Antiviral Effect of Guanidine

Abstract. It has been found that soluble guanidine salts show an antiviral effect on poliovirus and on some other enteroviruses. The activity was first detected in a cell culture screening system when the guanidine salt of hydroxyaminomethylene malononitrile inhibited the cytopathic effect of poliovirus in cell cultures. The studies were extended to various in vivo experiments in which the suggestive therapeutic activity of guanidine salts was again observed in monkeys infected with poliovirus. While these drugs cannot be considered for use in human disease because of severe toxicity, it is significant that potential antiviral compounds detected by the cell culture test show in vivo activity and point to the validity of such direct chemotherapeutic trials.

A tissue culture procedure has been developed which gives a semiquantitative estimate of the antiviral activity and cytotoxicity of chemotherapeutic agents in a single assay with the same cell culture (*1*). During the course of antiviral studies with this method, it was found that certain guanidine salts inhibited the cytopathic effect of poliovirus in mammalian cell cultures.

The antiviral activity was initially observed with the guanidine salt of hydroxyaminomethylene malononitrile (SV-870) (*2*) on type 2 poliovirus (MEF-1) with No. 2 human epithelial (H.Ep. 2) cells as the cell culture system. The effect of this compound was demonstrated by infecting cultures with 1000 $TCID_{50}$ doses of virus and immediately treating with known decreasing concentrations of SV-870. The mixtures of virus, cell, and drug were incubated at 37°C for 7 days, at which time the cell monolayers were examined microscopically.

The drug was toxic to the H.Ep. 2 cells at 630 μg/ml, but inhibited the cytopathic effect of poliovirus at nontoxic concentrations of 200 and 63 μg/ml. The compound was inactive at 20 μg/ml. Subsequent testing of this type was done with simpler guanidine salts, since it was shown that guanidine hydrochloride at a concentration of 60 μg/ml in cell culture reduced the infectivity titer of poliovirus 10^4- to 10^5-fold. Other guanidine salts showed activity that was approximately proportional to the solubility in the medium.

Delayed treatment of cultures infected with poliovirus showed that there was protection of cells when the monolayer was treated with SV-870 as long as 48 hr after exposures of cells to virus. In an application of a plaque-inhibition technique (*3*), a group of 48 cell cultures (in 32-oz bottles) was infected with 100 plaque-forming units (PFU) of type 1 (Mahoney) poliovirus, and a similar group of 48 bottles was inoculated with 1000 PFU doses of virus. After the virus was permitted to adsorb for 1 hr at 37°C, the cultures were washed once with 100 ml of media in order to dilute and remove the virus inoculum. The bottles were held at 37°C with 50 ml of maintenance media, and at intervals of 0, 1, 2, 8, 20, 30, and 48 hr six bottles of each group were removed and an agar overlay was added. Three of the bottles received an agar overlay containing 0.5 mg/ml of drug, and three received an overlay without drug. All cultures were held for 7 days, and the plaques from each group of bottles were totaled. There were no plaques in any culture in which the compound was incorporated in the agar overlay. In untreated controls at the 20- and 30-hr intervals, there was a total of 120 plaques and more than 500 plaques in cultures inoculated with the 100- and 1000-PFU dosages, respectively.

The guanidine salts have a limited spectrum of antiviral activity. Among a number of viruses studied in cell culture, the compound was effective against only members of the enterovirus groups, as shown in Table 1. Viruses not inhibited by guanidine salts were: (i) myxovirus–influenza A2 (Asian), parainfluenza 1 and 3 (Sendai and HA-1); (ii) herpesvirus–human and simian (HF strain and B virus, Yale strain); (iii) measlesvirus (Edmonston strain); (iv) poxvirus-vaccinia (CL strain); (v) arborvirus A–eastern equine encephalomyelitis (Massachusetts strain), arborvirus B–St. Louis encephalitis (Hubbard strain) and Japanese B encephalitis (Nak. strain); (vi) reovirus–Echo 10 (Lang strain); (vii) polyoma virus (Stewart-Eddy strain).

