Effects of Cations and Organic Compounds on Inactivation of Poliovirus with Urea, Guanidine, and Heat (34316)

ROGER FUJIOKA, HILDA KURTZ, AND W. WILBUR ACKERMANN
Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan 48104

The capsid of poliovirion is comprised of capsomeres held together by noncovalent bonds (1, 2). Urea, heat and guanidine, agents which are capable of breaking noncovalent bonds, have been used to inactivate and degrade poliovirus (3, 4, 5). Treatment of the virus with heat (45–60°) or high concentrations of urea produces inactivation, antigenic alteration, and release of RNA from a largely intact capsid (4-7). Scharff *et al.* (5), however, reported that treatment of poliovirus with 6.5 *M* guanidine resulted in soluble polypeptide units which have their own distinct antigenicity and are believed to be the subunits of the capsomeres. Together these findings indicate that the effects of heat and urea on poliovirus are similar but differ from that of guanidine.

Besides inactivating poliovirus, guanidine inhibits poliovirus replication, probably by action upon the viral RNA replicase (8, 9). Choline or methionine, but not betaine, reverse guanidine inhibition of poliovirus replication (10,11). Further, Wallis and Melnick (12) showed that heat inactivation of the poliovirions was prevented by cations. The present study compares the effects of the above organic compounds with those of the cations in regard to their capacity to moderate the reactivity of the poliovirion toward heat, urea, and guanidine.

Methods and Materials. Mahoney strain type 1 poliovirus was grown in HeLa cells, pelleted by centrifugation and resuspended to one-tenth its original volume with phosphate-buffered saline (PBS), pH 7.0. The virus was then treated twice with equal volumes of Genetron and then pelleted from the aqueous phase. The pellet was resuspended in PBS, sonicated and banded in a CsCl gradient. The viral band was collected, diluted 10 times with PBS and dialyzed against PBS. This virus suspension was stored at 4° in soft glass test tubes and used in all tests.

Sources. The sources of the various reagents are as follows: Ultrapure guanidine hydrochloride, ultrapure urea (Mann Research Laboratories, New York, N.Y.); Baker analyzed $MgCl_2$, $MnCl_2$, $CaCl_2$ NaCl, KCl (J T. Baker Chem. Co., Phillipsburg, N.J.); betaine, choline chloride (Nutritional Biochemicals Corp., Cleveland, O.); methionine (General Biochemicals, Chagrin Falls, O.); Genetron, trifluorotrichloroethane (Allied Chemicals, New York, N.Y.).

Experimental Methods. One ml of the prescribed reagent solution was combined with 0.1 ml of the virus suspension in soft glass test tubes and incubated for the specified time and temperature. The samples were then removed from the water bath, immersed in ice water and assayed for infectivity on monolayers of HeLa cells in 2-oz prescription bottles. Infectivity titer is expressed as plaque-forming units per ml. The degree of inactivation is expressed as the log V/V_0, where V is the infectivity titer of the treated virus sample. The control titer V_0 represents an unincubated sample in the heat-inactivation studies and a sample incubated at 37° for 1 hr in water in the urea and guanidine inactivation studies. Virus inactivation in water after 1 hr at 37° was not appreciable.

Results. Heat inactivation. The degrees of inactivation of poliovirus observed after 15 min at 45° in water, cations, and organic compounds are shown in Fig. 1. In water

