Biochemical Pharmacology, Vol. 23, pp. 649 660. Pergamon Press, 1974. Printed in Great Britain.

PATHWAYS OF NUCLEOTIDE METABOLISM IN *SCHISTOSOMA MANSONI*—V

ADENOSINE CLEAVAGE ENZYME AND EFFECTS OF PURINE ANALOGUES ON ADENOSINE METABOLISM *IN VITRO**

GERALD W. CRABTREE and ALFRED W. SENFT
Division of Biological and Medical Sciences, Brown University, Providence, R.I. 02912, U.S.A.

(*Received* 24 *May* 1973; *accepted* 13 *July* 1973)

Abstract—*Schistosoma mansoni* extracts have been found to possess an active anabolic pathway for nucleotide biosynthesis in which adenosine is cleaved to adenine followed by conversion of adenine to AMP via adenine phosphoribosyltransferase. A significant fraction of labeled adenosine was found to enter the nucleotide pool by this pathway; however, most of the nucleoside was converted to nucleotides by a pathway which employs adenosine deaminase, purine nucleoside phosphorylase and hypoxanthine phosphoribosyltransferase enzymes. Formycin A has been found to be a potent blocker of adenosine cleavage when tested in worm extracts. Arabinosyl-6-mercaptopurine and 6-thioguanosine are inhibitors of worm adenosine deaminase, and formycin B and 6-thioguanosine were found to inhibit the purine nucleoside phosphorylase of this parasite. Combinations of arabinosyl-6-mercaptopurine with either formycin A or formycin B result in substantial blockage of adenosine utilization for nucleotide synthesis. These studies thus suggest that adenosine analogues in combination might be useful *in vivo* for the chemotherapy of schistosomiasis.

THREE MAJOR groups of blood-dwelling parasites, the hemoflagellates (trypanosomes), plasmodia (malaria) and trematodes (schistosomes) have been shown[1–3] to lack the biosynthetic capacity to make purines *de novo*. In consequence, all three of these parasites must resort to "salvage mechanisms" in order to sustain nucleotide levels for nucleic acid synthesis and to supply energy for various biosynthetic processes. Consequently, the possibility of developing a new chemotherapeutic approach against these three parasites using purine analogues has presented itself. Experimental evidence regarding the usefulness of adenosine analogues such as cordycepin[4] and puromycin[5] for trypanosomiasis, or tubercidin for schistosomiasis,[6] is already available.

The use of purine analogues for the therapy of schistosomiasis necessitates prior knowledge of not only the complete sequence of the reactions of purine metabolism but also the relative contributions of each individual reaction to the over-all pathway. Previous work in this laboratory has recently revealed several reactions of purine nucleotide metabolism,[7] and the present study serves to extend these findings.

* This work has been supported by Grants GB-30646 from the National Science Foundation, CA-07340 from the United States Public Health Service and RF-72066 from the Rockefeller Foundation.

It has been shown[7] that a major route in the conversion of adenosine to AMP is via the shunt pathway, AR → In → Hx → IMP* in which the successive reactions are catalyzed by adenosine deaminase, purine nucleoside phosphorylase (PNPase*) and hypoxanthine phosphoribosyltransferase (HPRTase*). In contrast, the direct conversion of adenosine to AMP, mediated by adenosine kinase, appears to play a minor role in adenine nucleotide synthesis. Recent evidence has been obtained in *Schistosoma mansoni* extracts for the existence of an enzyme capable of converting adenosine to adenine which, in turn, may be converted to AMP in a reaction catalyzed by adenine phosphoribosyltransferase (APRTase*). The present communication demonstrates the existence of this alternative pathway of AMP synthesis from adenosine. In addition, initial efforts to inhibit the utilization of adenosine for nucleotide synthesis by purine analogues are described. Figure 1 illustrates the structural formulae of adenosine and the analogues used in the present study.

MATERIALS AND METHODS

Adenosine-8-^{14}C (28·8 mCi/m-mole) was purchased from Schwarz/Mann (Orangeburg, N.Y.). Sigma Chemical Co. (St. Louis, Mo.) supplied 5-phosphoribosyl-1-pyrophosphate. Papierwerke Waldhof-Aschaffenburg (Mannheim, West Germany) produced 6-thioguanosine. Arabinosyl-6-mercaptopurine was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio). Formycin B [7-hydroxy-3(β-D-rubofuranosyl)pyrazolo-[4, 3-*d*]pyrimidine] was from CalBiochem (San Diego, Calif.). Formycin A [7-amino-3(β-D-ribofuranosyl)pyrazolo-[4, 3-*d*]pyrimidine] was a gift from Dr. Hamao Umezawa, Institute for Microbial Chemistry, Tokyo, Japan.

Preparation of schistosome extracts. Adult *S. mansoni* were recovered from infected CF[1] mice and were washed free of plasma and erythrocytes. Fifty pairs of worms were homogenized in 0·75 ml incubation medium (see below) at 4° using an all-glass microhomogenizer. The resulting homogenate was centrifuged at 39,000 *g* for 45 min at 4° and the resulting supernatant was removed and used for the studies *in vitro*.

