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MACROMOLECULAR SYNTHESIS IN BLUETONGUE VIRUS INFECTED CELLS. II. HOST CELL METABOLISM

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ABSTRACT

HUISMANS, H. Macromolecular synthesis in bluetongue virus infected cells. II. Host cell metabolism. *Onderstepoort J. vet. Res.*, 37 (4), 199-210 (1970).

Infection of L-cells with bluetongue virus results in inhibition of protein and deoxyribonucleic acid synthesis shortly after infection. No inhibition of ribonucleic acid synthesis is observed before 7 hours after infection. The length of the lag phase before the initiation of the inhibition of protein synthesis is dependent upon the number of infecting virus particles. An increase in the multiplicity of infection results in a decrease in the length of the lag phase. No new macromolecular synthesis is required for the induction of inhibition. Inhibition of viral replication by interferon or UV inactivation does not prevent the induction of inhibition. Virus neutralized by antiserum or inactivated by heat or acid treatment is unable to induce the changes in host cell metabolism.

Introduction

Inhibition of host cell macromolecular synthesis is a common characteristic of many viruses. Infection with picorna viruses, e.g. poliovirus, results in a drastic inhibition of ribonucleic acid (RNA) and protein synthesis shortly after infection (Holland, 1964; Penman & Summers, 1965), whereas infection with a myxovirus, such as Newcastle disease virus, leads to inhibition of RNA and protein synthesis at a later stage in the infection cycle (Wilson, 1968). Relatively little is, however, known about the influence of double-stranded RNA viruses on host cell metabolism. The only double-stranded RNA virus which has been extensively studies in this respect is the reovirus. Infection with reovirus causes a marked inhibition of cellular deoxyribonucleic acid (DNA) synthesis about 6 hours after infection (p.i.) (Kudo & Graham, 1965; Ensminger & Tamm, 1969). No inhibition of RNA synthesis occurs, but inhibition of protein synthesis has been reported (Loh & Soergel, 1967). According to Ensminger & Tamm (1969), however, this inhibition of protein synthesis is only observed in suspension cultures.

Bluetongue virus (BTV) possesses many characteristics in common with reovirus. Both contain a fragmented double-stranded genome (Verwoerd, Louw & Oellermann, 1970), which is copied during replication into corresponding lengths of single-stranded messenger RNA molecules (Huismans, 1970). The main difference between them concerns the morphology of the virus particles. BTV particles are smaller and the single-layered capsids consist of a smaller number of capsomeres (Els & Verwoerd, 1969).

It is possible that the protein coat of a virus may play an important part in the induction of changes in host cell macromolecular synthesis. This motivated a study on the effect of BTV on cellular synthesis. From such a study it would be possible to compare the influence of two viruses with closely related nucleic acid moieties and similar modes of replication, but with very different protein coat structures.

In this paper results are presented illustrating the influence of BTV infection on host cell protein, DNA and RNA synthesis. It was found that, in contrast to reovirus, BTV caused a very drastic inhibition of macromolecular synthesis in the cell shortly after infection. Selective inhibitors of virus replication, protein and RNA synthesis respectively were used to determine whether replication of the virus or synthesis of new macromolecules were a prerequisite for inhibition. To obtain some information on the possible role of the protein coat and the nucleic acid in the induction of the observed inhibitory phenomena, a comparison was made between the influence of antiserum-neustralized virus and UV-inactivated virus on host cell macromolecular synthesis.

Materials and Methods

Virus

BTV Type 10 was used. Highly purified virus was obtained as described by Verwoerd (1969). The method for the partial purification of BTV has been described in the previous paper (Huismans, 1970).

UV-inactivation of the virus

A purified virus suspension in a Petri-dish was irradiated for 30 min with a 660 Watt UV lamp. The distance from the lamp to the meniscus of the suspension in the Petri-dish was 46 cm. Mechanical agitation of the suspension during irradiation ensured its homogeneity. After 30 min a residue of less than 0.01 per cent of the original plaque-forming units (PFU) remained in the suspension.

Neutralization of the virus

Purified virus was mixed with a suitable amount of Type 10 BTV antiserum and incubated for 30 min in a waterbath at 37°C. The neutralized virus suspension which was obtained contained only about 0.01 per cent of the original PFU's.

Cells

The origin and cultivation of the L-cells that were used have been described previously (Verwoerd, Oellermann, Broekman & Weiss, 1967).

Interferon

Mouse interferon was prepared by the procedure described by Huismans (1969). Primary mouse embryo cells were infected with BTV and the interferon harvested 24 hours later. The interferon was concentrated by two successive zinc acetate precipitations according to the method of Lampson, Tytell, Nemes & Hilleman (1963). Zinc ions were removed by dialysis against a solution of 0.9 per cent NaCl in diluted HCl, pH 2.5, followed by dialysis against phosphate-buffered saline, pH 6.8. The interferon concentration was determined by a plaque reduction method. Ecbovirus SA-I was used as indicator virus. Before use, the concentrated interferon solution was diluted at least 20-fold with leucine-free Eagle's medium to a final concentration of 300 interferon units/ml.

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Pulse labelling technique used to study the rate of macromolecular synthesis during infection

Macromolecular synthesis in infected cells was studied by measuring the amount of labelled precursor of protein, RNA and DNA synthesis built into trichloracetic acid (TCA)-precipitable material during a 15 min pulse at regular intervals after infection. Precursors ^{14}C-uridine (0.05 μCi/ml), ^{14}C-thymidine (0.025 μCi/ml) and ^{3}H-leucine (0.5 μCi/ml) were used for RNA, DNA and protein synthesis respectively.

Actively-growing, 2 or 3 day old monolayer cultures of L-cells in Roux flasks were incubated overnight with leucine-free Eagle's medium supplemented with 4 per cent bovine serum. These cells were then used to prepare a suspension culture in leucine-free Eagle's medium, containing 1.5×10^6 cells/ml. This cell suspension was divided into a number of smaller aliquots in Erlenmeyer flasks and incubated at 37°C. The cultures were stirred magnetically. To ensure a constant pH during the experiments the stoppered flasks were flushed from time to time with a 5 per cent CO_2/air mixture.

Unless otherwise indicated, cells were infected with purified BTV at an input multiplicity of 80 to 100 PFU/cell. Virus was allowed to adsorb for 60 min at 37°C and unadsorbed virus removed by low speed centrifugation at 4°C. The cells were washed once in ice cold medium and resuspended at the previous concentration in leucine-free Eagle's medium at 37°C. The actual time of infection was taken as zero hour and both the time immediately before removal of excess virus and the time when cells were resuspended in the medium at 37°C were taken as 1 hour after infection.

The labelled precursors of the different macromolecules were measured out in separate 10 ml screw cap tubes. At ½ hour intervals after infection 1 ml volumes of each of the relevant cultures were pipetted into the prewarmed tubes and the tubes incubated at 37°C in a small roller tube apparatus. After 15 min, further incorporation was stopped by precipitating the cells with 6 volumes of ice cold 10 per cent TCA. The precipitates were allowed to stand for at least 1 hour at 4°C and were then collected on Millipore filters. Precipitates were washed several times with ice cold 5 per cent TCA, followed by a single wash with 4 per cent acetic acid to remove any residual TCA. Precipitates were dried for 1 hour at 80°C and counted in toluene scintillator solution in a Tricarb scintillation counter.

