MOLECULAR AND CELLULAR BIOLOGY, Jan. 1982, p. 66–75
0270-7306/82/010066-10$02.00/0

Vol. 2, No. 1

Conditions Necessary for Inhibition of Protein Synthesis and Production of Cytopathic Effect in *Aedes albopictus* Cells Infected with Vesicular Stomatitis Virus

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Received 15 June 1981/Accepted 1 September 1981

The relationship between the development of cytopathic effect (CPE) and the inhibition of host macromolecular synthesis was examined in a CPE-susceptible cloned line of *Aedes albopictus* cells after infection with vesicular stomatitis virus. To induce rapid and maximal CPE, two conditions were required: (i) presence of serum in the medium and (ii) incubation at 34°C rather than at 28°C. In the absence of serum, incubation of infected cultures at 34°C resulted in a significant increase in viral protein and RNA synthesis compared with that observed at 28°C. However, when serum was present in the medium, by 6 h after infection protein synthesis (both host and viral) was markedly inhibited when infected cells were maintained at 34°C. RNA synthesis (host and viral) was also inhibited in vesicular stomatitis virus-infected cells maintained at 34°C with serum, but somewhat more slowly than protein synthesis. Examination of polysome patterns indicated that when infected cultures were maintained under conditions which predispose to CPE, more than half of the ribosomes existed as monosomes, suggesting that protein synthesis was being inhibited at the level of initiation. In addition, the phosphorylation of one (or two) polysome-associated proteins was reduced when protein synthesis was inhibited. Our findings indicate a strong correlation between virus-induced CPE in the LT-C7 clone of *A. albopictus* cells and the inhibition of protein synthesis. Although the mechanism of the serum effect is not understood, incubation at 34°C probably predisposes to CPE and inhibition of protein synthesis by increasing the amount of viral gene products made.

Clones of *Aedes albopictus* cells show great variation in their response to infection with Sindbis virus (10, 13). One clone, LT-C7, showed marked cytopathic effect (CPE) after infection with Sindbis virus if the cultures were maintained at 34°C but little CPE at 28°C, the temperature at which these cells are normally grown. Uninfected cells grow equally well at both temperatures. Other clones, on the other hand, showed little or no CPE at 28 or 34°C even though they produced approximately similar amounts of infectious virus (10).

To extend our studies of virus-induced CPE in *A. albopictus* cells, we decided to examine the response of the LT-C7 clone to infection with vesicular stomatitis virus (VSV). VSV grows to high titers (10^9 PFU/ml or higher) in these cells at both 28 and 34°C, but the initial rate of replication is greater at 34°C (1). As with Sindbis virus, CPE is much more pronounced at the higher temperature. An advantage of using this virus for such studies is that a considerable amount of work has been done concerning how VSV kills cells. For example, Marcus and coworkers (5–7) have shown that (i) VSV can kill mouse L-cells in the absence of infectious virus production and (ii) cell killing requires expression of the genes for the N and NS proteins. With the use of VSV temperature-sensitive mutants, these workers also demonstrated that cell killing and the ability to inhibit host protein synthesis were always jointly expressed. In this report we describe conditions which affect the induction of CPE in VSV-infected *A. albopictus* cells and examine the correlation between cell killing and the inhibition of host macromolecular synthesis.

MATERIALS AND METHODS

Cells and virus. The LT-C7 clone of *A. albopictus* cells was described by Sarver and Stollar (10), and the preparation of the VSV stock in BHK cells was described by Gillies and Stollar (2). Monolayer cultures of *A. albopictus* cells were infected at an input multiplicity of 10 PFU per cell (unless otherwise specified), also as described by Gillies and Stollar (2).

Cultures were maintained at 28 or 34°C in modified Eagle medium (E medium [10]) containing fetal calf serum at the concentrations indicated in the figure legends. Media are designated in the text by the percentage of serum they contain, e.g., E-0 or E-10.

Labeling and analysis of host and viral macromolecules. Infected cultures were pulsed with [^{3}H]leucine (25 μCi/ml; 55.9 Ci/mmol) for 2 h (except in the experiments shown in Fig. 5 and 8) beginning at the times indicated in the figure legends. At the end of the labeling period, whole-cell extracts were prepared and the labeled proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described before (1). In any given experiment, the different samples applied to a gel represented equal volumes of lysate and thus an equal number of cells.

To examine the synthesis of viral RNAs, infected cultures were treated with actinomycin D (4 μg/ml) for 1 h before the addition of [^{3}H]uridine (25 μCi/ml; 25.3 Ci/mmol). Cultures were then pulsed for 2 h, and the RNA was extracted from whole cells and analyzed by denaturing agarose gel electrophoresis (1). For the analysis of host RNA synthesis, actinomycin D treatment was omitted, and the RNA was analyzed on a 3.5 to 15% polyacrylamide gel containing 7 M urea (S. Gillies and V. Stollar, J. Biol. Chem., in press). As with the protein samples, equal volumes of the different samples of RNA were applied to a given gel and thus represented equal numbers of cells.

For experiments in which virus was adsorbed to cells at 0°C (Fig. 4 through 7), time after infection was measured beginning at the end of the adsorption period. In those experiments in which virus was adsorbed at 28 or 34°C, time after infection was measured from the time at which virus was added.

