Mechanism and Specificity of Action of Ribavirin

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Ribavirin at a concentration of 30 μg/ml added immediately after infection completely inhibited influenza A/Port Chalmers/1/73 (H3N2) virus hemagglutinin production in infected MDCK cells. Under these conditions, host cell protein synthesis was inhibited by only 10 to 20%. Polyacrylamide gel electrophoresis of [35S]methionine-labeled material from virus-infected cultures confirmed that ribavirin inhibited viral but not host cell protein synthesis. In parallel experiments, actinomycin D also preferentially inhibited viral protein synthesis. The possibility that ribavirin inhibited viral protein synthesis as a result of general inhibition of ribonucleic acid synthesis was therefore examined. In uninfected cells, ribavirin at 30 μg/ml inhibited the incorporation of [3H]uridine into ribonucleic acid but stimulated the incorporation of [3H]guanosine. The effects noted are consistent with an inhibition of the host cell enzyme inosine 5'-monophosphate dehydrogenase. This suggestion is supported by the finding that addition of guanosine, but not inosine, to the culture medium substantially reversed the antiviral effect of ribavirin. There was no separation between the concentration of ribavirin causing inhibition of influenza A viral protein synthesis or inhibition of MDCK cell ribonucleic acid synthesis, suggesting that ribavirin is not specifically antiviral in this system but inhibits viral protein synthesis as a result of the general inhibition of ribonucleic acid synthesis.

Ribavirin is a nucleoside analog which inhibits the replication of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) viruses, including influenza type A and type B (18). Despite many reports, it is not yet clear whether the compound is specifically antiviral in its mode of action or whether it inhibits virus replication as a result of its effect(s) on the host cell.

Thus, ribavirin has been reported to reduce the production of hemagglutinin (HA) by influenza type A in chicken embryo cells by 50% at a concentration of 3.2 μg/ml. The growth of these chicken embryo cells was reduced by only 50% at a ribavirin concentration of 1,000 μg/ml (8). Additionally, ribavirin has been reported to inhibit the growth of influenza type A virus while causing no significant inhibition of host cell protein and RNA synthesis, although DNA synthesis was affected (15, 16).

Data on the mode of action of this compound suggest that ribavirin, as ribavirin 5'-phosphate, affects virus-infected cells primarily by inhibiting the enzyme inosine 5'-monophosphate (IMP) dehydrogenase; whether this enzyme is virus specific or is a host cell enzyme stimulated on infection is not known (19). Scholtissek (17), however, has shown that ribavirin has no effect on the cellular guanosine 5'-triphosphate pool of chicken embryo cells. This suggests that IMP dehydrogenase is not inhibited by ribavirin. Cell-free DNA- and RNA-dependent polymerase assays have shown that a metabolite of ribavirin, ribavirin 5'-triphosphate, selectively inhibits the influenza RNA-dependent RNA polymerase (6).

In contrast to these reports, De Clercq et al. (5), using primary rabbit kidney cells and Vero cells, found that ribavirin inhibited host cell DNA and RNA synthesis at concentrations similar to those inhibiting the replication of a range of viruses. Ribavirin has also been shown to inhibit the replication of uninfected mouse L5178, cells at concentrations as low as 1.15 μg/ml. In the same cells, ribavirin also causes a marked decrease in DNA, RNA, and protein synthesis and also reduces the size of the cellular guanosine 5'-triphosphate pool (14). However, the effect of ribavirin on virus replication was not examined in this cell line.

The study reported here attempts to clarify the mechanism and specificity of action of ribavirin, using influenza A/Port Chalmers/1/73 (H3N2) virus grown in MDCK cells as a model system.

MATERIALS AND METHODS

Cell lines and virus stocks. Madin and Darby canine kidney (MDCK) cells were obtained from Flow Laboratories, Ltd., and passaged in a humidified at-
mosphere of 5% CO₂ in air in Eagle minimal essential medium containing: 10% fetal bovine serum; NaHCO₃ at 2.2 μg/ml; nonessential amino acids and penicillin at 100 IU/ml; and streptomycin at 100 μg/ml. The influenza A/Port Chalmers/1/73 (H₃N₂) virus was obtained from G. C. Schild, National Institute for Medical Research, Mill Hill, London, and was grown in fertile hen eggs. Stocks were assayed by plaquing serial dilutions on monolayers of MDCK cells (20).

