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EFFECTS OF EDEINE, HYGROMYCIN B AND ALPHA-SARCIN ON INFLUENZA VIRUS REPRODUCTION

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Summary. — The effects of edeine, hygromycin B and α-sarcin on the synthesis of virus-specific proteins and formation of infectious virions was studied in cells infected with fowl plague virus (FPV). The manifestation of the antiviral effect of edeine depended on the peculiarities of the FPV strains and the host-cell systems. Hygromycin B inhibited significantly the synthesis of virus-specific proteins and the formation of the infectious virions, but did not influence protein synthesis in uninfected cells. α-Sarcin in the concentrations tested neither showed a marked antiviral activity nor affected protein synthesis in the uninfected cells.

Key words: Influenzavirus A; protein synthesis; edeine; hygromycin B

Introduction

Carrasco (1978) reported that some inhibitors, not penetrating into the normal cells and not influencing their metabolism, might enter virus-infected cells and inhibit virus reproduction. Such results were obtained on Mengo, encephalomyelitis, Semliki forest and SV40 viruses with edeine, hygromycin B and some other inhibitors of protein synthesis (Carrasco, 1978; Contreras and Carrasco, 1979). According to Carrasco (1978), application of such preparations may represent a novel approach to chemotherapy of viral diseases.

We studied the effects of edeine, hygromycin B and α-sarcin on protein synthesis and reproduction of orthomyxoviruses in cell culture. Our results confirmed those of Carrasco (1978) concerning the possibility that preparations unable to penetrate into uninfected cells and influence the synthesis of cell macromolecules, inhibit viral protein synthesis in infected cells. But we found that this action is not universal and that it depends on the peculiarities of the viral strains and the host cell.

Materials and Methods

Viruses. Fowl plague virus (FPV) strains Weybridge (Hav1Neq1) and Dobson (Hav1Neq1) were used as a model of influenza A virus. The latter strain adapted to mammalian cells was kindly supplied by Dr. J. Závada (Institute of Virology, Bratislava). In addition, we used en-

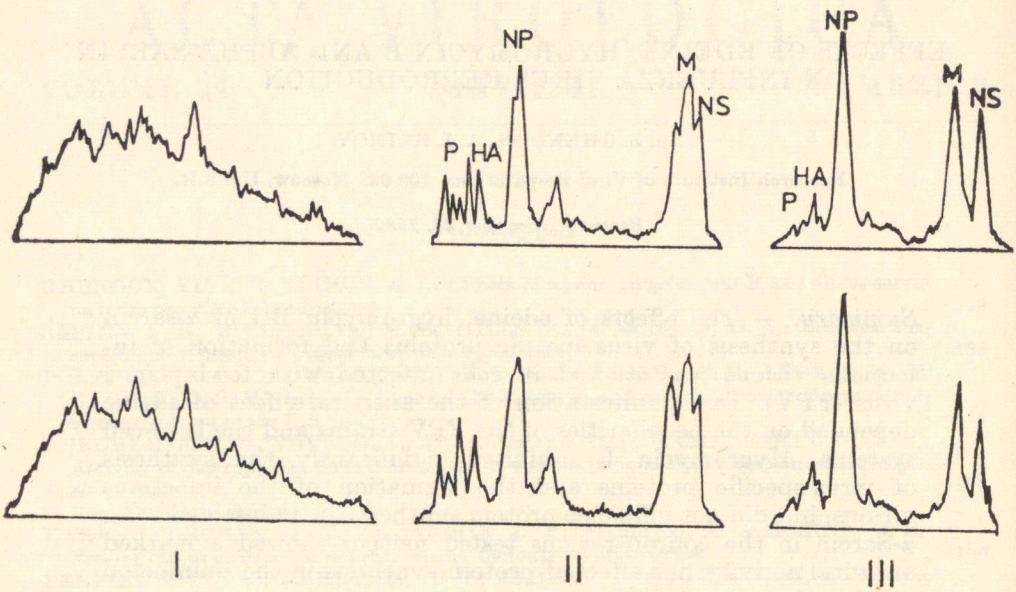

Fig. 2.

