Cells Persistently Infected with Newcastle Disease Virus

II. Ribonucleic Acid and Protein Synthesis in Cells Infected with Mutants Isolated from Persistently Infected L Cells

HARSHAD THACORE AND JULIUS S. YOUNGNER

Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Received for publication 11 March 1970

A comparison of the replication patterns in L cells and in chick embryo (CE) cell cultures was carried out with the Herts strain of Newcastle disease virus (NDV_o) and with a mutant (NDV_{pi}) isolated from persistently infected L cells. A significant amount of virus progeny, 11 plaque-forming units (PFU)/cell, was synthesized in L cells infected with NDV_o, but the infectivity remained cell-associated and disappeared without being detectable in the medium. In contrast, in L cells infected with NDV_{pi}, progeny virus (30 PFU/cell) was released efficiently upon maturation. It is suggested that the term "covert" rather than "abortive" be used to describe the infection of L cells with NDV_o. In both L and CE cells, the latent period of NDV_{pi} was 2 to 4 hr longer than for NDV_o. The delay in synthesis of viral ribonucleic acid (RNA) in the case of NDV_{pi} coincided with the delay in the inhibition of host RNA and protein synthesis. Although both NDV_o and NDV_{pi} produced more progeny and more severe cell damage in CE cells than in L cells, the shut-off of host functions was significantly less efficient in CE cells than in L cells. Paradoxically, no detectable interferon was produced in CE cells by either of the viruses, whereas in L cells most of the interferon appeared in the medium after more than 90% of host protein synthesis was inhibited. These results suggest that the absence of induction of interferon synthesis in CE cells infected with NDV is not related to the general shut-off of host cell synthetic mechanisms but rather to the failure of some more specific event to occur. In spite of the fact that NDV_{pi} RNA synthesis commenced 2 to 4 hr later than that of NDV_o, interferon was first detected in the medium 8 hr after infection with both viruses. This finding suggests that there is no relation between viral RNA synthesis and the induction of interferon synthesis.

A previous report from this laboratory described the properties of mutant viruses isolated from mouse L cells persistently infected with the Herts strain of Newcastle disease virus (NDV; 7). In contrast to the wild-type virus (NDV_o), the mutant virus (NDV_{pi}) replicated efficiently in L cells. The abortive infection of L cells by wild-type NDV_o seemed to be due in part to the inefficient release of the virus from infected cells. The present work was undertaken (i) to study the replication of clones of NDV_o and NDV_{pi} in primary chick embryo (CE) cells and mouse L cells in an attempt to elucidate further the defect in the L cell-NDV_o system, and (ii) to determine the effect of productive and abortive infections on host cell macromolecular synthesis.

MATERIALS AND METHODS

Cell cultures. Primary CE cell cultures and mouse L cells (clone 929) were propagated and maintained in Eagle's minimal essential medium containing 4% calf serum, as described earlier (10).

Viruses. The isolation and plaque purification of the wild-type Herts strain of NDV (NDV_o, clone 19) and the small plaque mutant (NDV_{pi}, clone 2) used in this study have been described elsewhere (7). The viruses were grown in chick embryos and concentrated as follows. Nine-day-old chick embryos were inoculated into the allantoic cavity with 10⁴ to 5 × 10⁴ plaque-forming units (PFU) of either NDV_o or NDV_{pi} and incubated at 37 C. The allantoic fluid from eggs inoculated with NDV_o was harvested at 48 hr when all of the embryos were dead. With NDV_{pi}, only those embryos surviving at 72 hr were used to make the virus pools because wild-type revertants were present in fluids from dead embryos (7). The infected allantoic fluid

was clarified by low-speed centrifugation and then centrifuged at $105,000 \times g$ for 60 min at 4 C. The pellets were resuspended to 5% of the original volume in phosphate-buffered saline (PBS, pH 7.2) and treated with sonic oscillation for 2 min to obtain a uniform suspension. When assayed in CE cell monolayers, NDV_o and NDV_{pi} characteristically produced large (3 mm) and small plaques (< 1 mm), respectively.

