Effect of Organic and Inorganic Acids on Poliovirus at 50°C.* (27775)

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Enteroviruses are stabilized at 50°C in high molar concentrations of monovalent and divalent cations(1-2), and some strains of poliovirus are also stabilized at 50°C by Al ions(3). In this paper we wish to report that a variety of acids, both inorganic and organic, may stabilize certain strains of poliovirus.

Materials and methods. Monkey kidney (MK) cells. Kidneys from immature rhesus monkeys were trypsinized and grown in M-H lactalbumin medium(4). To suppress adventitious agents, the growth medium contained 0.2 mM $AlCl_3$(5). Before use, on about the fifth day of cultivation, the growth medium was replaced with an Al-free maintenance M-E medium.

Viruses. The virulent strain was a plaque-purified line of type 1 poliovirus (Mahoney), and the attenuated strain (LSc) was Sabin's plaque-purified line as used in the oral poliovaccine. The virus harvests were stored frozen. Before use, they were thawed, centrifuged lightly, and, based on previous titrations, diluted to $10^{7.3}$ PFU/ml in distilled water.

Virus assays. All virus titrations were carried out by determination of plaque-forming units (PFU), using the bottle technic. The overlay medium contained $MgCl_2$ at a concentration of 25 mM to expedite early plaque counting(6). First counts were made after 24 hours and final counts at 48 hours. With this overlay medium, no significant increase occurs after this time(6).

Heating methods. The virus preparations described above were further diluted with an equal volume of distilled water or of acid, so that they now contained $10^{7.0}$ PFU/ml. The acid concentrations referred to in the text are final concentrations. Aliquots to be heated were delivered directly to the bottom of tubes and the top of each tube was flamed thoroughly to sterilize at least one inch of the uppermost surface of the tube. Test tubes used were uniform in size and thickness, measuring 13 × 100 mm, with an 0.8 mm wall. The tube was then rubber stoppered and plunged into the water bath, covering the tube to ¼ inch from the stopper. At the end of the heating period, the tubes were quickly transferred to an ice-water bath.

Results. Effect of organic acids on type 1 poliovirus. The viruses were diluted to contain 10^7 PFU/ml, as described above, in different molarities of acetic acid to attain pH levels from 3.0 to 6.5. Samples were heated at 50°C for 15 minutes and then titrated. The attenuated strain was stabilized between pH 4.0 and 5.0 (Fig. 1). On the other hand, the virulent strain was inactivated to a greater degree in all concentrations of acetic acid than samples heated in distilled water at pH 7.2. At pH 4.0, the optimal stabilizing zone for the attenuated strain (12 mM acetic acid), there was no detectable infectivity of the virulent virus. Thus, the attenuated virus was stabilized over 6.0 $\log_{10}$ greater than the virulent strain under similar conditions.

Oxalic and lactic acids were also tested as described above. Both these acids stabilized the attenuated virus, and inactivated the virulent virus to almost the same degree (at the same pH levels) as acetic acid. However, 4 other organic acids tested (citric, tartaric, benzoic and salicylic acids) presented a different pattern (Fig. 2). The attenuated strain was markedly stabilized in the zone of pH 4.0 (*1.5 mM citric acid*), and the virulent virus was slightly stabilized in the same pH zone.

To determine whether the stabilization was due to pH caused by the acid or to the citrate ion, sodium citrate at the same molarity (1.5 mM) was tested as described above. The type 1 attenuated and virulent viruses were diluted in 1.5 mM citrate (pH 7.0) and heated at 50°C. The attenuated and virulent strains were inactivated to the same degree as samples in distilled water. It is evi-