Hemagglutination assays were performed by the procedure of Minor and Dimmock (12), except that one cycle of freeze-thawing only was used to release cell-associated HA activity.

[³⁵S]Methionine labeling of infected cells. Confluent monolayers of MDCK cells were rinsed with phosphate-buffered saline (PBS) and inoculated with virus at a multiplicity of 1 plaque-forming unit per cell; adsorption was for 1 h at 4°C. The inoculum was removed, and the cell sheet was rinsed with PBS. Warm maintenance medium containing 2% fetal bovine serum was added and the cultures were incubated for 6 h at 33°C. The time of infection was measured from the start of the incubation at 33°C. The medium was then removed, and the cells were overlaid with PBS containing 5 μCi of [³⁵S]methionine per ml for 15 min at 33°C. At the conclusion of the labeling period, the cells were rinsed in ice-cold PBS, lysed in 0.2 ml of electrophoresis sample buffer (10), and heated at 100°C for 3 min before analysis by polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis. [³⁵S]Methionine-labeled samples of virus-infected cultures in electrophoresis sample buffer were analyzed by discontinuous polyacrylamide gel electrophoresis (10). Slab gels, 1.5 mm thick, consisting of an 11% running gel overlaid with a 4% stacking gel, were run for 16 h at a constant 50 V in a Shandon polyacrylamide gel electrophoresis apparatus. For resolution of the viral M and NS proteins, the running gel was increased to 13% polyacrylamide and electrophoresis was for 24 h. After electrophoresis, the gels were impregnated with scintillant, dried under vacuum onto Whatman 3MM chromatography paper, and exposed to Fuji-RX X-ray film at -70°C (1).

To quantitate the relative amount of [³⁵S]Methionine labeling of the M and NS proteins, the bands were first located by autoradiography, then excised and solubilized in 19 parts 30% H₂O₂ and 1 part 0.88 ammonia at 60°C before scintillation counting (1).

Incorporation of labeled precursors. Uninfected cells were examined for their ability to incorporate labeled precursors into trichloroacetic acid-soluble and -insoluble pools by a modification of the method of Minor and Dimmock (13). Cultures containing 2 x 10⁶ MDCK cells in a rapid phase of growth were incubated for 3 h at 37°C in fresh medium containing 2% fetal bovine serum and actinomycin D or ribavirin at the appropriate concentration. Labeling with RNA precursors was accomplished by adding [³H]guanosine (10 μCi/ml), [³¹C]inosine (1.0 μCi/ml), or [³H]uridine (20 μCi/ml) to the medium. For labeling protein, the medium was removed and replaced with warm PBS containing [³⁵S]Methionine (5 μCi/ml). Cultures were labeled for 30 min and then washed five times with 1 ml of ice-cold PBS. Scintillation counting of the trichloroacetic acid-soluble and -insoluble pools was performed as described by Minor and Dimmock (13).

Chemicals, film, and isotopes. L-[³⁵S]Methionine (>100,000 μCi/mmol), [⁵,⁶-³H]uridine (49 Ci/mmol), [³⁸-²H]guanosine (10,000 to 20,000 mCi/mmol), and [³⁴C]inosine were obtained from the Radiochemical Centre, Amersham, England. Actinomycin D, grade A, was obtained from Calbiochem Ltd., Bishop's Stortford, England. Ribavirin (1-B-d-ribofuranosyl-1,2,4-triazole-3-carboxamide) was a gift from R. W. Sidwell, ICM Nucleic Acid Research Institute, Irvine, Calif. Acrylamide, bisacrylamide, guanosine, and inosine were obtained from British Drug Houses Ltd., Poole, Dorset, England. Eagle minimal essential medium was obtained from Wellcome Reagents Ltd., Beckenham, Kent, England. Fuji-RX X-ray film was obtained from Hanimax (U.K.) Ltd., Dorcan, Swindon, Wiltshire.

RESULTS

Effect of ribavirin on cellular and viral protein synthesis. In this study, the behavior of ribavirin is compared to that of actinomycin D, a known inhibitor of DNA-dependent RNA synthesis.

