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# COMPARATIVE STUDIES ON GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS TYPES 0 AND ASIA 1 IN BHK-21 RAZI CELLS

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*Summary.* — Growth pattern of foot-and-mouth disease virus types 0 and Asia 1 in BHK-21 Razi cells was compared; while type 0 virus grew in high titre, Asia 1 virus was produced in low titre. Inhibition of host protein synthesis in type 0 virus-infected cells was more pronounced than in Asia 1 virus-infected cells. Foot-and-mouth disease virus type 0 infected cells showed higher lactic dehydrogenase activity when compared to Asia 1 virus. A significant decrease in virus yield was observed when Actinomycin D had been added at 50 μg/ml to infected cells.

*Key words: foot-and-mouth disease virus*; *BHK-21 cells*; *lactic dehydrogenase activity*

## *Introduction*

Foot-and-mouth disease (FMD) is an acute and highly contagious febrile disease affecting cloven-hooved animals. The use of BHK-21 clone 13 cells for the growth of foot-and-mouth disease virus (FMDV) has helped the large scale production of vaccine in many countries including India. As the evaluation of vaccine by animal testing is expensive, efforts have been made to evaluate the vaccine using in vitro laboratory tests. BHK-21 cells obtained from three laboratories showed different susceptibility profile to FMDV types 0, A, C and Asia 1 (Vasantha and Lal, 1982). Lactic dehydrogenase (LDH) activity as function of FMDV multiplication in BHK-21 suspension cell cultures was used to monitor the harvesting time in vaccine production (Spier, 1977). The LDH activity in infected cell cultures was shown to correlate with the infectivity titre of the virus (Spier, 1977). Inhibition of protein synthesis and stimulation of RNA synthesis in BHK-21 cells infected with FMDV were reported by Vande Woude *et al.* (1970).

In the present investigation BHK-21 Razi cells obtained from the Razi Institute Teheran, were used to compare the growth profile of FMDV types 0 and Asia 1. The effect of FMDV infection on host cell protein and RNA syntehsis and assay of LDH activity in infected cell cultures were carried

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out to understand the growth pattern of FMDV types 0 and Asia 1. Further, the effect of Actinomycin D on FMDV RNA synthesis and virus yield was studied to understand the mode of its action on virus replication.

## *Materials and Methods*

*Chemicals* were obtained from Sigma chemicals (St. Louis, USA), Difco Laboratories (Detroit, USA).

*Cells and viruses* were grown and maintained as described (Vasantha and Lal, 1982). Foot-and-mouth disease virus type 0 (subtype $0_5R_2/75$) and Asia 1 (subtype Pak 1/54, 63/72) isolated in India were used throughout.

*Infection of BHK-21 Razi cells with FMDV types O and Asia 1.* Confluent monolayer cells were infected with the virus at multiplicity of 10 PFU/cell. At each interval samples were collected starting from 4 to 24 hr post infection (p. i.). Infectivity titre ($TCID_{50}$) of each sample was determined according to Reed and Muench.

*Lactic dehydrogenase assay.* LDH assay was carried out as described by Spier (1977). One unit of enzyme activity is the amount of LDH which changes the optical density of NADH at 340 nm by 0.001/min in the 3 ml assay system (Wroblewski and La Due, 1955; Bergmeyer, 1963). LDH units/min/ml were calculated according to the formula:

$$\frac{(\text{E } 340/\text{min}) \times 10\,000}{3} = \text{LDH units/min/ml}$$

(E 340 is the decrease in OD at 340 nm)

