appear to have essentially the same activity against RNAse in the 2 systems it is quite reasonable to suggest that the effects on biosynthesis may be attributable to RNAse inhibition. It will be recalled, however, that the "unnatural" polymers reverse RNAse inactivation as well as prevent it. This observation suggests, but does not necessarily prove, that they function in a positive way by fulfilling a requirement of the system rather than by inhibiting RNAse. On the other hand, ALE is inactive against RNAse *in vitro*. It is difficult to see how its effect in biosynthesis can be explained on the basis of RNAse inhibition.

Summary. Liver homogenates preincubated with RNAse, in contrast to controls, do not incorporate mevalonic acid into nonsaponifiable material or cholesterol. This inactivation by RNAse is preventable or reversible with an autoclaved extract of liver or with certain anionic polymers including polyethylene sulfonate, heparin, DNA and even RNA. The polymers also inactivate RNAse *in vitro* at comparable ratios of inhibitor to enzyme used in the biosynthetic experiments. Inactivation of RNAse *in vitro* is not observed, however, with comparatively high levels of liver extract. It is concluded that liver extract does not function in the homogenate system merely by inactivation of RNAse but this interpretation may be applicable to the anionic polymers.

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Hemagglutination-Inhibition and Serum Neutralization Response of Horses To Eastern Equine Encephalomyelitis Virus.* (25592)

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Hemagglutinating activity for Japanese B encephalitis, one of the arthropod-borne group of viruses, was first reported in 1950(1). Subsequent work revealed that other members of this group of viruses also agglutinate erythrocytes(2-5), and methods for hemagglutination-inhibition (HI) tests were developed concurrently. Information, other than from this laboratory(6), on use of the HI test for clinical and epidemiological studies of eastern equine encephalomyelitis (EEE) has been found in only one instance. Groot *et al.*(7) testing human sera for EEE found none to be positive for either HI or neutralizing antibodies. The experiments reported here were designed to compare use of HI and neutralization tests in detecting the immunological response of horses experimentally infected with EEE virus.

Materials and methods. The EEE virus used was the V-52 strain, isolated from a naturally infected horse in Maryland in 1956. The strain had undergone 17 mouse brain passages prior to inoculation of the horses. The 4 young horses used for experimental work were obtained locally from non-enzootic areas. Three horses (#126, #127, #128) were inoculated intradermally and one (#115) was inoculated subcutaneously with a virus suspension containing approximately 10^6 mouse

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TABLE I. Serological Response of Horses Experimentally Infected with EEE Virus.

Time post-inoc.	Horse 115 HI titer	Horse 115 Neutralization index (log_{10})	Horse 126 HI titer	Horse 126 Neutralization index (log_{10})	Horse 127 HI titer	Horse 127 Neutralization index (log_{10})	Horse 128 HI titer	Horse 128 Neutralization index (log_{10})
0	N*	<1	N	<1	N	<1	N	<1
5 days	40†	"	10	1.2	20	"	20	"
7			20	1.4	80	"	40	"
10	1280	1.4						
14	"	2.4	320	1.9	320	2.1	320	1.7
4 wk	320	3.2	40	2.8	80	2.4	80	2.2
2 mo	160	3.5	"	2.6	"	"	"	2.4
4	"	3.2	"	2.9	40	2.7	40	2.3
6	"	3.6	"	3.1	"	3.4		
9	"	3.3	20	2.7	"	3.5		
12	40	3.6			160	3.2		

* No inhibition at 1:10 dilution of serum.
† Reciprocal of highest serum dilution causing complete inhibition.

LD_{50}'s. Serum was collected at various intervals listed in Table I. Neutralization tests were performed by combining equal amounts of undiluted serum and serial 10-fold dilutions of virus. Following incubation of serum-virus mixtures for 2 hours in 37°C water bath, 3-week old Swiss mice were inoculated intracerebrally. The inoculum was 0.03 ml and 5 mice used for each serum-virus mixture. Mice were observed for 10 days, and 50% mortality endpoints were calculated by the method of Reed and Muench(8). Appropriate controls were included and results reported as the log_{10} of neutralization index. Hemagglutinin was prepared according to the method described by Chanock and Sabin for WEE(3) with following modifications. The mouse brain suspension was made in pH 9.0 borate† and was given a single centrifugation at 4000 rpm for 30 min at 4°C. The hemagglutination (HA) titration and HI tests were conducted according to method described by Clarke and Casals(5). Day-old chick erythrocytes were suspended in a diluent‡ which when combined with equal amounts of pH 9.0 borate yielded a final pH of 6.3. Sera were extracted twice with acetone and the dried precipitate resuspended in pH 9.0 borate to yield an initial 1:10 dilution. Further dilutions were carried out in 2-fold steps to a dilution of 1:5120. Results are reported as the highest dilution of serum giving complete inhibition of hemagglutination.

