

THE RABICIDAL ANTIBODY CONTENT OF RABBIT IMMUNE SERUM AS AN INDEX OF ACQUIRED RESISTANCE TO RABIES INFECTION.

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INTRODUCTION.

THE degree of anti-rabies immunity possessed by rabbits actively immunised against rabies virus is ordinarily measured by an estimation of the rabicidal antibody content of their sera and by their acquired resistance to subsequent infection with rabies strains. On the subject of relationship between these two criteria of immunity, however, no general agreement has yet been reached. Biglieri and Villegas (1926), for example, consider that "anti-rabies immunity is not necessarily a blood immunity," and it is incontestable that a solid immunity occasionally exists in cases whose sera show no evidence of antibody formation. Marie (1927) also, while accepting the view that specific changes in the blood serum characteristic of all active immunisation follow anti-rabies treatment, maintains that the rabicidal antibodies so produced gradually disappear, without there being, however, any correlation with the degree or duration of immunity. On the other hand, Semple (1911) concluded from his experimental work that "the rabicidal action of the serum of animals treated with a rabies vaccine is one of the factors which indicate immunity." Again Pereira da Silva (1927) attributes the superiority of his etherised vaccine to the fact that in the sera of patients treated at his Institute (Camara Pestana) "anti-rabies substances appear most quickly, attain the highest value and persist the longest." We (1929) too have stated that usually "immunity and rabicidal action of the serum are produced concomitantly; they appear together, are present in greatest degree together and disappear at or about the same time," and further that "it may be concluded with reasonable certainty that the rabicidal properties of the serum of an animal are an indication of the immunity against rabies possessed by that animal." Moreover, from the experimental evidence of cross immunity following treatment with carbolised suspensions of exalted and classical strains of rabies virus, we (1931*b*) were forced to the belief that "a very definite, indeed an almost mathematical relationship was established in treated rabbits between their degree of acquired immunity and the rabicidal antibody content of their blood." Such close connection was also implied in the recommendation of the International Rabies Conference of

1927 that in the evaluation of methods of treatment "enquiries should be made into the rabicidal action of the serum...during and after immunisation."

In view of the divergence of opinion on this subject further work appeared to be necessary and the present investigation was undertaken, therefore, to determine if possible whether the rabicidal antibody content of the blood of rabbits treated by various methods affords a true index of the degree of their immunity to rabies virus.

ANIMAL EXPERIMENTATION.

This investigation was conducted in two stages and was at first concerned with an accurate determination of the relative content of anti-viral bodies present in the sera of three rabbit groups, immunised respectively with fresh-fixed virus, carbolised-fixed virus and etherised-fixed virus in equal quantities and over identical periods. From results obtained during the first part of the enquiry we (1931*a*) were able to show that the subcutaneous inoculation of 1400 grm. rabbits with a 2 per cent. suspension of these anti-rabies vaccines in a dosage of 5 c.c. daily on 14 consecutive days was followed by a development in the animals' sera of rabicidal antibodies which, in date of appearance, in quantity and in degree of persistence, varied according to the method of treatment employed. The principal findings may be summarised thus:

A. Serological results after treatment with living fixed virus:

(1) Rabicidal properties first appeared 18 days after the commencement of treatment and were retained for 222 days.

(2) Rabicidal antibody content reached its height 60 days after the last injection.

(3) Rabicidal power was developed to such an extent that one unit volume of serum proved able to neutralise, when antibody formation was at a maximum, 16 unit volumes of a 1 : 100 suspension of fresh-fixed virus in normal saline solution.

B. Serological results after treatment with killed carbolised-fixed virus:

(1) Rabicidal properties became demonstrable 18 days after the commencement of treatment and persisted for 131 days.

(2) Rabicidal antibody content was at a maximum 60 days after completion of treatment.

(3) Rabicidal power was such that one unit volume of serum neutralised 8 unit volumes of 1 : 100 fresh-fixed virus in normal saline solution.

C. Serological results after treatment with killed etherised-fixed virus:

(1) Rabicidal properties were present on the last day of treatment and only disappeared after 226 days.

(2) Rabicidal antibody content reached its maximum 60 days after the end of treatment.

(3) Rabicidal power was so exalted that one unit volume of serum was capable of neutralising as many as 24 volumes of a 1:100 fresh-fixed virus suspension in normal saline solution.

From these results it was concluded that in the immune sera of rabbits treated respectively with equal quantities by weight of etherised-fixed virus, fresh-fixed virus and carbolised-fixed virus the maximum rabicidal antibody content, reached in each group 60 days after completion of treatment, existed in the relative proportions of 3, 2 and 1. It remained, therefore, only to determine what degree of immunity was attained in each treated group at the time of maximum antibody production; if, for example, immunity were also to be present in the same proportions of 3, 2 and 1, then rabicidal antibody production would be identical with anti-rabies immunity; if not, at least some idea of their relationship would be established. The following experiment was devised to settle this point.

