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Host Range Restriction of Vaccinia Virus in Chinese Hamster Ovary Cells: Relationship to Shutoff of Protein Synthesis

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Chinese hamster ovary cells were found to be nonpermissive for vaccinia virus. Although early virus-induced events occurred in these cells (RNA and polypeptide synthesis), subsequent events appeared to be prevented by a very rapid and nonselective shutoff of protein synthesis. Within less than 2 h after infection, both host and viral protein syntheses were arrested. At low multiplicities of infection, inhibition of RNA synthesis with cordycepin resulted in failure of the virus to block protein synthesis. Moreover, infection of the cells in the presence of cycloheximide prevented the immediate onset of shutoff after reversal of cycloheximide. Inactivation of virus particles by UV irradiation also impaired the capacity of the virus to inhibit protein synthesis. These results suggested that an early vaccinia virus-coded product was implicated in the shutoff of protein synthesis. Either the nonpermissive Chinese hamster ovary cells were more sensitive to this inhibition than permissive cells, or a regulatory control of the vaccinia shutoff function was defective.

Vaccinia virus has a relatively broad host range in tissue culture. In our own experience, during studies with more than 10 cell lines or strains derived from seven different species, no cells displaying a nonpermissive phenotype were found. Moreover, few reports have appeared concerning abortive infections with wild-type vaccinia virus. Recently however, we have found that a Chinese hamster ovary (CHO) cell line is nonpermissive for our wild-type vaccinia virus strain. The level of arrest of vaccinia virus multiplication in these cells has been studied. The results have suggested that the very early and extensive inhibition of protein synthesis in CHO cells may be responsible for the fact that the infection is abortive. These findings have prompted us to reinvestigate some aspects of vaccinia virus-directed inhibition of protein synthesis.

MATERIALS AND METHODS

Cells. The baby hamster kidney cell line (BHK-21) was grown in monolayers in BHK medium (Eurobio, Paris, France) supplemented with 10% calf serum. The CHO cell line (CHO proline auxotroph) was grown in monolayers in alpha medium (Eurobio) supplemented with 10% calf serum.

Virus. The Lister strain of vaccinia virus, previously passaged in KB cells, was grown on BHK cells and purified by the method of Joklik (9). Virus titers were determined in PFU by plaque assay on monolayers of BHK cells.

UV irradiation of virus. Virus was irradiated at a distance of 35 cm with UV light from a 15-W germicidal lamp. Measurement of the energy emitted at the level of the irradiated samples gave a value of 20 ergs/mm^2 per s.

Infection of cells. Monolayers containing approximately 5×10^6 cells were infected with 1 ml of purified vaccinia virus at various multiplicities. Virus was adsorbed to the cells by gentle rocking of the cultures for 30 to 60 min as indicated in the experiments. At the end of the adsorption period, unadsorbed virus was removed, the cells were washed once with phosphate-buffered saline, fresh medium was added, and the cultures were incubated in a water bath at 37°C.

Labeling of proteins and RNA. Proteins of infected or uninfected cell cultures were labeled in a methionine-free medium with [^{35}S]methionine or in a normal medium with a mixture of ^{14}C-amino acids. After the labeling period the cells were collected by trypsinization, sedimented, and washed twice with phosphate-buffered saline. In some cases cytoplasmic fractions were prepared by suspending the cell pellets in 2 ml of reticulocyte swelling buffer containing Tris-chloride (pH 7.4, 0.01 M), KCl (0.01 M), and $MgCl_2$ (0.001 M). After 10 min the cells were broken with a Dounce homogenizer, cell breakage being monitored by microscopy, and the nuclei were pelleted by centrifugation for 10 min at 1,000 × g. The supernatants or whole-cell pellets were precipitated with 5% trichloroacetic acid and heated in a boiling-water bath for 20 min. The precipitates were then recovered on glass fiber filters (Whatman GF/C), and the radioactivity was determined by liquid scintillation counting.