Effect of edeine on the synthesis of FPV proteins in BHK cells
The cells were infected with FPV strain Weybridge or Dobson (30—50 PFU/cell) and incubated for 4 hr at 36 °C, after which edenine (2 mg/ml and 30 min later ^{35}S-methionine was added. After 30 min incubation at 36 °C the cells were solubilized, analyzed by PAGE and the autoradiograms subjected to densitometry.
Top row: cells incubated without edeine; bottom row: cells incubated with edeine
I — Uninfected cells; II — cells infected with FPV strain Weybridge; III — cells infected with FPV strain Dobson. P, HA, NP, M, NS — virus-specific polypeptides.

cephalomyocarditis virus (EMCV), kindly supplied by Dr. S. Maslova (Institute of Poliomyelitis and Viral Encephalitides, Moscow). FPV grown in chick embryos and EMCV grown in HeLa cells were purified and concentrated by differential centrifugation.

Cells. Primary chick embryo fibroblast (CEF) cultures and the continuous BHK-2113 cell line were used with FPV, as were HeLa cells with EMCV.

Protein synthesis. Cells were infected with the viruses at a multiplicity of 30—50 plaque forming units (PFU) per cell and left to adsorb for 30 min at room temperature. In experiments on edeine, the cells were incubated for 4 hr (FPV) or 4.5 hr (EMCV) at 36 °C. The medium was then changed for one without methionine, test concentrations of edeine were added and the cultures incubated for 30 min at 36 °C. Thereafter 20 μCi ^{35}S-methionine (^{35}S-methionine, Radiochemical Centre, Amersham, specific activity 1300 Ci/mmol) were added per sample and incubation was continued for 30 min at 36 °C. In experiments on hygromycin B and α-sarcin, after adsorption the cells were incubated for 1 hr at 36 °C, the medium was then changed for one without methionine, the test concentrations of the inhibitors were added and incubation was continued for 4 hr. ^{35}S-Methionine (20 μCi per sample) was added and the cultures were incubated for 30 min. In control experiments virus-infected cells were treated and incubated under similar conditions but without the inhibitors. In experiments on uninfected cells the conditions of treatment and incubation were the same as with infected cells. At the end of the experiment the cells were collected into solubilizing solution (5 M urea, 1 % sodium dodecyl sulphate, 0.1 % β-mercaptoethanol), boiled for 3 min and electrophoresed in 25 % and 15 % polyacrylamide gels (PAG) using the buffer

