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Basis for Variable Response of Arboviruses to Guanidine Treatment

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The effect of guanidine on the replication of the group A arboviruses, Sindbis virus, and Semliki Forest virus (SFV) was studied. Guanidine rapidly, but reversibly, inhibited SFV ribonucleic acid (RNA) synthesis. The synthesis of all species of viral RNA was inhibited, but that of ribonuclease-resistant forms was least affected. This inhibition occurred when the drug was added at any point during the log phase of virus growth. The growth of SFV was also markedly inhibited, but Sindbis virus growth was unimpaired. Infection of guanidine-treated cells with the viruses together resulted in a significant inhibition of the yields of both. It appears that, in the case of Sindbis virus, viral RNA is ordinarily produced in such excess that inhibition of its synthesis does not reduce virus yields. In the case of SFV, guanidine also markedly distorts the pattern of RNA synthesis by greatly decreasing the production of the 26*S* interjacent RNA form. This may account for the observed inhibition of SFV growth in the presence of guanidine.

Guanidine has been employed extensively as an inhibitor of picornavirus replication. Some of the data so far published suggest that its inhibitory action is due to a block in the initiation of new viral ribonucleic acid (RNA) chains (2, 3), although other mechanisms of action have also been suggested (1, 11). A recent study suggested that a structural protein of poliovirus is the primary site of guanidine action (4). Only one report has been published indicating that arboviruses are also sensitive to guanidine. Semliki Forest virus (SFV, arborirus group A) growth was reversibly inhibited by 30 mM guanidine (5), a concentration which is 20 times that usually employed to inhibit poliovirus replication.

In this investigation, the effect of guanidine on two group A arboviruses, SFV and Sindbis virus, was studied in some detail. Guanidine was found to inhibit SFV replication, probably as a result of an alteration in the pattern of SFV RNA production. On the other hand, despite a profound inhibition of Sindbis virus RNA synthesis in guanidine-treated cells, no significant inhibition of virus production was found.

MATERIALS AND METHODS

Viruses and cells. Primary chick embryo fibroblast (CEF) cultures and pools of SFV and Sindbis virus were prepared and assayed by previously described methods (8, 13).

Protein and RNA synthesis. Protein and RNA synthesis were estimated by incorporation of ^{3}H-leucine or ^{3}H-uridine into perchloric acid-precipitable radioactivity (8). Total protein was estimated by the method of Lowry et al. (9).

Viral RNA synthesis. RNA was extracted from virus-infected cells which had been treated with actinomycin D (1 μg/ml) by a phenol-sodium dodecyl sulfate method (8). The extracted RNA was analyzed on a 6 to 30% sucrose density gradient, and the acid-precipitable radioactivity in each fraction was estimated by previously described methods (8).

Polyacrylamide gel electrophoresis. Gels, 7.5 cm in length and containing 2.2% acrylamide and 0.5% agarose, were prepared by previously described methods (10). Since the buffer system used contained 0.5% sodium dodecyl sulfate, all operations were run at room temperature. Electrophoresis at 5 ma per gel was carried out for 30 min before the addition of the specimens. The specimens (in 50 μliters or less) were layered on the gels and the electrophoresis was carried out at 6 ma per gel for 4 hr. The gels were then fixed in 5% trichloroacetic acid and sliced into 1.3-mm segments. The slices were prepared for analysis in a liquid scintillation counter as previously described (10).

Reagents. Actinomycin D was a gift from Merck, Sharp & Dohme. Guanidine was purchased from Eastman Organic Chemicals. ^{3}H-uridine (20 Ci/mmole) was purchased from Schwarz BioResearch, Inc., and ^{3}H-leucine, (58.2 Ci/mmole) from New England Nuclear Corp.

RESULTS

Inhibition of SFV growth and RNA synthesis by guanidine. SFV replication was inhibited by treatment with 30 mM guanidine at the time of initia-

tion of virus infection (5). It was of interest to determine whether guanidine added during the course of infection would also inhibit virus growth. Therefore, CEF monolayers were infected with SFV, and samples of infected cells were frozen at various time periods after infection. At each period selected, guanidine (30 mM) was added to a duplicate set of monolayers. In all cases, the cells to which guanidine had been added were harvested with the last sample taken for the growth curve, at 8 hr after infection. All specimens were then titered for virus yield.

The results (Fig. 1) indicated that virus growth was inhibited at any time after infection by guanidine addition. These results resemble those previously reported in similarly conducted experiments in which cycloheximide or puromycin was added during the growth of SFV (7, 12).

Since guanidine probably blocks picornavirus replication by inhibiting viral RNA synthesis (1–3, 11) and guanidine has also been shown to inhibit arbovirus RNA synthesis (5), the dose-response curve of this inhibition was investigated. Various concentrations of guanidine were added to actinomycin D-treated monolayers of SFV-infected cells 4 hr after infection, early in the log phase of virus replication. After 10 min, ^{3}H-uridine was added to a concentration of 10 μCi/ml for 5 min. The cells were washed, and acid-precipitable radioactivity was determined. The results (Fig. 2) showed that a 3 mM concentration of guanidine effected only a 50% inhibition of viral RNA synthesis. Marked inhibition was seen at 10 and 30 mM concentrations.