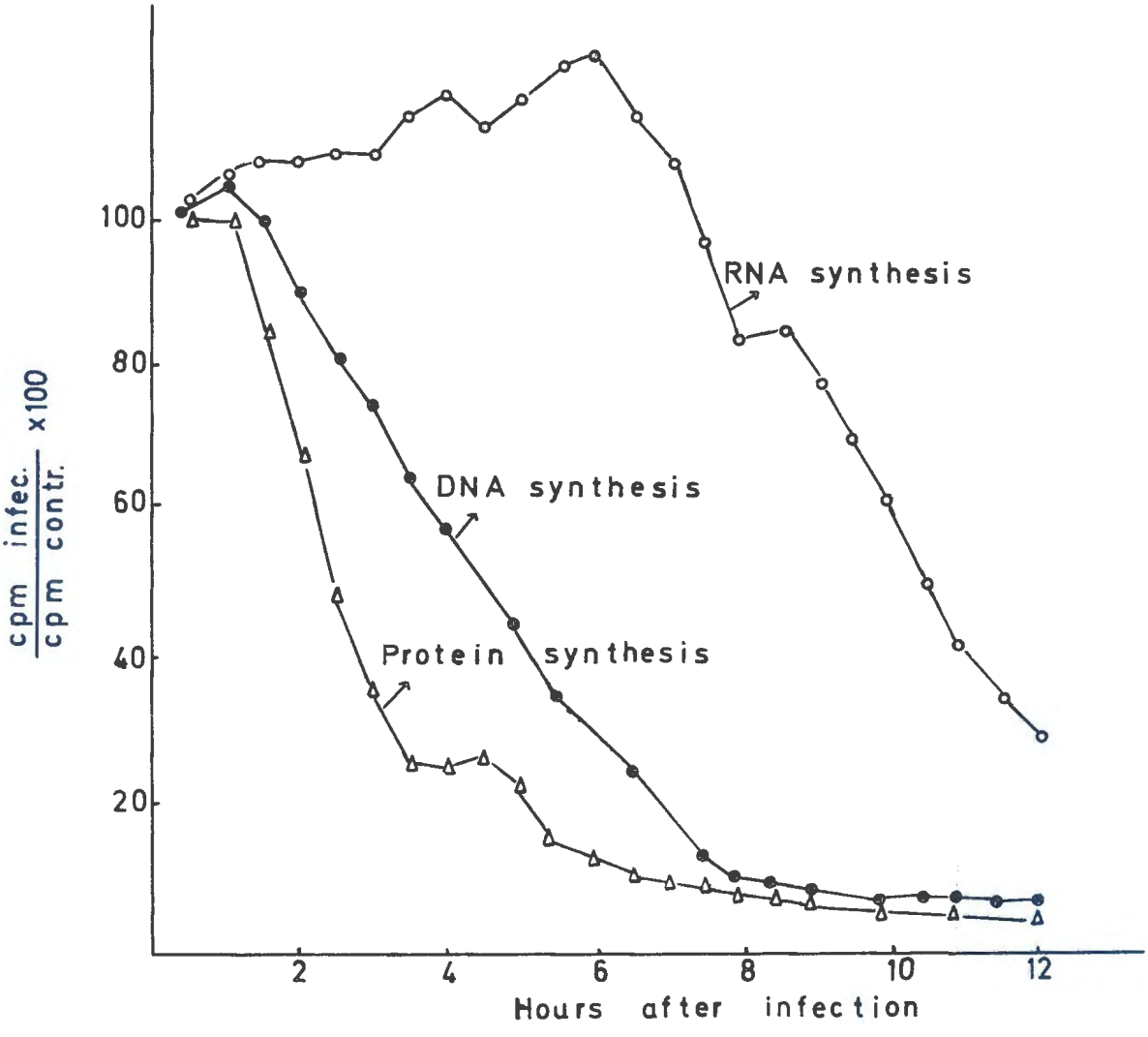

FIG. 1 Incorporation rate of labelled precursors of RNA, DNA and protein synthesis in BTV-infected L-cells. The rate of incorporation is expressed as a percentage of that in a non-infected control. ^{14}C-uridine incorporation, —○—; ^{14}C-thymidine incorporation, —●—; ^{3}H leucine incorporation, —△—

Presentation of the results

All results were referred to values obtained for non-infected control cultures, that had been treated throughout the experiments in the same way as the infected cultures. The results are presented by plotting the amount of label incorporated in the infected culture as a percentage of the label incorporated in the equivalent control culture against time after infection. A comparison of the results obtained in different repeat experiments was enhanced by this method due to elimination of variations in the absolute rate of macromolecular synthesis caused by small differences in cell concentration and metabolic state of the cells in different experiments.

Due to the difficulty of handling a large number of samples at the same time it was often impossible to obtain duplicate values for the incorporation of different precursors. To eliminate errors, the interval between succeeding 15 min pulses was made as short as possible. Every experiment was repeated at least three times.

Results

Protein, DNA and RNA synthesis in BTV-infected cells

Fig. 1 shows the rate of protein, DNA and RNA synthesis after infection of a L-cell suspension culture with purified BTV at an input multiplicity of 75 PFU/cell. There is obviously a marked inhibition of protein and DNA synthesis shortly after infection. The inhibition of protein synthesis commences at about 1 hour p.i. The rate of synthesis decreases rapidly and 3 hours p.i. protein synthesis is inhibited by 70 per cent. A very small increase in the rate of protein synthesis was usually observed between 3½ and 5 hours p.i. This increase could possibly be attributed to viral protein synthesis. Inhibition of DNA synthesis begins shortly after the inhibition of protein synthesis, but the inhibition is less drastic. After 3 hours the rate of DNA synthesis is inhibited by 20 per cent. Nine hours p.i. the inhibition of DNA synthesis is almost complete.

The pattern for RNA synthesis in the infected cells is somewhat different. There is no severe inhibition of RNA synthesis up to 7½ hour p.i. and between 2½ and 7½ hours p.i. the rate of RNA synthesis in the infected culture actually appears to be higher than in the control culture. However, inhibition of RNA synthesis does occur subsequently and 11 hours after infection the rate of RNA synthesis is inhibited by more than 50 per cent.

It has been shown by Huismans (1970) that virus-specific RNA synthesis commences soon after infection and continues until at least 14 hours p.i. This probably explains the higher rate of total RNA synthesis in the infected culture between 2½ and 7½ hours p.i. After 7 hours p.i. total RNA synthesis in the infected culture is inhibited. This must be due to inhibition of cell-specific RNA synthesis, because the rate of virus-specific RNA synthesis continues to increase rapidly at this stage and does not decline before 13 hours p.i. The large amount of virus-specific RNA that is formed between 8 and 13 hours p.i. undoubtedly masks to a certain extent the true inhibition of cell-specific RNA synthesis during this time. The inhibition is therefore probably more drastic than is reflected by the results in Fig. 1.

