antibody rises, particularly to Japanese virus. Those horses not previously immune to St. Louis virus showed no response. The response of these St. Louis immune hosts was identical to that shown by persons with naturally acquired immunity to Japanese virus. Normal individuals show no such response even after several injections of vaccine. This immediate, secondary type immunologic response to an injection of vaccine is not specific for Japanese virus but to an antigen common to Japanese and St. Louis viruses and probably to other viruses in this complex. It is suggested that immunity acquired by active infection with one member of the group may serve as good basic or primary immunization for other representatives and a high and probably effective protection response can be stimulated subsequently by giving one "booster" injection of a killed virus vaccine representing an immunologically related virus.

1. Hammon, W. McD., unpublished.

2. Sabin, A. B., Ginder, D. R., Matumoto, M., and Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 135.

3. Ginder, D. R., Matumoto, M., Schlesinger, R. W., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 130.

4. Casals, J., *J. Immunol.*, 1947, v56, 337.

5. España, C., and Hammon, W. McD., *ibid.*, 1948, v59, 31.

6. 406 Medical General Laboratory, Ann. Historical Rep., 1952, Professional Sect. p43.

7. Southam, C. M., and Moore, Alice E., *J. Immunol.*, 1954, v72, 446.

8. Hammon, W. McD., and Sather, G. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 521.

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Immunity of Hamsters to West Nile and Murray Valley Viruses Following Immunization with St. Louis and Japanese B.* (22314)

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In a previous paper(1) it was shown that human convalescents of St. Louis encephalitis (SLE) virus and horses with naturally acquired SLE immunity responded quickly with a secondary type of serological response to Japanese B (JBE) virus after one injection of JBE virus, killed vaccine. This paper deals with animal challenge by the subcutaneous route with West Nile (WN) and Murray Valley encephalitis (MVE) viruses following immunization with JBE and SLE viruses belonging to the same immunologic group. Serological crossing between SLE, JBE, WN, and MVE viruses has been demonstrated by neutralization, complement fixation, and hemagglutination tests by a large number of workers and antigenic overlapping of this group is well recognized. Cross-protection tests using the intracerebral challenge of immunized mice have not shown such crossing or have given inconclusive results with two exceptions as noted below. On the basis of the usual lack of cross-protection in mice has rested the principal method of differentiation of members of this group as distinct viruses. Recently, however, Ruchman reported that he had demonstrated cross-protection between SLE and WN using immune rabbits and guinea pigs as test animals(2). His criterion of demonstrated disease in those animals involving tests with recognized strains of SLE and WN was the development of fever. Cross vaccination studies by Pond, Russ, Rogers, and Smadel(3) have demonstrated the relationship between JBE and MVE, since JBE vaccinated mice were satisfactorily protected against peripheral challenge with MVE virus.

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MVE vaccinated mice, however, were not significantly protected against JBE, although there was a suggestion of slight crossing.

In our work, Syrian hamsters have been found susceptible to high dilutions of MVE virus by peripheral routes of infection. Previously, Smadel and others had shown them to be highly susceptible to WN by these routes(4). Because of the low degree of susceptibility of mice by peripheral routes, we felt that hamsters would be a more suitable laboratory animal for demonstrating cross-resistance between certain viruses of this group, if such existed.

Materials and methods. Viruses used were JBE, Nakayama strain, mouse passage 44; SLE, Webster strain, mouse passage number unknown; MVE, Strain #1, mouse passage 12; and WN, B956, mouse passage 28. Stock virus was prepared as 20% infected mouse brain suspension in 50% normal rabbit serum (inactivated at 56°C for 20 minutes) broth and stored in sealed glass ampoules in a CO_2 ice chest. Infectivity of the virus was determined by intracerebral inoculation of 3-week-old CFW mice with 0.03 ml of serial 10-fold virus dilutions made in 0.2% bovine albumen broth. Hamsters were obtained from the Lakeview Hamster Colony of New Jersey. Hamsters from this breed had been found susceptible to infection with this group of viruses. Animals previously tested from a local breeder had proved resistant to SLE virus by even the intracerebral route. Groups of hamsters were immunized when 6 weeks of age to either JBE or SLE viruses by a series of 3 intraperitoneal injections of live virus given as follows: *JBE*: first injection, 0.1 ml 10^{-2} dilution of virus; 10 days later, 0.1 ml 10^{-1} dilution of virus; 10 days later, 0.2 ml 10^{-1} dilution of virus. Challenge was made 12 days later. *SLE*: first injection, 0.1 ml 10^{-2} dilution of virus; 12 days later, 0.1 ml 10^{-1} dilution of virus; 7 days later, 0.2 ml 10^{-1} dilution of virus. Challenge was made 13 days later. For challenge controls, normal hamsters of the same shipment were set aside at the beginning of the immunization period. The neutralization index of serum was determined by employing serial dilutions of virus mixed with undiluted serum, incubated at 37°C for 2 hours, and inoculated by the intracerebral route into 3-4-week-old mice.

