

## Serological Relationship Between the Viruses of Japanese B and St. Louis Encephalitis.

MARGARET G. SMITH AND HAROLD R. REAMES. (Introduced by R. A. Moore.)

*From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.*

Although many points of resemblance between the viruses of St. Louis and Japanese B encephalitis have been emphasized, the evidence supporting their serological relationship is conflicting.

In neutralization tests in mice, Kawamura<sup>1</sup> and his associates found no cross protection with antisera prepared in mice, rabbits and goats by immunization with the viruses of Japanese and St. Louis encephalitis, nor with sera of patients convalescent of the two diseases. Kasahara<sup>2</sup> and his colleagues found that the Japanese hyperimmune serum of rabbits neutralized both the Japanese virus and the St. Louis virus, but the latter only to a slight degree. On the other hand, the St. Louis immune serum neutralized only the St. Louis virus. With one of the strains of St. Louis virus used, this slight cross protection was apparent when the virus-serum mixtures were instilled intranasally but not when inoculated intracerebrally.

Kudo<sup>3</sup> and his collaborators observed that sera of rabbits immunized with the Japanese encephalitis virus neutralized not only the homologous virus, but to some extent the St. Louis virus. Their tables show that protection of mice against 1 to 100 minimal lethal intracerebral doses of the St. Louis virus was afforded by immune sera from each of 3 rabbits.

In protection tests in mice, Webster<sup>4</sup> found no cross neutralization of the Japanese and St. Louis viruses with the sera of individuals convalescent from either Japanese B encephalitis or St. Louis encephalitis. However, he cites an instance of cross neutralization with human sera noted by Kuttner.<sup>5</sup> Sera obtained by Kuttner from two Europeans who had contracted encephalitis in China pro-

<sup>1</sup> Kawamura, R., Kodama, M., Ito, T., Yasaki, T., and Kobayakawa, Y., *Arch. Path.*, 1936, **22**, 510.

<sup>2</sup> Kasahara, S., Yamada, R., and Hamano, R., *Kitsato Arch. Exp. Med.*, 1937, **14**, 22.

<sup>3</sup> Kudo, M., Uraguchi, K., Matsuda, S., and Hashimoto, K., *J. Immunol.*, 1937, **32**, 129.

<sup>4</sup> Webster, L. T., *J. Exp. Med.*, 1938, **67**, 109.

<sup>5</sup> Kuttner, A., and T'ung, T., *J. Clin. Inv.*, 1936, **15**, 525.

tected against both the Japanese and the St. Louis viruses. Furthermore, Webster<sup>4</sup> obtained no cross protection against the Japanese virus with the serum of a monkey hyperimmunized with the St. Louis virus; but with serum from a monkey hyperimmunized with the Japanese virus, mouse protection was afforded not only against the Japanese virus but against at least one minimal lethal dose of the St. Louis virus. This cross protection with the Japanese serum is not emphasized by Webster, although it appears in the table of his results.

In contrast to the results of others Perdrau,<sup>6</sup> in experiments published posthumously, demonstrated neutralization of the homologous and the heterologous virus to approximately the same extent with antisera prepared in rabbits by hyperimmunization with either the virus of Japanese encephalitis or the virus of St. Louis encephalitis. These results are not in accord with the other published data.\*

In view of the conflicting evidence reported in the literature, it seems desirable to report our results obtained with the sera of mice and rabbits hyperimmunized with the viruses of St. Louis and Japanese encephalitis.

*Materials and Methods. Virus.* The Nakayama strain of Japanese encephalitis virus and the Hubbard strain of St. Louis encephalitis virus were employed in these experiments.

*Serum.* The serum was prepared in the following manner: Three groups of mice were immunized with the Japanese virus by repeated subcutaneous inoculations of .25 cc of dilutions of fresh infected mouse brain. Each group received 10 weekly inoculations. For the first inoculation a virus dilution of  $10^{-5}$  was employed. The strength of the virus dilution used was increased with subsequent inoculations, so that a dilution of  $10^{-2}$  was used for the final 4 inoculations. Each group of mice was bled one week after the last injection. The sera from the individual mice of each lot were pooled.

