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Cross-Protection in Hamsters Immunized with Group A Arbovirus Vaccines

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Cross-protection between Venezuelan, Eastern, and Western equine encephalomyelitis (VEE, EEE, WEE) viruses was studied in the hamster by using challenge responses and neutralizing antibody titers as indexes of protection. Formalin-inactivated vaccines induced only homologous protection regardless of the sequence of vaccination or the combination of vaccines employed. Use of attenuated VEE vaccine, singly, produced absolute homologous protection as well as 37 and 59% protection against WEE and EEE challenges, respectively. Neither deleterious nor enhancing interaction occurred when attenuated VEE and inactivated WEE and EEE vaccines were employed in various sequences of immunization and all possible combinations. The most rapid and simple immunization scheme eliciting excellent homologous protection consisted of a single dose of combined attenuated VEE and inactivated WEE and EEE vaccines. Studies with attenuated strains of VEE, EEE, and WEE viruses showed that all elicited excellent homologous protection when administered singly. However, use of these live strains in many combinations and sequences resulted in a significant ($P < 0.05$ to <0.001) decrease in the protective efficacy of the WEE or EEE strains. These results are discussed in relation to serum neutralization test data obtained on sera drawn pre- and postchallenge.

For several years this Institute has directed a major research effort towards the development and evaluation of group A arbovirus vaccines for use in man, namely, those against Venezuelan, Eastern and Western equine encephalomyelitis virus (VEE, EEE, WEE) infections (1-3, 5, 11, 17). Our ultimate goal was the development of an immunization scheme for the group A arboviruses which would elicit broad group protection while requiring as few vaccines and doses as possible. Such vaccination programs would be of greatest benefit to at-risk personnel such as laboratory workers, veterinarians, or troops stationed in endemic areas. To provide base-line data for future use in volunteer studies, vaccines were tested in hamsters singly, in combinations, and in various sequences to determine the degree of heterologous protection afforded. Reported here are the results of such studies.

MATERIALS AND METHODS

Challenge virus strains. California strain WEE virus (6), Cambridge strain EEE virus (6), and Trinidad strain VEE virus (21) were used to challenge hamsters. All three strains had a history of numerous passages in suckling mice, guinea pigs, or embryonated eggs; however, these viruses were lethal to high titers, i.e., 10^7 to 10^{10} median lethal doses (LD_{50}) per milliliter, when administered to hamsters via the intraperitoneal (ip) route.

Vaccines and attenuated virus strains. The Formalin-inactivated EEE and WEE vaccines currently in use in man have been described (2, 17), as has the attenuated VEE vaccine (5). With the techniques employed for the EEE and WEE vaccines, a Formalin-inactivated VEE vaccine was produced for use in these studies. In addition, a small plaque mutant of EEE virus, strain Arth 167, was obtained from P. H. Coleman of the Center for Disease Control, Atlanta, Ga., as culture fluid from the 13th passage in duck embryo cell (DEC) culture. A further passage was made in DEC in our laboratory. This strain had a titer of $10^{8.5}$ LD_{50}/ml in suckling mice and was infectious but avirulent for the hamster. The clone 15, B628 attenuated strain of WEE virus was obtained from Lederle Laboratories (Pearl River, N.Y.) as culture fluid from the second passage in chick embryo cell (CEC) culture (22). After an additional passage in CEC culture, this material had a titer of $10^{5.0}$ median ip immunizing doses (ID_{50}) per milliliter in hamsters against a WEE challenge. The latter two attenuated strains and the experimental, Formalin-inactivated VEE vaccine are for laboratory use and

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are not approved for use in man, in contrast to the first three vaccines (23).

Immunization and challenge schemes. All studies were conducted with Lakeview strain golden Syrian hamsters (85 to 95 g) obtained from the Lakeview Hamster Colony, Newfield, N.J. For simplicity, the following generalizations may be made. (i) All vaccinations and challenges were made via the ip route by using 10^2 hamster ID_{50} of the inactivated vaccines, 10^3 ID_{50} of the attenuated strains, and 10^3 LD_{50} of the challenge viruses. (ii) When more than one dose of an inactivated vaccine was given, there was a 7-day interval between doses, whereas there was a 21- to 30-day period between doses of different attenuated viruses. (iii) Challenges were made 21 to 30 days after the last vaccine dose.

