Title
Efficient, inefficient, and abortive infection of different mammalian cells by small RNA viruses.

Journal
Virology, 33(1)

ISSN
0042-6822

Authors
Buck, CA
Granger, GA
Taylor, MW
et al.

Publication Date
1967-09-01

DOI
10.1016/0042-6822(67)90091-8

VIROLOGY 33, 36–46 (1967)

Efficient, Inefficient, and Abortive Infection of Different Mammalian Cells by Small RNA Viruses[1]

C. A. BUCK,[2] G. A. GRANGER, M. W. TAYLOR,[3] AND J. J. HOLLAND

Department of Molecular and Cell Biology, University of California, Irvine, California 92664

Accepted May 12, 1967

We have compared the biochemical capacity of cultured cells from a variety of animal sources to replicate the genetic information of two small RNA viruses.

Mengovirus is shown to infect and kill cultured cells from many animal species regardless of whether the virus replicated well, moderately, or poorly in any cell type. The production of infectious viral RNA, and of viral protein by any cell type was proportional to the yield of intact virus produced by that cell type.

The virus yield per cell was compared in a variety of cells employing two small RNA viruses (mengovirus and bovine enterovirus 1) capable of adsorbing to, penetrating, and infecting all the cells tested. The yields varied over a range of more than a thousandfold with both viruses, but the patterns of cell susceptibility were markedly different for each virus. Both viruses caused profound inhibition of host cell protein synthesis even in cells which replicated the virus very inefficiently. Possible biochemical explanations for such highly specific host cell restriction are discussed.

INTRODUCTION

The small RNA animal viruses often show a strict host range specificity. In the case of certain human enteroviruses, this cell specificity is attributable in large measure to the presence or absence of cell receptors for virus attachment and entrance into the cell (McLaren and Holland, 1959; Holland and Hoyer, 1962). In such cases it is possible to infect many normally resistant cells merely by exposing cells to infectious nucleic acid (Holland *et al.*, 1959), or by enclosing viral nucleic acid in the protein coat of a virus which is able to adsorb to the resistant cell (Cords and Holland, 1964).

There are numerous reports in the literature of myxovirus infections in which the ultimate result of viral infection is cell destruction with concomitant production of large quantites of viral antigen without the production of mature virus particles (Prince and Ginsberg, 1957; Walker, 1960). Abortive infections are also characterized for adenovirus infection of monkey cells (Feldman *et al.*, 1966), simian virus 40 transformation of hamster cells (Gerber, 1966), avian tumor virus infection of mammalian cells (Vogt, 1965; Hanafusa *et al.*, 1966), and vesicular stomatitis virus (VSV) infection of human leukocytes (Edelman and Wheelock, 1966). In these cases, normal viral replication can be achieved either by superinfection with a helper virus (O'Conor *et al.*, 1963; Feldman *et al.*, 1965), cytoplasmic interaction with normally susceptible cells (Gerber, 1966; Sarma *et al.*, 1966; Vogt, 1965), or treatment of the infected cells with phytohemagglutinin (Edelman and Wheelock, 1966).

In the present study we have employed two small RNA virsuses, mengovirus and

[1] This work was supported by a grant from the U. S. Public Health Service, NIH Grant no. GM CA 14124-01, and postdoctoral grants nos. 1-F2-CA-30,113-01 (MWT); 5-F2-A1-13,382-02 (CAB), and 1-F2-CA-25,607-01 (GAG).

[2] Present address: Department of Therapeutic Research, University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

[3] Present address: Department of Microbiology, Indiana University, Bloomington, Indiana.

bovine enterovirus 1, which are able to adsorb to a wide variety of host cells and which reproduce with widely varying efficiencies in different cells. This paper describes some biological properties of abortive infections with these two small RNA viruses, the ultimate objective of such studies being to determine why different cells, even from the same species of animal, treat the same viral RNA genetic message in different ways. A study of the intracellular barriers to virus replication is important not only to an understanding of the problem of virus control of host-cell synthesis, but also to an understanding of general mechanisms of control of synthesis in animal cells.

MATERIALS AND METHODS

Virus stocks. The mengovirus used in these experiments was kindly provided by Dr. John Colter of the University of Alberta, and was grown in HeLa cell cultures unless otherwise stated. The bovine enterovirus type 1 was kindly provided by Dr. Torbjorn Moll of Washington State University, and grown on MBK cells.

Cell culture. HeLa cells were obtained from Flow Laboratories and from the San Diego Public Health Laboratories. The Maden strain of bovine kidney cells (MBK) was supplied by Mrs. Doris Jones of the Colorado Serum Company, Denver, and a second stock of this cell line was obtained from Dr. Jack Stevens of UCLA. A marsupial cell line, Ptk_1, and a strain of hamster cells transformed by adenovirus 3 (adeno-3) were purchased from Flow Laboratories. Mouse embryo fibroblasts (MEF) were grown from trypsinized 15 to 18 day embryos. Chick embryo fibroblasts (CEF) were obtained from 9- to 12-day trypsinized embryos. Whole chorioallantoic membranes (CAM) were removed from chicken eggs containing 12- to 15-day embryo. Mouse macrophages were washed from the peritoneum of mice with saline 24–72 hours after the intraperitoneal injection of 1 ml of a 1% suspension of corn starch in saline. The cells flushed from the mice were washed twice in isotonic saline before being placed in culture.

