Abortive Infection of L Cells by Fowl Plague Virus: Comparison of RNA and Protein Synthesis in Infected Chick and L Cells

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SUMMARY

When L cells were infected with fowl plague virus, virus haemagglutinin, neuraminidase and complement-fixing antigen, but no infectious virus, were produced. Comparison with infection of chick cells showed that, in both cases, there was an early period of sensitivity to actinomycin and that infection caused depression of host-cell protein synthesis. There was no difference in the distribution of newly synthesized virus RNA between the nucleus and cytoplasm in both chick cells and L cells, and all the virus proteins synthesized in chick cells were also synthesized in L cells. However, the two proteins found in the nucleus did not appear to move out of the nuclei of L cells.

INTRODUCTION

Influenza virus undergoes different patterns of multiplication depending not only on the host cell but also on the conditions of infection. Infection of primary chick embryo cells is followed by synthesis of virus RNA, and such virus specific antigens as haemagglutinin, complement-fixing antigen, and neuraminidase, and leads to formation of fully infectious progeny virus (Breitenfeld & Schafer, 1957). In contrast, influenza virus infection of various cell lines, such as L cells (Franklin & Breitenfeld, 1959; Bader & Morgan, 1961), HeLa cells (Hillis, Moffat & Holtermann, 1960) or BHK 21 cells (Fraser, 1967), results in synthesis of virus RNA and proteins but no mature virus is formed, and the growth cycle is abortive. Infection of chick embryo cells with influenza virus at high multiplicity leads to production of progeny of lowered infectivity (von Magnus, 1954). The production of von Magnus virus differs from abortive infection in that, in the latter case, no virus is either formed or released and the effect is not dependent upon infection with high multiplicities of virus. Pons & Hirst (1969) observed that, in contrast to the fully mature influenza virus, the particles of incomplete influenza virus prepared by two von Magnus passages lacked one of the pieces of RNA. This omission could be due to a block in the synthesis of this piece of RNA, or to its failure to be incorporated into the virus particle under conditions of multiple infection.

Much less is known about the basis of abortive infection. Using the fluorescent antibody technique, Franklin & Breitenfeld (1959) observed that the virus haemagglutinin and the soluble ribonucleoprotein antigen (the s-antigen) were synthesized in infected L cells of fowl plague virus but that no infectious virus was formed. However, in contrast to normal infection, the soluble ribonucleoprotein antigen could not be detected within the cytoplasm, and the authors suggested that the abortive infection of L cells with fowl plague virus was due to

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the failure of the soluble ribonucleoprotein antigen to leave the nucleus, thus preventing the combination with haemagglutinin and neuraminidase to form infective virus.

The work presented in this paper is an attempt to explain the abortive cycle of multiplication of fowl plague virus in L cells by comparison of virus nucleic acid and protein synthesis in normal and abortive infection.

METHODS

Materials. Actinomycin D was given by Merck, Sharp and Dohme Ltd. [5-3H]-uridine (27·6 c/m-mole), [3H]-DL-valine (250 to 515 mc/m-mole) and [U-14C]-valine (260 mc/m-mole) were obtained from the Radiochemical Centre, Amersham, Buckinghamshire, and acrylamide, NN'-methylenedisacrylamide and NN,N'N'-tetramethylethylenediamine from Eastman, Kodak. Sodium dodecyl sulphate (specially pure) was obtained from British Drug Houses Ltd, Poole, Dorset. Ether and ethanol were redistilled before use.

Tissue culture. Primary chick embryo cells were prepared and cultured as previously described (Waiters, Burke & Skehel, 1967). L cells were obtained from Flow Laboratories, Irvine, Scotland. The medium used was Eagle's Minimum Essential Tissue Culture Medium obtained from Wellcome Research Laboratories, Beckenham, Kent. Growth and maintenance medium contained 7 and 2 % calf serum (Flow Laboratories), respectively.

