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Inhibition of Mengo Virus by Interferon

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ABSTRACT

GAUNTT, CHARLES J. (The University of Texas, Austin), AND ROYCE Z. LOCKART, JR. Inhibition of Mengo virus by interferon. J. Bacteriol. **91**:176–182. 1966.—The inhibition of Mengo virus replication in L cells resulting from interferon was studied quantitatively. Interferon was titrated on L cells with Western equine encephalomyelitis (WEE) virus as the challenge virus. One protective unit (PU) of interferon is the least amount of interferon which prevents cytopathic effects when a large multiplicity of WEE virus is added subsequent to overnight incubation with interferon. Ten PU of interferon reduced the yields of Mengo virus by about 90%. Larger doses of interferon, up to 220 PU, caused no further reduction in the amount of virus produced. Plaque formation by Mengo virus was also reduced in number by about 85 to 90%, but could not be further reduced. The plaques which formed on interferon-treated cells were reduced in size. We were unable to obtain a virus population with increased resistance to interferon action by use of five successive growth cycles in interferon-treated cultures. Analysis of the cell population for the proportion of cells able to act as infectious centers revealed that incubation of cells with 10 PU of interferon decreased the proportion of virus-yielding cells by 80%. The yield of virus per virus-producing cell was decreased by 40 to 60%. Despite the reduction in yields, plaques, and infectious centers resulting from interferon, all doses of interferon failed to prevent the complete destruction of the cells. Experiments with puromycin indicated that the cytopathic effects observed in L cells infected with Mengo virus required that a virus-directed protein be synthesized between 4 and 5 hr postinfection. The evidence suggested, therefore, that the Mengo virus genome was able to code for new protein synthesis in the absence of the production of infectious virus.

It is commonly stated and known that interferons inhibit a wide range of viruses. The quantitative studies on the relationship between interferon dose and virus inhibition which have been done are mostly limited to viruses selected for their great sensitivity to interferon action. It is usually assumed that cell protection will result if cells are incubated long enough with sufficient concentrations of interferon. However, Levy (6) found that interferon concentrations which almost totally inhibited Mengo virus yields failed to protect the cells against resulting cytopathic effects (CPE). This would indicate that insufficient interferon for complete protection was used, that Mengo virus was partially resistant to interferon action, or that the virus could initiate events in cells which would result in CPE in the absence of replication. This report is a quantitative study of the inhibition of Mengo virus by interferon. The results favor the alternative that cells may be destroyed in the absence of the production of infective virus.

MATERIALS AND METHODS

The methods for the growth and maintenance of the L cell subline (designated Lts) used throughout this study were previously described (7, 9). For experiments involving interferon, cultures in 60-mm petri dishes were used which contained 1.5 to 2.0 million cells at the time of virus challenge. Virus titrations were performed on 100-mm petri dishes containing 8 to 10 million Lts cells.

From the original sample of Mengo virus, which was kindly provided by R. M. Franklin, a single-plaque type was isolated which had a diameter of 4 to 5 mm on Lts cell monolayers. Three consecutive single-plaque isolations were made before preparation of stock suspension by growth in Lts cells.

In all experiments, 20 plaque-forming units (PFU) of virus per cell was added to insure infection of all cells. Mengo virus infectivity was assayed by adding 0.2-ml samples of the appropriate dilutions to Lts cell monolayers, which had been washed twice with phosphate-buffered saline (4) containing 0.1% bovine serum albumin (PBSA). A 1-hr period at room temperature was permitted for adsorption. A 10-ml amount of agar overlay was added which consisted of