Incubation conditions. Reaction mixtures consisted of 50 μl worm supernatant added to incubation medium consisting of $MgCl_2$ (2 mM), KCl (20 mM), NaCl (88 mM), Tris-HCl buffer, pH 7·4 (40 mM), and potassium phosphate buffer, pH 7·4 (22 mM), to a total volume of 1·9 ml. After the solution had been equilibrated to 37° (5 min), 0·10 ml adenosine-8-^{14}C (28·8 mCi/m-mole) was added to give a final concentration of $1{\cdot}8 \times 10^{-5}$ M. Drugs, when utilized, were present at final concentrations of 1 mM with the exception of PRPP which was at 2 mM. Usually, drugs and adenosine-8-^{14}C were added concurrently; however, as noted in the text, in some cases worm extracts were preincubated (30 min, 37°) before addition of radioactive precursor.

Incubations were carried out in 10-ml Erlenmeyer flasks in a shaking water bath (approximately 50 oscillations/min) at 37° with air as the gas phase. Control flasks were run for each experiment. Results are presented as averages of duplicate experi-

* AR, adenosine; In, inosine; Hx, hypoxanthine; Ad, adenine; IMP, inosine 5′-monophosphate; AMP, adenosine 5′-monophosphate; PNPase, purine nucleoside phosphorylase (purine nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1); HPRTase, hypoxanthine phosphoribosyltransferase, EC 2.4.2.8); APRTase, adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7); PRPP, 5-phosphoribosyl-1-pyrophosphate; TGR, 6-thioguanosine; TG, 6-thioguanine; ara-6-MP, arabinosyl-6-mercaptopurine; GMP, guanosine 5′-monophosphate; 6-thioGMP, 6-thioguanosine 5′monophosphate.

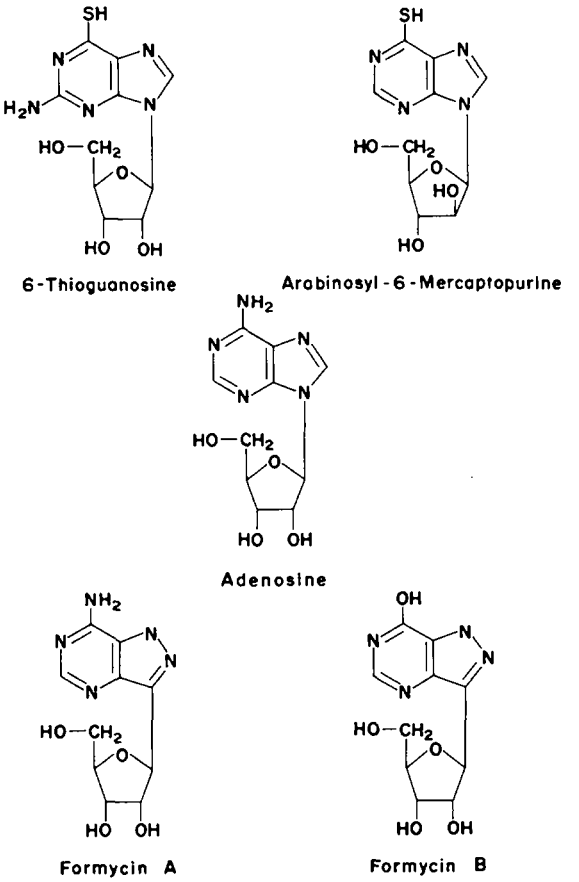

FIG. 1. Structural formulae of adenosine and purine analogues used in this study.

ments. Ranges were found to be within ± 3 per cent for these replicate determinations.

Extraction of reaction mixtures. At appropriate times after addition of adenosine-8-^{14}C, 0·2-ml aliquots of reaction mixture were removed and placed into chilled (4°) 15-ml centrifuge tubes containing 50 μl of 20% perchloric acid. After mixing well, the extracts were allowed to stand for about 30 min at 4°. All subsequent manipulations were carried out at 4°. The perchloric acid extracts were neutralized to a pH of 6·8 to 7·8 with cold KOH, mixed well, and centrifuged at top speed in a clinical centrifuge to remove insoluble potassium perchlorate. The clear neutralized supernatants were transferred via Pasteur pipet to 10 × 75 mm culture tubes; the tubes were sealed and kept at −20° until the contents were analyzed.

Analysis of neutralized samples. Aliquots (20 μl; approximately 6500 cpm) of the neutralized extracts were spotted on sheets of Whatman 3 MM chromatography paper together with appropriate carrier compounds. The chromatograms were developed in a descending manner for 55 hr with a solvent of water-saturated *n*-butanol–NH_4OH (100:1). In this solvent, the compounds migrate in the order Ad > AR- > Hx > In; purine nucleotides remain at the origin.

After chromatography, the sheets were air-dried, and ultraviolet-(2537 Å) absorbing areas were cut out and placed in scintillation vials to which 12 ml of a toluene-based scintillation fluid was added. The scintillation solution was prepared by dissolving one 4-g packet of Omnifluor [New England Nuclear; contains 98% PPO (2,5-diphenyloxazole) and 2% Bis-MSB (*p*-Bis-[*o*-methylstyryl]-benzene)] in 1 liter of scintillation-grade toluene. The samples were counted at 7° in a Packard Tri-Carb model 4000 liquid scintillation spectrometer.

RESULTS

In the presence of PRPP, cell-free extracts of *S. mansoni* are able to convert readily adenosine into nucleotides. Figure 2 illustrates that approximately 70 per cent of the initial adenosine radioactivity is present as nucleotides after 120 min of incubation. Little, if any, inosine or hypoxanthine had accumulated after 120 min, confirming the presence of rapid metabolic routes for adenosine conversion into nucleotides.[7] In contrast, when PRPP is omitted from the reaction mixture (Fig. 2), negligible amounts of radioactivity are present as nucleotides, even after 120 min. A transient accumulation of inosine radioactivity is apparent in the absence of PRPP; this falls to a low level in 120 min. At the end of the incubation period, about 35 per cent of the initial adenosine radioactivity is present as hypoxanthine. Of premier importance, however, is the significant accumulation of adenine during the incubation period in the absence of PRPP. Approximately 22 per cent of the total radioactivity can be found as adenine after 2 hr. Although the ultimate (i.e. after 120 min) conversion of adenosine to hypoxanthine via adenosine deaminase plus PNPase is greater than the cleavage of adenosine to adenine, the initial rates (i.e. after 5 min) of conversion by both routes are approximately the same.