Virus. The ROSTOCK strain was used throughout. The virus was grown in the allantoic cavity of 11-day-old chick embryos as described previously (Walters et al. 1967). Complement-fixation, neuraminidase, haemagglutinin and infectivity assays were carried out as described previously (Walters et al. 1967; Long & Burke, 1970).

Growth experiments in chick and L cells. Cells (10 × 10^6 chick cells; 2 × 10^6 L cells) were infected with virus (at m.o.i. of 5 and 25, respectively) for 1 hr at 37°. The cells were then washed twice with Medium 199 containing 2 % calf serum before incubation in medium (2 ml./culture) at 37°.

Estimation of virus-induced 'cut-off' of host cell protein synthesis. Triplicate cultures of virus-infected and control chick embryo cells or L cells were pulsed for 1 hr with [3H]-valine at different times after infection. At the end of the pulse, the cultures were harvested as described earlier (Skehel et al. 1967).

Cell fractionation. Nuclear and cytoplasmic fractions for the study of virus protein synthesis were prepared as described by Ben-Porat, Shimono & Kaplan (1969). This method was faster and less elaborate than that previously used (Joss et al. 1969) but the results obtained were essentially the same. It could not be used for the study of virus RNA synthesis since the presence of Triton X-100 interfered with the subsequent extraction of RNA by phenol, and the method of Taylor, Hampson & White (1969) was used instead. Contamination of the nuclear pellet with cytoplasmic material was negligible, using the presence of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase as criteria.

Measurement of virus RNA synthesis in infected cells was carried out as described by Gandhi & Burke (1970).

Extraction and polyacrylamide gel electrophoresis of proteins from infected cells was carried out as described by Joss et al. (1969).

RESULTS

Virus multiplication in chick and L cells

Cells were infected, washed and incubated at 37°, and at intervals infectivity, haemagglutinin, neuraminidase and complement-fixing titres were measured in both cells and supernatant fluids. The results (Fig. 1) showed that, in contrast to chick cells, very little infectious
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Virus was produced in L cells, although haemagglutinin, neuraminidase and complement-fixing antigen were produced. These results are similar to those obtained by Franklin & Breitenfeld (1959), for infected L cells and Franklin & Henry (1960) for infected chick cells, except that we found that haemagglutinin was released more quickly from infected L cells, and that there was some indication of very limited virus multiplication. Neuraminidase levels in infected chick and L cells were very similar.

Fig. 1. Growth of fowl plague virus in chick and L cells. The infected cells were harvested at different times after infection and assayed for released infectivity (●—●), cell-associated infectivity (○—○), released haemagglutinin (■—■), cell-associated haemagglutinin (□—□), neuraminidase (▲—▲) and complement-fixing antigen (▼—▼).

Fig. 2. The effect of infection with fowl plague virus on the rate of protein synthesis in chick and L cells. Rates are expressed as a % of that of the uninfected control.
Virus induced 'cut-off' in chick and L cells

Long & Burke (1970) investigated the inhibitory effect (cut-off) of fowl plague virus infection on the rate of chick cell protein synthesis and showed that inhibition depended upon the formation of a virus-directed protein early in infection. It was therefore of interest to see whether infection of L cells with fowl plague virus caused a similar effect. Fig. 2 shows that treatment with virus produced a profound inhibitory effect in both types of cells.

The actinomycin sensitive event in chick and L cells

Actinomycin inhibits the multiplication of influenza virus in chick cells by a mechanism which is not understood (Barry, Ives & Cruickshank, 1962). It is, however, only effective early in the multiplication cycle (Rott & Scholtissek, 1964; White et al. 1965). Table 1 shows that chick and L cells behaved similarly in this respect, actinomycin having little or no effect on the processes leading to production of haemagglutinin when added 3 hr after infection, or later.

Table 1. Effect of addition of actinomycin to infected chick and L cells at different times after infection

<table>
<thead>
<tr>
<th>Time of addition of actinomycin* (hr after infection)</th>
<th>Haemagglutinin titre produced between time of addition and 24 hr after infection</th>
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<tr>
<td></td>
<td>Chick cells</td>
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<td>Control</td>
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<td>0</td>
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* Fluids were removed at different times after infection and replaced by medium containing 0.5 μg/ml. of actinomycin.

