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ANTIGIARDIAL ACTIVITY OF GUANINE ARABINOSIDE

MECHANISM STUDIES

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Abstract—Guanine arabinoside (araG) inhibited the *in vitro* growth of *Giardia lamblia* WB with an ED_{50} value of 4 μM. The inhibition was prevented completely by 2′-deoxyguanosine, prevented partially by guanine and guanosine, and not prevented by adenine, adenosine or 2′-deoxyadenosine. Extracts of *G. lamblia* grown in the presence of [8-^{3}H]araG contained radiolabeled araGMP, araGDP and araGTP. The formation of araGTP during the exponential phase of cell growth increased with time and was dependent upon the araG concentration. AraG was incorporated into *G. lamblia* DNA in a time-dependent manner at a ratio of 1 araG for each 27 2′-deoxyguanosine residues. Short-term exposure of growing cultures to araG was inhibitory to DNA synthesis but not to RNA or protein synthesis. Over an extended period, synthesis of all three macromolecules was depressed. Attempts to measure araG phosphorylation by cell-free extracts of *G. lamblia* under a variety of nucleoside kinase and nucleoside phosphotransferase assay conditions were unsuccessful. In an attempt to understand further the action of araG, the metabolic pathways of guanine, guanosine and 2′-deoxyguanosine were delineated in detail. The presence of araG did not appear to cause any major alterations in the metabolism of these compounds; however, it was accompanied by a 3- to 4-fold increase in the endogenous pools of ATP and GTP.

Giardia lamblia is an aerotolerant anaerobic protozoan that infects man and a number of other mammals. Infection occurs via ingestion of the cyst from contaminated water supplies [1]. Although treatment of the infection with metronidazole or quinacrine is greater than 90% effective, both drugs have potentially serious side effects [2].

Since *G. lamblia* lacks *de novo* purine biosynthesis [3], it depends upon salvage of preformed purine bases to satisfy its growth requirement. Adenine and guanine are precursors for their respective nucleotides which cannot be interconverted. The parasite is also incapable of incorporating hypoxanthine into either adenine- or guanine-containing nucleotides [3, 4]. These findings suggest that *G. lamblia* requires a source of both adenine and guanine for growth.

Studies with intact cells and cell extracts indicate that the purine ribonucleosides, adenosine and guanosine, are cleaved to their free bases prior to incorporation into nucleotides. This incorporation is reported to proceed via a nucleoside hydrolase followed by a purine phosphoribosyltransferase (PRTase)† [3].

A study of the partially purified hydrolase suggests that it is a single enzyme that is capable of cleaving both adenosine and guanosine.‡ The PRT activity in this organism is attributable to separate enzymes for adenine and guanine, the latter having been purified to apparent homogeneity [5]. The *G. lamblia* guanine PRTase utilizes guanine much more efficiently than hypoxanthine. This substrate specificity contrasts with that of the mammalian enzyme which utilizes guanine and hypoxanthine with nearly equivalent efficiencies [6]. This difference in substrate specificity led to the suggestion that the guanine PRTase of this protozoan might serve as a potential target for chemotherapeutic agents.

Berens and Marr [4] have reported recently on the antigiardial activity of a number of adenosine analogs. The antigiardial activities of these compounds imply that, in addition to the PRT salvage pathway previously described [3], *G. lamblia* possesses a pathway for the direct phosphorylation of some purine nucleoside analogs. This pathway may represent an avenue for chemotherapeutic attack by purine-containing compounds. Herein, we report the *in vitro* antigiardial activity of guanine arabinoside (araG), its metabolism, and its effect on the metabolism of guanine and guanine nucleosides in this organism.

MATERIALS AND METHODS

Growth of the organism. Giardia lamblia WB (ATCC 30957) was obtained from the American Type Culture Collection, Rockville, MD, and grown in axenic culture using TYI-S-33 medium [7] supplemented with bile [8]. All cultures (13 ml) were maintained at 37° in 16 × 125 mm tubes capped with gas tight rubber septa. Under these conditions, the organism exhibited logarithmic growth up to a con-

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† Abbreviations: Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonate); Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate; araG, guanine arabinoside; PP-ribose-P, 5-phosphoribose 1-pyrophosphate; and PRT, phosphoribosyltransfer.

‡ R. L. Miller, unpublished.

centration of 0.9×10^6 organisms per ml of medium with a doubling time of 7–8 hr as previously reported [8]. Stationary phase of growth was attained at 1.5–2.0×10^6 organisms per ml.

Parasites were quantitated microscopically using a hemocytometer after cooling the culture tubes in an ice bath for 10 min to detach the organisms from the culture tube walls. To minimize gas exchange in the culture tubes, aliquots of 0.1 ml were removed by syringe. One milliliter of packed parasites represents approximately 3×10^9 organisms.

