Effect of Several Inorganic Salts on Infectivity of Mengo Virus.* (26352)

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There have been few systematic investigations of the effect of salts on inactivation of small animal viruses(1). Columbia SK, a strain of encephalomyocarditis (EMC) virus, was noted to be unstable when diluted in physiological saline(2). EMC virus was also inactivated in dilute chloride salts but was relatively stable in distilled water(3). Ribonucleic acid derived from Mengo infected tissues was biologically and physically more stable in hypertonic sodium chloride than in isotonic sodium chloride(4). This paper presents a study of inactivation of the Mengo strain of EMC virus in sodium chloride and certain other salts.

Materials and methods. Chemicals were all analytical reagent grade. Solutions were made with distilled water passed through a mixed bed ion exchange resin. Concentrations are expressed as molar (M) or millimolar (mM).

Mengo strain of EMC virus(5) was obtained through the courtesy of Dr. A. E. Moore. A single pool of 43rd mouse passage was prepared as a 20% W/V mouse brain homogenate in distilled water containing a final concentration of penicillin 80 units/ml and streptomycin 0.16 mg/ml. After centrifugation at 5000 g for 1 hour, the supernate was divided into 1 ml aliquots, sealed in glass ampules, frozen and stored at −70°C.

Serial 10-fold dilutions of virus were made using chilled diluents and glassware. Each dilution tube was subdivided into 2 or more 0.9 ml aliquots, thus making 2 or more replicate sets of each dilution series. One such set was immediately tested for infectivity in mice. The others were incubated in a water bath, without shaking, for times and temperatures indicated in the respective experiments, then tested for infectivity.

Titrations in 16-18 g Swiss-Webster mice were by intraperitoneal inoculation, 0.05 ml inoculum per mouse, 5 mice per dilution, using 5 successive dilutions chosen to include the LD_{50} as judged by previous experiments. Deaths occurred as early as day 2 and seldom later than day 5. LD_{50} dilutions were calculated from deaths through the 7th day(6).

Results. Replicate dilutions of Mengo-infected mouse brain in 150 mM sodium chloride were tested for infectivity before and after varying periods of incubation at 37°C. There was a progressive loss of infectivity during the first hour, with a consistent inactivation of approximately 10,000 LD_{50} units per unit volume by the end of 2 hours (Table I). The persistence of infective virus at 10^{-3} dilutions even after 2 hours of incubation probably represents the protective effect of 0.1% mouse brain extracts(3).

Parallel titrations incubated at different temperatures indicated that loss of infectivity varied directly with temperature of incubation (Table II). Relatively little inactivation occurred at 0°C in contrast to inactivation at 37°C. This result is in agreement with the study of Boesche and Drees(3) on EMC virus although a different method was used, and confirms the original observation of Jungeblut and Sanders(2).

Replicate dilutions of Mengo virus in distilled water and in various concentrations of

TABLE I. Results of Incubating Replicate Serial Dilutions of Mengo for Various Times in 150 mM NaCl at 37°C, and Injecting Intraper. into Mice. Fractional figures represent No. of mice dead/No. of mice inj.

Virus dilution	Time of incubation 0′	15′	30′	60′	90′	120′
10^{-2}			5/5	5/5	5/5	5/5
10^{-3}		5/5	5/5	4/5	5/5	4/5
10^{-4}		5/5	5/5	1/5	1/5	0/5
10^{-5}	5/5	3/5	1/5	0/5	0/5	0/5
10^{-6}	5/5	0/5	0/5	0/5	0/5	0/5
10^{-7}	4/5	0/5				
10^{-8}	1/5					
10^{-9}	0/5					
LD_{50}	$10^{-7.5}$	$10^{-5.2}$	$10^{-4.6}$	$10^{-3.5}$	$10^{3.6}$	$10^{-3.4}$

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TABLE II. Effect of Temperature on Infectivity of Mengo Incubated in 150 mM NaCl for 2 Hours.

Temp., °C	Virus LD_{50} $-\log_{10}$ 0′	120′
37	7.5	3.4
32	7.8	4.5
27	7.7	5.3
22	7.6	5.6
0	7.8	6.6

sodium chloride were tested for infectivity before and after incubation for 2 hours at 37°C. The virus was stable in markedly hypertonic sodium chloride and distilled water. Maximal inactivation occurred between 15 mM and 150 mM (Table III).

There was little or no inactivation of Mengo virus diluted and incubated in several other sodium salts (Table IV). The presence of Na+ in concentrations of 150 meq/l is not, in itself, a sufficient condition for inactivation. Monobasic sodium phosphate (pH 4.3) and dibasic sodium phosphate (pH 9.0) represent the minimum and maximum pH of diluents used in these studies suggesting that inactivation observed in the present studies is not primarily an effect of pH.

