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PREPARATION OF ANTIRABIES SERUM FROM THE MULE

by

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The Pasteur Institute of Iran was able, in 1955, (1) to confirm the value of the prophylactic treatment of human rabies with antirabies serum having a high antibody concentration. In 1956 the Institute requested the national health authorities to decentralize this method of treatment and establish serum inoculation posts in all the main provincial through which injured persons must pass before being sent on to Tehran.

The shortness of the time during which seroprophylaxis is effective, found experimentally to be not more than 72 hours, made such decentralization unavoidable, in view of the difficult communications in certain parts of the country, precisely those most threatened, and the length of the journey which the injured had, in most cases, to make before reaching Teheran.

However, this indispensable decentralization involved the immobilization in various parts of the country of large amounts of serum and made national production practically essential. The Razi Institute, responsible for the preparation of sera for human use, was requested to undertake this production by the national health authorities at the beginning of 1957.

★ WHO/Rabies/129.27 November 1959

1 Baltazard, M., Bahmanyar,M. et al. (1955) **Bull. Wld Hlth Org., 13, 747**

Two methods were tried out: that of the Paris Pasteur Institute, as described in the WHO Manual (2) and that of the Instituto Toscano (3) which one of us (H. M.) was able to study at Siena, thanks to the Professors D'Antona and Falchetti.

The first tests, carried out with two series of 3 and 7 horses, respectively, revealed the mediocre nature of the results obtained with this animal, as already observed by other workers. Out of 3 horses immunized by the Paris Pasteur Institute method, only one responded satisfactorily to immunization, the corresponding figures for the Istituto Toscano method being 4 out of 7. Such a wastage was inacceptable under our working conditions so that we decided to try some other species of animal. The results published by other workers on the immunization of sheep, goats or bovines did not seem encouraging, so we decided to try the only species which to our knowledge had not yet been employed, namely the mule.

IMMUNIZATION OF THE MULE

As the first results were encouraging, we established the following technique which is a slightly modified version of the Istituto Toscano method, as indicated below:

Vaccine: 5% suspension of sheep brain (Sassari virus) in Kaplan's phosphate buffer, phenolized (0.5%) and inactivated for 24 or 48 hours at 37°C, freshly prepared or kept for less than a week at + 4°C.

Virus: 5% sheep brain suspension (Sassari virus) in Kaplan's phosphated buffer, freshly prepared or kept for less than a week at + 4°C.

2 Laboratory Techniques in Rabies (World Health Organization: Monograph Series No. 23)

3 D'Antona D & Falchetti, E., (1953) Sixth International Congress of Microbiology, Rome, 3, 319

Toxoid: The animals are simultaneously immunized against tetanus with purified toxoid absorbed on aluminium phosphate (P.A.T.T.). 1

Days	Inoculum
1	20 ml phenolized vaccine + 10 ml P.A.T.T.
2-20 (= 19 days)	20 ml " each day.
21	30 ml " +25 ml P.A.T.T.
22-40 (= 19 days)	30 ml " each day
41	45 ml " + 40 ml P.A.T.T.
42-56 (= 15 days)	45 ml " each day
	Interval of 60 days
117	30 ml phenolized vaccine + 30 ml P.A.T.T.
118-126 (= 8 days	30 Ml " each day
127	45 ml " +45 ml P.A.T.T.
128-136 (= 9 days)	45 ml " each day
137	30 ml live virus suspension + 60 ml P.A.T.T.
138-143 (= 6 days)	30 ml live virus suspension each day
	Interval of 10 days
156	first harvest bleeding

A first series of 8 mules, aged from 12 to 15 years, was immunized in this way at the same time as a series of 7 horses of the same age. Several test bleedings were made in the course of immunization, one of them before the first inoculation. Serum-virus neutralization tests in mice, using the standard method, with these test bledings and the harvest bleeding gave titres which were already superior, from the qualitative viewpoint in the mule and also revealed a fact we regarded as particularly important, namely that all the 8 mules responded to immunization wheras the horses behaved in the usual way, 2 out of 7 not reacting to immunization and one only very feebly.