The effect of ribavirin on protein synthesis in uninfected MDCK cells was determined by measuring the incorporation of [³⁵S]Methionine into trichloroacetic acid-soluble and -insoluble pools after 3 h of ribavirin treatment. Ribavirin has a negligible effect on incorporation into the trichloroacetic acid-soluble pool and shows only a slight inhibition of protein synthesis below a concentration of 100 μg/ml (Fig. 1). Above 100 μg/ml, the inhibition of protein synthesis increases to 37% at a concentration of 1,000 μg/ml (Fig. 1). For comparison, the effect of actinomycin D at a concentration of 5 μg/ml is also shown. Actinomycin D causes a 24% increase in uptake of [³⁵S]Methionine into the trichloroacetic acid-soluble pool but inhibits protein synthesis by 33%. The inhibition caused by actinomycin D has been attributed to the effect actinomycin D has on RNA transcription rather than a direct effect on protein synthesis itself (4).

The effect of ribavirin on viral protein synthesis was measured by its inhibition of appearance of HA activity and by polyacrylamide gel electrophoresis of [³⁵S]Methionine-labeled virus-infected MDCK cell lysates. Table 1 shows that ribavirin markedly decreases the yield of HA activity (measured 6 h postinfection); this inhibition reaches 100% at a ribavirin concentration of 30 μg/ml. Figure 2 shows a fluorograph of an 11% polyacrylamide gel demonstrating the effect of ribavirin and actinomycin D on the synthesis of viral proteins in infected MDCK cells. Equal amounts of [³⁵S]Methionine-labeled protein were added to each gel track to compensate for the general inhibition of protein synthesis (Fig.
FIG. 1. Effect of actinomycin D and ribavirin on protein synthesis in uninfected MDCK cells. Cultures of cells were incubated in the presence of actinomycin D or ribavirin for 3 h at 37°C before pulse-labeling with [\(^{35}\)S]methionine for 30 min. Symbols: ○, trichloroacetic acid-soluble material; ●, trichloroacetic acid-insoluble material; AD, actinomycin D at a concentration of 5 \(\mu\)g/ml.

**TABLE 1.** Effect of ribavirin on HA yield

<table>
<thead>
<tr>
<th>Ribavirin (µg/ml)</th>
<th>HA titerb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.0</td>
</tr>
<tr>
<td>1</td>
<td>5.75</td>
</tr>
<tr>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>30</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>100</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>1,000</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

a MDCK cells were infected with 1 plaque-forming unit of virus per cell at 4°C for 1 h. Prewarmed maintenance medium containing the appropriate concentration of ribavirin was added, and the cultures incubated for 6 h at 33°C. Cultures were then freeze-thawed to liberate cell-associated HA activity as described in the text.

b Expressed as log\(_2\) of the reciprocal of the dilution at the endpoint of the titration.

1). Examination of the gel tracks A, B, and D through H (Fig. 2) shows a decline in viral protein synthesis on increasing the concentration of ribavirin and a concomitant resumption of host cell protein synthesis. Traces of the viral NP and M/NS proteins are still visible at a ribavirin concentration of 100 µg/ml.

Minor and Dimmock (13) have shown that a range of inhibitors of cellular DNA function have a differential effect on the synthesis of some influenza (fowl plague) viral proteins, notably the M and NS proteins. Further [\(^{35}\)S]methionine-labeled samples were therefore electrophoresed on 13% polyacrylamide gels to resolve the M and NS protein bands (Fig. 2, tracks I and J), and the relative amount of [\(^{35}\)S]methionine label in these bands was determined by scintillation counting. Synthesis of the M and NS proteins in this system is similar to that reported for growth of the WSN strain of influenza A\(_0\) virus in MDBK cells (11). Both proteins can be detected as early as 2 h postinfection. Initially, the rate of synthesis of the NS protein exceeds that of the M protein. In this system, however, the rate of synthesis of the M protein reaches equivalence at 3 to 4 h postinfection and overtakes NS protein synthesis at 5 to 6 h postinfection (data not shown). Ribavirin at 10 µg/ml preferentially inhibits M protein synthesis (Table 2). The scale of this inhibition is similar to that observed by Minor and Dimmock (13), using actinomycin D, echinomycin, or mithramycin at low concentrations.
The effect of actinomycin D (5 μg/ml only) on viral protein synthesis is shown in Fig. 2 track C as a control. Actinomycin D specifically inhibits viral protein synthesis, in agreement with previous findings (13).

The results shown in Fig. 1 and 2 and Table 1 demonstrate that actinomycin D and ribavirin both inhibit influenza A viral protein synthesis while having only minor effects on host protein synthesis. It has been shown that actinomycin D inhibits protein synthesis by its action on RNA transcription rather than by a direct effect on translation, (4) and thus it is possible that the effect of ribavirin on viral protein synthesis shown here and elsewhere (15, 16) is due to the operation of a similar mechanism. This possibility was explored by examining RNA synthesis in uninfected MDCK cells.

