JOURNAL OF BACTERIOLOGY, July, 1965
Vol. 90, No. 1

Printed in U.S A.

Protection of Measles Virus by Sulfate Ions Against Thermal Inactivation

FRED RAPP, JANET S. BUTEL, AND CRAIG WALLIS
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

Received for publication 1 March 1965

ABSTRACT

RAPP, FRED (Baylor University College of Medicine, Houston, Tex.), JANET S. BUTEL, AND CRAIG WALLIS. Protection of measles virus by sulfate ions against thermal inactivation. J. Bacteriol. 90:132–135. 1965.—The infectivity of measles virus in water is rapidly destroyed at temperatures of 37 C and above. More than 50% of the infectivity is lost after 1 hr at 25 C, and almost 90% loss of infectivity occurs within 24 hr at 4 C. Magnesium chloride enhances the inactivation of the virus at all temperatures tested. Addition of either magnesium or sodium sulfate protects the virus against thermal inactivation. The stabilizing effect is demonstrable at temperatures ranging from 4 to 56 C, but is especially pronounced through 45 C. Prolonged storage (up to 6 weeks) of the virulent virus at 4 C in 1 M magnesium sulfate permits retention of substantial infectivity, whereas storage at 4 C in either water or 1 M magnesium chloride results in a loss of infectivity approximating 99% after 2 weeks. Magnesium chloride also enhances inactivation of the attenuated vaccine strain of measles virus. The attenuated virus, however, is strongly protected by magnesium sulfate against thermal inactivation, and retention of infectivity for long periods of time at 4 C seems feasible when the virus is kept in 1 M magnesium sulfate.

The development of a rapid and precise plaque assay for measles virus (Rapp, 1964) afforded the opportunity to study the stability of the virus under various conditions. The known ability of 1 M $MgCl_2$ to stabilize enteroviruses and to labilize myxoviruses (Wallis and Melnick, 1961*a*, *b*, 1962; Wallis, Yang, and Melnick, 1962) suggested that the effects of this compound on the infectivity of measles virus should be investigated. During the course of these studies, it became apparent that $MgCl_2$ also enhances the inactivation of measles virus, but that addition of 1 M $MgSO_4$ protected the infectivity against thermal inactivation. This report is a detailed account of the effect of temperature on the infectivity of virulent and attenuated strains of measles virus maintained in the absence or presence of $MgCl_2$, $MgSO_4$, or other salts.

MATERIALS AND METHODS

Measles virus. The Edmonston virulent strain of virus used throughout these experiments was the same as that used in a previous study (Rapp, 1964). Stocks of virus were prepared in the stable African green monkey kidney cell line, BSC-1 (Hopps et al., 1963). All stocks used titered greater than 10^5 plaque-forming units (PFU) per ml in both BSC-1 and in primary cells derived from African green monkey kidneys. The attenuated vaccine virus has also been described (Rapp, 1964); it had undergone five passages in BSC-1 cells prior to the experiments reported here.

Tissue cultures and virus assays. The BSC-1 cells were grown in Eagle's basal medium supplemented with 10% calf serum. Plaque assays were carried out in 60-mm petri dishes under an agar overlay as previously described (Rapp, 1964). The overlay consisted of 1% agar in Eagle's basal medium and 10% calf serum. Cultures were incubated at 37 C and 5% CO_2. Plaques were routinely counted after 4 days of incubation of the cultures.

Inactivation and stability studies. The virus was diluted 2- to 10-fold in distilled water or the salts indicated; the salts had been prepared so that the concentrations given are final. Samples were equilibrated at *p*H 6.9 to 7.1 with HCl or NaOH; representative samples in small volumes (usually 1 ml) were placed at the temperatures indicated. The virus was carefully delivered to the bottom of tubes (13 × 100 mm), and the tops of the tubes were heated to destroy any possible residual virus. The tubes were rubber-stoppered and plunged into a water bath at the proper temperature to a depth sufficient to cover the tube to within 0.25 inch of the stopper. Samples were withdrawn at the time indicated and were immediately diluted at least 10-fold in cold tris(hydroxymethyl)aminomethane buffer (*p*H 7.2) and titered in BSC-1 cells. Virus harvested for one period of time only was chilled by immersion in an ice-water bath after the desired time at the indicated temperature.