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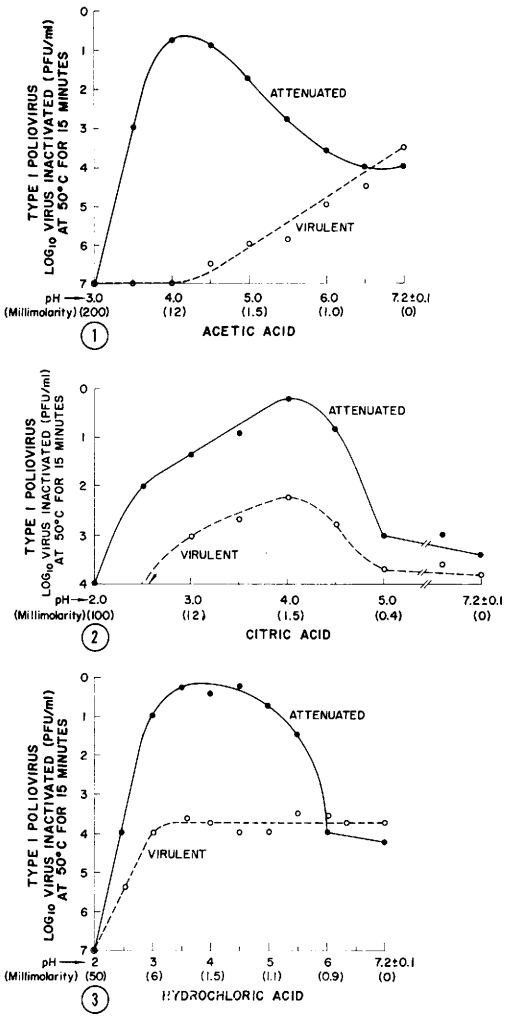

FIG. 1. Effect of acetic acid on poliovirus at 50°C. Type 1 attenuated and virulent strains diluted to contain 10^7 PFU/ml in different millimolarities of acetic acid to attain pH levels described. Oxalic and lactic acids gave results similar to acetic acid.

FIG. 2. Effect of citric acid on poliovirus at 50°C. Tartaric, benzoic and salicylic acids gave results similar to citric acid.

FIG. 3. Effect of inorganic acids on poliovirus. Other inorganic acids (nitric, sulfuric, and phosphoric) gave results similar to HCl.

dent, therefore, that the stabilizing effect is not due to the citrate ion.

Effect of inorganic acids on type 1 poliovirus. Hydrochloric, nitric, sulfuric and phosphoric acids were tested as described above. Since these acids were similar in their effects on the polioviruses at 50°C, a representative experiment is shown in Fig. 3. The attenuated strain was stabilized in a pH zone of 3.0 to 5.5. The virulent virus was not stabilized at any concentration of acid tested. Both strains were completely inactivated at 50°C at pH 2.0. Unheated samples of both strains stored at pH 2.0 at 4°C or 25°C for 15 minutes were not inactivated to any detectable degree.

Comparison of HCl and $AlCl_3$ as stabilizing agents. We have previously reported the stabilization of type 1 attenuated poliovirus and enhanced inactivation of the virulent strain at 50°C in dilute concentrations of $AlCl_3$, which renders the virus suspension acid (3). Type 1 attenuated virus is stabilized in 0.75 mM to 50 mM $AlCl_3$, a pH zone of 3.0 to 4.5.

The attenuated strain is stabilized in 10 mM $AlCl_3$ at pH 4.0 in distilled water, but on decreasing the concentration of $AlCl_3$ to 0.2 mM (pH 6.0) no stabilization occurs. Similarly, this strain is stabilized in 2 mM HCl at pH 4.0 and with decreasing concentrations of HCl (to pH 6.0) stabilization is lost. Therefore, experiments were carried out to determine to what extent the $AlCl_3$ stabilization might differ from that conferred by hydrogen ions. A heat sensitive strain of type 1 attenuated poliovirus grown in cystine-free medium(7) was diluted to contain 10^7 PFU/ml in buffered saline† to which $AlCl_3$ was added so that its final concentration was 10 mM. The pH was 3.5. By addition of $NaHCO_3$, samples at different pH steps from 4.0 to 7.0 were prepared. At the same time the virus was diluted to contain 10^7 PFU/ml in $AlCl_3$-free buffered saline containing $NaHCO_3$ at a pH of 7.0. HCl was added to attain samples at similar pH values. Samples at different pH levels, with and without Al ions, were then heated at 50°C for 15 minutes. Table I shows the results of such an experiment. At a pH zone of 4.0-4.5, virus samples containing $AlCl_3$ or HCl were stabilized to similar degrees. However, as the pH approached 6.0, the virus in HCl was almost completely inactivated (5.4 $\log_{10}$ units) as compared to only 1.0 $\log_{10}$ unit lost for the $AlCl_3$ sample