For labeling of vaccinia virus cytoplasmic RNA, the procedure described by Becker and Joklik (3) was

followed. Briefly, [^{14}C]uridine was added to the cultures for 10 min at various times after infection. The cytoplasmic fractions were prepared as described above, precipitated with 5% trichloroacetic acid, collected on glass fiber filters, and counted.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. [^{35}S]methionine-labeled cytoplasmic fractions from 5×10^6 cells were precipitated with 5% trichloroacetic acid, washed twice with acetone, and dissolved in 100 μl of a solution containing Tris-hydrochloride (0.05 M pH 6.8), sodium dodecyl sulfate (2%), β-mercaptoethanol (2%), glycerol (15%), and bromophenol blue (0.001%). The samples were submitted to electrophoresis on slab gels (5% acrylamide stacking gel, 10% acrylamide resolving gel) by the techniques described by Laemmli (12) and O'Farrell et al. (17). After electrophoresis, gels were stained with Coomassie brilliant blue, destained, dried, and exposed to Kodirex films.

Radioisotopes and chemicals. [^{35}S]methionine (400 Ci/mol), [^{14}C]uridine (400 Ci/mol), and the ^{14}C-amino acid mixture (40 Ci/carbon atom) were obtained from the Commissariat à l'Energie Atomique, Saclay, France. Cordycepin and cycloheximide were obtained from the Sigma Chemical Co., St. Louis, Mo.

RESULTS

Restriction of vaccinia virus multiplication in CHO cells. Vaccinia virus failed to grow in CHO cells, whereas frog virus 3 grew very efficiently (Fig. 1). Permissiveness for frog virus 3 implied that CHO cells did not display any intrinsic inability to support the growth of a cytoplasmic DNA virus. The multiplicities of infection (MOI) with vaccinia have been varied from 0.2 to 200 PFU per cell without resulting in any increase of the input titer. Although vaccinia did not multiply in CHO cells, infection resulted in cell killing with one-hit kinetics as measured by single-cell colony formation (data not shown). Successive passages of cells surviving high or low MOI did not allow the selection of any virus adapted for growth on CHO cells. Clearly the block in permissiveness of CHO cells, for vaccinia, is a stable one.

Protein synthesis in vaccinia-infected cells. Protein synthesis was followed by pulse-labeling with [^{35}S]methionine after infection of