Culture medium. All cells were grown or maintained in Eagle's minimal essential medium containing 10% calf serum; streptomycin, 100 μg/ml; penicillin, 100 μg/ml; mycostatin, 50 μg/ml; and at intervals, kanamycin, 100 μg/ml. Whenever cells were to be labeled with a radioactive amino acid, the cold amino acid corresponding to the one being used in the experiment was omitted from the medium, and the medium was supplemented with 2% dialyzed calf serum.

Virus assay and infection. All monolayers were infected with the designated amount of mengovirus adsorbed from 0.2 ml of Eagle's medium. Unless otherwise stated, the monolayers were washed 3–5 times with either warm medium or warm saline following infection. Two milliliters of medium was then added to the monolayers and they were incubated for a given period of time to allow virus replication. At stated times after infection, monolayers were frozen and thawed 3 times to disrupt cells and release virus. Mengovirus was assayed by plaque technique on HeLa cell monolayers, and bovine enterovirus 1 by plaque technique on MBK cells. Dilutions of the virus in 0.2 ml of medium were adsorbed to the monolayers at 37° with regular shaking for 1 hour. After this time, the monolayers were overlaid with medium containing 0.4% washed Difco Bacto agar. The monolayers were stained with crystal violet, and plaques were counted 40–48 hours after infection.

Determination of the incorporation of ^{14}C amino acids into protein. Cells were labeled as monolayers on glass. Following a pulse with a ^{14}C amino acid in Eagle's medium, the monolayers were removed from the bottles with 0.5 ml of 0.1 *N* NaOH. The volume was raised to 2.5 ml by the addition of saline, and equal volumes of 10% trichloroacetic acid were added. Following 3 trichloroacetic acid precipitations with intermittent heating to 100° for 5 minutes, the final precipitate was caught on a cellulose nitrate filter and counted in a gas flow counter. All amino acids used in these experiments were purchased from New England Nuclear Corporation, Boston, Massachusetts.

Extraction and assay of infectious RNA. A method described previously (Holland *et al.*, 1960) was used to extract the infectious

TABLE 1

GROWTH OF MENGOVIRUS IN VARIOUS CELL CULTURES[a]

Cell culture[c]	Total PFU of CAV[b]	Total PFU of virus yield
HeLa	1×10^6	1×10^{10}
CEF	10^4	9×10^7
MBK	3×10^5	8×10^6
Macrophage	8×10^2	3×10^3
PtK_1	$< 10^3$	3×10^6
CAM	1×10^3	4×10^5
Adeno-3	4×10^5	1×10^{10}
MEF	1×10^6	1×10^9
L cell	8×10^5	1×10^{10}

[a] In all cases, except that of the CAM, 2×10^6 cells were infected with 2×10^8 PFU of mengovirus. Whole CAM's were exposed to 2×10^8 mengovirus. Monolayers were washed 3–5 times with medium after infection. Virus yields were determined 24 hours after infection.

[b] CAV is cell-associated virus (cell-bound infectious inoculum virus).

[c] HeLa = a human carcinoma cell line, L cell = a mouse fibroblast cell line, CEF = chick embryo fibroblasts, MBK = Maden bovine kidney cell line, PtK_1 = a marsupial cell line (kangaroo rat), CAM = intact chorioallantoic membranes from chick embryos, Adeno-3 = an adenovirus-transformed hamster cell line, MEF = mouse embryo fibroblasts, BHK_{21} = a baby hamster kidney cell line, Macrophage = mouse peritoneal macrophages.

RNA, except that the cells were treated with 0.5% sodium dodecyl sulfate (SDS) immediately prior to phenol extraction. Infectious RNA was assayed using the DEAE-dextran procedure of Vaheri and Pagano (1965).

Preparation of fluorescent antiserum. Mengovirus antiserum was obtained from rabbits injected with concentrated suspensions of mengovirus plus Freund's complete adjuvant (Difco). Fluoroescein isothiocyanate 10% Celite (Calbiochem, Los Angeles, California) was conjugated to the γ-globulin fraction of antiserum according to the procedure of Rinderknecht (1962). After conjugation and removal of excess dye, the antiserum was adsorbed successively with acetone-extracted mouse liver, HeLa cells, chick fibroblasts, and MBK cells.

Direct staining with fluorescent antiserum. Cells grown on coverslips were infected as stated above. At designated times following infection, the coverslips were removed from the medium, washed once in saline, and fixed for 5 minutes in acetone. After acetone fixation, the cells were washed and stained according to the procedure of Kelloff and Vogt (1966). Stained cells were examined using an American Optical fluorescence microscope and a BG-12 exciter filter.

RESULTS

Growth of Mengovirus in Various Cells

Monolayers containing approximately 2×10^6 cells of various types were infected with 2×10^8 plaque-forming units (PFU) of virus. Parallel cultures were infected to determine the amount of cell-associated virus or bound uneclipsed inoculum (CAV). These cultures were handled identically to the normally infected cells except that they were incubated for 24 hours following infection in medium containing 100 μg of puromycin per milliliter. Whole chorioallantoic membranes (CAM) were exposed to 2×10^8 PFU of virus and washed and incubated exactly as were the cell cultures. Virus yields were determined 24 hours after infection.