The appearance of infective virus particles was also determined during the course of the experiment (Fig. 2), indicating that virus particles are not synthesized before 5 hours p.i. and that most of the virus is formed between 7 and 10 hours p.i. Inhibition of protein and DNA synthesis therefore commences long

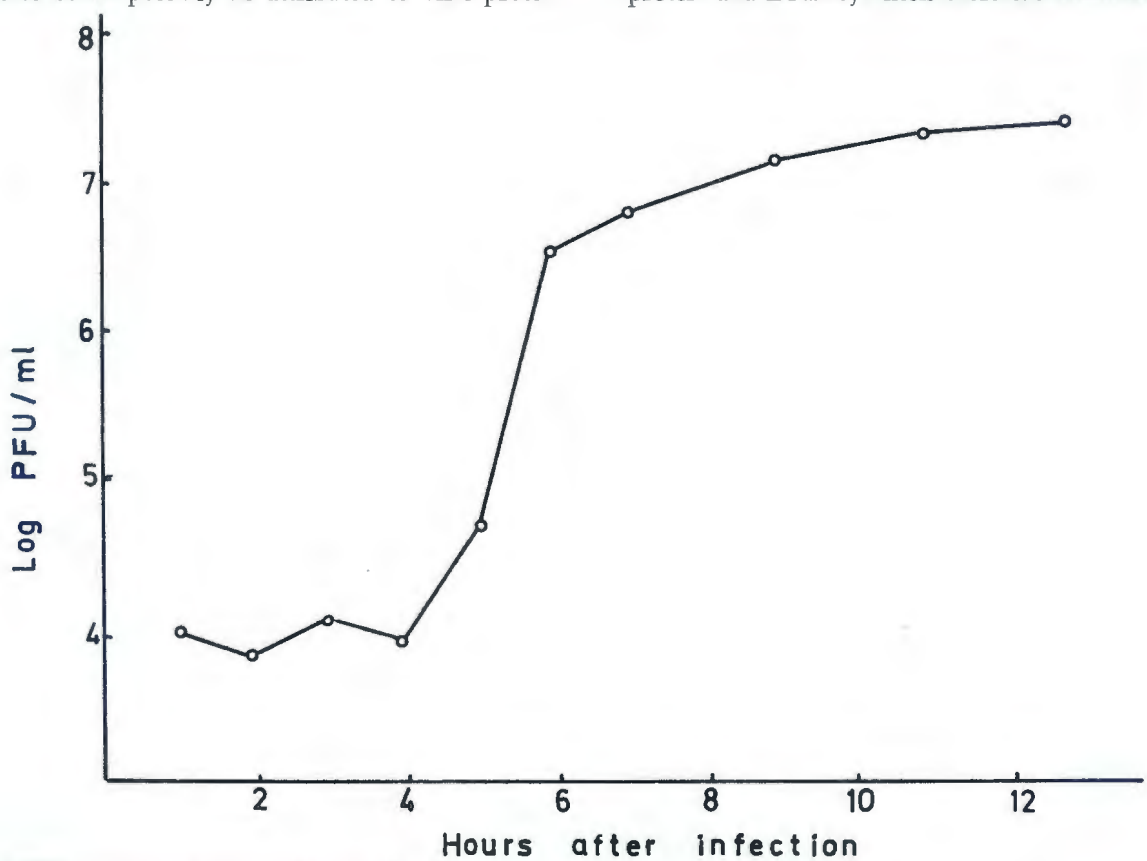

FIG. 2 BTV growth curve determined during the course of the experiment summarized in FIG. 1

before the appearance of new virus particles in the cell and only the inhibition of RNA synthesis could possibly be related to this event.

Infection with heat- and pH-inactivated virus

Due to the difficulty of obtaining large quantities of highly purified virus, many of the experiments were done with partially purified virus. In none of the experiments could any indication be found that the results obtained with highly purified virus differed from those obtained with partially purified virus. However, to provide some further proof that the inhibition was indeed caused by the virus itself and not by any impurity, the experiments were repeated using partially purified virus which had been selectively inactivated. BTV is very sensitive to high temperatures and low pH (Owen, 1964; Howell, Verwoerd & Oellermann, 1967). This provided a suitable means for inactivation of the virus and it was possible to destroy more than 99.9 per cent of the infectivity of a virus suspension by treatment of the virus for 30 min at 56°C or by suspending the virus for 30 min in a buffer at pH 4.0. Cells were infected with this pH- and heat-inactivated virus at an input multiplicity equivalent to about 200 PFU/cell. No inhibition of protein or DNA synthesis was observed with either the heat- or the pH-inactivated virus. The results indicate that participation of an intact virus particle is required for the induction of inhibition.

Effect of input multiplicity

It was observed in different experiments that the virus-induced inhibition of protein synthesis was influenced by the input multiplicity of the infecting virus. To investigate this phenomenon cell cultures were infected with input multiplicities ranging from 25 to 150 PFU/cell and the rate of protein synthesis measured at regular intervals thereafter. The result is shown in Fig. 3. It is clear that the length of the lag phase before the initiation of protein synthesis inhibition depends on the input multiplicity. At a very high input multiplicity (150 PFU/cell) inhibition commences almost immediately after infection whereas at an input multiplicity of 25 PFU/cell the initiation of inhibition is delayed by approximately 3 hours. From the results in Fig. 3 it is possible to determine for every input multiplicity investigated the time after infection when protein synthesis is inhibited by 50 per cent. This indicates that between 25 and 150 PFU/cell every two-fold increase in input multiplicity results in an approximate 45 min reduction in the time after infection when protein synthesis is inhibited by 50 per cent.

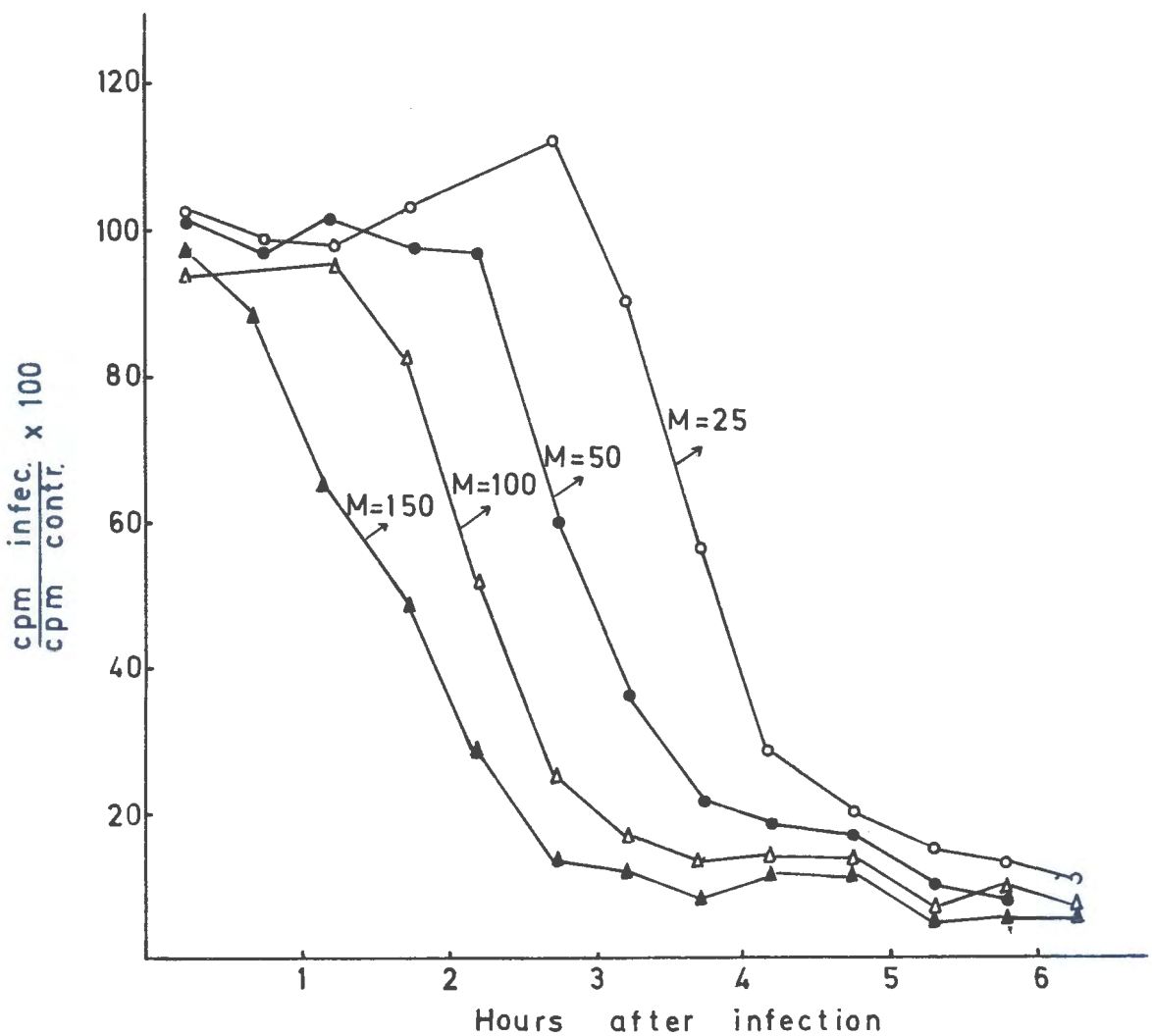

FIG. 3 Rate of ^{3}H-leucine incorporation in BTV-infected L-cells after infection at different input multiplicities (M) of virus. The incorporation rate is expressed as a percentage of the rate in a non-infected control