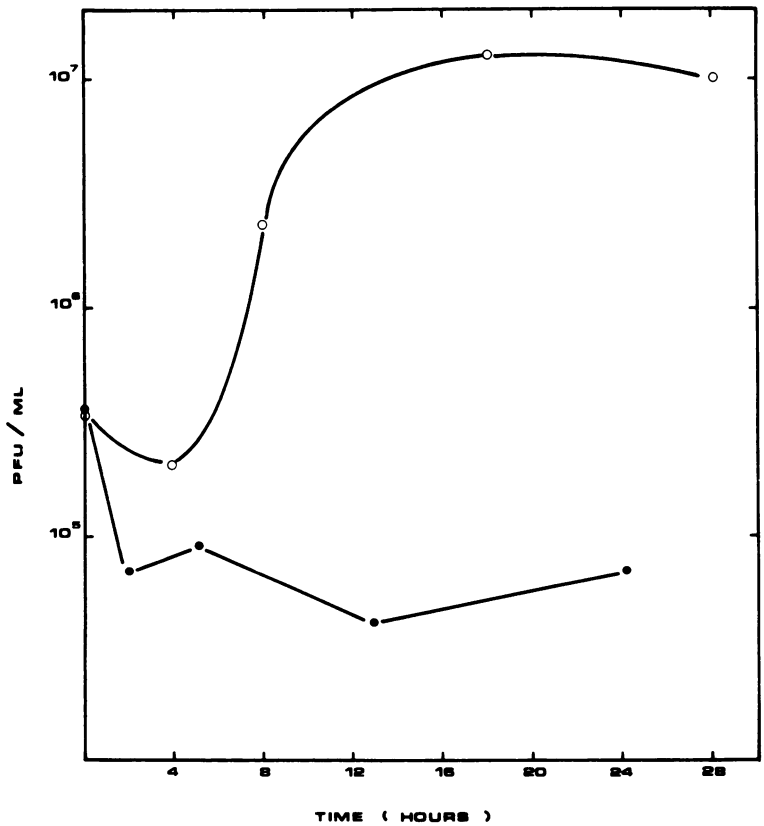

FIG. 1. *Multiplication of vaccinia virus (at 37°C) and frog virus 3 (at 29°C) in CHO cells. Cell monolayers were infected with 2 PFU/cell. At the indicated times the cultures were frozen and subsequently assayed for virus titers on BHK cells. Symbols: ●, vaccinia; ○, frog virus 3.*

permissive BHK or nonpermissive CHO cells (Fig. 2). In BHK cells, the rate of incorporation of radioactivity into acid-precipitable material decreased somewhat after infection but remained at a level at least 50% of the uninfected cells for 5 h. In CHO cells, the rate of incorporation into acid-precipitable material was rapidly inhibited by up to 95% as soon as 90 min after infection. The rate of uptake of methionine into the acid-soluble material of CHO cells increased by about 30% during the same period (data not shown); therefore, inhibition of protein synthesis was not a consequence of a defect in uptake. When a mixture of radioactively labeled amino acids was used instead of methionine, similar results were obtained. These experiments suggested that productive infection of CHO cells was restricted by the nearly total inhibition of protein synthesis that occurred very early after infection.

To ascertain whether any virus-induced polypeptides were synthesized during the first 2 h of infection, cytoplasmic extracts from infected cells were submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3). Several polypeptides with molecular weights differing from the cellular ones appeared both in CHO and BHK cells as soon as the first 30-min pulse-labeling after infection. These polypeptides, most likely coded for by the viral genome, displayed similar molecular weights in CHO and BHK cells. In CHO cells the overall inhibition of protein synthesis affected both cellular and virus-induced polypeptides from 30 min after infection on, whereas in BHK cells there was a slight decrease in labeling of cellular polypeptides. Inhibition of cellular polypeptides in BHK cells similar to that which occurred in CHO cells was only observed after 5 to 6 h of infection (data not shown). During the first hour of infection, when virus-induced polypeptides in CHO cells were best observed, no qualitative difference could be detected between the virus-induced polypeptides in CHO and BHK cells.

Effect of cordycepin and cycloheximide on vaccinia-induced shutoff of CHO protein synthesis. Cordycepin (3'-deoxyadenosine) completely inhibited vaccinia virus cytoplasmic RNA synthesis in CHO cells (Fig. 4). This has enabled us to determine whether viral RNA synthesis is necessary for the rapid inhibition of protein synthesis in these cells. As shown in Fig. 5, inhibition of protein synthesis (assayed 2 h after infection) was dependent on the MOI and reached a maximum level at 5 PFU per cell. At low MOI (below 5 PFU/cell) cordycepin clearly prevented inhibition of protein synthesis, whereas at higher MOI inhibition occurred, although to a lesser extent than in the cells infected without cordycepin.

Cycloheximide (20 μg/ml) inhibited protein synthesis in CHO cells by 93% within the first