Effects of analogue purine nucleosides on adenosine metabolism

6-*Thioguanosine.* The presence of TGR in worm extracts incubated with adenosine-8-^{14}C causes several significant changes compared to controls in the distribution

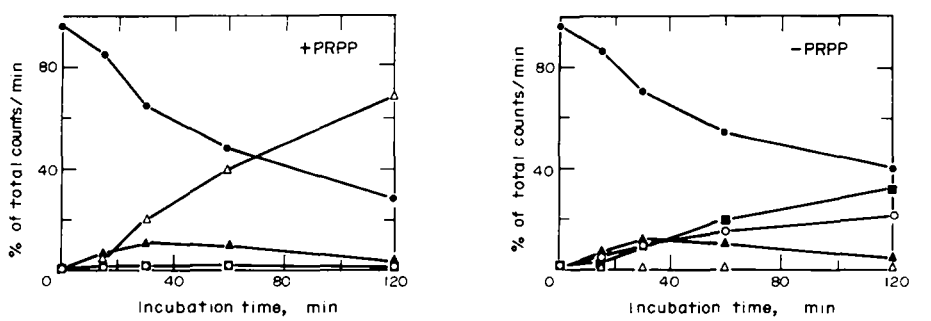

FIG. 2. Metabolism of adenosine-8-^{14}C by *S. mansoni* extracts. Adenosine-8-^{14}C ($1{\cdot}8 \times 10^{-5}$ M) was incubated at 37° with 50 μl of *S. mansoni* extract (see Materials and Methods for preparation) in a total volume of 2·0 ml. At the times indicated, 0·20-ml aliquots were removed, extracted with perchloric acid, neutralized and centrifuged, and 20-μl aliquots of the resulting supernatants were analyzed as outlined in Materials and Methods. When present, 5-phosphoribosyl-1-pyrophosphate (PRPP) was at 2 mM. AR = adenosine; In = inosine; Hx = hypoxanthine; Ad = adenine. Results, which are averages of duplicate determinations, are presented as per cent of total radioactivity in the individual compounds with 100 per cent being equivalent to about 6500 cpm.

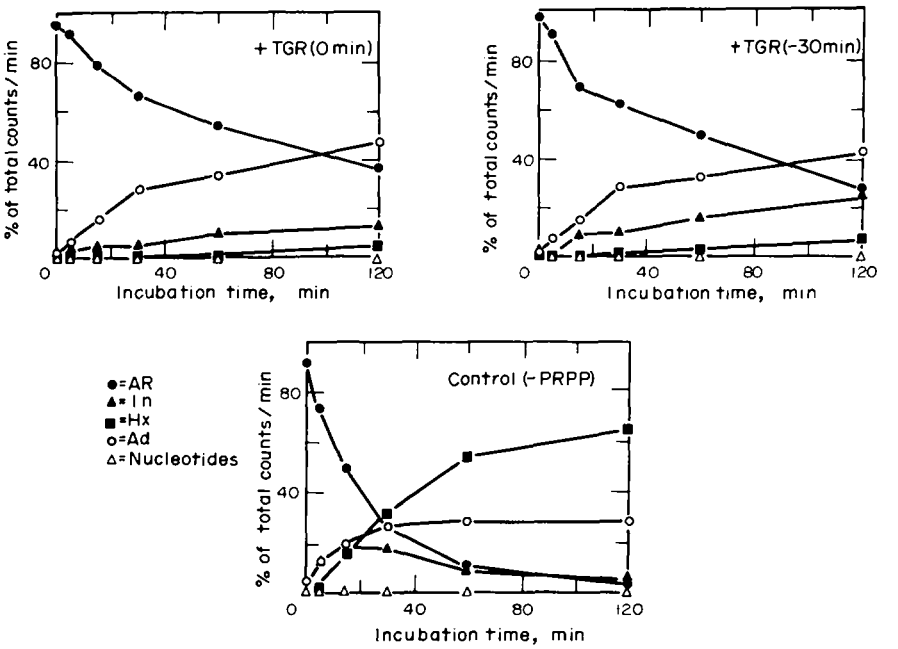

FIG. 3. Effect of 6-thioguanosine (TGR) on the metabolism of adenosine-8-^{14}C by *S. mansoni* extracts. Conditions for incubation, etc., are as outlined for Fig. 2. TGR (1 mM) was added concurrently with, or 30 min previous to, adenosine-8-^{14}C. PRPP was omitted from reaction mixtures.

of radioactivity. From Fig. 3 it is apparent that the rate of adenosine disappearance is much lower in treated than in control preparations; after 120 min, approximately 30–35 per cent of the adenosine is unchanged in treated as compared to about 5 per cent in controls. In the absence of added PRPP, nucleotide synthesis does not occur and bases and nucleosides accumulate. In controls, the total inosine plus hypoxanthine radioactivity accounts for approximately 75 per cent of the total radioactivity after 120 min compared to only about 18 per cent in TGR-treated samples. Apparently, TGR acts by inhibiting the adenosine deaminase step. In addition, whereas the ratio of hypoxanthine to inosine is about 12 in controls, it is only about 0·4 in treated samples. Accordingly, TGR appears to be blocking the action of PNPase on inosine in *S. mansoni* extracts; TGR is known to be a substrate for PNPase from other sources.[8] Significantly, the concentration of adenine rises from about 28 per cent of total radioactivity in controls to about 43–45 per cent in TGR-treated samples.

