Portland State University

PDXScholar

Dissertations and Theses

Dissertations and Theses

1990

# Methionine Metabolism in Fasciola Hepatica

Carol Theresa Ayer

*Portland State University*

Follow this and additional works at: https://pdxscholar.library.pdx.edu/open_access_etds

Part of the Biochemical Phenomena, Metabolism, and Nutrition Commons, and the Biology Commons

Let us know how access to this document benefits you.

## Recommended Citation

Ayer, Carol Theresa, "Methionine Metabolism in Fasciola Hepatica" (1990). *Dissertations and Theses.* Paper 3954.

https://doi.org/10.15760/etd.5838

This Thesis is brought to you for free and open access. It has been accepted for inclusion in Dissertations and Theses by an authorized administrator of PDXScholar. Please contact us if we can make this document more accessible: pdxscholar@pdx.edu.

AN ABSTRACT OF THE THESIS OF Carol Theresa Ayer for the Master of Science in Biology presented October 30, 1990.

Title: Methionine Metabolism in _Fasciola_ _hepatica_.

APPROVED BY THE MEMBERS OF THE THESIS COMMITTEE:

David T. Clark, Chair

Gary R. Brodowicz

Richard R. Peterson

Leonard Simpson

5'-Deoxy-5'-methylthioadenosine (MTA) is derived from S-adenosylmethionine (AdoMet) during the synthesis of the polyamines spermidine and spermine. Methionine can be regenerated from MTA by one of two mechanisms. In mammalian cells and some microorganisms, MTA is degraded to adenine and 5-methylthioribose-1-phosphate (MTR-1-P) via MTA phosphorylase. In certain other microbes, however, MTA is catabolized in two steps; first to adenine and

5-methylthioribose (MTR) via MTA nucleosidase followed by conversion of MTR to MTR-1-P via MTR kinase.

This study was to demonstrate the presence of MTA nucleosidase or MTA phosphorylase in both redia containing cercariae and adult *Fasciola hepatica* Linnaeus, 1758. If MTA nucleosidase was present, it was wanted to determine if MTR kinase was also present.

The phosphate-dependent cleaving activity of MTA phosphorylase was demonstrated in the cell-free extracts of adult *Fasciola hepatica* along with an unidentified MTR metabolizing activity. Redia containing cercariae showed MTA nucleosidase and MTR kinase activity.

# METHIONINE METABOLISM IN *FASCIOLA HEPATICA*

by

CAROL THERESA AYER

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University
1990

TO THE OFFICE OF GRADUATE STUDIES:

The members of the Committee approve the thesis of Carol Theresa Ayer presented October 30, 1990.

David T. Clark, Chair

Gary R. Brodowicz

Richard R. Peterson

Leonard Simpson

APPROVED:

W. Herman Taylor, Jr., Chair, Department of Biology

C. William Savery, Interim Vice Provost for Graduate Studies and Research

# ACKNOWLEDGMENTS

I wish to take this opportunity to thank the people who aided me in encouragement, support, and guidance in the completion of this research. First to Paula Tower who offered technical advice and guidance in the daily effort of the research. To Mike K. Riscoe who provided direction when the research appeared to be going in circles. To Robert Baldwin who supplied the infected snails free of charge and the information to keep them alive and healthy until needed. Finally to David T. Clark who took me on as a graduate student and supplied unlimited encouragement and direction. To all these people I say thanks for believing in me and the research I was trying to accomplish.