*Protein and RNA synthesis in FMDV infected BHK-21 Razi cells.* The BHK-21 Razi cells were grown to confluent monolayer and infected with FMDV types 0 and Asia 1 separately as described earlier. At 60 min intervals up to 360 min p. i., the medium was removed, cells were washed with PBS and labelled for 30 min with 2 ml of maintenance medium containing 10 μg/ml of ($^3$H)-uridine (BARC-Bombay-SP-Activity, 359 GBq/mmol). After 30 min, the cells were chilled on ice and the medium was descarded. The cells were washed with chilled PBS, and 1 ml Tris-magnesium buffer (100 mmol/l Tris, HCl buffer, pH 7.4 containing 1 mmol/l $MgCl_2$) containing 1% SDS was added. The TCA precipitable counts were determined in each case and the counts per milligram of RNA were used to determine the percentage of controls. To determine protein synthesis 2 ml of Hank's balanced salt solution containing 370 kBq/ml of ($^{14}$C)-chlorella protein hydrolysate (BARC-SP activity-1.554 GBq/matom C) was added and the samples were processed as described for RNA synthesis. Counts per milligram of protein were used to determine the percentage of control.

*Effect of Actinomycin D on FMDV RNA synthesis and virus yield.* The virus-specific RNA synthesis was monitored in the infected cells in presence of various concentrations (5—50 μg/ml) of Actinomycin D and the drug was added immediately after virus adsorption. Medium was removed from infected cells at 60 min intervals up to 360 min p. i. The cells were washed with PBS, 2 ml medium containing 370 kBq/ml of ($^3$H)-uridine were added and incubated at 37 °C. After 30 min the cells were chilled in ice, medium was discarded and processed as described earlier. Similarly, infected cells were treated with Actinomycin D (5—50 μg/ml) and the virus yield was determined at 20 hr p. i.

## *Results*

The results of the growth FMDV types 0 and Asia 1 in BHK-21 Razi cells are presented in Fig. 1. Type 0 virus multiplied better than Asia 1 as evidenced from the infectivity titre ($TCID_{50}$/ml). The maximum titre for type 0 virus at 20 hr p. i. was found 7.9, whereas in the case of Asia 1 it was 6.4 only.

The LDH activity in the supernatant from virus-infected and uninfected cells are shown in Fig. 2. It is evident that as high as 2 450 units of LDH

**Table 1. Effect of Actinomycin D on infectivity of FMDV types 0 and Asia 1**

| FMDV | Concentration of Actinomycin D (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 10 | 25 | 50 |
| Type 0 | 7.2* | 7.2 | 6.2 | 5.9 | 4.2 |
| Asia 1 | 6.2 | 6.2 | 5.4 | 4.9 | 3.9 |

*Mean infectivity titre = log 10 $TCID_{50}$/ml, 20 hr p.i.

activity were present at 20 hr p. i. in the case of type 0 virus, whereas only 1 950 units were present in the case of Asia 1 virus.

Inhibition of protein synthesis in BHK-21 Razi cells infected with type 0 and Asia 1 is shown in Fig. 3. It is observed that at 240 min p.i. protein synthesis was inhibited by 47% in the case of type 0 and by 30% in the case of Asia 1 virus. The profile of RNA synthesis in BHK-21 Razi cells infected with FMDV is shown in Fig. 4. At 240 min p. i. RNA synthesis was stimulated up to 221% in the case of type 0 and up to 153% in the case of Asia 1 virus.

The results on the effect of different concentrations of Actinomycin D on viral RNA synthesis are shown in Figs. 5 and 6. The results show that the synthesis of viral RNA was inhibited progressively with increasing concentration of Actinomycin D in both FMDV types 0 and Asia 1. At 300 min p. i. Actinomycin D at 50 μg/ml concentration inhibited RNA synthesis by 40% in the case of FMDV type 0 and by 27% in the case of Asia 1.

The results on the effect of various concentrations of Actinomycin D on the infectivity titre ($TCID_{50}$) of FMDV types 0 and Asia 1 are shown in Table 1. It is evident from the results that at concentration of 5 μg/ml of Actinomycin D no decrease in infectivity titre was observed in both FMDV types 0 and Asia 1 whereas at 50 μg/ml concentration the infectivity titre was decreased significantly in both FMDV types 0 and Asia 1.

