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α-Methyl-1-Adamantane-Methylamine Hydrochloride: Inhibition of Rous and Esh Sarcoma Viruses in Cell Culture* (34032)

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Our work and that of others, employing 1-adamantanamine hydrochloride (amantadine HCl, Symmetrel) to inhibit Bryan strain Rous sarcoma (RSV) and Esh sarcoma viruses (ESV) (1, 2) in cell culture led to our interest in α-methyl-1-admantanemethylamine hydrochloride (methyl amantadine HCl, MA). Studies indicate that MA was more effective in inhibiting influenza virus *in vitro* than was amantadine HCl (3). MA also inhibited the growth of other myxoviruses *in vitro,* especially rubella and rubeola, as well as respiratory syncytial, parainfluenza 2 and 3.

Materials and Methods. Bioassay of the two avian oncogenic viruses was similar to that of Rubin (4), except that chick embryo fibroblasts that had been frozen in a dimethyl sulfoxide-medium mixture and stored in ampules in liquid nitrogen were used for focus production (5). The growth medium contained, in addition to the original ingredients, Eagle's basal medium and calf serum (rather than chicken serum that might contain antibodies to avian leukosis viruses).

MA was added to the tubes of chick embryo cells, and the tubes were then placed in a water bath at 37° for 15 min. Immediately, the drug–cell suspension was pipetted into Falcon petri dishes (60 × 15 mm with 2-mm grids), and the various dilutions were added to the plates within 5 min. The next day, the cultures were overlaid with agar that contained MA and on day 4 postinoculation, feed medium (also containing MA) was added to all of the plates. After incubation in a CO_2 incubator (4%) and >85% relative humidity, the virus-induced foci were counted.

Results. Figure 1 shows the data obtained in graphic form. The mean concentration of MA to give 50% inhibition of virus with RSV was 7.4 μg/ml (range 6.0–9.7). For ESV, a mean concentration of 5.8 μg/ml (range 4.5–7.2) was necessary for 50% inhibition. The mean values for 50% inhibition using amantadine HCl was 18.4 μg/ml for RSV and 11.3 for ESV (1). Lower concentrations were also noted for the effect of MA with the myxoviruses (3).

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In all the experiments and at all concentration levels, the effect of MA on the cells was compared to cells that had not been treated with MA. Foci were difficult to count with higher concentrations of MA as they were with higher concentrations of amantadine