Eagle's medium supplemented with 1% calf serum, 0.08% protamine sulfate, and 1% agar. The monolayers were incubated at 37 C under increased CO_2 tension. After 2 days, the cultures were stained by addition of 0.01% neutral red in PBSA, and the resulting plaques were counted after about 2 hr of additional incubation. Virus titers were determined from the average number of plaques on two cell monolayers. Infectious centers were assayed as follows. Monolayer cultures were infected with Mengo virus at an input of 20 PFU per cell. After the 1-hr adsorption period, the cells were washed three times with PBSA and then incubated for 20 min at 37 C with 0.5 ml of a 1:100 dilution of anti-Mengo rabbit serum. Under similar conditions, this concentration of antiserum, when added to a 0.5-ml suspension of Mengo virus at a titer of 4×10^8 PFU/ml, was sufficient to neutralize 99.87% of the stock virus. The cells were washed three more times to remove residual Mengo antibodies, and then dispersed by incubation for 5 min with warm 0.05% trypsin followed by scraping. The cells were counted, diluted in PBSA, and 0.5-ml samples were added to two Lts cell monolayers which had been previously washed with PBSA. A 1-ml amount of agar overlay was added immediately, and an additional 9.0 ml was added when the initial layer had hardened. The medium from the most concentrated cell suspensions was assayed for free virus after removal of cells by centrifugation at $1,200 \times g$ for 10 min. These fluids were plated in the same manner as those containing cells. The results revealed that less than 1 plaque in 4,000 resulted from free virus.

Interferon was produced by the addition of Newcastle disease virus (NDV) at an input multiplicity of about 1.0 PFU per cell to Blake bottles containing Lts cells. The interferon was precipitated from the medium by the addition of ammonium sulfate to 80% saturation, and was redissolved in 0.2 M phosphate buffer (*p*H 7.2). This interferon preparation was dialyzed in the cold against 50 volumes of various buffers in the following order: 0.01 M phosphate, *p*H 7.2 (24 hr, two changes of buffer); 0.05 M tris(hydroxymethyl)aminomethane (Tris), *p*H 2.0 (12 hr, once); 0.05 M phosphate, *p*H 6.0 (24 hr, two changes of buffer); and Eagle's medium (24 hr, two changes of medium). Prior to use, 5 ml of interferon solution in a 100-mm petri dish was exposed for 5 min to an 8-w GE germicidal lamp at a distance of 15 cm. The titers of interferon were determined as previously described (9). Lockart (8) designated as 1 protective unit (PU) the highest dilution of interferon which prevents the appearance of CPE in Lts cultures (ca. 1.2 million cells) challenged with 10 PFU per cell of Western equine encephalomyelitis (WEE) virus at 12 to 15 hr after interferon addition. One PU of interferon by our assay is equivalent to about 5 units of interferon as determined by the method employing a 50% reduction in plaque number as the end point. For use in experiments, interferon was diluted in Eagle's medium with 5% calf serum, and 2 ml was added to Lts cell monolayers. In all experiments, the cells were incubated with interferon for 12 hr prior to the addition of virus.

Puromycin dihydrochloride was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

RESULTS

Effect of varying concentrations of interferon on yields of Mengo virus. The effect of varying concentrations of interferon on yields of Mengo virus obtained at 24 hr postinoculation (pi) is shown in Table 1. Maximal inhibition of virus yields was found when between 10 and 220 PU of interferon was added. At these concentrations of interferon, an average of approximately 100 PFU per cell was still produced. High doses of

TABLE 1. *Effect of varying concentrations of interferon on yields of Mengo virus**

Expt no.	Interferon (PU/culture)†	Virus yields (PFU/cell)‡	Virus yields (per cent of control)
1	220	118	17
	165	100	14
	110	119	17
	55	94	13
	13.7	88	12
	3.5	118	17
	None	720	100
2	165	73	7
	110	78	8
	55	94	9
	13.7	127	12
	3.5	206	20
	None	1,002	100
3	10	110	11
	1	528	51
	0.1	620	60
	0.01	890	86
	None	1,030	100

* Replicate monolayer cultures of Lts cells containing 1.4, 2.0, and 5.5 million cells for experiments 1, 2, and 3, respectively, were incubated for 12 hr with 2 ml of Eagle's medium containing the various concentrations of interferon. After removal of medium with or without interferon, the monolayers were washed with PBSA, and 20 PFU per cell of Mengo virus was added. After 1 hr of incubation at room temperature, free virus was removed by washing with PBSA. The cultures were incubated at 37 C for an additional 23 hr. Total virus was determined for pooled samples from two cultures in the following manner. The cells were scraped into the fluid. Virus was released by subjecting the samples to three cycles of alternate freezing and thawing. The samples were stored at −10 C until titrated. CPE at 24 hr postinoculation was complete for all concentrations of interferon.