Similar experiments were performed at various times after virus infection to determine to what extent viral RNA synthesis was inhibited. At each time period, the incorporation of ^{3}H-uridine (10 μCi/ml) into acid-precipitable counts was determined after a 5-min pulse in the presence or absence of 30 mM guanidine. In the controls (Fig. 3), the previously reported biphasic pattern of RNA synthesis in SVF-infected CEF was again observed (13). The same pattern was seen in guanidine-treated cells, but the specific activity of the RNA was only about 10% of the control in all cases. These results indicated that SFV RNA synthesis, like virus replication (Fig. 1), can be inhibited by guanidine addition at any time during the log phase of virus growth.

The rate at which SFV RNA synthesis was inhibited was also studied. Guanidine (30 mM) was added to SFV-infected cells. At various periods thereafter, monolayers were pulse-labeled for 2 min with 10 μCi of ^{3}H-uridine per ml, and the rate of viral RNA synthesis was estimated. The results (Fig. 4) indicated that viral RNA synthesis was rapidly inhibited after guanidine addition. Curiously, for up to 60 min after guanidine addition, no significant inhibition of virus-directed protein synthesis was observed in several experiments. At that time after infection, more than 80% of the protein synthesis is virus-directed (5).

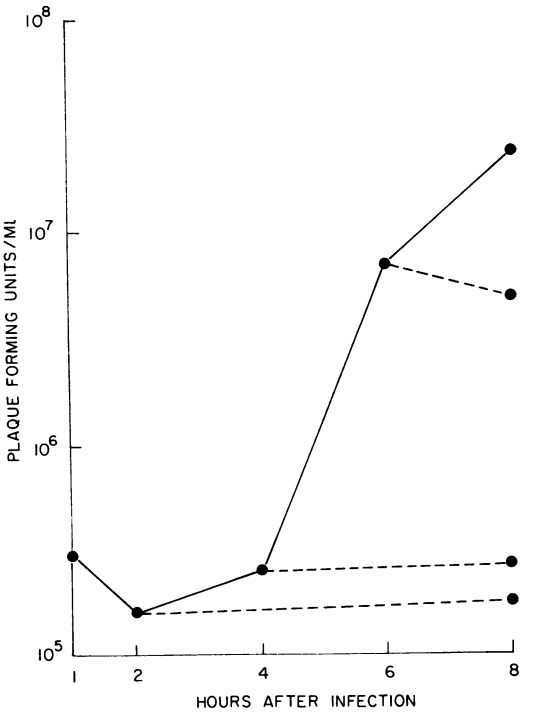

FIG. 1. *Effect of guanidine on Semliki forest virus yields. Chick cells were infected with SFV at a virus-cell multiplicity of 20:1. After 1 hr of infection, the cells were washed five times, and fresh medium was added. One pair of cultures was frozen at 2, 4, 6, and 8 hr after infection. Also, at each of these times, guanidine (30 mM) was added to an additional set of cultures which was incubated until 8 hr after infection and then frozen. All cultures were later thawed and assayed for virus titers.*

These results indicated that rapid inhibition of viral RNA synthesis, but not of viral protein synthesis, takes place after guanidine addition. In one other experiment performed with a different lot of guanidine, a 30 to 40% inhibition of virus protein synthesis was observed under the conditions employed in the experiment shown in Fig. 4. The results shown are probably more meaningful because they indicate that inhibition of virus RNA synthesis is not necessarily accompanied by inhibition of virus protein synthesis.

In a previous study, guanidine inhibition of SFV replication was shown to be a reversible phenomenon, if guanidine was added at the time of initiation of virus infection (5). The reversi-

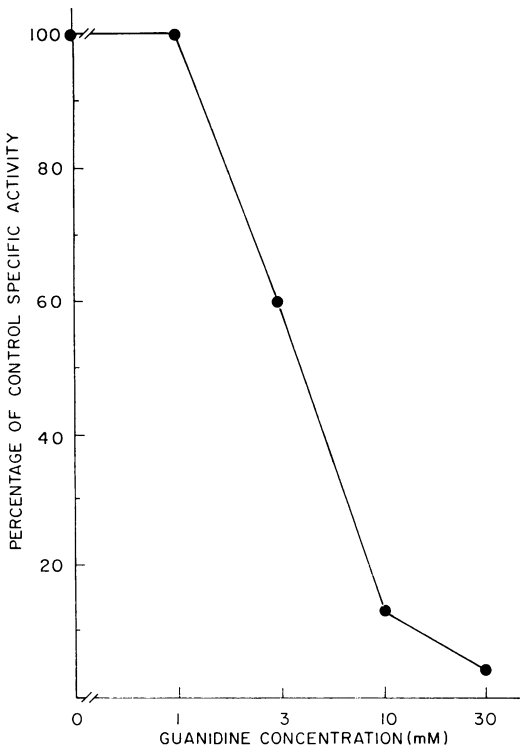

FIG. 2. *Effect of guanidine concentration on SFV-directed RNA synthesis. Chick cells were treated with actinomycin D (1 μg/ml) for 1 hr and were infected with SFV at a virus-cell multiplicity of 20:1. After 4 hr, pairs of cultures were treated with various concentrations of guanidine for 10 min; ^{3}H-uridine (10 μCi/ml) was then added for an additional 5 min, and the specific activity of acid-precipitable radioactivity was determined on each pair. Results are presented as the percentage of acid-precipitable radioactivity of cultures not treated with guanidine.*