Preincubation of homogenates with TGR for 30 min before addition of adenosine-8-^{14}C does not enhance the effects of TGR. As illustrated in Fig. 3, preincubation seems to reduce these effects. Not only is adenosine utilization increased in the preincubated samples (compared to no preincubation), but also adenine accumulation is decreased slightly after 120 min. In addition, although the accumulation of hypoxanthine at the end of the incubation period is the same in both cases, inosine accumulation is increased more than 2-fold in the pretreated samples.

Thioguanosine may exert its effects either at the level of TGR or at the level of the 5′-nucleotide, 6-thioGMP. In order to examine this problem in greater detail, *S. mansoni* extracts were incubated with adenosine-8-^{14}C in the presence of 1 mM GMP (6-thioGMP was not available at the time of the study) with the hope that

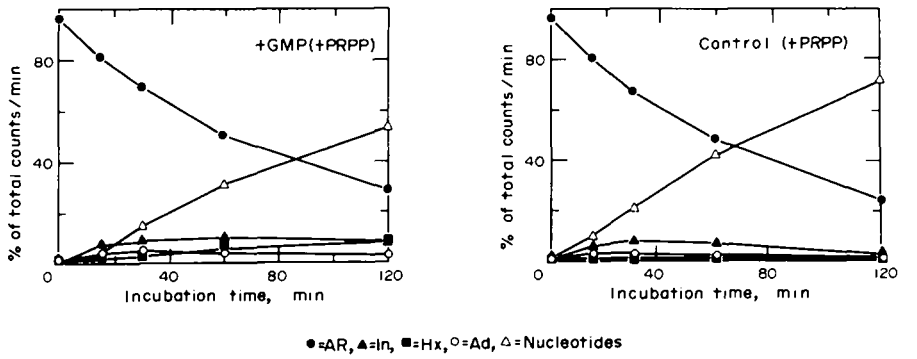

FIG. 4. Effects of GMP on the metabolism of adenosine-8-^{14}C by *S. mansoni* extracts in the presence of PRPP (2 mM). Conditions for incubation, etc., are outlined in Fig. 2. GMP (1 mM) was added concurrently with adenosine-8-^{14}C.

GMP might mimic any effects of 6-thioGMP that would occur. Figure 4 illustrates that in the presence of GMP (plus PRPP) only about 53 per cent of the total radioactivity is present as nucleotides after 120 min of incubation compared to about 70 per cent for controls. Adenosine utilization and adenine formation are the same in both treated and control samples. However, inosine plus hypoxanthine radioactivity increases to about 12 per cent above control values when extracts are treated with GMP. This increase in inosine plus hypoxanthine radioactivity reflects the decrease

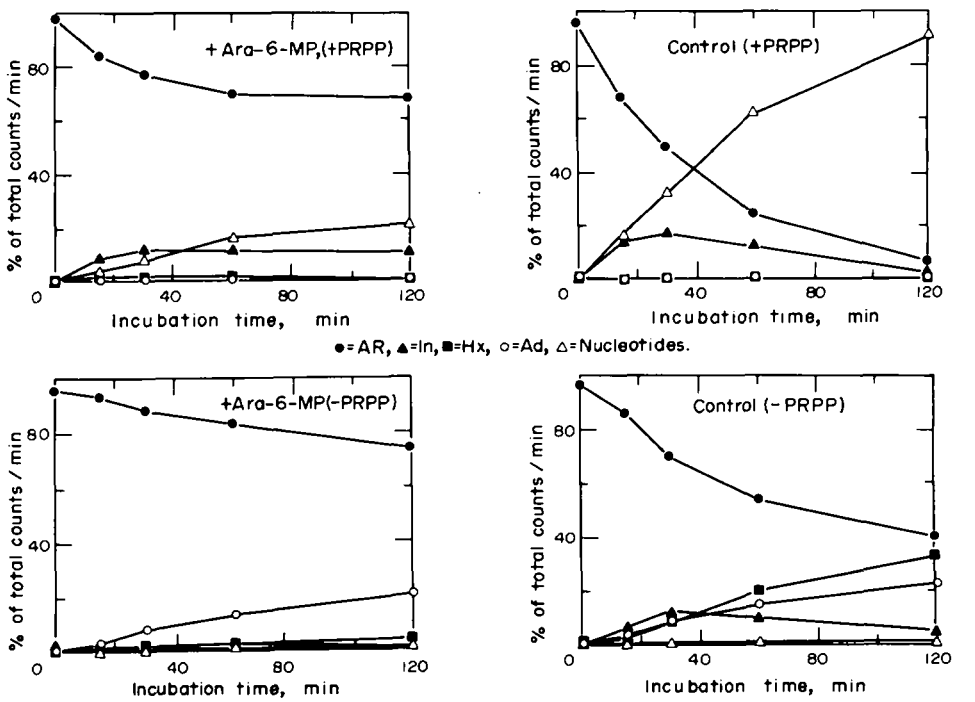

FIG. 5. Effect of arabinosyl-6-mercaptopurine (ara-6-MP) on adenosine-8-^{14}C metabolism by *S. mansoni* extracts in the absence and presence of PRPP (2 mM). Conditions for incubation, etc., are as outlined in Fig. 2. Ara-6-MP (1 mM) was added concurrently with adenosine-8-^{14}C.

in nucleotide formation noted above. Therefore, GMP appears to exert its effects by blocking the synthesis of nucleotides from inosine and hypoxanthine.

