7. Raymond, S., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 278.

8. Grassmann, W., and Hannig, K., *Hoppe-Seyler's Z. physiol. Chem.*, 1952, v290, 1.

9. McLeod, C. P., and Stokes, P. S., *Pub. Health Rep.*, 1955, v70, 379.

Received February 23, 1956. P.S.E.B.M., 1956, v91.

Serological Response to Japanese B Encephalitis Vaccine of Children and Horses Immune to St. Louis Virus.* (22313)

W. MCD. HAMMON, GLADYS E. SATHER, EDWIN H. LENNETTE, AND W. C. REEVES.

Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pa., Viral and Rickettsial Disease Laboratory, State of California Department of Public Health, Berkeley, and the George Williams Hooper Foundation for Medical Research and School of Public Health, University of California, San Francisco and Berkeley.

In the spring of 1946 an opportunity was afforded one of us (W. McD. H.) to make serological tests on adult Japanese and American troops given a single injection (1 ml) of Japanese B encephalitis (JBE) mouse brain vaccine, then in current use in American troops. Japanese with elevated neutralizing antibody titers to JBE virus and without detectable complement fixing (C.F.) antibody prior to vaccination responded with elevated C.F. titers within 10 days after injection of a single dose of vaccine(1). This observation was subsequently confirmed by Sabin, Ginder, Matumoto and Schlesinger(2). On the other hand, American troops usually had neither neutralizing nor C.F. antibody responses after such an interval and only about 50% responded with significant neutralizing antibodies after the regular course of 3 injections; also, with rare exceptions, there was no C.F. antibody detectable at any time(1,3). This led us to wonder whether a rapid C.F. antibody response after an injection of JBE vaccine might be a specific means of determining whether an individual found to have JBE neutralizing antibody had been previously infected with JBE virus or whether such antibodies might be the result of an infection with St. Louis encephalitis (SLE), West Nile (WN) or another virus in this closely related group. In this connection, 2 of us (W. McD. H. and W. C. R.), while at the Hooper Foundation, had repeatedly observed in performing sero-diagnostic work for the arthropod-borne viral encephalitides that many humans and horses with SLE virus neutralizing and C.F. antibodies also had elevated serological titers to JBE virus. In some instances the titers to the latter agent were higher than those to SLE virus. Similar diagnostic rises occurred with both viruses. Obviously, if both viruses should be present in the same area this would pose a serious diagnostic problem. Also, as has already occurred in our experience and in that of others, antibodies for several of these viruses have been found in populations of other countries prior to isolation of any virus. Which virus gave rise to the antibodies has not always been obvious. The experiments reported in this paper were undertaken to see whether administration of JBE vaccine would permit of specific serologic differentiation for JBE virus infection.

Materials and methods. All sera after collection were frozen in rubber stoppered Pyrex glass tubes until tested. They were shipped with dry ice, then held at −25°C. All sera in a series from the same host and pertaining to one experiment were tested simultaneously. Neutralization tests were performed both by the intracerebral route (in 3 to 4-week-old mice) and by the intraperitoneal route (in

* These studies were conducted under sponsorship of Commission on Viral Infections, Armed Forces Epidemiological Board, and were supported in part by Office of Surgeon General, Department of Army, and by research grant C-481(C-7) from National Microbiological Institute of National Institutes of Health, Public Health Service.

TABLE I. Serological Response of Human Encephalitis Cases from Kern County, Calif., during Disease and Convalescence and Finally before and after an Injection of Japanese B Vaccine.

Name	Time relation to onset or vaccination (days)	St. Louis Neutralization Intracer.	St. Louis Neutralization Intraper.	St. Louis CF	Japanese B Neutralization Intracer.	Japanese B Neutralization Intraper.	Japanese B CF
		× 1000					
Goodloe	8 P.O.†	(1)* 39	—	(1)<4	(1) 100	—	—
		(2)* 120		(2) "			
	20 P.O.	(2) 220	—	(1) 64	(1) 100	—	—
				(2) "			
	27 P.O.	(1) 285	(1) >100	(1) "	(1) 1,600	—	—
				(2) "			
	53 P.O.	(2) 770	(1) 1,000	(2) "	(1) 1,000	—	—
	0 P.V.†	(1) 3,200	(1) 160	(1) 4	(1)<100	(1) 80	(1)<4
	11 P.V.	(1)10,000	(1) >320	(1) 32	(1) 1,000	(1)10,000	(1) 8
Leflore	16 P.O.	(1) 32	—	(1) 32	(1) 75	—	—
	42 P.O.	(1) 2,000	>1,000	(1)128	(1) 1,000	—	—
	140 P.O.	—	—	—	(1) 100	—	—
	0 P.V.	>100	>10	(1) 8	(1) 630	(1) 3,200	(1)<4
	11 P.V.	1,000	>320	(1) 8	(1) 320	(1) 6,300	(1) 8
Bugni	Onset	(1) 155	(1) 10	(1)<4	(1) 150	—	—
	45 P.O.	(1) 1,900	—	(1) 64	(1) 3,200	—	—
	120 P.O.	—	(1) 1,000	—	(1) 320	—	—
	155 P.O.	—	(1) 1,000	—	(1) 1,000	—	—
	0 P.V.	(1) 1,000	(1) >320	(1) 16	320	800	(1) 4
	11 P.V.	(1) 5,000	(1) >320	(1) 32	1,300	32,000	(1) 32