† *Buffered saline*—Per liter: 8.0 g NaCl, 0.4 g KCl, 0.06 g Na_2HPO_4, 0.06 g KH_2PO_4, to which 7.5% $NaHCO_3$ was added as needed.

TABLE I. Effect of 10 mM $AlCl_3$ on Stabilization of Type 1 Attenuated Poliovirus at Different pH Levels.

	Log_{10} virus inactivated (PFU/ml) at 50°C after 15 min						
	pH						
Diluent	4.0	4.5	5.0	5.5	6.0	6.5	7.0
$AlCl_3$*	.2	.4	.7‡	.8‡	1.0§	2.0‖	3.2‖
HCl†	.3	.7	1.3	3.2	5.4	5.1	5.2

* Virus was diluted to contain 10^7 PFU/ml in buffered saline containing 10 mM $AlCl_3$, and different pH levels were attained by addition of $NaHCO_3$.

† Virus was diluted in Al-free buffered saline containing $NaHCO_3$, and HCl added to obtain pH levels indicated.

‡ Slight precipitate formed.

§ Gelatinous precipitate.

‖ Heavy gelatinous precipitate.

at pH 6.0. A gelatinous precipitate was formed in the Al samples at higher pH, and the inactivation rates increased at pH 6.5 to 2.0 log_{10} and 3.2 log_{10} at pH 7.0. With formation of the precipitate at the higher pH levels it is likely that insufficient Al^{+++} was available to protect the virus. Regardless, even at the higher pH levels, $AlCl_3$-treated virus was stabilized 100 to 1000 times more than virus in HCl.

Investigation of the precipitates formed in the Al^{+++} aliquots revealed that the attenuated virus but not the virulent strain had been selectively adsorbed. This differentiation will be reported later.‡

Although the effects of HCl and $AlCl_3$ on the attenuated strain were quite similar at 50°C, at pH levels of 3.0 to 5.0 these reagents did not have the same effect on the virulent strain. The virulent virus in 10 mM $AlCl_3$ was inactivated to a greater degree than virus in HCl at the same pH level at 50°C. In repeated tests the virulent strain was consistently inactivated 1.0 to 1.5 log_{10} more in $AlCl_3$ than in HCl at the same pH. The enhanced inactivating effect of $AlCl_3$ was evident more strikingly when a cystine-stabilized virus preparation was used. Type 1 virulent poliovirus was stabilized against heat by treating aliquots for 12 hours at 37°C in 50 μg/ml L-cystine(7). Control (heat sensitive) virus consisted of the same virus but without the cystine treatment. Samples were then diluted to contain 10^7 PFU/ml in 2 mM HCl and in 10 mM $AlCl_3$, each at pH 4.0, and heated at 50°C for 15 minutes. The results of such an experiment are shown in Table II. The virulent virus in $AlCl_3$ was inactivated to a much greater degree than virus in HCl in those samples stabilized with cystine.

‡ In preparation.

Discussion. Loring and Schwerdt(8) and Pollard and Connolly(9) tested certain virulent strains at pH 2 to pH 10 and found them resistant to inactivation at 25°C. Our report is concerned with inactivation at 50°C where a number of different acids have been tested on virulent and attenuated type 1 virus. The attenuated virus was stabilized by all inorganic and organic acids tested, with the optimal pH being 4.0. On the other hand, the virulent strain was inactivated either to a greater degree or to the same extent in all inorganic acids and in acetic, oxalic and lactic acids, as samples in distilled water. However, the virulent strain was slightly stabilized at pH 4.0 in citric, tartaric, benzoic and salicylic acids.