† A PU stands for 1 protective unit of interferon against WEE virus. For its determination, see Materials and Methods.

‡ Cell counts were made at the time of challenge.

interferon failed to prevent complete destruction of the cultures by the virus. Complete CPE was observed at about 16 hr pi in control cultures and those previously incubated with interferon. These observations are in agreement with those of Levy (6).

Growth curve of Mengo virus in Lts cells previously incubated with interferon. Growth curves of Mengo virus in Lts cells previously incubated for 12 hr in Eagle's medium with or without 10 PU of interferon are illustrated in Fig. 1. Replicate monolayer cultures were incubated for 12 hr with 10 PU of interferon or medium. After removal of incubation media, the monolayers were washed with PBSA and then challenged with 20 PFU per cell of Mengo virus. Adsorption was permitted to occur for 1 hr at room temperature, after which free virus was removed by washing. Total virus was determined at each time period by scraping the cells from two plates into the medium, pooling them, and then subjecting the samples to three cycles of rapid freezing and thawing. All samples were stored at −70 C until assayed for virus contents. In cultures incubated with interferon, an increase in infectious virus was delayed by 1 hr, and the yield at 24 hr was decreased by 85% from that of the controls. The 1-hr delay is also in good agreement with the data of Levy (6), who found that, in L cells incubated with interferon prior to infection, the rapid inhibition of normal cell ribonucleic acid (RNA) synthesis accompanying infection with Mengo virus occurred about 1 hr later than it did in the control cultures. The ever-present complete CPE, despite the reduction of virus yields in cultures previously treated with interferon, suggested two possibilities: (i) all the cells might merely be making reduced yields of virus, or (ii) only a small fraction of the cell population produces virus, but at nearly normal yields. These hypotheses were first investigated by studying the inhibition of plaque formation in cultures which had been incubated previously with interferon.

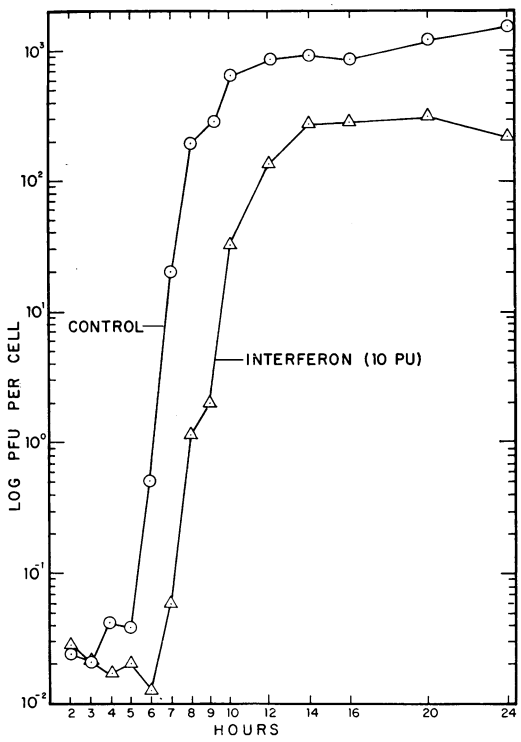

FIG. 1. *Growth curve of Mengo virus in Lts cells previously incubated with interferon. Replicate monolayer of 2.0 million cells were incubated for 12 hr with 10 PU of interferon or medium. After removal of incubation medium, the cultures were washed and virus was added. After 1 hr of adsorption, free virus was removed by three washes. Duplicate plates were scraped at the indicated times, and the contents were pooled and frozen until assayed for total virus content. See Table 1 for explanation of total virus*

Reduction in the number and size of Mengo virus plaques resulting from incubation of Lts cells with interferon. If all of the cells were producing reduced yields of virus, one would expect to find no reductions in the numbers of plaques in monolayers which had been incubated with interferon, but only reductions in plaque sizes. Table 2