Growth inhibition and prevention of growth inhibition. A sterile aqueous solution of araG was added through the septum to logarithmically growing triplicate cultures at a parasite density of $1–2 \times 10^4$ organisms per ml. After growth periods of 24 and 48 hr, the parasites were counted. For reversal of growth inhibition studies, the cultures were made 100 μM with respect to the potential growth inhibition reversal agent prior to the addition of the araG.

Incorporation of precursors into DNA, RNA or protein. [^{3}H-*Methyl*]2′-deoxythymidine, [6-^{3}H]-uridine, or [4,5-^{3}H]isoleucine were added to logarithmically growing cultures ($1–2 \times 10^4$ organisms per ml) in the presence or absence of various concentrations of araG and incubated for 30 min at 37°. After the parasites had been quantitated and collected by centrifugation as described above, 0.1 mg calf thymus DNA, 0.1 mg yeast RNA, or 0.1 mg bovine serum albumin was added, and the sedimented parasites were extracted with 2 ml of cold 0.5 N perchloric acid. The resulting suspension was incubated for 30 min in an ice bath, and the precipitate was collected on a glass-fiber filter. The precipitate was washed three times with 5 ml of cold 0.5 N perchloric acid and then twice with 5 ml of cold 95% ethanol. Radioactivity on the dried filters was quantitated by liquid scintillation in 15 ml of Scintilene (Fisher Scientific Co.).

Phosphorylation of araG and 9-deazaadenosine by extracts. Extracts of *G. lamblia* were prepared at 4° as follows: 0.5 ml of packed cells was suspended in 1.5 ml of 20 mM sodium Hepes, pH 7.7, containing 2 mM $MgCl_2$ and 2 mM dithiothreitol and stored at −70° until needed. Parasites were thawed, diluted with 3 ml of 50 mM sodium Pipes, pH 6.8, containing 5 mM $MgCl_2$ and 5 mM dithiothreitol and sonicated at maximum power for two 15-sec cycles using a Heat Systems Sonifier equipped with a microtip. The resulting suspension was centrifuged at 100,000 *g* for 60 min. The supernatant fraction (4.1 ml) was dialyzed for 2 hr against 1 liter of 25 mM sodium Pipes, pH 6.8, containing 2 mM $MgCl_2$ and 2 mM dithiothreitol (buffer A) and then again against 1 liter of buffer A for an additional 14 hr. The pellet from the above centrifugation was resuspended in 3 ml of buffer A.

Phosphotransferase reaction mixtures (50 μl) contained 50 mM potassium Pipes, pH 6.8, or 100 mM sodium acetate, pH 5.4; 0.1 mM [8-^{14}C]araG (18 Ci/mol) or 0.1 mM [G-^{3}H]9-deazaadenosine (2 Ci/mmol); 1 mM $MgCl_2$; 15 mM each of the phosphate donors, AMP, dTMP and *p*-nitrophenylphosphate; and either undialyzed/uncentrifuged sonicated extract, dialyzed high-speed supernatant, or the resuspended high speed centrifugation pellet. Reaction mixtures for nucleoside kinase were similar to those for the phosphotransferase measurements at pH 6.8 except that 5 mM ATP replaced the phosphate donors and each reaction contained an ATP-regenerating system consisting of 5 units of pyruvate kinase and 5 mM phosphoenolpyruvate. After preincubating the reaction mixtures for 3 min at 30°, reactions were initiated by the addition of one of the extract fractions. Aliquots (10 μl) were withdrawn at 5, 20 and 60 min and applied to a PEI-cellulose thin-layer chromatography plate that had been prespotted with 20 nmol each of appropriate carriers. The chromatographic plates were developed in water, dried, and scanned for radioactivity using a Berthold thin-layer chromatography radioactive plate scanner. In this system, the nucleotide product stayed at the origin and the nucleoside substrate travelled approximately one-half way up the plate.

Nucleoside metabolism studies. Logarithmically growing cultures, at a density of $1–2 \times 10^4$ organisms per ml, were incubated with 0.5 μM radiolabeled guanine, guanosine, 2′-deoxyguanosine or the appropriate concentration of araG. After quantitation, the parasites were separated from the culture medium by centrifugration at 2800 *g* for 10 min and immediately extracted with 2 ml of cold 0.5 N perchloric acid. After neutralization of the supernatant fraction with 3 M KOH, the ribonucleotides were analyzed by anion-exchange HPLC as previously described [9]. For deoxyribonucleotide analysis, extracts were treated with periodate and methylamine to remove ribonucleotides [10], acidified to pH 4 to stabilize dGTP, and separated using a linear gradient from 0.3 to 0.8 M potassium phosphate buffer developed over 30 min.