The Indiana strain of vesicular stomatitis virus (VSV) was used as the challenge virus in interferon assays. The propagation of VSV in L-cell cultures has been described (10).

Interferon assay. Interferon determinations were carried out in L-cell cultures by the plaque reduction method by using VSV as the challenge virus (10). All samples were treated with anti-NDV serum to eliminate infective virus.

Chemicals. ³H-uridine (specific activity, 2 c/mmole) and ³H-leucine (specific activity, 40 c/mmole) were purchased from Schwarz BioResearch, Inc., Orangeburg, N.Y. Actinomycin D was made available through the courtesy of H. B. Woodruff of Merck Sharp and Dohme Co.

Incorporation of ³H-uridine into virus-specific ribonucleic acid (RNA). Two methods were used to measure the synthesis of virus-specific RNA in monolayer cultures infected with NDV_o and NDV_{pi}. (i) The cumulative incorporation of ³H-uridine into viral RNA was determined as follows. Cell cultures (3×10^6 cells/dish) were infected with virus with an input multiplicity of infection (MOI) of 250. After adsorption for 60 min at 37 C, the cells were washed twice, medium was added, and the cultures were reincubated at 37 C. At 2.5 hr after infection, the fluid from each culture was replaced with 3 ml of medium containing 1 μ g of actinomycin D per ml. Fifteen minutes later, 0.5 μ Ci of ³H-uridine was added to each culture, and incubation was continued at 37 C. At appropriate times, medium was removed; the cells were washed twice, resuspended in 1 ml of cold Hanks solution, and disrupted by sonic oscillation for 5 min. The trichloroacetic acid-precipitable radioactivity was determined as follows. To each sample (1 ml) an equal volume of 10% trichloroacetic acid (containing 4% filteraid) was added, and the precipitate was collected on a Whatman filter paper. The precipitate was washed twice with 5% trichloroacetic acid, once with 95% ethanol, and once with ether, allowed to dry for 20 to 30 min, and transferred to a scintillation vial containing 0.5 ml of hydroxide of hyamine. The samples were allowed to solubilize overnight at 4 C, toluene scintillation fluid (10 ml/vial) was added, and the radioactivity was counted in a Packard Tri-Carb 3375 liquid scintillation spectrometer. (ii) The rate of viral RNA synthesis was determined as described above for cumulative RNA synthesis, with the following modifications. The cell cultures were infected with virus and treated with actinomycin D as described above. At various times after infection, the cultures were labeled for 50 min at 37 C with ³H-uridine (1 μ Ci/ml) in 3 ml of medium. The medium containing labeled precursor was removed, and the cells were washed, harvested, and processed as described for the determination of trichloroacetic acid-precipitable radioactivity.

Macromolecular biosynthesis in virus-infected cell cultures. The rates of cellular RNA and protein synthesis in cultures infected with NDV_o and NDV_{pi} were measured by the incorporation of radioactive precursors at various times after infection. At appropriate intervals, the uninfected (control) and virus-infected cultures were exposed at 37 C to 3 ml of medium containing either ³H-uridine (1 μ Ci/ml) or ³H-leucine (1 μ Ci/ml). Labeling times of 50 and 20 min were used for ³H-uridine and ³H-leucine, respectively. After removing the medium containing labeled precursors, the cells were washed, harvested, and processed for the determination of trichloroacetic acid-precipitable radioactivity as described above.

RESULTS

Replication of NDV_o and NDV_{pi} in CE cell monolayer cultures. A comparison of the replication of the two viruses was carried out in CE cell cultures as described in the legend to Fig. 1. A 2-hr difference was found in the latent period of the viruses; infective progeny virus first appeared at 4 and 6 hr with NDV_o and NDV_{pi}, respectively (Fig. 1). Although the rate of increase of virus infectivity appeared to be similar for the two viruses, the maximum titer of NDV_o (at 10 hr) was threefold greater than the maximum titer of NDV_{pi} (at 12 hr). The total virus yield (PFU) per cell for NDV_o and NDV_{pi} was calculated to be 320 and 107, respectively. Both viruses produced demonstrable cytopathic effect (CPE) 8 to 10 hr after infection, and both viruses were released rapidly from the cells upon maturation. No detectable interferon was produced by either NDV_o or NDV_{pi} in CE cell cultures.