Preparation and analysis of polysomes. Cell monolayers containing approximately 3.0×10^7 cells in a 100-mm dish were infected with VSV (10 PFU per cell) or mock infected and then maintained at 28 or 34°C in E medium for 6 h, with or without 10% fetal calf serum. Cytoplasmic extracts were prepared and analyzed by fractionation on 7.5 to 40% (wt/wt) sucrose gradients, as described elsewhere (3).

Labeling and analysis of phosphoproteins. Cell cultures growing in 100-mm plates (2×10^7 cells per plate) were infected or mock infected and then maintained for 4 h at 28 or 34°C in normal E medium containing 10% fetal calf serum. Carrier-free $^{32}PO_4$ was added to a concentration of 100 μCi/ml, and incubation was continued for another 4 h. Cultures were then chilled on ice, and cytoplasmic extracts were prepared as described above for polysomes. To obtain the total cytoplasmic phosphoproteins, the proteins in the extracts were precipitated with 5 volumes of acetone (16 h at −20°C). To determine whether specific phosphoproteins were polysome associated, cytoplasmic extracts were fractionated on sucrose gradients (see above) and the appropriate fractions were pooled. Polysomal phosphoproteins were defined as those sedimenting faster than 80S monosomes, whereas nonpolysomal phosphoproteins were those derived from the 80S monosomes, including the subunits as well as the more slowly sedimenting proteins. The polysomal and nonpolysomal proteins were precipitated with 5 volumes of acetone and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (see above).

RESULTS

Cell killing of *A. albopictus* LT-C7 cells by VSV. Figure 1 illustrates the CPE in VSV-infected LT-C7 cells which have been stained for viral antigen by the indirect fluorescent-antibody method. By 12 h after infection, the time at which these cells were fixed, all of the infected cells contained VSV antigens whether they were maintained at 28 or 34°C or in the presence or absence of serum. In the culture maintained at 34°C in E-10 medium, most cells showed cytopathic change, and some had begun to detach from the dish. In contrast, infected cells maintained without serum at 34°C or with 10% serum at 28°C showed little change, although they also contained abundant viral antigen.

Figure 2 illustrates a semiquantitative measurement of CPE at 34°C as determined by the detachment of cells. Confirming what was seen in Fig. 1, CPE was maximum in the presence of 10% serum, minimum in the absence of serum, and intermediate in 2% serum. Furthermore, in each case CPE, as scored at 20 h after infection, was clearly greater as the input multiplicity of virus was increased. Since at a multiplicity of infection of 10 PFU per cell the contrast between cells without serum and cells in 10% serum was the greatest, this multiplicity of infection was used in all subsequent experiments.

Inhibition of protein synthesis in VSV-infected *A. albopictus* cells. To determine whether the development of CPE was associated with an inhibition of cellular protein synthesis, VSV-infected *A. albopictus* cells were pulsed with [^{3}H]leucine from 6 to 8 h after infection, and the newly synthesized proteins were analyzed by gel electrophoresis. At 28°C uninfected cultures synthesized similar amounts of protein whether they were refed with E-0 or E-10 medium (Fig. 3, lanes A and B); infected cells, however, made somewhat higher amounts of viral proteins in the E-10 medium. When uninfected cells were maintained at 34°C, slightly higher amounts of proteins were made in the presence than in the absence of serum, but the differences were not striking (lanes E through G). In contrast, VSV-infected cultures maintained at 34°C synthesized progressively less protein (host and viral) as the serum concentration was increased (lanes H through J). Figure 3 also shows that, in the absence of serum, significantly larger amounts of viral protein were made at 34 than at 28°C (cf. lanes C and H).

Next, we examined the time course of protein synthesis in VSV-infected cells maintained at 34°C in either E-0 or E-10 medium. Cultures were pulsed for 2-h intervals from 0 to 8 h after infection, and the proteins were analyzed by electrophoresis. Host and viral protein synthe-