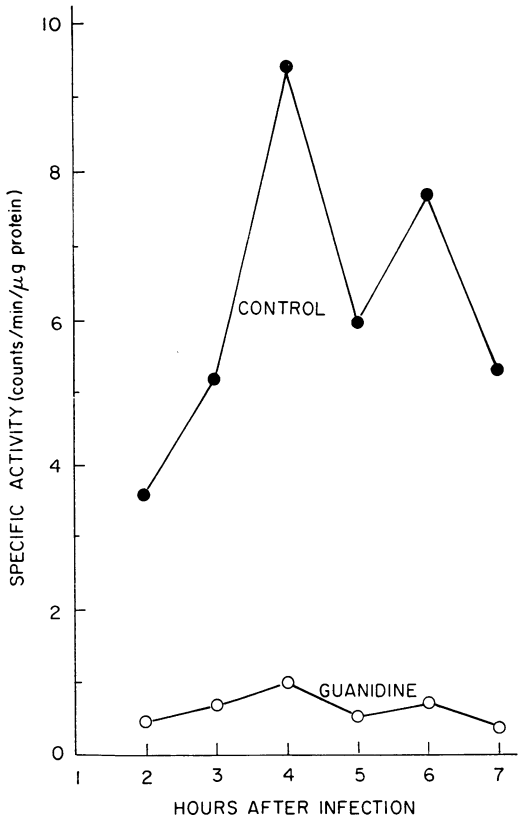

FIG. 3. *Effect of guanidine addition at various times after infection on SFV-directed RNA synthesis. Actinomycin D-treated chick cells were infected with SFV as previously described. At various times after infection, one pair of cultures was treated with guanidine (30 mM) for 10 min. To this and to an additional pair of untreated cultures, ^{3}H-uridine (10 μCi/ml) was added for 5 min and the specific activity of the acid-precipitable radioactivity was determined on each pair.*

bility of guanidine action at later times was studied (Fig. 5). Guanidine (30 mM) was added to six monolayers of chick cells at 3 hr after SFV infection and treatment with 1 μg of actinomycin D per ml. During the next 1 hr, the rate of RNA synthesis increased in two control cultures not treated with guanidine. In the guanidine-treated cells, a marked drop in RNA synthesis was seen between 3 and 3.5 hr after infection. At 3.5 hr after infection, two plates were washed five times to remove guanidine, and the rate of viral RNA synthesis in these was estimated 4 hr after infection. RNA synthesis rose in the washed cultures but remained at low levels in two cultures from which guanidine had not been removed; therefore, the inhibitory effect of guanidine on viral RNA synthesis seemed reversible.

It was of interest to study what effect guanidine addition had on the species of viral RNA produced in SFV-infected cells. Cultures were treated with 30 mM guanidine for 10 min, and ^{3}H-uridine (10 μCi/ml) was added for 1 hr, a sufficient period of time for all virus RNA species to become tritium-labeled (8). RNA was then extracted from the cells and analyzed on sucrose density gradients. The results (Fig. 6) showed a typical pattern of arbovirus RNA synthesis in control cells (Fig. 6A). Several species of RNA were seen—42*S* and 26*S* single-stranded RNA, the 18*S* core of the replicative intermediate form, and the replicative form (6). In the guanidine-treated cells, however, a different pattern was evident (Fig. 6B). In addition to a marked inhibi-

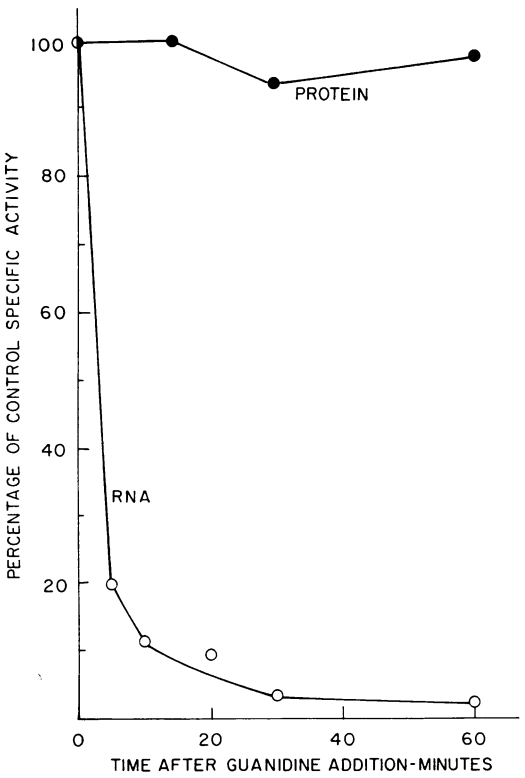

FIG. 4. *Rate of inhibition by guanidine of SFV-directed macromolecule synthesis. Actinomycin D-treated chick cells were infected with SFV as previously described. After 4 hr, pairs of cultures were treated with guanidine (30 mM) for the indicated periods of time, and then ^{3}H-uridine (10 μCi/ml) or ^{3}H-leucine (10 μCi/ml) was added for 2 min. Acid-precipitable radioactivity was determined for each pair of samples. Results are presented as specific activity of each sample as a percentage of controls which had not been treated with guanidine.*

