fresh material in dark field, impregnations and colouring did not prove the least successful.

The inoculated Guinea pigs who showed high temperatures were bled off at heart, or sacrificed, their blood and emulsions of their organs being inoculated to other young animals. The reactions, on the young Guinea pig however, disappear at the end of 3 passages as was already observed by STOKES, BAUER and HUDSON. In the blood and organs of such animals, no *Leptospira* is to be found. Also negative have remained the inoculations of the blood and organs of monkeys to Guinea pigs, and also without a result remained the direct examinations about *Leptospiras* in these animals.

PFEIFFER’s reaction effected with 8 different serums proceeding from patients with 8 to 15 days convalescence, remained likewise negative in vivo in young Guinea pigs, either with the *Leptospira icteroides* isolated in Bahia by NOGUCHI and his collaborators (Palmeira race) or with a race of *Leptospira icterohaemorrhagiae*, both parasites continuing alive, still 2 hours after the experiment, in the peritoneum of Guinea pigs injected with 1/2 c.c. of serum and 1/2 c.c. of culture.

In view of the negative results of these researches allied to those previously obtained in Africa (STOKES, BAUER and HUDSON, MATHIS and SELLARDS) as also in Brazil in man (GOMES DE FARIA, MARQUES DA CUNHA, OLYMPIO DA FONSECA, BORGES VIEIRA, BURLE DE FIGUEIREDO) we may conclude that the *Leptospira icteroides*, does not play any etiologic part in yellow fever. It is most probable the *Leptospira icteroides* appears in the cases in which it has been isolated, as a germ of secondary association, perhaps proceeding from the intestine of persons who ingest waters of bad quality and spoiled by *Leptospira*.

This hypothesis appears to us as being probable in view of the isolating, every time more and more frequent, of *Leptospiras*, pathogenic to Guinea pigs, directly from mine waters, pools, wells, etc. which renders it most probable, that such germs, being brought into the digestive tube through polluted waters, pass afterwards into the blood, in certain severe infectious cases like yellow fever.

As less probable would appear the hypothesis that, amongst the cases of yellow fever studied by NOGUCHI and his collaborators, there could exist some ones of the WEIL’s disease, which would not have been identified and in which, he would have isolated the *Leptospira* which was that named *icteroides*.

IDENTITY OF AFRICAN AND SOUTH AMERICAN YELLOW FEVER

The works realized between us during the actual epidemic of yellow
fever, under the most favourable conditions, enable us to leave aside, as far as we may believe, the etiologic part of the *Leptospira icteroides* in yellow fever. Also, in Africa, the results in this respect, were absolutely negative, so that, we may conclude that no etiologic difference exists between the two diseases, either the one reigning in Africa or the other existing among us, both being caused by a still unknown virus.

The identity between both diseases, is, besides, thoroughly established, by its clinic aspects, anatomo-pathologic epidemiologic lesions, as also because both are practically caused by a unic transmitter, the *Aedes aegypti*.

The experiments effected here have shown that the *Macacus rhesus* was sensible to the American, in the same manner as to the African virus, which still firmer identifies both diseases.

To these proofs we may still add one more, this one being the cruised immunity between the African and the American virus.

Dr. CARLOS CHAGAS having brought from the Pasteur Institute, preserved in ice, fragments of liver from *rhesus* infected with African virus, this material was inoculated 20 days later to a *rhesus* and further to another one. The first monkey had a febrile reaction of 40° on the 5th day, and the second had no accentuated febrile reaction, the virus either having lost its virulence, or having such so attenuated as to be unable to cause the animal’s death.

It thus gave way to suppose, that this virus, however attenuated, would be sufficient for conferring the *rhesus* immunity against the inoculation of an homologue virus. It was in fact, what was controlled, and the two above mentioned *Macacus rhesus*, being injected with an emulsion of liver of a *rhesus* infected with the virus isolated here, underwent no reaction, whereas a witness died within 7 days, which demonstrates that the first inoculation with the African material, however in attenuated form, was sufficient to protect it against our own virus, thus proving their identity.

V

ATTEMPTS TO OBTAIN A SEROLOGIC DIAGNOSIS OF YELLOW FEVER.

A question of the highest importance in yellow fever is to succeed in obtaining a sure method for its diagnosis, being given the high number of cases which evolve atypically, and of those which, owing to their benignancy do not differ from a common influenza and which for this reason and their infectiousness are more dangerous, under the hygienic point of view. Hence the interest there is in discovering them in time. For this purpose, we have tried, since the beginning of our researches about experimental yellow fever, to verify whether it could not be possible to obtain a serologic diagnose of the illness, in making use of the fixation complement test.

