Effect of Foot-and-Mouth Disease Virus on Protein Synthesis and Ribonucleic Acid Polymerase Activity at Various Temperatures (34715)

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(Introduced by H. L. Bachrach)

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Foot-and-mouth disease virus (FMDV) infection of baby hamster kidney (BHK 21) cells alters normal protein and nucleic acid metabolism (1), RNA methylation (2), and induces the formation of an RNA-dependent FMDV-RNA polymerase (3). The present investigation was initiated to determine the effect of temperature on the sequence of activities associated with virus production, protein metabolism, and FMDV-RNA polymerase formation in infected cells incubated at 25 to 45°. Data will also be presented on the *in vitro* activity of FMDV-RNA polymerase at various temperatures.

Materials and Methods. Cell culture and virus infection. Baby hamster kidney-21 cells, clone 13 (American Type Culture Collection, Rockville, Maryland) were grown at 37° in monolayers in 2-liter Baxter bottles on a rotating mill as previously described (4). The growth medium was a modified Eagle's, 0.02 *M* in Tris, containing 10% tryptose phosphate broth and 10% bovine serum. Experiments were done on 6- to 7-day-old cells with each bottle containing from 6 to 8 $\times$ 10^8 cells. Cells were infected at a multiplicity of 10 plaque-forming units (PFU) of FMDV, type A, strain 119/cell as described earlier (1), using a partially purified "aqueous phase" virus (5). When virus production was to be measured, unadsorbed inoculum was removed after 30-min incubation by five rinses with 0.16 *M* Tris buffer pH 7.5. At the desired time, cells were lysed with 0.5% sodium dodecylsulfate, and a portion of the resulting solution (1) was assayed in primary calf kidney cell cultures (6).

Protein synthesis. Cells were pulse-labeled for 15 min before harvest with 1 μCi of ^{14}C reconstituted protein hydrolyzate (Schwarz Bioresearch, Inc., Orangeburg, New York). As described in detail before (1), the bottles at harvest were cooled in an ice bath, and cells were collected by scraping with a rubber policeman. The cells were precipitated with 5% trichloracetic acid, dissolved in 1 *N* NaOH and a portion was taken for measurement of radioactivity. Protein concentrations were determined by the biuret reaction.

Polymerase preparation and assay. The enzyme was prepared and assayed as described previously (3). Briefly, cells were harvested 3.5 hr postinfection. The cytoplasm from lysed cells was spun at 100,000 *g*, and the pellet containing the enzyme activity was suspended in 0.25 *M* sucrose, 0.001 *M* magnesium ion at a protein concentration of 5–10 mg/ml. The assay measured the incorporation of tritiated uridine triphosphate into acid-insoluble material in a cell-free system containing the four ribonucleoside triphosphates, magnesium ion, and an ATP generating system.

Temperature control. Cells were incubated in water baths maintained at $\pm$0.5° of the desired temperatures.

Results. The protein synthesis of normal cells as a function of temperature is shown in Table I. Cells did not tolerate exposure to 45°. Cells exposed to 41° frequently metabolized greater amounts of protein than at 37°, but results were inconsistent. As expected, protein synthesis continued to decrease with falling temperatures. The value obtained at

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TABLE I. Changes in Protein Synthesis in Normal and FMDV-Infected BHK Cells After 5-hr Incubation at Various Temperatures, Correlated with Changes in Virus Production.

	Protein synthesis (PS)			
	Normal cells		Infected cells; inhibition of PS of normal cells at same temp[c] (%)	
Temp (°C)	Cpm/mg of protein[a]	% of 37° value[b]		Virus titer (log PFU/ml)
25	735	20.4 ± 3.3	33.4 ± 4.8	5.2[d]
30	1937	55.5 ± 6.9	43.8 ± 1.8	7.2
33	3020	74.4 ± 9.0	53.8 ± 7.9[e]	8.4
37	3689	100.0	76.6 ± 5.4	8.8[d]
41	4316	95.8 ±21.2	79.2 ± 6.0	8.5
45	—[f]			3.9[f]

[a] Data from a representative experiment.

[b] Mean and average deviation of 4–6 experiments.