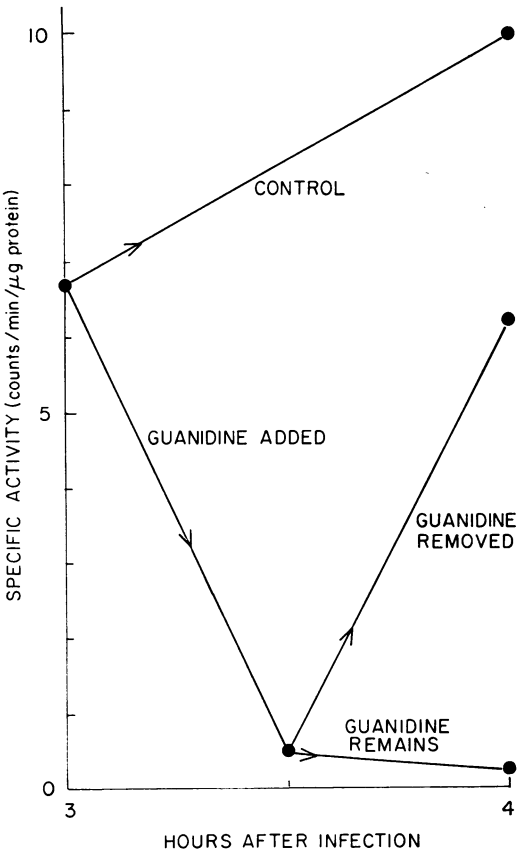

FIG. 5. *Reversibility of guanidine inhibition of SFV-directed RNA synthesis. Actinomycin D-treated chick cells were SFV-infected as previously described. After 3 or 4 hr of infection, 10 μCi/ml of uridine was added to each of two sets of monolayers for 10 min (controls). At 3 hr after infection, three pairs of cultures were also treated with 30 mM guanidine (guanidine added), and after 3.5 and 4 hr of infection one pair each of guanidine-treated cultures was also pulse-labeled with ^{3}H-uridine (guanidine remains). The additional set of guanidine-treated cultures was washed five times after 3.5 hr of infection and incubated in fresh medium until 4 hr after infection, when it too was pulse-labeled with ^{3}H uridine (guanidine removed). Acid-precipitable radioactivity was then determined on all cultures.*

tion of viral RNA synthesis (note the change in scale), the major radioactive species of RNA present was an 18S form which was quite resistant to ribonuclease treatment. In addition, some 42S RNA was evident. It was not possible to determine by sucrose density analysis whether traces of 26S RNA were present.

To analyze further the RNA forms of SFV produced in guanidine-treated cells, samples of the tritiated SFV-RNA preparation used in Fig. 6B were subjected to co-electrophoresis on 2.2% acrylamide gels with ^{32}P-SFV RNA from control cells. The result (Fig. 7) showed in controls a complex pattern which is currently being investigated (R. Friedman and J. Levin, *unpublished data*). The four RNA forms previously described were identified: the replicative intermediate, 42S and 26S single-stranded RNA, and the replicative form. In acrylamide gels, the replicative form has consistently been shown to separate into at least two distinct species (replicative forms). In addition, two intermediate minor species of single-stranded RNA (I_1 and I_2) have consistently been identified. Finally, small molecular weight RNA species which are present in uninfected cells were also seen. As in the case of the poliovirus replica-

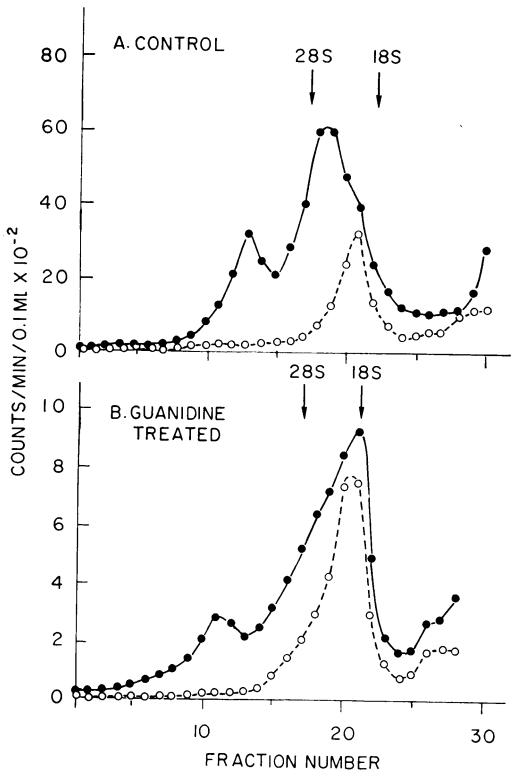

FIG. 6. *Viral RNA synthesis in the presence and absence of guanidine. Actinomycin D-treated cells were infected with SFV as previously described. After 4 hr, 2 cultures (~4 × 10⁷ cells) were treated with guanidine (30 mM) for 10 min; two additional cultures served as controls. ³H-uridine (10 μCi/ml) was then added to the medium of these four cultures. After 1 hr, the cells were washed and RNA was extracted from them by a phenol-sodium dodecyl sulfate method. The RNA was layered over a 6 to 30% sucrose density gradient and sedimented for 1 hr at 300,000 × g. Fractions were collected and analyzed for acid-precipitable radioactivity (●). Part of each fraction was treated with 2 μg of ribonuclease per ml (37 C, 30 min, 0.1 M NaCl) and also analyzed for acid-precipitable radioactivity (○). The top of the gradient in this and other sucrose density gradient analysis patterns shown is to the right. The designations 28S and 18S indicate the positions of ribosomal RNA in the gradient as determined by optical density readings at 260 nm.*

