Differences in Infectivity of Neurotropic Viruses Incubated in Distilled Water, Isotonic NaCl and Hypertonic NaCl.* (27109)

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The Mengo strain of encephalomyocarditis virus (EMC) lost infectivity, as judged by lethality for mice, when diluted in isotonic NaCl and incubated at 37°C, but was stable in distilled water (DW) or hypertonic NaCl (1). Several other animal viruses were studied and differences observed which may be of value in preliminary classification and identification.

Materials and methods. Virus stocks were 20% (2×10^{-1}) homogenates of infected mouse brain in 0.9% NaCl containing 5% rabbit serum, penicillin 100 units/ml and streptomycin 0.2 mg/ml. After centrifugation at 5000 g for one hour the supernate was frozen in 1 ml aliquots and stored at −70°C.

The preparation of diluents and virus dilutions has been described(1). Aliquots from an ampule of virus were serially diluted in 1 M (molar) NaCl, 0.15 M NaCl or DW, and each dilution was tested for infectivity before and after incubation at 37°C for one hour by intracerebral (i.c.) injection into groups of 5 adult Swiss-Webster mice. Adult mice tolerate DW or 1 M NaCl i.c. with little mortality or apparent morbidity; however more concentrated NaCl i.c. may cause appreciable mortality. LD_{50} calculations were based on deaths from the 2nd through the 14th day after inoculation(2).

Results. When the MM and Mengo strains of EMC were serially diluted in DW, 0.15 M NaCl and 1 M NaCl and immediately inoculated i.c. in mice, the level of virus infectivity, as judged by the LD_{50}, was approximately the same in all 3 diluents (Table I) and was similar to the LD_{50} in 0.9% NaCl containing 0.75% bovine plasma albumen or 5% rabbit serum. This indicates that, initially at least, there is no inhibitory or enhancing effect of these diluents on virus infectivity. After incubation for one hour virus infectivity was preserved at very nearly its preincubation level in DW or 1 M NaCl, but when incubated in 0.15 M NaCl there was an appreciable loss of infectivity (Table I). Although some infective virus persisted at 10^{-3} dilutions after incubation in isotonic NaCl, the amount present (as determined by further serial dilutions in 0.5% bovine serum albumen in DW) was diminished by more than 2 logs as compared with preincubation titrations. There was no such decrease in the titer of virus incubated at 10^{-3} in DW or 1 M NaCl. Mouse brain preparations of Mengo incubated at 10^{-1} or 10^{-2} in isotonic NaCl did not show such a decrease in infectivity, possibly due to the protective effect of tissue extracts on such inactivation(3). Mengo virus grown in HEp #2 tissue culture or partially purified from mouse brain preparations by ultracentrifugation and elution from calcium phosphate columns(4) was completely inactivated in isotonic NaCl at 10^{-3} and even 10^{-2} dilutions.

Both the GDVII and FA strains of mouse encephalomyelitis had similar titers before incubation when diluted in DW, isotonic NaCl or 1 M NaCl (Table I). After incubation at 37°C for one hour infectivity was maintained in all 3 diluents. In contrast to EMC there was little loss of infectivity after incubation in isotonic NaCl.

When arbor viruses were titrated before incubation similar LD_{50} levels were obtained in all 3 diluents (Table I). When serial dilutions were incubated at 37°C there was loss of infectivity in all 3 diluents, although more marked in DW and 0.15 M NaCl than in hypertonic NaCl. Semliki Forest Virus, which is highly pathogenic for mice by systemic inoculation, showed the same pattern of greater loss of infectivity in DW and isotonic NaCl than in hypertonic NaCl when assayed by intraperitoneal injection.

Discussion. The difference in stability of EMC and mouse encephalomyelitis in isotonic NaCl may be a convenient non-serologi-

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TABLE I. Serial Dilutions of Several Neurotropic Viruses in 1M NaCl, 0.15M NaCl and Distilled Water Assayed in Mice before and after Incubation at 37°C for 1 Hour. Figures refer to $-\log_{10}$ i.c. MLD_{50}.

	Diluent					
	1M NaCl		0.15M NaCl		DW	
	Before	After	Before	After	Before	After
EMC						
Mengo	8.2	7.6	7.6	3.5	8.6	8.6
MM	7.6	7.5	6.8	3.5	7.7	7.5
Mouse encephalomyelitis						
GDVII	6.8	6.5	7.4	7.2	7.4	7.4
FA	7.5	7.6	7.5	7.2	7.8	7.8
Arbor viruses						
Semliki Forest	6.6	5.5	6.5	3.5	6.8	3.5
Western equine encephalitis	8.0	5.4	7.6	3.8	7.5	3.5
Ilheus	6.4	4.8	6.2	3.5	7.2	3.8
West Nile	7.6	4.4	7.5	3.6	7.8	3.5

cal method of differentiating the two groups, while arbor viruses are characterized by a loss of infectivity in distilled water, distinguishing them from both EMC and mouse encephalomyelitis.