Arabinosyl-6-mercaptopurine. From Fig. 5 it is apparent that ara-6-MP severely limits the metabolism of adenosine-8-^{14}C in worm extracts. After 120 min of incubation, about 64 per cent of the total radioactivity is present as adenosine in treated samples as compared to about 10 per cent for controls. Since PRPP was present in these samples, nucleotide synthesis should have proceeded. Instead, this synthesis is substantially blocked by ara-6-MP in the treated samples. Whereas almost 90 per cent of radioactivity in the controls is detected as nucleotides after 2 hr of incubation, only about 21 per cent of original radioactivity is present as nucleotides in the treated samples. Ara-6-MP appears to be exerting its inhibition on the adenosine deaminase in the worm extracts. Accordingly, most of the nucleotides formed from adenosine in the presence of ara-6-MP probably arise via the AR → Ad → AMP pathway.

Two other observations support this conclusion. First, Fig. 5 shows that significant amounts of inosine, hypoxanthine and adenine are formed from adenosine in the absence of PRPP; inosine accumulation is transient, being maximal at 30 min. In the presence of ara-6-MP (no PRPP), accumulations of inosine and hypoxanthine are negligible throughout the incubation period, reflecting inhibition of adenosine deaminase by the analogue. In contrast, adenine accumulation is not significantly affected by ara-6-MP; apparently the adenosine cleavage enzyme is unaffected by this drug. Second, the amount of radioactivity present as nucleotides after 120 min in samples treated with both ara-6-MP and PRPP is equivalent to the amount of adenine which accumulates in samples treated with ara-6-MP alone.

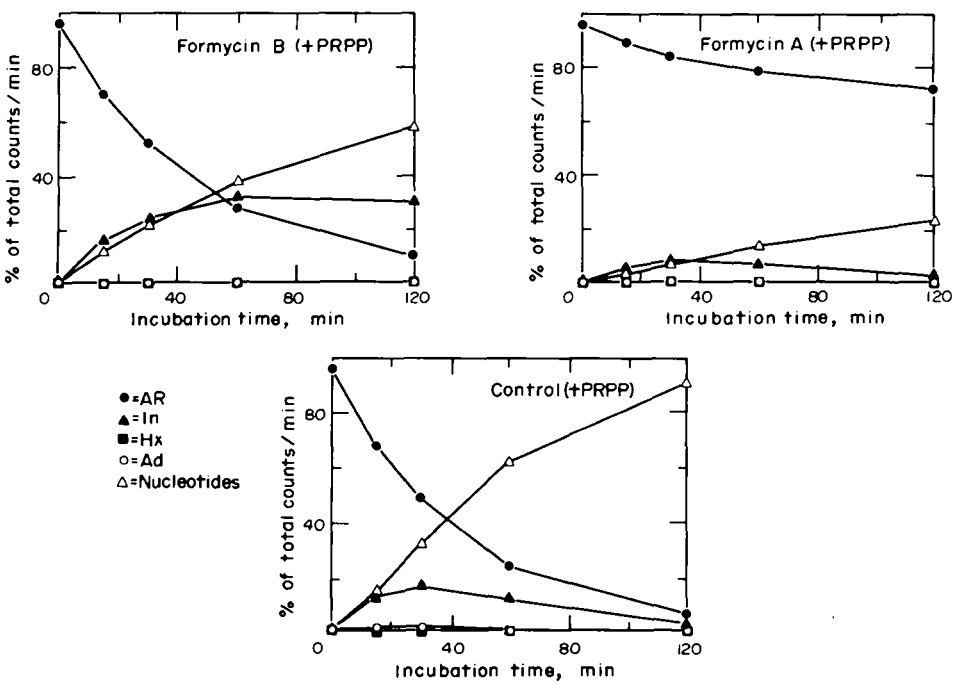

FIG. 6. Effects of formycin B and formycin A on the metabolism of adenosine-8-^{14}C by *S. mansoni* extracts in the presence of PRPP (2 mM). Conditions for incubation, etc., are outlined in Fig. 2. Formycin B (1 mM) or formycin A (1 mM) were added concurrently with adenosine-8-^{14}C.

Formycin B. Figure 6 illustrates the effects of formycin B on the metabolism of adenosine-8-^{14}C by extracts of *S. mansoni* in the presence of PRPP. The utilization of adenosine in both treated and control samples is approximately the same. However, much larger amounts of nucleotides accumulate in control samples compared to treated samples. In the presence of formycin B, about 60 per cent of the total radioactivity is present as nucleotides after 120 min of incubation as compared to about 90 per cent for controls. Approximately 28 per cent of the total radioactivity accumulates as inosine by the end of the incubation period in treated extracts. In control samples, inosine shows transient accumulation with a peak (about 19 per cent of total radioactivity) at 30 min, but only about 1 per cent of the total radioactivity is present as inosine after 120 min. In treated, as in control samples, insignificant amounts of hypoxanthine or adenine accumulate in the presence of PRPP.