Further studies were conducted to correlate the difference in the latent period of the two viruses with the initiation of the synthesis of viral RNA.

Cumulative incorporation of ³H-uridine into virus-specific RNA in CE cell monolayers infected with NDV_o and NDV_{pi}. Cell cultures were infected with NDV_o or NDV_{pi} at an input MOI of 250, treated with actinomycin D, and then incubated in medium containing radioactive precursor as described above. The amount of actinomycin D (1 μ g/ml) used in these experiments inhibited over 95% of cellular RNA synthesis. Table 1 presents the cumulative incorporation of labeled precursor into viral RNA in cells infected with NDV_o and NDV_{pi}. Synthesis of viral RNA was first detected between 3 and 4 hr after infection with NDV_o and between 6 and 8 hr with NDV_{pi}. The delay in the synthesis of viral RNA in cells infected with NDV_{pi} corresponds to the longer latent period of this virus described in Fig. 1. The data in Table 1 also show that almost 30% more viral RNA was synthesized in cells infected with NDV_o than in cells infected with NDV_{pi} under similar conditions.

FIG. 1. Replication of NDV_o and NDV_{pi} in CE cell cultures. Monolayer cultures (2.2×10^6 cells per dish) were infected with virus at an input MOI of 250. After adsorption for 60 min at 37 C, the cells were washed twice, 3 ml of medium was added, and the cultures were reincubated at 37 C. At various times after infection, cell-associated virus and released virus were determined as follows. The culture fluid was harvested and assayed for released virus. The cells were washed twice, scraped from the glass, and suspended in 3 ml of medium. The cell suspension was disrupted with sonic oscillation for 1 min and then assayed for cell-associated virus. Infectivity assays were performed in CE cell monolayers. Symbols: NDV_o, (○) cell-associated, (●) released; NDV_{pi}, (△) cell-associated, (▲) released.

RNA and protein synthesis in CE cell monolayer cultures infected with NDV_o and NDV_{pi}. A study of host RNA and protein synthesis was carried out to determine whether a correlation exists between the time of appearance of viral RNA and the initiation of the inhibition of cellular functions (9). CE cell cultures were infected with virus at an input MOI of 250. At various times after infection, the rate of incorporation of radioactive precursors into RNA or protein was measured as described above. Replicate cultures were infected in a similar manner and treated with actinomycin D; the rate of viral RNA synthesis was measured by the incorporation of ³H-uridine at various times after infection.

In cells infected with NDV_o, no change in the rate of either cellular RNA or protein synthesis was observed during the first 4 hr after infection (Fig. 2). During the next 6.5 hr, the rates of total RNA and protein synthesis declined gradually to about 80 and 60%, respectively, of the rates in control uninfected cultures. In cells infected with NDV_{pi}, no significant change in the rate of

TABLE 1. Cumulative incorporation of ³H-uridine into CE cell cultures infected with NDV_o and NDV_{pi}^a

Time after infection hr	Counts/min		
	Uninfected cells (control)	Cells infected with NDV _o	Cells infected with NDV _{pi}
3	26	22	20
4	101	159	94
6	120	744	130
8	135	1664	430
10	162	2647	1460
12	121	2455	1941

^a Cell cultures were infected (MOI = 250) in the usual manner, treated with actinomycin D, and exposed to ³H-uridine as described in the text. Uninfected cells treated with actinomycin D were used as the control. The radioactivity incorporated into trichloroacetic acid-precipitable material is expressed as counts per 3×10^6 cells.