RESULTS

Stability of measles virus in water. Approximately 90% of the infectivity of the virulent strain of measles virus was destroyed in 10 min at 45 or 56 C (Fig. 1). Virus could not be detected after 18 min at 56 C, but a very small fraction of virus continued to persist at 45 C through 2 hr. Inactivation proceeded more slowly at 37, 25, and 4 C (Fig. 1). Nevertheless, 90% of the virus was inactivated in less than 2 hr at 37 C (Fig. 1). Similar results were obtained when progeny of virus that had been plaque-purified three times was compared with the parent strain at 37 C.

Effect of various salts on stability of measles virus. The effect of 1 or 2 M concentrations of various salts on the stability of virulent measles virus was tested at 50 C (Table 1). A loss of more than 3 logs of infectivity was observed when virus was suspended in water, $MgCl_2$, $CaCl_2$, or Na_2HPO_4. Both 1 M $MgSO_4$ and 1 M Na_2SO_4 protected the virus against thermal inactivation. Addition of NaCl, KCl, and K_2SO_4 protected the virus to some extent, but, nevertheless, 90 to 99% of the virus infectivity was lost after 15 min at 50 C (Table 1).

The stability of the virulent strain of measles virus in various concentrations of $MgSO_4$ and

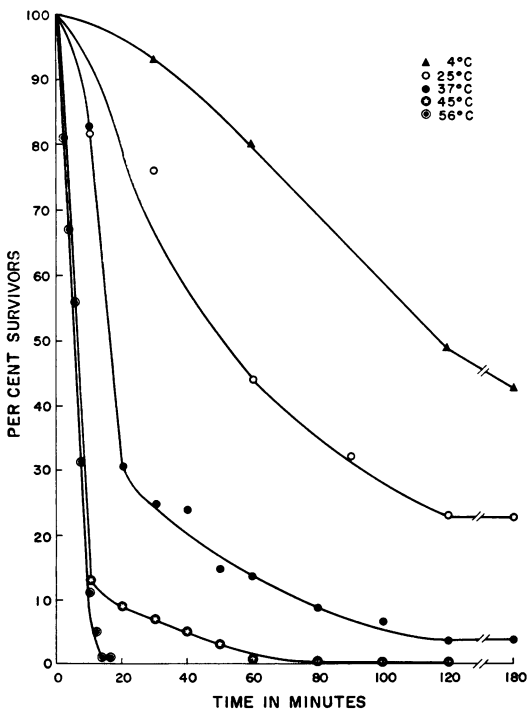

FIG. 1. *Inactivation of virulent strain of measles virus in water at various temperatures.*

TABLE 1. *Effects of different salts on stability of measles virus* at 50 C*

Diluent	pH	Log_{10} titer (PFU/ml)	
		Unheated control	After heating for 15 min at 50 C
$MgSO_4$, 1 M	6.9	5.7	5.5
$MgCl_2$, 1 M	6.9	5.7	<2.0
$CaCl_2$, 1 M	6.9	5.6	<2.0
Na_2SO_4, 1 M	7.0	5.6	5.2
NaCl, 2 M	7.2	5.5	3.5
KCl, 2 M	7.0	5.4	4.4
K_2SO_4, 1 M	7.0	5.6	4.5
Na_2HPO_4, 1 M	6.9	5.4	<2.0
Water	7.2	5.5	<2.0

* Virulent strain.