The delay in the inhibition of protein synthesis with decrease in input multiplicity as reflected by this result is not due to incomplete infection of the cells at the lower input multiplicities. This can also be deduced from Fig. 3, where it is shown that the degree of inhibition that is eventually obtained after about 6 hours p.i. is more or less the same in the different infected cultures. This indicates that in each case complete inhibition of cell-specific protein synthesis occurred in approximately the same percentage of the cells. As inhibition will only be induced in infected cells it must be assumed that about the same number of cells was infected under the different conditions. The differences between these cultures must therefore be attributed to the actual number of particles that entered the cell. This result suggests some direct participation of the virus particles in the induction of inhibition.

Difference between macromolecular synthesis in cells that had been infected as monolayers and as suspension cultures

Several authors have reported differences between the virus-induced inhibition of macromolecular synthesis in suspension and monolayer cell cultures (Levy, 1964; Levy, Snellbaker & Baron, 1966; Martin & Kerr, 1968; Ensminger & Tamm, 1969). These results could indicate that the host cell has a modifying effect on the virus-induced inhibition of cellular synthesis. Some preliminary experiments suggested a similar effect with BTV. To investigate this phenomenon, cells were infected as growing monolayers cultures instead of the normal procedure of infecting the cells in suspension. After a suitable adsorption period suspension cultures were prepared from the infected cells and the different rates of macromolecular synthesis measured.

This procedure of infecting the cells under different conditions, but measuring the rate of macromolecular synthesis thereafter under exactly the same suspension growth conditions, had certain advantages. The procedure limited the difference between suspension and monolayer cells to the differences in the growth conditions of the cells during the actual process of infection. During this time initiation of the inhibition of protein synthesis is likely to occur and any influence that the cell may have on the induction of this inhibition would be reflected in the macromolecular synthesis of the suspension culture prepared from these cells.

Cultures were infected under the above-mentioned conditions at an input multiplicity of 150 PFU/cell. After a 60 min adsorption period a suspension culture was prepared from the infected cells. The rate of protein and DNA synthesis was measured and compared to the synthesis in a non-infected control culture. The result is illustrated in Fig. 4 and reveals that no inhibition of protein or DNA synthesis occurs before $4\frac{1}{2}$ hours p.i. To determine if the inhibition that did occur after $4\frac{1}{2}$ hours p.i. was also influenced by the input multiplicity,

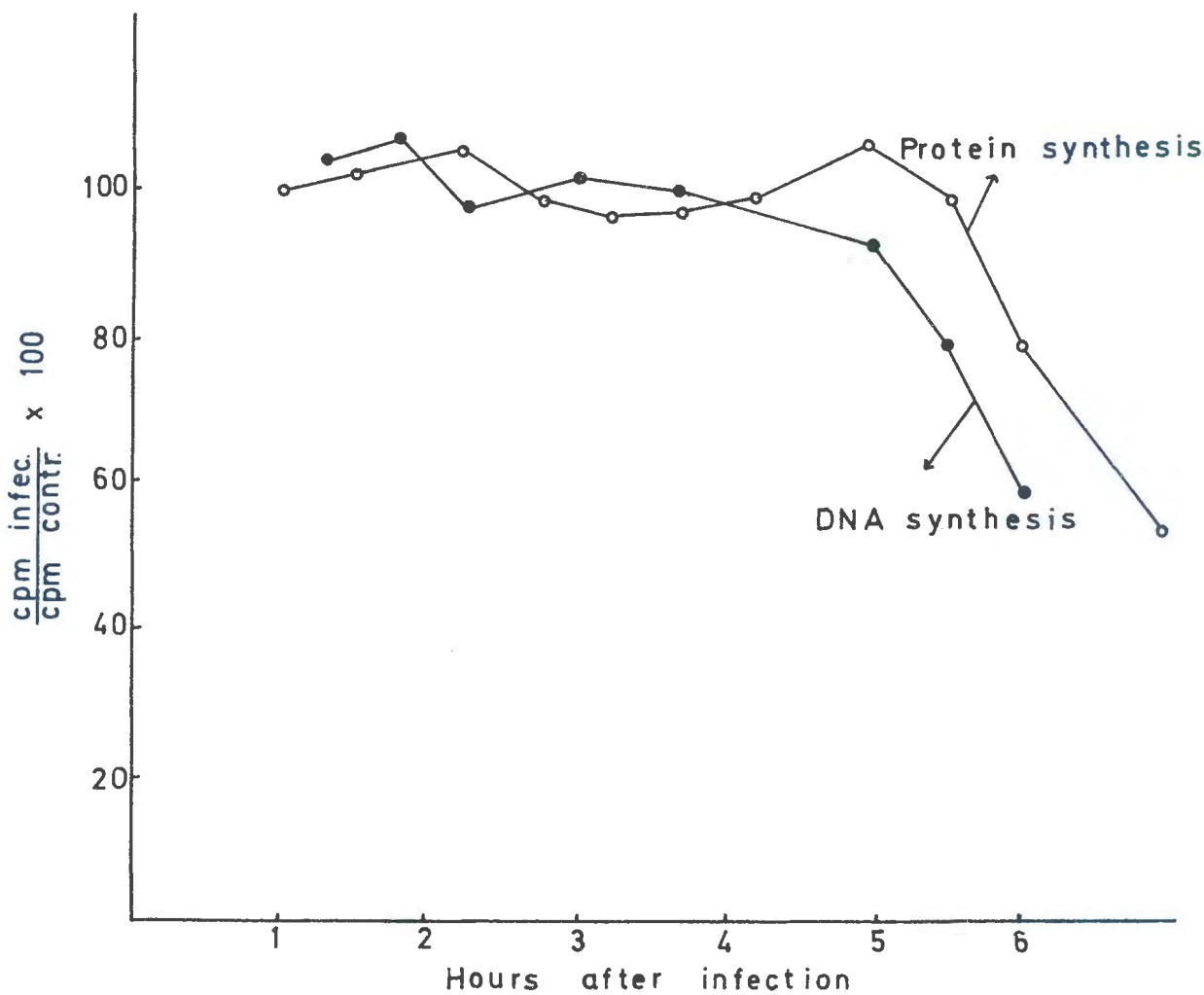

FIG. 4 Rate of ^{14}C-thymidine and ^{3}H-leucine incorporation in monolayer infected L-cells. ^{14}C-thymidine incorporation, —●—, ^{3}H-leucine incorporation, —○—