Serological procedures. Randomly selected hamsters were bled at various periods pre- and postvaccination or postchallenge. All sera were stored at −20 C until tested for hemagglutination-inhibiting (HI) or neutralizing antibody, or both. HI antibody was determined by the method of Clarke and Casals (9), as modified by French and McKinney (12). Serum neutralizing antibody was measured by the constant serum-varying virus method, with intracerebral inoculation of 1- to 3-day-old mice (13). These are expressed as $\log_{10}$ serum neutralization indexes (LNI).

TABLE 1. *Response to intraperitoneal challenge of hamsters vaccinated with combined attenuated (atten) VEE or inactivated WEE, EEE, and VEE vaccines, or both*[a]

Vaccination[b] schedule	No. of doses	Per cent surviving challenge with 10^3 LD_{50} of		
		WEE	EEE	VEE
WEE	1	78	11	0
EEE	1	15	72	0
WEE-EEE	1	85	88	0
VEE (atten)-WEE-EEE	1	84	84	100
WEE	2	100	5	0
EEE	2	20	95	0
WEE-EEE	2	100	96	0
WEE	3	87	4	—[c]
EEE	3	4	83	
WEE-EEE	3	93	85	
VEE (atten)-WEE	1	76	46	100
VEE (atten)-EEE	1	40	86	100
VEE (atten)	1	37	59	98
VEE	1	3	4	83
VEE (5× doses)	2	1	2	100

[a] Data compiled from results of several experiments.

[b] Unless otherwise noted, vaccines were Formalin-inactivated.

[c] Blank denotes not tested.

RESULTS

Immunization with combined vaccines. Table 1 is a summary of data from several experiments in which hamsters were given inactivated WEE, EEE, and VEE vaccines, either singly, or combined, both with and without attenuated VEE vaccine. Each vaccination group (minimum of 150 hamsters per vaccination group) was then randomly subdivided into three equal groups and challenged with the indicated viruses. As shown, neither deleterious nor enhancing interaction occurred when the vaccines were given in combined form. For example, combined WEE and EEE vaccines elicited 85% protection against WEE challenge and 88% protection against EEE challenge, which was not substantially different from the per cent homologous protection observed when the WEE and EEE vaccines were administered singly. Nor did combination with attenuated VEE vaccine appreciably alter these results.

In no instance did WEE or EEE vaccines given singly or combined, regardless of the number of doses, protect against challenge with VEE virus. Excellent homologous protection was induced by attenuated VEE vaccine when administered in any combination with the other vaccines. The degree of homologous and heterologous protection elicited by the VEE vaccine given singly has been relatively constant in separate experiments in which more than 500 hamsters have been challenged with these and other strains of WEE and EEE viruses (*unpublished data*). In contrast, inactivated VEE vaccine administered in a single 0.5-ml dose or in two 2.5-ml doses protected against homologous challenge only.

In preliminary studies on the practical aspects of human immunization with combined vaccines,

TABLE 2. *Response to intraperitoneal challenge of hamsters immunized with a single dose of combined attenuated VEE or inactivated WEE and EEE vaccines, or both*

Vaccination schedule	Per cent surviving (no./total) challenge with 10^3 LD_{50} of		
	WEE	EEE	VEE
VEE-WEE-EEE	93 (65/70)	99 (69/70)	100 (10/10)
WEE-EEE	100 (50/50)	100 (50/50)	4 (2/50)
None (challenge control)	5 (1/20)	0 (0/20)	0 (0/20)

hamsters were simultaneously immunized with attenuated VEE and inactivated WEE and EEE vaccines. The latter two vaccines were prepared and packaged in the freeze-dried form. Attenuated VEE vaccine was diluted to contain 10^3 hamster ID_{50} per 0.5 ml; this material was then used to reconstitute the freeze-dried WEE vaccine. The resulting VEE-WEE vaccine mixture was used to reconstitute the EEE vaccine. Thus the 0.5-ml hamster ip dose contained 10^3 doses of attenuated VEE vaccine as well as the human doses of WEE and EEE vaccines (i.e., 10^2 hamster ID_{50} each). A second group of hamsters received only the combined WEE and EEE vaccines. The results in Table 2 illustrate, again, the absence of interaction in both the trivalent and divalent vaccine groups; namely, excellent homologous protection was induced by each vaccine in the two vaccination groups, but no VEE protection was induced by the WEE-EEE combination.