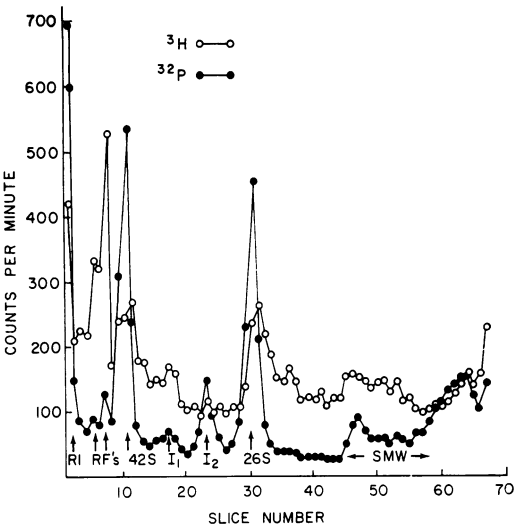

FIG. 7. *Polyacrylamide gel electrophoresis of SFV-RNA forms. Chick cells were infected as previously described. After 4 hr, the cells were incubated for 1 hr in the presence of otherwise phosphate-free medium containing 2 mCi/ml of $H_3{}^{32}PO_4$ for 1 hr. RNA was then extracted as previously described. A mixture of 45 μliters of the viral ³H-RNA preparation from guanidine-treated cells described in Fig. 6B was subjected to co-electrophoresis on a 2.2% acrylamide gel with 5 μliters of the above-described ³²P-SFV RNA. After 4 hr at 37 C, the gels were fixed, sliced, and counted as described in Materials and Methods. The species of RNA present have been identified as replicative intermediate form (RI), replicative forms (RF's), 42S RNA, two single-stranded intermediate forms (I_1 and I_2), 26S RNA, and small molecular weight RNA species (SMW).*

tive intermediate (11), the replicative intermediate of SFV did not enter the gel under the conditions employed.

In the guanidine-treated cells, the major SFV RNA forms appeared to be present (Fig. 7). The striking change was in their distribution, since the major species seen were the replicative forms. Some replicative intermediate RNA was present. The single-stranded RNA forms (42S, 26S, I_1, and probably I_2) appeared to be present, but in relatively reduced amounts. The 42S RNA formed in the presence of guanidine may be abnormal, as its peak lacked the sharpness consistently noted in that of controls (Fig. 7).

The fate of viral RNA, the synthesis of which had already been initiated at the time of guanidine addition, was also studied. Cells were pulse labeled for 1 min with 100 μCi of ³H-uridine per ml. At that time, as observed previously (8), only the replicative intermediate form of the virus was prominently labeled (Fig. 8A). The cells were then washed five times with cold medium, and medium containing uridine (3×10^{-4} M), with or without guanidine (30 mM), was added to the cultures. The cells were then incubated for an additional 1 hr.

In controls (Fig. 8C), additional radioactivity over that incorporated into the replicative intermediate of the 1-min pulse (Fig. 8A) was found

