

Observations on Acquired Cellular Resistance to Equine Encephalomyelitis Virus.

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In animals vaccinated with formalin-inactivated Eastern (E.E.E.) or Western (W.E.E.) viruses, degree of resistance to intracerebral inoculation of homologous virus is correlated with titer of neutralizing antibody in the serum¹ and with neutralizing capacity of cerebrospinal fluid.² In contrast to this specific immunity, a non-specific type of resistance has been observed which cannot be explained on the basis of serological findings. It has been encountered only in animals whose CNS has been previously in contact with certain encephalitic viruses. For study of this phenomenon animals were prepared in the following manner.

"Abortive Infections" with Equine Encephalomyelitis Virus in Guinea Pigs and Rabbits. Guinea pigs vaccinated with formalin-inactivated W.E.E. virus were, after suitable intervals, injected intracerebrally with 1 to 1,000 m.l.d. of W.E.E. virus. In vaccinated animals which survived, the response during the first 24 hours after inoculation was indistinguishable from that of non-vaccinated control animals: a steep rise in temperature occurred, first observed 2 hours after inoculation, which persisted for about 24 hours. While in control animals fever lasted until prostration or death, in immune animals the temperature dropped after 24 to 30 hours and remained normal thereafter. In both normal and vaccinated guinea pigs the intensity of febrile reaction on the first day was proportional to the amount of virus injected. Rabbits vaccinated against E.E.E. or W.E.E. reacted similarly when tested with homologous virus. No such characteristic rise in temperature occurred in guinea pigs or rabbits after intracerebral inoculation of formalin-inacti-

vated W.E.E. virus, of 1% normal mouse brain suspension, or of a neutral mixture of W.E.E. virus and homologous antiserum.

It was concluded that the "fever spike" of vaccinated animals reflected viral action on susceptible tissue and may indicate an "abortive infection."

Resistance of Guinea Pigs and Rabbits after Abortive Infection with E.E. Virus. Guinea pigs vaccinated with formalin-inactivated W.E.E. virus remained fully susceptible to intracerebral inoculation of E.E.E. virus. Others, similarly vaccinated but in addition convalescent from an abortive infection with W.E.E. virus, resisted 2 weeks later intracerebral re-inoculation of 10 or 1,000 m.l.d. of E.E.E. virus. The degree of this non-specific resistance depended on the intensity of the abortive infection. Similar observations were made in rabbits.

Abortive infection with W.E.E. virus rendered guinea pigs resistant to New Jersey strain of vesicular stomatitis virus injected intracerebrally. This resistance was less pronounced than that to E.E.E. virus, and when the interval between the two inoculations was 2 weeks, death occurred, as a rule, although after considerable delay. At 3 weeks, full susceptibility was restored.

Resistance in Mice. (1) Mice, after vaccination with formalized W.E.E. virus and intracerebral test inoculation with homologous virus, were found to resist 10,000 to 100,000 cerebral m.l.d. of E.E.E. virus, while others which had been vaccinated but not tested remained fully susceptible to E.E.E. virus. (2) Mice inoculated intracerebrally with 10 m.l.d. of Theiler's virus (T.V.) developed during the incubation period (13-14 days) an increasing degree of resistance to W.E.E. virus, as shown in Table I. Several mice paralyzed by T.V. for 8 to 9 days were re-inoculated with 10 to 100 m.l.d. of W.E.E. virus and survived.

¹ Morgan, I. M., and Olitsky, P. K., *J. Immunol.*, 1941, **42**, 445.

² Morgan, I. M., Schlesinger, R. W., and Olitsky, P. K., *J. Exp. Med.*, 1942, **76**, 357.

TABLE I.
Fate of Mice Inoculated Intracerebrally with Theiler's Virus and Re-inoculated at Varying Intervals with W.E.E. Virus.

Outcome	Dilution W.E.E. inoculated	Interval between T.V. and W.E.E. virus in days					Total 4-10-day intervals	Total controls†
		2	4	6	8	10		
Dead of W.E.E. infection	10-3	N.T.	N.T.	N.T.	N.T.	1/4	1/4	N.T.
	10-5	4/5*	5/5	3/5	1/5	0/5	9/20	19/20
	10-7	5/5	0/5	0/5	0/4	N.T.	0/14	17/20
Theiler's disease	$\left\{ \begin{array}{l} 10-3 \\ 10-5 \\ 10-7 \end{array} \right\}$	1†	5	7	8	8	28/28	20/20

* 4/5 = 4 of 5 inoculated mice died of W.E.E. infection.

† 1 = All mice inoculated with both viruses which did not die of W.E.E. became paralyzed with Theiler's disease after the usual incubation period.

‡ = Control mice receiving at same time as test mice W.E.E. virus or T.V.

N.T. = Not tested.

Discussion. The experimental results recall the interference phenomena described for other neurotropic viruses.^{3,4,5} Although "cross-immunity" between Eastern and Western equine encephalomyelitis had been observed before in horses^{6,7} and in guinea pigs and rabbits,⁸ the conditions under which it arose had not been defined. Non-specific resistance, as described, developed following exposure of the CNS to virus and was demonstrated in the absence of neutralizing antibody; it is believed, therefore, to be due to a change in the susceptibility of the cell. This differentiates it from specific immunity induced by vaccination^{1,2} and by passive immu-

nization.^{8,9} Attempts are under way to demonstrate resistance to equine encephalomyelitis virus following intracerebral inoculation of inactivated virus. It has been shown for bacteriophage¹⁰ and for influenza virus^{11,12} that ultraviolet irradiation does not destroy the interfering capacity. A fuller description will be published elsewhere; meanwhile, the practical significance of the findings should be pointed out. An animal, vaccinated against one of the equine viruses is specifically immune, but the specificity of its immunity is masked, at least temporarily, once it has been tested with an intracerebral challenge dose. This should be borne in mind when vaccinated and tested animals are used for the identification of newly isolated strains of virus.

Summary. Acquired, non-specific cellular resistance to equine encephalomyelitis virus has been described and differentiated from specific immunity induced by vaccination or passive immunization.

³ Dalldorf, G., Douglass, M., and Robinson, H. E., *J. Exp. Med.*, 1938, **67**, 333; Dalldorf, G., and Douglass, M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 294; Dalldorf, G., *J. Immunol.*, 1939, **37**, 245.

⁴ Theiler, M., *Medicine*, 1941, **20**, 443.

⁵ Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 127.

⁶ Records, E., and Vawter, L. R., *J. Am. Vet. Med. Assn.*, 1934, **85**, 89.

⁷ Meyer, K. F., Wood, F., Haring, C. M., and Howitt, B., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 56.

⁸ Howitt, B. F., *J. Immunol.*, 1935, **29**, 319.

⁹ Olitsky, P. K., Schlesinger, R. W., and Morgan, I. M., *J. Exp. Med.*, 1943, **77**, 359.

¹⁰ Luria, S. E., and Delbrück, M., *Arch. Biochem.*, 1942, **1**, 207.

¹¹ Henle, W., and Henle, G., *Science*, 1943, **98**, 87.

¹² Ziegler, J. E., Jr., personal communication.