Materials. [8-^{3}H]AraG (0.67 Ci/mmol), [G-^{3}H]9-deazaadenosine (16 Ci/mmol), [8-^{3}H]guanosine (11 Ci/mmol), [2′-^{3}H]2′-deoxyguanosine (18 Ci/mmol) and [8-^{3}H]2′-deoxyguanosine (16 Ci/mmol, further purified by reverse-phase HPLC) were purchased from Moravek Biochemicals, Brea, CA; [6-^{3}H]uridine (20 Ci/mmol), [^{3}H-*methyl*] thymidine (20 Ci/mmol) and [4,5-^{3}H]isoleucine (105 Ci/mmol) were purchased from New England Nuclear, Boston, MA; [8-^{3}H]guanine (6 Ci/mol), [5′-^{3}H]guanosine (22 Ci/mol; further purified by reverse-phase HPLC) and [5′-^{3}H]2′-deoxythymidine (14.6 Ci/mmol) were purchased from Amersham, Arlington Heights, IL. After purification as noted above, all radiochemicals were >99% radiochemically pure as judged by reverse-phase HPLC. 9-Deazaadenosine was a gift from D. R. S. Klein, Sloan–Kettering Institute, New York, NY. Rabbit muscle pyruvate kinase and phosphoenolpyruvate were purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN; yeast extract and casein digest peptone were from BBL Microbiology Systems, Cockeysville, MD; L-ascorbic acid, bovine bile, L-cysteine, ATP, AMP, dTMP, *p*-nitrophenylphosphate, Pipes, Hepes, dithiothreitol, calf thymus DNA, yeast RNA, bovine serum albumin, and calf intestinal alkaline phosphatase were from the Sigma Chemical Co., St. Louis, MO. *Escherichia coli* 2′-deoxythymidine phosphorylase (4000 mU/ml) was purified in these laboratories by W. Hall [11]. AraG and [8-^{14}C]araG (50.5 Ci/mol)

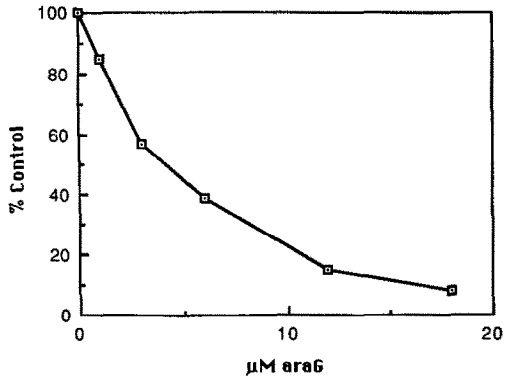

Fig. 1. Effect of the concentration of araG on the growth of *G. lamblia*. Parasites were grown in triplicate in 13 ml of TYI-S-33 medium (see Materials and Methods). Cultures at 2×10^4 parasites/ml were grown in the presence and absence of the appropriate amount of araG. After 48 hr at 37°, parasites were quantitated by hemocytometer (see Materials and Methods). At this point, control cultures contained 9×10^5 parasites/ml. Points represent the average of the triplicate samples. In no case was there greater than 10% difference in the number of parasites among the triplicate samples.

were synthesized in these laboratories by Dr. G. W. Koszalka as previously described [12]. 9-DeazaAMP was synthesized by the methods previously described for acycloGMP [13]. GF/A glass-fiber filters were purchased from Whatman, Clifton, NJ. All other materials were of analytical reagent grade.

RESULTS AND DISCUSSION

Growth inhibition by araG and its prevention by purines and purine nucleosides. AraG exhibited an apparent inhibition of growth of *G. lamblia* WB with an EC_{50} value of 4 μM (Fig. 1). This apparent growth inhibition appeared to be reversible, at least in the short term, since parasites cultured in the presence of 10 μM araG for 48 hr and then subcultured into fresh medium lacking araG attained a normal growth rate within 12–24 hr. However, parasites cultured for 96 hr in 10 μM araG or for 48 hr in 100 μM araG did not grow when transferred to fresh, non-araG-containing medium. In addition, the total number of parasites was noted to decrease in the presence of 100 μM araG over the 48-hr time period. These results indicate that araG had a pronounced cidal effect. The apparent growth inhibition caused by araG was prevented completely by 2′-deoxyguanosine, was prevented partially by guanine and guanosine, and was unaffected by adenine-containing compounds (Table 1). Adenine, adenosine and 2′-deoxyadenosine caused an apparent growth stimulation, suggesting a possible shortage of adenine-containing compounds in the TYI-S-33 medium used for the growth of these organisms. At present, no completely defined media exist for the axenic culture of *G. lamblia*. The medium of choice is TYI-S-33 [7] as modified by Keister [8]. This medium, which contains yeast extract, provides purine for the organism. However, even in the presence of exogenous purines, araG produced an EC_{50} value in the micromolar range. Considering the high level of purine nucleoside hydrolase activity present in these organisms [3], it would be expected that purine bases and not ribonucleosides would predominate in the medium. Since the present study indicates that araG was phosphorylated without going through a purine intermediate (see below), prevention of the growth inhibition caused by araG would be expected by other nucleosides which serve as poor substrates for the hydrolase (i.e. 2′-deoxyguanosine), thus possibly explaining the low EC_{50} values observed in the presence of the exogenous purines. The partial prevention of growth inhibition observed in the presence of guanine and guanosine (Table 1) may be indicative of an additional mode of action of araG which is not immediately evident from the present study.

