Effect of Virus Input Multiplicity and Tissue Cell Concentration on Growth of Rift Valley Fever Virus

MICHAEL D. ORLANDO, RICHARD D. DeLAUTER, AND JEAN M. RILEY

Department of the Army, Fort Detrick, Frederick, Maryland 21701

The effects of virus input multiplicity and of tissue cell concentration upon the growth of Rift Valley fever virus in L cells (Earle) were determined. The titers obtained in suspension cultures with cells obtained from two separate laboratories were significantly different. With both monolayer culture and suspension culture systems, a virus input multiplicity of 2.5 resulted in the greatest proliferation of virus. Optimal viral yields were obtained in suspension cultures containing 4×10^5 tissue cells per ml of suspension.

Although many papers have been presented on Rift Valley fever (RVF) virus since its isolation by Daubney, Hudson, and Garnham (3), the work reported on the growth of RVF virus in tissue culture systems has been meager. Easterday and Murphy (4) described the growth of RVF virus in five established cell lines under a limited set of conditions. Coackley (1) described the alteration in virulence that occurs during serial passage in lamb testis cells. Tribble and Boyle (*unpublished data*) studied the growth of RVF virus in L cells (Earle) in suspension cultures, of 30-ml volume, in Nagle's defined medium. However, the effects of virus input multiplicity (ratio of infectious inoculum to tissue cells) and tissue cell concentration were not reported.

This report describes the growth of RVF virus in L cells (Earle) obtained from two separate sources and the effect of virus input multiplicity and tissue cell concentration upon that growth.

MATERIALS AND METHODS

Virus strain. The pantropic Van Wyk strain of RVF virus described by Kashula (*unpublished data*) was used. Infected lamb serum stored at -70 C was the working seed stock. It had a titer of about $10^{8.3}$ mouse intracerebral median lethal doses (MICLD$_{50}$) per ml.

Tissue culture systems. The established L cell (Earle) line was obtained from two laboratories. In laboratory 1, the cell line had been maintained in suspension cultures (2) in Eagle's minimum essential medium (double strength) with Earle's balanced salt solution supplemented with bovine serum (10%), cysteine (260 μg/ml), and ascorbic acid (50 μg/ml). In laboratory 2, the cell line had been maintained as monolayers in medium 199 (6) containing calf serum (10%).

Cultures were maintained in our laboratory as either monolayers or suspension cultures at 36 C. The monolayer cultures were prepared in 650-ml roller bottles (Strumia blood plasma bottles; Arthur Thomas Co., Philadelphia, Pa.) rotated at 7 rev/min. The total cell concentration in the average roller bottle was 2.8×10^7 cells with 80 ml of growth medium. Suspension cultures were prepared in 100-ml volumes in centrifuge bottles (250-ml) fitted with Teflon-coated, suspended bar magnets and removable vents (Rosensteel and Daniels, *personal communication*). Medium 199 containing 10% calf serum was used as the growth medium. Penicillin (250 units/ml), dihydrostreptomycin (250 μg/ml), and kanamycin (50 μg/ml) were added to the growth medium only during the viral infectivity studies.

Infection of tissue cultures. The roller bottles and suspension cultures were infected with sufficient inoculum in a total volume of 10 ml of growth medium to obtain virus input multiplicites of 1, 2.5, 5, and 10.

The suspension cultures were started with total cell populations of 20, 40, 80, 120, 160, or 200 million cells. The amount of virus inoculum varied with the total cell population so that a constant virus input multiplicity of 2.5 was maintained.

The culture systems were incubated 36 C.

Assay. Ten-fold serial dilutions were made in medium 199 containing 10% calf serum. Mice (Swiss-Webster, 10 to 14 g) were injected intracerebrally with 0.03-ml amounts of appropriate virus dilutions. Ten mice were injected per dilution, and deaths were recorded during the next 6 days. Deaths prior to 24 hr were assumed to be traumatic. The LD$_{50}$ values were calculated by the method of Reed and Muench (6).