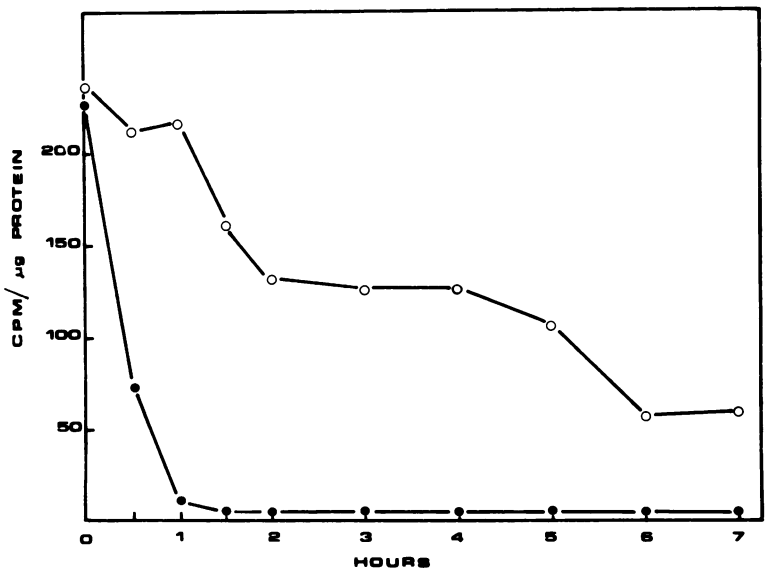

FIG. 2. *Rate of protein synthesis in BHK and CHO cells infected with vaccinia virus. Cell monolayers were infected with approximately 10 PFU of vaccinia virus per cell. The adsorption period lasted 30 min at room temperature, and then the cells were incubated at 37°C and labeled with [^{35}S]methionine (4 μCi/ml) for 30 min at the times indicated after adsorption. The cells were collected, cytoplasmic fractions were prepared, and the acid-precipitable radioactivity was determined. Symbols: ○, BHK; ●, CHO.*

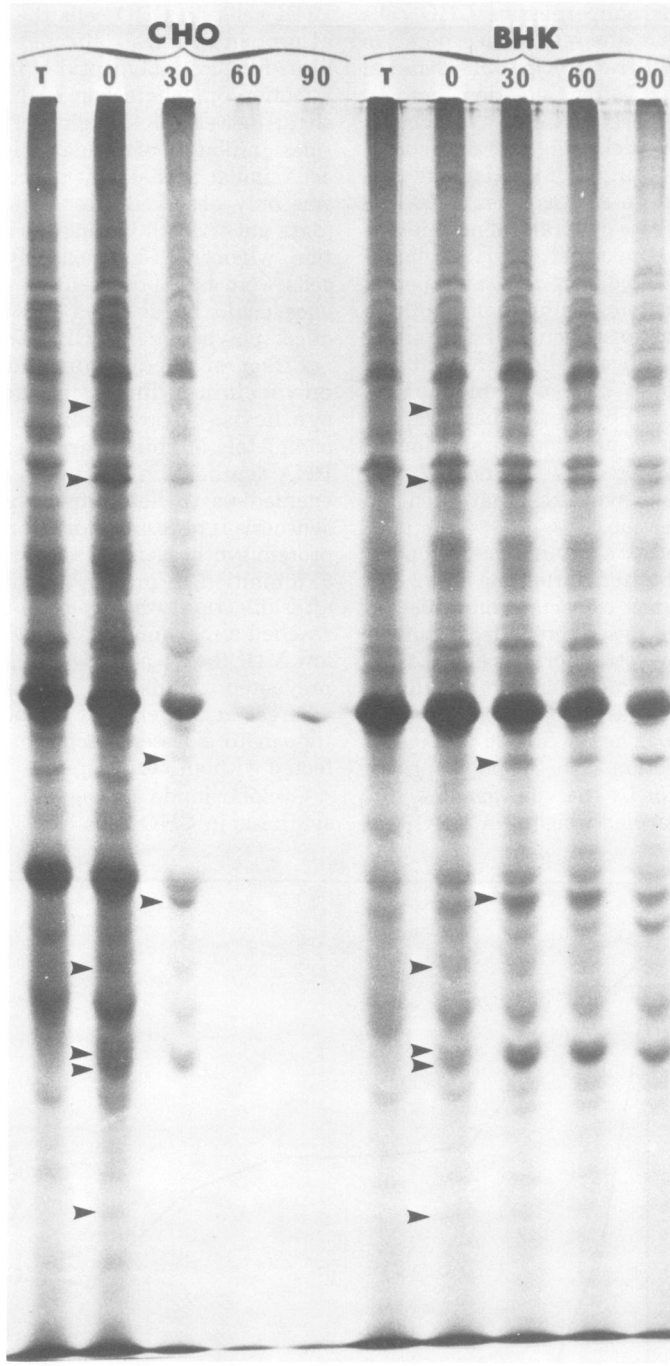

FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of polypeptides from CHO and BHK cells infected with vaccinia virus. Cells were infected as described in the legend to Fig. 2. At the indicated times after infection (in minutes) the cells were labeled with [^{35}S]methionine (4 μCi/ml) for 30 min. After the labeling the cells were collected, and cytoplasmic fractions were prepared for gel electrophoresis which was carried out as previously described. T, Mock-infected cells. The positions of a number of virus-induced polypeptides found in CHO and BHK cells are designated with arrows.*