**Effect of ribavirin on [3H]uridine utilization.** Rapidly dividing cultures of MDCK cells were incubated in the presence of ribavirin for 3 h at 37°C before labeling with [3H]uridine for 30 min. Ribavirin exerts a complex effect on incorporation of the label into the trichloroacetic acid-soluble and -insoluble pools (Fig. 3a). Ribavirin causes a 25% inhibition of [3H]uridine uptake into the MDCK cells at 30 μg/ml, but this is reversed at higher levels. Incorporation of label into RNA is inhibited to a much greater extent (74% at 30 μg/ml). However this, too, is largely reversed at high levels of ribavirin.

It was considered possible that the behavior of the [3H]uridine labeling was an artefact due to depletion of extracellular levels of [3H]uridine or perhaps due to saturation of the transport of the isotope into the cells. The kinetics of labeling were therefore reexamined. MDCK cells were pulse-labeled with [3H]uridine at the times indicated (Fig. 3b), and the amount of isotope incorporated was measured. It can be seen that, under the conditions of the assay, incorporation of label into the trichloroacetic acid-soluble and -insoluble pools has not reached completion after 30 min, thus ruling out the possible artefacts outlined above.

The inhibition of RNA synthesis contrasts with previous reports (15, 16) that ribavirin has little effect on host cell RNA synthesis. The unexpected complexity of the results obtained in this study (Fig. 3a) may explain why an inhibition has not been observed in some systems (15, 16). The inhibition of labeling of RNA in MDCK cells by the lower concentrations of ribavirin is very similar to the findings of De Clercq et al. (5) and Muller et al. (14). These workers, however, did not examine the effect of higher concentrations of ribavirin.

The effect of actinomycin D (5 μg/ml) is also shown in Fig. 3a. Although it stimulates [3H]uridine uptake by 8%, RNA synthesis is inhibited by 96% in this system. This finding is in agreement with the results obtained by Minor and Dimmock (13).

**Effect of ribavirin on guanosine and inosine utilization.** As a result of the effect of ribavirin on the [3H]uridine labeling, it was decided to examine the effect of ribavirin on RNA synthesis in greater depth. The RNA precursors [3H]guanosine and [3H]inosine were chosen (i) because they are purine nucleosides, whereas uridine (studied above) is a pyrimidine nucleoside and (ii) because one of the putative sites of action of ribavirin is the enzyme IMP dehydrogenase, which lies on the pathway between inosine monophosphate and guanosine monophosphate (19).
Rapidly growing cultures of MDCK cells were incubated for 3 h in the presence of ribavirin before labeling with [3H]guanosine or [14C]inosine (Fig. 4). Ribavirin inhibits the uptake of both nucleosides into the trichloroacetic acid-soluble pool. This inhibition is presumably due to competition for transport into the cell and is consistent with the suggestion that ribavirin is a purine nucleoside analog. This finding contrasts with a previous report that ribavirin has no effect on the uptake or incorporation of labeled guanosine into chicken embryo cells (17).

Ribavirin also inhibits the incorporation of [14C]inosine into trichloroacetic acid-insoluble material (Fig. 4a). This inhibition is more than could be accounted for on the basis of an inhibition of uptake alone. Thus, incorporation of [14C]inosine into the trichloroacetic acid-soluble pool is depressed 50% by a ribavirin concentration of 160 μg/ml, whereas incorporation into the trichloroacetic acid-insoluble pool is depressed by 50% at a ribavirin concentration of 4 μg/ml. The inhibition of RNA synthesis as measured by [14C]inosine utilization does not undergo a reversal at higher ribavirin concentrations.

Despite the fact that the uptake of [3H]guanosine into the trichloroacetic acid-soluble cellular pool is inhibited (Fig. 4b), the incorporation of label into RNA is in fact stimulated by ribavirin concentrations up to 100 μg/ml. This is presumably because the (putative) inhibition of IMP dehydrogenase causes a decrease in the size of the cellular guanosine 5'-monophosphate pool, thus creating an increase in the specific activity of [3H]guanosine label.

As with [3H]thymidine labeling (described above), the effect of ribavirin on [3H]guanosine incorporation is also reversed by higher concentrations of ribavirin (Fig. 4b).