* No. in parentheses represent the date on which tests were performed. In any one section (post onset, or pre or post vaccination) in the same vertical column, all those tests with the same numeral in parentheses were performed at one time.

† P.O. = Post onset; P.V. = Post vaccination.

suckling mice) by the virus dilution method, using undiluted and unheated serum. The virus strains used were: for the intracerebral test, SLE, Webster strain, and JBE, Okinawa strain; and for the intraperitoneal test, SLE, Hubbard strain, and JBE, Nakayama strain. Incubation of serum and virus was for 2 hours at 37°C. All mice were of the CFW strain. Complement fixation tests were done by the method of Casals(4), employing serial dilutions of serum, 2 exact units of complement and incubation overnight at 4°C. The antigens were of the benzene-extracted type, using the method of España and Hammon(5). JBE chick embryo type of formalin inactivated vaccine of suitable potency was obtained from Doctor J. E. Smadel of the Army Medical Service Graduate School. Through the courtesy of Doctor W. A. Longshore of the California State Department of Public Health, 3 children whom we had diagnosed by serological methods in 1949 as cases of St. Louis encephalitis were bled on March 8, 1950, then given 1 ml of vaccine and bled 11 days later. Sera for retest taken during the acute and convalescent phase of their illnesses were still present in our deep freeze. Fourteen horses resident in Kern County were selected on the basis of earlier serological testing and antibody status. Two control horses were selected from a non-endemic area on the basis of an absence of detectable antibodies. Through the kindness of Doctor Ben H. Dean of the California State Department of Public Health the horses were bled, injected with 5 ml of vaccine each, then bled again 10 and 30 days later. Aliquots of serum samples from these horses were tested in 3 different laboratories, California State Department of Public Health (EHL), Hooper Foundation, U. of California (WCR) and University of Pittsburgh (WMH and GES), by a variety of methods. The results in the 3 laboratories were essentially alike, and those reported here are the results of tests performed at the University of Pittsburgh.

Results. 1. *Convalescent Patients.* The results of tests made during illness and convalescence, followed by those just before and after vaccination of the 3 children, are pre-

sented in Table I. It will be observed that just before vaccination all had detectable neutralizing and C.F. antibody to SLE virus. Goodloe had essentially no antibody of either type to JBE virus. Leflore had only neutralizing antibody, while Bugni had a detectable level of both types of antibody. Following vaccination all but Leflore showed an increase in JBE neutralizing antibody and all showed significant rises in JBE C.F. antibody. In

TABLE II. Serological Studies on Japanese B and St. Louis Antibody Response of California Horses Vaccinated with Japanese B Vaccine.

	Neutralization tests				C.F. tests	
	St. Louis		Japanese B		St.	
Horse	Intracer.	Intraper.	Intracer.	Intraper.	Louis	Jap B
Red	* 4,000	>50,000	20	1,800	<4§	<4
	† 800	"	130	160,000	32	32
	‡				16	16
Boots	4,000	"	1,000	3,000	<4	<4
	8,000	"	10,000	4,000	8	8
					4	<4
Pat	1,000	320,000	12	<100	<4	<4
	500	6,000,000	100	16,000	8	8
					4	4
King	1,000	500,000	250	6,000	<4	<4
	4,000	6,000,000	16,000	1,000,000	8	4
					<4	<4
Jackass	800	>1,000,000	200	<100	8	<4
	1,600	"	400	84,000	16	16
Black	6,300	"	700	200,000	<4	<4
	6,300	"	700	8,400,000	32	32
Marge	1,600	"	500	500	<4	<4
	16,000	"	2,000	>6,300	8	8
					4	<4
Sally	1,000	200,000	25	250	<4	<4
	5,000	400,000	400	250,000	64	64
					16	16
Boots	800	700,000	40	160	<4	<4
	500	1,600,000	25,000	1,000,000	16	16
					8	8
Apache	5,000	1,000,000	70	100	<4	<4
	1,000	>1,000,000	700	6,000,000	16	16
					8	8
Chief	6,000	>50,000	180	1,300	<4	<4
	6,300	"	4,000	1,000,000	16	16
					<4	8
Thunder	4,000	"	40	50	<4	<4
	4,000	"	800	100,000	32	32
					8	8
Pinto	500	>4,000,000	160	6,300	<4	<4
	2,500	"	16,000	1,000,000	16	16
					8	8
Easter	1	<600	16	<100	<4	<4
	50	"	16	"	8	8
					<4	<4
#1564 Cutter	1	<30	1	<130	<4	<4
	1	"	8	"	<4	<4
					<4	<4
#1556 Cutter	1	100	1	<130	<4	<4
	1	<32	8	"	<4	<4
					<4	<4