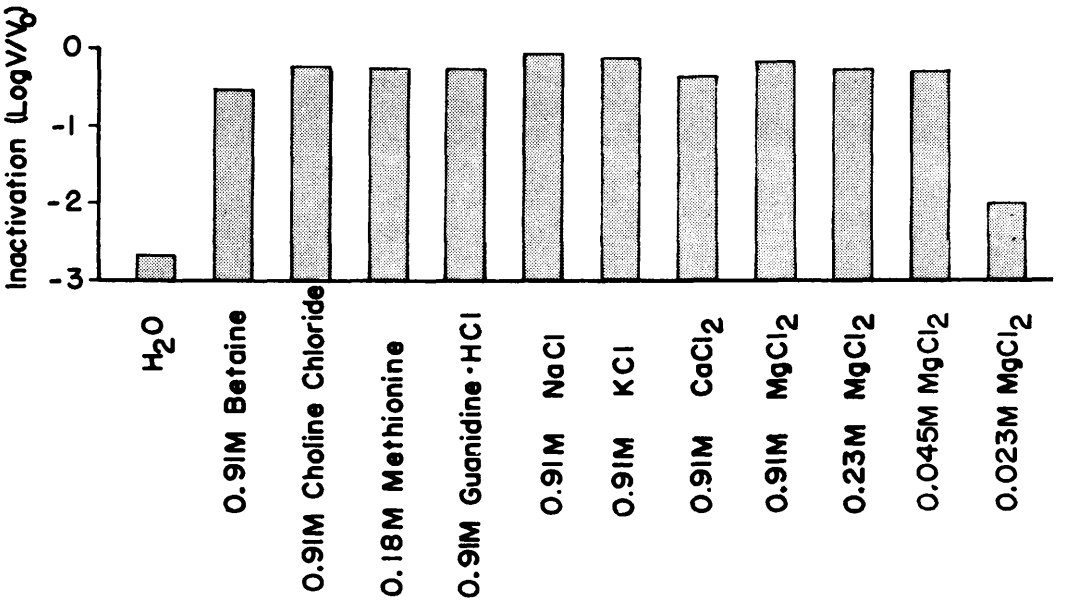

FIG. 1. Stabilization of poliovirus infectivity against heat (45°, 15 min) by cations and some organic compounds; inactivation expressed as log reduction in virus titer (PFU/ml).

alone, the virus titer was reduced by 99.9%. However, the presence of 0.91 *M* $MgCl_2$, $CaCl_2$, KCl, NaCl, betaine, choline chloride, guanidine–HCl, or 0.18 *M* methionine resulted in nearly complete protection. The minimum concentration of $MgCl_2$ needed for this protection was between 0.023 and 0.045 *M*. Preliminary results showed that purified virus was more stable in soft glass than in Pyrex or cellulose nitrate tubes, but always more sensitive than crude virus samples.

Urea inactivation. The degrees of inactivation of poliovirus observed after incubation for 1 hr at 37° in various concentrations of urea with and without 0.91 *M* $MgCl_2$ are shown in Fig. 2. The inactivation curve without Mg ions shows a shoulder at low concentrations of urea followed by an exponential decrease in infectivity as the urea concentrations are increased. $MgCl_2$ (0.91 *M*) completely stabilized poliovirus against inactivation by 2.7 *M* urea, the highest concentration included. In other experiments stabilization to 4.5 *M* urea was obtained. The effect of cations and organic compounds on poliovirus inactivation by 1.8 *M* urea observed after 1 hr at 37° is shown in Fig. 3. Aqueous 1.8 *M* urea reduced the virus titer 99.9%, however, the presence of 0.91 *M* $MgCl_2$, $MnCl_2$, KCl, NaCl, betaine, choline chloride, guanidine hydrochloride, or 0.18 *M* methionine resulted in nearly complete protection. The minimum concentration of $MgCl_2$ needed to stabilize poliovirus against 1.8 *M* urea inactivation is

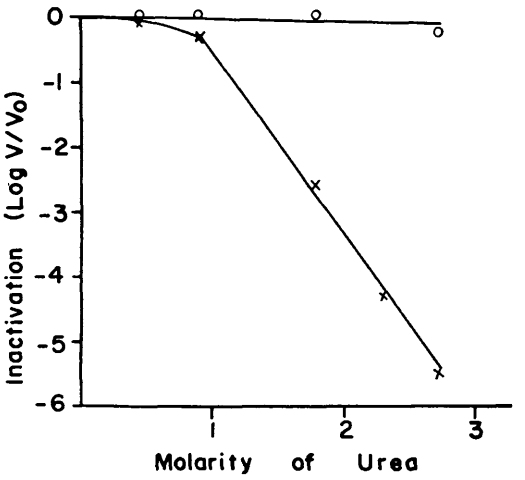

FIG. 2. Inactivation of poliovirus infectivity in various concentrations of urea in the absence (x) and presence (O) of 0.91 *M* $MgCl_2$ observed after 1 hr at 37°; inactivation expressed as log reduction in virus titer (PFU/ml).