TABLE 2. *Reduction in the numbers and sizes of Mengo virus plaques resulting from incubation of Lts cells with interferon**

Expt no.	Interferon (PU/culture)	Avg no. of plaques†	Plaques as per cent of control	Plaque size
				mm
1	50	41	15	1
2	50	27	17	1
1	10	52	19	1
2	10	38	24	1
1	1	136	51	2–3
2	1	77	48	2–3
1	None	267	100	4–5
2	None	159	100	4–5

* Replicate monolayer cultures containing 4.5 and 6.0 million Lts cells in experiments 1 and 2, respectively, were incubated for 12 hr with 6 ml of the various concentrations of interferon or medium. All cultures were washed before the addition of a known number of PFU of Mengo virus. Resulting plaques were counted after 48 hr of additional incubation.

† Average number of plaques was determined by counts from three replicate monolayers.

shows that incubation with interferon effected a reduction in plaque numbers, as well as a reduction of plaque sizes. The reduction in plaque sizes suggested that virus yields were reduced. The reduction in numbers of plaques suggested that the number of cells producing virus per culture was reduced by the action of interferon. A further analysis of both hypotheses was made by determining the proportion of virus-producing cells per culture.

Reduction in the number of virus-producing cells by incubation with interferon. The number of cells able to produce virus was determined by the ability of the cells to act as infectious centers when plated under agar on confluent monolayers of Lts cells. The data in Table 3 show the number of infectious centers produced from plating a specified number of cells which had been incubated in either medium or interferon prior to challenge with 20 PFU of Mengo virus per cell. Assuming that all the cells in the control cultures were infected, the techniques employed resulted in an efficiency of plating of 0.40 and 0.84 for experiments 1 and 2, respectively. The per cent of infectious centers decreased with higher concentrations of interferon, showing that the proportion of virus-yielding cells in cultures incubated with interferon was dependent on the concentration of interferon.

Reduction by interferon of the virus yield per virus-producing cell. A calculation of the average virus yield per virus-producing cell was made from a knowledge of the following parameters: the proportion of cells producing virus per culture, the total number of cells per culture, the efficiency of the assay, and the total virus yield per culture. The incubation of cultures with interferon reduced the number of virus-producing cells and also reduced the average amount of virus produced per virus-producing cell (Table 4). Cultures incubated with 50 PU of interferon contained 14 and 28% of the number of virus-producing cells found in control cultures. The yield per virus-producing cell in cultures incubated with 50 PU of interferon was about 40% of the yield produced per cell in control cultures.

Attempts to isolate a Mengo variant resistant to the action of interferon. The inability of high concentrations of interferon to prevent the production of virus in 14 to 28% of the interferon-treated cells suggested two alternatives. (i) The cell population is heterogeneous with respect to susceptibility to the antiviral action of interferon. This hypothesis seems unlikely in view of the work of Lockart and Horn (9). They found, using WEE virus in the Lts cell line, that incubation of cells overnight with interferon prior to infection reduced the per cent infectious centers below 1% of the controls, and reduced the PFU per yielder ratio to less than 1. Therefore, greater than 99% of the cells are susceptible to the action of interferon. Control experiments performed during the course of these studies confirmed the above findings. (ii) An alternative hypothesis is that the virus population is heterogeneous and contains a variant which is insensitive or only slightly sensitive to the inhibitory action of interferon. We sought to find such a variant by using the enrichment culture technique. Stock virus was added at a multiplicity of 20 PFU per cell to

TABLE 3. *Reduction in the number of virus-producing cells by incubation with interferon**

Expt no.	Interferon (PU/culture)	Cells plated	Infectious centers		Virus yield (per cent of control)
			No.†	Per cent	
1	50	2,883	180	6.3	7.8
	10	955	129	13.5	12.0
	1	311	97	31.2	43.0
	None	308	123	40.0	100.0
2	50	765	184	24.0	10.9
	10	775	203	26.2	14.4
	1	243	147	59.8	43.7
	None	265	222	83.8	100.0

* Monolayer cultures containing 3.7×10^6 and 3.2×10^6 Lts cells in experiments 1 and 2, respectively, were incubated for 12 hr with interferon. See Materials and Methods for details.

