Competitive Inhibition of Uridine Incorporation by 6-Azauridine in Uninfected and Mengovirus-Infected Novikoff Hepatoma Cells[1] (34266)

MICHAL KORBECKI[2] AND PETER G. W. PLAGEMANN
(Introduced by Dennis W. Watson)
Department of Microbiology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

The replication of many RNA viruses requires the induction in the host cell of the formation of an RNA-dependent RNA polymerase (replicase) which is the enzyme responsible for the synthesis of viral RNA [see (1, 2)]. We have shown previously (2) that in mengovirus-infected Novikoff rat hepatoma cells viral RNA polymerase activity as measured in cell-free extracts becomes detectable about 2.5-hr after infection and that its appearance coincides with the beginning of the synthesis of the bulk of the viral RNA. Little is known about the biochemical processes occurring during this 2.5-hr lag period except that continuous protein synthesis is required between 1 and 2.5-hr after infection for RNA polymerase activity to appear at the normal time (3). In attempts to determine whether only the parental viral RNA serves as messenger for the synthesis of viral RNA polymerase or whether some early RNA synthesis is required for viral RNA polymerase formation, we examined the effect of 6-azauridine on the formation of viral RNA polymerase, viral RNA, and mature virus. Azauridine is a general inhibitor of cellular and viral RNA synthesis in both bacteria (4, 5) and mammalian cells (6–8) and consequently inhibits the growth of cells or the replication of viruses in them (4–10). RNA synthesis is inhibited by azauridine since the phosphorylated form of azauridine, azaUMP, is a competitive inhibitor of OMP decarboxylase and thus inhibits *de novo* synthesis of pyrimidine nucleotides (6, 11).

Preliminary experiments showed that azauridine has no effect on the formation of RNA polymerase in mengovirus-infected cells but also did not affect viral yield, in spite of the fact that it markedly inhibited the incorporation of labeled uridine into viral specific RNA and into mature virus. Although it is known that uridine or cytidine at high concentrations reverse the toxic effects of azauridine on cell growth (12–15), uridine or uracil in low concentrations have been employed in a number of studies to evaluate the effect of azauridine on RNA synthesis (6–8, 15). Wilson and his co-workers (7, 15) have shown that azauridine inhibits the incorporation of uridine into Newcastle disease virus RNA in chick embryo monolayer cultures and interpreted this finding to indicate an inhibition of viral RNA synthesis. In contrast, Pasternak and Handschuhmacher (6) reported that azauridine stimulates the incorporation of uridine by L5178Y lymphoma cells and suggested that this stimulation was due to an increased utilization of the scavenger pathway by cells in which *de novo* synthesis of pyrimidine nucleotides was blocked by treatment with azauridine.

The present investigation shows that azauridine inhibits uridine incorporation into both cellular and mengovirus RNA in Novikoff cells by interfering with incorporation of uridine into free nucleotides. It acts as a competitive inhibitor of both the entry of uridine into the cell and its subsequent phosphorylation to UMP. When host cell and viral RNA synthesis are measured by the

[1] This work was supported by Public Health Service research grant AI 07250. Michal Korbecki was recipient of a fellowship from the Ford Foundation.

[2] Permanent address: Department of Medical Microbiology, School of Medicine, Warsaw, Poland.

incorporation of adenosine, both processes are found to be highly resistant to inhibition by azauridine.

Materials and Methods. ^{3}H-5-uridine, ^{3}H-adenosine (generally labeled) were purchased from New England Nuclear Corp., Boston, Mass.; ^{3}H-6-azauridine from Schwarz BioResearch, Orangeburg, N.Y.; and 6-azauridine from Sigma Chemical Corp., St. Louis, Missouri.

Cell culture and virus infection. The cultivation of Novikoff rat hepatoma cells (line N1S1-67) in medium 67 in suspension culture and their infection with mengovirus were described previously (16–18). Cells were collected from cultures in the exponential phase of growth between 1.4 and 2.5 $\times$ 10^6 cells/ml (18). Infected and uninfected cells were suspended in basal medium 42 (BM42, 16) to about 2 $\times$ 10^6 cells/ml. Methods for the assay of virus-induced RNA polymerase (19) and of virus concentration by hemagglutination (HA) titration (16) have been described. Plaque assays were conducted on monolayer cultures of L-67-G cells as described elsewhere (Plagemann, in preparation).