Table 1. Prevention of araG growth inhibition of *G. lamblia*

Agent (100 μM)	% of Control growth rate	
	No araG	+10 μM araG*
None	100	20
Guanine	105	60
Guanosine	110	55
2′-Deoxyguanosine	100	120
Adenine	150	30
Adenosine	140	30
2′-Deoxyadenosine	130	30

* Values in the presence of araG are relative to the corresponding cultures in the absence of araG. All measurements were made in triplicate on logarithmically growing cultures at 48 hr of growth (see Materials and Methods). Control cultures had a doubling time of 7.8 hr.

Stability of the glycosidic bond of araG. Logarithmically growing cultures of *G. lamblia* exposed to [8-^{3}H]araG exhibited no detectable radioactivity in endogenous purine ribonucleotide pools (Table 2). Since exogenous [8-^{3}H]guanine was readily incorporated into GTP (see below), radioactivity would have been found in the GTP pool if the arabinosyl bond of araG had been cleaved. These data indicate that the arabinosyl bond of araG was stable throughout the 48-hr incubation period. In addition, analysis of the culture medium by reverse-phase HPLC revealed no [8-^{3}H]guanine. Only the starting material, [^{3}H]araG, was found, thus confirming the observation that [8-^{3}H]araG was not cleaved by the parasite or the medium. The apparent stability of araG in the giardial cultures contrasts to that in the mouse where considerable hydrolysis, deamination and oxidation produced xanthine and uric acid [14].

Formation of araGTP from araG. Parasites grown in the presence of [8-^{3}H]araG exhibited three radiolabeled species eluting in the mono-, di-, and triphosphate regions of the HPLC chromatogram in addition to a large radioactive component of unmetabolized araG (Fig. 2). These metabolites of araG eluted with retention times identical to those of authentic araGMP, araGDP and araGTP respectively. Alkaline phosphatase treatment of the putative "araGTP" yielded only [^{3}H]araG upon analysis by reverse-phase HPLC; no guanosine or 2′-deoxyguanosine was detected. Subsequent treatment of

Table 2. Metabolites of guanine, guanosine and 2′-deoxyguanosine in *G. lamblia*; effect of araG*

Isotope	Radiolabeled pools				Unlabeled pools	
	ATP	dATP	GTP	dGTP	ATP	GTP
	(pmol/10^6 cells)				(pmol/10^6 cells)	
			(No araG)			
None	—	—	—	—	340	89
[8-^{3}H]Gua	<0·001	<0.001	0.056	<0.001	430	100
[8-^{3}H]Guo	<0.001	<0.001	0.680	<0.001	410	120
[5′-^{3}H]Guo	0.060	<0.001	0.020	<0.001	370	85
[8-^{3}H]dGuo	<0.001	<0.001	0.180	0.039	370	91
[5′-^{3}H]dGuo	<0.001	<0.001	<0.001	0.042	390	89
			(+10 μM araG)			
[8-^{3}H]araG†	<0.002	<0.002	<0.002	<0.002	1620	400
[8-^{3}H]Gua	<0.004	<0.004	0.098	<0.004	1640	380
[8-^{3}H]Guo	<0.003	<0.003	0.500	<0.003	1800	410
[5′-^{3}H]Guo	0.049	<0.002	0.018	<0.002	1490	370
[8-^{3}H]dGuo	<0.002	<0.002	0.270	0.094	1690	470
[5′-^{3}H]dGuo	<0.002	<0.002	<0.002	0.089	1370	280

* All values are the average of duplicate samples. In no case did the values vary by more than 15%.

† [8-^{3}H]AraG, 10 μM, replaced the non-radiolabeled araG. Radiolabeled concentrations of araGTP and araATP were 20 and <0.1 pmol per 10^6 parasites respectively.