Putting this method in practice, we used, in the beginning, as an antigen, the serum of patients in the 1st and 2nd days of the illness, at a dosage of 0.5 c.c. and, as an antibody, the serum of convalescents of 8 to 15 days, at a dosage of 0.2 to 0.5 c.c. The complement we used was that of Guinea pig and as a hemolytic system, we made use of rabbit anti-sheep serum and washed sheep’s red cells.
The results of the experiments in 8 convalescent patients having been negative, we decided to substitute to the antigen, serum of a patient at the beginning of the disease, a phenicated extract of rhesus liver, prepared by emulsionating 5 grams of liver pulp, finely trituated, in 100 c.c. of saline solution, phenicated at 0.5%. The emulsion thus obtained was filtrated on paper, afterwards left to rest for a few days, being then controlled its preventive power. This antigen was used at a dosage of 0.2 to 0.4 c.c. with the same serums of convalescents as used in the former case, still not giving the least positive result.

The results got at till now in experiments by mean of deviation of complement have not been absolutely favourable, but it is possible that, using other technic methods, the viewed aim will be at last reached.

The serum of convalescent has no influence whatever on the WASSERMANN's reactions, which remain negative and as well at the beginning as during the convalescence of the illness, when syphilitic antecedents are not to be thought of, as we have been able to make certain in 8 cases we observed.

As an element for establishing a diagnosis of yellow fever, we can still avail ourselves, in certain cases, of the preventive properties acquired by serum from persons having recovered from the illness.

The serum of convalescent prevents easily the infection of the monkey, as demonstrated by STOKES, BAUER and HUDSON, whereas 2 c.c. serum of a normal man have no action whatever. Thus, as far as we shall be in presence of a case, suspected to be one of yellow fever, whose benignancy however, or the fact of having been seen rather late, did not allow a thorough diagnosis to be made, we can bring it into light in a rather easy way, searching the preventive properties of the serum. Take 2 c.c. of serum from the suspected person and inject it to a Macacus rhesus; 24 hours later, inoculate the same animal with a mortal dosage of virus, and if it does not contract the disease, this will mean that the serum is a preventive one, and there will be a high probability, if not a certainty, that the case was one of yellow fever; if, on the contrary, the animal gets ill, the case is to be considered as negative. This is a method to be experimented and which may lend useful services to the hygienist, explaining certain dubious cases, allowing the relation of some cases to others, and providing the hygienist with more precise epidemiologic data, a method of great advantage in certain cases.

This process was already used in three cases of a certain epidemiologic importance in this city, being observed on two occasions, positive results together with the verification of the preventive properties of the serum from suspected persons, and in the third case, the proof was considered negative, showing plainly the suspicion of yellow fever was to be rejected, owing to the serum having protected the rhesus against the inoculation of the virus.

The results obtained in using this process of diagnose were of great usefulness for the epidemiologic works of the Public Health Department, for making clear the three cases above referred to.
VACCINATION AGAINST YELLOW FEVER

The vaccination against yellow fever, can be effected by two different methods, as well by way of passive immunisation through inoculation of serum from convalescents or from a monkey, immunized by repeated inoculations of virulent material, as by inoculating a vaccine prepared with blood or organs of infected monkeys.

We have used in many occasions, the serum from convalescents as a temporary protection method against the infection. It was at first used in Manguinhos to protect those working in the laboratories with infected monkeys and later on, its use was extended to people living amongst foci and to others who required a protection against any emergency. The number of such protected persons amounts to 50 or 60, all having well undergone the injections, which, only in one or another case, would cause a certain very short, quite individual depression. The serum used was that of persons who were with 15 days to one month recovering from mild and severe attacks of the illness. Between the persons thus protected against yellow fever, no case of the illness broke out up to this day, being used in same 1 to 2 c.c. serum, sub-cutaneously, as a vaccinating dosis.

The use of serum of convalescents is however a limited one, in view of the difficulties prevailing in getting it in big quantity allowing it to be used on a large scale.

We are immunising with blood and organs of infected rhesus, 1 horse and 2 sheep, 6 rhesus with a view to obtain in same, a serum having eventually a sufficient immunizing power, so as to be possibly advantageously used more extensively, in certain foci of the illness.

It is possible also that the serum of immunized animals whose preventive action has just been proved at the Pasteur Institute in Paris, may have also an efficient curing power in man, when used during the first days of the illness and in large quantities.

The vaccine against yellow fever, which is likely to obtain a big success, is obviously the one prepared with emulsion of organs of Macacus rhesus or cynomolgus and sterilized by either of the well known processes.