FIG. 2. RNA and protein synthesis in CE cell cultures infected with NDV_o or NDV_{pi}. Monolayer cultures were infected (MOI 250) and at appropriate intervals the rates of RNA and protein synthesis were measured by the incorporation of radioactive precursors as described. Uninfected cells were used as the control. The average radioactive counts incorporated by uninfected cultures were 13,400 counts/min for ³H-uridine and 37,660 counts/min for ³H-leucine (per 3×10^6 cells). The rate of viral RNA synthesis was determined in cultures treated with actinomycin D (1 μ g/ml) after infection. Symbols: total RNA (●), total protein (○), and viral RNA (△) synthesis.

cellular RNA or protein synthesis was noted during the first 6 hr of infection. During the next 2.5 hr, the rate of RNA synthesis was rapidly inhibited to 25% of the rate in control cells, whereas the rate of protein synthesis was inhibited to only 60% of the control rate. In the lower curves of Fig. 2, the rates of viral RNA synthesis in cells infected with NDV_o or NDV_{pi} are in accord with the results obtained by cumulative incorporation methods (Table 1). The 2- to 4-hr delay in the initiation of the inhibition of host RNA and protein synthesis in cells infected with NDV_{pi} correlated with the delay in the synthesis of viral RNA. These results suggest a correlation between the time of appearance of viral RNA and the inhibition of cellular RNA and protein synthesis.

The experiments described above were conducted in CE cells, a permissive host for both viruses. Since NDV_{pi} replicates efficiently in L cells and NDV_o does not, experiments similar to those described above were carried out in L cells in an attempt to elucidate the defect in NDV_o replication.

Replication of NDV_o and NDV_{pi} in L-cell monolayer cultures. Cell cultures were infected with virus at an input MOI of 250, and, at various times after infection, cell-associated virus and released virus were determined as described in the legend to Fig. 1. The latent period in L cells for NDV_{pi} was 2 hr longer than for NDV_o (Fig. 3);

FIG. 3. Replication of NDV_o and NDV_{pi} in L cell cultures. Monolayer cultures were infected with either NDV_o or NDV_{pi} (MOI 250), and cell-associated virus and released virus were determined at various times after infection as described in the legend of Fig. 1. Symbols: NDV_o, (○) cell-associated, (●) released; NDV_{pi}, (△) cell-associated, (▲) released.

this finding is in agreement with the results obtained in CE cell cultures (Fig. 1). In L cells infected with NDV_o, infective progeny virus appeared at 5 hr and reached a maximum 6 hr after infection. With NDV_{pi}, an increase in virus progeny was first observed between 6 and 8 hr after infection, and the maximum virus yield was reached at 10 hr. In contrast to the efficient release of both viruses from CE cells (Fig. 1), a marked difference was observed in the efficiency of release of infective virus from L cells. Whereas NDV_{pi} was efficiently released from L cells after maturation, essentially all infective NDV_o remained cell-associated and gradually disappeared over a period of 8 hr without appearing in the medium. The nature of this loss of cell-associated infectivity is not understood at the present time. When virus yield per cell is calculated on the basis of released infectivity, the number of PFU produced per cell is 1 for NDV_o and 22 for NDV_{pi}. On the basis of these data, the infection of L cells with NDV_o can be considered to be abortive and L cells can be classed as a "nonpermissive" host for this virus. On the other hand, if the yield per cell is calculated from the total virus produced, i.e., from the sum of the maximum observed cell-associated and released infectivity, the number of PFU produced per cell is 11 for NDV_o and 30 for NDV_{pi}. Further interpretation of these findings will be considered below. No CPE was observed at 14 hr postinfection with either virus; with further incubation at 37 C, complete cell destruction was observed between 24 and 30 hr. Interferon synthesis was induced by both viruses in L cells, but no significant difference was found in the rate or the time of appearance of the viral inhibitor in the medium. The data for the production of interferon in L cells by NDV_o and NDV_{pi} will be presented below.