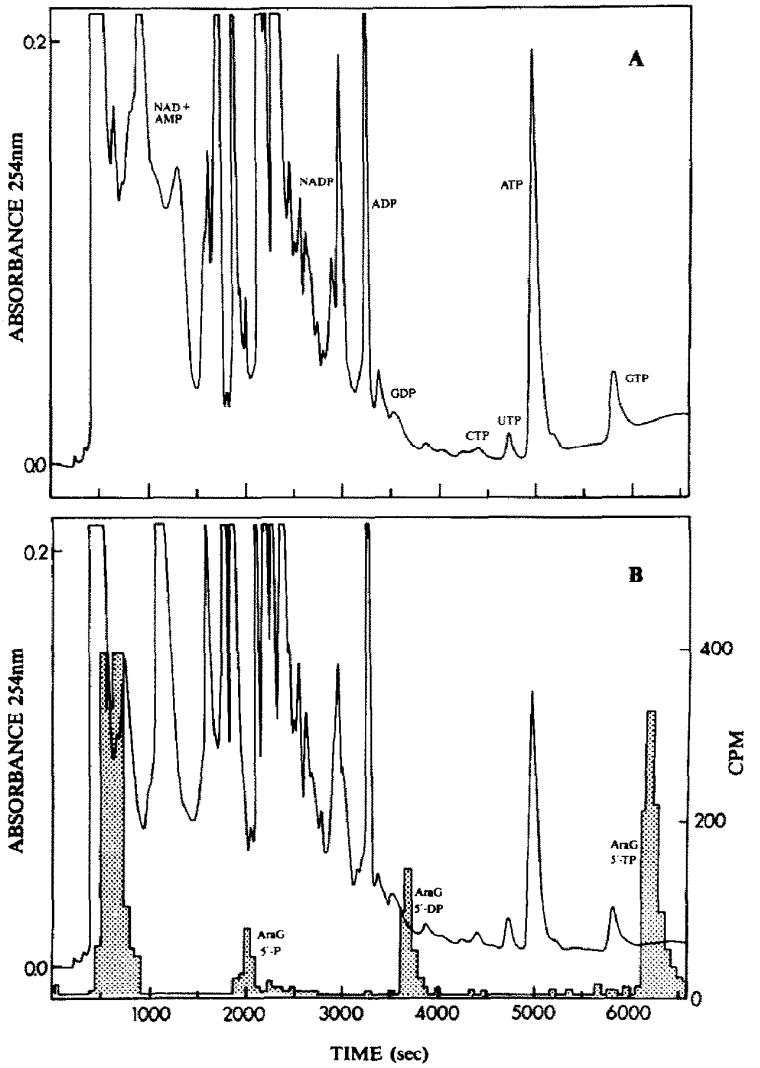

Fig. 2. HPLC separation of nucleotides in perchloric acid extracts of *G. lamblia*. The samples represent 1.4 × 10^6 parasites. Separation was accomplished on a Partisil SAX column with a linear gradient elution of KH_2PO_4, pH 3.5, from 0.015 to 1.0 M at a flow rate of 1.0 ml/min. Log phase cells were incubated for 48 hr in the absence (A) and presence (B) of 10 μM [8-^{3}H]araG.

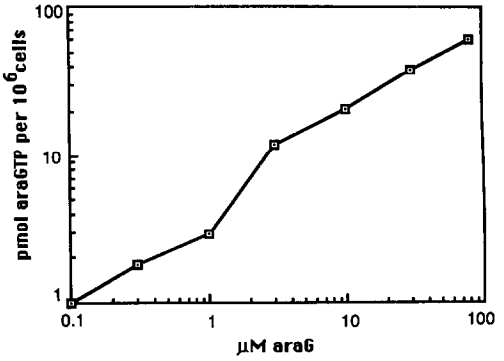

Fig. 3. Formation of araGTP as a function of araG concentration. Logarithmically growing *G. lamblia* at 2×10^4 parasites/ml were inoculated with araG at the appropriate concentrations. After 48 hr, the parasites were quantitated and extracted with perchloric acid. AraGTP was quantitated by HPLC analysis, and measurement of the area under the eluted peak was compared to that of standards.