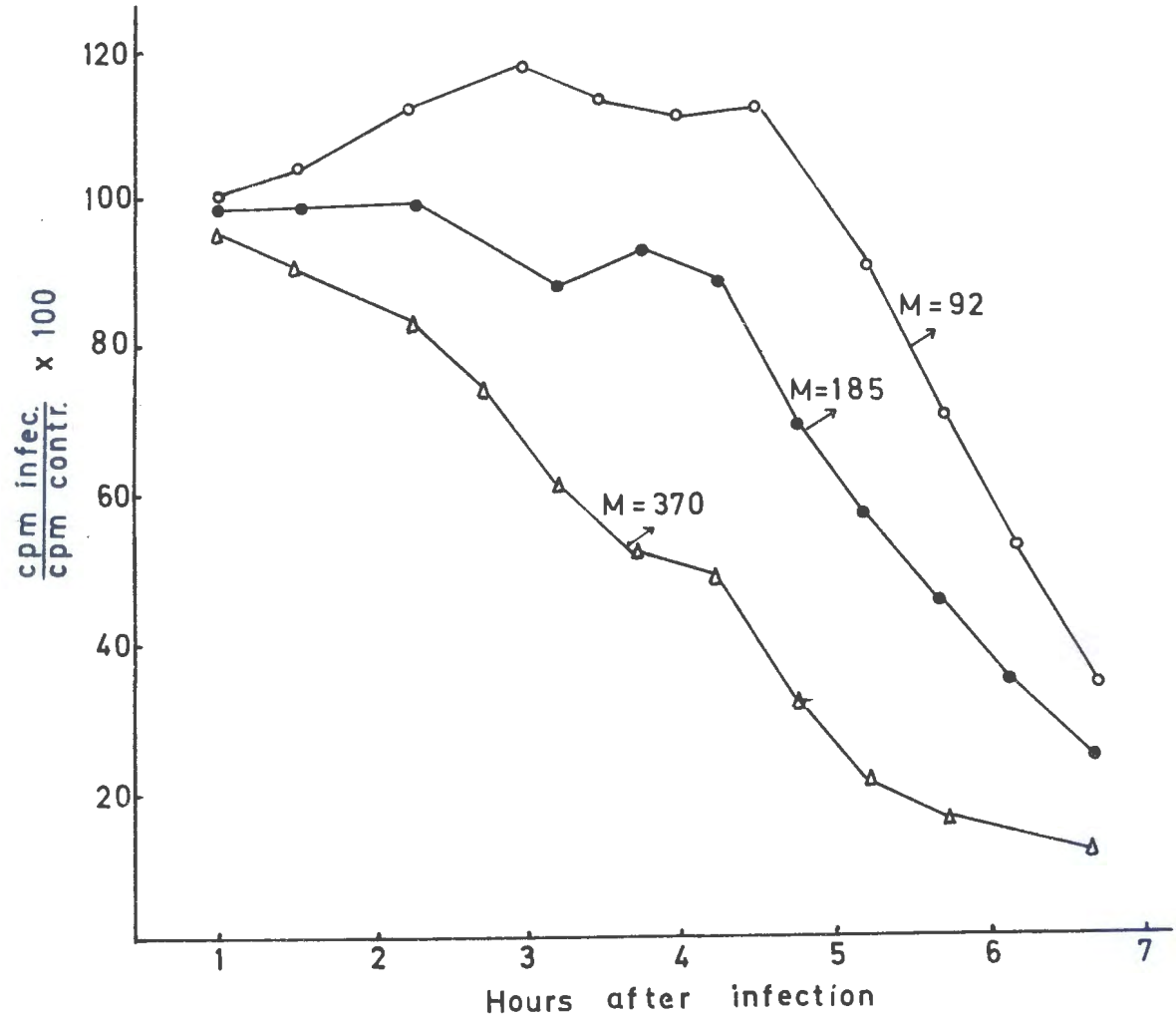

FIG. 5 Rate of ^{3}H-leucine incorporation in L-cells after infection of monolayer L-cells with BTV at different input multiplicities (M)

the experiment was repeated, infecting the monolayer cultures at different input multiplicities, using a small volume of concentrated virus for adsorption.

The influence of infection under these conditions on cellular protein synthesis is shown in Fig. 5. Again the inhibition seems to be dependent on input multiplicity. At an input multiplicity of 370 PFU/cell a 50 per cent inhibition of protein synthesis is obtained about 3½ hours p.i. This value is in agreement with the inhibition which is obtained after infection of suspension cultures at an input multiplicity of about 30 PFU/cell (Fig. 3). This means that the difference between monolayer and suspension cultures could possibly be explained by a difference in the actual infection multiplicity. It would imply that monolayer cells are infected with a much lower efficiency than suspension cultures.

Effect of inhibition of RNA synthesis

It was important to determine whether or not new synthesis of macromolecules is a prerequisite for inhibition. The first aspect that was investigated was whether new RNA synthesis was necessary. Cells were treated 30 min before infection with 0.25 μg/ml Actinomycin D. This concentration inhibited cell-specific RNA synthesis without interfering with virus-specific RNA synthesis. Incorporation of label in the infected culture was expressed as a percentage of the label incorporated in a non-infected control culture treated with Actinomycin D. The result (Fig. 6) shows that the normal inhibition of protein and DNA synthesis occurred. The experiment was repeated using azauridine (30 mg/ml) to inhibit total RNA synthesis. An essentially identical result was obtained, indicating that no new RNA synthesis is required for the induction of inhibition.

Effect of inhibition of protein synthesis

To establish whether new protein synthesis is required for the induction of inhibition, a somewhat different experimental approach was used. The experiment utilized the complete reversibility of cycloheximide-induced inhibition of protein synthesis (Moss, 1968). Four identical L-cell suspension cultures were prepared. Two were used as controls and two were infected with BTV. One of the control cultures and one of the infected cultures were treated 30 min before infection with cycloheximide (final concentration of 7 μg/ml). At 1, 2½ and 4½ hours after infection a suitable amount of cell suspension was removed from each of the four different cultures. The cells were collected by low speed centrifugation, washed in ice cold medium and resuspended at the same concentration in cycloheximide-free medium at 37°C, containing 0.62 μCi/ml ^{3}H-leucine. Care was taken to ensure that the cells were kept at a very low temperature during removal of the cycloheximide to prevent any protein synthesis