TABLE OF CONTENTS

PAGE

ACKNOWLEDGEMENTS.................................. iii

LIST OF TABLES.................................... vi

LIST OF FIGURES................................... vii

INTRODUCTION...................................... 1

REVIEW OF THE LITERATURE.......................... 5

MATERIALS AND METHODS............................. 11

Tematode Collection........................... 11

Extracts of Tissue............................ 11

Methylthioadenosine Phosphorylase Assay...... 13

Methylthioadenosine Nucleosidase Assay....... 15

Methylthioribose Kinase Assay................ 17

Thin Layer Chromatography.................... 19

Paper Chromatography......................... 19

Protein Concentration........................ 20

RESULTS........................................... 21

Methylthioadenosine Phosphorylase Assay...... 21

Methylthioadenosine Nucleosidase Assay....... 24

Methylthoribose Kinase Assay................. 26

Thin Layer Chromatography.................... 30

Paper Chromatography........................ 33

DISCUSSION.................................. 34

LITERATURE CITED............................ 39

## LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| I | MTA phosphorylase, MTA nucleosidase, and MTR kinase activity in cell extracts... | 9 |
| II | MTA phosphorylase activity in cell free extracts of <u>Fasciola</u> <u>hepatica</u> .......... | 22 |
| III | MTA nucleosidase activity in cell free extracts of <u>Fasciola</u> <u>hepatica</u>.......... | 25 |
| IV | MTR kinase activity in cell free redia containing cercariae extracts of <u>Fasciola</u> <u>hepatica</u> ...................... | 28 |
| V | MTR kinase activity in cell free adult fluke extracts of <u>Fasciola</u> <u>hepatica</u> incubated with variable pH's........... | 29 |
| VI | MTR kinase activity in cell free adult fluke extracts of <u>Fasciola</u> <u>hepatica</u> incubated with variable temperatures... | 31 |
| VII | MTR kinase activity in cell free adult fluke extracts of <u>Fasciola</u> <u>hepatica</u> incubated at variable times........... | 32 |

## LIST OF FIGURES

FIGURE PAGE

1. Methionine recycling pathway.............. 3

2. Synthesis of 5'deoxy-5'methylthioadenosine (MTA)................................ 7

# INTRODUCTION

The digenetic trematode, Fasciola hepatica Linnaeus, 1758, has been historically used to demonstrate to herdsman of cattle, sheep, and goats the parasitological effects of a trematode. The worm has been used extensively for teaching parasitology because of its global distribution and easily demonstrated pathology of the main bile ducts and the liver parenchyma of the infected hosts.

Fasciola hepatica has a complex life cycle with larval stages developing in several species of snails of the genus Lynmaea. These stages are the sporocyst and the two stages of rediae, in the second one of which the cercariae develop. The cercariae attach to smooth surfaces such as submerged grass, water cress, water chestnuts, and other plants, and secrete protective protein membranes around themselves and become metacercaria. These are the infective, larval stage for domestic animals when they graze the vegetation with the metacercariae encysted on their surfaces. Human beings also consume the vegetation on which Fasciola hepatica metacercariae may have encysted, including water cress and water chestnuts. For more historical information about Fasciola hepatica, the reviews by Sinclair, 1967, and Boray, 1969 are especially important.

The role of Fasciola hepatica for research investigations is also significant. Parasitologists continue to study several aspects of this trematode, including: the snail host, diagnosis of the disease, and adult fluke immunity in the host. Additional areas of study involving both adult and larval forms include investigating physiological parameters and biochemical pathways.

This research was directed toward a selected group of enzymes responsible for metabolism of methionine. The purpose of this study was to demonstrate the presence or absence in Fasciola hepatica of an enzyme sequence known to occur in several eubacteria and protozoa (Sakayu, 1988). The enzyme sequence is also present in green plants (Abeles, 1973; Yang, 1974).

The enzymes constitute part of the recycling pathway for 5'-deoxy-5'-methylthioadenosine (MTA) (Figure 1). Methionine can be regenerated from MTA by one of two mechanisms. In mammalian cells and some miroorganisms, MTA is degraded to adenine and 5-methylthioribose-1-phosphate (MTR-1-P) via MTA phosphorylase. In certain other microbes, MTA is catabolized in two steps; first to adenine and 5-methylthioribose (MTR) via MTA nucleosidase, followed by conversion of MTR to MTR-1-P via MTR kinase. MTR-1-P is recycled into methionine via 1-phospho-2,3-diketo-5-methylthiopentane and 2-keto-4-methylthiobutyrate (Adams &