Incorporation of ^{3}H-5-uridine, ^{3}H-adenosine, and ^{3}H-6-azauridine. Suspensions of cells were supplemented with radioactive precursors as indicated in the appropriate experiments. Duplicate samples of cell suspension were analyzed as follows: (i) for total radioactivity associated with cells. The samples were centrifuged at 500*g* for 2 min at 0°. The cells were rapidly washed with 5 ml of cold (0°) BSS (20) and suspended in 0.2 ml of 0.5 *N* trichloroacetic acid. The mixtures were heated at 70° for 30 min, transferred to bottles containing 15 ml of a scintillator–solvent mixture described previously (16) and the radioactivity was measured with a Packard liquid scintillation spectrometer. (ii) for radioactivity in acid-insoluble material. Other samples were quickly frozen in a bath of solid CO_2 in ethanol. Later the samples were thawed, mixed with perchloric acid at 0°, and the precipitates were washed repeatedly with perchloric acid and trichloroacetic acid as described previously (16, 18). The precipitate of each sample was suspended in 0.1 ml 0.5 *N* trichloroacetic acid and heated and analyzed for radioactivity as described under (i). The counting efficiency of ^{3}H was approximately 12% for all samples as estimated by the external standard method. Acid-extracts to be analyzed chromatographically were prepared from labeled cells as described previously (21). The incorporation of uridine into virus-specific single-stranded and double-stranded RNAs and into mature virus was measured as described previously (22).

Chromatography. Acid-extracts from labeled cells and uridine kinase reaction mixtures were analyzed by ascending chromatography on 3 MM Whatman paper (21). The paper was developed with a mixture of 1 *M* ammonium acetate (pH 5.0) and 95% ethanol (3:7; v/v) at 30° for 18 hr. Mixtures of appropriate standards were co-chromatographed and located after chromatography by examining the paper under ultraviolet light. Chromatograms of the experimental samples were cut into 1-cm segments at right angles to the direction of migration. Each segment was rocked with 1 ml of H_2O for 1 hr and then mixed with 15 ml of scintillation fluid and analyzed for radioactivity as described above. The counting efficiency was approximately 12% and the recovery of radioactivity from the paper was close to 100%.

Results. Effect of azauridine on uridine and adenosine incorporation by infected cell and on virus production. The data in Fig. 1B demonstrate that 24 m*M* 6-azauridine strongly inhibited the actinomycin-resistant incorporation of uridine by infected cells into acid-insoluble material. Results from additional studies showed that the incorporation of uridine into virus-specific single-stranded and double-stranded RNA and into virus were reduced to about the same extent by azauridine. However, this inhibition of incorporation of uridine into viral RNAs was probably a direct consequence of a failure of the azauridine-treated cells to convert uridine into nucleotides, since the data in Fig. 1A show that the incorporation of uridine into total cell material was inhibited to about the same extent as its incorporation into RNA.

TABLE I. Distribution of ^3H among Uridine Nucleotides in Acid-Extracts from Mengovirus-Infected Cells Labeled with ^3H-Uridine in the Presence of Actinomycin D and in the Presence and Absence of Azauridine.[a]

	UTP		UDP + UDP-G[b]		UMP	
Azauridine (m*M*)	(cpm)[c]	(%)[d]	(cpm)[c]	(%)[d]	(cpm)[c]	(%)[d]
0	2750	76	636	18	225	6
24	137	63	48	22	31	15

[a] The details of the experiment are described in the legend to Fig. 1. Cells were treated with azauridine at zero time of infection and with actinomycin D at 2.5 hr and labeled with ^3H-uridine from 3 to 3.5 hr after infection. Acid-extracts (0.4 ml) were prepared from 5×10^6 cells and analyzed chromatographically as described in "Materials and Methods."

[b] UDP-G refers to those components which migrate in chromatography and electrophoresis identical to authentic UDP-glucose (21). We have not attempted further identification.

[c] Total amount of radioactivity associated with the various nucleotides after chromatography of 25 μl of acid-extract.

[d] Percentage of total radioactivity associated with nucleotides.

This is also indicated by the results from chromatographic analyses of acid-soluble extracts prepared from these cells (Table I) which show that only small amounts of labeled nucleotides were present in infected cells that were labeled with uridine in the presence of azauridine. Results from previous experiments (21) have shown that actinomycin D has no effect on the incorporation of uridine into free nucleotides for at least several hours, and the data in Table I show that, as in uninfected cells (21), most of the uridine incorporated by infected cells was further phosphorylated to the triphosphate level.