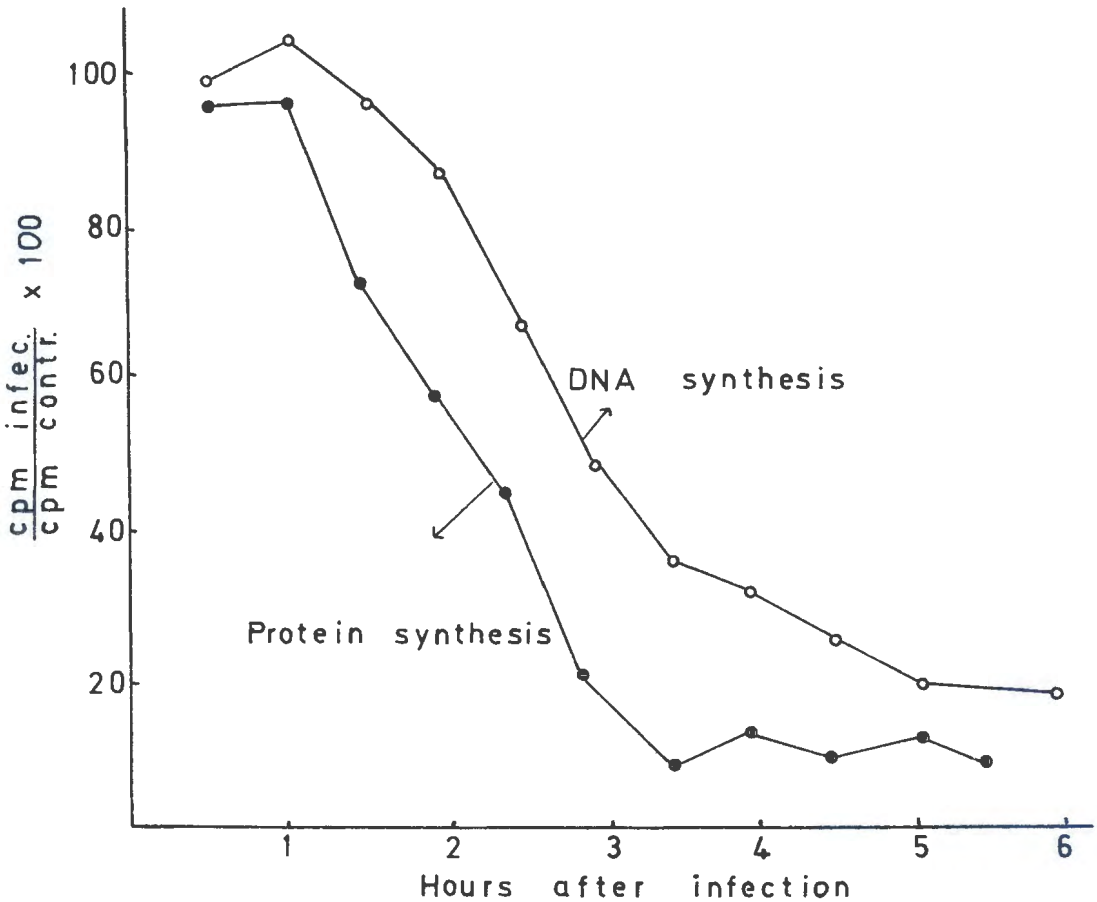

FIG. 6 Rate of ^{14}C-thymidine and ^{3}H-leucine incorporation in L-cells after infection with BTV in the presence of Actinomycin D. The rate of incorporation is expressed as a percentage of that in a non-infected, Actinomycin-treated control culture. ^{14}C-thymidine incorporation, —○—; ^{3}H-leucine incorporation, —●—

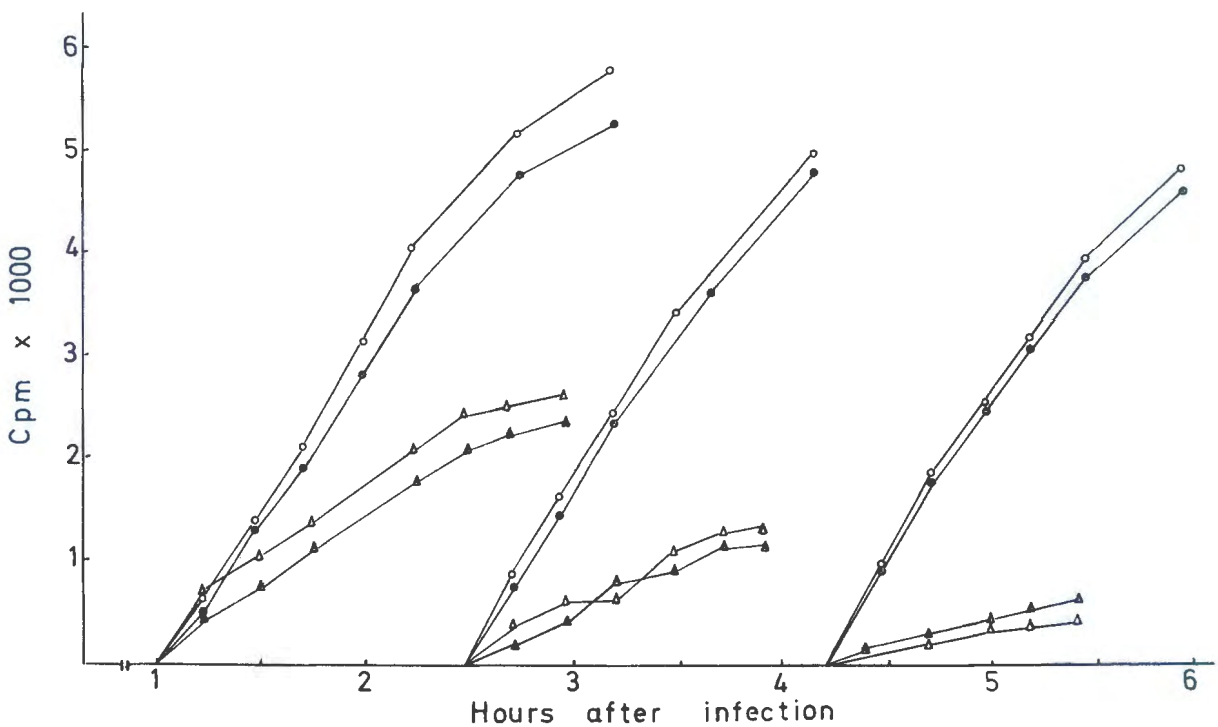

FIG. 7 Influence of cycloheximide on the cumulative incorporation of ^{3}H-leucine in infected and non-infected L-cells after removal of the drug at different intervals p.i. Cumulative ^{3}H-leucine incorporation in the cycloheximide treated infected culture, —▲—; the cycloheximide treated control, —●—; the normal infected culture, —△—; the normal control, —○—