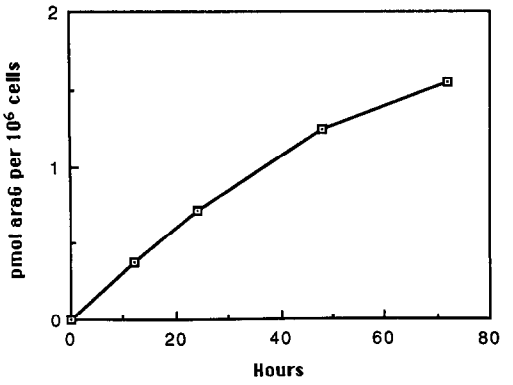

Fig. 5. AraG incorporation into *G. lamblia* DNA as a function of time. Cultures at 2×10^4 parasites/ml were inoculated with 10 μM [8-^{3}H]araG and incubated at 37°. At the appropriate times, parasites were extracted with perchloric acid, and the radioactivity in the acid-insoluble fraction was quantitated.

the material recovered from reverse-phase HPLC with purine nucleoside phosphorylase, an enzyme known to poorly catalyze the phosphorolysis of araG [15], failed to yield guanine under conditions where guanosine and 2′-deoxyguanosine were cleaved completely. These data verify that the ^{3}H-labeled metabolite of araG, eluting in the nucleoside triphosphate region of the HPLC chromatogram, was araGTP.

Formation of araGTP by logarithmically growing parasite cultures was proportional to araG concentration (Fig. 3) and to time up to 48 hr (Fig. 4). When parasites were grown in medium containing araG for 48 hr and then resuspended in fresh, non-araG-containing medium, the araGTP pool decreased by 90% within 2 hr (data not shown). This finding suggests a short half-life for araGTP in the absence of araG.

Radiolabel was not transferred from araG into araATP, indicating that interconversion of guanine to adenine arabinonucleotides did not occur (Table 2). The lack of incorporation of the guanine-containing compounds into adenine nucleotides indicated that there was also no interconversion of guanine and adenine ribonucleotides. This observation is in agreement with those previously reported in *G. lamblia* [3, 4].

Incorporation of araG into DNA. Incorporation of radiolabel from araG into the acid-insoluble fraction was time dependent (Fig. 5). The radioactivity in this fraction, after repeated ethanol extraction, was not solubilized by KOH. In a parallel study, the radiolabeled acid-insoluble material derived from parasites grown in the presence of [^{3}H]uridine was solubilized by the KOH treatment. These findings are consistent with incorporation of araG into DNA and not RNA. The quantitative measurement of

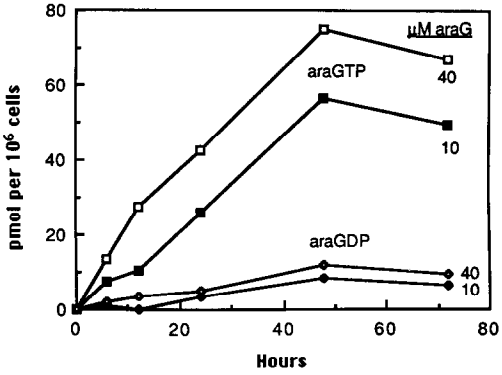

Fig. 4. Formation of araGDP and araGTP by *G. lamblia* cultures as a function of time at 10 and 40 μM araG. Cultures at a concentration of 2×10^4 parasites/ml were inoculated with the appropriate amount of araG and incubated at 37°. At the appropriate times, parasites were extracted with perchloric acid, and the nucleotide pool sizes were determined by HPLC.

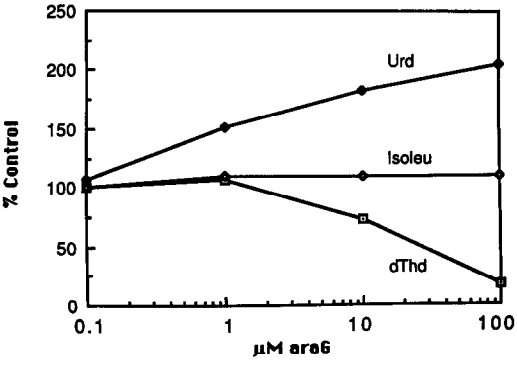

Fig. 6. Effects of araG on DNA, RNA and protein synthesis. Logarithmically growing *G. lamblia* at 6×10^5 parasites/ml were inoculated with the appropriate amount of araG and 0.5 μM [^{3}H]uridine, [^{3}H]thymidine or [^{3}H]isoleucine. Cultures were incubated at 37° for 30 min. Parasites were extracted with perchloric acid, and the radioactivity in the acid-insoluble fraction was quantitated. Thymidine, uridine and isoleucine incorporation values in the absence of araG were (pmol): 0.84, 0.63 and 0.05 respectively.

araG incorporation indicated that, for each 27 residues of 2′-deoxyguanosine in DNA, 1 araG was incorporated. These calculations are based on a DNA content of 3.1×10^7 base pairs per giardial cell and giardial DNA having a 42% GC content [16].

