

COMPARATIVE VIRULENCE OF ST. LOUIS ENCEPHALITIS VIRUS
CULTURED WITH BRAIN TISSUE FROM INNATELY
SUSCEPTIBLE AND INNATELY RESISTANT MICE

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There is reason to believe that the response of normal mice to experimental inoculation of St. Louis encephalitis virus depends largely upon properties of the brain tissue itself. Thus, virus dropped into the nares of innately resistant mice reaches the brain as promptly as virus similarly instilled into closely related, yet innately susceptible mice (1-3). Again, serum from innately resistant mice is identical with that from susceptible mice in having no deleterious effect upon the virus. The main point, however, is that virus reaching the brain of resistants, no matter by what route, fails to increase in quantity beyond a certain point at which it is still harmless to the animal, whereas in the brain of susceptibles it increases 10,000-fold in amount above the point reached in resistant animals and brings about widespread nerve cell destruction and death.

To gain further information on the behavior of this virus in the brain tissue of susceptible and resistant mice, we turned to tissue culture methods and compared the titres of virus in different combinations of tissue plus serum mixtures.

Technique

Culture media consisting of serum-Tyrode solution plus tissue were prepared according to the methods of Rivers and Ward (4), and Lloyd, Theiler, and Ricci (5), except that embryo mouse brains were employed rather than whole mouse or chick embryo.

Tyrode solution from stock was measured according to the needs of each experiment and to it serum was added in a proportion of 10 per cent. Three types of serum were used—normal rabbit, normal adult mouse of a genetically resistant strain, and normal adult mouse of a closely related, yet genetically susceptible strain (3). This 10 per cent serum-Tyrode solution was then filtered through a Seitz pad and transferred in 4.0 cc. quantities to 50 cc. Erlenmeyer flasks. Brain tissue from embryo or 1-day-old mice of three types was prepared and added to the filtered serum-Tyrode solution in flasks in the following manner. Embryos from pregnant females of the W-Swiss strain of mice were removed close to term, their brains enucleated, washed in saline, and minced with scissors. Brains from 1-day-old genetically susceptible and resistant mice (3) were also prepared in the same manner. Enough serum-Tyrode was added

to the minced brain to make a 33 per cent suspension. 3 gtt. or about 0.15 cc. of this tissue suspension was added to each flask containing 4.0 cc. of serum-Tyrode.

The virus inoculum consisted originally of 1 per cent suspension of infected brain tissue of a mouse injected intracerebrally with St. Louis encephalitis virus. The flasks were incubated for 3 or 4 days at 37°C. and 1 cc. of the supernatant was then transferred to the next flask.

The cultures were carried in triplicate with suitable tests for bacterial contaminants.

The activity of the culture virus was measured after 3 to 4 days incubation by preparing serial dilutions of a centrifuged supernatant and injecting 0.03 cc. of each intracerebrally into 3-weeks-old W-Swiss mice. Two, and occasionally four or six mice, were used for each dilution.

Under these conditions we have cultivated the St. Louis encephalitis virus successfully since early 1935.¹

EXPERIMENTS

The behavior of St. Louis encephalitis virus in culture media containing brain tissue from (*a*) susceptible and (*b*) resistant mice was compared in the following manner.

A control medium was set up consisting of Tyrode plus 10 per cent adult rabbit serum plus brain tissue from embryo W-Swiss mice near term. A "susceptible medium" was also prepared consisting of Tyrode plus 10 per cent pooled serum from adult susceptible mice and brain tissue from 1-day-old susceptible mice, and a "resistant medium" of Tyrode plus 10 per cent pooled serum from adult resistant mice and brain tissue from 1-day-old resistant mice.

Three flasks of each medium were inoculated with 1 cc. of culture virus from a previous control mouse embryo passage. Following 3 or 4 days incubation, 1 cc. from each flask was transferred to another flask of similar medium. At least eight serial passages were made and certain of these cultures were titrated for virus activity.

The comparative activity of St. Louis encephalitis virus in media containing resistant and susceptible sera and brain tissue is shown in Tables I and II. Table I records the duration of life of mice injected with eighth-passage materials. Mice dying of St. Louis encephalitis virus infection did so after 5 to 9 days with rare exceptions. Titration end-points were clear-cut. In this test, the Swiss embryo culture titred 0.03 cc. of the 1:10,000 dilution or better, the susceptible mouse culture, 1:10,000, and the resistant mouse culture, 1:160 dilution, a 62-fold difference. Table II summarizes the results of four series of tests. It shows, first of all, unpredictable variations in titre from test to test but consistency as to relative titres. Thus, in series 4, the eighth-passage

¹ Jerome T. Syverton and George P. Berry reported the successful cultivation of St. Louis encephalitis virus in *Science*, 1935, **82**, 596.

tests were consistently tenfold lower than the fourth-passage tests.² Secondly, the tests show that titres of virus in embryo brains are generally higher than those in 1-day-old brains. Finally, and of greatest interest to the present study, is the fact that the virus in the resistant media in each of eight tests showed a titre 1/10th to 1/100th of that in the susceptible media.