The effect of actinomycin D (5 μg/ml) on the utilization of the guanosine and inosine was measured in parallel (Fig. 4) to ensure that the label incorporated into trichloroacetic acid-insoluble material represented RNA (i.e., the process was actinomycin D sensitive) and that the label used had not been sequestered elsewhere. Thus, the appearance of 99% of the guanosine label and 93% of the inosine label in trichloroacetic acid-insoluble material was inhibited by actinomycin D (5 μg/ml).

**Antagonism of the action of ribavirin by added nucleosides.** The results obtained in this study using labeled nucleosides suggest that ribavirin acts primarily by inhibition of the enzyme IMP dehydrogenase. As independent confirmation of this hypothesis, it was decided to attempt to reverse the effect of ribavirin on viral HA synthesis by addition of extra nucleosides to the medium. If the suggested mechanism of action of ribavirin is correct, then the effects of ribavirin should be counteracted by guanosine but not by inosine.

MDCK cells were preincubated for 30 min at 37°C in the presence of ribavirin (30 μg/ml). The cells were then infected with 1 plaque-forming unit of virus per cell, and virus was adsorbed at 4°C for 1 h in the presence of ribavirin. Cultures were then incubated for 6 h at 33°C in maintenance medium containing ribavirin (30 μg/ml), in the presence or absence of added guanosine or inosine, before freeze-thawing to release cell-associated HA (Table 3). The results

**TABLE 3. Effect of guanosine or inosine on the activity of ribavirin**

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Conc (μg/ml)</th>
<th>Ribavirin concn (μg/ml)</th>
<th>HA titer^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>30</td>
<td>&lt;1.0</td>
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<tr>
<td>Guanosine</td>
<td>105</td>
<td>30</td>
<td>2.75</td>
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<tr>
<td>Guanosine</td>
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<tr>
<td>Guanosine</td>
<td>106</td>
<td>0</td>
<td>5.5</td>
</tr>
<tr>
<td>Guanosine</td>
<td>210</td>
<td>0</td>
<td>5.0</td>
</tr>
<tr>
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<td>30</td>
<td>&lt;1.0</td>
</tr>
<tr>
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<td>200</td>
<td>30</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Inosine</td>
<td>100</td>
<td>0</td>
<td>5.25</td>
</tr>
<tr>
<td>Inosine</td>
<td>200</td>
<td>0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

^a MDCK cells were treated with ribavirin 30 μg/ml (where appropriate) for 30 min at 37°C. Cells were infected with 1 plaque-forming unit per cell at 4°C in the presence of ribavirin (where appropriate). Cultures were then incubated at 33°C for 6 h in maintenance medium containing nucleosides as described in the text. The cells were then freeze-thawed to release cell-associated HA.

^b See footnote b to Table 1.
in Fig. 4 show that ribavirin inhibits uptake of these nucleosides. It is therefore likely that simultaneous addition of ribavirin with an excess of guanosine or inosine would prevent entry of ribavirin into the cells. It would not then be possible to interpret the results of an experiment by using simultaneous addition of compounds in terms of inhibition of an intracellular enzyme(s) alone.

The results in Table 3 show that inosine at 100 or 200 μg/ml (three or six times molar excess) is completely ineffective in reversing the effects of ribavirin. On the other hand, the same molar quantity of guanosine causes a substantial reversal of the inhibition of HA production, even though it is added later than ribavirin. Neither guanosine nor inosine alone causes any significant inhibition of HA production.

The results obtained in this experiment are in good agreement with the findings of Streeter et al. (19) and Oxford (15, 16).

**DISCUSSION**

The results shown in Fig. 1 and 2 and Table 1 demonstrate that ribavirin inhibits viral protein synthesis, while having only minor effects on host protein synthesis. Quantitation of the effect of ribavirin on the viral M and NS proteins shows that the M protein is preferentially suppressed. This differential effect is similar to that obtained by treatment of influenza (fowl plague) virus-infected cells with actinomycin D, echinomycin or mithramycin (13). Comparison of the effects of ribavirin with those of actinomycin D, a known inhibitor of DNA-dependent RNA synthesis (Fig. 1 and 2), suggested that the "specific" inhibition of viral protein synthesis could be dependent on an inhibition of RNA synthesis rather than a direct inhibition of protein synthesis itself (4). The effect of ribavirin on the utilization of [3H]guanosine, [14C]inosine, and [3H]uridine is consistent with this suggestion. At ribavirin concentrations less than 100 μg/ml, the effect on MDCK cell RNA synthesis is indicative of a specific inhibition of the cellular enzyme IMP dehydrogenase. Thus, the incorporation of [14C]inosine and [3H]uridine is inhibited approximately equally (Fig. 3a and 4a). This might be expected, because both purines and pyrimidines are required in the synthesis of RNA. The stimulation of incorporation of [3H]guanosine into RNA (Fig. 4b) is consistent with a decrease in the size of the cellular guanosine 5'-monophosphate pool causing an increase in the specific activity of isotopic labeling, and is in agreement with the observed reduction in the guanosine 5'-triphosphate pool size of L5178y cells reported by Muller et al. (14). The ability of guanosine, but not inosine, to reverse the inhibitory effect of ribavirin on HA synthesis (Table 3) provides further evidence for the enzyme IMP dehydrogenase being the primary site of action of ribavirin.