The results of these experiments are presented in Table 1. All infected cells except mouse macrophages produced some virus. However, only adeno-3 cells and mouse fibroblasts gave yields of virus approaching that of HeLa cells. The other cells lines produced yields of virus ranging from 1 to 0.01% of the HeLa cell yield. All infected cultures, regardless of the amounts of virus produced, showed marked cytopathic effect. Attempts to plaque mengovirus on CEF and MBK monolayers were unsuccessful.

Despite the variable yields obtained with various cell lines, we found that mengovirus adsorption occurred with all cells tested (10^7 cells/ml adsorbed over 90% of exposed mengovirus in 2 hours at 37°, when HeLa cells, L cells, macrophages, mouse embryo fibroblasts, or hamster cells were employed).

Effect of Mengovirus Infection on Host Protein Synthesis

Microscopic examination indicated that despite relative low yields of virus, most of

TABLE 2

INHIBITION OF PROTEIN SYNTHESIS RESULTING FROM MENGOVIRUS INFECTION[a]

Cell culture[b]	CPM control	CPM 24 hours post-infection	Ratio CPM-infected cells: CPM controls
HeLa	23,700	5,800	0.24
CEF	13,000	4,000	0.31
MBK	44,200	413	0.01
Macrophages	4,900	400	0.08
PtK_1	7,543	1,773	0.23
CAM	103,000	23,000	0.27
Adeno-3	12,900	800	0.06
MEF	19,600	105	0.01

[a] 2×10^6 cells or whole CAM's (chorioallantoic membranes) were infected with 10^8 PFU of virus. At 24 hours after infection, infected and control cells were pulsed with ^{14}C amino acids. All cells except MBK_1 PtK_1 and CAM were pulsed for 20 minutes with valine-^{14}C, 0.5 μC/ml (208.5 mC/mmole). The other cells were labeled for 60 minutes with phenylalanine-^{14}C, 0.5 μC/ml (393 mC/mmole).

[b] See Table 1, footnote *c*.

the cells in a monolayer were killed as a result of mengovirus infection. To measure the extent of damage to cell synthetic capacity, cultures containing 2×10^6 cells were infected with 10^8 PFU of mengovirus, as previously described, and checked for ability to synthesize proteins. The virus was allowed to replicate for 18–24 hours. At this time, the medium was poured off the monolayers, and replaced with 1 ml of medium warmed to 37° containing 0.5 μC/ml of a ^{14}C amino acid. All cells except MBK, PtK_1, and CAM were labeled for 20 minutes with valine-^{14}C (208.5 mC/mmole); CAM, PtK_1 and MBK cells were labeled for 60 minutes with phenylalanine-^{14}C (393 mC/mmole).

The incorporation of ^{14}C amino acids into protein was reduced by 70% or more in all cultures despite the fact that HeLa, adeno-3, and MEF cells were the only ones that produced maximal yields of virus (Table 2). These results indicate that most of the cells in the monolayer are killed by mengovirus regardless of the virus yield obtained from any cell.

Viral Antigen Synthesis

The lower yield of virus by certain cells might indicate a rather inefficient utilization of viral message by the host. Therefore, it was of interest to determine the extent of viral antigen production by cells which failed to produce normal amounts of virus.

For these experiments, coverslip cultures of chick fibroblasts and HeLa cells were infected at a multiplicity of 2000:1 and allowed to incubate for 10 hours. At this time the coverslips were removed and treated with fluorescent antiserum as described above. The results of one such experiment are shown in Figs. 1 and 2. In the infected HeLa cell monolayer (Fig. 1), every cell exhibited intense cytoplasmic fluorescence. In infected CEF monolayers (Fig. 2), there were normal-appearing cells, cells that were rounding up indicating CPE, but showing little flourescence, and cells that fluoresced brightly. All flourescence could be greatly reduced with anti-mengo serum or prevented by incubating the infected cultures in medium containing puromycin. Uninfected cells gave no fluorescence. It was concluded that CEF cells produced significant amounts of virus protein, but considerably less than did HeLa cells. Similar experiments were performed with MBK cells and mouse macrophages. Infected MBK cells showed very faint, but definite fluorescence as compared to controls, whereas macrophages produced so little viral antigen that it was very difficult to distinguish between control and infected cells. Thus, it appears that the amount of viral antigen produced in the various cells reflects roughly their capacity to produce infectious virus.

Synthesis of Infectious RNA

Monolayers containing 2×10^6 cells were infected with 4×10^8 PFU of virus. Ten hours after infection, the medium was poured off the cells and centrifuged to recover any cells that had floated off the monolayer. The cells and monolayers were washed 3 times with saline, and the infectious RNA extracted. Parallel cultures were infected and harvested at 10 hours in order to determine the amount of whole virus synthesized.