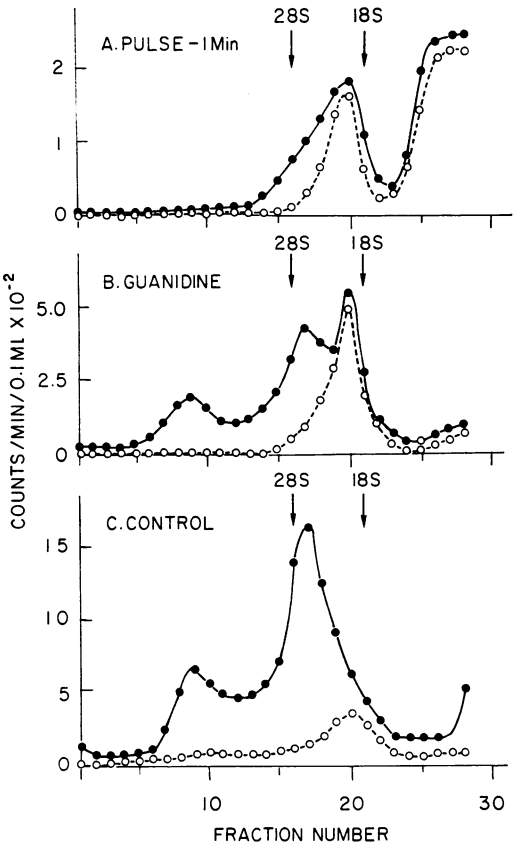

FIG. 8. *Fate of pulse-labeled RNA in guanidine-treated cells. Actinomycin D-treated cells were infected with SFV as previously described. After 4 hr, six cultures were treated with ^{3}H-uridine (100 μCi/ml) for 1 min. The cells were quickly washed with iced Eagle's medium, and RNA was extracted from two of the cultures. Warm Eagle's medium containing guanidine (30 mM) and uridine (3 × 10^{-4} M) or only uridine was added to each of two other cultures. After 1 hr, the RNA was also extracted from these cultures. All three RNA preparations were then analyzed on sucrose density gradients as described in the legend to Fig. 6. Fractions were treated with ribonuclease as in Fig. 6. Symbols: (●), acid-precipitable radioactivity; (○), acid-precipitable radioactivity after ribonuclease treatment.*

associated with RNA forms similar to those shown in Fig. 6A. In cells treated with guanidine after the 1-min pulse (Fig. 8B), additional ^{3}H-uridine incorporation (over that in Fig. 8A) was also seen. Again, as in the case of Fig. 6B, a change in the pattern of RNA synthesis was noted. The 18*S* species was most prominent and the 42*S* single-stranded RNA was seen. In addition, at variance with the findings in Fig. 6B, a 26*S* RNA peak was also consistently evident.

Although pulse-chase conditions could not be established, this result suggested that RNA synthesis initiated before guanidine addition may be carried out normally in its presence, since all RNA species of SFV were noted in cells treated in this manner (Fig. 8B). In contrast, RNA synthesis initiated after guanidine addition appears to be distinctly abnormal in that the replicative forms were the most heavily labeled forms (Fig. 6B and 7).

Inhibition of Sindbis virus RNA synthesis in the absence of growth inhibition. Since SFV and Sindbis virus are closely related, it was anticipated that the effect of guanidine on Sindbis virus would be similar to its effect on SFV. This proved to be an incorrect assumption, for, although guanidine treatment did inhibit Sindbis virus RNA synthesis (Table 1), and this inhibition was rapid and reversible, as in the case with SFV, guanidine had no effect on Sindbis virus growth (Table 2). In the same experiment, SFV growth was inhibited by 40-fold. The growth of SFV in the presence of guanidine was not greatly enhanced in cells also infected with Sindbis virus, since no significant portion of the virus produced in co-infected cells could be neutralized by SFV specific antibody (Table 2). In fact, a significant inhibition of Sindbis virus growth was seen in the co-infected cells.

The inhibition of Sindbis virus growth in the presence of SFV, the growth of which was blocked by guanidine treatment, was repeatedly observed (Table 3). The inhibition was not significantly enhanced by preinfecting the cells with SFV. This would appear to be an intrinsic interference phenomenon not dependent on interferon production, since it took place in the presence of actinomycin D at concentrations high enough to inhibit completely interferon production induced by virus infection in chick cells (13).

The ability of Sindbis virus to grow under con-

TABLE 1. *Inhibition of Sindbis virus RNA synthesis by guanidine*

Sample	Counts per min per μg of protein[a]
Control	6·67
Guanidine-treated	0·62

[a] Four monolayers of chick cells (~8 × 10^7 cells) were treated for 1 hr with 1 μg of actinomycin D per ml and were infected with Sindbis virus at a multiplicity of 20 to 40 plaque-forming units/cell. After 4 hr, two plates were treated with guanidine (30 mM) for 10 min, and then to all plates ^{3}H-uridine (10 μCi/ml) was added for 10 additional min. The plates were washed, and acid-precipitable radioactivity was determined.

TABLE 2. *Growth of Sindbis virus and SFV in the presence of guanidine*

Sample[a]	Yield (plaque-forming units/ml) after infection with		
	Sindbis virus	SFV	SFV + Sindbis virus
Control	19×10^7	11×10^7	10×10^7
Guanidine-treated	15×10^7	27×10^5	40×10^6
Guanidine treated + anti-SFV antibody	13×10^7	24×10^4	42×10^6

[a] Actinomycin D-treated chick cells were infected with SFV, Sindbis virus, or a mixture of equal titers of the two viruses in the absence or presence of guanidine. In all cases, infection was at the same virus to cell multiplicity. After 8 hr, the cultures were frozen, and they were later assayed under standard conditions. Samples from cultures of guanidine-treated cells were treated with a 1:10 dilution of rabbit anti-Semliki Forest virus (anti-SFV) antibody for 1 hr at 25 C before assay.

TABLE 3. *Effect of SFV infection on yield of Sindbis virus in guanidine-treated cells*

Guanidine concn[a]	Time of addition of SFV	Sindbis virus yield[b]
mM		
0	None added	40×10^7
30	None added	28×10^7
30	2 hr before Sindbis virus	27×10^6
30	At the time of Sindbis virus	43×10^6

[a] Chick cells were treated with actinomycin D (1 μg/ml) for 1 hr, washed, and divided into four groups. One set was infected with SFV in the presence of guanidine (30 mM) for 2 hr. The cells were then infected with Sindbis virus. Another set was infected with both SFV and Sindbis virus in the presence of guanidine; a third, with Sindbis virus in the presence of guanidine; and the fourth, with Sindbis virus in the absence of guanidine. After 8 hr of Sindbis virus infection, the cultures were frozen and thawed, and the culture fluids were assayed for Sindbis virus yields.