The data obtained in this study suggest that ribavirin inhibits RNA synthesis by depletion of the cellular purine nucleotide pools. If this is the case, synthesis of all RNA species (host and viral) should be equally inhibited. Actinomycin D, however, has been shown to inhibit influenza virus replication in the intact cell but does not affect the virion-associated polymerase in vitro (2). It is thus possible that influenza RNA synthesis would be able to tolerate the reduced nucleotide concentrations in ribavirin-treated cells but that inhibition of DNA-dependent (host cell) RNA synthesis, which is necessary for influenza virus transcription, prevents this. Both mechanisms would be expected to produce the same inhibition of viral protein synthesis (Fig. 2), and it is therefore not possible to distinguish which mechanism is operating here.

At concentrations of ribavirin greater than 100 μg/ml, the stimulation of [3H]guanosine labeling and the inhibition of [3H]uridine labeling of RNA undergo a reversal (Fig. 3a and 4b). The lack of reversal of the effects of ribavirin on viral and host cell protein synthesis and the continued inhibition of [14C]inosine labeling (Fig. 1, 2 and 4a; Table 1) suggest that these reversals do not reflect a genuine cessation of the "antiviral" effect of ribavirin. It is possible that at higher concentrations ribavirin becomes less specific in its action and causes changes in aspects of RNA metabolism other than the inhibition of IMP dehydrogenase. Thus, a decrease in the uridine 5'-monophosphate pool size could cause an increase in the specificity of labeling by [3H]uridine. A reduction in the amount of [3H]guanosine entering RNA may be caused by the direct action of ribavirin triphosphate on the cellular RNA polymerase(s). Cell-free assays, however, show that these enzymes are not inhibited by ribavirin triphosphate (6, 14). The cause of the reversal of [3H]guanosine labeling therefore remains unclear. The complexity of the effects of ribavirin, particularly at concentrations above 100 μg/ml, at least suggests a reason why some workers using [3H]uridine labeling (5, 14) may have found that ribavirin inhibited RNA synthesis in uninfected cells whereas others also using [3H]uridine (15, 16) or [3H]guanosine (17) did not.

The results above indicate that the antiviral effect of ribavirin is due to its inhibition of RNA synthesis and, in particular, to inhibition of the normal host cell enzyme IMP dehydrogenase.
Cell-free assays however have shown that ribavirin triphosphate selectively inhibits the influenza A RNA-dependent RNA polymerase (6). This mechanism is unlikely to be important in the system used in this study, because there is no separation between concentrations of ribavirin inhibiting viral HA synthesis and those inhibiting RNA synthesis in the uninfected MDCK cell. Thus, although viral HA activity is reduced by 50% by a ribavirin concentration of ca. 5 μg/ml (Table 1), host cell RNA synthesis is inhibited to the same degree by as little as 3 to 4 μg of ribavirin per ml (Fig. 3a and 4a). Indeed, the inhibition of host cell metabolism rather than a "specific antiviral" mode of action would be consistent with the broad spectrum of activity of this compound (8). A further line of argument against ribavirin being specifically antiviral is based on the hypothesis of Herrman and Herrman (7), which suggests that compounds may be classified as being specifically antiviral only if resistant mutants of the target virus can be produced. Thus, influenza virus mutants resistant to amantadine have been reported (3), whereas none has yet been reported in the case of ribavirin.

In conclusion, the results obtained in this study suggest that ribavirin exerts its antiviral effect on influenza A virus as a result of its inhibition of MDCK cell RNA synthesis rather than by specific inhibition of virus replication.

ACKNOWLEDGMENTS

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LITERATURE CITED