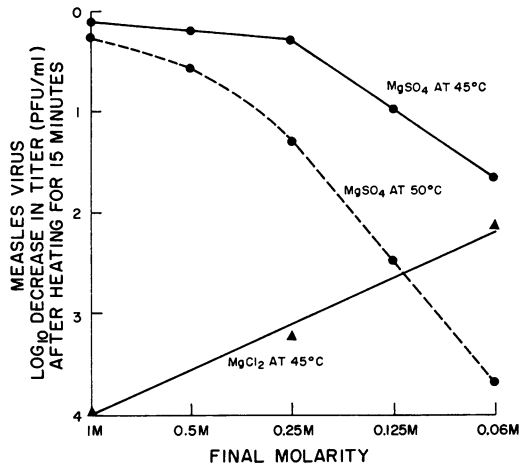

FIG. 2. *Effect of concentration of $MgSO_4$ and $MgCl_2$ on the stability of virulent strain of measles virus at 45 and 50 C.*

$MgCl_2$ was tested at 45 and 50 C (Fig. 2). As the molarity of $MgSO_4$ decreased below 0.25, a sharp drop in infectivity at 45 C occurred. Higher concentrations of $MgSO_4$ protected almost completely. Conversely, a direct relationship was observed at 45 C between the concentration of $MgCl_2$ and the inactivation of measles virus. As the molarity decreased, enhancement of inactivation also decreased (Fig. 2).

The sparing effect of $MgSO_4$ and enhanced inactivation by $MgCl_2$ were also observed at other temperatures (Fig. 3). Almost no loss of infectivity occurred in $MgSO_4$ at 45 C for 30 min; at higher temperatures, progressively greater inactivation occurred. However, in either water or $MgCl_2$, inactivation of the virus proceeded rapidly at 45 C and significant inactivation occurred at 37 C (Fig. 3).

A thermal inactivation time curve carried out on the virulent strain of virus in water, 1 M $MgSO_4$, and 1 M $MgCl_2$ is summarized in Fig. 4. At both temperatures 45 and 50 C, 1 M $MgSO_4$ greatly reduced inactivation. Though no detectable infectivity was retained after 15 min at 50 C in water or $MgCl_2$, only 0.4 $\log_{10}$ decrease in titer occurred in $MgSO_4$. After 60 min at 50 C in $MgSO_4$, only 1 $\log_{10}$ had been lost. Comparable results were obtained at 45 C (Fig. 4).

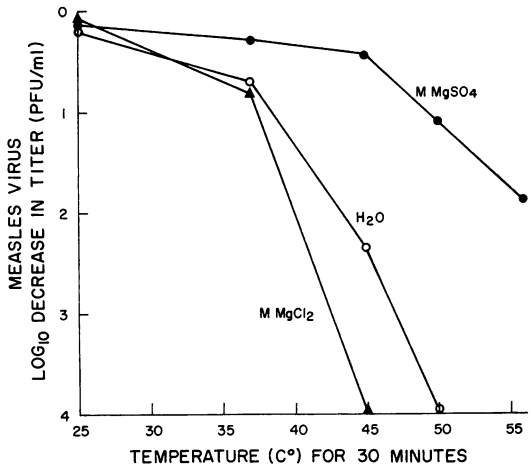

FIG. 3. *Effect of $MgSO_4$ and $MgCl_2$ on the stability of virulent strain of measles virus at various temperatures.*

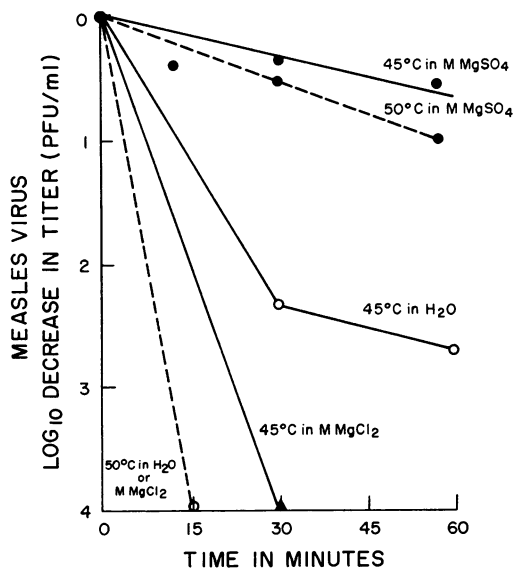

FIG. 4. *Rate of inactivation of virulent strain of measles virus at 45 and 50 C in water, $MgCl_2$, or $MgSO_4$.*