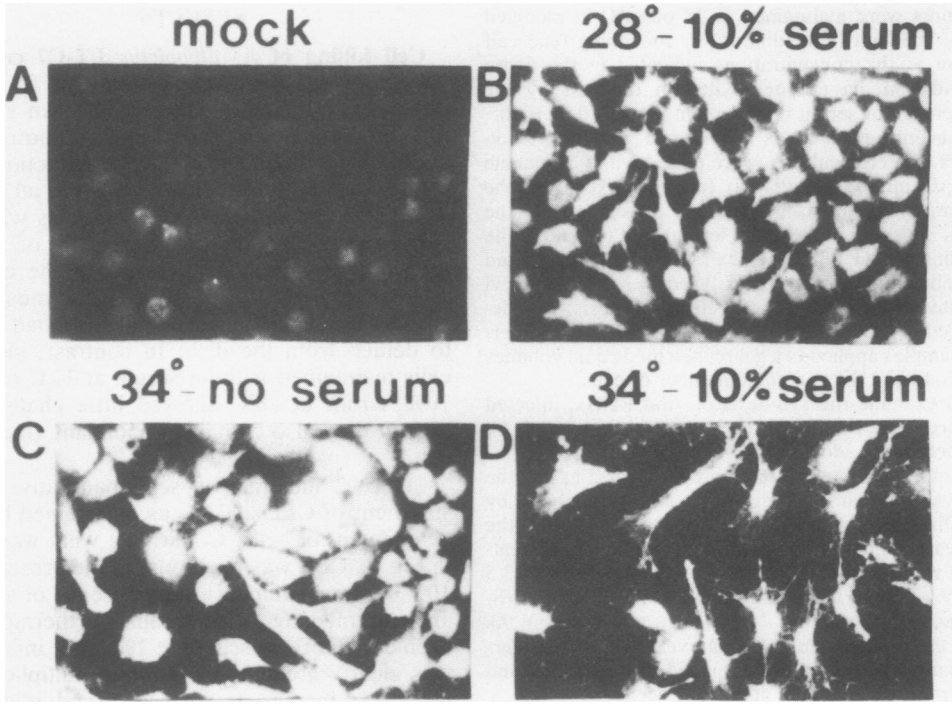

FIG. 1. Immunofluorescent staining of VSV-infected *A. albopictus* cells. Coverslip cultures of *A. albopictus* LT-C7 cells were mock-infected (A) or infected with VSV (10 PFU per cell) (B, C, and D) and maintained at 28 or 34°C for 12 h. Cells were then fixed in acetone at −20°C and were reacted with rabbit anti-VSV antiserum (1/50 dilution), followed by fluorescent-labeled goat anti-rabbit antiserum (Cappel Laboratories).

ses from 0 to 2 h after infection were not significantly different in cultures maintained in E-0 or E-10 medium (Fig. 4, lanes A and E). Between 2 to 4 h, however, the synthesis of host proteins began to decline in the culture maintained in E-10 medium (lane B), and there was also less of the VSV proteins made compared with the culture lacking serum (lane F). After 4 h, protein synthesis in the VSV-infected cultures maintained in E-10 decreased rapidly (lanes C and D), whereas that in cells maintained without serum remained relatively constant (lanes G and H).

Effect of actinomycin D on VSV protein synthesis at 28°C. Treatment of uninfected *A. albopictus* cells with actinomycin D results in a rapid decrease of translatable host mRNA (as determined by polysome content) and in an increase in the amount of available 80S monosomes (unpublished data). Thus, if in actinomycin D-treated cells the translation of VSV mRNA were markedly increased relative to untreated cells, it might be concluded that the translation of viral mRNA was limited by competition for ribosomes by host mRNA.

We have already shown that, in VSV-infected *A. albopictus* cells maintained at 34°C without serum, actinomycin D does not significantly increase the already high rate of viral protein synthesis (3). We show here (Fig. 5), on the other hand, that in VSV-infected *A. albopictus* cells maintained at 28°C (also without serum) the synthesis of VSV proteins was markedly stimulated by low concentrations of actinomycin D (0.1 μg/ml; Fig. 5, lanes d through f) and was further increased at higher concentrations (1 μg/ml; lanes g through i). As can be seen, viral protein synthesis increased as the translation of host proteins decreased, suggesting that at 28°C translation of the relatively low concentration of VSV mRNA (see below) was limited by competition for ribosomes.

RNA synthesis in VSV-infected *A. albopictus* cells. The time course of VSV-specific RNA synthesis in infected *A. albopictus* cells is shown in Fig. 6. In the cultures that were incubated under conditions in which CPE did not occur, i.e., cultures maintained at 28°C (lanes A through D) or at 34°C without serum (lanes E through H), VSV RNA synthesis increased from 0 to 6 h after infection and then leveled off. The amount of VSV RNA synthesis was, however, much greater at 34 than at 28°C. Although not shown, RNA synthesis at 28°C in the absence of serum was similar to that seen in the presence of serum (lanes A through D). In the infected

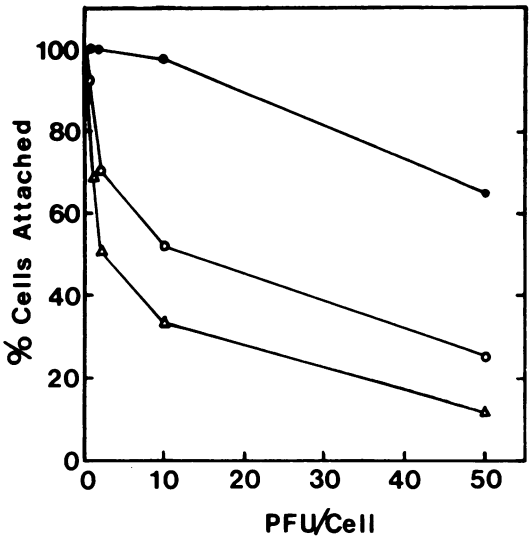

FIG. 2. CPE in VSV-infected *A. albopictus* cells as measured by cell detachment: effect of varying the multiplicity of infection and the concentration of serum in the medium. *A. albopictus* cells growing in 35-mm plates (1.6×10^6 cells per plate) were infected at the indicated multiplicities and were maintained at 34°C in E medium containing 10% (△), 2% (○), or no (●) fetal calf serum. At 20 h after infection, duplicate cultures were gently rinsed twice with cold phosphate-buffered saline to remove floating cells, after which the cells which remained attached were removed by vigorous pipetting and were counted with a Coulter counter. The percentage of cells remaining attached in a given culture was calculated relative to the number in mock-infected cultures maintained in the same medium.

cultures maintained at 34°C in E-10 medium (lanes I through L), the amount of VSV RNA synthesized in the first 4 h was similar to that seen in cells maintained at 34°C in E-0 medium. Between 4 and 6 h (lane K) after infection, however, RNA synthesis began to decline, and between 6 and 8 h it had become markedly reduced compared with that in cells maintained without serum.