Furthermore, when assayed at 8 hr after infection, the infectivity titers (about 1×10^8 plaque forming units/ml) and the hemagglutination titers (about 5120 HA units/ml) were about the same in azauridine-treated and untreated suspensions of infected cells. Results from other studies showed that azauridine had no effect on the induction of viral RNA polymerase. Similar results were obtained when cells were incubated in BM42 in the presence and absence of azauridine for 4 hr prior to infection, except that the viral yield was reduced about 30% in both suspensions.

The effect of azauridine on viral RNA synthesis was further investigated by labeling actinomycin-treated infected cells with ^3H-adenosine (Fig. 2). Uninfected cells treated with actinomycin D were similarly labeled to estimate the normal actinomycin-resistant incorporation of adenosine into nucleic acids. Actinomycin-treated uninfected cells incorpo-

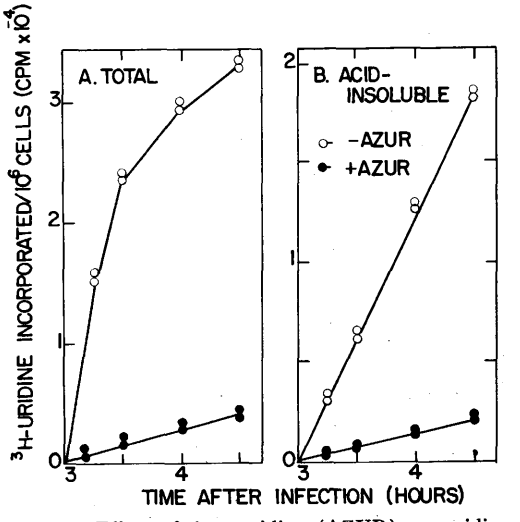

FIG. 1. Effect of 6-azauridine (AZUR) on uridine incorporation into total cell material (A) and into acid-insoluble material (B) by actinomycin-treated, mengovirus-infected cells. One half of a suspension of infected cells (2×10^6 cells/ml) in BM42 was supplemented with 24 m*M* azauridine at 0 time of infection, and the other half was not treated with azauridine. Actinomycin D was added to both suspensions to 2 μg/ml at 2.5 hr after infection and 0.037 μM ^{3}H-5-uridine (27,000 μCi/μmole) at 3 hr. At various times thereafter, 0.5-ml samples were analyzed for radioactivity in total cell material (A) or in acid-insoluble material (B) as described in "Materials and Methods."