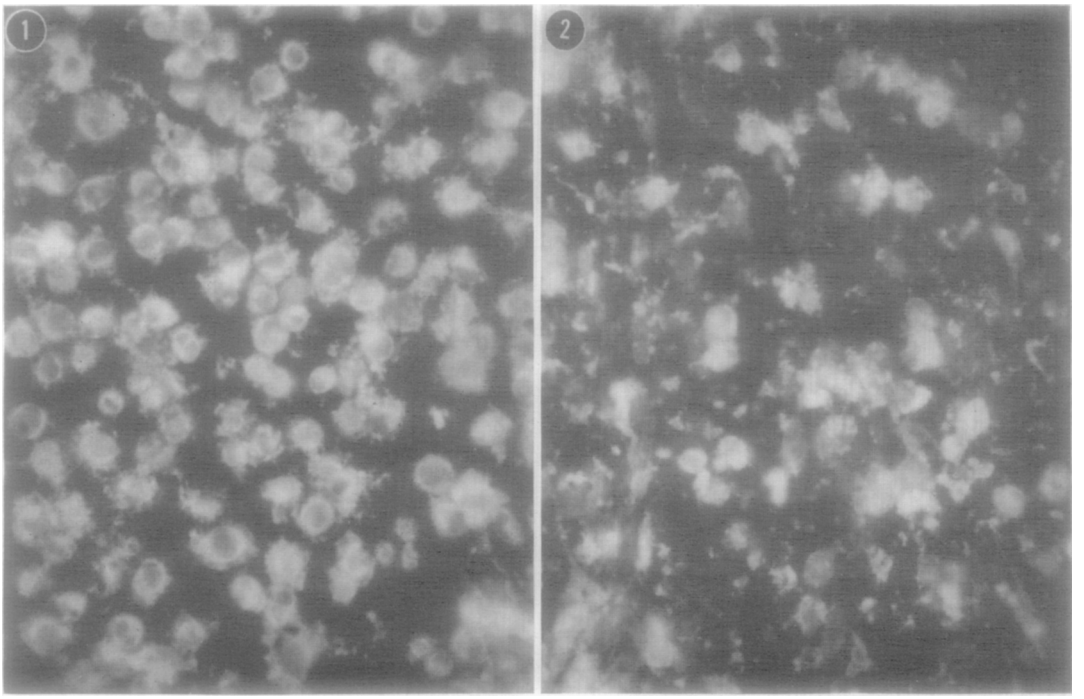

FIG. 1. HeLa cells stained with fluorescent anti-mengo serum 10 hours after infection. Cells were infected with 2×10^9 mengovirus; 10 hours after infection the cells were fixed in acetone and stained. Magnification: × 250.

FIG. 2. Chick fibroblasts stained with fluorescent anti-mengo serum 10 hours after infection. Cells were infected with 2×10^9 mengovirus; 10 hours later they were fixed in acetone and stained. Magnification: × 250.

It is evident from Table 3 that the ratio of infectious virus to PFU of infectious RNA is the same in both HeLa cells and chick embryo fibroblasts. There is, therefore, not an excess of unencapsidated infectious viral RNA synthesized in CEF cultures.

Passage of Mengovirus in Various Cell Lines

Since some mengovirus was produced upon infection of all cells tested except mouse macrophages, serial propagation of mengovirus in cell cultures was attempted. For the first passage, 2×10^6 cells were infected with 10^8 PFU of virus. The virus yield from each passage was used to inoculate subsequent cultures. Puromycin CAV controls were employed at every passage. To further decrease the amount of parental virus present, proflavine-containing mengovirus, 10^8 PFU/ml, was used in the inital infection of chick fibroblasts.

TABLE 3

INFECTIOUS MENGOVIRUS RNA PRODUCTION BY HELA CELLS, CHICK EMBRYO FIBROBLASTS, AND MOUSE PERITONEAL MACROPHAGES[a]

Cells employed[b]	Total PFU virus produced	Total PFU infectious RNA	Ratio PFU infectious RNA: PFU virus yield
HeLa	8×10^9	3×10^5	3.8×10^{-5}
HeLa	7×10^9	2×10^5	2.9×10^{-5}
Chick	5×10^8	2×10^4	4.0×10^{-5}
Chick	4×10^8	1×10^4	2.5×10^{-5}
Macrophages	2×10^8	$< 10^1$	—

[a] 2×10^6 cells were infected with 4×10^8 virus. Ten hours a fter infection, the infectious RNA was extracted with SDS-phenol, and assayed using DEAE-dextran. Cultures for virus yield were treated in exactly the same way except that they were frozen at the end of 10 hours and whole virus was subsequently assayed.

[b] See Table 1, footnote *c*.

TABLE 4

SERIAL PASSAGE OF MENGOVIRUS ON VARIOUS CELL LINES[a]

Cell line[b]	Total PFU of virus produced per passage				
	Passage 1	Passage 2	Passage 3	Passage 4	Passage 7
HeLa	7×10^9	1×10^{10}	—	—	—
Adeno-3	7×10^8	9×10^9	—	—	—
CEF	4×10^7	NA[c]	3×10^4	1×10^3	0
MBK	5×10^6	4×10^4	$< 10^2$	0	—
PtK_1	3×10^6	2×10^5	$< 10^3$	—	—

[a] 2×10^6 cells were infected with 10^8 PFU of virus except for Adeno-3 passage 1, where only 5×10^5 cells were infected. Proflavin mengovirus (10^8 PFU) was used in the initial inoculum of CEF's. All virus yields were assayed 24 hours after infection.

[b] See Table 1, footnote *c*.

[c] Not assayed.