Formycin A. In the presence of PRPP, formycin A treatment of schistosome extracts results in greatly reduced utilization of adenosine for nucleotide synthesis (Fig. 6). Only about 23 per cent of the total radioactivity is present as nucleotides after 120 min of incubation in treated samples compared to about 90 per cent in untreated samples. Formycin A-treated and control samples both showed transient inosine accumulation.

The greater inhibition of nucleotide synthesis from AR by formycin A as compared to formycin B must be due to blockage of the AR → Ad step by formycin A. Formycin A would first have to be deaminated to formycin B before it could be an effective inhibitor of PNPase. Thus, if formycin A does not inhibit adenosine cleavage, but

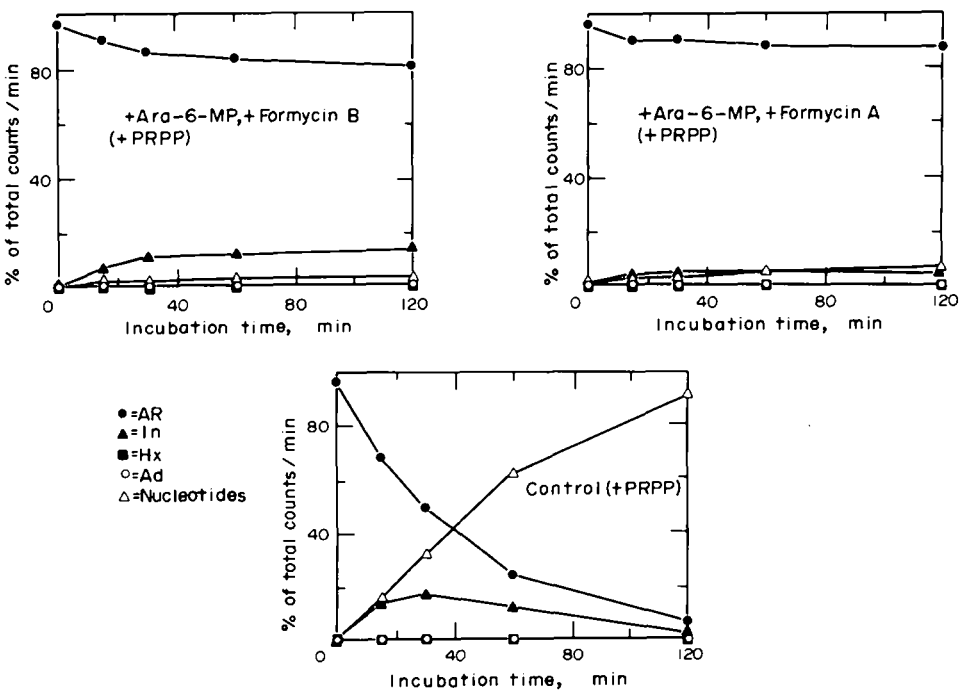

FIG. 7. Effects of combinations of ara-6-MP plus formycin B and ara-6-MP plus formycin A on adenosine-8-^{14}C metabolism by *S. mansoni* extracts in the presence of PRPP (2 mM). Conditions for incubation, etc., are as outlined in Fig. 2. All analogues (1 mM) were added concurrently with adenosine-8-^{14}C.

acts merely through its conversion to formycin B, formycin B should reduce nucleotide synthesis from adenosine to a greater extent than formycin A. In fact, the opposite is true, i.e. formycin A is a more potent inhibitor of nucleotide synthesis than formycin B. Therefore, the major inhibition of the conversion of adenosine to nucleotides by formycin A appears to occur at the AR → Ad step, although some contribution at the PNPase step (as formycin B) cannot be completely eliminated, since formycin A can be deaminated by *S. mansoni* adenosine deaminase.[7]

Arabinosyl-6-mercaptopurine plus formycin B or formycin A. From the above data it seemed likely that combinations of ara-6-MP plus one of the formycins might be useful as chemotherapeutic agents against schistosomiasis. Ara-6-MP plus formycin A should be most effective in this regard in light of the inhibition of the AR → In → Hx pathway by ara-6-MP and the inhibition of AR → Ad by formycin A. Figure 7 illustrates that little, if any, differences in adenosine metabolism are observed whether worm extracts are treated with combinations of ara-6-MP plus formycin B or ara-6-MP plus formycin A in the presence of PRPP. When compared with controls, however, it is apparent that adenosine utilization is very low in treated samples. Only very small amounts of nucleotides accumulate even after 120 min in treated samples. Small amounts of inosine which accumulate are presumably due to incomplete inhibition of adenosine deaminase by the combination treatments. As expected in the presence of PRPP, no hypoxanthine or adenine is seen at any of the times examined throughout the incubation period.

DISCUSSION

Although *S. mansoni* are deficient in the *de novo* pathway of purine nucleotide biosynthesis,[3] they possess multiple pathways for the utilization of preformed purine bases and nucleosides for nucleotide production. Of particular emphasis in recent studies are methods by which schistosomes can utilize adenosine for nucleotide biosynthesis. Previous to the present study, two pathways for nucleotide biosynthesis from adenosine were postulated as follows:

$$AR \rightarrow AMP \tag{1}$$

$$AR \rightarrow In \rightarrow Hx \rightarrow IMP \rightarrow AMP. \tag{2}$$

Although pathway 2 involves several steps, it was believed to be the major route of adenosine utilization on the basis of relative enzyme activities in worm homogenates.[7]

