Notes

Production of Hyperimmune Antirabies Serum in Horses

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In 1955 a report was issued [a] describing a method for the preparation of antirabies vaccines of high immunizing potency, based on the use of brain tissue of suckling mice infected with fixed virus and inactivated by ultraviolet irradiation. The results reported in that publication, subsequently confirmed many times, have led us to use this type of antigen in horses for producing antirabies sera of high neutralizing potency.

In experiments in our laboratory we compared the antibody responses of guinea-pigs inoculated with antirabies vaccines prepared from the nervous tissue of suckling mice with those elicited by vaccine prepared from nervous tissue of adult mammals. Contrary to vaccine made from adult brain, vaccine made from the tissue of suckling mice did not produce complement-fixation antibodies of the organ-specific type, and produced only a small amount of antibodies of the species-specific type. However, the suckling-mouse-brain vaccine did induce high titres of virus-specific antibodies (unpublished results).

Since organ-specific antigen is not experimentally demonstrable in the brains of suckling mice, this makes it possible to combine the suckling-mouse-brain vaccine with adjuvant for the immunization of horses with less risk of producing allergic encephalitis. This method was tested for the production of hyperimmune antirabies horse serum to be used in the prophylaxis of rabies in man.

Method

Four horses, over 10 years of age, were hyperimmunized. They were divided into two groups. Group A consisted of two animals in poor physical condition and weighing approximately 250 kg, and group B of animals in good physical condition, weighing approximately 450 kg.

Both groups were given injections of inactivated antirabies vaccine containing varying concentrations of infected suckling-mouse brain. After 20 days' rest, they were inoculated with active virus in the form of suspensions of infected suckling-mouse brain incorporated into Freund's adjuvant without mycobacteria.

TABLE 1

INOCULATION SCHEDULE FOR PRIMARY HYPERIMMUNIZATION OF HORSES WITH SUCKLING-MOUSE-BRAIN RABIES-VIRUS ANTIGEN

Antigens	Volume (ml)	Inoculation route [a]	Days since first inoculation
1 % ultraviolet-inactivated vaccine	40	SC	
2 % " " "	40	SC	7
5 % " " "	40	SC	14
8 % suspension virulent suckling-mouse brains in adjuvant	5	IP	34
	4	SC	
	1	ID	
16 % suspension virulent suckling-mouse brains in adjuvant	5	IP	41
	4	SC	
	1	ID	
20 % suspension virulent suckling-mouse brains in adjuvant	8	IP	48
	6	SC	
	1	ID	

[a] SC = subcutaneous; IP = intraperitoneal; ID = intradermal.

The hyperimmunization schedule followed, as regards vaccine concentration, amount of virulent nervous tissue, inoculation routes, and intervals between inoculations, is given in Table 1. The schedule was not fully applied to one horse in group B (No. 4), which received only the first inoculation of live virus as we wished rapidly to obtain a reference serum for use in our laboratory. Twenty days after the last inoculation, the horses of both groups were bled, followed by the refusion of red blood cells.[b]

[a] Fuenzalida, E. & Palacios, R. (1955) *Bol. Inst. bact. Chile*, **8**, 3.

[b] The refusion method consisted in the bleeding of horses, using 1 % neutral potassium oxalate as anticoagulant, and the immediate reinoculation of the sedimented red blood cells.

TABLE 2

SERUM NEUTRALIZATION TITRES OF BLEEDINGS DURING AND AFTER HYPERIMMUNIZATION OF HORSES WITH INFECTED SUCKLING-MOUSE-BRAIN ANTIGEN

Experiment No.	Horse No.	Serum tested	Neutralization titre [a]	LD_{50} virus used	Relation to International Standard
1	A-1	Primary hyperimmunization	3 200	62	Not done
	A-2	Primary hyperimmunization	1 200	62	Not done
2	A-2	After 5 months' rest period	440	47	Not done
3	A-1	30 days after second booster dose of inactive vaccine	2 800	5	Not done
	A-2	30 days after second booster dose of inactive vaccine	3 200	5	Not done
4	A-1	20 days after booster dose of virulent brain suspension	4 840	68	2.5 : 1
	A-2	20 days after booster dose of virulent brain suspension	4 900	68	2.5 : 1
	—	International Standard	1 940	68	Not done
5	A-2	Pool of 5 daily bleedings starting with that used in Experiment 4	1 420	428	2.8 : 1
	B-4	Bled 20 days after only one inoculation of virulent virus in primary hyperimmunization	520	428	1 : 1
		International Standard	510	428	Not done
6	B-3	Primary hyperimmunization, 1st daily bleeding	11 380	20	8.5 : 1
	B-3	Primary hyperimmunization, 5th daily bleeding	9 040	20	7.6 : 1
	B-3	Primary hyperimmunization, 10th daily bleeding	4 520	20	3.4 : 1
	—	International Standard	1 344	20	Not done

[a] Reciprocal of that serum dilution calculated to protect 50 % of the mice against the LD_{50} used in each test.