The chemotherapeutic effect of these compounds was studied in monkeys infected with poliovirus. The combined results from four experiments with guanidine hydrochloride and from three experiments with SV-870 are given in Table 2. In these studies *Macaca mulatta* (rhesus) monkeys, weighing from 2.0 to 4.5 kg, were challenged intramuscularly with type 1 (Mahoney) poliovirus in the left gastrocnemius muscle. The drugs were administered orally in one dose at daily intervals. The treatment schedule was initiated 3 days before virus challenge and con-

Table 1. Antiviral activity of SV-870.

Virus tested	$TCID_{50}$ virus titer with drug levels (mg/ml) at					
	Control (no drug)	0.015	0.03	0.06	0.125	0.250
Poliovirus, type 1 (Mahoney)	$10^{-8.5}$	$10^{-8.0}$	$10^{-7.5}$	$10^{-5.5}$	$<10^{-5.0}$	$<10^{-5.0}$
Poliovirus, type 2 (MEF-1)	$10^{-7.5}$	$10^{-6.5}$	$10^{-5.5}$	$10^{-4.5}$	$<10^{-4.5}$	$<10^{-4.5}$
Poliovirus, type 3 (Saukett)	$10^{-7.5}$	$10^{-6.9}$	$10^{-6.2}$	$10^{-5.0}$	$<10^{-5.0}$	$<10^{-5.0}$
ECHO-6 (D'Amori)	$10^{-7.3}$	$10^{-7.0}$	$10^{-6.0}$	$10^{-4.0}$	$<10^{-4.0}$	$<10^{-4.0}$
ECHO-9 (Bourn)	$10^{-8.0}$	$10^{-8.2}$	$10^{-7.8}$	$10^{-7.0}$	$10^{-6.8}$	$10^{-6.5}$
Coxsackie A-9 (Grigg)	$10^{-8.7}$	$10^{-8.5}$	$10^{-8.3}$	$10^{-6.7}$	$10^{-6.0}$	$<10^{-6.0}$
Coxsackie B-1 (Connecticut-5)	$10^{-6.8}$	$10^{-6.3}$	$10^{-5.9}$	$10^{-5.5}$	$<10^{-5.5}$	$<10^{-5.5}$

Table 2. Effect of guanidine salts administered orally to rhesus monkeys challenged intramuscularly with type 1 (Mahoney) virus.

Daily dose of drug (mg/kg)*	No. tested	Toxicity		No. satisfactory for polio test	Pathology typical of polio	
		Total	%		Total	%
		Guanidine hydrochloride				
10–20	11	0	0	11	11	100
30–50	23	2	9	21	13	62
60–75	12	2	17	10	5	50
120–240	12	6	50	6	1	17
Control (no drug)	23			23	19	83
		SV-870				
12–25	8	0	0	8	5	63
33–50	24	3	13	21	9	23
100	6	2	33	4	0	0
Control (no drug)	13			13	10	77

*Values are the calculated amount of free guanidine base administered.

tinued 14 days after challenge for a total of 17 daily doses. Since clinical observations would not permit clear-cut differentiation between poliovirus infection and drug toxicity, the evaluation of the results was based upon the histopathological analysis of spinal cord and brain stem sections. There is an indication that both drugs were effective in decreasing the incidence of disease in the polio-infected monkeys, although the activity was seen only at marginal toxic levels.

It is significant that, in additional experiments in which the drugs were administered intramuscularly to monkeys at near toxic levels, no sparing effect similar to that seen with oral treatment was noted. In a limited study the activity of the drugs was not altered or enhanced by the oral administration of these compounds in divided dosages when compared with the same total dose at a single daily interval.