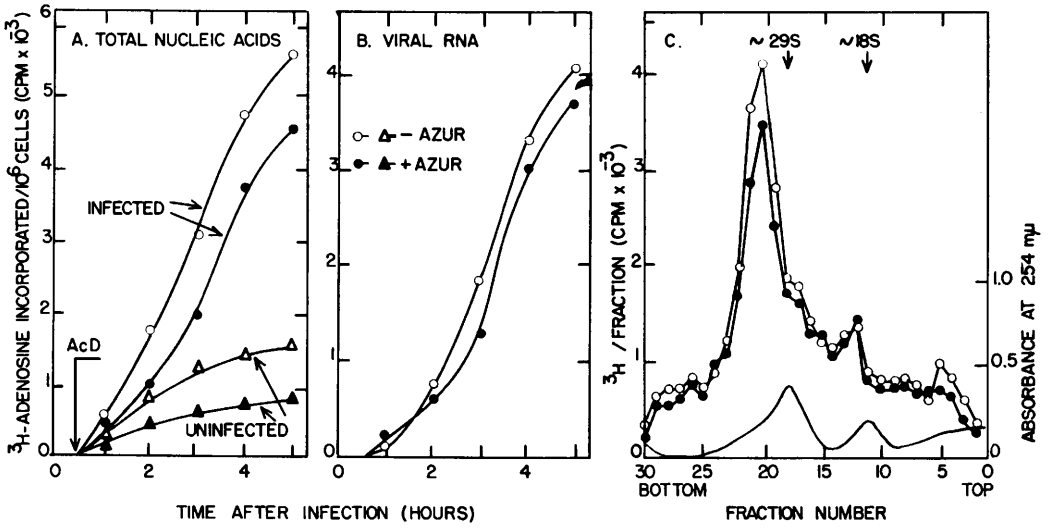

FIG. 2. Incorporation of ^{3}H-adenosine into nucleic acids by actinomycin-treated uninfected and infected cells in the presence and absence of azauridine (AZUR) and sedimentation analysis of RNA from infected cells. A portion of a suspension of mengovirus-infected cells (2×10^6 cells/ml) in BM42 was supplemented with 24 m*M* azauridine at the time of infection (0 time) and another portion remained without azauridine. Suspensions of uninfected cells were prepared and treated in an identical manner. At 0.5 hr, all suspensions were supplemented with 2 μg of actinomycin D/ml and 5 min later with 0.01 m*M* ^{3}H-adenosine (100 μCi/μmole). At various times thereafter, duplicate 0.5-ml samples were analyzed for radioactivity in acid-insoluble material as described in "Materials and Methods," and the average values of the duplicate samples are presented in (A). The amount of radioactivity in virus-specific RNA (B) was estimated by subtracting the values for azauridine-treated and untreated cells from the corresponding values for infected cells. At 5 hr after infection 2×10^7 cells were collected by centrifugation from each of the infected cultures, treated with sodium dodecyl sulfate and the deproteinized samples were analyzed by gradient centrifugation (C) as described previously (17). After centrifugation, the gradients were analyzed for absorbancy at 254 mμ (—) and 1-ml fractions were collected by means of an ISCO gradient fractionator which was attached to a continuously recording spectrophotometer. The fractions were analyzed for radioactivity in acid-insoluble material (○), (●).

rated only about 10% as much adenosine into acid-insoluble material as uninfected cells not treated with actinomycin D (compare Figs. 2A and 3A). The amount of adenosine incorporated into viral RNA (Fig. 2B) was estimated by subtracting the radioactivity in actinomycin-treated uninfected cells from that in virus-infected cells (Fig. 2A). The data in Fig. 2B clearly show that 24 m*M* azauridine had no significant effect on the incorporation of adenosine into viral RNA. This conclusion is substantiated by sedimentation analyses of the RNA isolated from labeled azauridine-treated and untreated infected cells (Fig. 2C). In both preparations most of the label was recovered in RNA with the sedimentation properties (37S) typical for mengovirus RNA (2). Smaller amounts of label sedimented at 18–20S and probably represented mainly viral double-stranded RNA (2).

The data in Fig. 2A also show that in contrast to the synthesis of viral RNA, the incorporation of adenosine by actinomycin-treated uninfected cells was inhibited about 40% by azauridine. In the following experiments the effect of azauridine on the incorporation of uridine and adenosine by uninfected cells was determined to further compare the effect of the drug on cellular and viral RNA synthesis and to obtain information with respect to the mechanism of resistance of Novikoff cells to azauridine.

Effect of azauridine on the incorporation of

TABLE II. Distribution of ^3H among Adenosine Nucleotides in Acid-Extracts from Uninfected Cells Labeled with ^3H-Adenosine in the Absence and Presence of Azauridine.[a]

Azauridine (m*M*)	ATP		ADP		AMP	
	(cpm)[b]	(%)[c]	(cpm)[b]	(%)[c]	(cpm)[b]	(%)[c]
0	6117	62	2573	26	1189	12
24	5710	58	2423	24	1663	18

[a] The details of the experiment are described in the legend to Fig. 3A. After 90 min of labeling with ^3H-adenosine, acid-extracts were prepared from 5×10^6 cells and analyzed chromatographically as described in "Materials and Methods."

[b] Total cpm associated with the various nucleotides after chromatography of 25 μl of acid-extract.

[c] Percentage of total radioactivity associated with nucleotides.

uridine and adenosine by uninfected cells. As indicated by the data in Fig. 3A, 24 m*M* azauridine had no effect on the incorporation of adenosine into total cell material, whereas the incorporation into acid-insoluble material was inhibited about 45%. The inhibition of the latter was not due to a failure of the cells to phosphorylate adenosine to the triphosphate level since about the same proportion of the total acid-soluble label in the cells (about 60%) was present in ATP whether the cells were labeled in the presence or absence of azauridine (Table II). Thus, the inhibitory effect was probably due to an inhibition of the *de novo* synthesis of pyrimidine nucleotides by azauridine.