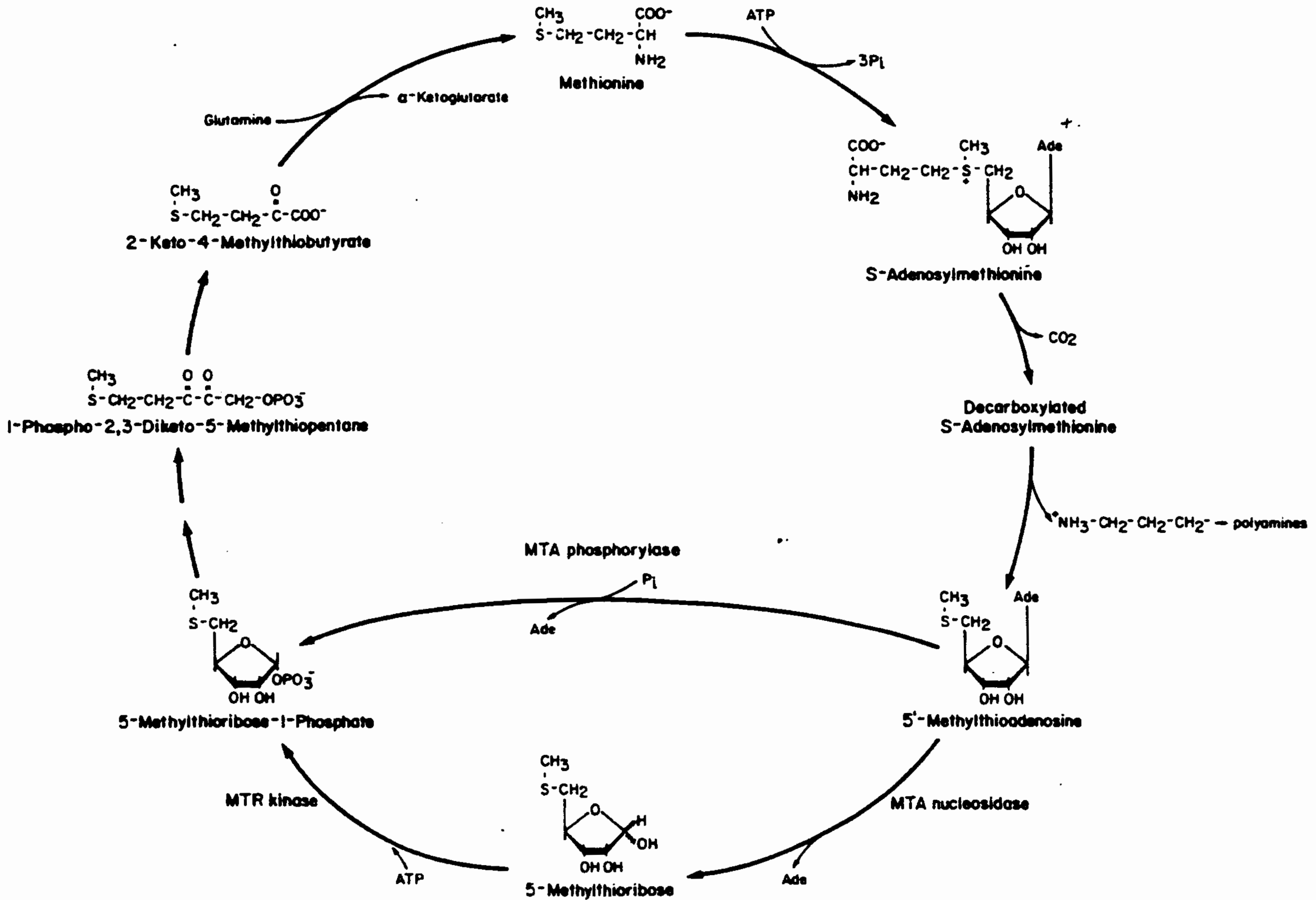


Figure 1. Methionine recycling pathway.

Yang, 1977; Shapiro & Barrett, 1981; Sugimoto, et al., 1976; Wang, et al., 1982) (Figure 1).

The purpose of this study was to demonstrate the presence of MTA nucleosidase or MTA phosphorylase in both redia containing cercariae and adult *Fasciola hepatica*. If MTA nucleosidase was present, further steps were taken to determine if MTR kinase was also present.

## REVIEW OF THE LITERATURE

*Fasciola hepatica* has been widely studied since it is a large trematode which causes pathology to the bile ducts and hepatocytes of the infected host (Isseroff, et al., 1977). The pathology may include a striking enlargement of the main bile duct due to cellular proliferation that results in thickened duct walls. An increase in folding of the endothelium surrounding the enlarged duct lumen is also seen.