The evidence indicates that the effective agent is guanidine itself and not a derivative. Chromatographic studies were used to follow the fate of 2-C^{14} guanidine hydrochloride when it was administered orally in mice. The animals were given radioactive guanidine corresponding to 20 μc of activity, and then samples of blood, feces, and urine were taken at various intervals after administration of the compound. Two-dimensional paper chromatograms were prepared, and autoradiograms showed that the drug was adsorbed and excreted rapidly as unaltered guanidine with the urine containing many times more guanidine than either blood or feces. There was no apparent conversion of guanidine to other compounds.

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References and Notes

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Foliar Retention of Strontium-90 by Wheat

Abstract. Wheat harvested from the University of Maryland Agronomy Farm in June 1959 contained 20 to 50 micromicrocuries of strontium-90 per kilogram of grain. More than 90 percent of the strontium-90 came from deposition on aboveground plant parts, and less than 10 percent was taken up through the soil. About 1 to 2 percent of the strontium-90 fallout during the time the heads were exposed was retained in the grain.

In order to further understanding of the mechanisms contributing to strontium-90 contamination of plants, Russell (*1*) has suggested the consideration of two components, one of which is absorbed through leaves, stems, and surface roots after lodging on them. The other component is absorbed through roots after incorporation into the soil. Specific activity measurements are helpful in separating the two components under natural fallout conditions, since the Sr^{90} deposited in fallout is essentially carrier-free, while that which is incorporated into the soil is diluted by the exchangeable strontium in the soil.

Specific activity determinations were made on four samples of mature winter wheat plants collected on 17 and 18 June 1959 from the University of Maryland Agronomy Farm, Beltsville, Md. Three samples were of the Leapland variety and one of Knox. The plants were separated into chaff, grain, leaves, stems, and roots, the roots being obtained from the surface 4 in. of soil and thoroughly washed with tap water to remove the soil adhering to them. The samples were dry ashed and dissolved in HCl. Calcium was determined in a small portion of the sample by titration with ethylenediaminetetraacetic acid, following double precipitation as calcium oxalate to remove interfering amounts of phosphate. Strontium was determined in the same portion by flame photometry. Strontium-90 was determined in the main part of the sample by separating and counting its radioactive daughter yttrium-90 (see Table 1).

The flame photometric strontium determination was not sensitive enough to detect the strontium content of the grain. While each plant sample contained a total of 5 to 10 mg of strontium, the grain contained less than 0.15 mg of strontium. This is in the lower range of results reported by Duckworth and Hawthorn (*2*), who showed that the grain contained 2 to 5 percent of the total Sr^{89} taken up by wheat plants grown in sand culture. Our analyses showed much lower strontium-calcium ratios in the grain than in other parts of the plant, which indicates a marked discrimination

Table 1. Content of Ca, Sr, and Sr^{90} in wheat plants harvested on University of Maryland Agronomy Farm, 17 and 18 June 1959. Samples A, C, and D were Leapland variety; sample B was Knox variety.

Sample	Wt. (g)	Ca (g)	Sr (mg)	Sr^{90} ($\mu\mu$c)	Specific activity ($\mu\mu$c/mg)	Calculated Sr^{90} from the air (%)
			Chaff			
A	293	0.19	0.39	128	328	93
B	374	0.34	0.71	211	297	91
C	434	0.47	1.87	217	116	80
D	264	0.30	1.61	124	77	70
			Grain			
A	1029	0.44	<0.09	22	>245	>90
B	1515	0.66	<0.14	80	>570	>95
C	1666	0.73	<0.12	61	>510	>95
D	974	0.39	<0.08	28	>350	>93
			Leaves			
A	175	0.62	1.24	368	297	92
B	178	1.02	1.76	351	199	87
C	286	1.54	2.68	494	184	88
D	189	0.93	2.60	304	117	80
			Stems			
A	556	0.54	1.96	126	64	62
B	496	0.86	3.00	175	58	55
C	714	1.04	4.83	187	39	41
D	528	0.73	3.34	97	29	21
			Roots			
A	54	0.08	0.67	16	24	
B	63	0.14	0.95	25	26	
C	39	0.10	0.78	18	23	
D	59	0.11	0.77	18	23	