TABLE 5

GROWTH OF MENGOVIRUS ON VARIOUS CELL LINES AFTER PREVIOUS PASSAGE IN A HETEROLOGOUS HOST[a]

Cell line[b]	MBK-Mengo[c] $(3 \times 10^5)^f$	PtK_1-Mengo[d] $(3 \times 10^5)^f$	CEF-Mengo[e] $(6 \times 10^5)^f$
HeLa	6×10^7	4.6×10^7	3×10^7
MBK	8×10^3	1.3×10^5	—
PtK_1	3×10^5	2×10^5	—
CEF	2×10^5	—	2×10^4

[a] 2×10^6 cells were infected with mengovirus grown on the designated cell lines. CAV (bound infectious inoculum virus) was less than 10^3 in all cases.

[b] See Table 1, footnote *c*.

[c] Mengovirus obtained after growth on MBK.

[d] Mengovirus obtained after growth on PtK_1.

[e] Mengovirus obtained after growth on CEF.

[f] PFU of virus used as inoculum for these experiments.

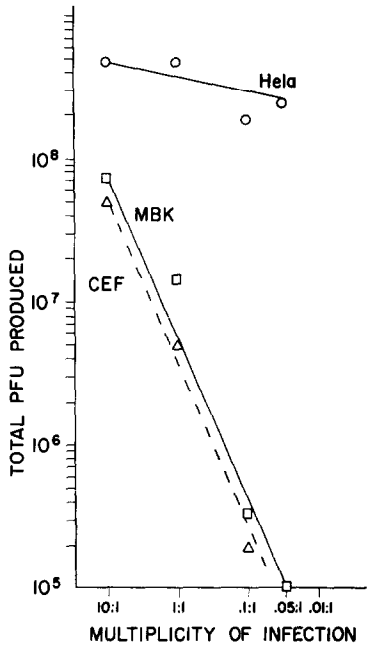

FIG. 3. Mengovirus produced as a result of different multiplicities of infection. 2×10^6 cells were infected with sufficient mengovirus to give the above ratios of PFU per cell. Thirty-six hours after infection, the virus yields were determined.

The results of one series of passages are shown in Table 4. It was never possible to select a mutant of mengovirus that could be passed in CEF, MBK, or PtK_1 cells. As shown in Table 5, even after growth on MBK, PtK_1, or CEF, mengovirus still replicated more efficiently in HeLa cells than in either of the other three cell lines. Again, proflavine mengovirus was used as the initial inoculum in CEF cultures.

To eliminate a possible role of interferon that might be introduced with the virus inoculum and to show that the virus itself was not altered after passage in chick fibroblasts so as to make it difficult for the virus to replicate a second time in these cells (host-induced modification), the following experiment was performed: Chick fibroblasts were infected as described above. After 24 hours, the infected cells were frozen and thawed 3 times, and cell debris was removed

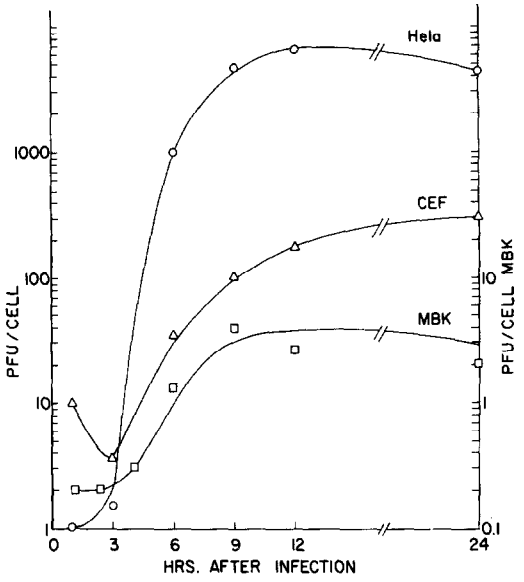

FIG. 4. Growth curves of mengovirus on CEF, MKB, and HeLa cells. 2×10^6 cells were used in all cases. CEF and HeLa cells were infected with 4×10^8 PFU of mengovirus. MBK cells were infected with 10^7 PFU of virus. Note the different scale used to plot MBK yields.

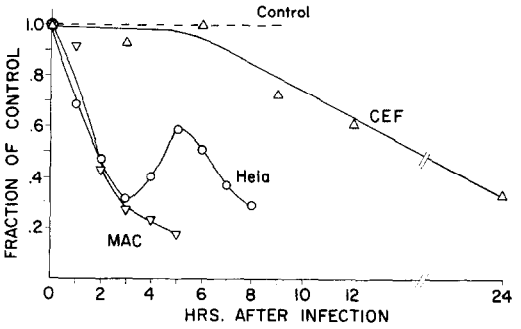

FIG. 5. Inhibition of protein synthesis as a result of mengovirus infection. 2×10^6 cells were infected with 2×10^8 PFU of virus. At the stated times after infection, monolayers were pulsed for 20 minutes with 1μC of valine-^{14}C (208.5 mC/mmole). Control cultures were mock infected and pulsed for 20 minutes at various intervals after infection. Counts per minute of valine-^{14}C incorporated into protein were determined after hot trichloroacetic acid precipitation.

by centrifugation at 10,000 *g* for 10 minutes. The virus was then pelleted by centrifugation at 105,000 *g* for 3 hours and resuspended in Eagle's medium to give a final titer of 2×10^8 PFU/ml. CEF monolayers were then infected with 2×10^7 PFU of this pelleted CEF-mengovirus and allowed to incubate 24 hours. Parallel cultures were infected with 2×10^7 PFU of mengovirus from HeLa cells. Total virus produced from the cells infected with CEF-grown mengovirus was 7×10^6 PFU, and that produced in the control cultures infected with HeLa grown mengovirus was 3×10^6 PFU.