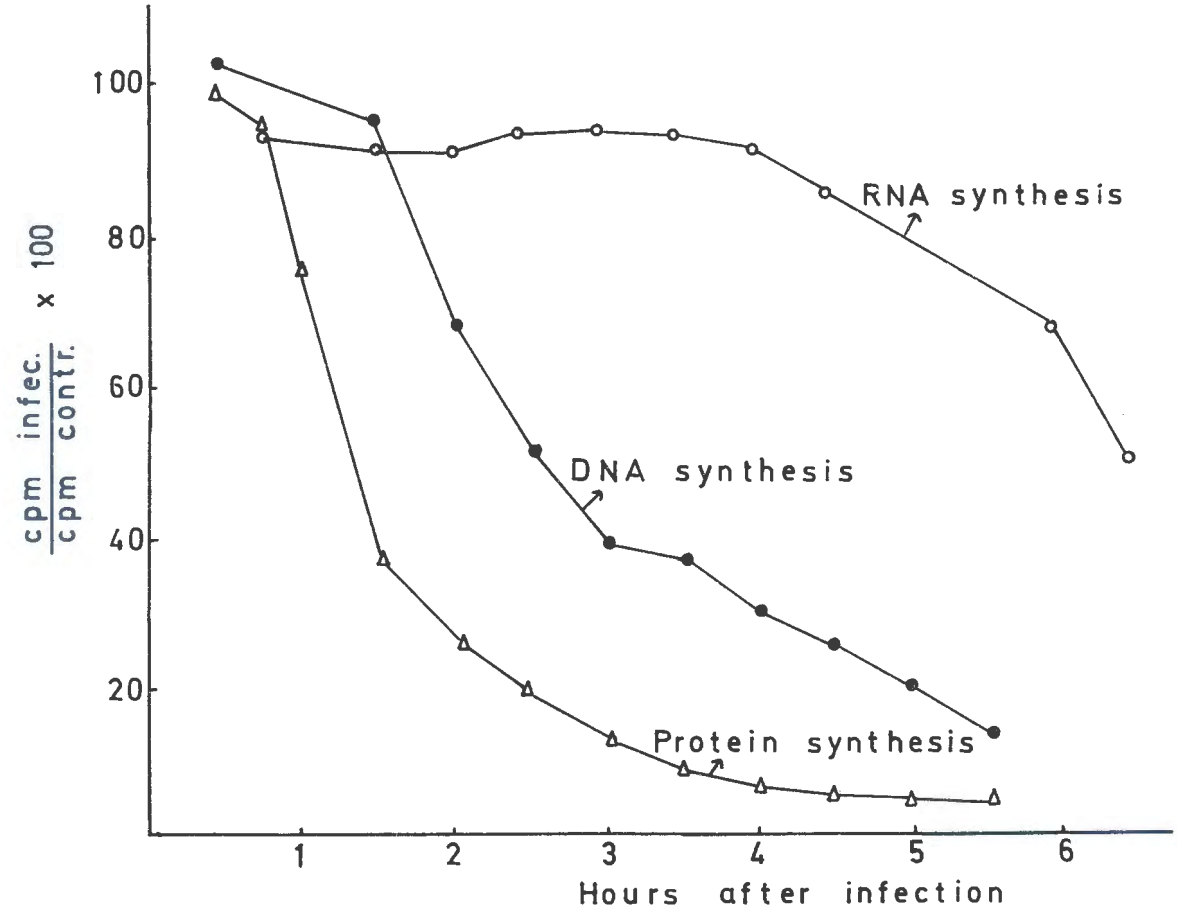

FIG. 8 Rate of ^{14}C-thymidine, ^{3}H-leucine and ^{14}C-urdine incorporation after infection of interferon-treated L-cells with BTV. The rate of incorporation is expressed as a percentage of that in a non-infected, interferon-treated control culture. ^{14}C-thymidine incorporation, —●—; ^{3}H-leucine incorporation —△—; ^{14}C-urdine incorporation, —○—

during this stage. The cells were kept in suspension at 37°C after the drug had been removed and at 15 min intervals after resuspension, 1 ml duplicate samples were removed from the different reaction mixtures and precipitated with ice cold TCA. Radio-activity in the samples was determined as usual. The results shown in Fig. 7 indicate that the virus-induced inhibition of protein synthesis occurs in the presence of cycloheximide. There is very little difference between the rate of protein synthesis in the infected cultures which had been treated and those which were not treated with cycloheximide. This indicates that cycloheximide had no effect on the virus-induced inhibition of cellular protein synthesis. The degree of inhibition in the infected culture after the cycloheximide had been present for 4¼ hours is also significantly higher than the degree of inhibition after cycloheximide had been present for 1 hour. Inhibition, therefore, progressed normally in the presence of the drug, thus excluding the possibility that the difference between the controls and the infected cultures could be the result of an effect of the virus on the normal reversibility of the cycloheximide inhibition of protein synthesis.

The experiment was repeated using a higher concentration of cycloheximide (20 μg/ml). The same result was obtained. Protein synthesis is inhibited at these cycloheximide concentrations by more than 95 per cent and it can therefore be deduced that no protein synthesis is required for the initiation of the virus-induced inhibition of protein synthesis.

Unfortunately cycloheximide also inhibited cellular DNA synthesis and as this inhibition was only partially reversible, the virus-induced inhibition of DNA synthesis could not be investigated in the presence of the drug.

Effect of interferon

The different results obtained suggested that no new synthesis was required for the induction of inhibition. Viral replication is therefore probably not a prerequisite for inhibition. This result was verified by investigating the macromolecular synthesis in virus-infected cells in the presence of interferon.

L-cells were incubated overnight in medium containing 300 interferon units/ml and then suspended in interferon-containing medium. After infection, the rate of protein, DNA and RNA synthesis was measured. After 20 hours the virus yield in the interferon treated cells was also determined. Interferon inhibited viral yield by more than 99.9 per cent but did not prevent the inhibition of protein, DNA or RNA synthesis (Fig. 8). The inhibition of macromolecular synthesis therefore occurs irrespective of whether virus replication takes place or not.

Infection with virus inactivated by UV light and neutralized by antiserum

If new synthesis is not required for the induction of inhibition, it is very likely that the inhibition is induced by the whole or by a component of the infecting

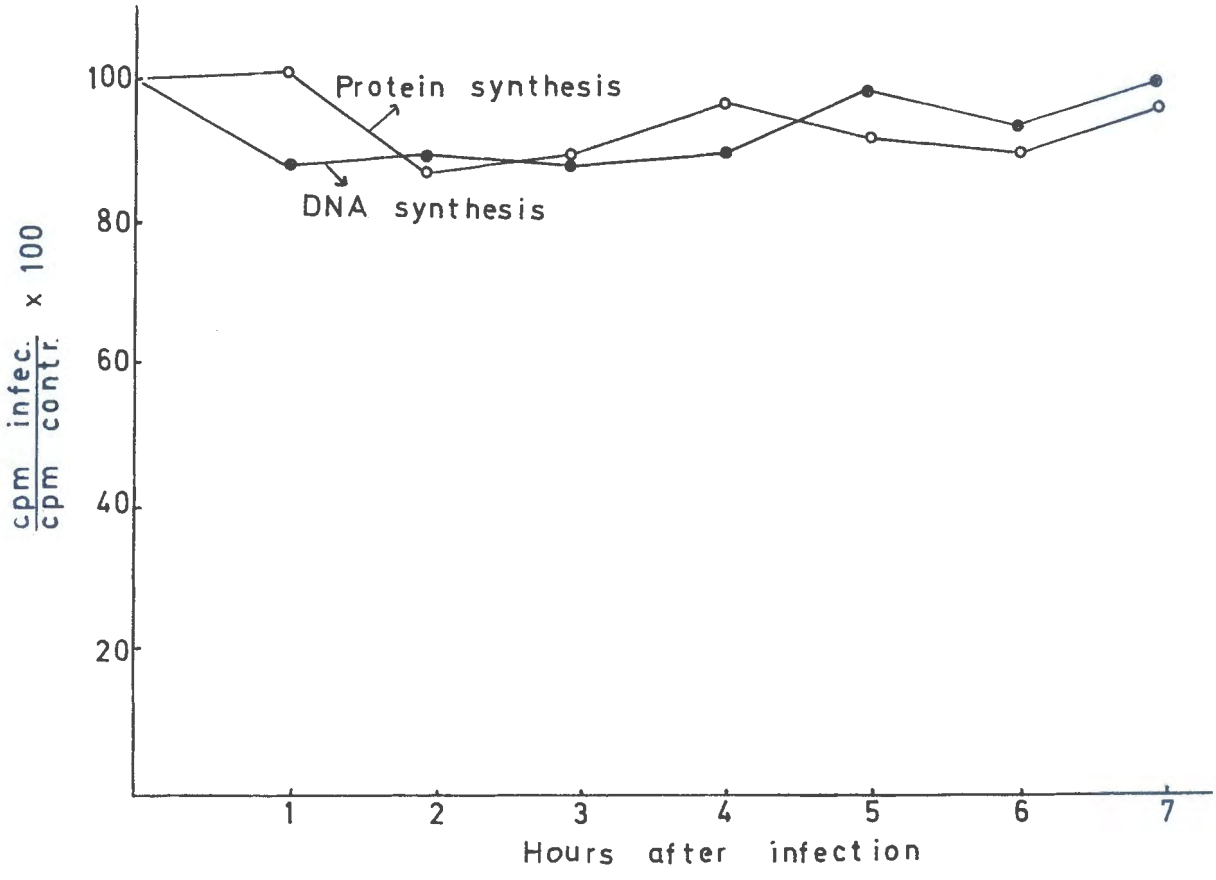

FIG. 9 Rate of ^{14}C-thymidine and ^{3}H-leucine incorporation in L-cells after infection with neutralized BTV at an input multiplicity which is equivalent to 200 PFU/cell. ^{14}C-thymidine incorporation, —●— ;^{3}H-leucine incorporation, —○—

virus. This means that the inhibition can be initiated by either the protein coat or the nucleic acid of the virus. To differentiate between these two possibilities, cells were infected with UV-irradiated or with antiserum-neutralized virus. UV irradiation mainly affects the nucleic acid of the virus leaving the protein coat intact Neutralization on the other hand involves the protein coat. Complex formation between the virus protein and antibody could be expected to influence the normal activity of the protein coat.

