Vaccination with serum-virus in yellow fever (*)

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The solution of the problem of vaccination against yellow fever has great practical importance, not only as a means of individual protection, but also on account of the great part it may contribute to make easy and more economical the prophylaxis of that disease. Therefore, it is not astonishing to see the considerable amount of efforts put forth to discover a sure method which might afford a useful and efficient solution of so important a question. (Aragão H. B. Brasil Medico 17-1-31).

The processes, used up to now, may be classified into 3 different groups, as follows:

1°.—Passive immunization that is obtained, in man or animal, by means of an injection of serum from an individual recovered from yellow fever or from a monkey immunized against the virus.

2°.—Active immunization with the aid of vaccines constituted by emulsions of the organs of infected monkeys, after previous treatment of the material with an appropriate antiseptic substance, such as formol, chloroform, carbolic acid, etc.

3°.—The vaccination with serum-virus, i. e. an active vaccination obtained by means of inoculation of serum from an individual or animal immunized against yellow fever and inoculation of virus of that disease; the latter has to be made 24 hours after the former.

The first method, that of passive immunization with the aid of human or animal serum, immune against yellow fever, was employed here, by us, in a fair number of cases, either to protect the persons who are working in laboratories with yellow fever virus, or to protect against the disease those who live in foci of the disease and are liable to be bitten by

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infected mosquitoes. Here, in Rio, the number of persons protected by such a mean amounts to 200, and among them there was no case of yellow fever. The dose of serum varied between 1 and 4 cc.

The method of vaccination against yellow fever by means of vaccines prepared with emulsions of organs of infected monkeys is that which, up to now, has been the most widely employed in clinical practice and laboratory experience.

The method was set out by Hindle, and was also used by us, Petit, and Stephanopoulos, Lemos Monteiro and other researchers. Before then, the French Commission had verified that the heated serum of a yellow fever patient in the first days of disease, i.e. when still containing the virus, has a vaccinating action, when inoculated into a non-immune individual. The vaccination with organs of infected monkeys had been fairly widely experimented, not only in the laboratory but also in clinical practice.

At the beginning, the results of its employment in the laboratory and in man, were fairly encouraging, but after its use was made on a large scale, its action revealed itself to be somewhat irregular. In certain cases the immunity obtained was very good, while it failed in others, which fact might be imputed to insufficiency of the dose used (2 cc. in man) and possible immunizing variations of the different emulsions employed. The human vaccinations applied here, during the epidemic outbreak of 1928-1929, amounted to about 25 thousand.

As this method of vaccination still continues to be studied in laboratory, it is possible that new technical processes and a still greater experience may render its action more efficient, in animal as well as in man.

The vaccination with serum-virus is a method of immunization against yellow fever which we have been trying for some time and, judging from the experiments up to now performed, we believe that it may give sure and useful results in practice.

Two elements are necessary in order to make the vaccinations by this method: a serum endowed with immunizing properties against yellow fever and the virus of that disease. The serum to be used may come from a man recovered from yellow fever as well as from a monkey also recovered from that disease. The process being very practical, it is explicitly recommendable to employ the hyperimmunization of the givers of serum
(man or monkey) with the aid of successive inoculations of virulent blood by the bite of infected mosquitoes.

The virus necessary to complete the immunization by this process has to be that which exists in blood of infected monkeys; the latter, when dried and kept at low temperature, is preserved from alteration for about one year. Therefore the virus can be easily transported anywhere; it is only necessary to dilute it on the occasion of its employment.

The practical performance of the process is of the great simplicity; it consists in inoculating 1 or 2 cc. of serum into a monkey and 24 hours afterwards 0.1 cc. of virus (blood at natural state or a corresponding dilution of dried blood). One cubic centimeter of blood, by drying, is reduced to 0.165 grs. of dry material, and 0.1 cc. of blood to 0.016 grs. In man, the dose of serum to be used has to be 4 cc. and that of virus must not exceed 0.1 cc., which dose revealed itself to be perfectly tolerated by monkeys. Twelve days after the vaccination with serum-virus, it is commendable to repeat the inoculation of the same quantity of virus (0.1 cc.) in order to reinforce the first immunization. Our experience showed that even several months after the employment of the vaccination by the serum-virus the immunization of monkeys is fairly sure, inasmuch as, inoculating into them 0.1 to 1 cc. of virulent blood, they presented no reaction.

Consequently, the practical performance of the process does not present any insuperable difficulty, it only being necessary, in case of its wider employment, to organize a corp of serum givers, such as already exist in many countries for the prophylaxis of measles, etc., and a service for immunization of monkeys for the same purpose. Virus, under the form of dried blood, is very easily obtainable on a large scale, without any greater difficulty, on account of the small quantity required for use.

The technique of vaccination with serum-virus against yellow fever may be resumed as follows:

1°—Inoculation into a rhesus of 1 to 2 cc. of immunize human serum or of that from a monkey immune against yellow fever (4 cc. for a human individual).