The data in Table 4 suggest that the amount of virus obtained from MBK and CEF cultures was proportional to the inoculum even at multiplicities considerably in excess of 1:1.

Figure 3 shows that the 36-hour yield of mengovirus by MBK and CEF cultures was proportional to the inoculum. Also, despite the fact that there was sufficient time for 3 cycles of replication to take place in all cultures, only in HeLa cells did significant reinfection and replication of virus occur. Similar results were obtained when virus replication was terminated after one cycle of infection (10–12 hours). Regardless of the multiplicity of infection, the yield of virus from HeLa cells was always at least ten times that found in the MBK or CEF cultures.

Figure 4 shows one-step growth curves for mengovirus in HeLa cells, MBK cells, and CEF. Despite the wide disparity in yield, the growth curves are similar except that chick fibroblasts replicate virus at a much slower rate than do the other two cell types.

Kinetics of the Inhibition of Host Protein Synthesis

To examine the inhibitory effect of mengovirus infection of host cell protein synthesis, three cell types were pulse labeled with valine-^{14}C.

The results of these experiments on HeLa, CEF, and mouse macrophages show (Fig. 5) that protein synthesis was inhibited rapidly from the first hour after infection in HeLa cells and macrophages. Between 4 and 6 hours after infection, there is a peak of protein synthesis in HeLa cells which probably represents viral protein synthesis. This would correspond to the pattern of protein

synthesis following mengovirus infection of L cells reported by Franklin and Baltimore (1962). There is no significant inhibition of protein synthesis in chick fibroblasts until 6 hours after infection. In this respect, chick fibroblasts respond to mengovirus infection in a manner similar to strain 67 of Novikoff hepatoma cells (Plagemann and Swim, 1966). In other experiments it was found that the kinetics of inhibition of protein synthesis in MBK cells resembled that seen for mouse macrophages. It is clear that mengovirus can inhibit protein synthesis profoundly in cells in which it replicates poorly or not at all.

Growth of Bovine Enterovirus 1 in Various Cells

All the above findings with mengovirus were subject to the trivial interpretation that certain cells are simply biochemically inefficient in replicating any small RNA virus in comparison to other cells. To examine this possibility, we studied another picornavirus, bovine enterovirus 1. Like mengovirus, this virus was able to adsorb to and infect all tested cells. Table 6 shows that at both high and low multiplicities this small RNA virus replicated in different cells with efficiencies varying over more than one thousandfold. The pattern of cell sensitivities to this virus is quite unlike that seen for mengovirus. As with mengovirus, however, the replication of bovine enterovirus and its cytopathic effect in resistant cells is increased by infecting with higher virus multiplicities. Table 7 contrasts the sensitivities of various animal cells to the two viruses. The two right-hand columns in Table 7 normalize the per cell yield of each virus in different cells by arbitrarily assigning a 100% value to the yield in the most sensitive cell examined. Note for example that L cells produced 3% as many PFU of bovine enterovirus per cell as did MBK cells, whereas the situation is reversed with mengovirus (MBK cells produced 0.06% as many PFU/cell of mengovirus as did L cells).

TABLE 6

GROWTH OF BOVINE ENTEROVIRUS 1 IN VARIOUS CULTURED ANIMAL CELLS AT HIGH AND LOW MULTIPLICITIES

Cell culture[a]	Multiplicity of infection 3:1		Multiplicity of infection 50:1	
	Virus yield[b]	CPE[c]	Virus yield[b]	CPE[c]
MBK	2×10^7	4+	1×10^8	4+
HeLa	5×10^6	4+	8×10^7	4+
BHK_{21}	1×10^6	2+	3×10^7	4+
Adeno-3	2×10^6	4+	1×10^7	4+
CEF	1×10^2	0	6×10^5	3+
Macrophage	—	—	8×10^4	4+
L cell	3×10^5	3+	5×10^6	4+

[a] See Table 1, footnote *c*.

[b] Virus yield is expressed as total PFU virus produced by 2×10^6 cells harvested 18 hours after infection at either multiplicity.

[c] Cytopathic effect (CPE) was determined 18 hours post-infection. 4+ = total cell involvement, 3+ = extensive cytopathology, 2+ = definite involvement of one-third or more of the cells.

TABLE 7

CONTRASTING REPLICATIVE EFFICIENCIES OF MENGOVIRUS AND BOVINE ENTEROVIRUS 1[a] IN VARIOUS CELLS IN CULTURE

Cell employed[b]	Yield per cell		Percent of yield in maximally yielding cell type[c]	
	PFU/Cell BEV1	PFU/Cell Mengo	BEV1	Mengo
L cell	2	3300	3%	100%
HeLa cell	40	3100	66%	94%
Chick fibroblast	0.3	200	0.5%	6%
MBK	60	2	100%	0.06%
BHK	2	490	3%	15%
Adeno-3 hamster	6	2000	10%	65%
Mouse macrophage	<0.001	<0.01	<0.01%	<0.01%

[a] Cell monolayers containing 2×10^6 cells were infected with mengovirus at a multiplicity of 100 and with bovine enterovirus 1 at a multiplicity of 50, and unattached inoculum virus was removed after 1 hour of adsorption. Virus was harvested at 20 hours post-infection and assayed. Cells were counted in a hemacytometer.