* Pre-inoculation bleedings. † 10-day bleeding. ‡ 30-day bleeding. § Reciprocal of dilution giving 2+ or higher complement fixation.

general, rises also occurred in SLE antibodies. However, end-points were not always attained, but in some where titrations were complete, significant rises were not demonstrated.

2. *Horses.* Results of the tests on the horses are presented in Table II. The prevaccination sera of 13 of the 16 horses contained significant neutralizing antibody to SLE virus and most of these 13 also had detectable JBE neutralizing antibody titers. Easter had none detectable by the intracerebral method but the intraperitoneal test was not performed with a small enough amount of virus to eliminate the possibility of a trace of antibody. The last 2 horses (controls) in the Table can be considered negative for neutralizing and C.F. antibodies.

Following vaccination, C.F. antibody titers rose rapidly to both SLE and JBE in all those with previous SLE and/or JBE neutralizing antibody. Higher titers were found after 10 days than after 30. Easter gave this same type of response. However, those unequivocally negative for SLE neutralizing antibody prior to vaccination showed no response. Neutralizing antibody response to JBE in the 10-day interval (not tested after 30 days) also appears to have been very pronounced in most horses originally immune to SLE, and the rise was more conspicuous than that to SLE. Easter and the two with no previous immunity showed no detectable response.

Discussion. Both children and horses (resident in the Western United States) and naturally immune to what we assume must be St. Louis virus responded serologically to a single injection of a killed-virus JBE vaccine. This response was indistinguishable from that of persons immune to JBE. The C.F. antibody response was most dramatic, since nonimmunes fail to develop any antibody of this type, regardless of time or even after repeated injections. If one assumes that the horses and children had not been previously infected with JBE virus, the secondary type response demonstrated by JBE vaccine is not specific for previous JBE infection, and the test does not serve any useful purpose in differentiating between those two viruses. It is, however, in all probability a specific secondary immunologic response to a common antigen present in both JBE and SLE viruses.

It will be recalled, however, that in addition to the rapid rise in C.F. antibody a very striking and rapid rise generally occurred also in JBE neutralizing antibodies. In view of the low response rate (7 to 30%) to even 3 injections of vaccine of this same type then in use in normal American troops(6), one of the factors leading to its discontinuance in military preventive medicine, the response in St. Louis immunes, assumes particular interest. The following questions are raised: (1) Is it possible that the few American adults (troops) who had good serological responses to JBE, *i.e.*, high neutralizing antibody titers and positive C.F. tests, represented those few individuals with immunity to SLE, and (2) could this heterologous, booster phenomenon be used for a practical purpose in immunization?

Since JBE immunization with currently available killed vaccine has not been used recently in military groups because its effectiveness has been questioned, it is possible that another antigenically related virus of a more benign nature might be employed as an infecting agent to give basic primary group immunity. This then could be supplemented with an injection of killed JBE vaccine to obtain the response noted in these experiments. Certain strains of West Nile virus have already been demonstrated to be quite benign(7) and suggest themselves for this purpose.

With this in view, experimental work in laboratory animals has been undertaken by the University of Pittsburgh group and will be the subject of subsequent reports(8).

Summary and conclusions. Three children convalescent from St. Louis encephalitis and a group of 16 horses, 13 of which had naturally acquired antibodies to St. Louis virus, were given one injection with a killed Japanese B encephalitis virus vaccine. After 10 or 11 days all individuals previously infected with St. Louis virus showed complement fixing antibody rises to Japanese B and St. Louis viruses and most also showed neutralizing

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.

Received February 2, 1956. P.S.E.B.M., 1956, v91.

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

W. McD. HAMMON AND G. E. SATHER.

Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pa.

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.

* These studies were conducted under sponsorship of Commission on Viral Infections, Armed Forces Epidemiological Board, and supported in part by Office of Surgeon General, Department of Army.