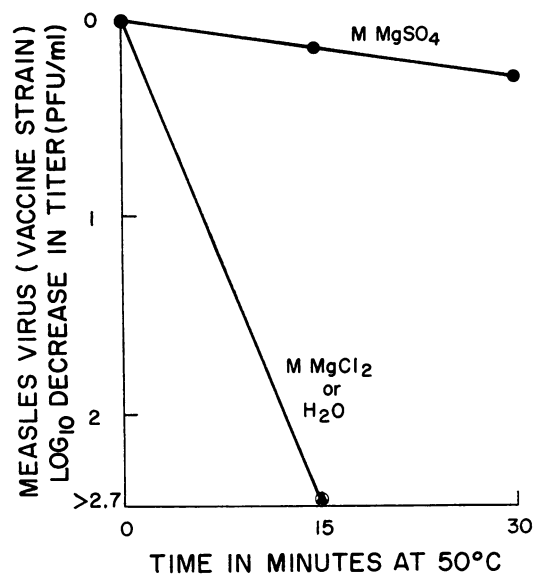

FIG. 5. *Rate of inactivation of attenuated strain of measles virus at 50 C in water, $MgCl_2$, or $MgSO_4$.*

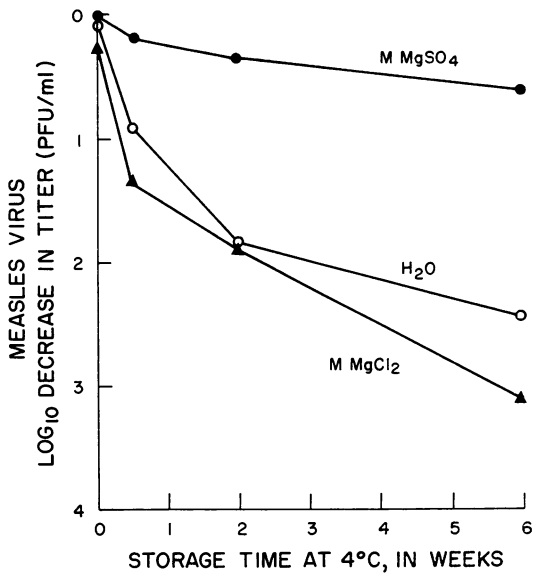

FIG. 6. *Stability of measles virus after prolonged periods at 4 C in water, $MgCl_2$, or $MgSO_4$.*

Similar results were obtained when the attenuated strain of measles virus was tested at 37, 45, and 50 C (Fig. 5) in water or in 1 M $MgSO_4$ or $MgCl_2$. In each instance, the $MgSO_4$ protected the virus against inactivation, whereas $MgCl_2$ enhanced inactivation of virus infectivity.

The addition of 1 M $MgSO_4$ also protected virus infectivity for prolonged periods at 4 C (Fig. 6).

Greater than 99 and 99.9% of the infectivity were lost in water and $MgCl_2$, respectively, after 6 weeks of storage at 4 C, but only 75% of the infectivity of the virulent virus had been lost in $MgSO_4$ after a comparable period under similar conditions. Similar results have been obtained with the attenuated strain of virus.

Freezing and subsequent thawing of the virus in either water or 1 M $MgSO_4$ does not affect infectivity; the same treatment in 1 M $MgCl_2$ destroys 99.9% of the infectivity of both virulent and attenuated strains of measles virus.