The time course of host cell RNA synthesis (predominantly rRNA) was also examined in VSV-infected cells. rRNA and tRNA syntheses during the first 4 h of infection were very similar in both the absence (Fig. 7, lanes A and B) and the presence (lanes E and F) of serum. As was the case for viral RNA synthesis, host RNA synthesis also began to decline between 4 and 8 h after infection in serum-grown cells (lanes G and H), but not as rapidly as did host protein synthesis.

Late addition of serum to VSV-infected *A. albopictus* cells. The results described above indicated that both host and viral macromolecular syntheses were inhibited in VSV-infected cells if serum was present in the culture medium and if cultures were incubated at 34°C, and the time course experiments were consistent with the idea that the protein-synthetic machinery of the cell was the primary target of this inhibition. To test the effect of the addition of serum to infected cells which already contained high levels of viral products, serum (10% final concentration) was added to VSV-infected cultures which had been maintained without serum at 34°C for 5 h. The rate of protein synthesis was then determined in untreated cultures and in the cultures which received serum.

During the first hour after the addition of serum, the rates of host and viral protein syntheses were the same as in the control culture to which serum had not been added (Fig. 8, lanes F and A, respectively). Between 1 and 2 h, however, protein synthesis was drastically inhibited in the cultures to which serum was restored, but continued at the same rate in the absence of serum (cf. lanes G and B). Thus, when viral RNA and protein syntheses had been allowed to proceed for 5 h, it took only 1 h after the addition of serum for a marked inhibition of protein synthesis to occur. This contrasts with the 4 h or more required to observe inhibition when serum was added immediately after infection.

Polysomes in uninfected and VSV-infected *A. albopictus* cells. The proportion of ribosomes which are in polysomes in uninfected and VSV-infected *A. albopictus* cells was examined at 6 h after infection. By this time protein synthesis in VSV-infected cells maintained at 34°C in the presence of serum was drastically reduced compared with similarly infected cells maintained without serum (see Fig. 4).

The distributions of ribosomes in uninfected cells maintained at 28°C with serum, at 34°C without serum, and at 34°C with serum are shown in Fig. 9A, C, and E, respectively. In each case good polysome patterns were obtained. It can be seen, however, that when cells were maintained at 34°C without serum (Fig. 9C) more of the larger polysomes and less of the smaller polysomes were obtained than when cells were maintained at the same temperature with serum. We attribute these differences to the fact that *A. albopictus* cells contain high levels of endogenous RNase activity and that in some manner this RNase activity is markedly reduced when cells are maintained in the absence of serum (S. Gillies and V. Stollar, unpublished data).

Polysome patterns in VSV-infected cells are also shown in Fig. 9. When infected cells were maintained at 28°C with serum, there was no detectable change in the distribution of ribosomes (Fig. 9B) compared with the uninfected

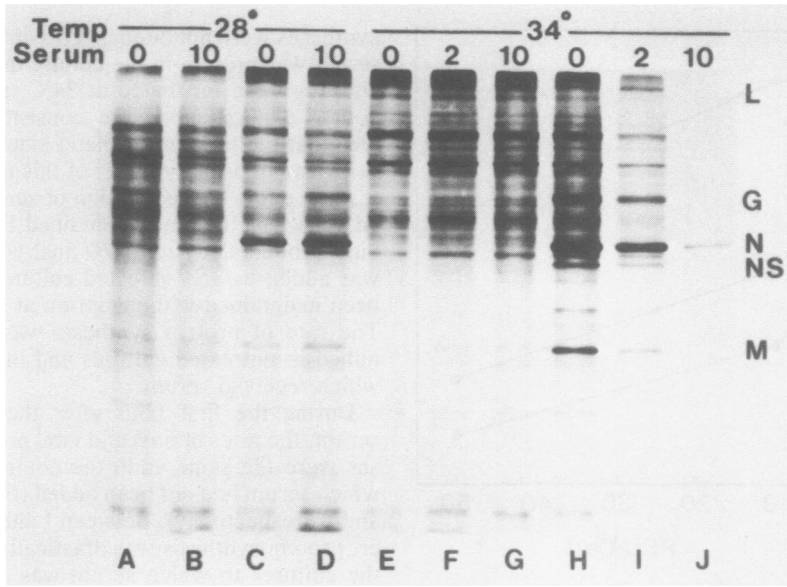

FIG. 3. Effect of temperature and serum concentration on protein synthesis in uninfected and VSV-infected *A. albopictus* cells. *A. albopictus* LT-C7 cells growing in 35-mm plates (1.6×10^6 cells per plate) were mock-infected (lanes A, B, E, F, and G) or infected (lanes C, D, H, I, and J) with VSV (10 PFU per cell) and maintained at 28 or 34°C in E medium containing 0, 2, or 10% fetal calf serum. At 6 h after infection, [^{3}H]leucine (25 μCi/ml) was added, and incubation was continued for 2 h. Cell extracts were prepared and the labeled proteins were analyzed on a 10% polyacrylamide gel. L, G, N, NS, and M are the five VSV proteins and can best be seen in lane I.