The question next arose of whether the relatively low virus titre in the resistant media might possibly be due to the presence of resistant serum rather than to resistant brain tissue.

Culture media were set up and inoculated as in the previous experiment except that two additional types of media were added, one containing sera from susceptible adult mice plus brain tissue from resistant 1-day-old mice, and the

TABLE I
Virulence of St. Louis Encephalitis Virus Cultured in Susceptible and Resistant Media
Series 1—8th Passage

Medium	Fate of mice injected with 0.03 cc. culture virus in dilution										Titre. 0.03 cc. of dilution	Dif- fer- ence in titre
	1:10	1:20	1:40	1:80	1:100	1:160	1:320	1:640	1:1,000	1:10,000		
Rabbit serum—Swiss embryo brain	5*, 5	—	—	—	5, 5	—	—	—	6, 7	8, 10	1:10,000+	
Susceptible mouse serum and brain	5, 5	—	—	—	4, 5	—	—	—	7, 7	8, S	1:10,000	
Resistant mouse serum and brain	6, 6	7, 8	6, 8	7, 8	—	7, S	7, S	S, S	S, S	S, S	1:160	62

* Mouse dead of St. Louis encephalitis 5 days following injection.

S = mouse survived 30 days following injection.

other, sera from resistant and brain tissue from susceptible mice. The comparative titres of virus in these several media are shown in Table III.

As in previous tests, the virus titred higher in embryo brain than in brain cultures from newborn mice, and different tests showed different end-points. Of chief concern, however, was the fact that virus cultured with susceptible brain showed similar titres, regardless of whether the serum came from susceptible or resistant mice, and virus cultured with resistant brain showed similar titres regardless of whether the serum came from resistant or susceptible mice. The titre of virus in resistant brain cultures remained 1/10th to 1/100th as high as that in susceptible brain cultures. In all, seven comparative tests have been made with similar results.

² F. Howell Wright, who tested the variability of titre of St. Louis encephalitis virus during and between experiments in our laboratory, noted that between experiments titres often varied tenfold in either direction and occasionally one hundredfold, but that the relation of titres of virus in different media remained consistent.

Satisfied that the superior multiplication of St. Louis encephalitis virus in the brain tissue of susceptibles as contrasted with that of resistants is due to differences in the brain tissue itself, regardless of whether the tests are carried out *in vivo* or *in vitro*, we next attempted to determine whether the difference in titre in tissue culture was due to stimulating factors in the susceptible brain or to inhibiting factors in the resistant brain. The following protocol is an example of one of these tests.

TABLE II
Virulence of St. Louis Encephalitis Virus Cultured in Susceptible and Resistant Media
Summary of Results

Series	Medium	4th passage		6th-8th passage	
		Titre 0.03 cc. of dilution	Differ- ence in titre	Titre 0.03 cc. of dilution	Differ- ence in titre
1	Rabbit serum—Swiss embryo brain	1:1,000	} 100	1:10,000+	} 62
	Susceptible mouse serum and brain	1:1,000		1:10,000	
	Resistant mouse serum and brain	1:10		1:160	
3	Rabbit serum—Swiss embryo brain	1:1,000	} 10	1:100,000	} 50+
	Susceptible mouse serum and brain	1:1,000		1:1,000	
	Resistant mouse serum and brain	1:100		<1:20	
4	Rabbit serum—Swiss embryo brain	1:10,000	} 100	1:1,000	} 100+
	Susceptible mouse serum and brain	1:1,000		1:100	
	Resistant mouse serum and brain	1:10		0	
5	Rabbit serum—Swiss embryo brain	1:100,000+	} 20	1:100,000	} 10
	Susceptible mouse serum and brain	1:10,000		1:10,000	
	Resistant mouse serum and brain	1:500		1:1,000	

Cultures were prepared as above with the addition of two more sets, one of susceptible mouse serum and susceptible brain tissue from newborn mice plus 0.5 cc. of the supernatant from centrifuged 10 per cent resistant brain tissue, and the other of resistant mouse serum and resistant newborn brain tissue plus 0.5 cc. of the supernatant from centrifuged 10 per cent susceptible brain tissue.

This procedure was planned to demonstrate a possibly enhancing effect of susceptible extract containing relatively few cells or an inhibiting effect of resistant extract. Table IV shows that virus in the susceptible media titred 1:10,000 and in susceptible media plus resistant extract, the same,—1:10,000. Virus in resistant media titred 1:500 and in resistant media plus susceptible extract, slightly higher,—1:1,000 to 1:5,000. This slight difference occurred on repetitions of the test but it remains of questionable significance.