[b] Expressed as plaque-forming units per milliliter.

ditions inhibitory to SFV replication was puzzling. A clue to the explanation for this observation was found in the effect of guanidine on the pattern of Sindbis virus RNA synthesis. Normally this pattern is identical to that shown in Fig. 6A and 8C for SFV. In guanidine-treated cells, however, under conditions which markedly distorted the RNA pattern of SFV (Fig. 9A), the pattern of RNA synthesis in Sindbis-infected cells was close to normal (Fig. 9B) in that a peak of 26S RNA was evident and the replicative-form peak was not dominant.

Sindbis virus RNA produced in the presence of guanidine (30 mM) was also subjected to co-electrophoresis with ^{32}P-SFV RNA (Fig. 10). Again, less of a distortion of the normal pattern of viral RNA synthesis was noted than was seen in the case of SFV-RNA produced under the same conditions (Fig. 7). Although the replicative forms were much more in evidence in guanidine-treated cells than in normal cells (Fig. 10),

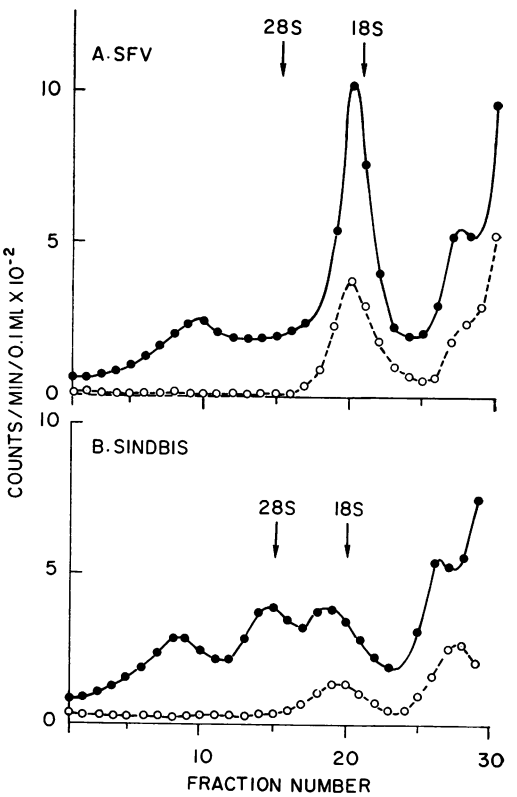

FIG. 9. *Sindbis virus and SFV-directed RNA synthesis in the presence of guanidine. Actinomycin D-treated cells were infected with SFV or Sindbis virus as previously described. After 4 hr, four cultures (two infected with Sindbis virus, two with SFV) were treated with guanidine; four similar cultures served as controls. After 10 min, ^{3}H-uridine was added to each of the cultures, and RNA was extracted and analyzed on sucrose density gradients as previously described (Figs. 6 and 8). Results in cultures not exposed to guanidine closely resembled those shown in Fig. 6A and 8C. Symbols are as in Fig. 6 and 8.*

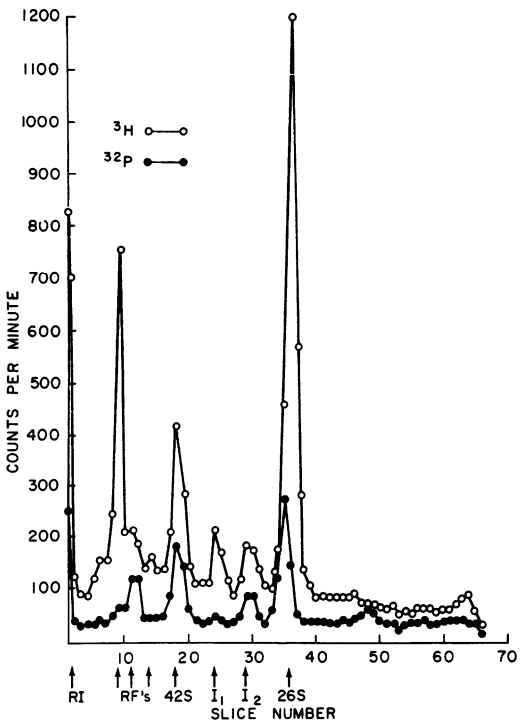

FIG. 10. *Polyacrylamide gel electrophoresis of Sindbis virus RNA forms. Sindbis virus ^{3}H-RNA was prepared as described for ^{3}H-SFV RNA in the legend to Fig. 7. It was co-electrophoresed with the same preparation of ^{32}P-SFV RNA as was employed in Fig. 7. The gels were analyzed as previously described (Fig. 7). RI, replicative intermediate form; RF's, replicative forms; 42S, I_1, I_2, and 26S indicate single-stranded RNA forms.*

a large amount of replicative intermeditate was present, together with sharp peaks of all four single-stranded RNA forms (42*S*, I_1, I_2, and 26*S*). The 26*S* RNA was the dominant peak of radioactivity.

