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A Safety Test for Eastern Equine Encephalomyelitis Vaccine

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Since the development of the procedures for producing and testing Eastern equine encephalomyelitis (EEE) vaccine for human use (R. J. Randall, J. W. Mills, and L. L. Engel, J. Immunol. **55**:41, 1947), the work of other investigators on the susceptibility of various host or cell systems to EEE virus has indicated that there might be an advantage to testing this vaccine for safety in systems other than by the prescribed intracerebral inoculation of Swiss white mice. These later investigations have shown that 12-hr-old chicks (R. W. Chamberlain, R. K. Sikes, and R. B. Kissling, J. Immunol. **73**:106, 1954) or chick embryo cell cultures (D. W. Medairis and S. Kibrick, Proc. Soc. Exptl. Biol. Med. **97**:152, 1958) were more sensitive than the adult mouse as a means of detecting strains of EEE virus for use in either etiological diagnosis or in epidemiological studies.

It has also been shown that in some host systems the expression of live organisms could be masked either by interference due to presence of dead organisms (R. W. Schlesinger, p. 157, in F. M. Burnet and W. M. Stanley [ed.], *The Viruses*, vol. 3, Academic Press, Inc., New York, 1959) or by neutralization of the live organism as a result of antibodies elicited by the safety test dose (S. Berman et al., J. Bacteriol. **79**:747, 1960).

To compare the efficacy and relative sensitivity of various host systems for the purpose of testing EEE vaccine for safety (ability to detect minimal numbers of live virus in the presence of large numbers of dead virus), parallel titrations were performed by inoculating these systems with our egg-adapted vaccine strain of EEE virus diluted in either appropriate tissue culture growth medium or EEE vaccine. Serial 10-fold dilutions of the virus were made in medium 199 for the inoculation of chick embryo fibroblast cells (in tubes), 2-week-old suckling and 3-week-old mice, 6- to 12-hr-old Rhode Island Red chicks and 7-day-old embryonating eggs. With the use of Eagle's minimal essential medium as diluent, a second series of virus dilutions were prepared

TABLE 1. *Relative sensitivity of mice, embryonating eggs, chicks, and cell culture to EEE virus*

Test system	Route[a]	Dose (ml)	Virus diluted in		Observation (days)
			Medium	Vaccine[b]	
3-week-old mice	ic	0.03	7.9[c]	7.9	21
Suckling mice	ic + ip	0.03 + 0.2	8.6	8.5	21
Embryonating eggs	Allantoic	1.0	7.4	7.3	12
Chicks	sc	0.03	9.7	10.0	7
	sc	0.5	11.2	11.1	7
Cell culture					
Chick embryo		0.1	9.5	8.5	3
Monkey kidney		0.1	10.2	9.2	3
Hamster kidney		0.1	10.5	9.8	3
Dog kidney		0.1	10.5	9.5	3
WI-38		0.1	10.5	9.5	3

[a] Intracerebral, ic; intraperitoneal, ip; subcutaneous, sc.
[b] Results were similar with either whole chick embryo vaccine or cell culture vaccine.
[c] Infectivity titer ($\log_{10}$) per indicated dose.

for the inoculation of African green monkey kidney, dog kidney, hamster kidney, and WI-38 cell culture systems. To determine the possibility of interference with the expression of the live virus by inactivated virus, freeze-dried EEE vaccine, prepared at this laboratory, was rehydrated with distilled water and used as a third diluent for the titration of the EEE virus. The virus diluted in vaccine was inoculated into all the above-mentioned test systems.

The results of the virus titrations (Table 1) demonstrated that the 3-week-old mouse inoculated with 0.03 ml intracerebrally and the 7-day-old embryonating egg inoculated with a 1.0 ml dose by the allantoic route were the least sensitive of the test systems. The suckling mouse inoculated with 0.03 ml intracerebrally plus 0.2 ml intraperitoneally was approximately 1 log higher in sensitivity. The responses in the tissue culture systems to a 0.1-ml inoculation of the virus dilutions were greater than in the above-mentioned systems. However, in the presence of vaccine, all cell culture systems showed evidence of interference between the inactivated and the live virus, thus making these systems unsatisfactory for safety test purposes. The 6- to 12-hr-old chicks, on the other hand, inoculated with 0.5 ml subcutaneously gave the greatest response to the virus, with no evidence of interference from the inactivated virus.

The results indicate that, of the safety test systems evaluated, the 6- to 12-hr-old chick is the most sensitive for detecting residual live EEE virus in EEE vaccine. In addition, the use of the chick instead of the mouse permits a 17-fold increase in the volume of vaccine tested per animal, with results available in 7 instead of 21 days.