Results. Results of serological tests on 4 experimentally infected horses are shown in Table I. None of these horses had significant EEE neutralizing or HI antibody prior to their inoculation, and clinical disease did not develop in any exposed animals. Evidence of infection was measured by appearance of HI antibody which became detectable the fifth day after inoculation in all 4 horses, whereas neutralization tests on these same sera failed to yield any significant antibody levels. It appears from Table I that significant levels of neutralizing antibody, *i.e.,* neutralization index of ≥ 50, were attained between 7th and 14th days. The HI titers of the 7-day sera available from 3 horses showed a 2-fold or greater increase over the 5-day sera, and by the 14th day had reached their maximum values. The HI titers began to decline after 14th day but were still detectable one year after inoculation, though at a low titer. The level of neutralizing antibody, on the other hand, reached its maximum level several months post-inoculation, the time varying with each horse. In contrast to the HI titers, high levels of neutralizing antibody persisted for at least one year.

Results of HI tests performed on sera from survey horses and ponies having significant neutralizing antibody levels are shown in Table II.

† *Boric Acid Stock Solution*: 0.2M H_3BO_3; 0.2M KCl in 0.12M NaCl. *pH 9.0 Borate*: Stock solution: 100 ml; 0.2N NaOH: 42.6 ml; qs to 400 ml with 0.12M NaCl.

‡ To prepare 1 liter of diluent, combine 100 ml of 1.5M NaCl with 70 ml of 0.05M Na_2HPO_4, and 151.5 ml of 1.0M NaH_2PO_4, and qs to volume with demineralized water.

TABLE II. HI Test Results on Equine Sera Containing Significant EEE Neutralizing Antibody Obtained 8 or More Months Following an Epizootic.

Neutralization index (log_{10})	Serum HI titer with varying units of antigen 2	4	8
2.6	N*	N	N
3	320†	80	40
2	N	N	N
2.1	N	N	N
2	10	N	N
3	N	N	N
2.5	N	N	N
2.3	320	160	80
2.1	80	40	10
2.5	"	"	20
2.7	160	80	40
2.9	"	40	N
2.2	40	N	N
2.8	"		

* Negative at 1:10 serum dilution.

† Reciprocal of highest serum dilution causing complete inhibition.

Note: These sera were collected in areas of Maryland and Virginia where EEE was known to have occurred the previous year. Fifty-nine of the 73 sera tested had no measurable HI or neutralizing antibodies.

Discussion. Early detection of HI antibodies reported here is in agreement with findings of other investigators studying other arthropod-borne encephalitides(3,9,10). The HI test, therefore, is particularly well-suited for serodiagnosis of EEE since high mortality in this disease usually precludes the opportunity to obtain second serums with which an increase in antibody titer can be demonstrated. Unpublished results of more extensive use of the HI test in a recent EEE epizootic in the Middle Atlantic states further supports the latter observation. The problem of cross-reactions of EEE with other members of Group A Arbor viruses could conceivably be a problem in areas where other members of the group are prevalent. This would not be a serious problem in the U.S.A. where EEE and WEE are the only Group A members known to occur at present. Further, these 2 agents do not crossreact to a high degree(12, 13).

TABLE III. Results of Laboratory Tests Used to Diagnose EEE in 3 Horses.

Serology*		Examination of brain	
Neutralization index (log_{10})	HI titer	Virus isolation	Histopathology
1.8	1:640	Yes	Pos.†
1.3	"	No	"
1	1:2560	Yes	"

* Serum specimen obtained during acute stage of disease within 24 hr of death.

† Lesions characteristic of viral encephalitis.

The fact that none of the experimental horses developed clinical EEE may be attributed to several factors. Results of epidemiological surveys indicate that many horses undergo subclinical infections with EEE virus. Furthermore, other investigators(11) found that only 5 of 13 horses inoculated with EEE virus succumbed to clinical infection. The possibility also exists that the pathogenicity of this EEE virus strain for the horse was reduced by the number of mouse brain passages.

Summary. 1. Four horses inoculated with EEE virus remained asymptomatic following injection but did develop measurable HI and neutralizing antibodies as a result of infection. HI antibodies were detectable earlier than neutralizing antibodies but the levels tended to drop more rapidly. 2. Of 14 horses and ponies having significant levels of neutralizing antibody, 5 to 9 had measurable HI antibody depending on number of units of antigen employed in the test. 3. Sera from 3 clinical cases of EEE were positive on the HI test while only one of these animals had a significant neutralizing antibody level.

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