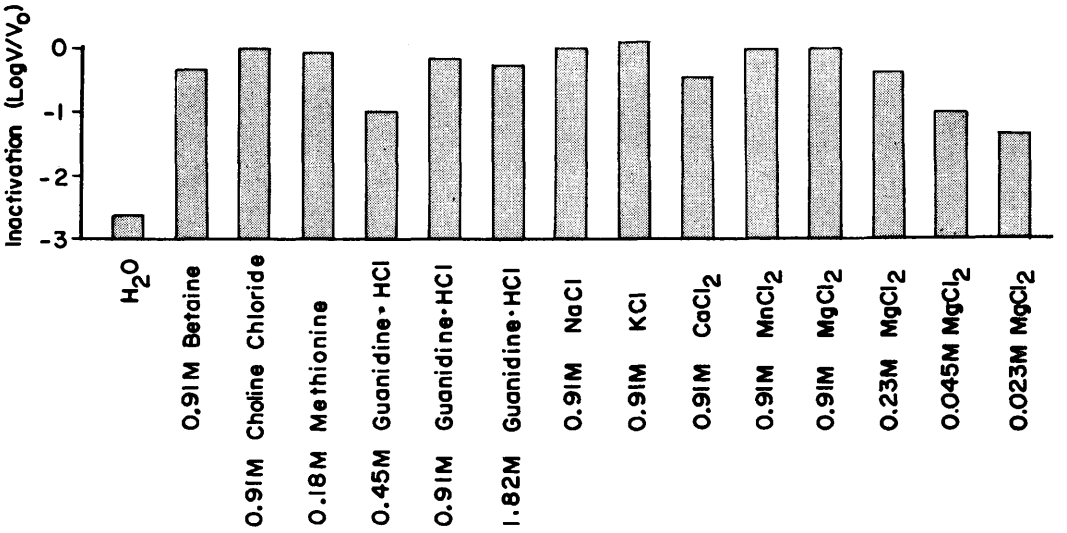

FIG. 3. Stabilization of poliovirus infectivity against 1.82 *M* urea observed after 1 hr at 37° by cations and some organic compounds; 1.82 *M* urea was incorporated into all the solutions listed; inactivation expressed as log reduction in virus titer (PFU/ml).

similar to that needed to stabilize poliovirus against heat inactivation. Thus, the effect of cations, organic compounds, and guanidine hydrochloride on both heat and urea inactivation appears to be identical, suggesting that heat and urea inactivates by affecting the same sites on the virion.

Guanidine inactivation. The degrees of inactivation of poliovirus observed after incubation for 1 hr at 37° in various concentrations of guanidine with and without 0.91 *M* $MgCl_2$ are shown in Fig. 4. The guanidine inactivation curve without Mg ions also has a multistage pattern with an initial shoulder. However, much higher concentrations of guanidine than urea are needed to inactivate poliovirus. $MgCl_2$ (0.91 *M*) greatly enhanced the inactivating effect of guanidine, in contrast to its effect on urea inactivation. However, the enhancing effect of $MgCl_2$ appears to be restricted to those concentrations of guanidine which are capable of inactivating poliovirus and to be negligible at low guanidine concentrations which do not inactivate the virus. Although lower concentrations of guanidine do not inactivate poliovirus, it can be seen from Figs. 1 and 3 that 0.91 *M* guanidine stabilized the virus to both heat and urea inactivation indicating that even lower concentrations of guanidine definitely affect the virion and that this effect is similar to that of cations.