RNA synthesis in chick and L cells

Pons & Hirst (1969) have suggested that incomplete virus differs from normal virus in the absence of one of the pieces of RNA. Since it was possible that the abortive cycle in L cells was due to the failure to synthesize one or more pieces of the virus RNA, virus RNA synthesis in the infected cells was compared. It was found impossible to detect virus RNA synthesis by an isotopic incorporation method unless actinomycin was added to depress host cell RNA synthesis. An attempt to detect virus RNA synthesis by mixing infected cells which had been labelled with [14C]-uridine and uninfected cells labelled with [3H]-uridine, and determination of 14C/3H ratios was unsuccessful since no increases in the ratio (due to virus RNA synthesis) could be detected in nuclei or cytoplasm. Since actinomycin inhibits influenza virus multiplication if added before 2 1/2 hr after infection (Rott & Scholtissek, 1964; White et al. 1965), [3H]-uridine could not be added before 3 hr after infection and by this time virus RNA synthesis, both in nucleus and cytoplasm, is declining (Bell & Maasab, 1969; Scholtissek & Rott, 1970). Therefore this method only studied the later phase of virus RNA synthesis. It has also been demonstrated that addition of actinomycin depresses synthesis of minus strands of RNA and distorts the pattern of virus RNA synthesis (Scholtissek & Rott, 1970).
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Despite these difficulties, it was possible to detect virus RNA in both chick and L cells (Fig. 3). The RNA was electrophoresed on polyacrylamide gels in order to remove the low molecular weight RNA synthesized in the presence of actinomycin (Gandhi & Burke, 1970) and to demonstrate the characteristic pattern due to the presence of several components in the virus RNA. RNA extracted from L cells appeared to contain more of the lightest com-

![Polyacrylamide gel electrophoresis of the RNA extracted from infected chick and L cells.](image)

Fig. 3. Polyacrylamide gel electrophoresis of the RNA extracted from infected chick and L cells. Actinomycin (5 μg./ml.) was added at 3 hr after infection and [3H]-uridine (50 μC/culture) was present from 3½ to 5½ hr after infection. The arrows show the positions of the ribosomal markers.

![Polyacrylamide gel electrophoresis of the RNA extracted from the cytoplasm and nuclei of infected chick and L cells.](image)

Fig. 4. Polyacrylamide gel electrophoresis of the RNA extracted from the cytoplasm and nuclei of infected chick and L cells. Actinomycin (5 μg./ml.) was added at 3 hr after infection and [3H]-uridine (200 μC/culture) was present from 3½ to 4½ hr after infection. The arrows show the positions of the ribosomal markers.
ponent, but in other respects the patterns were similar. Lerner & Hodge (1969) have reported
a similar result using chick cells and HeLa cells.

Despite the nuclear localization of the virus nucleoprotein, it appears that virus RNA
synthesis proceeds mainly in the cytoplasm of infected cells (see review by Robinson &
Duesberg, 1968). There are also several reports of nuclear virus RNA synthesis (Robinson &
Duesberg, 1968; Bell & Maasab, 1969), although in no case is it possible to rule out cyto-
plasmic synthesis followed by transport into the nucleus. It was possible that in the abortive
cycle no virus RNA was associated with L cell nuclei, and the distribution of pulse-labelled
virus RNA between both chick and L cell nuclei and cytoplasm was therefore compared.
Newly synthesized virus RNA was detectable in both nuclei and cytoplasm (Fig. 4), although
the low counts in the nuclear fraction made resolution of the different RNA molecules more
difficult. Since the total nuclear radioactivity amounted to only 5% of the cytoplasmic
radioactivity, it was necessary to show that this small amount of RNA was not due to cyto-
plasmic contamination of the nuclei. No labelled RNA was detectable in the nuclear frac-
tion when uninfected cells were treated with actinomycin and then pulsed with [3H]-uridine,
nor was any found when cells were infected with Newcastle disease virus (NDV), although in
this case large amounts of labelled cytoplasmic RNA were readily detectable. Since NDV
multiplies exclusively in the cytoplasm this demonstrates that the labelled nuclear RNA in
cells infected with fowl plague virus was not due to cytoplasmic contamination. When L cells,
infecte with fowl plague virus, were treated in similar fashion, virus RNA was found in
both nuclear and cytoplasmic fractions (Fig. 4). Thus the normal and abortive cycles showed
no difference in the distribution of newly synthesized virus RNA, although the results were
not sufficiently precise to determine whether the relative amounts of the different RNA
molecules were the same in the two types of cell.