[b] See Table 1, footnote *c*.

[c] The cell type which gave the highest yield per cell with either virus was arbitrarily assigned a value of 100% yield.

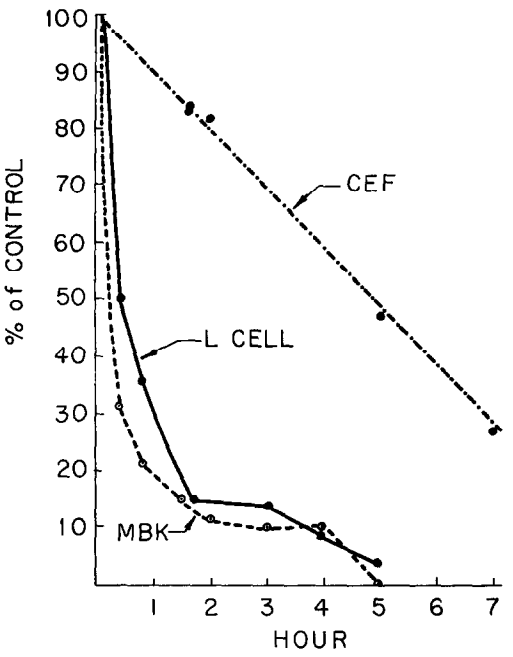

FIG. 6. Inhibition of protein synthesis as a result of bovine enterovirus 1 infection. 2×10^6 cells were infected at a multiplicity of 50. At the stated times after infection, the monolayers were pulsed for 15 minutes with 1μC of phenylalanine-^{14}C (393 mC/mmole), as were uninfected control monolayers. Counts per minute of phenylalanine-^{14}C incorporated into protein were determined after hot trichloroacetic acid precipitation.

Inhibition of Host Cell Protein Synthesis by Bovine Enterovirus 1

It is demonstrated in Fig. 6 that bovine enterovirus, like mengovirus, profoundly inhibits host cell protein synthesis in cells which replicate the virus inefficiently (L cells, CEF) as well as in a very sensitive cell (MBK). It can be seen that there is a remarkably rapid inhibition of protein synthesis in MBK and L cells, and a more gradual but definite inhibition in chick embryo fibroblasts.

It is clear from these comparative results with the two viruses that the wide variation in cellular replication efficiency for either virus cannot be explained by such simple mechanisms as exclusion at the cell surface, failure of uncoating, or simple biochemical inefficiency of certain cells to replicate any small RNA virus genome.

DISCUSSION

We have demonstrated that different cell lines may utilize the same genetic message (viral RNA) with varying degrees of efficiency. This efficiency varies specifically for two different RNA viruses. When virus production was restricted in certain cells, the synthesis of infectious RNA and viral antigens was correspondingly limited, even though the virus inhibited cell protein synthesis in all cases. Failure of early stages (attachment, penetration, or uncoating) cannot explain the extent of virus resistance seen, since these viruses clearly enter and kill the host cell, and grow to limited degrees in them.

A possible explanation for these results is that some host restrictive mechanism with complex specificities is operative in animal cells. It is possible that the more resistant cells break down viral RNA more readily than do sensitive cells, as has been observed in phage systems. Dussoix and Arber (1965) demonstrated that host modification alters phage DNA, and in addition Arber (1965) obtained indirect evidence that specific methylation of viral DNA may be involved. A similar mechanism cannot be ruled out in our studies, although host-induced modification is clearly not a factor here (Table 5). Nor, does interferon appear to play a role here. We have not observed detectable induction of interferon during infection of cells in which virus replication is restricted.

We are presently investigating in detail the possibility that the above results might be due to inefficient or aberrant translation of the viral RNA message in less efficient virus-cell interactions. Qualitative or quantitative variability in tRNA molecules among various animal species or among differentiated tissues and organs could explain the above results in a manner analogous to the case of *amber* and *ocher* suppressor mutant bacteria (Kaplan *et al.*, 1965; Garen *et al.*, 1965). We have recently observed chromatographic variations among certain aminoacyl tRNA species from different animal cells (Taylor *et al.*, 1967). However, the biochemical mechanisms for the virus results above remain to be established.

Of particular interest is the observation that various cells from the same species of animal respond differently to mengovirus infections. Mouse macrophages are killed, but produce no virus, whereas mouse fibroblasts are killed, but produce large quantities of virus (Table 1). This implies that as a result of differentiation, the ability of cells to replicate certain viral genes is altered. The increased vesicular stomatitis virus production resulting from treatment of human leukocytes with phytohemagglutinin (Edelman and Wheelock, 1966), and the support of VSV replication in the vaginal tract of mice only following carcinoma induction by benzpyrene (Koprowska and Koprowski, 1957) are also compatible with this idea. These investigations are being extended to determine to what extent the biochemical ability of cells to support mengovirus replication varies in the various tissues of the mouse. It is already clear that there are wide variations in mengovirus tropisms for cells of various tissues, and that these are not due to failure of viral adsorption, penetration, or uncoating (Buck *et al.*, manuscript in preparation).