DISCUSSION

The results indicated that guanidine rapidly, but reversibly, inhibited SFV and Sindbis virus RNA synthesis at any time during logarithmetic phase of virus growth. Growth of SFV was also depressed, but Sindbis virus growth was unaffected. Co-infection of guanidine-treated cells with SFV and Sindbis virus resulted in significant inhibition of the yields of both viruses.

The mechanism of guanidine inhibition of arbovirus RNA synthesis was not completely elucidated by these studies. The synthesis of all species of viral RNA was inhibited, but the inhibition of single-stranded RNA forms was more significant than that if ribonuclease-resistant RNA. The results taken together indicated that, in the presence of guanidine, viral RNA synthesis is slowed, and newly formed viral RNA leaves the template on which it was synthesized more sluggishly than under normal conditions. Since the number of templates is limited, this situation would soon lead to an inhibition of viral RNA synthesis, greater in the case of single-stranded RNA, which appears to be formed on the templates, than of the ribonuclease-resistant replicative form.

The lack of effect of guanidine on Sindbis virus yields, despite its inhibition of Sindbis virus RNA synthesis and of both RNA synthesis and virus yields in SFV infection, was surprising. This result suggests that, under normal conditions, excess viral RNA is produced during Sindbis virus infection, since marked inhibition of virus RNA synthesis resulted in no significant decrease in virus yield. T. Sreevalsan (*personal communication*) has independently confirmed the herein reported findings on the lack of sensitivity of Sindbis virus yields to doses of guanidine which markedly inhibit SFV replication. At 29 C, however, both viruses are equally sensitive to the drug.

The difference noted between SFV and Sindbis virus with respect to sensitivity of virus yields to guanidine would appear to be due to the nature of the RNA produced rather than the actual amount. In the case of SFV, a gross distortion of the normal pattern of RNA synthesis was seen in guanidine-treated cells (Fig. 6 and 9A). Other forms of RNA were produced, but most of the RNA was in the ribonuclease-resistant replicative forms. The 26*S* RNA form, which was one of the dominant species in the controls, was greatly decreased in guanidine-treated cells. In cultures pulse-labeled with uridine before addition of guanidine, however, 26*S* viral RNA was consistently found, even after 1 hr of guanidine treatment (Fig. 8). This suggests that SFV RNA, the synthesis of which was initiated before guanidine addition, may eventually give rise to 26*S* RNA; however, during SFV infection, the synthesis of 26*S* RNA was greatly inhibited in RNA initiated after guanidine addition. On the other hand, in Sindbis virus infection in the presence of guanidine, the distortion of the normal pattern of RNA synthesis was not as great as in SFV infection. Some 26*S* RNA was apparent along with 42*S* and ribonuclease-resistant RNA (Fig. 9B and 10). The ribonuclease-resistant species were not as dominant in Sindbis infection in the presence of guanidine as in SFV.

The results with both SFV and Sindbis virus indicate that inhibition of 26*S* RNA synthesis by

guanidine is relatively more marked in the case of SFV-infected cells. This suggests that this species of RNA is in some way associated with normal virus maturation leading to production of infectious virions. A similar conclusion was recently reached as a result of studies on a temperature-sensitive Sindbis virus mutant, TS-24 (12).

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LITERATURE CITED

1. Baltimore, D. 1968. Inhibition of poliovirus replication by guanidine, p. 340–347. *In* M. Sanders and E. H. Lennette (ed.), Medical and applied virology. Warren H. Green, St. Louis, Mo.
2. Caliguiri, L. A., and I. Tamm. 1968. Action of guanidine on the replication of poliovirus RNA. Virology 35:408–417.
3. Caliguiri, L. A., and I. Tamm. 1968. Distribution and translation of poliovirus RNA in guanidine-treated cells. Virology 36:223–231.
4. Cooper, P. D., B. B. Wentworth, and D. McCahon. 1970. Guanidine inhibition of poliovirus: a dependence of viral RNA synthesis on the configuration of structural protein. Virology 40:486–493.
5. Friedman, R. M. 1968. Structural and nonstructural proteins of an arbovirus. J. Virol. 2:1076–1080.
6. Friedman, R. M. 1968. Repicative intermediate of an arbovirus. J. Virol. 2:547–552.
7. Friedman, R. M., and P. M. Grimley. 1969. Inhibition of arbovirus assembly by cycloheximide. J. Virol. 4:292–299.
8. Friedman, R. M., H. B. Levy, and W. B. Carter. 1966. Replication of Semliki Forest virus. Three forms of viral RNA produced during infection. Proc. Nat. Acad. Sci. U.S.A. 56:440–446.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
10. Moss, B., and R. Filler. 1970. Irreversible effects of cycloheximide during the early period of vaccinia virus replication. J. Virol. 5:99–108.
11. Noble, J., and J. Levintow. 1970. Dynamics of poliovirus-specific RNA synthesis and the effects of inhibitors of virus replication. Virology 40:634–642.
12. Scheele, C. M., and E. R. Pfefferkorn. 1969. Inhibition of interjacent ribonucleic acid (26*S*) synthesis in cells infected by Sindbis virus. J. Virol. 4:117–122.
13. Taylor, J. 1965. Studies on the mechanism of action of interferon. I. Interferon action and RNA synthesis in chick embryo fibroblasts infected with Semliki Forest virus. Virology 25:340–349.