† Values for infectious centers represent the average number of plaques on two plates.

TABLE 4. *Reduction by interferon of the virus yield per virus-producing cell*

Expt no.	Interferon (PU/culture)	No. of cells producing virus*	Total virus produced (PFU+)	PFU per virus-producing cell
1	50	5.2×10^5	3.4×10^8	653
	10	1.3×10^6	5.2×10^8	400
	1	2.9×10^6	1.9×10^9	655
	None	3.7×10^6	4.3×10^9	1,160
2	50	9.0×10^5	5.0×10^8	556
	10	9.9×10^5	6.6×10^8	666
	1	2.1×10^6	2.0×10^9	953
	None	3.2×10^6	4.6×10^9	1,437

* Calculated from the proportion of cells able to form infectious centers, corrected for the efficiency of the assay of infectious centers, i.e., the number of yielders per culture = (per cent IC) (1/efficiency assay) (total cells per culture). Total virus was determined by the method given in Table 1.

Lts monolayers which had been incubated with 10 PU of interferon, and to control cultures which had been incubated only in Eagle's medium. At 24 hr pi, the cells on two plates were scraped into the medium; the contents were pooled and then subjected to three cycles of rapid freezing and thawing prior to assay for infectious virus by plaque formation. Virus that was harvested from monolayers which had been incubated with 10 PU of interferon was used as stock virus to infect a subsequent set of interferon-treated and control monolayer cultures. In this manner, virus obtained from cultures incubated with 10 PU of interferon was passaged five times in interferon-treated cells. Using this technique, we were unable to find a fraction of the stock virus which was more resistant to the action of interferon than the initial virus suspension.

Inhibition by puromycin of the CPE resulting rom Mengo virus. We have shown that incubation of Lts cells with high concentrations of interferon prior to infection with Mengo virus could effect a reduction in virus-producing cells, but not a reduction in CPE. This fact suggested that viral replication and CPE were not interdependent. Levy (6) suggested that the death of L cells infected with Mengo virus may be due to a toxic material in the virus preparation or to a "toxic" action of the virus itself. Observations favoring the second hypothesis were made from the following experiments.

Monolayers of Lts cells were incubated with 20 PFU per cell of Mengo virus which had been exposed for 5 min to an 8-w GE germicidal lamp at a distance of 15 cm. No morphological changes were observed in these cells up to 48 hr pi. This implied that the virus preparation was free from toxic material. Two kinds of experiments were then designed to demonstrate that CPE was a virus-directed phenomenon.

Monolayer cultures of Lts cells were infected with 20 PFU per cell of Mengo virus. Some of the cultures had been incubated for 1 hr with actinomycin (10 μg/ml) to block cellular RNA synthesis. After 13 hr, CPE was almost complete regardless of whether the cells had been exposed to actinomycin. Monolayer cultures of Lts cells similarly infected and incubated in the presence of puromycin at a concentration of 50 μg/ml showed only a very slight amount of CPE at 13 and 27 hr pi. The small changes seen were probably due to the puromycin, because control monolayers incubated only with puromycin also showed the very small amount of CPE after the same periods of incubation. Removal of puromycin at 13 hr pi from infected cultures resulted in the complete destruction of all the cells by 27 hr pi. The results of these experiments show that CPE is due to a protein which is synthesized under the genetic control of the virus.