DISCUSSION

The observation that $MgSO_4$ protects measles virus against thermal inactivation and that $MgCl_2$ enhances inactivation is surprising, since previous studies with enteroviruses suggested that the Mg ions were responsible for protection of these viruses. The reasons for the differing activity of these salts with different viruses are not known at present, but the effect of $MgSO_4$ on other viruses is now under investigation; preliminary results suggest that at least one myxovirus, influenza, and a number of other nonenteroviruses are stabilized by the addition of $MgSO_4$, but not $MgCl_2$ (Wallis, Melnick, and Rapp, Virology, *in press*). While this manuscript was in preparation, Nakamura and Ueno (1964) also reported that $MgSO_4$ and Na_2SO_4 partially stabilized the infectivity of Japanese B encephalitis virus but not the infectious ribonucleic acid extracted from that virus.

The thermal inactivation data obtained in this study with the virulent strain of measles virus are somewhat different from those previously reported (Black, 1959; Musser and Underwood, 1960). The virus in our test was more thermolabile, but this difference can be accounted for by the inclusion of 10% serum or rich nutrient fluids in the studies carried out earlier. Black (1959) also noted, however, that the inactivation of measles virus at 46 and 56 C was extremely rapid, and he reported results comparable to ours.

The suggestion that stabilization of poliovirus with $MgCl_2$ might lead to a more potent (antigenically) formalin-treated vaccine (Wallis and Melnick, 1961*a*) led Ozaki and Melnick (1963) to produce a $MgCl_2$-stabilized formalin-inactivated poliovirus vaccine of higher potency than the vaccines prepared by treatment with formalin alone. The incorporation of $MgSO_4$ to stabilize measles virus during the procedures carried out to prepare the inactivated measles vaccine may also enhance the antigenic potency of the killed measles vaccine.

ACKNOWLEDGMENTS

We are grateful for the technical assistance of Darlena Vanderslice and Fred Morales in carrying out the tests described.

This investigation was supported by Public Health Service grants AI 05398 and AI 05382 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

BLACK, F. L. 1959. Growth and stability of measles virus. Virology **7**:184–192.

HOPPS, H. E., B. C. BERNHEIM, A. NISALAK, J. H. TJIO, AND J. E. SMADEL. 1963. Biologic characteristics of a continuous kidney cell line derived from the African green monkey. J. Immunol. **91**:416–424.

MUSSER, S. J., AND G. E. UNDERWOOD. 1960. Studies on measles virus. II. Physical properties and inactivation studies of measles virus. J. Immunol. **85**:292–297.

NAKAMURA, M., AND Y. UENO. 1964. Infectious ribonucleic acid (RNA) of Japanese B encephalitis (JBE) virus: High yields of RNA and its stabilization. Proc. Soc. Exptl. Biol. Med. **117**:700–704.

OZAKI, Y., AND J. L. MELNICK. 1963. Reaction of poliovirus and formaldehyde in magnesium chloride solution to enhance potency of killed-virus vaccine. J. Immunol. **90**:429–437.

RAPP, F. 1964. Plaque differentiation and replication of virulent and attenuated strains of measles virus. J. Bacteriol. **88**:1448–1458.

WALLIS, C., AND J. L. MELNICK. 1961*a*. Stabilization of polioviruses by cations. Texas Rept. Biol. Med. **19**:683–700.

WALLIS, C., AND J. L. MELNICK. 1961*b*. Cationic inactivation of vacuolating virus (SV_{40}) in poliovirus suspensions. Texas Rept. Biol. Med. **19**:701–705.

WALLIS, C., AND J. L. MELNICK. 1962. Cationic stabilization—a new property of enteroviruses. Virology **16**:504–525.

WALLIS, C., C. YANG, AND J. L. MELNICK. 1962. Effect of cations on thermal inactivation of vaccinia, herpes simplex and adenoviruses. J. Immunol. **89**:41–46.