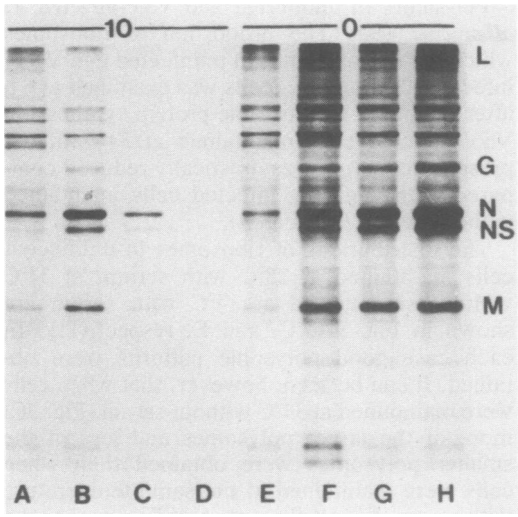

FIG. 4. Time course of protein synthesis in VSV-infected *A. albopictus* cells in the presence or absence of serum. *A. albopictus* cells were infected with VSV (10 PFU per cell) as in the legend to Fig. 3 except that adsorption was at 0°C for 60 min. Prewarmed E medium containing 0 or 10% fetal calf serum (as indicated) was added, and the cultures were maintained at 34°C. Protein synthesis was analyzed as in the legend to Fig. 3 except that labeling was from 0 to 2 (lanes A and E), 2 to 4 (lanes B and F), 4 to 6 (lanes C and G), or 6 to 8 (lanes D and H) h after the end of the adsorption period.

control culture (Fig. 9A). In VSV-infected cells maintained at 34°C without serum (Fig. 9D) there was a slight decrease in the average size of polysomes (cf. Fig. 9C) as a result of the increased translation of the relatively small VSV mRNA molecules. The majority of ribosomes, however, were still present in polysomes, indicating active initiation of protein synthesis. In contrast, in VSV-infected cells maintained at 34°C in the presence of serum (under which conditions protein synthesis is markedly inhibited [Fig. 3 and 4]), there was a large reduction in the polysome content and a corresponding increase in the 80S monosomes (cf. Fig. 9F and 9E). These results strongly suggest that the inhibition of protein synthesis in such cells is the result of a reduced rate of initiation relative to the rate of elongation of polypeptide chains.

Effect of virus infection and temperature of incubation on protein phosphorylation in serum-grown *A. albopictus* cells. Since the inhibition of host and viral protein syntheses in VSV-infected *A. albopictus* cells was greatly enhanced by the presence of serum in the medium and many phosphorylation reactions can be regulated by hormones contained in serum (see, for example, reference 14), we compared the phosphorylation patterns of proteins from infected and uninfected cells maintained in serum-containing media. Mock- and VSV-infected cultures were incubat-

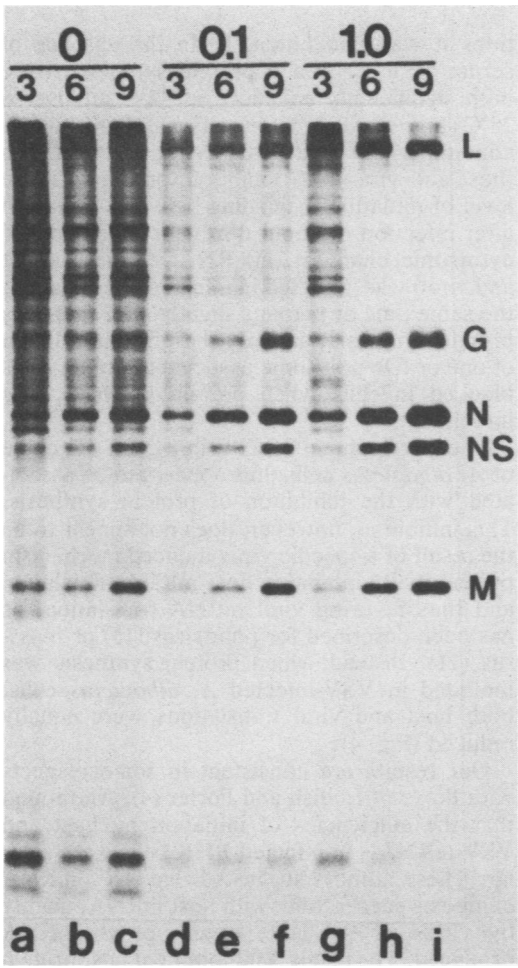

FIG. 5. Effect of actinomycin D on VSV protein synthesis in *A. albopictus* cells. *A. albopictus* cells were infected with VSV as described in the legend to Fig. 4 except that incubation was at 28°C. E medium (without serum) contained 0, 0.1, or 1.0 μg of actinomycin D per ml, as indicated. Labeling with [^{3}H]leucine was for 3 h, ending at the times indicated at the top of each lane. Proteins were analyzed on a 10% polyacrylamide gel.

ed in E-10 medium at either 28 or 34°C and were labeled with $^{32}PO_4$ from 4 to 8 h after infection. Cytoplasmic extracts were prepared and analyzed by gel electrophoresis.

In uninfected cells the patterns of ^{32}P-labeled proteins were very similar at 28°C (Fig. 10, lane a) and 34°C (lane d). The only new phosphoprotein seen in infected cells (lanes b and e) comigrated with the NS protein (lane c), a viral protein known to be phosphorylated (9).

A major ^{32}P-labeled band (designated p2, p3 in Fig. 10) was seen in extracts from both uninfected and VSV-infected cells maintained at 28°C (lanes a, b, and d). Very little labeling of this species was seen in extracts from VSV-infected cells maintained at 34°C with serum (lane e), i.e., conditions which lead to the disaggregation of polysomes (Fig. 9). Thus, the inhibition of protein synthesis appeared to be associated with the decreased phosphorylation of a specific protein or proteins. Except for p4, a minor band, the labeling of most other phosphoproteins was affected very little under these same conditions.