RESULTS

The results of the study (means of four replicates) on the effect of virus input multiplicity are presented in Table 1. In the roller bottles (mono-

TABLE 1 *Effect of virus input multiplicity on the growth of Rift Valley fever virus*

Virus input multiplicity	Titer (MICLD$_{50}$/ml) at various times (hr)						
	Monolayer cultures[a]		Suspended cultures[b]				
	0	72	0	24	48	72	96
1	6.00	7.60	6.60	—[c]	6.44	6.30	5.34
2.5	6.40	7.99	6.90	6.51	6.51	7.17	5.65
5	6.70	7.65	7.20	7.50	6.80	6.74	5.82
10	7.00	7.69	7.50	7.26	6.70	6.73	6.27

[a] Results are the means of the titers obtained from cells secured from both laboratories (no significant difference between cell sources).

[b] Results are those obtained with cells secured from laboratory 2 only.

[c] Samples lost.

TABLE 2. *Effect of tissue cell concentration of suspension cultures on the growth of Rift Valley virus*

Cell concn (millions per suspension)	Titer (MICLD$_{50}$/ml)		Virus output factor			
			Per tissue culture cell		Per viral LD$_{50}$ of inoculum	
	1[a]	2	1	2	1	2
20	6.30	6.81	10	32	4	13
40	6.87	7.45	19	70	8	28
80	6.56	7.16	5	18	2	8
120	6.31	7.42	2	22	1	13
160	6.26	7.16	1	9	>1	4
200	—[b]	7.16	—	7	—	3

[a] Numbers 1 and 2 denote the two different laboratory sources of L cell (Earle) lines.

[b] Samples were gelatinous and could not be resuspended in diluent.

layer system), the effect of virus input multiplicity could readily be seen by comparing the virus output factors (yield titer divided by inoculum titer) as shown in the following: at an input multiplicity of either 1 or 2.5, the output factor was 40; at an input of 5, the output was 8; at an input of 10, the output was 5.

Judged by the virus output factors, the use of either the 1- or 2.5-virus input multiplicities produced the maximal growth ratios; however, based on the largest virus yield per unit volume (MICLD$_{50}$/ml), the 2.5-virus input multiplicity was optimal.

The results of the study on virus input multiplicities in suspension cultures were disappointing. Apparently, the only virus input multiplicity that resulted in a proliferation of the virus occurred

with the 2.5 virus input dose after 72 hr (an increase of only 0.27 log).

The results of the study (means of four replicates) on the effect of various tissue cell concentrations are summarized in Table 2; only the virus titers obtained after 72 hr of incubation are presented. No significant differences in titers were observed when the cultures were prepared with cells secured from the same source. However, significant differences were observed when the cultures were prepared with cells secured from two different laboratories. Thus, at each cell concentration used, the virus titers obtained with the cells secured from laboratory 2 were significantly greater than the titers obtained with cells secured from laboratory 1.

The effect of tissue cell concentration can be seen more readily by comparing the virus output factors. The optimal virus yield was obtained at a concentration of 40 million cells per 100 ml (4 × 10^5/ml) with cells originally obtained from laboratory 2.

DISCUSSION

Although the source of L cells (Earle) did not affect the growth or yield of RVF virus in monolayers, a significant effect was seen in the suspension cultures. With either the monolayer or the suspension culture system, the optimal virus input multiplicity was apparently 2.5. Although the difference in virus input multiplicity varied 10-fold, there was complete destruction of the tissue cells by 72 hr in the monolayer cultures.

The optimal cell concentration in the suspension culture system is apparently 40 × 10^6 cells per 100 ml (4 × 10^5/ml). Many factors may influence proliferation of the virus. Since the suspension culture system was not designed with precise controls, the reasons for optimal growth at this cell concentration cannot be specified. If the suspension culture study had been conducted in a "controlled" manner as described by Daniels et al. (2), a better understanding of the metabolic effects of viral infection could probably have been reached. Since cytolytic viruses alter the biosynthetic processes of the tissue cells, one could then study the biochemical changes that occur in the RVF virus-tissue cell infection.

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