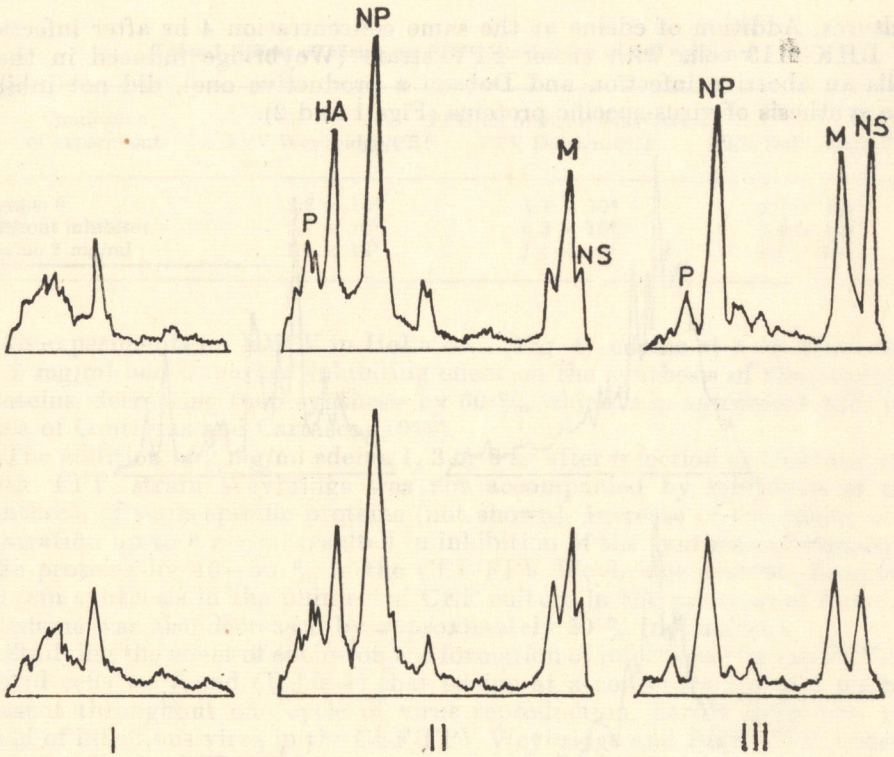

Fig. 3.

Effect of edeine on the synthesis of FPV proteins in CEF
Explanations as in Fig. 2.

system described by Laemmli (1970). Autoradiography was performed as described by Russel and Skehel (1972). The autoradiograms were evaluated in a Joyce Loebel microdensitometer.

Virus reproduction. The cell cultures were infected at a multiplicity of 1-3 PFU/cell. After 30 min adsorption at room temperature the cells were washed 5 times with a cold buffer solution, warm medium 199 was added and one sample was immediately frozen (sample 0) and the others were incubated for 1 hr at 36 °C. Then the test concentrations of the inhibitors were added and incubation was continued for 15 hr at 36 °C, after which the cells were once frozen and thawed and the virus titre was determined by the plaque technique in CEF cultures.

Reagents. Edeine (Calbiochem) was received through the courtesy of Dr. E. Yakobson (Imperial Cancer Research Fund, London), and hygromycin B and α-sarcin through the courtesy of Dr. L. Carrasco (Universidad Autonoma de Madrid).

Results

Effect of edeine on protein synthesis and FPV reproduction in cell cultures

The data presented in Figs 1—3 show that edeine at a concentration of 2 mg/ml hardly affected protein synthesis in uninfected CEF and BHK cell

cultures. Addition of edeine at the same concentration 4 hr after infection of BHK-2113 cells with either FPV strain (Weybridge induced in these cells an abortive infection and Dobson a productive one), did not inhibit the synthesis of virus-specific proteins (Figs 1 and 2).

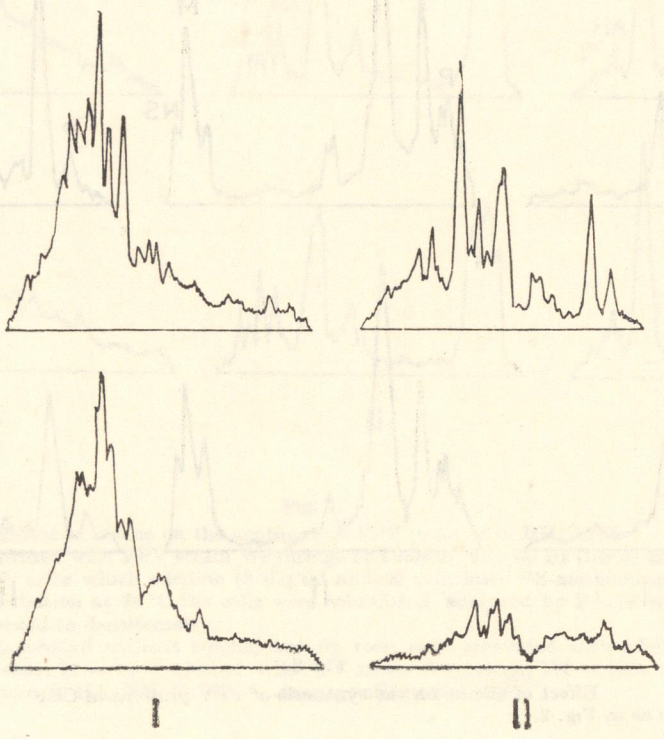

Fig. 4.