RNA and protein synthesis and interferon production in L-cell monolayer cultures infected with NDV_o and NDV_{pi}. Cell cultures were infected with virus in the usual manner at an input MOI of 250. At various times after infection, the rates of (i) cellular RNA and protein synthesis, (ii) viral RNA synthesis, and (iii) interferon production were determined (Fig. 4). In cells infected with NDV_o, the rate of cellular RNA synthesis began to decline 1 hr after infection, and, by 16 hr, 85% of host RNA synthesis was inhibited. Protein synthesis in these cells was not significantly affected for 4 hr and then was precipitously inhibited to 5% of the control rate by 8 hr. In the case of cells infected with NDV_{pi}, the shut-off of cellular RNA and protein synthesis was delayed considerably compared to cells infected with NDV_o. No significant decrease in the rate of RNA synthesis was observed for 10 hr after

FIG. 4. RNA and protein synthesis and interferon production in L-cell cultures infected with NDV₀ and NDV_{pi}. Monolayer cultures were infected in the usual manner (MOI 250), and at designated intervals the rates of RNA and protein synthesis were measured by the incorporation of radioactive precursors as described. Uninfected cultures were used as the control. The average radioactivity incorporated by uninfected cultures was 83,456 counts/min for ³H-uridine and 43,365 counts/min for ³H-leucine (per 3×10^6 cells). The rate of viral RNA synthesis was determined in cultures treated with actinomycin D (1 μ g/ml) after infection. Culture fluids harvested at the appropriate intervals were tested for interferon activity. Symbols: total RNA (●), total protein (○), viral RNA (Δ) synthesis, and interferon (□) activity.

infection; by 16 hr, the rate of RNA synthesis had declined to 55% of the rate in control cells. Shut-off of cellular protein synthesis was not detected in cells infected with NDV_{pi} for 6 hr after infection; by 10 hr, protein synthesis in these cells had declined to 10% of the control rate.

The delay in the initiation of shut-off of host RNA and protein synthesis in cells infected with NDV_{pi}, compared to those infected with NDV₀, can be correlated with the first detectable synthesis of viral RNA. The incorporation of ³H-uridine into NDV₀ RNA was first detected at about 4 hr, and the rate of this synthesis reached a maximum 8 hr after infection (Fig. 4). In contrast, the incorporation of the label into NDV_{pi} RNA was not detected until 8 hr after infection, and the rate of synthesis of this viral RNA reached a maximum at 14 hr. Significantly more radioactivity was incorporated into NDV_{pi} RNA than into NDV₀ RNA.

Despite the fact that NDV₀ RNA synthesis commenced 2 to 4 hr earlier than that of NDV_{pi}, interferon was first detected in the medium 8 hr after infection with both viruses and interferon continued to increase in concentration for the next 8 hr. This finding, which suggests that the initiation of synthesis of viral RNA is not directly

related to the induction of interferon synthesis, will be discussed in detail below.

It seems paradoxical that most of the interferon activity appeared in the medium after the rate of incorporation of ³H-leucine into trichloroacetic acid-precipitable material in infected cells was reduced to 10% or less of the rate in control cells (Fig. 4). This is more evident in cells infected with NDV₀, since this virus shuts off cellular protein synthesis more rapidly than does NDV_{pi}.

To eliminate the possibility that the uptake of radioactive precursors by the cells was impaired by virus infection, experiments were conducted to measure the amount of trichloroacetic acid-precipitable and trichloroacetic acid-soluble radioactivity at various times after infection. L-cell cultures were infected with NDV₀ in the usual manner, and at various times the cells were labeled with ³H-leucine for 20 min at 37 C. The incorporation of radioactive counts into trichloroacetic acid-precipitable and trichloroacetic acid-soluble materials was determined as described in footnote *a* of Table 2. In uninfected control cells, 62 to 69% of the total counts taken up by the cells were incorporated into trichloroacetic acid-precipitable material. In infected cells, 56% of the total recoverable counts were incorporated into trichloroacetic acid-precipitable material at 2 hr after infection; this value decreased to 33% at 5 hr and to 12% at 8 hr. This decrease in trichloroacetic acid-precipitable counts was correlated with an increase in the trichloroacetic acid-soluble counts from 44% at 2 hr to 88% at 8 hr. The fact that most of the radioactive ³H-leucine taken up by infected cells remained acid-soluble and was not incorporated into trichloroacetic acid-precipitable material suggests that the inhibition of protein synthesis in infected cells is not due to an impaired uptake of ³H-leucine.