TABLE I. Susceptibility of Hamsters by Route of Inoculation.*

Virus	Log LD_{50} of endpoint dilution		
	Intracer.	Intraper.	Subcut.
Japanese B	7.0	<1.0	—
St. Louis	7.8	<1.0	—
Murray Valley	8.0	8.1	7.0
West Nile	9.5	9.2	8.7

* Inoculum—.1 ml.

Results. 1. The susceptibility of hamsters to these viruses as determined by route of inoculation (Table I). The inoculum was 0.1 ml. Both MVE and WN viruses were shown to be highly pathogenic by the peripheral routes tested, while neither JBE nor SLE exhibited this peripheral pathogenicity. The four viruses, JBE, SLE, MVE, and WN were all pathogenic by the intracerebral route.

2. *Immunization and Cross Challenge.* Two groups of hamsters were immunized, one to JBE virus and the other to SLE by the methods described. The results of homologous and cross challenge tests are shown in Table II. Since neither JBE nor SLE were pathogenic by a peripheral route, it was necessary to use the intracerebral route of inoculation for challenge with the homologous viruses. For both MVE and WN viruses (heterologous challenge) the *subcutaneous* route of inoculation was used. It will be observed that animals immunized with JBE virus were solidly immune not only to the homologous virus (JBE) but also to both the MVE and WN viruses (protection index greater than 5 to 7 logs). This experiment was repeated with similar results. Immunization with SLE also resulted in complete protection against challenge with the homologous (SLE) virus, but it may be observed that SLE immunization did not result in complete protection against either MVE or WN viruses but that significant protection, nevertheless, did occur. In the case of both MVE and WN viruses protection was most obvious against the most concentrated virus inoculum. This phenomenon frequently observed after ho-

TABLE II. Results of Virus Challenge of Immunized Hamsters.

Immunizing virus	Challenge virus	Route of challenge	Virus dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	Log LD_{50}	Log protection index
JBE	JBE	i.c.	0/4*	0/4	0/4	0/4							<1.0	>7.5
None	"	"					4/4	4/4	4/4	3/4	1/4		8.5	
JBE	MVE	s.c.	0/4	0/4	0/4	0/4	0/4	0/4					<1.0	>5.2
None	"	"					4/4	3/4	1/4	0/4			6.2	
JBE	WN	"	1/4	0/4	0/4	0/4	0/4	0/4					<1.0	>7.3
None	"	"						4/4	2/4	4/4	0/4	0/4	8.3	
SLE	SLE	i.c.	0/4	0/4	0/4	0/4	0/4						<1.0	>6.8
None	"	"					4/4	4/4	3/4	4/4	2/4		7.8	
SLE	MVE	s.c.	0/4	1/4	2/4	3/4	3/4	2/4	0/4				?	?
None	"	"				4/4	4/4	2/4	0/4	0/4			6.0	
SLE	WN	"	0/4	1/4	3/4	2/4	2/4	1/4	1/4				?	?
None	"	"					4/4	4/4	3/4	3/4	4/4	0/4	9.3	

* Mortality ratio = No. dying/No. inoculated.

mologous challenge of incompletely immunized animals has been discussed by Schlesinger(5). Because of this zone-like reaction the LD_{50} and the protection index were not calculated.