Effect of edeine on the synthesis of EMCV in HeLa cells

The cells were infected with EMCV (30—50 PFU/cell) and incubated for 4.5 hr at 36 °C, after which edeine (2 mg/ml) and 30 min later ^{35}S-methionine was added. Further procedure as indicated in Fig. 1.

Top: cells incubated without edeine; bottom: cells incubated with edeine.

I — Uninfected HeLa cells; II — EMCV-infected HeLa cells.

In CEF cultures (Fig. 3) infected with FPV strain Weybridge (which induced productive infection in this culture), edeine at a concentration of 2 mg/ml had no effect on the synthesis of virus-specific proteins. On the other hand, in CEF cultures infected with the Dobson strain (productive infection), the synthesis of virus-specific proteins was decreased in the presence of 2 mg/ml of edeine by 34 % (in other tests by 42 % and 39 %, not shown).

Table 1. Effect of edeine on FPV reproduction in cell cultures

Conditions of experiment	PFU/ml in virus/cell systems		
	FPV Weybridge/CEF	FPV Dobson/CEF	FPV Dobson/BHK
Sample 0	2.2×10^4	1.1×10^4	3.7×10^4
Without inhibitor	2.7×10^7	6.3×10^6	2.4×10^6
Edeine 2 mg/ml	1.6×10^7	1.1×10^5	1.1×10^6

In experiments on EMCV in HeLa cells (Fig. 4), edeine at a concentration of 2 mg/ml had a marked inhibiting effect on the synthesis of virus-specific proteins, decreasing their synthesis by 60 %, which is in agreement with the data of Contreras and Carrasco (1979).

The addition of 2 mg/ml edeine 1, 3 or 6 hr after infection of CEF cultures with FPV strain Weybridge was not accompanied by inhibition of the synthesis of virus-specific proteins (not shown). Increase of the edeine concentration up to 8 mg/ml resulted in inhibition of the synthesis of virus-specific proteins by 40—50 % in the CEF/FPV Weybridge system. However, protein synthesis in the uninfected CEF culture in the presence of 8 mg/ml of edeine was also decreased by approximately 50 % (not shown).

Studying the effect of edeine on the formation of infectious virus in FPV-infected cells we found (Table 1) that edeine at a concentration of 2 mg/ml, present throughout one cycle of virus reproduction, hardly influenced the yield of infectious virus in the CEF/FPV Weybridge and BHK/FPV Dobson systems. In the CEF/FPV Dobson system, the yield of virus in the presence of 2 mg/ml of edeine was decreased by more than 1 $\log_{10}$ unit. These experiments were repeated four times with similar results.

Effects of hygromycin B and α-sarcin on protein synthesis and FPV reproduction in cell cultures

The results presented in Figs 5 and 6 show that both hygromycin B and α-sarcin in the concentrations tested practically did not influence protein synthesis in uninfected CEF cultures. An analysis of total cell protein synthesis based on ^{35}S-methionine incorporation into the acid-insoluble fraction also showed that hygromycin B at a concentration of 2 mM and α-sarcin at a concentration of 2 μM did not inhibit the synthesis of cell proteins (uninfected CEF culture — 1.39×10^6 count/min; the same culture incubated for 4 hr in the presence of 2 mM hygromycin B — 1.48×10^6 count/min and in the presence of 2 μM α-sarcin — 1.33×10^6 count/min). The results presented in Figs 5 and 6 showed that hygromycin B at a concentration of 2 mM decreased the synthesis of virus-specific proteins in CEF cultures infected with FPV strain Weybridge by 65 % (in other tests by 73 and 58 %). At the same time α-sarcin at a concentration of 2 μM had a small inhibiting effect (22 % decrease; in other tests 10 and 26 % decrease) on the synthesis of FPV proteins. An increase of the α-sarcin concentration