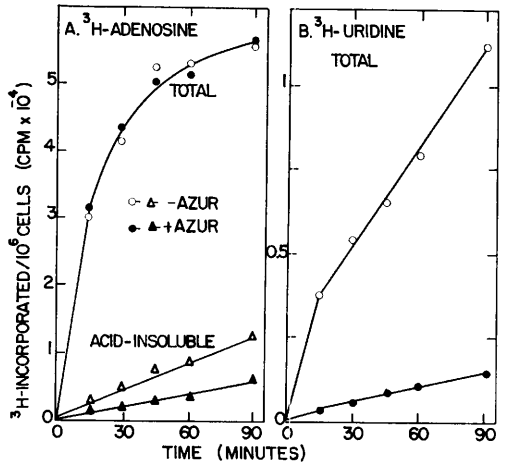

FIG. 3. Effect of azauridine (AZUR) on the incorporation of ^3H-adenosine (A) and ^3H-uridine (B) by uninfected cells. Portions of suspensions of uninfected cells in BM42 (2×10^6 cells/ml) were supplemented with 24 m*M* azauridine and other portions remained untreated. The ^3H-uridine (10 μCi/μmole) was added to 0.2 m*M* (0 time) immediately after the addition of azauridine (B). The ^3H-adenosine (100 μCi/μmole) was added to 0.01 m*M* (0 time) after 2 hr of preincubation with or without azauridine (A). At various times thereafter, duplicate 0.5-ml samples were analyzed for radioactivity in total cell material (○), (●); or in acid-insoluble material (△) (▲) as described in "Materials and Methods." All values are averages of the duplicate determinations.

In contrast to adenosine and similarly as in actinomycin-treated infected cells, the incorporation of uridine into total cell material by uninfected cells was markedly inhibited by azauridine (Fig. 3B), and the incorporation of uridine into acid-insoluble material was equally affected (not shown). Results from a previous study (Plagemann and Roth, submitted for publication) indicate that the entry of uridine into the cell is a reaction distinct from the uridine kinase reaction. This conclusion was based on the findings that uridine incorporation by whole cells is inhibited competitively by adenosine, persantin, and phenethyl alcohol, whereas the *in vitro* uridine kinase reaction is not affected by adenosine or persantin and is inhibited by phenethyl alcohol in a noncompetitive manner. It was also observed that the K_m for the incorporation of uridine by whole cells is over one order of magnitude lower than that for the phosphorylation of uridine by cell-free extracts (see Table III). Further, the finding that the V_m for the latter reaction is about 15 times greater than that for the incorporation of uridine by whole cells suggested that the rate of incorporation of uridine into the

TABLE III. Kinetic Constants for the Phosphorylation of Uridine by Whole Cells and Cell-Free Extract and Their Inhibition by Azauridine.[a]

Preparation	K_m[a] (M)	K_i[a] (M)	$\frac{K_m}{K_i}$
Whole cells	1.3×10^{-5}	8×10^{-4}	1.6×10^{-2}
Cell-free	2.1×10^{-4}	6×10^{-3}	3.5×10^{-2}

[a] The values were calculated from the slopes of the curves in Figs. 4 and 5, assuming simple competitive inhibition.

nucleotide pool by whole cells is limited by the rate with which uridine is taken up by the cells. It was concluded that the rate of incorporation of uridine by whole cells is an effective measure of the entry reaction. The data in Fig. 4 demonstrate that azauridine

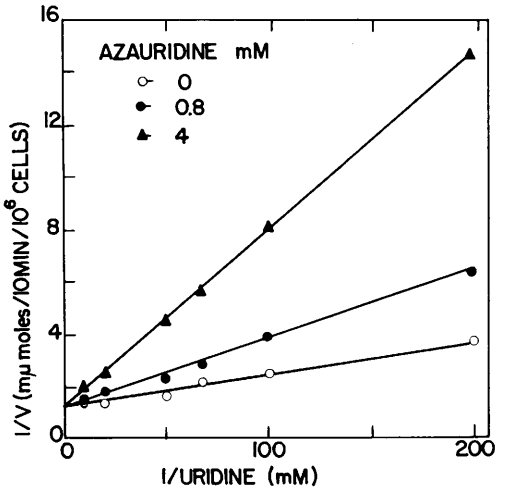

FIG. 4. Lineweaver-Burk plot of the incorporation of uridine into total cell material by whole cells in the absence and presence of various concentrations of azauridine. Portions of a suspension of 2×10^6 cells/ml of BM42 were mixed with azauridine to the indicated concentrations; and immediately thereafter, 10-ml samples were supplemented with 5, 10, 15, 20, or 50 μM ^{3}H-5-uridine (40 cpm/$\mu\mu$mole) or with 50, 100, or 200 μM ^{3}H-5-uridine (4 cpm/$\mu\mu$mole) and incubated on a gyrotory shaker at 37°. At 5 and 10 min of incubation, duplicate 1-ml samples were analyzed for radioactivity in total cell material as described in "Materials and Methods." The initial rates of incorporation were estimated from these values and taken as an estimate of the rates of incorporation of uridine into the acid-soluble pool since the free nucleotides are obligatory intermediates in the incorporation of uridine into RNA.