Experiment.

To provide against the possibility of breakdown from the occurrence of fatalities or of abnormal response such as non-reaction among the animals undergoing active immunisation, it was essential to employ at the commencement of the investigation a minimum of 75 rabbits. These rabbits were divided into three groups A, B and C, each group consisting of 25 animals of 1400 gm. average weight. Each rabbit in group A received on 14 consecutive days, 5 c.c. of a 2 per cent. suspension of fresh-fixed virus in normal saline solution, each rabbit in group B received an identical dosage of killed carbolised virus and each rabbit in group C an identical dosage of killed etherised virus administered over the same period. The survivors in each group were bled 57 days after the completion of treatment and tested for immunity 3 days later by subdural inoculation with a 1:1000 suspension of fresh-fixed rabies virus, the true minimal lethal dose of the strain. It was of course imperative that in this experiment each survivor's serum had to be investigated in respect of rabicidal antibody content so that final calculations might be based only on those rabbits whose sera possessed the normal neutralising power of their respective groups, viz. the capability in the etherised group of neutralising, volume by volume, 24 centesimal fixed virus suspensions, in the fresh-fixed virus group 16 and in the carbolised group 8. Eventually 12 rabbits in each group fulfilled the requisite conditions of admittance and, therefore, became available for statistical consideration. The results of immunity tests carried out on these representatives and on their controls in group D are given in Table I and are complementary to those previously recorded (1931 *a*).

The results recorded in Table I show that, whereas the rabicidal antibody content after three different courses of anti-rabies treatment was respectively 3-, 2- and 1-fold, the corresponding degrees of immunity conferred were in the relative proportions of 1·2, 1 and 1.

Table I. *Experiments to show the relative immunity acquired by the sera of various rabbit groups treated with fresh-fixed virus, carbolised-fixed virus and etherised-fixed virus respectively and showing maximum rabicidal antibody formation on the 60th day after completion of treatment.*

Group A: immunised with a 2 per cent. emulsion of living-fixed virus in normal saline solution in a dosage of 5 c.c. daily on 14 consecutive days.
 Group B: immunised with a 2 per cent. emulsion of killed carbolised virus in a dosage of 5 c.c. daily on 14 consecutive days.
 Group C: immunised with a 2 per cent. emulsion of killed etherised virus in a dosage of 5 c.c. daily on 14 consecutive days.
 Group D: not immunised but serving as controls for groups A, B and C.

Group	Rabbit no.	Method of treatment	Result of subdural inoculation of 0.2 c.c. of a 1 : 1000 fresh-fixed virus in normal saline solution 60 days after completion of treatment	% of immunity
A	1	Fresh-fixed virus in normal saline solution	Died of F.V. rabies in 9 days	41.66
	2		Lived	
	3		Died of F.V. rabies in 8 days	
	4		" " 8 "	
	5		Lived	
	6		Died of F.V. rabies in 10 days	
	7		Lived	
	8		"	
	9		Died of F.V. rabies in 9 days	
	10		" " 8 "	
	11		Lived	
	12		Died of F.V. rabies in 8 days	
B	1	Carbolised-fixed virus	Died of F.V. rabies in 8 days	41.66
	2		" " 9 "	
	3		" " 9 "	
	4		Lived	
	5		"	
	6		Died of F.V. rabies in 10 days	
	7		" " 9 "	
	8		Lived	
	9		"	
	10		Died of F.V. rabies in 8 days	
	11		Lived	
	12		Died of F.V. rabies in 7 days	
C	1	Etherised-fixed virus	Died of F.V. rabies in 8 days	50
	2		" " 7 "	
	3		Lived	
	4		"	
	5		"	
	6		"	
	7		Died of F.V. rabies in 8 days	
	8		" " 9 "	
	9		Lived	
	10		"	
	11		Died of F.V. rabies in 8 days	
	12		" " 8 "	
D	1	Nil	Died of F.V. rabies in 7 days	0
	2		" " 7 "	
	3		" " 7 "	

SUMMARY.

1. Opinions upon the relationship between anti-rabies immunity and the rabicidal power of rabies-immune serum have been stated.

2. The rabicidal antibody content of the sera of rabbits immunised with etherised virus, living fixed virus and carbolised virus has been determined while at its maximum and compared with the degree of immunity acquired after each method of treatment.

CONCLUSIONS.

1. A practically identical degree of anti-rabies immunity can be secured by the exhibition of equal quantities by weight of etherised virus, fresh-fixed virus or carbolised virus over equal periods of time.

2. Although, in general, some indication of anti-rabies immunity is afforded in treated rabbits by the rabicidal antibody content of their blood, no mathematical relationship can be established.

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