Effect of Multiple Inoculations on Interference Between St. Louis Encephalitis Virus and Equine Encephalomyelitis Virus. (19595)

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In a previous communication from this laboratory it was reported that young rats, which are more or less uniformly susceptible to intranasally inoculated Western equine encephalomyelitis (W.E.E.) virus, were much less susceptible to nasally instilled W.E.E. virus if a single intranasal inoculation of St. Louis encephalitis (S.L.E.) virus had preceded by 72 hours the inoculation of W.E.E. virus(1). It was found that 50% of 3- to 4-week-old rats which had had S.L.E. virus instilled into their noses survived a subsequent intranasal inoculation of W.E.E. virus. Since a single intranasal inoculation of S.L.E. virus protected 50% of the animals against W.E.E. virus, further experiments were conducted to determine whether a greater degree of protection would be afforded by multiple intranasal inoculations of S.L.E. virus and also to determine whether heat-inactivated S.L.E. virus would afford protection. The purpose of this communication is to report the results obtained in these studies.

Materials and methods. Viruses and animals. The strain of S.L.E. virus, as well as the strain of W.E.E. virus, used in the present study has already been described(1). The virus suspensions used were prepared from virus-infected brains of 3- to 4-week-old Swiss mice. The infected brains were ground in a mortar with sand and sufficient inactivated, undiluted rabbit serum to make a 10% suspension of the infected brains. The suspensions were centrifuged at about 2000 r.p.m. for 5 minutes to sediment gross particles. Immediately after centrifugation the supernatant fluid was removed and distributed in 2 ml amounts in screw-capped vials. The virus suspension in the vials was "quick-frozen" by rolling the vial in a mixture of dry-ice and alcohol. The frozen virus was then stored at about —70°C in dry-ice cabinet. When needed it was thawed by placing a vial of frozen virus in a water bath at 37°C until the material was thawed. A single suspension of each of the 2 viruses was used. The mouse intracerebral LD/50 titer(2) of the suspension of W.E.E. virus, after storage at about —70°C for 4 days, was $1 \times 10^{-7.8}$. The S.L.E. virus, after 5 days storage in the dry-ice cabinet, had a 50% end point of $1 \times 10^{-8.5}$. The 10% suspension of normal mouse brain (N.M.B.) used was prepared and stored as indicated above except that normal mouse brains were used. The heat-inactivated S.L.E. virus was prepared by heating approximately 5 ml of S.L.E. virus at 56°C for one hour. This virus had been stored at about —70°C for 20 days before it was heated. Eight 3- to 4-week-old mice survived for 21 days following intracerebral inoculation of 0.03 ml of this heated virus. The rats used were male Sprague-Dawley. The mice used in the studies were obtained from Rockland Farms.

Experimental. In the present series of experiments 95 rats 3 to 4 weeks of age were used. The various materials (Table I), inoculated intranasally into these animals, were in the form of 10% suspensions. The 95 rats were divided into 5 groups. One group, consisting of 18 rats, received 0.05 ml of the N.M.B. suspension and 72 hours later received 0.05 ml of W.E.E. virus. A second group composed of 20 rats, received 0.05 ml of the suspension of S.L.E., and 72 hours later 0.05 ml of W.E.E. A third group, consisting of 19 rats, received 3 daily inoculations of 0.05 ml of N.M.B. followed 24 hours after the third inoculation with 0.05 ml of W.E.E. A fourth group of 19 animals was inoculated on each of 3 successive days with 0.05 ml of heat-inactivated S.L.E. and 24 hours later received 0.05 ml of W.E.E. The last group, consisting of 19 rats, received 0.05 ml of S.L.E. virus on

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TABLE I. Interference Between S.L.E. and W.E.E.* Comparison between single and multiple inoculations of interfering virus.

No. of rats tested	Material instilled into nose		Interval (days) between W.E.E. and death																		Results	% mortality
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
18	N.M.B.×1	W.E.E.	0‡	0	16	1	0	0	0	0	1										18/18§	100
20	S.L.E.×1	"	0	0	1	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	7/20	35
19	N.M.B.×3	"	0	0	17	1	0	0	1												19/19	100
19	S.L.E.†×3	"	0	0	14	1															15/19	79
19	"	"	0	0	0	0	0	0	0	0	0	0	0	1							1/19	5

* N.M.B., normal mouse brain; W.E.E., Western equine encephalomyelitis virus; S.L.E., St. Louis encephalitis virus.

† St. Louis encephalitis virus heated 56°C 60 min.

‡ Zero indicates no deaths that day. Other number indicates No. of rats which died that day.