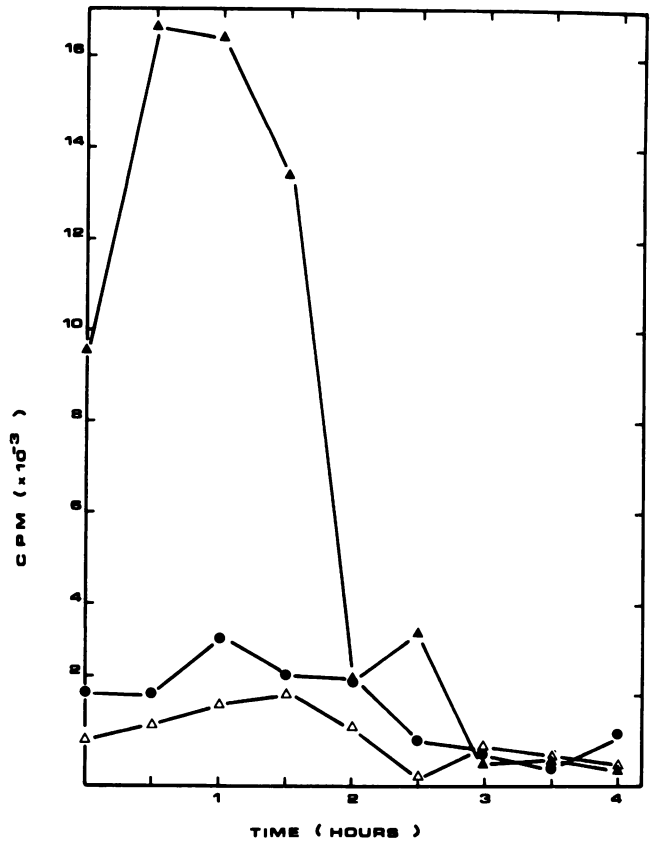

FIG. 4. *Vaccinia virus RNA synthesis in CHO cells. Cells were infected with vaccinia virus (20 PFU/cell) at room temperature. At 90 min before infection cordycepin (100 μg/ml) was added to some of the cultures and maintained in the medium during and after the adsorption period. After 30 min of adsorption the cells were incubated at 37°C. At the indicated times after adsorption, the cultures were labeled with [^{14}C]uridine (2.5 μCi/ml) for 10 min. Cytoplasmic fractions were prepared, and the acid-precipitable radioactivity was determined. Symbols: ▲, infected cells in the absence of cordycepin; △, infected cells with cordycepin; ●, mock-infected cells.*

20 min of its addition (data not shown). Infected (5 PFU/cell) or mock-infected cells were treated with 20 μg of cycloheximide per ml before and during infection, and the recovery of the protein synthetic capacity was followed after the removal of the cycloheximide-containing medium from the cultures (Fig. 6). Under these conditions protein synthesis immediately increased in the uninfected cells, although to a rate only 25% that of cells not previously treated with cycloheximide. In the infected cells the rate of protein synthesis was similar to that of the uninfected cells for the first two pulses after reversal (30-min interval) and declined thereafter. It appears, therefore, that the presence of cycloheximide during the first 150 min of infection prevented the appearance of a state in which protein synthesis could be inhibited after the removal of cycloheximide. Resumption of protein synthesis for at least 30 min was required for inhibition to occur.