St. Louis virus immune serum was prepared in mice in a similar manner. Two groups of mice were immunized. One group received 11 inoculations; and the other, 14 inoculations. A virus dilution of  $10^{-3}$  was used for the first few inoculations; and for later inoculations, a stronger dilution,  $10^{-2}$ . The sera from the mice in each group were pooled.

Immune sera were prepared in rabbits by immunizing them with

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<sup>6</sup> Perdrau, J. R., *J. Path. and Bact.*, 1940, **50**, 545.

\* Webster has recently published the results of a study of the strains of virus used by Perdrau. He reached the conclusion that some of the material had been mislabeled and that Perdrau had been dealing only with strains of the St. Louis virus. Webster, L. T., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 499.

the Japanese and the St. Louis virus respectively by repeated intraperitoneal inoculations of active virus. Approximately 5 cc of a 10% dilution of virus in broth was used for each inoculation. Two animals immunized with the Japanese virus received 17 inoculations over a period of 10 months. Sera from these 2 rabbits were pooled. Another animal received 4 biweekly inoculations. Two rabbits immunized with the St. Louis virus received 20 inoculations over a period of 10 months. All rabbits were bled 2 to 3 weeks after the final inoculation.

*Neutralization Test.* Brains of mice infected with the St. Louis or Japanese encephalitis virus were removed aseptically and stored in the frozen state for 1 or 2 days. At the time of the test, one brain was warmed at room temperature and made into a 10% emulsion with broth of pH 7.4. After light centrifugation the supernatant fluid was used to make serial tenfold dilutions. One part of each dilution of virus used was added to 2 parts of serum, and the virus-serum mixture was incubated for 2 hours at 37°C. Swiss mice under light ether anesthesia were inoculated intracerebrally with .03 cc of this mixture. Four mice were used for each dilution of virus.

*Results.* The results of the cross neutralization tests appear in the tables. It is apparent that no protection was afforded against the Japanese virus with either of the 2 lots of pooled St. Louis immune mouse sera, although good protection was afforded against the homologous virus (Table I). The same was true of the St. Louis immune rabbit sera (Table II). On the other hand, with each of the 3 lots of pooled Japanese immune mouse sera protection was

TABLE I.  
Neutralization Tests Using St. Louis Immune Mouse Sera with the Japanese B Encephalitis Virus and with the St. Louis Encephalitis Virus.

Pooled mouse sera	Dilution of Japanese virus added to serum			
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
Normal No. 366	—	4, 6, 6, 8	(1), 6, 7, 8	6, 7, 7, 8
St. Louis Imm. No. 342	—	(1), 6, 7, 8	4, 5, 7, 8	(1), 6, 8, 8
Normal No. 386	—	(1), 5, 5, 5	5, 6, 6, 7	6, 6, 6, 6
St. Louis Imm. No. 406	—	5, 6, 6, 6	5, 5, 6, 6	5, 7, 7, 7
	Dilution of St. Louis virus added to serum			
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
Normal No. 386	—	—	5, 5, 5, 5	5, S, S, S
St. Louis Imm. No. 342	—	S, S, S, S	S, S, S, S	S, S, S, S
St. Louis Imm. No. 406	S, S, S, S	13, S, S, S	S, S, S, S	S, S, S, S

Numerals indicate day of death of mouse; S = survival; ( ) = accidental death.

afforded not only against the Japanese virus, but to a slight extent against the St. Louis virus (Table III). This protection against the heterologous virus, although slight against only one to 10 minimal lethal intracerebral doses, appears to be of significance since it occurred with all 3 sera. With the Japanese immune rabbit serum, the same slight neutralization of the heterologous virus was observed (Table IV). These results are in agreement with those obtained by Kudo and by Kasahara with immune rabbit sera. They are also in agreement with the findings recorded by Webster with immune

TABLE II.  
Neutralization Tests Using St. Louis Immune Rabbit Sera with the Japanese B Encephalitis Virus and with the St. Louis Encephalitis Virus.