DISCUSSION

The results presented in this report shed additional light on the nature of the inefficient replication of wild-type NDV₀ in L cells. Although a considerable amount of progeny virus is produced (11 PFU/cell), this infectivity remains cell-associated. After reaching a maximum at 6 hr, cell-associated virus gradually disappears for the next 8 hr and fails to appear as infective units in the medium. This finding accounts for the so-called "abortive" infection and for the overt "nonpermissiveness" of L cells for NDV₀. In light of the findings reported here, these designations are not as accurate as one would like. The fact that there is a significant production of mature virions intracellularly is not compatible with a

TABLE 2. Incorporation of ^3H -leucine into acid-precipitable protein and acid-soluble pool in L-cell monolayer cultures infected with NDV_o^a

Time after infection <i>hr</i>	Counts/min			
	Uninfected cells		Infected cells	
	Acid-precipitable	Acid-soluble	Acid-precipitable	Acid-soluble
2	85,340 (69) ^b	37,920 (31)	38,520 (56)	29,680 (44)
5	45,880 (61)	29,120 (39)	21,900 (33)	44,160 (67)
8	56,880 (62)	34,800 (38)	8,940 (12)	60,960 (88)

^a Cell cultures were infected with NDV_o at an input MOI of 300. After adsorption for 60 min at 37 C, the cells were washed twice, medium was added, and the cultures were reincubated at 37 C. At designated times after infection, the cultures were exposed to 1 μC of ^3H -leucine per ml for 20 min at 37 C. The cells were washed twice, suspended in 2 ml of Hanks solution, and treated with sonic oscillation for 5 min. The trichloroacetic acid-precipitable radioactivity was determined in the disrupted cell suspension by adding an equal volume of 10% trichloroacetic acid and incubating the mixture for 15 min at 4 C. The precipitate was collected onto a membrane filter (Millipore Corp.) and dried. The filter paper was transferred to a scintillation vial to which 10 ml of Bray's fluid (1) was added, and the radioactivity was measured. The trichloroacetic acid-soluble radioactivity in the disrupted cell suspension was determined by precipitating with trichloroacetic acid as described above. After the mixture was centrifuged at $1,000 \times g$ for 20 min, the supernatant fluid (0.05 ml) was added to 10 ml of Bray's fluid and the radioactivity was determined.

^b Per cent of total counts.

"nonpermissive" environment in the host cell. Alternatively, the process that we have described, i.e., intracellular maturation followed by faulty release and then by a gradual loss of infective units, can be designated a "covert" infection. It is apparent that if infective units were sought in the extracellular fluid at any time during the replication cycle or after 8 hr in a cell-associated state, the result would give the false impression that virus replication had not occurred. The situation which has been described pertains thus far only to the Herts strain of NDV. Experiments are in progress to determine how far this pattern of "covert" replication can be applied to the infection of L cells with other strains of NDV. In addition, we are investigating the events following

the "covert" infection of L cells with low multiplicities of NDV_o. Infection under these conditions results in the establishment of persistent infection and the ultimate selection of NDV_{pi} mutants (7). The rate and frequency of emergence of the mutant viruses which are fully infective for L cells, as well as the fate of the progeny NDV_o particles which disappear, are being studied.