In agreement with the effects of cycloheximide and cordycepin, it was found that irradiation of vaccinia with UV light also resulted in loss of the ability to inhibit protein synthesis (data not shown). The 37% survival dose for this function, determined at a MOI of approximately 10 PFU/cell, was 2,400 ergs/mm^2. As compared to the UV sensitivities of other targets (8, 13, 14, 19) the 37% survival dose for inhibition of protein synthesis by vaccinia suggests that loss of the inhibitory activity was due to inactivation of viral nucleic acid rather than structural protein.

DISCUSSION

Abortive infection of CHO cells with vaccinia virus may be related to the particular virus strain or cell line that we have employed. Indeed,

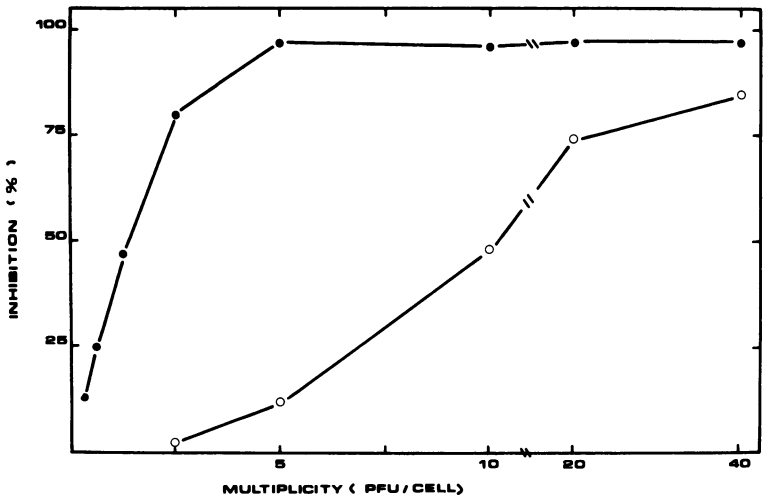

FIG. 5. *Effect of cordycepin on inhibition of protein synthesis in CHO cells infected with vaccinia. Cells were infected at the indicated multiplicities, and adsorption was carried out at 4°C for 45 min. At the end of the adsorption period, the cells were incubated at 37°C either in the presence* (○) *or absence* (●) *of cordycepin (100 μg/ml). At 2 h after infection, the cells were pulse-labeled for 45 min with a mixture of* ^{14}*C-amino acids (2 μCi/ml). The cells were then collected, and the total acid-precipitable counts were determined. The percent inhibition was calculated relative to mock-infected cells either treated or not treated with cordycepin. (In uninfected cells cordycepin reduced protein synthesis by 20%.)*

our particular "wild-type" vaccinia strain may be a naturally occurring host range mutant. Other vaccinia strains should be assayed on CHO cells to answer this question. Moreover, the CHO cell line is not characteristic of Chinese hamster cells in general, because we have found that the Chinese hamster lung cell line (V 79) is permissive for vaccinia virus. Nevertheless, abortive infection of CHO cells with vaccinia virus displays several interesting features. Most surprisingly, inhibition of protein synthesis in CHO cells is much more rapid and extensive than in the BHK permissive cell line. Unlike the inhibition normally observed after virus infection, vaccinia did not selectively inhibit translation of cellular mRNA but affected both the synthesis of viral and cellular polypeptides. Shutoff of protein synthesis was maximal after 90 min of infection when viral RNA was still being synthesized at a high rate (Fig. 4). It seems reasonable to suggest that the nonselective inhibition of protein synthesis in CHO cells is the explanation for restriction of vaccinia multiplication in this cell line. However, it cannot be excluded that other steps in vaccinia multiplication would not be defective if infection could proceed.

A better understanding of this abortive infection should be obtained by studying the processes involved in vaccinia-induced shutoff of protein synthesis. A number of reports dealing mainly with the inhibition of cell protein synthesis by vaccinia virus have appeared (1, 2, 4, 5, 11, 15, 18, 20–22). In two of these reports, experiments carried out with cordycepin suggested that viral RNA synthesis is required for shutoff of host polypeptide synthesis (1, 2), whereas others have presented similar experiments implying that viral RNA synthesis is not a requisite for expression of the shutoff function (7, 15, 18). The experiments in CHO cells suggest that at low MOI shutoff requires viral RNA synthesis, whereas at high MOI a vaccinia virus constitutive inhibitor may be at a sufficient concentration to act directly on protein synthesis. There is some in vitro evidence for such a constitutive vaccinia virus inhibitor of protein synthesis (4, 5).