The French Commission of the Pasteur Institute had demonstrated that the warmed serum of a patient in the first days of the illness, when the virus is existing in him, had an obvious vaccinating power, in relation to the yellow fever infection in man.

It was then to be expected that the organs of the Macacus rhesus or cynomolgus full with virus should offer more favourable conditions for preparing a vaccine, and, accordingly, since we infected the first Macacus rhesus, we used the organs of the animal to prepare it, reducing them to a pulp which, after being emulsified in sterilized water in the proportion of 1 to 9, was filtrated on gauze and sterilized by formol vapours, in accordance with the technic which has been in use amongst us since 1911 to sterilize the vaccine against fowl's spirkoetusis.

The vaccine was already prepared and being tested in rhesus, and man, when we received HINDLE's work, giving an account of his interesting and precursory attempts with vaccines used by him against
yellow fever, one merely formulated, the other glycerinated and phenicated, using in both one part of liver against four of the sterilizing solute, in the first case formol at 1 o/oo and in the second a solute of 60/o/o glycerine in phenicated water at 0.5/o/o.

We decided then to prepare the glycerine and phenol vaccine, as recommended by HINDLE and to compare with another formulated 2 o/oo and phenicated at 0.5/o/o with a view to obtain therewith a more perfect guaranty as to sterility as regards bacteria and fungi, without prejudice as to conservation of the virus. Both vaccines provided good results in the rhesus, protecting them against experimental infections and were, consequently, inoculated to man.

As the glycerinated vaccine would have shown more painful, we began to use in man, on a large scale the phenicated and formulated vaccine which is more easily borne, being hardly a little sensible at the moment of inoculating, and showing no posterior reaction.

The technic we nowadays apply to, for preparing the vaccine, is the following. We infect a sound rhesus or cynomolagus, with a doubtlessly mortal quantity of the virus, and when the animal, after having undergone the characteristic thermic rise, enters the stage of hypothermy, and sometimes even before this, it is then sacrificed by chloroform. The organs are immediately extracted with the maximum of asepsis, making use, for preparing the vaccine, of the liver, kidneys, spleen and brain, which are placed on large PETRI's dishes, sterilized and carefully examined. As soon as they prove to be perfectly sound, they are washed in a saline solution, wiped on sterile filter paper and weighed.

Afterwards the organs are cut into small fragments which are placed into a mortar with sterile washed sand, being then triturated carefully. This being done, to one part of organ, are added 5 of distilled water, sterilized, formulated at 2 per o/oo and phenicated at 0.5/o/o shaking and stirring off the material until a fine and homogeneous emulsion is got at, which then is filtrated on 4 leaves of gauze and collected into a sterile matrass. This filtrate is the vaccine which then is kept in the ice-box for 5 days, after which delay, its complete sterility is verified, which is obtained by inoculations into anaerobian and aerobian mediums, and consequent inoculation of 0.5 c.c. to two Guinea pigs.

Since the culture mediums remain sterile, the vaccine is then distributed into ampules with the usual care and inoculated anew. If its sterility is perfect and the Guinea pigs, after the due time, remain sound, the vaccine may be considered as fit and ready for use in man.

The dosis in which it is used in an adult man is 2 c.c. sub-cutananeously, care being taken however to shake the ampule to emulsify the material it contains, which deposits when not in use.

The formulated and phenicated vaccine, prepared as aforesaid, is a roseeous yellowish liquid, trouble, giving a light deposit and without disagreeable smell, neither that of the formol nor of the phenic acid it contains.

This vaccine began to be used in the Oswaldo Cruz Institute between the people who deal there with yellow fever, and having shown no disagreeable reactions, began to be of common use, and the Public Health Department already received it, and is being using it widely, in small
isolated foci of yellow fever in the State of Rio de Janeiro, the total of vaccinated persons being already computed in more than three hundred. It was always easily borne. It did not give troublesome reactions, and amongst the vaccinated people, there has been no case of yellow fever. However, as the question has been dealt with in a small scale only, and not richly observed, there is no possibility, for the time being, to make oneself a definite opinion at this respect.

Presently, about 2 thousand doses are ready for experiments to be undertaken by the Public Health Department.

With our pursuance of Laboratory experiments with this vaccine, and its being used practically on a large scale, we may nourish the hope of reaching, in an early time, to positive and definite results as to its practical advantages, time of keeping, duration of the immunization, etc.

These are, here resumed, the results which up to this day, we have obtained from our researches, on yellow fever in Rio de Janeiro.