Protein synthesis in chick and L cells

Virus-specific protein synthesis was studied by the double-labelling method of Hay,
Skehel & Burke (1968). A preliminary experiment showed that there were no significant
differences in the virus proteins synthesized either in chick embryo or L cells. The abortive
infection of L cells is therefore not a consequence of the failure of the virus to synthesize one
of the virus proteins in L cells, and the intracellular distribution of virus protein synthesis
was therefore examined.

Nuclear and cytoplasmic fractions

Previous work (Joss et al. 1969) demonstrated that even though four or five virus proteins
were synthesized in infected chick cells, only two proteins were present in purified nuclei,
and Taylor et al. (1969, 1970), showed that these proteins were synthesized in the cytoplasm
and then migrated to the nucleus. When nuclear and cytoplasmic fractions of infected chick
and L cells were examined, these two proteins were also found in L cell nuclei (Fig. 5),
although cytoplasmic synthesis in L cells was at a much lower level than in chick cells. It is
clear that the abortive infection of L cells is not due to the absence of these two proteins from
the nucleus.

Virus proteins synthesized following different pulse lengths

Franklin & Breitenfeld (1959) suggested that the abortive cycle of fowl plague virus
multiplication in L cells was due to the failure of virus antigen to leave the nucleus, and
attempts were made to test this by 'pulse-chase' experiments in which it was hoped to follow
the movement of the two proteins associated with the nucleus, from the cytoplasm to the
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nucleus and back into the cytoplasm in the process of virus maturation. However, satisfactory results could not be obtained and attention was turned to a study of virus protein synthesis, using different pulse lengths.

A 5 min. pulse (Fig. 6) showed the presence of two virus proteins in the nucleus in both chick and L cells. The cytoplasmic proteins were less well resolved but the same two proteins

![Diagram](image)

**Fig. 5.** Polyacrylamide gel electrophoresis of the proteins extracted from the cytoplasm and nuclei of infected chick and L cells. The isotopes were present between 5 and 7 hr after infection.

![Diagram](image)

**Fig. 6.** Polyacrylamide gel electrophoresis of the proteins extracted from the cytoplasm and nuclei of infected chick and L cells at 5 hr after infection, when the isotopes were present for 5 min. Electrophoresis of the chick cell extracts was for a shorter time than of the L cell extracts.
could be distinguished. Thus, if all the virus proteins are synthesized within the cytoplasm they migrate into the nucleus within 5 min. Longer pulses of 30 min. (Fig. 7) and 2 hr (Fig. 5) all showed the presence of the two nuclear proteins. However, they revealed a striking difference between chick and L cells in that the two proteins associated with the nucleus are also present in chick cell cytoplasm but absent from L cell cytoplasm. If these two proteins are synthesized within the cytoplasm and move into the nucleus in both infected chick and L cells, but only migrate out of the chick cell nucleus, then they would be expected to be present in chick cell cytoplasm but almost absent from L cell cytoplasm.