ACKNOWLEDGMENT

The authors wish to thank Mr. James Massey for his technical assistance.

REFERENCES

ARBER, W. (1965). Host Specificity of DNA produced by *Escherichia coli*. *V*, The role of methionine in the production of host specificity. *J. Mol. Biol.* **11**, 247–256.

CORDS, C. E., and HOLLAND, J. J. (1964). Replication of poliovirus RNA induced by heterologous virus. *Proc. Natl. Acad. Sci. U. S.* **51**, 1080–1082.

DUSSOIX, D., and ARBER, W. (1965). Host specificity of infectious DNA from bacteriophage lambda. *J. Mol. Biol.* **11**, 238–246.

EDELMAN, R., and WHEELOCK, E. F. (1966). Vesicular stomatitis virus replication in human leukocyte cultures: enhancement by phytohemagglutinin. *Science* **154**, 1053–1055.

FELDMAN, L. A., BUTEL, J. S., and RAPP, F. (1966). Interaction of adenovirus tumor antigen during abortive infection of simian cells. *J. Bacteriol.* **91**, 813–818.

FELDMAN, L. A., MELNICK, J. L., and RAPP, F. (1965). Influence of SV40 genome on the replication of an adenovirus-SV40 "hybrid" population. *J. Bacteriol.* **90**, 778–782.

FRANKLIN, R. M., and BALTIMORE, D. (1962). Patterns of macromolecular synthesis in normal and virus-infected mammalian cells. *Cold Spring Harbor Symp. Quant. Biol.* **27**, 175–198.

GAREN, A., GAREN, S., and WILHELM, R. C. (1965). *J. Mol. Biol.* **14**, 167–178.

GERBER, P. (1966). Studies on the transfer of subviral infectivity from SV40-induced hamster tumor cells to indicator cells. *Virology* **28**, 501–509.

HANAFUSA, H., and HANAFUSA, T. (1966). Determining factor in the capacity of Rous sarcoma virus to induce tumors in mammals. *Proc. Natl. Acad. Sci. U S.* **55**, 532–538.

HOLLAND, J. J. and HOYER, B. H. (1962). Early stages of enterovirus infection. *Cold Spring Harbor Symp. Quant. Biol.* **27**, 101–112.

HOLLAND, J. J., MCLAREN, L. C., and SYVERTON, J. T. (1959). The mammalian cell-virus relationship. IV. Infection of naturally insusceptible cells with enterovirus nucleic acid. *J. Exptl. Med.* **110**, 65–80.

HOLLAND, J. J., MCLAREN, L. C., HOYER, B. H., and SYVERTON, J. T. (1960). Enteroviral ribonucleic acid. II. Biological, physical, and chemical studies. *J. Exptl. Med.* **112**, 841–864.

KAPLAN, S., STRETTON, A. O. W., and BRENNER, S. (1965). *J. Mol. Biol.* **14**, 528–533.

KELLOFF, G., and VOGT, P. K. (1966). Localization of avian tumor virus group-specific antigen in cell and virus. *Virology* **29**, 377–384.

KOPROWSKA, I., and KOPROWSKI, H. (1957). Enhancement of susceptibility to virus infection in the course of neoplastic process. *Ann. N.Y. Acad. Sci.* **68**, 404–418.

MCLAREN, L. C., and HOLLAND, J. J. (1959). The mammalian cell-virus relationship. I. Attachment of poliovirus to cultivated cells of primate and non-primate origin. *J. Exptl. Med.* **109**, 475–485.

O'CONOR, G. T., RABSON, A. S., BEREZESKY, I. K., and PAUL, F. J. (1963). Mixed infection with simian virus 40 and adenovirus 12. *J. Natl. Cancer Inst.* **31**, 903–917.

PLAGEMANN, P. G. W., and SWIM, H. E. (1966). Replication of mengovirus. I. Effect on synthesis of macromolecules by host cell. *J. Bacteriol.* **91**, 2317–2326.

PRINCE, A. N., and GINSBERG (1957). Studies of the cytotoxic effect of Newcastle disease virus (NDV) on Ehrlich ascites tumor cells. I. Characteristics of the virus-cell interaction. *J. Immunol.* **79**, 94–103.

RINDERKNECHT, H. (1962). Ultra-rapid fluorescent labeling of proteins. *Nature* **193**, 167–168.

SARMA, P. S., VASS, W., and HEUBNER, R. J. (1966). Evidence for the *in vitro* transfer of

defective Rous sarcoma virus genome from hamster tumor cells to chick cells. *Proc. Natl. Acad. Sci. U. S.* **55,** 1435–1442.

TAYLOR, M. W., GRANGER, G. A., BUCK, C. A., and HOLLAND, J. J. (1967). Similarities and differences among specific t-RNA's in mammalian tissues. *Proc. Natl. Acad. Sci. U. S.* in press.

VAHERI, A., and PAGANO, J. S. (1965). Infectious poliovirus RNA: A sensitive method of assay. *Virology* **27,** 434–436.

VOGT, P. K. (1965). Avian tumor viruses. *Advan. Virus Res.* **11,** 293–385.

WALKER, D. L. (1960). *In vitro* cell-virus relationships resulting in cell death. *Ann. Rev. Microbiol.* **14,** 177–196.