[c] Mean and average deviation of 3–4 experiments.

[d] Control titers obtained by harvesting infected cells immediately after removal of unadsorbed inocula at 30-min postinfection yielded values of 5.1–5.4.

[e] Metabolism of infected cells at 33° was always the highest of the temperatures tested; e.g., protein synthesis at 33° in infected cells was, on the average, $74.4/100.0 \times (100-53.8)/(100-76.6) = 147\%$ of the 37° value.

[f] About ½ the cells sloughed off from glass.

25° was only 20% of that at 37°. Inhibition of this normal protein synthesis was studied in infected cells incubated for 5 hr (Table I). It had previously been established that this time period was necessary for maximum virus production at 37° (7). In infected cultures, protein synthesis was inhibited at all temperatures. Infected cells incubated at 25° showed about 33% reduction in normal protein synthesis, while synthesis of cells maintained at 37° was inhibited by about 77%. The protein synthesis of cells infected at 33° was always higher than at the other temperatures tested (see footnote, Table I), because of a high normal protein synthesis and a less than maximal viral inhibition of this synthesis.

As shown in Table I, virus production was highest in cells incubated at 37° for 5 hr. Virus production was not detectable at 25°. Control titers obtained by harvesting infected cells immediately after removal of unadsorbed inocula yielded values of 5.1 to 5.4 PFU/ml compared to the value of 5.2 PFU/ml for the 25° incubated cells. Nevertheless, as pointed out previously, protein synthesis was still inhibited by about 33% in these cells.

Table II presents data establishing the time required for cells incubated at various temperatures to produce maximum amounts of virus. The same maximum virus titer of about $10^{8.8}$ PFU/ml was ultimately reached in cells incubated at 30, 33, 37, and 41° for 12, 6, 5, and 4 hr, respectively. Cells incubated at 25° did not apparently produce virus for at least 12 hr, but did shown 7.1 PFU/ml of activity at 24 hr.

It previously had been established that maximum synthesis of FMDV-RNA dependent RNA polymerase occurred 3.5 hr postinfection in BHK cells incubated at 37° (3). Table II shows, contrary to the results on virus production, that viral RNA polymerase production was always highest at 37°. For example, cells incubated at 41° possessed only about one-fourth the maximal RNA polymerase activity of 37° incubated cells, although as shown before, virus production at this elevated temperature was the same as at 37°. The time of maximal enzyme activity always preceded that of maximal virus production.

TABLE II. Production of Virus[a] and Virus-Induced RNA Polymerase[b] in BHK Cells as a Function of Time and Temperature.

	25°		30°		33°		37°	41°	
Postinfection (hr)	Virus	Polymerase	Virus	Polymerase	Virus	Polymerase	Virus	Virus	Polymerase
2									5.1 ± 2.3
2.5								6.4	7.1 ± 1.6
3									19.4 ± 2.3
3.5		<0.3		1.9 ± 0.6		19.2 ± 3.7		7.8	25.0 ± 4.2
4						28.7 ± 5.0		8.7	22.5 ± 3.8
4.5				7.9 ± 1.1		50.3 ± 7.2	8.6	8.6	
5	5.2	<0.3	7.2		8.4	62.6 ±10.1	8.8	8.5	
5.5				35.5 ± 0.8	8.7	54.9 ± 4.4	8.8		
6			8.0		8.8				
6.5					8.6				
7			8.4	46.4 ± 3.7					
8.5			8.6	33.8 ± 0.4					
10			8.5	26.7 ± 2.2					
12	5.4		8.7						
24	7.1		8.3						

[a] Log PFU/ml units.

[b] Values, averages of 2–4 experiments, are percentages of activity of enzyme (cpm/mg of protein) related to enzyme prepared at the same time from cells infected for 3.5 hr at 37°. Activities of the 37° enzymes ranged from 42,100 to 69,086 cpm/mg of protein.