2°—Inoculation 24 hours afterwards, into animal or man, of 0.1 cc. of virulent blood in its natural state or the corresponding quantity of diluted dry blood.

3°—Inoculation 12 days afterwards, into animal or man already vaccinated with serum-virus, of 0.1 cc. of virus in order to consolidate the immunization obtained.

The bases of the process of vaccination with serum-virus against
yellow fever being thus established, we pass to report the experiments upon which we are basing our process, as it is here explained.

EXPERIMENT I

*Rhesus* 160.—Inoculated on 19-IX-28 with 2 cc. of serum from a boy (H.) suspected of having suffered from yellow fever (protective test) and on the next day with 0.1 cc. of an emulsion of virulent liver from a *rhesus* monkey. Temperatures: 19-IX, 39°; 20-IX, 38°8; 21-IX, 39°,5; 22-IX, 39°; 23-IX, 38°,7; 24-IX, 39°,4; 25-IX, 39°; 26-IX, 39°; 27-IX, 39°,4; 28-IX, 39°,5; 29-IX, 39°,2; 30-IX, 39°,3. Afterwards, the animal continued keeping well, the protective test performed confirming that the case under study was one of yellow fever. The observation of the animal was then interrupted and on 2-IV-29, i. e. more than 6 months afterwards, it was inoculated with 1 cc. of virulent blood from *rhesus* 359 which was fatal for other monkeys. Temperatures: 2-IV, 39°,3; 3-IV 39°,4; 4-IV, 39°,3; 5-IV, 39°,5; 6-IV, 39°,5; 7-IV, 39°,5; 8-IV, 39°,2; 9-IV, 39°; 10-IV, 39°,8; 11-IV, 39°; 12-IV, 39°,6. The animal continued keeping well, thus proving that the initial vaccination with serum-virus had conferred a very solid immunity on it.

EXPERIMENT II

*Rhesus* 213.—Inoculated on 23-XI-28 with 2 cc. of serum from a man (F.) suspected of having suffered from yellow fever (protective test) and on the next day with 0.1 cc. of an emulsion of virulent liver from *Macacus rhesus*. Temperatures: 23-XI, 39°; 24-XI, 39°,2; 25-XI, 39°,7; 26-XI, 39°,1; 27-XI, 39°; 28-XI, 38°,9; 29-XI, 38°,5; 30-XI, 39°,4; 1-XII, 39°,2; 2-XII, 39°,3; 3-XII, 39°,2. Afterwards, the animal continued keeping well, the protective test performed confirming that the case under study was one of yellow fever. On 8-XII-28, the animal was inoculated with 0.1 cc. of virulent blood from *rhesus* 212 which killed other monkeys. Temperatures: 8-XII, 39°,1; 9-XII, 39°; 10-XII, 39°,6; 11-XII, 39°,3; 12-XII, 39°,2; 13-XII, 38°,9; 14-XII, 39°,3; 15-XII, 39°,4; 16-XII, 39°,2. After this second inoculation of virus, the animal always was keeping well, proving, as the precedent monkey, that the initial vaccination with serum-virus had conferred on it a sure immunity against yellow fever.

EXPERIMENT III

*Rhesus* 626.—Inoculated with 2 cc. of serum from *rhesus* 12 (hyperimmunized) subcutaneously on 21-XII and on the next day with 0.1
cc. of virulent blood from *rhesus* 620. Temperatures: 21-XII, 39°,1; 22-XII, 39°; 23-XII, 39°,2; 24-XII, 39°,6; 25-XII, 39°,5; 26-XI, 39°,8; 27-XII, 39°,9; 28-XII, 39°,6; 29-XII, 39°,4; 30-XII, 39°; 31-XI, 39°. The animal continues without alteration until 27-1-30, when it was inoculated with 1 cc. of virulent blood from *cynomolgus* 644. Temperatures: 27-I, 39°,1; 28-I, 39°; 29-I, 38°,8; 30-I, 39°; 31-I, 38°,9; 1-II, 39°,1; 2-II, 39°,2; 3-II, 39°,5; 4-II, 39°,5; 5-II, 39°,4; 6-II, 39°,3; 7-II, 39°,5. On 8-II-30 the temperature of the animal rises to 39°,8, on the next day to 39°,9, later on to 40° and so it continues until the 15-II-30, when the animal is sacrificed; it is then verified that it presents bronchopneumonia, tuberculous foci, but no lesions of yellow fever; this proves that the immunization by vaccination of serum-virus in this case was complete and sure as it was showed by an ulterior inoculation of pure virus.