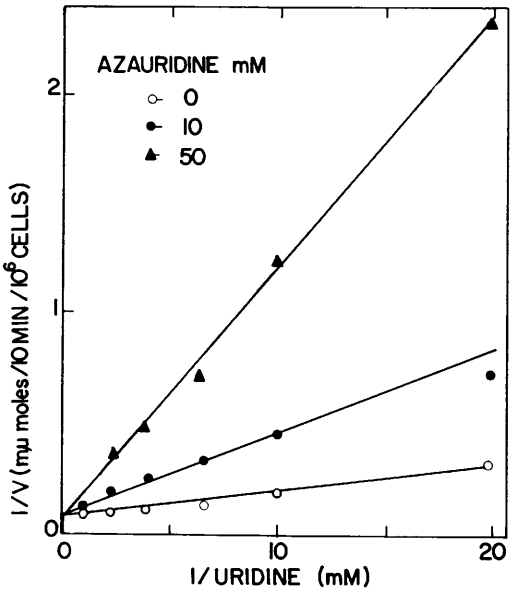

FIG. 5. Lineweaver-Burk plot of the phosphorylation of uridine by cell-free extract in the absence and presence of azauridine. A cell-free extract was prepared from 4×10^7 cells in 2 ml of B4 and assayed for uridine kinase activity as described previously (21). Samples of 0.2 ml of cell-free extract were mixed with equal volumes of a reaction solution containing: 20 mM ATP; 10 mM $MgCl_2$; 200 mM Tris–HCl (pH 8.2); 10 mM mercaptoethanol; 100 mM KCl; 50 μCi/ml of ^{3}H-5-uridine (27,000 μCi/μmole) and 50, 100, 150, 250, 400, and 1000 μM unlabeled uridine and in addition the indicated concentrations of azauridine. The reaction mixtures were incubated at 37° and at 2.5, 5, 10, 20, 30, and 45 min, 50-μl samples were transferred to tubes in a boiling water bath and heated for 1 min. The samples were rapidly cooled, clarified by centrifugation and 10-μl samples of each supernatant fluid were analyzed chromatographically. Reaction rates were estimated from the linear portions of plots of the amount of uridine converted into total uridine nucleotides as a function of time [see (21)].

acted as a competitive inhibitor of the incorporation of uridine by whole cells, probably by interfering with the entry of uridine into the cells. However, azauridine also inhibited in a competitive manner the phosphorylation of uridine by cell-free extracts (Fig. 5), as would be expected if azauridine is phosphorylated by the same kinase as uridine (12). Thus, azauridine inhibits uridine incorporation into RNA in N1S1-67 cells by interfer-

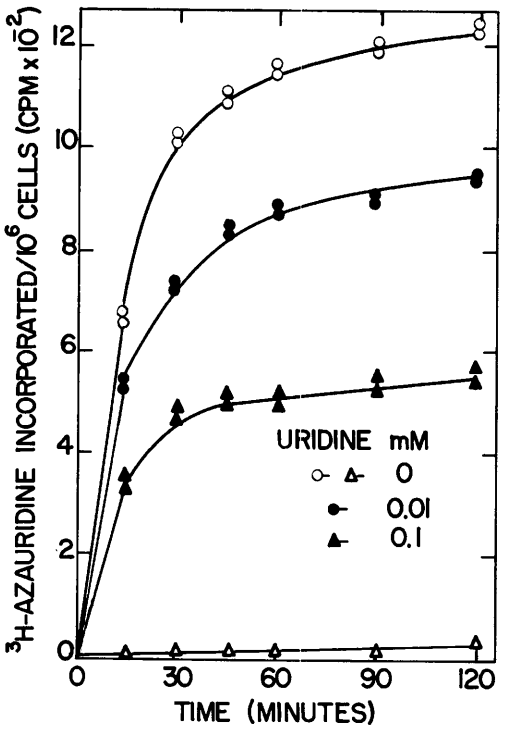

FIG. 6. Incorporation of ^{3}H-6-azauridine by uninfected cells. Samples of a suspension of 2 × 10^6 cells/ml of BM42 were supplemented with the indicated concentrations of unlabeled uridine (0 time) and immediately thereafter, with 1.3 μM ^{3}H-6-azauridine (330 cpm/$\mu\mu$mole). At various times thereafter, duplicate 0.5-ml samples were analyzed for radioactivity in total cell material (○), (●), (▲); and acid-insoluble material (△) as described in "Materials and Methods."

ing both with entry of uridine into the cells and its subsequent phosphorylation. The apparent K_m's for the incorporation of uridine by whole cells and for the *in vitro* uridine kinase reaction and the K_i's for the inhibition of these processes by azauridine are listed in Table III.