Sequential immunization. A two-dose schedule of inactivated vaccines was chosen for studies on the effect of sequence of vaccine administration, since this schedule is generally employed in human vaccination. As indicated in Table 3, protection against WEE virus challenge was complete whether WEE vaccine was given alone, in combination with EEE vaccine, sequentially after or before attenuated VEE vaccine, or in combination with EEE vaccine. Protection against EEE virus challenge was also unaffected by combinations or sequences. Similarly, protection against VEE challenge was virtually absolute when attenuated VEE vaccine was used, regardless of combinations and sequences. Moreover, the cross-protection induced by attenuated VEE vaccine was unaffected by sequence of vaccine administration (use line 1 as base-line data). As in prior experiments, the inactivated WEE and EEE vaccines failed to induce protection against VEE virus challenge.

TABLE 3. *Effect of sequential immunization of hamsters with attenuated VEE and inactivated WEE and EEE vaccines*

Vaccination schedule[b]	Per cent surviving intraperitoneal challenge with[a]		
	WEE	EEE	VEE
VEE	37	59	98
WEE(2)[c]	100	5	0
EEE(2)	20	95	0
WEE-EEE(2)	100	96	0
VEE, WEE(2)	98	54	99
WEE(2), VEE	100	60	100
VEE, EEE(2)	31	100	100
EEE(2), VEE	39	97	100
VEE, WEE-EEE(2)	100	100	98
WEE-EEE(2), VEE	98	100	100

[a] Data compiled from results of several experiments.

[b] Comma indicates sequential administration of vaccines; hyphen indicates combined administration.

[c] Number of doses given in parentheses.

Combined and sequential immunization with attenuated virus. In separate experiments, hamsters were inoculated via the ip route with attenuated strains of VEE, WEE, and EEE viruses by using various sequences of administration and all possible combinations. Sequential doses were administered 21 to 30 days apart, and all challenges were given ip 21 to 30 days after the last vaccine dose.

As shown in Table 4, the three strains elicited excellent homologous protection when administered singly. VEE elicited the highest degree of heterologous protection and EEE elicited the lowest. Sequence of immunization had a significant effect ($P < 0.001$) in a few schema. For example, use of attenuated VEE with attenuated WEE virus resulted in a significant increase in protection against EEE challenge regardless of sequence. However, reversing the sequence of WEE and VEE virus vaccinations depressed homologous protection against WEE virus challenge.

In vaccination schedules employing attenuated WEE and EEE viruses, sequence had no significant effect on homologous protection but had a marked effect ($P < 0.001$) on protection against VEE challenge; i.e. WEE followed by EEE elicited 23% protection against VEE challenge, whereas the reverse order resulted in 68% protection.

Simultaneous administration of the three attenuated strains resulted in a significant ($P < 0.001$) decrease in the homologous protective efficacy of the WEE virus but had no appreciable effect on the homologous protection induced by EEE or VEE viruses. When WEE virus was given simultaneously with either VEE virus or EEE virus, there was a similar depression in its capacity to elicit homologous protection ($P < 0.001$). The homologous protection induced by EEE virus was depressed ($P < 0.05$) when administered with VEE virus but was unaffected by simultaneous administration with WEE virus.

Serology. Results of HI antibody determinations proved to be of little value in these studies. With the exception of attenuated VEE vaccine, all attenuated viruses and inactivated vaccines failed to induce significant HI titers consistently (i.e., >1:20). Further, no correlation could be made between the HI titers and the results of

TABLE 4. *Response to challenge in hamsters vaccinated with attenuated VEE, EEE, and WEE viruses*

Vaccination schedule[b]	Per cent survivors (no./total) after ip challenge with 10^3 LD_{50}[a]		
	WEE	EEE	VEE
WEE, VEE	100 (20/20)	82 (45/55)	100 (10/10)
VEE, WEE	84 (42/50)	82 (41/50)	100 (13/13)
EEE, VEE	32 (16/50)	100 (25/25)	100 (10/10)
VEE, EEE	28 (14/50)	94 (47/50)	100 (12/12)
WEE, EEE	100 (45/45)	85 (69/81)	23 (63/275)
EEE, WEE	96 (24/25)	100 (25/25)	68 (123/180)
VEE-WEE-EEE	40 (32/80)	84 (67/80)	98 (59/60)
VEE-WEE	70 (21/30)	40 (12/30)	100 (30/30)
VEE-EEE	10 (3/30)	73 (23/30)	97 (29/30)
WEE-EEE	80 (28/35)	89 (31/35)	11 (11/100)
EEE	6 (8/128)	90 (75/83)	7 (5/70)
WEE	95 (133/140)	35 (45/130)	1 (1/94)
VEE	37 (97/260)	59 (158/268)	98 (190/194)

[a] Data are a compilation of results of several experiments.
[b] Comma indicates sequential administration of vaccines; hyphen indicates combined administration.