Several biochemical investigations have been directed toward understanding the respiratory metabolism (Grembergen, 1949), the metabolism of lipids (Moss, 1970; Lahoud, et al., 1971), and carbohydrates (Mansour, 1959; Von Brand, 1966; Pantelouris, 1965; Barrett, et al., 1978; Lloyd, 1986). Amino acid metabolism studies have included one by Moss (1970) who analyzed one gram samples of the saline in which individual whole adult flukes had been incubated for thirty minutes. He found 18 amino acids released into the saline. The most abundant were proline, phenylalanine, tyrosine, and histidine, found to comprise 70% (um/g wet wt) of the amino acid released in this study. Methionine was the sixth most common amino acid, but only accounted for 7% (um/g wet wt) of the total amino acids released. Kurelec and Rijavec (1966) demonstrated that in homogenized, pooled adult

Fasciola hepatica over 50% (mg/100 g wet wt) of total amino acid was represented by three amino acids: proline, alanine, and histidine. Methionine comprised 3% (mg/100 g wet wt) of the total amino acid content of the worm.

Methionine is a sulfur-containing amino acid that plays an important role in a variety of cellular functions including protein synthesis, transmethylation, and polyamine biosynthesis (Cooper, 1983). The methionine recycling pathway begins with methionine being converted to S-adenosylmethionine (AdoMet) in a condensation reaction involving ATP as the adenosyl donor (Figure 1).

AdoMet is decarboxylated, followed by a propylamino group being transferred from decarboxylated AdoMet and combining with putrescine (1,4-diaminobutane) to form the polyamines spermidine and spermine (Figure 2). A co-product of polyamine biosynthesis is MTA, which is produced in stoichiometric amounts with the formation of spermidine and spermine. MTA is found only in trace amounts in bacterial cells (Chu, et al., 1968) and mammalian tissue (Rhodes and Williams-Ashman, 1964).

Normal cells do not accumulate MTA in amounts equal to the concentration of polyamines produced, due to hydrolysis of MTA to adenine and either MTR or MTR-1-P. MTA accumulation in the cell would lead to inhibition of several important intracellular reactions (Schlenk, 1983).

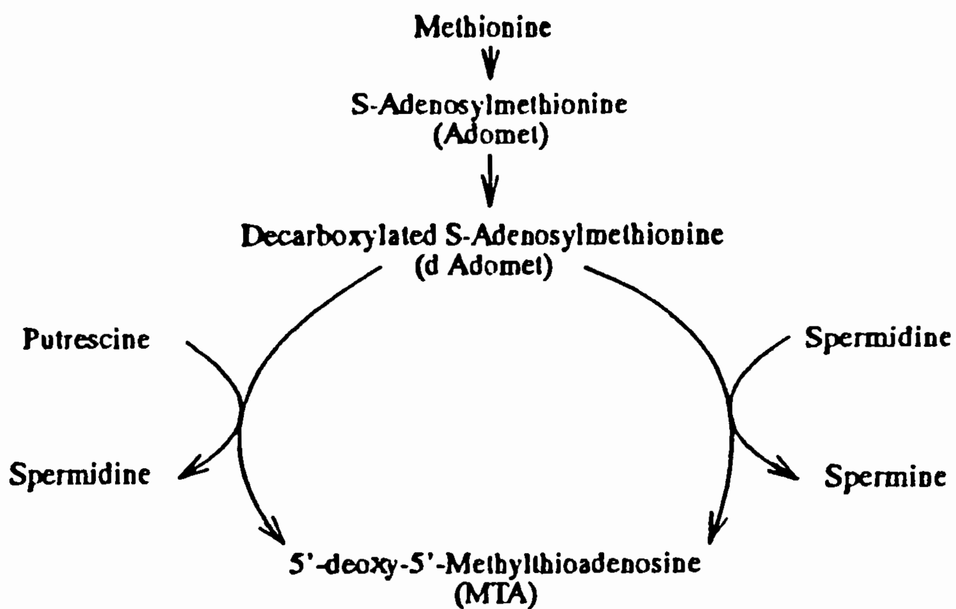


Figure 2. Synthesis of 5'deoxy-5' methylthioadenosine (MTA).