§ Denominator denotes No. of rats tested, numerator No. of deaths.

each of 3 successive days and then 0.05 ml of W.E.E. 24 hours after the last injection of S.L.E. The experiments were so designed that all of the animals shown in Table I were inoculated on the same day and with the same batch of W.E.E. virus. Reference to Table I shows that the previous intranasal inoculation of N.M.B. did not protect the rats against W.E.E. virus. This was true for the animals which received 3 inoculations of N.M.B. as well as for those which received a single inoculation, since of 37 rats receiving either single or multiple inoculations of N.M.B., all succumbed to a fatal infection caused by W.E.E. virus. These findings were expected and merely confirm results already reported (1). On the other hand it will be seen that animals which had S.L.E. virus instilled into the nose before the instillation of W.E.E. virus were much less susceptible to W.E.E. virus. Of the 20 rats which received a single intranasal inoculation of S.L.E. virus 72 hours before the inoculation of W.E.E. only 7 or 35% of the animals succumbed to infection with the latter virus. These results compare favorably with the results previously published(1), where it was found that a single intranasal inoculation of S.L.E. would protect 50% of rats so treated against W.E.E. inoculated intranasally. While a high degree of protection against W.E.E. was noted when a single inoculation of S.L.E. preceded the W.E.E. a still greater degree of protection was noted when 3 daily inoculations preceded the W.E.E. Only 1 of the 20 animals which received S.L.E. 3 times before the W.E.E. was inoculated, died. It should be pointed out that in this series of experiments, as well as in those already published(1), that when rats which had had S.L.E. virus instilled into the nose prior to nasal instillation of W.E.E. do succumb they survive for a longer period than rats which had not received S.L.E. Reference to Table I shows that rats which had received 3 intranasal inoculations of heat-inactivated S.L.E. were still highly susceptible to W.E.E. virus although 4 of the 19 animals in this group survived. The explanation for these results may be that all of the virus was not completely inactivated during the heating period and that the rats which were protected were protected by active virus. It will be recalled, however, that the heated S.L.E. virus was tested for activity by inoculating the material intracerebrally into each of 8 mice and was found to be inactive.

Discussion. The data reported here confirm and extend the observation that St. Louis encephalitis virus, instilled into the noses of young rats, changes the susceptibility of these animals to Western equine encephalomyelitis virus subsequently inoculated by the nasal route. In a previous communication(1) it was reported that 50% of rats which had had St. Louis encephalitis virus instilled into their noses resisted infection with Western equine encephalomyelitis virus inoculated intranasally 72 hours later. It was postulated that, in those animals which survived, the St. Louis encephalitis virus, which had been dropped into the nose, had penetrated all of the olfactory nerve endings which project into the olfactory mucosa and had "blocked" the entry into these fibers of the subsequently inoculated

Western equine encephalomyelitis virus. On the other hand, however, if the St. Louis encephalitis virus did not penetrate all of the nerve endings, in certain of the rats, the subsequently injected Western equine encephalomyelitis virus could reach the brains of these rats over a limited number of nerve fibers and cause a fatal encephalitis. That the Western equine encephalomyelitis virus did reach the brain by passing over a limited number of nerve fibers was suggested by the observation that in those animals which did succumb, the incubation period was longer and the animals survived for a longer period than did controls which had not received St. Louis encephalitis virus before the Western equine encephalomyelitis virus was injected. In the experiments reported here it was found that a single intranasal inoculation of St. Louis encephalitis virus protected 65% of the rats against Western equine encephalomyelitis virus. However, when rats were given 3 inoculations of St. Louis encephalitis virus before the equine encephalomyelitis virus was inoculated a greater degree of protection was noted since 95% of rats so-treated survived. From the findings reported here, as well as elsewhere(1) it would appear that St. Louis encephalitis virus, having passed over the fibers of the olfactory nerve and being present in the brain, "interferes" with the subsequent passage of Western equine encephalomyelitis virus over these fibers and thus prevents the latter virus from reaching the brain. The degree of interference and thus the percentage of animals that survive can be increased if 3 rather than 1 inoculation of St. Louis encephalitis virus precedes the inoculation of Western equine encephalomyelitis virus.

Summary. A single intranasal inoculation of St. Louis encephalitis virus protected 65% of 3- to 4-week-old rats against Western equine encephalomyelitis virus subsequently inoculated by the same route. The percentage of animals protected was increased to 95% if 3 inoculations of St. Louis encephalitis virus preceded the Western equine encephalomyelitis virus.

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A Biological Method for Determining Small Quantities of Sodium Retaining Substances.* (19596)

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Numerous investigations on the retention of urinary sodium for the quantitative estimation of adrenal cortical extracts and desoxycorticosterone have been made in adrenalectomized dogs(1-5), mice(6), and rats(7,8). Several workers(9,10) have taken advantage of the increased potassium excretion in rats treated with desoxycorticosterone as a means of estimating salt-regulating activity. Widespread investigation of the effect of desoxycorticosterone on sodium and potassium excretion has been somewhat restricted by the lack of a practical test, and a satisfactory experimental animal.

This report is concerned with sodium excretion in the adrenalectomized rat in response to various dose levels of desoxycorticosterone acetate with a constant load of administered sodium.

Material and methods. Biological. Male albino rats, obtained from Holtzman-Rolfsmeyer Co. (Madison, Wis.) and weighing from 150-155 g, were adapted to general laboratory conditions for 48 hours in group

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