It is generally agreed that one of the prerequisites for the induction of interferon synthesis by virus infection is that cellular RNA and protein synthesis must not be inhibited to a degree which interferes with the synthesis of interferon (2, 5, 8). More specifically, it has been reported that NDV fails to induce interferon synthesis in CE cell cultures because of the high virulence of the virus for this host and the consequent rapid inhibition of host-cell functions (3, 4). The data which we have presented make this view untenable. Our results show that, although the shut-off of host RNA and protein synthesis in CE cells by both NDV_o and NDV_{pi} is less rapid and significantly less efficient than the shut-off seen in L cells, virus production is greater and cell damage is more severe in CE cells than in L cells. Despite this difference in speed and efficiency of shut-off, it is in L cells, the more severely affected host, that interferon synthesis occurs. These results lead us to suggest that the defect in induction of interferon synthesis in CE cells infected with NDV is not related to the general shut-off of host cell synthetic mechanisms but rather to the failure of some more specific event to occur. In regard to the relation of shut-off of host protein synthesis and interferon induction, it is apparent from the observations in L cells that only a small residual fraction of the host protein-synthesizing activity is necessary for significant synthesis of interferon. Most of the interferon activity in the medium of cells infected with NDV_o or NDV_{pi} appeared after more than 90% of L cell protein synthesis was inhibited (Fig. 4). If one assumes that there probably is no selective inhibition of the synthesis of protein, it can be speculated that, in the absence of any inhibition of host-cell synthesis, NDV infection of L cells might produce 10 times more interferon. What we are measuring is the amount of interferon synthesis under the restrictive conditions imposed by virus infection.

The observations we have made also have some bearing on the relationship between initiation of viral RNA synthesis and the induction of interferon synthesis. Our results indicate that there may be little relationship of the two events. Viral RNA synthesis was detected in L cells infected with NDV_o at 4 hr and with NDV_{pi} at 8 hr. Despite a delay of up to 4 hr in the initiation of NDV_{pi} RNA synthesis, interferon was first

detected in the medium 8 hr after infection of L cells with either NDV_o or NDV_{pi}. Interferon concentration continued to increase for the next 8 hr with both viruses. This finding suggests that the initiation of viral RNA synthesis is not directly related to the induction of interferon synthesis and supports the conclusions of Lockart et al. (6), who studied interferon production in CE cells infected with RNA⁺ and RNA⁻ temperature-sensitive mutants of Sindbis virus. These workers concluded from their results that viral RNA replication or its accumulation in the cell is not related to the induction of interferon synthesis.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-06264 from the National Institute of Allergy and Infectious Diseases. It was conducted under the sponsorship of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under research contract 17-67-C7046.

LITERATURE CITED

1. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* 1:279-285.
2. Franklin, R. M., and D. Baltimore. 1962. Patterns of macromolecular synthesis in normal and virus-infected cells. *Cold Spring Harbor Symp. Quant. Biol.* 27:175-195.
3. Gandhi, S. S., and D. C. Burke. 1970. Interferon production by myxoviruses in chick embryo cells. *J. Gen. Virol.* 6:95-103.
4. Ho, M. 1964. Identification and "induction" of interferon. *Bacteriol. Rev.* 28:367-381.
5. Holland, J. J., and J. A. Paterson. 1964. Nucleic acid and protein synthesis during poliovirus infection of human cells. *J. Mol. Biol.* 8:556-573.
6. Lockart, R. Z., Jr., N. L. Bayliss, S. T. Toy, and F. H. Yin. 1968. Viral events necessary for the induction of interferon in chick embryo cells. *J. Virol.* 2:962-965.
7. Thacore, H., and J. S. Youngner. 1969. Cells persistently infected with Newcastle disease virus. I. Properties of mutants isolated from persistently infected L cells. *J. Virol.* 4:244-251.
8. Wagner, R. R., and A. S. Huang. 1966. Inhibition of RNA and interferon synthesis in Krebs-2 cells infected with vesicular stomatitis virus. *Virology* 28:1-10.
9. Wilson, D. E. 1968. Inhibition of host-cell protein and ribonucleic acid synthesis by Newcastle disease virus. *J. Virol.* 2:1-6.
10. Youngner, J. S., A. W. Scott, J. V. Hallum, and W. R. Stinebring. 1966. Interferon production by inactivated Newcastle disease virus in cell cultures and in mice. *J. Bacteriol.* 92:862-868.