TABLE III
Virulence of St. Louis Encephalitis Virus Cultured in Media Containing Combinations of Susceptible and Resistant Brain Tissue and Serum

Series	Passage	Medium	Fate of mice injected with 0.03 cc. culture virus in dilutions								Titre. 0.03 cc. of dilution	Difference in titre	
			Undiluted	1:10	1:50	1:100	1:500	1:1,000	1:5,000	1:10,000			1:100,000
2	8	Rabbit serum—Swiss embryo brain.....	—	—	—	7, 8	—	8, 8	—	S, S	S, S	1:1,000	} 100
		Susceptible mouse serum and brain.....	—	—	—	7, 8	—	S, S	—	S, S	S, S	1:100—	
		Resistant mouse serum—susceptible mouse brain.....	6, 6	6, 6	6, 7	8, 10	S, S	S, S	S, S	S, S	S, S	1:100	
		Resistant mouse serum and brain.....	S, S	S, S	S, S	S, S	S, S	S, S	S, S	S, S	—	0	
		Susceptible mouse serum—resistant mouse brain.....	S, S	S, S	S, S	S, S	S, S	S, S	S, S	S, S	—	0	
3	4	Rabbit serum—Swiss embryo brain.....	—	—	—	5, 6	—	6, 7	—	8, 8	11, S	1:100,000	} 10-20
		Susceptible mouse serum—resistant mouse brain.....	4, 4	5, 7	5, 8	5, 8	6, 8	S, S	S, S	S, S	—	1:500	
		Resistant mouse serum—susceptible mouse brain.....	4, 4	5, 5	5, 5	5, 6	6, 6	6, 8	9, S	11, S	—	1:5,000— 1:10,000	
3	8	Rabbit serum—Swiss embryo brain.....	—	—	—	5, 6	—	7, 7	—	7, 8	S, S	1:10,000	} 50
		Susceptible mouse serum—resistant mouse brain.....	4, 5	4, 6	4, 6	8, 10	S, S	S, S	—	—	—	1:100	
		Resistant mouse serum—susceptible mouse brain.....	4, 5	4, 5	4, 5	4, 4	5, 8	7, 8	8, 10	S, S	—	1:5,000	

Footnotes as in Table I.

TABLE IV
Virulence of St. Louis Encephalitis Virus Cultured in Susceptible and Resistant Media Plus Extracts

Medium	Fate of mice injected with 0.03 cc. of culture virus in dilutions									Titre 0.03 cc. of dilution
	Undiluted	1:10	1:50	1:100	1:500	1:1,000	1:5,000	1:10,000	1:100,000	
Rabbit serum—Swiss embryo brain.....	—	—	—	6, 6	—	6, 7	—	7, 8	7, 10	1:100,000+
Susceptible mouse serum and brain.....	—	—	—	7, 7	—	7, 7	—	7, S	S, S	1:10,000
Susceptible mouse serum and brain plus resistant extract....	4, 5	6, 6	6, 6	6, 7	7, 7	8, 8	10, S	10, S	—	1:10,000
Resistant mouse serum and brain.....	5, 5	6, 6	6, 6	6, 7	8, 8	S, S	S, S	S, S	—	1:500
Resistant mouse serum and brain plus susceptible extract.....	5, 5	5, 6	6, 7	6, 7	7, 7	8, S	8, S	S, S	—	1:5,000

Footnotes as in Table I.

SUMMARY

We find that St. Louis encephalitis virus cultured in 10 per cent serum-Tyrode solution plus brain tissue from 1-day-old innately susceptible mice attains a higher titre than when cultured in a similar solution plus brain tissue from 1-day-old closely related, yet innately resistant mice. This difference in titre persists regardless of whether the serum comes from innately susceptible or resistant mice. The relatively high titre of virus in the susceptible media is not affected by the addition of an extract (not cell-free) from the resistant brain; the relatively low titre of the virus in the resistant media may possibly be slightly enhanced by the addition of an extract from the susceptible brain.

The findings as a whole show that the marked difference in the increase of St. Louis encephalitis virus in the brain tissue of innately susceptible and resistant mice, on culture *in vitro*, is due to some difference in the brain tissue itself.

BIBLIOGRAPHY

1. Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 433.
2. Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 827.
3. Webster, L. T., *J. Exp. Med.*, 1937, **65**, 261.
4. Rivers, T. M., and Ward, S. M., *J. Exp. Med.*, 1933, **58**, 635.
5. Lloyd, W., Theiler, M., and Ricci, N. I., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1936, **29**, 481.