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Host-Dependent Restriction of Mengovirus Replication

IV. Effect of Some Quaternary Ammonium Ions on the Restricted Replication of Mengovirus in Madin-Darby Bovine Kidney Cells

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The quaternary ammonium ions, choline, hexamethonium, and tetraethylammonium, partially relieve the restricted replication of mengovirus in Madin-Darby bovine kidney cells.

The restricted replication of mengovirus in Madin-Darby bovine kidney (MDBK) cells is characterized by a premature cessation of viral RNA synthesis at 4 to 5 h postinfection and by the accumulation of a large amount of 125*S* and 80*S* subviral particles. There is a resemblance between the mengovirus restriction phenomenon and the effect of guanidine-hydrochloride on poliovirus multiplication (2). Although guanidine has not been reported to have an effect on mengovirus replication itself, several antagonists of the guanidine effect (4-6, 8) were tested for their ability to relieve mengovirus restriction in MDBK cells.

After 1 h of adsorption at 37 C, MDBK cell monolayers infected with mengovirus were washed with sterile saline, and fresh medium containing the compound (75 to 80 mM) being tested was added. Eight hours after infection the cells were subjected to three cycles of freeze-thaw and assayed for virus yield on L cell monolayers. The relative yield of mengovirus from untreated MDBK cells and cells to which guanidine antagonists have been added are compared in Table 1. No effect of these compounds on the host cell was noted. Two of the more effective compounds, choline and hexamethonium, were further tested, and Fig. 1 shows the dose-response curves for these two compounds. Since hexamethonium and choline relieve mengovirus restriction to the same extent (Fig. 1; Table 1) and over the same range of concentrations (Fig. 1), they were used interchangeably. Figure 2 illustrates the ability of hexamethonium to relieve mengovirus restriction in MDBK cells when added at increasingly

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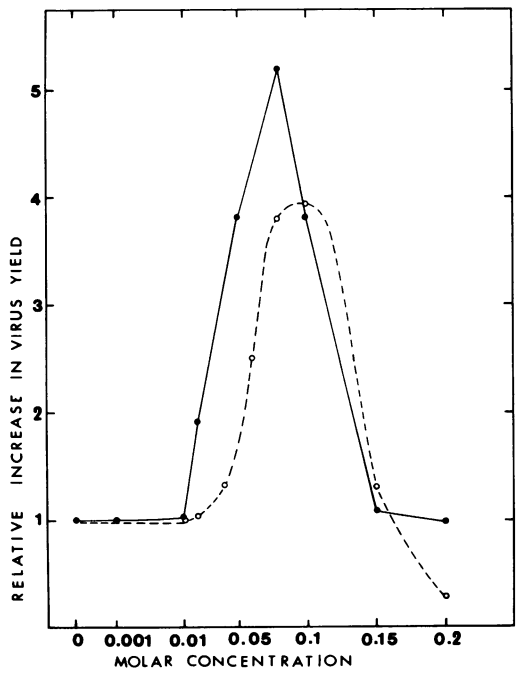


FIG. 1. *Dose-response curves for hexamethonium and choline in mengovirus-infected MDBK cells. MDBK cell monolayers, infected with 100 PFU of mengovirus/cell, were washed several times with sterile saline at 1 h after infection to remove unadsorbed virus, and from fresh medium containing the indicated concentrations, choline or hexamethonium was added. At 8 h after infection samples were subjected to three freeze-thaw cycles and then assayed on L cell monolayers for virus yield. Data are expressed as virus yield relative to the yield in untreated mengovirus-infected MDBK cells. Symbols:*●, *hexamethonium;* ○, *choline.*

later times in the virus replicative cycle. It should be noted that hexamethonium-chloride and choline partially relieve the restriction block up to 3 h postinfection, about the time of the initiation of the restrictive phenomenon (9a).