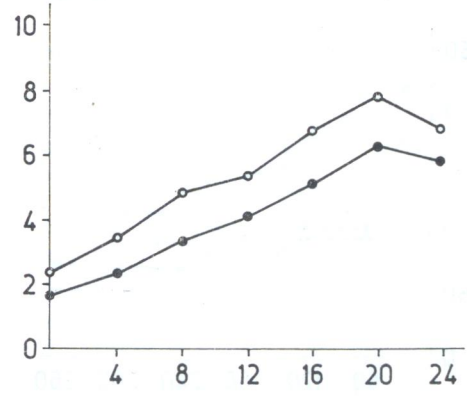


**Fig. 1.**
Growth profile of FMDV types 0 and Asia 1 in BHK-21 Razi cells
Type 0 virus (○); Asia 1 virus (●)
Abscissa: time (in hr p.i.); ordinate: log $TCID_{50}$/ml

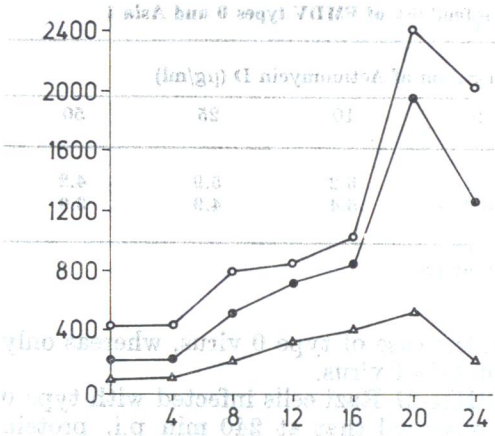


**Fig. 2.**
LDH activity in BHK-21 Razi cells infected with FMDV types 0 and Asia 1 (Each value is average of three determinations). Uninfected cells (△); Type 0 virus (○); Asia 1 virus (●)
Abscissa: time (in hr p. i.); ordinate: LDH units/min/ml

*Discussion*

The susceptibility of BHK cells to FMDV types 0, A, C and Asia 1 was different when cells were obtained from different laboratories (Vasantha and Lal, 1982). A high virus yield in a given cell culture system is the pre-requisite for the large scale vaccine production (Rweyemamu, 1978). The growth profile of FMDV types 0 and Asia 1 indicates the susceptibility of BHK-21 Razi cells to FMDV types 0 and Asia 1. In addition, LDH activity has been used as an indicator of virus growth (Spier, 1977; Whiteside and Spier, 1981). LDH assay in the Auto Analyser was used to monitor virus production in a large scale production unit (Whiteside and Spier, 1981). Furthermore, LDH activity can be used as a parameter to determine the susceptibility of BHK-21 cells to FMDV infection (Spier, 1977). The LDH activity profile shows that Asia 1 is a slow growing virus when compared to type 0. LDH activity in FMDV-infected cells can also be used as a marker to study the effect of antiviral agents.

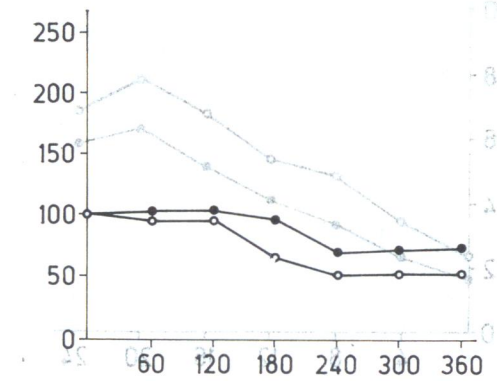


**Fig. 3.**
Effect of FMDV infection on BHK-21 Razi cell protein synthesis
Type 0 virus (○); Asia 1 virus (●)
Abscissa: time (min p.i.); ordinate: per cent of control (cpm/mg protein)