Incorporation of ^{3}H-azauridine by uninfected cells. The foregoing results showed that azauridine has no effect on viral RNA synthesis and, at 24 mM, inhibits cellular nucleic acid synthesis only about 40%. It is clear that this relative resistance of N1S1-67 cells to the action of azauridine was not due to a lack of uridine kinase [Fig. 5 (21)]. To obtain further information on the mechanism of this resistance, we determined the time course of the incorporation of ^{3}H-azauridine into the nucleotide pool and the nature of the labeled nucleotides. The data in Fig. 6 show that the incorporation of azauridine into total cell material ceased after about 60 min of incubation when only about 0.55% of the total azauridine had been incorporated or 2.7 $\mu\mu$moles/10^6 cells. In contrast, at 1 μM, the cells incorporate approximately 100 $\mu\mu$moles of uridine/10 min/10^6 cells (Plagemann, submitted for publication). As reported for other cell systems (6, 12), azauridine was not incorporated into nucleic acids (Fig. 6). Thus all the cell-associated label was present in acid-soluble material and chromatographic analysis of an acid-extract prepared after 120 min of labeling showed that about 93% of the total label was present in azaUMP, about 5% in azauridine and little, if any, in azaUDP, and azaUTP. The data in Fig. 6 also show that azauridine incorporation was inhibited by the presence of uridine in the medium.

Discussion. Although the principle objective of the study could not be realized because RNA synthesis in uninfected or mengovirus-infected Novikoff cells was found to be highly resistant to the inhibitory effects of azauridine, the results have some practical significance with respect to the general effects of 6-azauridine on the metabolism of cells and the mechanism of resistance of N1S1-67 cells to its action. The reason for this resistance has not been ascertained unequivocally, but it is probably related to the failure of the cells to accumulate significant amounts of azaUMP, the active form of azauridine (Fig. 6). This failure to accumulate azaUMP is certainly not due to a lack of uridine kinase in N1S1-67 cells [Fig. 5 (21)] as has been suggested as the mechanism of resistance of certain strains of *Escherichia coli* (23) or of L5178Y lymphoma cells (24). It seems more likely that the failure to accumulate azaUMP is the result of the induction of some regulatory control on the uptake or phosphorylation of azauridine, since the cells cease to incorporate ^{3}H-6-azauridine after 1 hr of incubation when less than 1% of the azauridine in the medium has been incorporated (Fig. 6). The results of Pasternak *et al.*

(24) also suggest that a mechanism other than loss of uridine kinase might be involved in establishing resistance of certain lines of cells to azauridine. These investigators reported that some lines of lymphoma cells were 1500 times more resistant to azauridine than the wild type line while they still phosphorylated uridine at 3–5% of the wild type rate. The phosphorylation of uridine by mammalian cells appears to be under feedback control by UTP (Plagemann, submitted for publication) and that of thymidine by dTTP (25, 26). A similar mechanism might be regulating the phosphorylation of azauridine, although we have been unable to detect the formation of azaUTP in N1S1-67 cells. Similarly, Pasternak and Handschuhmacher (6) reported that azaUMP is not further phosphorylated by L5178Y lymphoma cells, but it cannot be ruled out that the absence of azaUTP from acid-soluble extracts of azauridine-treated cells is not due to its degradation during preparation of the extracts. Higher phosphorylated derivatives of azauridine are formed in bacteria and indeed predominate (6).

The higher resistance of viral RNA synthesis to azauridine than cellular RNA synthesis cannot be explained satisfactorily at present. It may be related, however, to the fact that cellular RNA synthesis is largely confined to the nucleus whereas viral RNA synthesis occurs in the cytoplasm [see (2)], and that the cells possess two relatively independent nucleotide pools, only one of which serves as the direct precursor pool for cellular RNA synthesis in the nucleus (Plagemann, submitted for publication).