Picornavirus RNA and protein synthesis has been shown to be associated with separate cytoplasmic membranous complexes. Following mengo infection there is an increase in the proliferation of newly formed membranes, with a resulting stimulation in choline incorporation. Table 2 indicates that the choline effect is somewhat host specific, as has also been reported for the effect of guanidine (6) and guanidine antagonists (8) on poliovirus replication. Hexamethonium-chloride had no effect

TABLE 1. *Compounds tested for their possible ability to relieve restricted mengovirus replication in MDBK cells*

Compound tested[a]	Relative virus yield[b] (normal yield = 1.0)
Choline-chloride	2.25, 3.8, 6.0, 2.25
Hexamethonium-chloride	7.2, 5.2, 4.8, 2.8, 5.5, 2.15
Tetraethylammonium-bromide	4.4
Arginine	2.0
Methionine sulfoxide	1.875
Urea	1.8
Carbamyl choline	1.36
Acetylcholine-chloride	1.0
NaCl	1.0
$NaClO_4$	0.825
Methionine	0.81, 1.0
KCl	0.7
Guanidine-hydrochloride	
0.0025 M	0.56
0.08 M	0.14, 0.1
Ethionine	0.56
Betaine	0.5
Sodium thiocyanate	0.45, 0.57
Trimethylamine	0.34
Ouabain (10^{-3} M)	0.24
Ethanolamine	0.24
Valinomycin	
2×10^{-6} M	0.17
2×10^{-5} M	0.14
Perchloric acid	0.125
S-adenosyl methionine	0.11
Dimethyldilaurylammonium-chloride	0.11
Nicotinamide	0.075
Spermine	0.06
Dimethylglycine	0.054
Monomethylglycine (sarcosine)	0.036
$K_2Cr_2O_7$	0.015
Cystine	<0.01

[a] Compounds were tested at a concentration of 0.075 to 0.08 M unless otherwise indicated and were added at 1 h after infection.

[b] Data are expressed as relative virus yield when compared with the mengovirus yield in untreated MDBK cells, which was always equal to 1. Each number indicates a screening test performed on a different day with a different batch of MDBK cells.

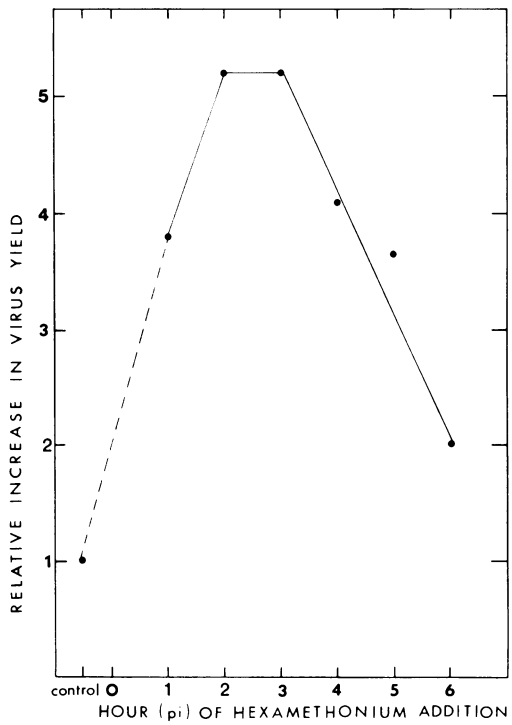


FIG. 2. *The effect of time of addition of 80 mM hexamethonium on the relative yield of mengovirus in MDBK cells. Experiments were done as described in the legend to Fig. 1, except that fresh medium containing hexamethonium was added at the indicated times.*

TABLE 2. *Host specificity of the choline effect on mengovirus yield*

Experimental condition	Virus yield (PFU/ml)[a]
HeLa cells + mengovirus	8.5×10^8
	9.2×10^8
HeLa cells + mengovirus + 0.08 M choline at $t = 1$	2.3×10^8
	3.4×10^8
MDBK cells + mengovirus	1.0×10^7
	3.2×10^7
MDBK cells + mengovirus + 0.08 M choline at $t = 1$	4.5×10^7
	1.25×10^8

[a] Virus yield was determined by plaque assay on L-cell monolayers.

(Fig. 3) on the increased membrane proliferation resulting from picornavirus infections as measured by [*methyl*-^{3}H]choline uptake into phospholipids, so that membrane proliferation does not appear to be affected (7, 9).