Neutralized and UV-inactivated virus were obtained as described under "Materials and Methods". The result of the infection with UV-inactivated virus agrees in every respect with the result obtained for interferon (Fig. 8). Normal inhibition of cellular synthesis occurs after infection with the irradiated virus. The result obtained with neutralized virus is shown in Fig. 9. Even though the cells were infected with the equivalent of 200 PFU/cell no inhibition of protein or DNA synthesis occurred. This result is suggestive that the virus coat protein is involved in the BTV-induced inhibition of cellular synthesis.

Discussion

Infection of L-cells at an input multiplicity of 75 PFU/cell results in a drastic inhibition of cellular protein and DNA synthesis shortly after infection. RNA synthesis is also inhibited from approximately 7 hours p.i. The latter effect is masked to large extent by virus-specific RNA synthesis. This inhibition pattern differs from the inhibition that occurs as the result of infection with members of the picorna virus group and also from the influence that the double-stranded RNA reovirus has on cellular metabolism. In the case of reovirus inhibition of DNA synthesis occurs from 6 to 8 hours p.i. without inhibition of RNA synthesis (Ensminger & Tamm, 1969). The inhibition pattern induced by BTV agrees, however, in almost every respect with the inhibition of cellular synthesis in HeLa cells after infection with vaccinia virus (Moss, 1968).

It was shown that pH- and heat-inactivated virus were unable to induce the inhibition. This result suggests that infection with an intact BTV particle is required for the induction of inhibition. It was also found that the lag phase before the start of protein synthesis inhibition is dependent on the number of virus particles that enter the cell. The lag phase decreases with increase in the infection multiplicity. The influence of input multiplicity on the virus-induced inhibition of macromolecular synthesis has been reported for DNA viruses such as adenovirus (Ginsberg, Bello & Levine, 1967) and vaccinia virus (Moss, 1968). This relationship between the infection multiplicity and the degree of inhibition provides some evidence for a direct participation of the virus particle, or a virus component, in the mechanism of the induced inhibition of cellular synthesis.

The effect of input multiplicity can also be used to explain in the case of BTV the observed difference between suspension and monolayer infected cells. It was found that the characteristics of inhibition in monolayer cells, infected at an input multiplicity of 370 PFU/cell, corresponded to the characteristics of inhibition that took place after infection of suspension

cells at an input multiplicity of about 30 PFU/cell. It is possible that these results can be attributed to a much lower efficiency of virus adsorption in monolayer cells than in suspension cells. In suspension the cells are in constant motion and a large surface of the cell membrane is available for adsorption of the virus. Monolayer cells on the other hand have, due to their attachment to the glass, a much smaller part of the membrane available for virus adsorption and the probability of attachment of a virus to this specific part is also lower due to the fact that the cells are in a fixed position on the glass. Whether a difference in the adsorption efficiency alone can account for the whole of the difference between monolayer and suspension infected cells is still uncertain, and other factors such as the metabolic state of the cell or the physical characteristics of the cell membrane at the time of infection may well be of importance in this respect.

It was proved that the inhibition is unrelated to viral replication in the cell. Interferon, an inhibitor of viral replication, does not prevent the induction of inhibition. The inhibition of protein synthesis also starts long before any new virus particles are synthesized. It was further shown that whereas the inhibition of protein synthesis by mengo- or ME-virus requires new protein synthesis (Baltimore, Franklin & Callender, 1963; Verwoerd & Hausen, 1963), the BTV-induced inhibition does not. Inhibition proceeds normally in the presence of cycloheximide which inhibits protein synthesis. It was also shown that new-RNA synthesis is not a prerequisite for inhibition. These results exclude the possibility that BTV induces the cellular synthesis of a protein or RNA macromolecule with a specific function in the initiation of inhibition. The direct participation of a component of the parental virus, e.g. the protein coat or the nucleic acid, is therefore indicated.

Inhibition of the normal expression of the viral genome by inactivation with UV-irradiation did not influence the ability of the virus to induce the changes in cellular metabolism. No intact virus RNA is therefore required. A participation of the virus RNA in the mechanism of macromolecular synthesis inhibition which does not require any specific expression of the genome is also unlikely. Otherwise, early inhibition of cellular synthesis would probably have been a common characteristic of all double-stranded RNA viruses, which is not the case. Direct participation of the protein coat of the virus in the mechanism of inhibition is more likely.

Neutralization of BTV, which specifically involves the protein coat of the virus, prevents the induction of inhibition. This, of course, could be due to the fact that the neutralized virus particles are prevented from entering the cell. Evidence obtained for other viruses suggests that neutralized viruses can adsorb and be taken into the cell (Joklik, 1964; Granoff, 1965; Mandel, 1967). If this is also true for BTV, it would indicate that the inhibition is not merely triggered by the physical presence of the virus particle in the cell, but that initiation of the inhibition requires some participation of the normal protein coat of the virus. This activity of the protein coat is most probably exerted while the protein coat is still part of the virus. Uncoating normally involves enzymatic degration of the protein and it is difficult to ascribe such a specific inhibitory effect to degraded protein.

Another fact which seems to indicate a participation of the protein coat of the virus is the difference between the inhibitory effects of reovirus and BTV, which could probably best be explained by the differences in their protein coats. In this connection the results obtained with UV-inactivated reovirus are interesting. Infection with UV-irradiated reovirus results in an inhibition of host cell macromolecular synthesis, which is similar in many respects to the inhibition caused by BTV infection (Loh & Oie, 1969). The reason for this similarity is at the moment uncertain. One possible explanation is that UV irradiation inhibits in one way or another the uncoating of reovirus particles. This would result in an accumulation of reovirus particles in the cell which could be responsible for the induction on inhibition. In the case of BTV it is possible that the uncoating process is either slower than for normal reovirus or that only a limited number of particles can be uncoated at a certain time. This would, in either case, result in an accumulation of BTV particles in the cell if the cells are infected at a high input multiplicity of virus. An interaction of the protein coats of these accumulated particles with a step in the biosynthesis of cellular protein could then be responsible for the observed inhibition.

The actual mechanism of such an interaction is quite unknown. The fact that inhibition occurs very soon after infection suggests that the block in protein synthesis occurs at the translation level.

It is uncertain whether the inhibition of DNA and RNA synthesis is the result of the protein synthesis inhibition or induced independently. The fact that DNA synthesis is inhibited by inhibitors of protein synthesis (Kim, Gelbard & Perez, 1968) suggests that the same mechanism is involved in the case of DNA synthesis inhibition.

Summary

Infection with bluetongue virus results in a drastic inhibition of protein and DNA synthesis in the host cell shortly after infection. A delayed inhibition of RNA is also observed from 7 hours after infection onwards. The length of the lag phase before the initiation of the inhibition of protein synthesis is dependent upon the number of infecting virus particles. An increase in the multiplicity of infection results in a corresponding decrease in the length of the lag phase. Inhibition of viral replication by interferon and UV inactivation does not affect the ability of the virus to induce the inhibition of macromolecular synthesis. It has also been shown that no RNA or protein synthesis is required for inhibition. Virus neutralized by antiserum or inactivated by heat or acid treatment is unable to induce the inhibition. The probability that the protein coat of bluetongue virus plays an important part in the mechanism of the inhibition is discussed.

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