3. *Antibody Response as Related to Survival.* To determine whether resistance to challenge with any virus correlated with the neutralizing antibody titer for that virus, certain animals prepared for use in the above experiments were sacrificed and bled. This was planned after completion of the challenge of the SLE immunized hamsters, so in this instance 4 hamsters surviving homologous challenge were bled. Four JBE immune hamsters which had not been challenged were sacrificed and bled at the time all the others were challenged. Sera from these 4 animals were pooled. All these sera were tested for neutralizing antibody titer against MVE and WN viruses as well as against the homologous viruses. The results are shown in Table III. It will be noted that in the case of those animals immunized with SLE virus, even though bled after intracerebral challenge with homologous virus, thus receiving more immunization than those that were immunized with JBE virus, the antibody titer to MVE and WN viruses was much lower than in those animals immunized with JBE virus. In a parallel manner, the SLE immunized group were less resistant to challenge inoculation with the two heterologous viruses than were those immunized with JBE.

Discussion. The importance of demonstrating cross-protection amongst this group of viruses, especially by peripheral challenge, is evident when we realize that this route more nearly represents the type of natural exposure expected in an arthropod-borne disease. The possible practical application of such a finding was conceived as a result of earlier work on the antibody response to one injection of JBE virus vaccine of children and horses in California who had had a previous, naturally acquired SLE infection(1). Since it was not practical to challenge either the children or the horses with a peripheral inoculation of JBE to demonstrate whether they were really immune, as suggested by the antibody response, it was necessary to find a suitable small laboratory animal that was susceptible to peripheral challenge. The hamster has served such a purpose for challenge with MVE and WN viruses, and cross immunity to each of these has been shown following immunization with either JBE or SLE virus.

TABLE III. Neutralization Indices of Sera from Immunized Hamsters Used in Challenge Tests.

Immune hamster serum	Virus: Homologous JBE	Homologous SLE	Heterologous MVE	Heterologous WN
Japanese B (pool)	2000	—	214	11300
St. Louis				
Serum 1	—	514	<5	650
2	—	163	15	1180
3	—	<163	8	650
4	—	163	10	<206

The development of cross immunity in hamsters, together with the antibody findings with SLE immune children and horses injected with JBE vaccine lend further support to the hypothesis that any one virus in the group (possibly a benign strain of WN) if used as a living immunizing agent, might furnish basic, partial protection against all and could be effectively supplemented by a single injection of a specific killed vaccine for the virus found in the area under consideration. Repeated injections of killed vaccine alone, for JBE, the only member of this group of viruses used extensively for immunization, have been shown to be relatively ineffective in producing a neutralizing or complement fixing antibody response in adult man, and hence of questionable value in affording clinical protection. Further work along these lines, using hamsters and monkeys with other combinations of viruses in this group, is now in progress and will be reported shortly.

Summary and conclusions. An available strain of Syrian hamsters was found to be highly susceptible to WN and MVE viruses by peripheral routes of inoculation. Groups of animals of this species were then immunized with JBE and SLE viruses and subsequently challenged subcutaneously with serial dilutions of WN and MVE viruses. Complete protection against these two agents was afforded by JBE immunization, and protection of a lesser degree obtained by SLE immunization. Neutralizing antibody titers of the immunized hamsters to the 2 heterologous challenge viruses paralleled their peripheral resistance in that the JBE virus immunes had higher neutralizing antibody titers to WN and MVE viruses than did the SLE virus immunes. These findings together with previously reported data support the hypothesis that cross immunity to these viruses probably occurs in man and that there might be some practical application of this concept in the field of human immunization. Further work with animals using other virus combinations is in progress.

1. Hammon, W. McD., Sather, G. E., Lennette, E. H., and Reeves, W. C., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 517.

2. Ruchman, I., *Fed. Proc.*, 1953, v12, 456; personal communication.

3. Pond, W. L., Russ, Sudie B., Rogers, Nancy G., and Smadel, J. E., *J. Immunol.*, 1955, v75, 78.

4. Watson, D. W., and Smadel, J. E., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 101.

5. Schlesinger, R. Walter, *J. Exp. Med.*, 1949, v89, 507.

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