The instability of other strains of EMC at high dilution in isotonic NaCl(5,3) and stability in DW(3) is consistent with the present study. Of interest is the report that rabies virus at high dilution is inactivated more rapidly in physiologic saline than in DW(6). The presence of serum or tissue extracts prevents such deterioration, as with EMC(3).

The persistence of high titers of poliovirus at 37°C in complex isotonic chloride salts in absence of protein(7) suggests that poliovirus resembles mouse encephalomyelitis rather than EMC in this regard. At least 2 strains of tissue culture adapted type 2 poliovirus hominis maintained infectivity at 37° at high dilution in 1 M NaCl and DW as well as isotonic NaCl (Speir and Messore unpublished). Both EMC and poliovirus are relatively stable in hypertonic salt at higher temperatures(8).

Inactivation of yellow fever, a group A arbor virus, at high dilution in isotonic NaCl has been known for many years(9), and arbor viruses are reported as generally unstable in aqueous solution at room temperature(10). They are also inactivated by bile salts(11) and in some cases by proteolytic enzymes (12). The inhibitory effect of high concentrations of NaCl on the inactivation of rabbit pox by enzymes and surface active agents has been demonstrated by Joklik *et al.*(13) and such a phenomenon may in part underlie the pattern of inactivation of arbor viruses presented here.

Summary. Three groups of viruses were found to differ in their stability, as judged by infectivity for mice, when diluted in DW, isotonic and hypertonic saline and held at 37°C for 1 hour. Mouse encephalomyelitis (GDVII,FA) was stable in all 3 diluents. EMC virus (Mengo, MM) lost infectivity in isotonic NaCl only. Arbor viruses lost infectivity in all 3 diluents.

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Action of Adenine and Related Compounds on Replication of Encephalomyocarditis Virus in Tissue Culture.* (27110)

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The study by McFall and Magasanik(1) on control mechanisms of purine biosynthesis in L cells and Ehrlich ascites cells encouraged us to examine the effect on virus growth of purines that alter metabolic pathways in host cells.

Because of extensive published data on the action of purine analogues on virus growth, it was of interest to find that adenine itself could suppress the growth of encephalomyocarditis virus (EMC) in mouse ascites cells. This positive finding was in striking contrast to essentially negative results in some other systems and with other purine nucleic acid precursors.

Materials and methods. Primary tissue cultures consisting of suspensions of 7-day Krebs 2 (K2) and Ehrlich ascites tumor cells (EAC) propagated by IP inoculation in mice were used with a final cell concentration of 1.3-1.5 $\times$ 10^6/ml and maintained up to 48 hours in roller tubes in a simple medium of Hanks' BSS plus 0.1% glutamine 0.1% bovine albumen, 0.1% lactate, 0.05% Na_2HPO_4, 0.02% glucose, 0.05% $NaHCO_3$, and antibiotics. For K_2 media, glucose and $NaHCO_3$ were omitted. Roller tubes in groups of 3 or 4, each containing 0.1 ml 10% cell suspension, 2 ml appropriate medium, 0.1 ml virus dilution or control inoculum, were set up and incubated at 35°C in a roller drum at ½ rpm. At appropriate time intervals, pH and eosin counts(2) were recorded whenever needed and the contents of tubes in each group pooled for titration.

The L cell clone 929 was used as monolayers in both bottles and stationary tubes. Waymouth's medium(3) was used for stock cultures, but bactopeptone was omitted during experiments because of its high purine content.

The L-adapted EMC virus, obtained from Dr. Habel (with a titer of 8.5-9.0 log $TCID_{50}$/ml) was used. In addition, 4 to 6 passages were done in K_2, EAC, and mouse brain.

Virus quantitation was done by a) LD_{50} by IC inoculation of mice and b) $TCID_{50}$ in L cells as determined by cytopathic effect and presence of hemagglutinins. Titrations were usually in duplicate and all readings were done by coding to eliminate any bias by the observer. In the earlier experiments, cells and fluids were combined, but in later experiments they were separated. Cells were washed with BSS, frozen and thawed three times with BSS to give a 10% cell extract considered as 10^{-1} dilution.

Results. The toxicity of the compounds under study was determined. For the adenine experiments the eosin counts(2), by 24 hours 5-20% stained and at 48 hours 15-30% stained, were not significantly different in non-infected cells except at the toxic level of 1000 μg/ml. The same result was obtained with the infected groups, but cells were killed by large inocula of virus. Sometimes, in the presence of virus or under adverse conditions such as high pH, adenine seemed to have a protective effect on the cells. From this pre-

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