The effects of cations and organic compounds on poliovirus inactivation by 5.5 *M* guanidine observed after 1 hr at 37° are shown in Fig. 5. In 5.5 *M* guanidine, over 90% of the virus was inactivated. However, the presence of 0.91 *M* of all the cations tested and also 1.8 *M* choline chloride and

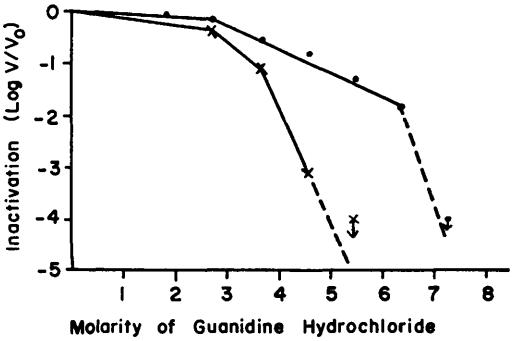

FIG. 4. Inactivation of poliovirus infectivity in various concentrations of guanidine hydrochloride in the absence (O) and presence (x) of 0.91 *M* $MgCl_2$ observed after 1 hr at 37°; symbols attached to descending arrows indicate that the final titer is something less than that level recorded; inactivation expressed as log reduction in virus titer (PFU/ml).

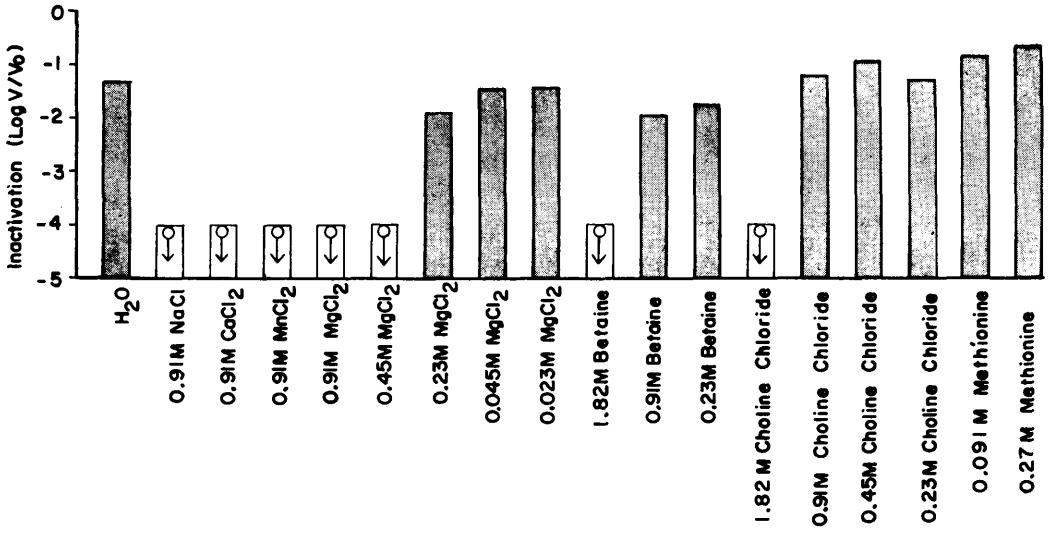

FIG. 5. Sensitization or stabilization of poliovirus infectivity by cations and some organic compounds to inactivation by 5.46 guanidine hydrochloride observed after 1 hr at 37°; 5.46 *M* guadine hydrochloride was incorporated into all the solutions listed; symbols attached to descending arrows indicates that the final titer is something less than that level recorded; inactivation expressed as log reduction in virus titer (PFU/ml).

betaine greatly enhanced the inactivation of 5.5 *M* guanidine. However, Fig. 5 also shows that certain low concentrations of choline and methionine, but not betaine, partially protected against 5.5 *M* guanidine inactivation.