When replicate dilutions of Mengo virus in four chloride salts were tested for infectivity before and after incubation at 37°C for 2 hours, there was marked inactivation in both monovalent and divalent salts (Table V). Attempts to modify the degree of inactivation by varying the ratio of monovalent to divalent cation(7,8) have so far been unsuccessful.

TABLE III. Effect of Different Concentrations of NaCl on Infectivity of Mengo Incubated at 37°C for 2 Hours.

Diluent		Virus LD_{50} $-\log_{10}$ 0′	120′
DW		8.3	7.5
NaCl	.15 mM	7.7	7.5
"	1.5	8.7	≥8.2
"	15	7.6	3.8
"	50	7.8	3.4
"	75	7.6	3.5
"	100	7.8	2.8
"	150	7.6	3.6
"	300	7.6	5.4
"	500	7.4	≥7.0
"	1000	7.6	7.3
"	1500	8.0	7.2

Inactivation in sodium halide salts occurred most extensively in chloride and bromide, somewhat less in iodide, and very little in fluoride, the pseudo halogen sodium thiocyanate, or distilled water (Table VI). At-

TABLE IV. Effect of Sodium Salts on Infectivity of Mengo Incubated at 37°C for 2 Hours.

Diluent		Virus LD_{50} $-\log_{10}$ 0′	120′
NaCl	150 mM	7.7	3.5
$NaClO_4$	"	8.4	7.5
$NaNO_3$	"	8.7	7.8
NaH_2PO_4	"	7.5	6.5
Na_2HPO_4	"	7.8	7.1
Na_2SO_4	"	7.8	7.2
Na formate	"	8.2	6.8
Na acetate	"	7.8	6.5
DW		8.3	7.5

TABLE V. Effect of Chloride Salts on Infectivity of Mengo Incubated at 37°C for 2 Hours.

Diluent		Virus LD_{50} $-\log_{10}$ 0′	120′
NaCl	150 mM	7.5	3.4
KCl	150	8.0	3.5
$MgCl_2$	75	7.8	3.5
$CaCl_2$	75	7.1	3.5
DW		8.5	7.8

tempts to differentiate between bromide and chloride by varying time and temperature of incubation were unsuccessful within the sensitivity of this method. Iodide was consistently less inactivating than either chloride or bromide and, with the lack of inactivation in sodium fluoride, may represent a dependence of inactivation on anionic size or hydration.

Discussion. The use of purified virus for

TABLE VI. Effect of Sodium Halide Salts on Infectivity of Mengo Incubated at 37°C for 2 Hours.

Diluent		Virus LD_{50} $-\log_{10}$ 0′	120′
NaF	150 mM	8.3	7.4
NaCl	"	7.8	3.5
NaBr	"	7.6	3.6
NaI	"	7.7	4.5
NaSCN	"	7.0	6.5
DW		8.2	7.6

inactivation studies has obvious advantages. However, crude virus preparations are convenient for use in a preliminary survey such as this, the information gained may be usefully applied to further purification procedures, and some of the inherent disadvantages of crude virus preparation may be lessened by the choice of methodology.

Diluting a concentrated crude virus preparation to a convenient lesser concentration in a diluent of known composition(3,7) decreases the concentration of non-viral material—thus possibly decreasing the influence of such chemically ill-defined material on the type of inactivation under study(9). A further advantage of diluted virus preparations is the increased sensitivity in detecting absolute changes in infectivity by assaying an all or none response to further serial dilutions. If rate of inactivation is not directly dependent upon virus concentration, as might occur in certain types of enzymatically limited reactions, such problems of assay sensitivity may be significant. Incubating serial dilutions of virus made in sodium desoxycholate and then testing for infectivity is an even more sensitive method used by Theiler(10), upon which the method of the present study is based. It is usually quite apparent (Table I) that an LD_{50} lies between 2 of the dilutions tested. However, application of the classic method of Reed and Muench(7) to such data the calculation of what would be an LD_{50} after incubation under the specified conditions, is admittedly an approximation.

The intraperitoneal route for titrations in these studies was chosen because of the toxicity of certain of the diluents when administered to mice intracerebrally, or when used in tissue culture.

It is of interest that inactivation is maximal at approximately the chloride concentrations of the extracellular fluid. It is conceivable that inactivation by chloride at body temperature is a mechanism of host defense. Whether inactivation is a direct effect of chloride on the virus or is mediated through some tissue component is unknown. The stability of this virus in both distilled water and hypertonic salt may in part determine the circumstances under which it is transmitted and persists in nature.

Summary. Mengo infected mouse brain preparations were stable, as judged by mouse lethality, when diluted in 1 molar sodium chloride or distilled water and incubated at 37°C. Mengo virus was inactivated when incubated in 150 mM sodium chloride. Inactivation was directly related to time and temperature of incubation and was demonstrable at concentrations of sodium chloride between 15 and 300 millimolar. Inactivation appeared to be related to the presence of Cl–, or similar anions, rather than Na+.

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