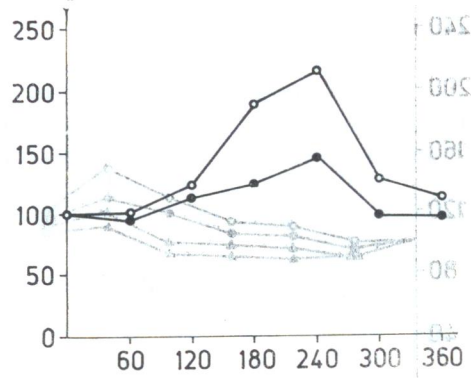


**Fig. 4.**
Effect of FMDV infection on BHK-21 Razi cell RNA synthesis
Type 0 virus (○); Asia 1 virus (●)
Abscissa: time (in min p.i.); ordinate: per cent of control (cpm/mg RNA)

Host cell protein synthesis was suppressed by the virus before replication and transfer of FMDV RNA (Bachrach, 1977). Polatnick *et al.* (1968) have reported that protein synthesis was inhibited in BHK-21 cells infected with FMDV which is in agreement with the present investigation. It is suggested that the inhibition of protein synthesis in infected cells could also be used as a parameter to determine the susceptibility of cells to FMDV infection (Polatnick *et al.*, 1968). Vande Woude *et al.* (1970) have observed 2—3 fold increase of RNA synthesis in BHK-21 cells infected with FMDV which was also observed in present investigation. This increase in RNA synthesis in BHK-21 cells infected with FMDV has been attributed to the synthesis of viral RNA.

Actinomycin D at a concentration of 50 μg inhibited virus-induced RNA dependent RNA polymerase in BHK-21 Glasgow cells infected with FMDV types 0 and SAT (Black and Brown, 1968; 1969). In the present investigation Actinomycin D at a concentration of 50 μg/ml (2 = $10^6$ cells) inhibited synthesis of virus-specific RNA up to 40% in the case of FMDV type 0, and up to 27% in the case of FMDV type Asia 1. The virus titre of FMDV

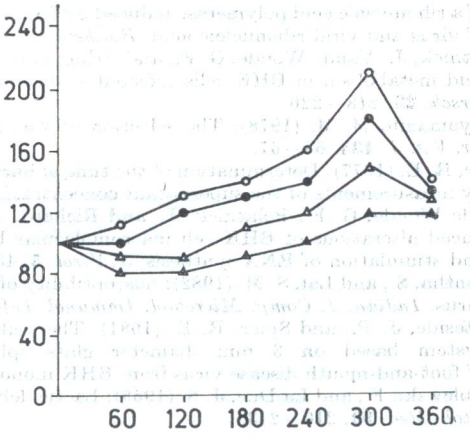


**Fig. 5.**
Effect of Actinomycin D on the synthesis of virus-specific RNA in BHK-21 Razi cells infected with FMDV type 0
Actinomycin concentration: 5 μg/ml (○); 10 μg/ml (●); 25 μg/ml (△); 50 μg/ml (▲)
Abscissa: time (in hr p.i.); ordinate: per cent control

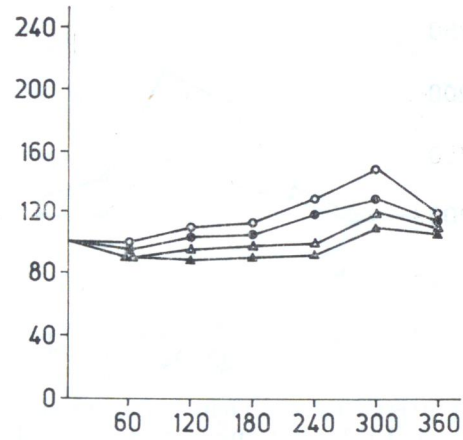


**Fig. 6.**
Effect of Actinomycin D on the synthesis of virus specific RNA in BHK-21 Razi cells infected with FMDV Asia 1
For legend see Fig. 5.

types 0 and Asia 1 infected BHK-21 Razi cells decreased significantly at a concentration of 50 μg/ml of Actinomycin D. In conclusion, the growth profile, LDH activity, and inhibition of host protein synthesis could be used as parameters for determining the susceptibility of BHK-21 cells to FMDV and for selecting the seed virus in large scale vaccine production.

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