Since changes in temperature significantly affected viral RNA polymerase activity in infected cells, the effect of temperature on enzyme activity in a cell-free assay system was also studied. The data in Table III show that the enzyme system is active from 25 to 50°, with enzyme activity increasing with temperature. However, enzyme activity decreased after extended incubation at 45 and 50°. Maximum incorporation of labeled substrate was always reached in the assays performed for 60 min at 33, 37, 41, and 45°, while assays at 30 and 25° required 120 and 180 min, respectively. Enzyme activity at 50° was high, but never reached the 60 min, 37° value.

In a further attempt to detect possible differences in the various polymerase preparations, the reaction products were separated on 5–25% sucrose gradients. Profiles of the

TABLE III. Effect of Incubation Time and Temperature on Cell-Free Polymerase Assay.[a,b]

	Temp (°C)					
Time (min)	25	30	33	41	45	50
10	44.7 ± 4.2	56.9 ± 1.9	80.6 ± 3.5	109.0 ± 1.0	138.0 ±10.5	152.0 ±10.7
30	47.0 ± 0.2	66.1 ± 5.5	100.0	100.0	113.0 ± 1.3	88.1 ± 7.6
60	66.0 ± 4.5	96.0 ± 3.7	100.0	100.0	100.0	73.7 ± 2.2
120	85.1 ± 1.8	100.0	100.0	100.0	83.2 ± 5.1	50.2 ± 4.5
180	100.0					

[a] Data, averages of 2–3 experiments, are percentages of values obtained at 37° for the same incubation time. Representative assay results at 37° for 10, 30, 60, and 120 min incubation are 21,840; 44,464; 62,133; and 61,760 cpm, respectively.

[b] Maximum incorporation always occurred in the 60-min assay at 37°. Assays performed at temperatures other than 50° reached this same maximum value in 60 to 180 min. At 50°, incorporation was maximal at 45 min but equal only to 78% of the 60 min, 37° value.

products of enzymes prepared from infected cells incubated at different temperatures did not show any qualitative differences. Gradient profiles of cell-free assay products from different incubation temperatures were also qualitatively similar. The typical RNA profile consisted of 37 S viral RNA, 20 S RNase-resistant RNA, and heterogeneous 26–28 S components (3).

Discussion. The inhibition of protein synthesis in infected cells incubated at 25° in the absence of any significant RNA polymerase and infectious virus production may have one or more explanations. It is known from earlier work that protein synthesis is inhibited in FMDV-infected cells in the absence of viral RNA synthesis. For example, protein synthesis was inhibited in infected cells at a time before viral RNA appears (90 min post infection) (1), or in cells treated with an inhibitor (guanidine) of viral RNA polymerase formation (3, 8). Translation of the viral RNA message is apparently occurring in cells incubated at 25° for 5 hr, forming products capable of interrupting host-cell protein synthesis. Alternatively, the agent responsible for hindering protein synthesis may be associated with and enter the cell with the viral inoculum.

The data in Table II showed that production of infectious virus was comparable in cells incubated at temperatures from 30 to 41°, although viral RNA polymerase formation was highest at 37°. At the latter temperature, the enzyme probably produces viral RNA in excess of that required for incorporation into infectious units. Earlier work with actinomycin D treatment of FMDV-infected BHK cells showed that at 35–40% inhibition of viral RNA polymerase formation did not affect the amount of infectious virus subsequently formed (9).

The lowered values of polymerase activity in cells incubated at temperatures other than 37° cannot be attributed to inactivation of the enzyme at those temperatures. Assays performed on enzyme preparations between 25 and 50° all showed that the enzyme was fully active. Thus, temperature probably affected mechanisms involved in synthesizing the enzyme *in vivo,* rather than enzyme activity itself.

Summary. Protein synthesis in FMDV-infected BHK cells was inhibited in cells incubated at 25 to 41°. The metabolism of infected cells was always highest at 33°. Infected cells incubated at 30 to 41° produced the same high levels of virus, but in times ranging from 12 to 4 hr, respectively. Virus-induced RNA polymerase activity was always highest at 37°, being about twice as great as that formed at any other temperature. The cell-free polymerase system was active from 25 to 50° and showed maximal activity after 60-min incubation at 37°. Sucrose gradient profiles of the products formed under all the conditions employed did not reveal any qualitative differences.

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