The correlation between reduced protein phosphorylation and reduced protein synthesis suggested that the phosphoproteins, p2 and p3, might be associated with polysomal ribosomes. To test this possibility, uninfected *A. albopictus* cells were labeled with $^{32}PO_4$ and a cytoplasmic extract was fractionated into polysomal and nonpolysomal fractions, as described in Materials and Methods.

When the cytoplasmic extract was analyzed by gel electrophoresis, the ^{32}P-labeled protein band described above (Fig. 10, lanes a, b, and d) was resolved into two bands, labeled p2 and p3, which were found predominantly in the polysomal fraction (lane h). These results suggest that the phosphorylation of protein p2 or p3 or both may be coupled to the entry of ribosomes into polysomes. We cannot tell, however, whether the reduced phosphorylation of p2 and p3 is the cause or the effect of a block at the level of initiation.

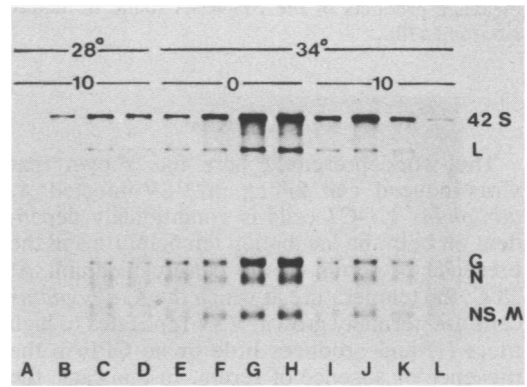

FIG. 6. Effect of temperature and serum concentration on VSV RNA synthesis in *A. albopictus* cells. *A. albopictus* cells were infected as described in the legend to Fig. 4 (virus was adsorbed to cells at 0°C for 60 min) and were maintained at 28 to 34°C in E medium containing 0 or 10% fetal calf serum, as indicated. Cells were labeled after a 1 h pretreatment with actinomycin D (4 μg/ml) with [^{3}H]uridine (25 μCi/ml) from 0 to 2 (lanes A, E, and I), 2 to 4 (lanes B, F, and J), 4 to 6 (lanes C, G and K), or 6 to 8 (lanes D, H, and L) h after the end of the adsorption period. Cells labeled from 0 to 2 h were treated with actinomycin D during the 1-h adsorption period. RNA was extracted and analyzed by gel electrophoresis as described in the text. The 42S genome RNA and the mRNA's coding for the L, G, N, NS, and M proteins are indicated.

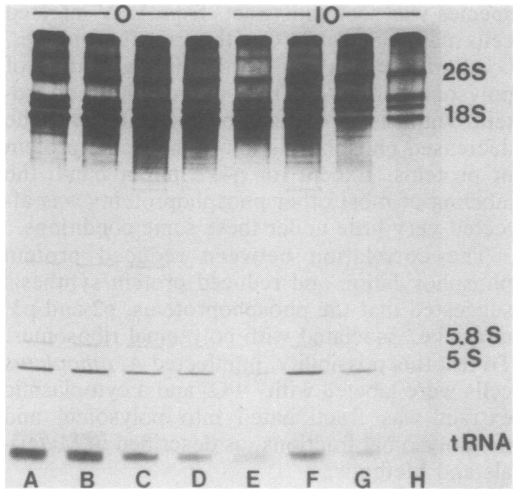

FIG. 7. Host cell RNA synthesis in VSV-infected *A. albopictus* cells in the absence or presence of fetal calf serum. *A. albopictus* cells were infected as described in the legend to Fig. 4 and were maintained at 34°C in E medium containing 0 or 10% fetal calf serum, as indicated. Labeling with [^{3}H]uridine was from 0 to 2 (lanes A and E), 2 to 4 (lanes B and F), 4 to 6 (lanes C and G), or 6 to 8 (lanes D and H) h after infection. RNA was extracted and analyzed by gel electrophoresis as described in the text. The small ribosomal subunit (18S) RNA, 5.8S, 5S, tRNA, and the unprocessed large subunit 26S RNA are indicated. The two major bands migrating above the 18S RNA are the two cleavage products of the 26S RNA found in mature ribosomes (8).

DISCUSSION

The work presented here has shown that virus-induced cell killing in VSV-infected *A. albopictus* LT-C7 cells is conditionally dependent on both the incubation temperature and the presence of serum in the culture medium. At 28°C, the temperature at which the *A. albopictus* cells are normally grown, VSV replicates to high titers (1) and produces little or no CPE in the presence or absence of serum. In this case, the high titers that are eventually reached by about 24 h after infection are the result of sustained viral replication at a moderate rate, and it appears that the level of viral products at any given time probably does not reach levels cytotoxic to the cell. In contrast, at 34°C, a temperature at which these cells grow as well as at 28°C, the rate of VSV replication is markedly increased (1) and results in the destruction of cell cultures by about 16 h after infection, but only if serum is present. Infected cultures maintained at 34°C without serum show almost no cytopathic changes in the first 24 h of infection.