TABLE 5. *Serological responses of hamsters vaccinated with attenuated WEE, EEE, and VEE viruses and challenged with virulent strains*

Immunization schedule	Mean $\log_{10}$ serum neutralization index						
	21–30 days post-vaccination			Challenge received	14–21 days post-challenge		
	WEE	EEE	VEE		WEE	EEE	VEE
VEE	<0.3	<0.2	3.9	WEE	2.2	<0.3	3.9
				EEE	<0.3	2.8	4.0
				VEE	<0.3	0.5	3.9
WEE	3.2	<0.3	<0.3	WEE	3.4	0.6	<0.3
				EEE	3.1	>4.1	<0.3
				VEE	—[a]		
EEE	<0.5	≥3.3	<0.3	WEE	3.5	2.9	
				EEE	<0.2	≥3.6	<1.0
				VEE			≥3.8
VEE-WEE-EEE[b]	1.1	2.0	4.0	WEE	2.4	2.5	3.9
				EEE	1.6	3.0	4.1
				VEE	1.4	1.8	3.8

[a] Blank denotes not tested.
[b] Hyphen = simultaneous administration of vaccine.

neutralization tests or host response to challenge. In all subsequent studies, only the neutralization test was employed for immunological evaluation of hamster sera.

Shown in Table 5 are representative results of neutralization tests on sera obtained from hamsters vaccinated by various schedules; LNI values were determined with pooled normal hamster sera paired with pools of sera (five hamsters per pool) from animals vaccinated or vaccinated and challenged. All three attenuated strains induced significant homologous LNI values but no measurable heterologous neutralizing antibody. Subsequent to challenge with the viruses indicated, homologous titers were essentially unchanged, whereas the LNI against the challenge virus increased to a significant level. However, challenge with one heterologous virus did not effect an increase in neutralizing antibody against the vaccine virus nor the other heterologous virus.

Simultaneous administration of the three attenuated viruses resulted in a depression of the LNI values against WEE and EEE viruses when compared to the base-line data obtained when these two viruses were administered singly. In this trivalent group, homologous titers against WEE and EEE viruses increased upon homologous challenge, whereas the VEE titer remained

TABLE 6. *Serological responses of hamsters vaccinated with inactivated WEE, EEE, and VEE and attenuated VEE vaccines and challenged with virulent strains*

Immunization schedule[a]	Mean $\log_{10}$ serum neutralization index						
	21–30 days post-vaccination			Challenge received	14–21 days post-challenge		
	WEE	EEE	VEE		WEE	EEE	VEE
WEE inact (2)	2.1	<0.5	<0.5	WEE	3.0	<0.5	<0.5
				EEE	—[b]	2.7	
				VEE			
EEE inact (2)	<0.5	1.8	<0.5	WEE	3.4	3.8	<0.5
				EEE	0.6	3.0	<0.5
				VEE			
WEE inact (2)-EEE inact (2)	2.5	2.0	<0.5	WEE	≥4.1	2.4	0.0
				EEE	3.4	3.0	0.0
				VEE			
VEE atten (1)-WEE + EEE inact (2)	2.4	≥2.9	4.0	WEE	3.1	2.8	≥3.8
				EEE	2.7	2.7	3.5
				VEE	2.3	2.1	>4.0
VEE inact (1)	0.0	<0.1	3.5	WEE			
				EEE			
				VEE			3.3
VEE inact (2)[c]	<0.5	<0.5	2.7	WEE			
				EEE			
				VEE			2.9

[a] Atten = attenuated virus; inact = inactivated vaccine; hyphen = simultaneous vaccination; ( ) = number of doses.

[b] Blank denotes not tested.

[c] Standard dose administered was 10×.

unchanged; as with the monovalent vaccination groups, there were no significant increases in heterologous antibody after challenge.

As seen in Table 6, the inactivated WEE and EEE vaccines given singly and in combined form elicited only homologous neutralizing antibody responses. Unlike the live vaccines, these killed preparations permitted limited replication of even the homologous challenge viruses, as indicated by viremia (*unpublished data*), with a resulting significant increase in antibody titers to the challenge viruses. Induction of heterologous antibody as a result of challenge was not noted. Combination of the attenuated VEE vaccine with these two killed vaccines resulted in an increase in prechallenge titers to EEE virus and, further, apparently prevented the increase in homologous antibody titer after challenge that was observed when the killed WEE and EEE vaccines were employed singly or in combined form.