Because $AlCl_3$ solutions are acidic, we have re-examined the stabilizing action of this salt on attenuated type 1 virus, and its labilizing action on virulent virus(3). At pH 4.0, both HCl and $AlCl_3$ stabilized attenuated virus to about the same degree but virulent virus was labilized to a greater extent by $AlCl_3$. As the pH approached neutrality, attenuated virus in $AlCl_3$ was protected to a higher degree than virus in HCl.

Another series of experiments has shown that the action of $AlCl_3$ on poliovirus is defi-

TABLE II. Effect of HCl and $AlCl_3$ on Type 1 Virulent Poliovirus at 50°C.

	Log_{10} virus inactivated (PFU/ml) at 50°C for 15 min		
Virus treatment*	HCl pH 4.0	$AlCl_3$ pH 4.0	H_2O pH 7.2
Untreated	3.8	5.2	4.0
Cystine	1.9	4.2	.9

* Mahoney virus was stored at pH 7.5 and a duplicate sample treated with 50 μg/ml L-cystine was held at pH 7.5 for 12 hr at 37°C. The samples were then diluted 10× in distilled H_2O and mixed with equal volume (a) distilled H_2O, (b) 20 mM $AlCl_3$ and (c) 4 mM HCl. Samples were then heated at 50°C for 15 min.

nitely not due to acidity alone. Virulent type 1 poliovirus can be attenuated by heating in 10 mM $AlCl_3$ at pH 4.‡ Virus survivors of $AlCl_3$-heat treatment were propagated further, and the virus harvests were heated again in $AlCl_3$ at 50°C. After numerous passages, with intervening heating in $AlCl_3$ and passage of survivors, we obtained virus that was completely stabilized to heat in 10 mM $AlCl_3$, and that was no longer paralytogenic for monkeys by intracerebral inoculation even with 10^8 PFU/dose. By similar procedures using 2 mM HCl at pH 4, an HCl-heat resistant line was developed after the same number of passages. However, this virus line retained the neurovirulence of the parent, as it paralyzed monkeys after intracerebral inoculation.

Summary. Type 1 attenuated poliovirus (LSc) was stabilized at 50°C at pH 4.0 to 5.0 in acetic, oxalic and lactic acids. The type 1 virulent strain (Mahoney) was inactivated to a greater degree in all concentrations of these acids at 50°C than in distilled water. Citric, tartaric, benzoic and salicylic acids at pH 4.0 stabilized the attenuated strain to a high degree but the virulent strain only slightly. Inorganic acids (HCl, HNO_3, H_3PO_4 and H_2SO_4) stabilized the attenuated strain from pH 3.0 to 5.5, but had no effect on the virulent strain. Thus another *in vitro* character, the acid marker, is available for distinguishing between attenuated and virulent polioviruses.

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Pharmacological Action of Tyramine on the Cardiovascular System in Man.* (27776)

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Tyramine bears a close structural relationship to norepinephrine and since 1909 has been known to have a pressor effect. Its action has been shown in animals to depend entirely upon the release of norepinephrine from tissue stores(1) since, when these are absent following denervation or reserpine administration, tyramine is without effect. Studies in man have confirmed its sympathomimetic action(2,3). In view of its mode of action, tyramine has been used to gauge the state of tissue norepinephrine stores in the cardiovascular system of human subjects(4). This study describes some cardiovascular effects of tyramine in man, the reproducibility of the pressor response, and a dose-response curve.

Materials and methods. Thirty-nine subjects were studied. Except for one healthy volunteer, all had been hospital in-patients for a considerable time and were free of obvious cardiovascular disease. They were not receiving hypotensive agents, sympathomimetic amines or monoamine oxidase inhibitors.

Eleven patients were the subjects of the first study. Observations were made while

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