Effect of araG on protein, DNA and RNA synthesis. Parasite-mediated incorporation of 0.1 μM [^{3}H-*methyl*]2′-deoxythymidine, [6-^{3}H]uridine and [4,5-^{3}H]isoleucine was linear for 2, 4 and 4 hr respectively. Parasites grown in the presence of 10 μM araG for 48 hr exhibited a greater than 80% decrease in incorporation of a 2-hr pulse of 2′-deoxythymidine, uridine or isoleucine into the acid-insoluble fraction. In contrast, when araG and the labeled precursors were added simultaneously and incorporation was allowed to proceed for only 30 min, araG inhibited DNA synthesis but not RNA synthesis or protein synthesis (Fig. 6). The explanation for the apparent increase in the synthesis of RNA which was observed in these experiments is not immediately obvious. However, it may be related to the increase in the ribonucleotide pools observed in parasites cultured in the presence of araG (Table 2).

Measurement of araG phosphorylation in extracts. In an attempt to identify the enzyme(s) involved in the phosphorylation of araG, studies were conducted using extracts of *G. lamblia*. 9-Deazaadenosine, a compound known to be phosphorylated by extracts of *G. lamblia* [4], was included as a control. In nucleoside kinase type reaction mixtures with ATP serving as the phosphate donor, no phosphorylation of araG or 9-deazaadenosine was observed with any of the extract fractions, i.e. uncentrifuged/undialyzed suspension, centrifuged/undialyzed supernatant, dialyzed high-speed supernatant or resuspended high-speed centrifugation pellet. This finding is in agreement with that previously reported for 9-deazaadenosine and extracts of this parasite [4]. In reaction mixtures in which ATP was replaced with a mixture of AMP, dTMP and *p*-nitrophenylphosphate, all of which are known to act as phosphate donors for nucleoside phosphotransferases [17, 18], no detectable phosphorylation of araG (<1 nmol/hr/mg protein) was observed regardless of the extract fraction used at either pH 6.8 or at pH 5.4, a pH which has been reported to be optimal for the mammalian and bacterial phosphotransferases [18, 19].* In contrast, the phosphorylation of 9-deazaadenosine was easily measured at pH 6.8. The majority of the 9-deazaadenosine phosphotransferase activity was found in the high-speed pellet fraction and had a specific activity of 13 nmol/hr/mg protein, a value in close agreement with the value of 16 nmol/hr/mg protein that has been reported previously [4]. At pH 5.4, no phosphorylation of 9-deazaadenosine was detected (<1 nmol/hr/mg protein), suggesting that the giardial nucleoside phosphotransferase differs from the bacterial and mammalian enzymes in its pH/activity profile. In an attempt to block the phosphorylation of 9-deazaadenosine with araG, it was found that the rate of 9-deazaadenosine phosphorylation was unaffected by a 7-fold concentration excess of araG. This observation suggests that these compounds are phosphorylated via different pathways. The outcome of these studies was that we were unable to determine which enzyme catalyzes the phosphorylation of araG. Even though the phosphorylation of araG is easily measured in intact giardial parasites, no phosphorylation was detected once the parasites were broken.†

Metabolites of guanine, guanosine and 2′-deoxyguanosine. In light of the growth inhibitory properties of araG, the metabolic fate of guanine-containing compounds within *G. lamblia* was delineated in detail. Studies on the incorportion of guanine, guanosine and 2′-deoxyguanosine into the nucleotide pools in the presence and absence of araG were conducted. The results of these studies are presented in Table 2. The use of nucleosides radiolabeled in either the purine or sugar moiety enabled the metabolic fate of both the heterocycle and pentose of guanosine and 2′-deoxyguanosine to be investigated. A summary of the pathways involved is presented in Fig. 7. From these studies, several observations were made. First, compounds labeled with tritium in the 8-position of the guanine ring were all incorporated into GTP. This observation is consistent with each nucleoside being at least partially cleaved to guanine and the [8-^{3}H]guanine being incorporated into the ribonucleotide pool. There was no evidence for conversion of guanine ribonucleotides to deoxyribonucleotides, i.e. no radioactivity appeared in dGTP in the experiments with [8-^{3}H]guanine or [8-^{3}H]guanosine, suggesting the possible absence of

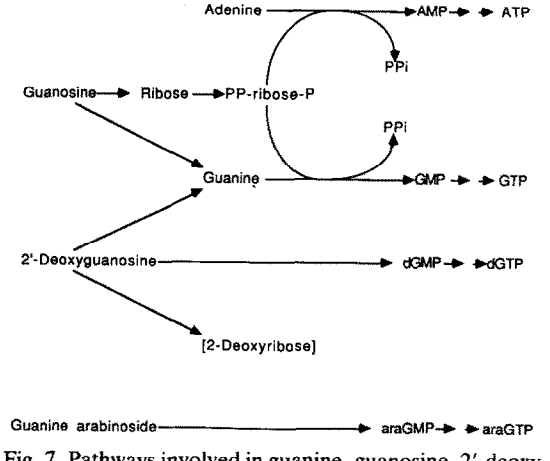

Fig. 7. Pathways involved in guanine, guanosine, 2′-deoxyguanosine and guanine arabinosine metabolism in *Giardia lamblia*.