Rabbit sera	Dilution of Japanese virus added to serum				
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>
Normal	—	—	5, 6, 7, 8	6, 8, S, S	—
St. Louis Imm. No. 391	—	—	(1), 6, 7, 7	7, S, S, S	—
Normal	—	—	5, 6, 6, 7	6, S, S, S	—
St. Louis Imm. No. 395	—	—	6, 7, S, S	S, S, S, S	—

  

Rabbit sera	Dilution of St. Louis virus added to serum				
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>
Normal	—	—	4, 4, 5, 5	4, 5, 5, 6	4, 5, 6, S
St. Louis Imm. No. 391	6, S, S, S	S, S, S, S	—	—	—
Normal	—	—	3, 4, 4, 5	4, 4, 5, 5	—
St. Louis Imm. No. 395	6, 6, 9, S	S, S, S, S	—	—	—

TABLE III.  
Neutralization Tests Using Japanese Immune Mouse Sera with Japanese B Encephalitis Virus and with St. Louis Encephalitis Virus.

Pooled mouse sera	Dilution of St. Louis virus added to serum			
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
Normal No. 386	—	4, 5, 5, 5	(2), 5, 5, 8	5, 8, S, S
Japanese Imm. No. 385	—	5, 5, 5, 5	5, 5, 8, S	S, S, S, S
Normal No. 366	—	4, 4, 5, 5	5, 5, 7, 8	5, 6, S, S
Japanese Imm. No. 365	—	4, 5, 6, 10	5, 8, S, S	S, S, S, S
Normal No. 386	—	—	5, 5, 5, 5	5, S, S, S
Japanese Imm. No. 405	—	—	6, S, S, S	S, S, S, S

  

Pooled mouse sera	Dilution of Japanese virus added to serum			
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
Normal No. 386	—	6, 6, 6, 6	6, 6, 6, 6	12, S, S, S
Japanese Imm. No. 385	—	8, 11, S, S	S, S, S, S	S, S, S, S
Normal No. 386	—	—	5, 6, 6, 7	6, 6, 6, 6
Japanese Imm. No. 405	6, 7, 8, 9	S, S, S, S	7, S, S, S	S, S, S, S

TABLE IV.  
Neutralization Tests Using Japanese Immune Rabbit Sera with Japanese B Encephalitis Virus and with St. Louis Encephalitis Virus.

Rabbit sera	Dilution of St. Louis virus added to serum				
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>
Normal serum	—	—	4, 4, 5, 5	4, 5, 5, 6	4, 5, 6, S
Japanese Imm. No. 399 (pooled)	—	—	5, 5, 5, 6	(1), 5, 6, S	S, S, S, S
Normal serum No. 267	—	5, 5, 5, 5	5, 5, 5, 5	—	—
Japanese Imm. No. 270	—	5, 5, 5, 10	5, S, S, S	—	—
	Dilution of Japanese virus added to serum				
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>
Normal serum	—	—	5, 6, 7, 8	6, 8, S, S	—
Japanese Imm. No. 399 (pooled)	9, S, S, S	S, S, S, S	S, S, S, S	—	—

serum of the monkey, although the latter investigators apparently did not attach any significance to the findings.

An accumulation of evidence shows that in protection tests in mice, a definite though slight protection against the St. Louis virus by Japanese immune serum prepared in a number of animal species can be demonstrated. On the other hand, no protection against the Japanese virus is afforded by St. Louis immune serum.

This partial serological relationship between two viruses resembling each other in many other respects is analogous to the relationship which has been demonstrated among the 3 viruses—virus B, the virus of herpes, and that of pseudorabies.<sup>7</sup> The 3 latter possess many properties in common, and the antisera to these viruses give a slight cross neutralization of the viruses. However, with the antisera to the viruses of pseudorabies and of herpes, the partial cross neutralization is in one direction only. The immune antiherpes serum protects slightly against the virus of pseudorabies, while the anti-pseudorabies serum has no demonstrable effect against the herpes virus. It has been suggested that a generic relationship exists between these 3 viruses. It is possible that a similar group relationship exists between the 2 viruses producing the St. Louis and the Japanese type of encephalitis respectively.

A definite though slight protection against the virus of St. Louis encephalitis by serum of animals immunized with the Japanese virus has been demonstrated. On the other hand, no protection against the virus of Japanese encephalitis has been afforded by St. Louis immune serum.

<sup>7</sup> Sabin, Albert B., *Brit. J. Exp. Path.*, 1934, **15**, 372.