The data presented here verify that *S. mansoni* extracts are capable of rapidly converting adenosine into nucleotides when supplied with PRPP. The observation that little nucleotide synthesis occurs in the absence of PRPP indicates not only that the concentration of endogenous PRPP is low in such extracts, but also that the activity of the adenosine kinase pathway (1 above) is very limited under the conditions used in this study. However, the adenosine analogue, tubercidin (7-deaza-adenosine), which is not a substrate for adenosine deaminase or purine nucleoside phosphorylase of *S. mansoni*, can be phosphorylated in intact *S. mansoni* to form the triphosphate nucleotide derivative, indicating that the adenosine kinase pathway is operative to some extent in whole worms.[9]

A previous communication from our laboratories reported the accumulation of trace amounts of adenine within worm supernatants when intact worms were incubated with adenosine. The existence of an enzyme capable of cleaving adenosine was therefore considered.[10] The present study with *S. mansoni* extracts confirms this earlier observation, since about 25 per cent of the radioactivity from adenosine is isolated as adenine after a 2-hr incubation period in the absence of PRPP. Accordingly, a third major route of adenosine utilization for nucleotide synthesis in *S. mansoni* extracts is proposed, namely:

$$AR \rightarrow Ad \rightarrow AMP. \tag{3}$$

Of the two enzymes required for this pathway to function, APRTase, which catalyzes the second step, has been shown to be extremely active in *S. mansoni* extracts.[7]

The adenosine cleavage reaction may be due to: (1) the ability of adenosine to act as a substrate for worm PNPase, as has been previously postulated;[10] (2) hydrolytic cleavage of adenosine; or (3) phosphorolysis of adenosine by an enzyme previously undetected in *S. mansoni* extracts. Preliminary experiments have indicated that the adenosine cleavage reaction is phosphorolytic rather than hydrolytic and that the enzyme catalyzing this cleavage is a separate entity from PNPase.*

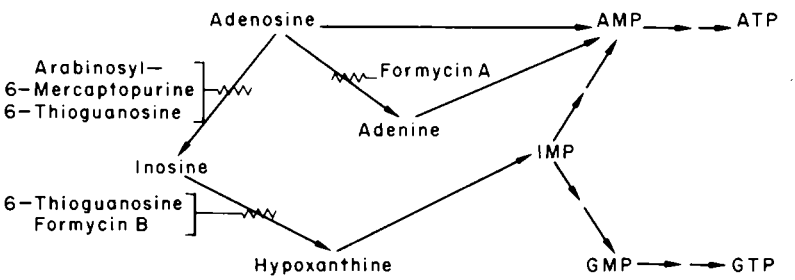

FIG. 8. Adenosine metabolism in *S. mansoni* extracts; effects of purine analogues.

The data obtained by using purine analogues in initial attempts to inhibit the synthesis of nucleotides from adenosine are summarized in Fig. 8. Thus, TGR appears to have significant inhibitory effects at the adenosine deaminase and PNPase steps. TGR has previously been shown to inhibit human erythrocytic adenosine deaminase[11] and to act as an alternative substrate for human erythrocytic PNPase.[8] The inhibition of adenosine deaminase by TGR appears to be quite potent in *S. mansoni* extracts.

TGR may exert its effects at the level of the free base (TG), the nucleoside itself, or the nucleotide (6-thioGMP). It is believed that the analogue nucleotide is the active form of TG or TGR when these compounds are used for cancer chemotherapy, and, indeed, 6-thioGMP is known to inhibit enzymes catalyzing reactions at the nucleotide level.[12] The results reported here, however, indicate that TGR exerts its effects at the level of the nucleoside. Preincubation of extracts with TGR before the addition of adenosine reduced the effects of the analogue. Presumably, cleavage of TGR to the less active TG form by PNPase during the preincubation period results in less unchanged TGR for inhibition of adenosine deaminase at the time the precur-

* R. P. Miech, personal communication.

sor is added. Similarly, 6-thioGMP does not appear to be responsible for the effects shown by TGR for two reasons. First, GMP has no effects other than to inhibit nucleotide synthesis from inosine plus hypoxanthine in the presence of PRPP, i.e. actual adenosine utilization was not inhibited. GMP is known to inhibit HPRTase from various sources.[13–15] Second, TGR has marked effects even in the absence of exogenous PRPP. Endogenous PRPP levels are probably too low in worm extracts to allow for significant synthesis of 6-thioGMP from TGR since nucleotides are not synthesized from either adenine or hypoxanthine in the absence of added PRPP.

Ara-6-MP also appears to block adenosine utilization at the adenosine deaminase step (Fig. 8), since neither inosine nor hypoxanthine accumulated in treated samples, whereas adenine accumulation is unaffected by ara-6-MP in the absence of PRPP. Ara-6-MP has been shown to competitively inhibit the adenosine deaminases from calf intestine and L1210 murine leukemia cells.[16] This analogue is not converted to the nucleotide derivative [17] nor is it cleaved by PNPase.[18] These properties indicate the potential usefulness of ara-6-MP as a chemotherapeutic agent since participation in such metabolic reactions might result in detoxification of this analogue.

Although formycin B and formycin A are structurally quite similar (Fig. 1), their sites of action appear to be different. Formycin B, an inosine analogue in which the N–C glycosidic bond is replaced by a C–C linkage, has been shown to inhibit PNPase from human erythrocytes.[19] Likewise, *S. mansoni* PNPase appears to be inhibited by formycin B.