Inactivated VEE vaccine whether given in a single (1×) dose or two 5× doses stimulated only homologous neutralizing antibody, the level of which was unaffected by homologous challenge. Sera from the few animals that survived heterologous challenge were not available for determination of antibody titers.

DISCUSSION

The inability of the inactivated WEE, EEE, and VEE vaccines to induce cross-protection was not totally unexpected for two reasons. First, the group A arboviruses are not as closely related serologically as are those in other groups (7). Second, with inactivated viruses of animal tissue origin as immunizing agents, other investigators have also failed to demonstrate cross-protection (4, 18, 19). Indeed, the significant group A cross-protection that has been reported by other investigators, in general, was induced by virulent virus infections or by repeated immunizations with one or more virulent group A viruses (15, 16, 20; W. P. Allen, Bacteriol. Proc. p. 152, 1962). Studies such as these, while contributing to the understanding of group A relationships, have not provided guidance for the development of a safe and effective immunization scheme for use in man.

Although amply demonstrated by our data, the cross-protection induced by the attenuated VEE vaccine is an incompletely understood phenomenon. However, the absence of cross-protection in hamsters given two 5× doses of inactivated VEE vaccine suggests that the cross-protection induced by live vaccine may be due only in small part to major antigen(s) common to the

three viruses. Quite apparent are the possibilities that total antigenic mass (presumably lower in inactivated vaccines), exposure of the host to minor antigens through vaccine virus replication in vivo, latent infection of target cells with the vaccine virus (16), or other factors may be involved in the VEE-WEE-EEE one way cross-protection phenomenon.

Experience with live vaccines indicates that they may be expected to rapidly produce long-lasting immunity with only one dose. Moreover, as previously mentioned, immunization with these live viruses would be expected to elicit broadened group A protection. However, the results obtained with the attenuated VEE, WEE, and EEE viruses in the present study were quite discouraging. With the exception of some sequential immunization schemes, use of these three strains resulted in a deleterious effect on the protective efficacy of the attenuated WEE and EEE viruses.

The neutralization test data show that the level of circulating heterologous antibody at the time of challenge does not play a role in our system. Both inactivated and live vaccines induced only homologous neutralizing antibody, the level of which remained unchanged upon challenge. As has been the experience of others, heterologous challenge resulted in a significant rise in titer to the specific challenge virus alone (7, 20). Although temporal antibody studies were not performed here, perhaps as suggested by Casals (7), preexperienced animals may produce cross-reacting antibodies at an accelerated rate upon challenge. In this case, the temporal relationship between the invasiveness of the virus and the rate at which the animal synthesizes antibody may spell the difference between survival and death.

As a result of the studies described in this paper, our concept for the prophylaxis of group A arbovirus diseases has been changed to one of immunization with combined vaccines for specific geographical areas. Our major effort will be directed toward the development of vaccines against those group A virus diseases that are moderately or severely incapacitating and which have occurred with some frequency. The obvious need for immunization against other arbovirus diseases will not be discussed in this paper. Thus, for example, within the continental United States, vaccination would be required for WEE and EEE with the possible later addition of VEE (8, 10). Immunization for Central and South America, including the Caribbean, would require vaccines for VEE and Mayaro. For Africa and the Near East, vaccines for Chikungunya and O'nyong nyong would be required. Finally, in Asia, Australia, and New Zealand, only a vaccine for Chikungunya would be required. At present, techniques for vaccine production are available for WEE (2), EEE (3), VEE (5), and Chikungunya viruses (14). To effect the geographical immunization program described, vaccines must therefore be developed for Mayaro and O'nyong nyong; studies are now in progress to achieve this end.