At low MOI, in addition to the requirement for RNA synthesis, the experiment carried out with cycloheximide suggests that protein synthesis is also a requisite for shutoff of translation in CHO cells. This result is in disagreement with a similar experiment in HeLa cells (15), although the discrepancy could be due to the differences in the cell line or the MOI employed. It has also been reported that in $LLCMK_2$ cells host protein synthesis was not immediately inhibited after removal of cycloheximide (1, 2); however, in these cells apparently little viral RNA was synthesized in the presence of cycloheximide. In the case of CHO cells, cycloheximide stimulated the rate of cytoplasmic RNA synthesis after infection (data not shown) as has been previously

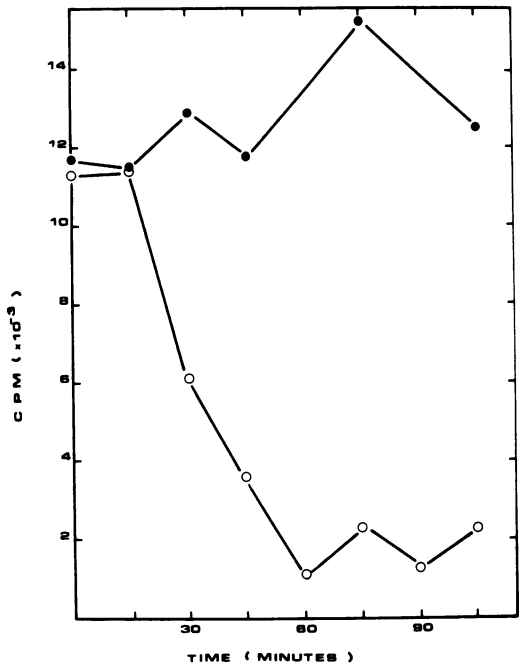

FIG. 6. *Effect of cycloheximide on inhibition of protein synthesis in CHO cells infected with vaccinia. Cells were treated for 1 h before infection with cycloheximide (20 μg/ml). They were then infected with 5 PFU/cell (○) or mock infected (●), and adsorption was carried out at room temperature for 30 min in the presence of cycloheximide. The cells were then incubated at 37°C with cycloheximide for 2.5 h. At the end of this period, the medium containing cycloheximide was removed, and the cells were washed once with phosphate-buffered saline and fresh medium without cycloheximide was added. The cultures were incubated at 37°C and pulse-labeled for 15 min with a mixture of ^{14}C-amino acids (4 μCi/ml) at the times indicated. At the end of each pulse, the cells were collected, and the acid-precipitable counts were determined. Control culture counts not shown on the figure were the following: mock infected without cycloheximide, 45,300; mock infected before the removal of cycloheximide, 1,700; infected before the removal of cycloheximide, 1,500.*

reported for vaccinia RNA synthesis (10, 23), although it is known that in the presence of cycloheximide a limited region of early sequences is not transcribed (6, 10).

These studies imply that an early vaccinia virus-induced product is directly or indirectly involved in inhibition of host protein synthesis. In the absence of expression of the viral genome a high MOI is required to achieve a comparable inhibitory effect. The rapid and nondiscriminative shutoff of protein synthesis by vaccinia virus in CHO cells may be due either to intrinsic sensitivity of the CHO translational machinery to a vaccinia virus inhibitor or to a defect in this cell system of a regulatory control of the shutoff function that normally works in permissive cells. It has been previously suggested on the basis of in vivo and in vitro experiments that vaccinia virus codes for an early viral protein factor necessary for translation of viral mRNA (5, 16). Defectiveness of such a factor in the CHO cell line would logically lead to nondiscriminative shutoff of protein synthesis.

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