Choline, hexamethonium, and tetraethylammonium, which partially relieve mengovirus restriction in MDBK cells (Table 1), are structurally related (Fig. 4) and are functionally cholinergic blocking agents (3, 10, 11). Dimethylaurylammonium, with a structure common to classical ammonium surfactants, on the other hand, reduced mengovirus yield (Table 1), suggesting that the effective compounds are not acting as surface active agents. Negative results with *S*-adenosyl methionine, methionine, monomethylglycine, dimethylglycine, and betaine suggest that the effective compounds are not acting as methyl donors although these compounds have been shown to reverse the guanidine effect (5). Ions which disrupt the structure of water, such as urea, guanidine, thiocyanate, perchlorate, and dichromate, also gave negative results. The strong positive charge of the effective quaternary ammonium ions, the host specificity of their effect (Table 2), the relatively large concentration necessary for their effect to occur (Fig. 1), the fact that they have no effect on the amount of new membrane proliferation resulting from mengovirus infection (Fig. 3), and the documented effect of these specific quaternary ammonium ions on model membrane systems (3, 10, 11) suggest that these ions may be acting in some way to affect the conformation of MDBK replicative complexes. Further analysis, including the effect of these compounds on viral RNA synthesis and viral maturation, needs to be done.

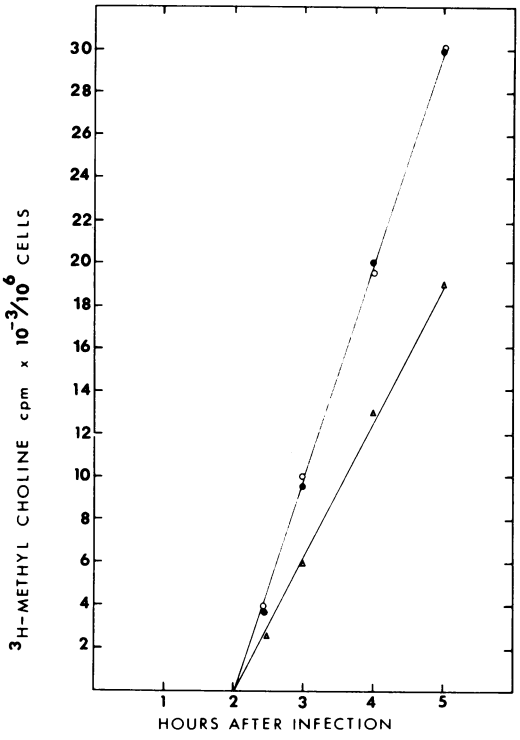
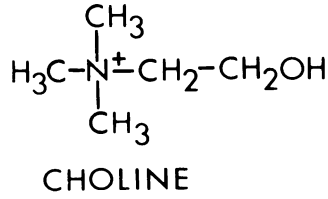
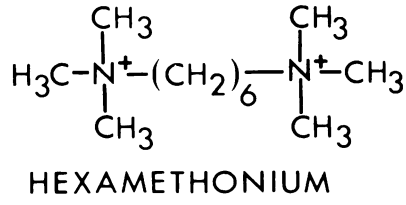


FIG. 3. *Membrane proliferation in MDBK cells. Uninfected MDBK cells and MDBK cells infected with 100 PFU of mengovirus/cell were labeled with 2 μCi of [methyl-^{3}H]choline in the presence of 0.1 mM unlabeled choline (specific activity of 20 μCi/mmol), and samples were analyzed for incorporation of isotope into acid-insoluble material. Symbols: Δ, uninfected MDBK cells; ●, mengovirus-infected MDBK cells; ○, mengovirus-infected MDBK cells treated with 80 mM hexamethonium at 2 h after infection.*





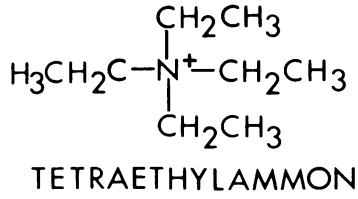


FIG. 4. *Structure of the quaternary ammonium ions most effective in relieving the restricted replication of mengovirus in MDBK cells.*

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