The horses of group A received a course of re-immunization five months later. This consisted of two subcutaneous doses of inactive vaccine, one at 5% brain concentration and the second, one week later, at 10%. Thirty days later an 8% suspension of active-virus-infected suckling-mouse brains suspended in Freund's adjuvant was given in a single dose. These animals were bled 20 days after the last immunizing inoculation.

The horse sera were tested for neutralizing antibody content by the methods described by Koprowski & Johnson.[c] When the International Standard Antirabies Serum was made available through the courtesy of WHO, it was included in every test. Each serial serum dilution mixed with challenge virus was inoculated intracerebrally into 5-7 Swiss mice weighing 10-14 g. Calculations of the end-point were made according to the Reed & Muench method,[d] and are expressed as the reciprocal of that serum dilution calculated as necessary to protect 50% of the mice. The CVS fixed-virus strain, Lot 23, obtained from the Division of Biologics Standards, National Institutes of Health, Bethesda, Md., USA, was used as challenge virus; and in each test the LD_{50} value of virus mixed with the serum dilutions was determined.

Results

The neutralizing antibody titres of the various serum specimens obtained during the course of hyperimmunization of the horses are summarized in Table 2. It is apparent that the antibody titres produced are in excess of those present in the International Standard Antirabies Serum in all instances where the full course of hyperimmuniza-

[c] Koprowski, H. & Johnson, H. N. (1954) *Serum-virus neutralization test.* In: *Laboratory techniques in rabies*, Geneva, p. 69 (*World Health Organization: Monograph Series*, No. 23).

[d] Reed, L. J. & Muench, H. (1938) *Amer. J. Hyg.*, **27**, 493.

tion was carried out. In experiment 6, where horse B-3 was bled daily with refusion of red blood cells, it can be seen that even the tenth bleeding produced serum having a satisfactory antibody level.

As stated above, in experiment 5 horse B-4 was bled before completing the full course of immunization in order that our laboratory could have its own standard reference serum to be used for standardization purposes in future tests, since the supply of the International Standard Antirabies Serum was limited. This B-4 serum, which was equal in potency to the International Standard in our tests, was also tested by Dr Karl Habel at the National Institutes of Health, Bethesda, Md., USA, who found essentially the same results.

Conclusion

The high neutralizing antibody titres produced in the sera of horses hyperimmunized with suckling-mouse-brain rabies-virus antigen have been obtained after immunization schedules which are shorter than those previously required when antigens from infected adult mammalian brains were used. These experiments, besides offering a more efficient method of producing antirabies horse serum for use in the prophylaxis of rabies in man, also give further evidence of the high level of antigenicity of the vaccine made from infected suckling-mouse brains.

The Use of Toxoid for the Prevention of Tetanus Neonatorum: Preliminary Report of a Double-blind Controlled Field Trial*

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Tetanus neonatorum is reported to be a major cause of death in many tropical and subtropical countries. Estimates of the mortality due to this cause vary from insignificant numbers to more than 10% of births.[a]

A number of possible methods of preventing this disease have been put forward. They have ranged from the provision of obstetrical services to the protection of babies at birth with tetanus antitoxin or antibiotics. The introduction of services has obvious advantages over and above the prevention of tetanus neonatorum, and has been successful in markedly decreasing the incidence rate in some areas. However, it is clear that even minimal services will not reach some populations for a period of years.

Conscious of the difficulties of providing these services, a number of workers have considered other methods of prevention. Most work has centred upon the passive protection of the baby at birth. Two groups of workers in 1923 [b] and 1927 [c] described the passage of tetanus antitoxin across the placenta, from mother to baby, and suggested that this antitoxin might protect the baby. The success or failure of this method was unconfirmed in the field until the 1961 New Guinea study.[a] This was not a strictly controlled trial and is therefore open to some methodological criticism. However, it appeared to demonstrate that three injections of CSL formolized tetanus toxoid given to pregnant women at six-week intervals protected their babies from tetanus neonatorum, while two injections gave less protec-

* Research study conducted by the International Center of Medical Research and Training, Cali, Colombia, supported by Grant E-4178 of the National Institutes of Health, Public Health Service, US Department of Health, Education, and Welfare, under the authority of the International Health Research Act of 1960 (US Public Law 86-610). This communication was presented to the Seventh International Congresses of Tropical Medicine and Malaria, Rio de Janeiro, Brazil, September 1963.

[a] Schofield, F. D., Tucker, V. M. & Westbrook, C. R. (1961) *Brit. med. J.*, **2**, 785-789.

[b] Broeck, C. T. & Bauer, J. H. (1923) *Proc. Soc. exp. Biol. (N.Y.)*, **20**, 399-400.

[c] Nathan-Larrier, L., Ramon, G. & Grasset, E. (1927) *Ann. Inst. Pasteur*, **41**, 848-861.