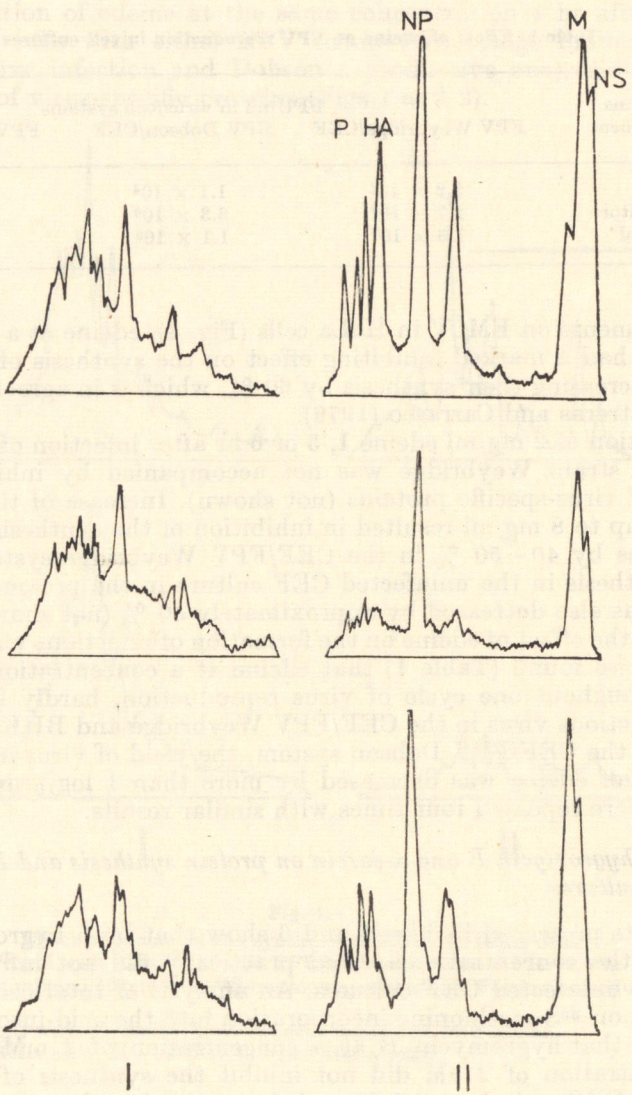

Fig. 6.
Effect of hygromycin B and α-sarcin on the synthesis of FPV proteins in CEF
The cells were infected with FPV strain Weybridge (30—50 PFU/cell) and incubated for 1 hr at 36 °C; hygromycin B (2 mM) or α-sarcin (2 μM) were then added and incubation was continued for 4 hr at 36 °C. ^{35}S-Methionine was then added and after 30 min incubation the cells were solubilized, analyzed by PAGE and the autoradiograms subjected to densitometry.
Top row: incubation without inhibitors; middle row: incubation in the presence of hygromycin B; bottom row: incubation in the presence of α-sarcin.
I — Uninfected CEF; II — FPV-infected CEF
P, HA, NP, M and NS — virus-specific polypeptides

Table 2. Effects of hygromycin B and α-sarcin on the reproduction of FPV strain Weybridge in CEF

Inhibitor		Virus titre (PFU/ml)
Sample 0		4.4×10^4
None		3.8×10^7
Hygromycin B	2 mM	5.8×10^5
	4 mM	3.4×10^5
	6 mM	2.0×10^5
α-Sarcin	1 μM	3.1×10^7
	2 μM	9.6×10^6
	3 μM	8.9×10^6

up to 3 and 4 μM caused no increase of the inhibition of viral protein synthesis and at higher concentrations (8 μM) protein synthesis in the uninfected cells decreased significantly (not shown).

As to the effect of hygromycin B and α-sarcin on the reproduction of FPV strain Weybridge in CEF cultures (Table 2), hygromycin B at concentrations from 2 to 6 mM inhibited virus reproduction by nearly 2 $\log_{10}$ units; α-sarcin at all the concentrations tested showed a moderate antiviral activity and inhibited the formation of the infectious virus in the range of 0.5 $\log_{10}$ unit. These experiments were repeated three times with similar results.