Another type of experiment was performed in which 2 ml of Eagle's medium containing puromycin at a concentration of 50 μg/ml was added at 15-min intervals pi to two plates in a series of replicate monolayers of Lts cells which had been infected with 20 PFU per cell of Mengo virus. The plates were examined for CPE at 18 hr pi, a time at which infected monolayers which had not received puromycin exhibited complete CPE. The results are reported in Table 5. All infected cultures of the series which had received additions of puromycin up to 4 hr pi demonstrated only a slight amount of CPE at 18 hr pi. Cultures which received puromycin at 4.25 to 4.75 hr exhibited 3+ CPE. Cultures which received puromycin additions at 5 hr or later underwent almost complete or complete CPE. Uninfected cultures which received puromycin at zero-time exhibited only a change in cell morphology, which was reversed by removing the puromycin. These results demonstrated that a protein whose synthesis must begin in Mengo virus-infected Lts cells at about 4 hr pi is in some manner respon-

TABLE 5. *Inhibition by puromycin of the CPE resulting from Mengo virus**

Time (pi) of puromycin	Addition of Mengo virus (20 PFU/cell)	CPE† at 18 hr pi
hr		
0	−	−‡
0.25–4	+	+
4.25–4.75	+	+++
5–5.25	+	+++ to ++++
>5.25	+	++++
None	+	++++
None	−	−

* Monolayer cultures containing 4.0 million Lts cells were infected with Mengo virus at an input of 20 PFU per cell. A 1-hr period at room temperature was allowed for virus adsorption, after which 2 ml of Eagle's medium plus 5% calf serum was added to the monolayers. Puromycin was diluted to a concentration of 50 μg/ml in Eagle's medium containing 5% calf serum. At 15-min intervals, Eagle's medium was removed from duplicate plates and replaced by 2 ml of the puromycin preparation. The plates were incubated at 37 C.

† Cytopathic effects: − = none, + = 5 to 25% cells destroyed, ++ = 25 to 75% cells destroyed, +++ = 75 to 95% cells destroyed, and ++++ = complete destruction

‡ A change in cell morphology was observed which is not to be confused with CPE.

sible for the CPE. An increase in infectious virus is not observed in infected cultures until after 5 hr pi (see growth curve, Fig. 1); therefore, the protein responsible for CPE appears in infected cells 0.75 to 1.0 hr prior to virus maturation.

DISCUSSION

The overnight incubation of L cells with a sufficient dose of interferon completely prevents viral replication and the occurrence of CPE when the cells are subsequently exposed to large multiplicities of WEE virus (7, 9). The report by Levy (6) that Mengo virus yields could be markedly inhibited by interferon without any amelioration of CPE was surprising. We were able to confirm these results easily. In addition, we found that doses of interferon (1 PU of interferon by our CPE inhibition assay method is equivalent to 5 to 8 units of interferon if assayed by the plaque inhibition method) many times greater than those necessary to completely inhibit the replication of WEE virus failed to reduce Mengo virus titers by more than 85 to 90% and infectious centers by more than about 80%. It was established that, after incubation of Lts cells with between 10 and 220 PU of interferon, 10 to 20% of the cells produced slightly reduced yields of virus, whereas 80 to 90% of the cells failed to produce any infectious virus but, nevertheless, were destroyed. Two alternatives are suggested to explain the virus-producing cells: the presence of (i) a heterogeneous response to interferon or (ii) a heterogeneous virus population. The first alternative can probably be ruled out by noting that virus production by cells from this subline can be inhibited almost completely when WEE virus is used as the challenge virus. The fact that plaque formation by Mengo virus could not be completely inhibited indicated that virus multiplicity is not involved, and would seem to point to the presence of an interferon-resistant fraction as hypothesized in the second alternative. However, this possibility, too, was ruled out, as we were unable to isolate an interferon-resistant virus, even after five consecutive passages in cells incubated with interferon prior to infection. Thus, we can offer no explanation at this time for a seemingly interferon-resistant persistent fraction. Such a persistent fraction has been found in populations of EMC and VSV viruses by Takemoto and Baron (*personal communication*).

