cellular RNA. Reduplication of viral RNA is not necessarily inhibited by factors capable of interfering with RNA synthesis which is governed by viral or host DNA. Presumably, therefore, the two RNA synthetic processes are enzymatically or topographically distinct (*11*).

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Rapid Development of Drug-Resistant Mutants of Poliovirus

Abstract. Guanidine hydrochloride is a potent inhibitor of poliovirus synthesis in cell culture. However, the viral progeny which do grow in the presence of guanidine may become approximately 10,000 times more resistant to the drug. The phenomenon of drug resistance poses yet another problem in the search for a satisfactory viral chemotherapeutic agent.

Because guanidine is a potent inhibitor of poliovirus multiplication in tissue culture (*1, 2*), we tested the drug on monkeys infected orally with poliovirus (*3*). This provided us with virus which had multiplied in vivo in the presence of guanidine. In the present report we wish to call attention to the fact that when virus highly susceptible to the drug is grown in the presence of the drug either in vitro or in vivo, the progeny of the virus become drug resistant.

Resistance produced in vitro. Wild Mahoney poliovirus was carried for five passages in monkey kidney tube cultures in the presence of guanidine hydrochloride; MG_1 and MG_5 are abbreviations for Mahoney passed once and five times, respectively, in the presence of guanidine. Controls passed in aliquots of the same cultures but in the absence of guanidine were labeled MC_1 and MC_5. Increasing concentrations of guanidine from 20 μg/ml to 75 μg/ml (at the fifth passage) were used.

At each passage the viruses were harvested when over 75 percent of the cells were showing pathologic changes, and 0.1 ml was transferred to new cultures. Table 1 shows that virus many thousand times more resistant than the original virus has been obtained by selecting out guanidine-resistant variants. Since the first passage specimen already contained virus which was considerably more resistant than controls, it appears that a selection of spontaneously occurring mutants occurred in the first few cycles of multiplication in the presence of the drug.

Attenuated LSc strain, the very virus used in the oral polio vaccine, was treated in a similar manner. Again the first and fifth passage materials were found to be considerably more resistant to guanidine, the results being similar to those shown in Table 1.

Resistance produced in vivo. Cynomolgus monkeys fed three times daily with guanidine hydrochloride at near toxic levels (60 to 80 mg/kg per day) and fed virulent type 1 Mahoney poliovirus 3 days after the initiation of the drug course developed paralysis about as frequently as monkeys not treated with guanidine (*3*). On the 5th day after virus feeding, which was the 8th day of drug administration, virus was isolated from the blood of two monkeys. The resistance of these viruses to guanidine was compared with that of the original virus. The recovered progeny strains were grown in cultures free of guanidine before their drug resistance was measured.

A plaque titration was done with the above samples with an overlay containing 28 μg of guanidine per milliliter (*2*). One set of controls was set up containing the progeny viruses with normal overlay. A further set of controls included Mahoney virus from the same sample as that used in inoculating the monkeys. The original virus was inhibited 100 to 1000 times more than either of the viruses which had multiplied in the monkeys fed guanidine. Plaques of resistant virus under an overlay of 28 μg of guanidine per milliliter were picked and passed in tube cultures without guanidine. On subsequent titrations this virus was found to be as resistant as before, indicating that the property is stable for several cycles of multiplication in the absence of guanidine.

Table 1. Emergence of mutants of poliovirus resistant to guanidine hydrochloride. PFU, plaque-forming units; MC_1, MC_5, Mahoney virus passed one and five times, respectively, in control cultures without guanidine; MG_1, MG_5, Mahoney virus passed one and five times, respectively, in the presence of guanidine.

Concn. of drug in overlay (μg/ml)	Titers of virus (PFU/ml)			
	MC_1	MG_1	MC_5	MG_5
30	$<10^2$	$10^{5.7}$	$<10^2$	$10^{6.3}$
None	$10^{7.7}$	$10^{7.5}$	$10^{8.0}$	$10^{7.8}$

Comment. Strains of poliovirus have been produced that are over a thousandfold more resistant to guanidine than the original viruses. The mechanism of the resistance is unknown. Nevertheless, the development of resistance to guanidine by poliovirus may be likened to the development of resistance to chemotherapeutic agents by certain bacteria, and it creates a potential difficulty in the development of an efficient viral chemotherapeutic agent.

The production of resistant strains which are easily distinguished from the parent strain offers a useful tool in the study of viral genetics. The fact that drug-resistant strains were as readily selected from progeny of an attentuated vaccine strain as from progeny of a wild strain emphasizes again the genetic pliability of the polioviruses (*4, 5*).

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