Formycin A, also a C–C glycoside, is an adenosine analogue. This compound can be phosphorylated to the polyphosphate nucleotides by the same kinases which utilize adenosine and adenosine nucleotides as substrates, and such conversions have been thought to be responsible for the effects of formycin A on nucleotide metabolism.[20] These analogue nucleotides have also been shown to be able to replace the corresponding adenosine derivatives in many reactions of cellular metabolism.[21] However, the apparent low activity of the adenosine kinase route of nucleotide synthesis from adenosine in *S. mansoni* extracts seems to preclude such metabolic phosphorylations of formycin A and indicates that the nucleoside itself is the active compound in such extracts. Formycin A appears to exert its primary effect on the adenosine cleavage reaction in worm extracts, and thus this effect appears to be analogous to the effect of formycin B on PNPase. Since formycin A may be deaminated by *S. mansoni* adenosine deaminase to form formycin B,[7] some inhibition at the PNPase step in formycin A-treated samples probably occurs concurrently.

From the information obtained in these studies, TGR appears to be a good choice for combination therapy with an adenosine cleavage blocker such as formycin A. Preliminary trials* using TGR alone on mice bearing *S. mansoni* indicate, however, that this drug is very toxic to mice at the dosage used (6 mg/kg/day; s.c.) since three of six mice died within 5 days. In addition, schistosomes recovered from treated mice appeared to be quite active and normal in appearance. Combinations of ara-6-MP with formycin A or B also appear to be potent blockers of nucleotide synthesis from AR in the cell-free system studied here. Experiments are presently under way to examine the effectiveness of such drug combinations on intact *S. mansoni* both *in vitro* and *in vivo*.

* A. W. Senft, unpublished data.

Interpretation of the data from these studies must be made with reservation. It should not be inferred that the quantitative amounts of enzymic blockage by analogues are absolutely reproducible. It must be noted that, because of the nature of the parasite life cycle and the difficulties of obtaining batches of live worms in amounts suitable for enzyme analysis, some variation in age, development and physiological condition of the biological material is unavoidable. Therefore, our experimental data show some variability in enzyme reactions as well. However, controls were run with each experiment, and it is of interest to note that duplicate points generally agreed within ± 3 per cent.

As is the case with studies on cell-free systems in general, severe limitations must be placed on the extrapolation of data from *S. mansoni* extracts to the intact worms *in vivo*. Host drug toxicity, transport of drugs into worms, metabolism of drugs within worms, compartmentation of enzymes within worms, and regulation of enzyme activities all play crucial roles in studies using intact worms; these were not considered in the work reported here.

REFERENCES

1. G. J. BONÉ and M. STEINERT, *Nature, Lond.* **178,** 308 (1956).
2. C. J. WALSH and I. W. SHERMAN, *J. Protozool.* **15,** 763 (1968).
3. A. W. SENFT, R. P. MIECH, P. R. BROWN and D. G. SENFT, *Int. J. Parasit.* **2,** 249 (1972).
4. J. WILLIAMSON, *Trans. R. Soc. trop. Med. Hyg.* **60,** 8 (1966).
5. R. J. HEWITT, A. R. GUMBLE, W. S. WALLACE and J. H. WILLIAMS, *Antibiot. Chemother.* **4,** 1222 (1954).
6. J. J. JAFFE, H. M. DOREMUS, H. A. DUNSFORD, W. S. KAMMERER and E. MEYMARIAN, *Am. J. trop. Med. Hyg.* **22,** 62 (1973).
7. A. W. SENFT, G. W. CRABTREE, K. C. AGARWAL, E. M. SCHOLAR, R. P. AGARWAL and R. E. PARKS, JR., *Biochem. Pharmac.* **22,** 449 (1973).
8. A. F. ROSS, K. C. AGARWAL, S.-H. CHU and R. E. PARKS, JR., *Biochem. Pharmac.* **22,** 141 (1973).
9. R. J. STEGMAN, A. W. SENFT, P. R. BROWN and R. E. PARKS, JR., *Biochem. Pharmac.* **22,** 459 (1973).
10. A. W. SENFT, D. G. SENFT and R. P. MIECH, *Biochem. Pharmac.* **22,** 437 (1973).
11. R. P. AGARWAL, S. M. SAGAR and R. E. PARKS, JR., *Fedn. Proc.* **32,** 512 (1973).
12. R. P. MIECH, R. E. PARKS, JR., J. H. ANDERSON, JR. and A. C. SARTORELLI, *Biochem. Pharmac.* **16,** 2222 (1967).
13. J. F. HENDERSON, L. W. BROX, W. N. KELLEY, F. M. ROSENBLOOM and J. E. SEEGMILLER, *J. biol. Chem.* **243,** 2514 (1968).
14. T. A. KRENITSKY and R. PAPAIOANNOU, *J. biol. Chem.* **244,** 1271 (1969).
15. D. L. HILL, *Biochem. Pharmac.* **19,** 545 (1970).
16. D. L.-S. CHAO and A. P. KIMBALL, *Cancer Res.* **32,** 1721 (1972).
17. G. A. LEPAGE, J. P. BELL and M. J. WILSON, *Proc. Soc. exp. Biol. Med.* **131,** 1038 (1969).
18. A. P. KIMBALL, G. A. LEPAGE and B. BOWMAN, *Can. J. Biochem.* **42,** 1753 (1964).
19. M. R. SHEEN, B. K. KIM and R. E. PARKS, JR., *Molec. Pharmac.* **4,** 293 (1968).
20. I. C. CALDWELL, J. F. HENDERSON and A. R. P. PATERSON, *Can. J. Biochem.* **47,** 901 (1969).
21. D. C. WARD, A. CERAMI, E. REICH, G. ACS and L. ALTWERGER, *J. biol. Chem.* **244,** 3245 (1969).