The results also indicate that azauridine inhibits the entry of uridine into the cells and its subsequent phosphorylation. Similarly, Skoda and Sorm (12) found that uridine inhibits the phosphorylation of azauridine by cell-free extracts of *E. coli*. It is not surprising that the inhibition is of the competitive type because of the close structural similarity between the two compounds. It seems likely that azauridine is taken up by the same cellular system as uridine and is phosphorylated by uridine kinase (12), but the low K_m/K_i values indicate that it is a less efficient substrate than uridine. These findings also indicate that the inhibition of uridine incorporation into RNA by azauridine is mainly a consequence of its inhibition of the uptake or phosphorylation of uridine by the cells, or of both. Thus under these conditions uridine incorporation cannot be employed as a measure of the synthesis of either viral or cellular RNA.

Summary. The incorporation of uridine into cellular RNA or viral RNA by uninfected and mengovirus-infected Novikoff rat hepatoma cells (line N1S1-67) is inhibited about 90% by 24 m*M* 6-azauridine in the medium. This inhibition is a direct consequence of an interference by azauridine with the conversion of uridine to free nucleotides and is not a reflection of an inhibition of RNA synthesis. Azauridine acts as competitive inhibitor of both the entry of uridine into the cell and its subsequent phosphorylation to UMP. Both, host cell and viral RNA synthesis are highly resistant to inhibition by azauridine as measured by the incorporation of adenosine, and the production of virus-induced RNA polymerase and mature virus are also not affected by 24 m*M* azauridine. The resistance of the cells to azauridine appears to be related to a failure of the cells to accumulate significant amounts of azaUMP, not because they lack uridine kinase but probably because azauridine uptake or phosphorylation is under some regulatory control.

The authors thank John Erbe for valuable technical assistance.

1. Spiegelman, S., Am. Scientist **55**, 3 (1967).

2. Plagemann, P. G. W. and Swim, H. E., Bacteriol. Rev. **30**, 288 (1968).

3. Plagemann, P. G. W., Virology **34**, 319 (1968).

4. Pardee, A. B. and Prestidge, L. S., Biochim. Biophys. Acta **37**, 544 (1960).

5. Lodish, H. F., Cooper, S., and Zinder, N. D., Virology **24**, 60 (1964).

6. Pasternak, C. A. and Handschuhmacher, R. E., J. Biol. Chem. **234**, 2992 (1959).

7. Wilson, D. E. and LoGerfo, P., J. Bacteriol **88**, 1550 (1964).

8. Rada, B. and Blaskovič, D., Acta Virol. **10**, 1 (1966).

9. Schäfer, W., Pister, L., and Schneider, R., Z. Naturforsch. **22b**, 1319 (1967).

10. Korbecki, M., Luczak, M., and Klimowicz, Z., Bull. Acad. Polon. Sci. **16**, 539 (1968).

11. Handschuhmacher, R. E., J. Biol. Chem. **235**, 2917 (1960).

12. Skoda, J. and Sorm, F., Collection Czech. Chem. Commun. **24**, 1331 (1959).

13. Schindler, R. and Welch, A. D., Science **125**, 548 (1957).

14. Elion, G. B., Bieber, S., Nathan, H., and Hitchings, G. H., Cancer Res. **18**, 802 (1958).

15. Bolognesi, D. P. and Wilson, D. E., J. Bacteriol. **91**, 1896 (1966).

16. Plagemann, P. G. W. and Swim, H. E., J. Bacteriol. **91**, 2317 (1966).

17. Plagemann, P. G. W., J. Virology **2**, 461 (1968).

18. Ward, G. A. and Plagemann, P. G. W., J. Cell. Physiol. **73**, 213 (1969).

19. Plagemann, P. G. W. and Swim, H. E., J Bacteriol. **91**, 2327 (1966).

20. Plagemann, P. G. W., Arch. Biochem. Biophys. **128**, 70 (1968).

21. Plagemann, P. G. W., Ward, G. A., Mahy, B. W. J., and Korbecki, M., J. Cell. Physiol. **73**, 233 (1969).

22. Plagemann, P. G. W., Virology **34**, 319 (1968).

23. Handschuhmacher, R. E., Biochim. Biophys. Acta **23**, 428 (1957).

24. Pasternak, C. A., Fischer, G. A., and Handschuhmacher, R. E., Cancer Res. **21**, 110 (1961).

25. Ives, D. H., Morse, P. A., and Potter, V. R., J. Biol. Chem. **238**, 1467 (1963).

26. Kornberg, A. and Okazaki, R., J. Biol. Chem. **239**, 275 (1964).

Received June 18, 1969. P.S.E.B.M., 1969, Vol. 132.