![Fig. 7. Polyacrylamide gel electrophoresis of the proteins extracted from the cytoplasm and nuclei of infected chick and L cells at 5 hr after infection, when the isotopes were present for 30 min.](image)

**DISCUSSION**

This paper analyses the infective and abortive cycles of growth of fowl plague virus in chick embryo and L cells respectively. Comparison of the growth curves showed that haemagglutinin, neuraminidase and complement-fixing antigen were produced in infected L cells, but that no infectious virus was produced. The growth curves also show an early period of sensitivity to actinomycin followed by insensitivity. The virus-induced 'cut-off' of host-cell protein synthesis seen in chick embryo cells (Long & Burke, 1970) was also observed in
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L cells. There is no difference in the synthesis of virus RNA associated with the nucleus and cytoplasm, and all the virus-specific proteins that are synthesized in chick embryo cells are also synthesized in L cells. Previous results (Joss et al. 1969) showed that there is another virus specific antigen besides the ribonucleoprotein antigen associated with the nuclei of chick embryo cells, and this has now also been shown for L cells. This is in agreement with the observation of Taylor et al. (1969, 1970) that these two proteins were synthesized in the cytoplasm before movement into the nuclei of the HeLa cells. It is not known whether the movement of these two proteins out of the nucleus is essential for influenza virus multiplication, but the movement of soluble antigen from nucleus to cytoplasm in successful but not abortive infection (Breitenfeld & Schafer, 1957), coupled with our observation that these two proteins are found in the nucleus and cytoplasm of chick cells but are almost exclusively limited to the nuclei in L cells, suggests that movement out of the nucleus is essential for successful infection and that the L cell cycle is abortive because this process is inoperative. Fraser (1967) has reported an abortive cycle of influenza in BHK 21 cells where the soluble antigen does leave the nucleus. It is possible that there is another event in the virus assembly process after exit from the nucleus that fails to operate in this case.

In conclusion, two questions may be raised. Why do the proteins migrate to the nucleus in infected cells and subsequently leave it, and why does this latter process not occur in L cells?

We suggest that synthesis of plus-strand influenza RNA is restricted to the nucleus, that minus-strand RNA is synthesized in the cytoplasm, where like NDV it acts as the messenger RNA for virus protein synthesis. Further, we suggest that the two proteins migrate to the nucleus and interact with plus-strand RNA to form the ribonucleoprotein core of the virus, which then leaves the nucleus and interacts with the components of the lipoprotein envelope in the cytoplasm. It is known that one of these proteins is the complement-fixing antigen, which is closely associated with the RNA, and recently Schulze (1970) isolated a subvirus particle from influenza virus which contains these two proteins, and a very small amount of a third. It is known that both plus- and minus-strand virus RNA are synthesized in chick cells (Scholtissek & Rott, 1970), although nothing is known about the subcellular localization of the plus and minus strands. Nor is anything known about the factors controlling the synthesis of the complementary strands, and it is possible that the actinomycin sensitivity of influenza virus multiplication is connected with the synthesis of plus strands in the nucleus. C. Scholtissek (personal communication) has observed that, in contrast to the polymerase isolated from the microsomal fraction of influenza virus infected cells which synthesizes 100% minus strands (Scholtissek & Rott, 1969), the polymerase associated with the nuclear fraction synthesizes about 20% of plus strands. The restriction of plus-strand synthesis to the nucleus may offer an explanation as to why, in contrast to the particles of parainfluenza viruses (Robinson, 1970), the particles of fowl plague virus contain RNA which shows no self-hybridization but is 100% plus strand (Scholtissek & Becht, 1971). Thus, exclusion of minus strands from the virus particle could be achieved by topological separation of plus- and minus-strand synthesis.

This hypothesis predicts that in chick cells, virus RNA in the nucleus should be plus and that in the cytoplasm, should be plus (from completed subvirus particles) and minus (from polysomes). In contrast, since the virus nucleoprotein does not leave the nuclei of L cells there should be no plus-strand RNA in the cytoplasm of infected L cells. This prediction is open to experimental test. This hypothesis does not explain why the virus proteins do not leave the nuclei of L cells; it may be that the assembly of the virus nucleoprotein is faulty or that factors are required for migration of the nucleoprotein out of nuclei, which are present in chick cells but not in L cells.
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