When macromolecular synthesis was compared in cells maintained under different conditions it was found that: (i) in the absence of serum VSV RNA and protein syntheses were both significantly enhanced at 34°C relative to 28°C (Fig. 3 and 6); (ii) in cells maintained under conditions that lead to CPE, protein synthesis (host and viral) was inhibited, probably at the level of initiation, beginning between 4 and 6 h after infection or about 6 h before the onset of cytopathic changes; (iii) RNA synthesis (host and viral) was inhibited in infected cells at about the same time or perhaps slightly later than was protein synthesis; and (iv) the phosphorylation of one or two polysome-associated proteins was blocked in cells when protein synthesis was inhibited.

The development of CPE in the LT-C7 clone of *A. albopictus* cells thus appears to be associated with the inhibition of protein synthesis. This inhibition, however, does not appear to be the result of a specific virus-induced mechanism preferentially inhibiting host mRNA translation and thus favoring viral mRNA translation, as has been described for poliovirus (16) or reovirus (11). Instead, when protein synthesis was inhibited in VSV-infected *A. albopictus* cells, both host and viral translations were equally reduced (Fig. 4).

Our results are consistent in some respects with those of Lodish and Porter (4), who found that the efficiencies of initiation by host and VSV mRNA's in infected BHK cells were similar. These authors suggested that VSV mRNA competes successfully with host mRNA, mainly by virtue of the large excess of viral RNA produced. The result is inhibition of host protein synthesis and eventual cell death.

In our system it is possible to control the rate of VSV macromolecular synthesis by maintaining infected cultures at different temperatures and thus to examine individually some of the factors related to the inhibition of protein synthesis. At 28°C we observed that the rate of VSV mRNA synthesis was much lower than at 34°C (Fig. 6); at this temperature, then, it is unlikely that VSV mRNA could compete effectively with host mRNA (Fig. 5). At 34°C, on the other hand, viral mRNA synthesis was markedly increased and resulted in the increased synthesis of viral proteins, probably at the expense of some host protein synthesis. It is possible, therefore, that two different mechanisms operate to inhibit protein synthesis in this system; one would involve competition by excess viral mRNA for a limiting number of ribosomes, leading to the inhibition of host protein synthesis, and the other would involve cytotoxic effects resulting from the buildup of viral products in the presence of serum. This second mechanism would lead to the inhibition of both viral and host protein syntheses.

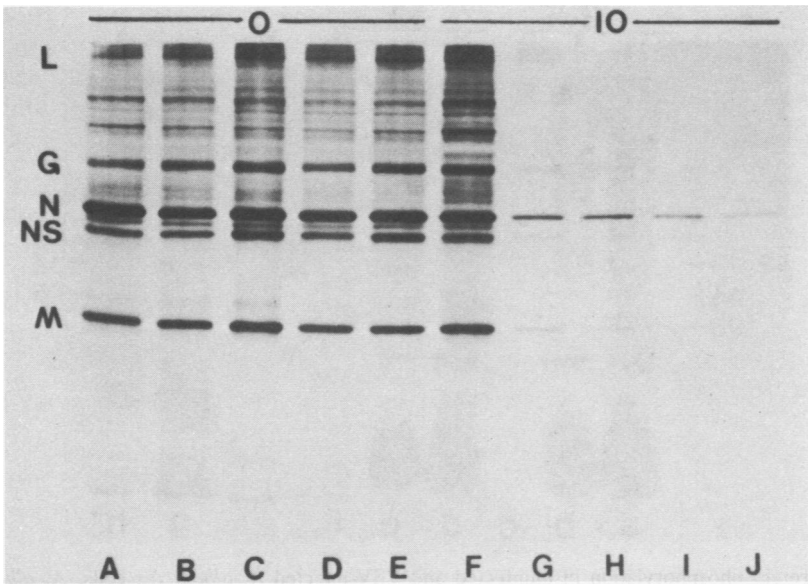

FIG. 8. Effect of the late addition (5 h after infection) of serum on protein synthesis in VSV-infected *A. albopictus* cells. Cultures of *A. albopictus* cells were infected with VSV and maintained in serum-free E medium for 5 h at 34°C. Fetal calf serum was then added to half of the cultures (lanes F through J; 10% final concentration), and incubation was continued at 34°C. No serum was added to the other cultures (lanes A through E). Protein synthesis was determined as in the legend to Fig. 3 except that the labeling time was 1 h: 0 to 1 (lanes A and F), 1 to 2 (lanes B and G), 2 to 3 (lanes C and H), 3 to 4 (lanes D and I), or 4 to 5 (lanes E and J) h after after the addition of serum.

Our results suggest that mosquito cells may survive infection with VSV under conditions in which viral RNA (and protein) synthesis is limited, such as occurs at 28°C. A similar state of affairs likely exists when *A. albopictus* cells are infected with Sindbis (10,13) or Semliki forest (15) virus. After infection of clonally derived cell variants, which permit increased rates of viral RNA synthesis, with either of these viruses, the outcome resembles the highly cytopathic infection usually seen with mammalian or avian cells. In the system described in this report, we have shown that the increased rate of viral RNA synthesis and the resulting accumulation of viral macromolecules were not sufficient, alone, to inhibit protein synthesis. In fact, the greatest amounts of viral proteins and RNAs were found in cells maintained at 34°C, without serum, conditions which do not lead to an early inhibition of cellular or viral macromolecular synthesis. The shutoff of protein synthesis and cell killing required a combination of serum in the medium and the accumulation of high levels of viral macromolecules. This was demonstrated most clearly by adding serum to cells which already contained high levels of viral protein and RNA (Fig. 8). In this case the inhibition of protein synthesis occurred quite rapidly, by 1 h, indicating that the serum-stimulated state of the cell in some way affected the ability of viral products to influence a translational control mechanism.