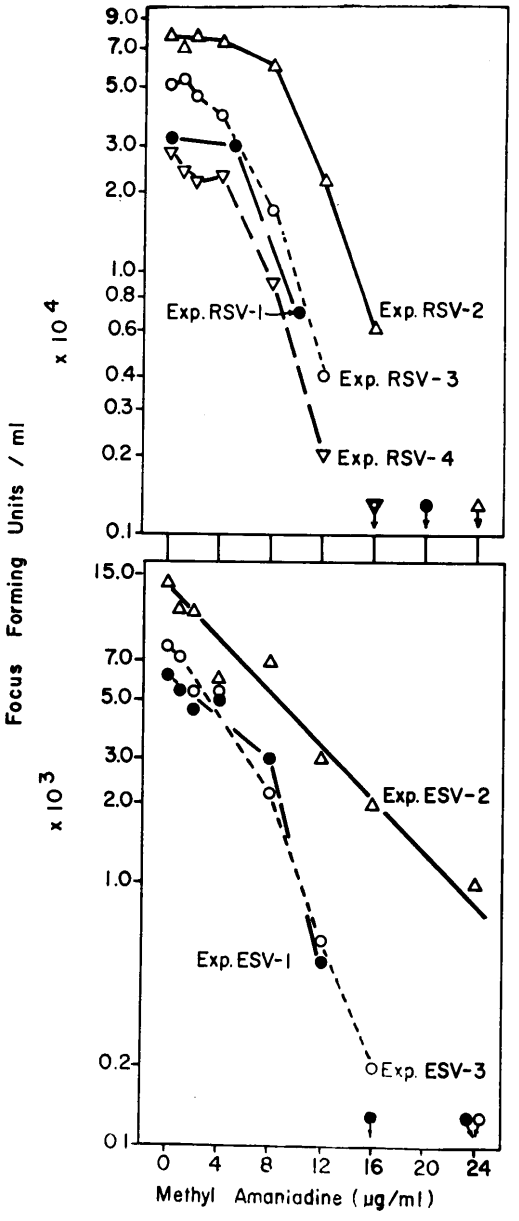

FIG. 1. Number of foci per milliliter produced by Rous sarcoma virus (RSV) and Esh sarcoma virus (ESV) in chick embryo fibroblasts in the presence of methyl amantadine HCl.

TABLE I. Effect of Methyl Amantadine HCl on Susceptibility of Chick Embryo Fibroblasts to Newcastle Disease Virus, GB Strain.

Methyl amantadine HCl (µg/ml)	Plaque-forming units/ml
12.5	2.4×10^5
25.0	2.3×10^5
37.5	2.1×10^5
50.0	2.2×10^5
0	2.4×10^5

HCl (1). Tests of cell intoxication with MA in cell culture using the same liquid medium, but without adding an agar overlay, indicated that cells did not attach as readily and started to come off the dishes in about 3 days at concentrations of 40–50 µg/ml of MA.

A direct toxic effect on the cells, which might prevent replication by MA, was tested by employing Newcastle disease virus (NDV), GB strain, a member of the myxoviruses known to be resistant to amantadine HCl (1). Again, chick embryo fibroblasts that had been frozen were used for bioassay. The cells were treated with MA and then NDV was added to the cells. The agar overlay contained MA. The results shown in Table I indicate that MA did not stop NDV replication at 12.5–50.0 µg/ml.

The possibility of a virucidal effect was determined by the addition of MA to RSV

TABLE II. Effect of Methyl Amantadine HCl on Rous Sarcoma (RSV) and Esh Sarcoma Viruses (ESV) at 37° for 2½ Hr.

Virus	Methyl amantadine HCl (µg/ml)	Focus-forming units/ml
RSV	10	1.4×10^4
	20	1.2×10^4
	30	1.2×10^4
	50	1.3×10^4
	0	1.4×10^4
	0[a]	3.1×10^4
ESV	10	3.2×10^3
	20	4.4×10^3
	30	4.0×10^3
	50	3.8×10^3
	0	4.2×10^3
	0[a]	3.9×10^3

[a] 2° for 2½ hr.

and ESV. The data in Table II indicates that even 50.0 μg/ml had no virucidal effect.

Discussion. It is of interest that less MA was necessary to cause 50% inhibition of RSV and ESV than with amantadine HCl. Employing the therapeutic index as suggested by Stuart-Harris and Dickinson (6) the value used for the minimum amount of drug showing antiviral activity was the mean amount inhibiting 50% of the virus. The values obtained were 5.4 for RSV and 6.9 for ESV. Both of these values are low, as 10 is suggested as a value that would possibly indicate an effective compound. Yet MA is about twice as effective against RSV and ESV than is amantadine HCl. Fletcher *et al.* (7) believed these compounds interfere with the penetration process by causing ionic changes at the cell surface. Evidently these two avian tumor viruses penetrate cells at the same site and penetration is prevented by the same ionic conditions as the affected myxoviruses.

Summary. Rous and Esh sarcoma viruses are inhibited at the 50% level by 7.4 and 5.8 μg/ml in cell cultures of chick embryo fibroblasts by α-methyl-1-adamantanemethylamine hydrocholoride as determined by an enumeration method. There was no virucidal effect at 50 μg/ml.

Note added in proof. A probable mechanism for the inhibitory activity of amantadine is given in Kato, N. and Eggers, H. J., Virology **37**, 632 (1969).

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