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LITERATURE CITED

1. Alevizatos, A. C., R. W. McKinney, and R. D. Feigin. 1967. Live, attenuated Venezuelan equine encephalomyelitis virus vaccine. I. Clinical effects in man. Amer. J. Trop. Med. Hyg. 16:762–768.
2. Bartelloni, P. J., R. W. McKinney, F. M. Calia, H. H. Ramsburg, and F. E. Cole, Jr. 1971. Inactivated Western equine encephalomyelitis vaccine propagated in chick embryo cell culture: clinical and serological evaluation in man. Amer. J. Trop. Med. Hyg. 20:146–149.
3. Bartelloni, P. J., R. W. McKinney, T. P. Duffy, and F. E. Cole, Jr. 1970. An inactivated Eastern equine encephalomyelitis vaccine propagated in chick embryo cell culture. II. Clinical and serologic responses in man. Amer. J. Trop. Med. Hyg. 19:123–126.
4. Beck, C. E., and R. W. G. Wyckoff. 1938. Venzuelan equine encephalomyelitis. Science 88:530.
5. Berge, T. O., I. S. Banks, and W. D. Tigertt. 1961. Attenuation of Venezuelan equine encephalomyelitis virus by *in vitro* cultivation in guinea pig heart cells. Amer. J. Hyg. 73:209–218.
6. Byrne, R. J., G. R. French, F. S. Yancey, W. S. Gochenour, P. K. Russell, H. H. Ramsburg, O. A. Brand, F. G. Scheider, and E. L. Buescher. 1964. Clinical and immunologic interrelationship among Venezuelan, Eastern, and Western equine encephalomyelitis viruses in burros. Amer. J. Vet. Res. 25:24–31.
7. Casals, J. 1963. Relationships among arthropod-borne animal viruses as determined by cross-challenge tests. Amer. J. Trop. Med. Hyg. 12:587–596.
8. Chamberlain, R. W., W. D. Sudia, T. H. Work, P. H. Coleman, V. F. Newhouse, and J. G. Johnston, Jr. 1969. Arbovirus studies in South Florida with emphasis on Venezuelan equine encephalomyelitis virus. Amer. J. Epidemiol. 89:197–210.
9. Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7:561–573.
10. Ehrenkranz, N. J., M. C. Sinclair, E. Buff, and D. O. Lyman. 1970. The natural occurrence of Venezuelan equine encephalitis in the United States. N. Engl. J. Med. 282:298–302.
11. Feigin, R. D., R. F. Jaeger, R. W. McKinney, and A. C. Alevizatos. 1967. Live, attenuated Venezuelan equine encephalomyelitis vaccine. II. Whole-blood amino acid and fluorescent-antibody studies following immunization. Amer. J. Trop. Med. Hyg. 16:769–777.
12. French, G. R., and R. W. McKinney. 1964. Use of β-propiolactone in preparation of inactivated arbovirus serologic test antigens. J. Immunol. 92:772–778.
13. Hammon, W. McD., and T. H. Work. 1964. Arbovirus infection in man. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial diseases, 3rd ed., p. 268–311. Amer. Pub. Health Assoc., New York, N. Y.
14. Harrison, V. R., L. N. Binn, and R. Randall. 1967. Comparative immunogenicities of chikungunya vaccines prepared

in avian and mammalian tissues. Amer. J. Trop. Med. Hyg. 16:786–791.
15. Hearn, H. J., Jr. 1961. Cross-protection between Venezuelan equine encephalomyelitis and Eastern equine encephalomyelitis virus. Proc. Soc. Exp. Biol. Med. 107:607–610.
16. Hearn, H. J., and C. T. Rainey. 1963. Cross-protection in animals infected with group A arboviruses. J. Immunol. 90:720–724.
17. Maire, L. F., III, R. W. McKinney, and F. E. Cole, Jr. 1970. An inactivated Eastern equine ecnephalomyelitis vaccine propagated in chick embryo cell culture. I. Production and testing. Amer. J. Trop. Med. Hyg. 19:119–122.
18. Morgan, I. M. 1941. Influence of age on susceptibility and on immune response of mice to Eastern equine encephalomyelitis virus. J. Exp. Med. 74:115–132.
19. Morgan, I. M., R. W. Schleisinger, and P. K. Olitsky. 1942. Induced resistance of the central nervous system to experimental infection with equine encephalomyelitis virus. I. Neutralizing antibody in the central nervous system in relation to cerebral resistance. J. Exp. Med. 76:357–369.
20. Parks. J. J., and W. H. Price. 1958. Studies on immunologic overlap among certain arthropod-borne viruses. I. Cross-protection relationships among group A viruses. Amer. J. Hyg. 67:187–206.
21. Randall, R., and J. W. Mills. 1944. Fatal encephalitis in man due to the Venezuelan virus of equine encephalomyelitis in Trinidad. Science 99:225–226.
22. Roca-Garcia, M., E. J. Jungherr, H. N. Johnson, and H. R. Cox. 1965. An attenuated strain of Western equine encephalitis virus as a possible live immunizing agent. p. 22–40. *In* 68th Annu. Proc. U.S. Livestock Sanitary Ass., 19–23 October 1964. Memphis, Tenn.
23. U.S. Army Regulation 40-7. 1969. Clinical use of investigational drugs. Department of the Army, Washington, D.C.