Discussion

Carrasco (1978) showed that some inhibitors, unable to enter normal eukaryotic cells, can enter cells infected with a virus as a result of a change of the permeability of the surface membrane of the uninfected cells. Contreras and Carrasco (1979) further found that both edeine and hygromycin B at the concentrations tested had a small effect on the synthesis of proteins in uninfected cells. At the same time edeine at concentrations from 0.5—3 mg/ml inhibited synthesis of EMCV and Mengo virus proteins in 3T6 cells, Semliki forest virus proteins in BHK cells, and SV40 proteins in CV-1 cells by 60——90 %. Hygromycin B at a concentration of 5×10^{-4} — 10^{-3} M inhibited synthesis of Semliki forest virus proteins in BHK cells and SV40 proteins in CV-1 cells by nearly 90 %.

Based on his results, Carrasco (1978) suggested that application of preparations unable to penetrate into uninfected cells, but inhibiting virus reproduction in infected cells, might represent a new approach to chemotherapy of viral diseases.

In our experiments on the picornavirus EMCV, like in experiments of Contreras and Carrasco (1979), edeine exerted a marked inhibitory effect on the synthesis of virus-specific proteins in HeLa cells. In parallel experiments on FPV strain Weybridge, a representative of influenza A viruses, edeine at a concentration of 2 mg/ml did not inhibit the formation of virus-specific

proteins in primary CEF cultures or in continuous BHK cells infected by the FPV strain Weybridge. But with FPV strain Dobson, differing from the Weybridge strain by the degree of homology of three genes and several biological characteristics (Markushin *et al.*, to be published), edeine did not influence the synthesis of virus-specific proteins in BHK cells but significantly inhibited their formation in CEF cultures. These results were in good agreement with those on the effect of edeine at a concentration of 2 mg/ml on the formation of infectious virus: a significant decrease of the infectious virus yield was observed only in the FPV Dobson/CEF system (Table 1).

As to its effect on the metabolism of uninfected cells, edeine at a concentration of 2 mg/ml practically did not inhibit the synthesis of cell proteins. An increase in edeine concentration up to 8 mg/ml was accompanied by a 50 % inhibition of cell protein synthesis.

Our results thus showed that the ability of edeine to inhibit the synthesis of virus-specific proteins might depend on the peculiarities of both the virus strains and the host-cell systems used. In fact, edeine at a concentration of 2 mg/ml inhibited synthesis of the proteins of FPV strain Dobson in CEF but not in BHK cells. On the other hand, in CEF cultures, edeine inhibited synthesis of Dobson strain proteins but did not affect synthesis of Weybridge strain proteins.

Of the other inhibitors of protein synthesis examined, hygromycin B — a preparation of the streptomycin group (Vazquez, 1974) — significantly inhibited both virus protein synthesis and the formation of infectious virions in CEF infected with FPV strain Weybridge. Hygromycin B at concentrations significantly inhibiting the synthesis of viral proteins, did not affect protein synthesis in uninfected cells. α-Sarcin in the concentrations tested proved to be a weak inhibitor as far as protein synthesis and formation of infectious virions of the FPV strain Weybridge in CEF is concerned. This could be due to the fact that the molecular weight of this preparation (about 12 000 daltons) is more than twofold that of both hygromycin B and edeine. According to Carrasco (1978) preparations with a molecular weight lower than 750 daltons might enter the infected cells, but substances with a molecular weight higher than 10 000—30 000 daltons cannot do so.

Our results thus confirmed those of Carrasco (1978) and Contreras and Carrasco (1979) on the ability of some inhibitors, not entering the uninfected cells and not changing the synthesis of cell proteins, to penetrate into virus-infected cells and to inhibit the synthesis of virus-specific proteins and the yield of infectious virions. But our experiments with edeine showed that the ability of such substances to inhibit the synthesis of virus-specific macromolecules is not universal and that it might depend on both the peculiarities of the virus strains and the host-cell systems. If the virus inhibitory effect of such substances as edeine actually is a consequence of leakiness of the surface cell membrane, as Carrasco (1978) assumes, the data obtained indicate that the change in the permeability of surface cell membrane following virus infection might depend on both the properties of the virus and the host cell itself.

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For Figs 1 and 5 see Plates XX and XXI.