**EXPERIMENT IV**

*Rhesus* 785.—Inoculated subcutaneously with 1 cc. of a mixture of serum hyperimmunized *rhesus* (ns. 12 and 20) on the 20-X-20 and on the following day with 1 cc. of a dilution of dry blood corresponding to 0,1 cc. of fresh blood. Temperatures: 20-X, 39°,2; 21-X, 39°; 22-X, 39°; 23-X, 39°,3; 24-X, 39°,4; 25-X, 39°,5; 26-X, 39°,6; 27-X, 39°,4; 28-X, 39°,6. The monkey did not present any reaction until the 26-XII-30, when it was inoculated again with 1 cc. of a dilution of dry virulent blood corresponding to 0,1 of fresh blood. Temperatures: 26-XII, 38°,4; 27-XII, 38°,3; 28-XII, 38°,1; 29-XII, 38°,4; 30-XII, 39°,8; 31-XII, 38°,6; 1-1-31, 38°,4; 2-I, 38°,8. The monkey, perfectly well remained as it can be seen and resisted to an new inoculation of virus, thus proving to be firmly immunized.

**EXPERIMENT V**

*Rhesus* 786.—Inoculated subcutaneously with 2 cc. of a mixture of serum from 2 individuals recovered from yellow fever on 20-X-30 and on the next day with 1 cc. of a dilution of dry blood corresponding to 0,1 cc. of fresh blood. Temperatures: 20-X-30, 39°,3; 21-X, 39°,5; 22-X, 39°,3; 23-X, 39°,4; 24-X, 39°,1; 25-X, 39°; 26-X, 39°,2; 27-X, 39°,1; 28-X, 39°,3. The rhesus did not present any alteration until the 26-XII-30, and on that day it was again inoculated with virus: 1 cc. of a dilution of dry blood corresponding to 0,1 cc. of fresh blood. Temperatures: 26-XII, 38°,4; 27-XII, 38°,1; 28-XII, 38°,6; 29-XII, 38°; 30-XII, 38°,5; 31-XI, 38°; 1-I-31, 37°,9; 2-I, 37°,8. Just as in the case of the precedent mon-
key, this one resisted perfectly against a new inoculation of virus, proving the solidity of immunization conferred on it by the initial vaccination with serum-virus.

VARIOUS EXPERIMENTS

Apart from the experiments mentioned in detail above, which bring into evidence the advantages of the serum-virus vaccination by the technique used, as a means of obtaining a sure immunity against yellow fever, we had occasion to perform some more experiments with various objectives, which we will here briefly mention, although they do not present any greater importance:

1º.—Experiments with vaccination of serum, using a smaller quantity of serum, intravenously. We inoculated into the vein of rhesi (605 and 606) 0,5 cc. of serum from a hyperimmunized monkey and 24 hours afterwards 0,1 cc. of virulent blood. Both animals tolerated the injection of virus, but the second had some fever reactions between 40 and 41 degrees, during 4 days, after which it returned to its normal state. The two monkeys inoculated again, 22 days afterwards, with a new dose of 0,1 cc. of virulent blood, presented again fever reactions during some days, after which they returned to their normal state. A new inoculation into rhesus 605 of 2 cc. of virulent blood proved that the immunization of that animal was solid, since it did not present any reaction.

2º.—Experiments performed with small quantities of serum from animal hyperimmunized against yellow fever. Some experiments made with vaccination of serum-virus, using 0,01, 0,1, 0,5, 1 and 2 cc. in order to verify which could be the minimal quantity necessary to protect and to immunize a rhesus against yellow fever, proved that the animals invariably presented a reaction, except with 1 cc. or more serum. With 0,5 cc., there are still fever reactions, and with 0,01 and 0,1 cc. the animals succumb to yellow fever. So, the dose of 1 cc. has to be considered as the useful one for monkeys, while, for man, we should use not less than 4 or 5 cc. of serum coming from an individual recovered from yellow fever or from a hyperimmunized monkey.

3º.—Apart from the serum of man and of monkey, we tried to verify, whether the same results could be obtained by using serum from horses immune against yellow fever, a circumstance which would be of great advantages in practice; but the results, unfortunately, were not good, owing to the weak protective power of the serum used.

4º.—Some experiments were made by substituting the injections of serum and virus on different days with simultaneous subcutaneous in-
jections of both elements in different parts of the body of the same animal, or by using mixtures of serum (0.5 cc.) and virus (0.1 cc.) subcutaneously. In both cases the animals, though having resisted against the inoculations, presented fever reactions, more or less protracted, so that a favourable result perhaps might be obtained by this process only by the use of greater quantities of serum.

CONCLUSIONS

Examining the results, up to now obtained by the use of the vaccination with serum-virus in yellow fever, we believe we may admit that the process is of easy performance in practice, that it confers a sure immunization on the animals on which it is used, and that in the practice of human vaccination it will give the results which are hoped from this method.

The facility in the transport of the serum and the dry virus from one place to another will secure to this process the possibility of its employment in any place, wherever this method of protection becomes necessary.

We hope that the divulgation of the method will induce the trial of the same in the different centres of yellow fever research.