The 80 to 90% of the cells which did not act as infective centers, but nevertheless were destroyed, may be explained by two hypotheses: (i) they produced too few viruses to initiate a focus of infection, or (ii) they failed to produce any infectious virus. If the second explanation is correct, it would appear that the virus genome causes cell destruction in the absence of the production of infective virus. This is, of course, the classical case of "viral toxicity." By use of puromycin, we have provided some evidence indicating that a protein which is synthesized somewhere between 4 and 5 hr pi is responsible for the CPE resulting from infection with Mengo virus. Similar studies with puromycin by Bablanian et al. (1) demonstrated that, in poliovirus-infected human embryonic lung cells (diploid), a protein is synthesized which is responsible for cell damage. Levy (6) was unable to demonstrate that interferon could prevent the inhibition of cellular protein synthesis which results from infection of L cells with Mengo virus (5). A probable explanation is that some products under the direction of the Mengo virus genome are formed in the absence of the formation of infective virus in interferon-treated cells. One of these products is a protein which we have shown is responsible for the eventual destruction of the cell; the other, the protein responsible for the inhibition of the cells' synthetic processes (2). Experiments are now in progress to determine the quantities of viral RNA and coat antigens produced by interferon-treated cells infected with Mengo virus.

It is striking to note the difference in the response of the two different RNA viruses to interferon action in the same kind of cells. It is generally thought that interferon action prevents the synthesis of foreign RNA preparations. This is the reason usually given for its wide spectrum of action. However, an RNA virus incapable of being completely inhibited by interferon suggests that some specificity is involved. This may be due either to the kind of nucleic acid involved or to the fact that different viruses replicate at different sites in the cell. Studies with NDV tend to favor the first possibility. We have found that the Texas GB strain of NDV can be inhibited only slightly (about 20%) by several hundred times the minimal amount of interferon required to completely inhibit the replication of WEE virus in chick embryo cells. Although most of the strains of NDV are probably relatively resistant to interferon (3), some strains of NDV are quite susceptible to the action of interferon (Bayliss and Lockart, *unpublished data*). A difference in susceptibility between strains of vesicular stomatitis virus has been reported by Wagner et al. (10). The most likely explanation for such a difference resides in the nature of the viral genomes rather than the postulation that each strain of virus multiplies in some different site or manner.

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LITERATURE CITED

1. BABLANIAN, R., H. J. EGGERS, AND I. TAMM. 1965. Studies on the mechanism of poliovirus-induced cell damage. II. The relation between poliovirus growth and virus-induced morphological changes in cells. Virology **26**:114–121.
2. BALANDIN, I. G., AND R. M. FRANKLIN. 1964. The effect of Mengo virus infection on the activity of the DNA-dependent RNA polymerase of L-cells. II. Preliminary data on the inhibitory factor. Biochem. Biophys. Res. Commun. **15**: 27–32.
3. BARON, S. 1964. Newcastle disease virus, an evolving pathogen, p. 205–220. Univ. Wisconsin Press, Madison.
4. DULBECCO, R., AND M. VOGT. 1954. One step growth curve of Western equine encephalomyelitis virus on chicken embryo cells grown *in vitro* and analysis of virus yields from single cells. J. Exptl. Med. **99**:183–199.
5. FRANKLIN, R., AND D. BALTIMORE. 1962. Patterns of macromolecular synthesis in normal and virus-infected mammalian cells. Cold Spring Harbor Symp. Quant. Biol. **27**:175–198.
6. LEVY, H. B. 1964. Studies on the mechanism of interferon action. II. The effect of interferon on some early events in Mengo virus infection in L cells. Virology **22**:575–579.
7. LOCKART, R. Z. 1963. Production of an interferon by L cells infected with Western equine encephalomyelitis virus. J. Bacteriol. **85**:556–566.
8. LOCKART, R. Z. 1964. The necessity for cellular RNA and protein synthesis for viral inhibition resulting from interferon. Biochem. Biophys. Res. Commun. **15**:513–518.
9. LOCKART, R. Z., AND B. HORN. 1963. Interaction of an interferon with L cells. J. Bacteriol. **85**: 996–1002.
10. WAGNER, R. R., A. H. LEVY, R. M. SNYDER, G. A. RATCLIFF, JR., AND D. F. HYATT. 1963. Biologic properties of two plaque variants of vesicular stomatitis virus (Indiana serotype). J. Immunol. **91**:112–122.