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1 This double immunization seemed indicated in view of the almost constant soiling of the wounds by earth or animal dejecta.

Immunization was then continued according to the following schedule:

Days	Inoculum
243	25 ml phenolized vaccine + 25 ml P.A.T.T.
244-253 (10 days)	25ml "
254-266 (13 days)	35 ml "
	Interval of 12 days
278	Harvest bleeding

The titres showed a spectacular difference: whereas the antibody level in the horses remained unchanged or increased only very slightly, all the 8 mules showed a considerable and regular increase.

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Immunization was continued following the same schedule after a new rest interval of 100 days, the series of control horses being now abandoned.

The attached table shows the increase of antibody during these immunization cycles.

In this table, the antibody titres are expressed in international units per ml, in the international refernce serum having a titre of 87 units per ml. The titrations were carried out with 70-140 LD50, the reference serum giving a final neutralizing dilution of 1/800 - 1/1200 and the mule serum reaching 1/6000.

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A second series of 10 mules, including 5 immunized with rabies antigen alone and 5 others simultaneously with tetanus antigen, gave results of the same order, also distinctly in favour of combined immunization. At present 14 mules have been immunized or are undergoing immunization, without a single one of these animals proving refractory to antibody production. The highest antirabies titre reached by certain of these animals is 350 U/ml and the minimum titre 80 U/ml at the end of hyperimmunization.

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Animals	No.	After basic immunization	After first hyperimmunizing series	After second hyperimmunizing series	Anti-tetanus titre A U/ml in vivo
Horses	4	40	60		100
	5	40	abandoned		
	6	0	< 10		60
	7	40	120		100
	9	20	abandoned		
	10	0	< 10		45
	12	40	40		110
	13	40	40		50
Mules	16	>40	240	> 320	230
	17	>40	210		
	18	>40	210		200
	19	>40	240	Mixture after	200
	20	>40	200	concentration	330
	21	>40	320	800	240
	22	>40	140		200
	23	>40	280	> 320	240

We felt that the value of the mule as a rabies antibody producer as compared with the horse might be linked with a genetic factor. Because of this we decided to immunize the donkey. Six donkeys were immunized, 3 with rabies antigen alone and 3 simultaneously with tetanus antigen, in parallel with 2 series of 5 mules immunized in the same manner. (However, because of their smaller size, the donkeys were given lower inocula, namely 10, 20 and 30 ml of phenolized vaccine, respectively.)

The donkeys behaved in appreciably the same manner as the horse: 2 out of 6 proved refractory to immunization and no animal exceeded a final hyperimmunization figure of 60 U/ml.

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The drawback to this type of immunization is the possibility of paralytic accidents: thus we lost 4 mules during hyperimmunization, one as a result of ascending paralysis while the others had to be killed when paraplegia appeared.

Consequently, like other authors, we attempted to inoculate them with antigens less rich in brain tissue. A series of 4 mules were immunized in this way (following our ordinary schedule) with a phenolized vaccine prepared with the supernatant liquid from an emulsion of sheep brain in distilled water, according to the method recommended by D'Silva et al. (4) Another series of 4 mules received a vaccine prepared by the method of LoGrippo and Hartman (5) with beta-propiolactone.

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In both series the rabicidal power of the sera increased very slowly, reaching 80 U/ml only after 2 series of hyperimmunizations and not exceeding this level subsequently.

In conclusion, the mule seems to be the animal of choice for the preparation of antirabies serum. None of the animals are refractory to immunization, antibody rapidly appears, there is a regular increase in titre during hyperimmunization to a maximum very much greater than that

4 D'Silva, G. B., Brooks, A. G., Thomas, A. K. & Ahuja, M. L., (1951) Indian J. med. **Res., 39, 423**

5 LoGrippo, G. A. & Hartman, F. W. (1955) J. Immunol. 75, 123

given by other animals used to produce antirabies serum. The serum-proteins are not allergenic in subjects rendered sensitive to horse serum. It is possible to prepare, by concentration, sera with an antibody titre 2 to 10 times higher than the international minimum standard.