* Under these conditions, using the high-speed supernatant fraction, the kinase reactions with guanosine and 2-deoxyguanosine exhibited phosphorylation rates of 5 and 0.08 nmol/hr/mg and the phosphotransferase reactions exhibited rates of >200 and 6 nmol/hr/mg respectively.

† In control experiments, when araGMP was incubated in the presence of the dialyzed extract, no dephosphorylation to araG was observed (<1 nmol/hr/mg). However, when ATP was added to these reaction mixtures, araGTP was formed at a rate of 84 nmol/hr/mg, with only a minor amount of araGDP being detected. These results indicate that rapid cleavage of araGMP was not the reason for being unable to detect araG phosphorylation and that araG phosphorylation must be the rate-limiting step.

ribonucleotide reductase in this organism. Second, 2′-deoxyguanosine labeled in the 8-position of guanine entered both GTP and dGTP. This observation suggests that some of the 2′-deoxyguanosine was cleaved to guanine and some was directly phosphorylated. Third, guanosine labeled in the 5′-position of the ribosyl moiety was incorporated into GTP and ATP. Radioactivity was also observed in CTP and UTP (data not shown). These data suggest that the [5-^{3}H]ribose released by the cleavage of [5′-^{3}H]guanosine entered the pentose pool of the parasite and was used in the synthesis of purine and pyrimidine ribonucleotides, probably via PP-ribose-P. Fourth, radioactivity from 2′-deoxyguanosine labeled in the 5′position of the deoxyribosyl moiety was only incorporated into dGTP, indicating that 2′-deoxyguanosine could be directly phosphorylated and that any 2-deoxyribose liberated from the hydrolysis of 2′-deoxyguanosine was not reutilized in the salvage of purines within this parasite.* Fifth, although araG did not cause major alterations in the metabolic fate of these compounds, it did cause a significant increase of incorporation of [8-^{3}H]guanine into GTP and either [8-^{3}H] or [5′-^{3}H]2′-deoxyguanosine into dGTP. It also caused a time-dependent† 3- to 4-fold increase in the endogenous (unlabeled) ATP and GTP pool sizes. The mechanism by which araG caused ATP and GTP pools to increase is not known at the present time. A negligible effect of araG on the incorporation of [8-^{3}H]guanosine into GTP, [5′-^{3}H]guanosine into ATP or GTP, or [8-^{3}H]2′-deoxyguanosine into GTP was observed. The recent report that adenosine analogs [4] and a guanosine analog (reported here) possess antigiardial activity has revealed a route to potential chemotherapeutic attack on this parasite. This type of chemotherapeutic approach, use of nucleoside analogs, has been reported in *Leishmania donovani* for allopurinol 1-ribonucleoside [20]. In *Leishmania*, allopurinol 1-ribonucleoside, once phosphorylated, was aminated via the adenylosuccinate synthetase/adenylosuccinate lyase system [21], converted to the ATP analog, and incorporated into RNA [22]. Unlike *L. donovani*, *Giardia* is incapable of utilizing hypoxanthine [3]. Therefore, utilization of the nucleosides as potential antigardial agents will most likely be limited to analogs of adenosine and guanosine which are poor substrates for the giardial nucleoside hydrolase.

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* In a separate experiment with [5′-^{3}H]2′-deoxythymidine, radioactivity only entered the dTTP pool. Generation of [5-^{3}H]2-deoxyribose by pretreatment of the [5′-^{3}H]2′-deoxythymidine with *E. coli* 2′-deoxythymidine phosphorylase and calf intestinal alkaline phosphatase, prior to addition to the parasite cultures, resulted in no radioactivity being detected in any nucleotide as analyzed by HPLC analysis. This finding further supports the observation that, once formed, 2-deoxyribose does not appear to be reutilized in purine or pyrimidine salvage.

† Logarithmically growing cells exposed to 10 μM araG for 0, 24 and 48 hr exhibited increasing concentrations of ATP and GTP (pmol/10^6 parasites) of 420 and 110; 850 and 180; 1620 and 410 respectively. Parasites grown in the absence of araG exhibited no such change with time or cell density.

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