An interesting and possibly related observation has been made with a mutant of VSV,

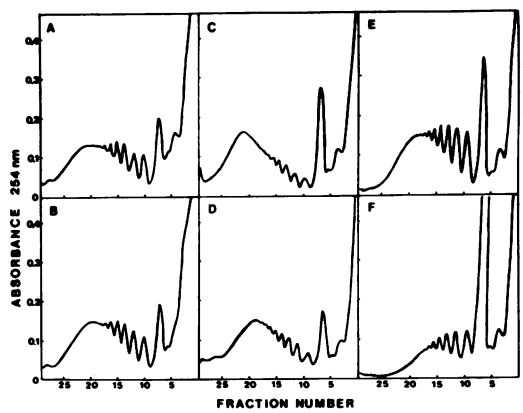

FIG. 9. Polysomes in uninfected and VSV-infected *A. albopictus* cells. Cytoplasmic extracts from mock- or VSV-infected *A. albopictus* cells were prepared at 6 h after infection as described in the text and analyzed by sucrose gradient centrifugation. (A) Mock-infected cells maintained at 28°C in E-10 medium; (B) VSV infected, 28°C, in E-10 medium; (C) mock infected, 34°C, E-0 medium; (D) VSV infected, 34°C, E-0 medium; (E) mock infected, 34°C, E-10 medium; (F) VSV infected, 34°C, E-10 medium.

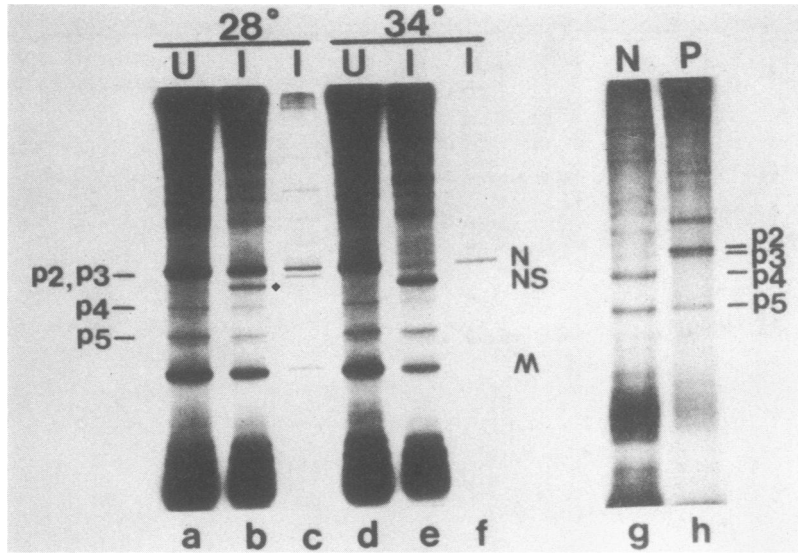

FIG. 10. Protein phosphorylation in uninfected and VSV-infected *A. albopictus* cells. *A. albopictus* cells were mock infected or infected with VSV and maintained at 28 or 34°C in E medium containing 10% serum. Cultures were then labeled with $^{32}PO_4$ from 4 to 8 h after infection, and the cytoplasmic phosphoproteins were analyzed by gel electrophoresis as described in the text (lanes a, b, d, and e). Extracts of [^{35}S]methionine-labeled, VSV-infected cells were analyzed in parallel (lanes c and f) to identify the position of the viral phosphoprotein, NS. Phosphoproteins from uninfected cells were also fractionated into nonpolysomal (N) and polysomal (P) phosphoproteins (lanes g and h) as described in the text. These fractions were analyzed on a separate gel that was run for a shorter time. Under these conditions we were able to resolve the phosphoproteins p2 and p3. Phosphoprotein p1 (not shown) is detected only in cell-free extracts labeled in vitro (Gillies and Stollar, in preparation). U, Uninfected cells; I, VSV-infected cells. The symbol ◆ (between lanes b and c) designates the position of the viral NS phosphoprotein.

termed P^-, that does not inhibit protein synthesis or rapidly kill mammalian cells (12). With this mutant viral protein synthesis is sustained over a long period of time and at the same initial rate as with the wild-type virus, but without the inhibition of total protein synthesis that normally accompanies the later stages of infection. Such a result was also seen in our system when infected cells were maintained at 34°C without serum. It is possible that a specific interaction (necessary to cause CPE) between a viral product and some host function is blocked in both cases. In the case of the P^- mutant, this might be due to the mutation in the viral product and, in our system, to the lack of serum in the culture medium.

A possible control mechanism involved in the shutoff of protein synthesis in these cells might be the phosphorylation of one or two polysome-associated proteins, as shown in Fig. 10. It appears that this phosphorylation reaction is blocked in serum-stimulated but not in serum-starved cells when a critical level of viral macromolecules is reached. Further experiments related to this finding have been carried out with cell-free extracts prepared from VSV-infected *A. albopictus* LT-C7 cells and confirm a correlation between protein synthesis and phosphorylation of phosphoprotein p2 (S. Gillies and V. Stollar, manuscript in preparation).

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-11290 from the National Institute of Allergy and Infectious Diseases, by the U.S.-Japan Medical Science Program through Public Health Service grant AI-05920, and by Institutional National Research Service Award CA-09069 from the National Cancer Institute.

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