Discussion. While the infectivity of poliovirion is retained in a wide range of concentrations of cations, methionine, choline, betaine, and even guanidine, the present data indicate that these produce profound alterations in the structure of the virion. Thus, at low concentrations of cations, the virion first becomes resistant to heat and urea. As the cation concentration is further increased, the stability of the virion to heat and urea is retained but the viron now becomes increasingly sensitive to guanidine inactivation. Similar effects were produced by the organic compounds tested. Since the same compounds stabilize poliovirion to both heat and urea but sensitize the virion to guanidine, it appears that urea and heat affect the same site on the virion which is different from those affected by guanidine. This conclusion is supported by earlier observations that an empty capsid results from heat or urea treatment of the poliovirion whereas a soluble product believed to be polypeptides resulted from guanidine treatment. Further, the concentration of guanidine required for inactivation of purified virus is in the range known to denature proteins while the concentration of urea required for inactivation was much lower than that usually needed for protein denaturation. These results suggest that guanidine inactivates the virion by denaturing the viral capsid protein whereas urea and heat inactivate by attacking a selective site on the poliovirion and support Cooper's theory that urea triggers a nucleic acid-liberating mechanism somewhere on the virus surface (4). It is concluded that the positive charges of cations as well as the selected organic compounds induce a series of conformational changes on the poliovirion which first stabilize the sites preferentially attacked by urea and heat and subsequently expose sites preferentially attacked by guanidine.

When inactivation of poliovirus was plotted against increasing concentrations of guanidine, an initial shoulder was obtained followed by a rapid decrease in virus infectivity as the guanidine concentrations exceeded 2.7 *M*. However, in the presence of 0.91 *M* $MgCl_2$, the guanidine inactivation curve re-

tained the initial shoulder but the subsequent decrease in virus infectivity was progressively enhanced as the concentrations of guanidine exceeded 2.7 *M*. Since, 0.91 *M* guanidine hydrochloride protected poliovirus against heat and urea inactivation to the same degree as 0.91 *M* $MgCl_2$, low concentrations of guanidine seem to induce the same changes on the virion as cations. These results suggest that guanidine acts in a two-stage manner to inactivate poliovirus. First by virtue of an allosteric effect associated with its positive charge, low concentrations of guanidine as well as cations expose critical bonding sites and sensitize the virion without inactivating it. At higher guanidine concentrations, these exposed sites are secondarily attacked by the hydrogen bonding effect of guanidine so as to disrupt and inactivate the virion. The dual properties of guanidine, *i.e.*, its charge and ability to hydrogen bond, have been evoked also to explain its effect upon the stability of double-stranded DNA (13).

Lwoff suggested that guanidine (10^{-3} *M*) inhibits poliovirus replication by preventing the conformation of the viral RNA-replicase (8). This effect is prevented by low concentration ($10^{-3}M$) of methionine and choline but not betaine (10,11). However, this antagonism is not competitive and is restricted to a narrow range of concentrations. Likewise, choline and methionine, but not betaine, at certain low concentrations were shown to partially prevent guanidine inactivation of poliovirus. These results indicate that the action of these compounds on poliovirus replication and inactivation may be similar. Guanidine may react with the same kind of bonds found in the virion as in the RNA replicase so as to change their conformation and reactivity. However, the concentrations of guanidine affecting the folding of a polypeptide chain may be considerably less than those required to break preformed bonds. Within a limited range of concentrations, choline and methionine may be able to stabilize these kinds of bonds.

Summary. Cations stabilized poliovirus against inactivation by urea (0.5–4.5 *M*) and heat (45°) but to sensitize it to inactivation by guanidine, indicating that urea and heat affect the same structure on the virion which is different from those affected by guanidine. Choline, methionine, betaine, and even guanidine had similar effects as cations. However, certain low concentrations of choline and methionine were also able to partially prevent guanidine inactivation. The action of these compounds on the reactivity of poliovirion is discussed in terms of induced conformational changes of the viral capsid protein.

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