There are two known mechanisms whereby MTA is metabolized to MTR-1-P (Figure 1). In mammalian cells and some microorganisms, MTA is degraded in one step to adenine and MTR-1-P by MTA phosphorylase (Pegg and Williams-Ashman, 1969). In some other organisms and green plants (Kushad, et al., 1982; Yung & Yang, 1982; Kushad, et al., 1983; Guranowski, 1983; Kushad, et al., 1985), MTA is catabolized in two steps; first to adenine and MTR via MTA nucleosidase (Duerre, et al., 1969), followed by conversion of MTR to MTR-1-P via MTR kinase (Ferro, et al., 1978). MTR-1-P is recycled into methionine via 1-phospho-2,3-diketo-5-methylthiopentane and 2-keto-4-methylthiobutyrate (Figure 1).

MTA phosphorylase activity was first noted by Pegg and Williams-Ashman, (1969) in rat ventral prostate tissue where the requirement for phosphate ion suggested the formation of MTR-1-P. Further studies demonstrated the presence of MTA phosphorylase in various rat tissues (Garbers, 1978), human placenta (Cacciapuoti, et al., 1978), human prostate (Zappia, et al., 1978), _Trypanosoma_ _brucei_ (Ghoda, et al., 1988), _Traypanosoma_ _cruzi_ (Miller and Toorchen, 1988), and some eubacteria (Sakayu, et al., 1988) (Table I).

Both MTA nucleosidase and MTR kinase are involved in one sequence for MTA metabolism. Ferro, et al., (1978) identified and classified MTR kinase as a kinase on the basis of its ATP-dependent phosphorylation. Both MTA

TABLE I

MTA PHOSPHORYLASE, MTA NUCLEOSIDASE, AND MTR KINASE ACTIVITY IN CELL EXTRACTS

| Source of Extract | MTA Nucleosidase | MTR Kinase | MTA Phosphorylase |
|---|---|---|---|
| **Bacteria:** | | | |
| Enterobacter aerogenes[1] | + | + | - |
| Escherichia coli[2] | + | - | - |
| Klebsiella pnuemoniae[9] | + | + | - |
| **Parasities:** | | | |
| Plasmodium falciparum[2] | + | + | - |
| Giardia lamblia[2] | + | + | - |
| Ochromonas malhamensis[6] | + | + | - |
| Trypanosoma brucei[3] | - | - | + |
| Trypanosoma cruzi[4] | - | - | + |
| **Mammals:** | | | |
| Human erythrocytes[2] | - | - | + |
| Human liver[2] | - | - | + |
| Rat liver[7] | - | - | + |
| Rat prostate[7] | - | - | + |
| **Plants:** | | | |
| Persea americana[5] | + | + | - |
| Malus sylvestris[8] | + | + | - |
| Lycopersicum esculentum[5] | + | + | - |

References:
1 Kushad, et al., 1982
2 Fitchen, et al., 1987
3 Ghoda, et al., 1988
4 Miller, et al., 1988
5 Kushad, et al., 1983
6 Sugimoto, et al., 1976
7 Garbers, et al., 1978
8 Yung & Yang, 1982
9 Gianotti, et al., 1990

nucleosidase and MTR kinase enzymes are found in some eubacteria, protozoa, and in most plants (Table I).

The role of MTA nucleosidase and MTR kinase or MTA phosphorylase in the degradation of MTA of the methionine recycling pathway appears to be widespread in all normal cells. However, Fitchen, et al., (1987) showed that Esherichia coli had the presence of MTA nucleosidase but apparently lacked MTR kinase (Table I). Determination of which MTA metabolism mechanism Fascoila hepatica employs would be of value in furthering the quest for a possible chemotherapeutic target for this parasitic disease.

## MATERIALS AND METHODS

### TREMATODE COLLECTION

Adult Fasciola hepatica used for this study were obtained from infected livers found in freshly killed cattle being slaughtered at the Carlton Packing Company, Carlton, Oregon. The flukes were collected from the liver within fifteen minutes of slaughter and were transported in cold (4°C) 0.9% saline to the laboratory. Redia containing developing cercariae of Fasciola hepatica were obtained from laboratory raised infected Lymnaea columbus snails, supplied by Robert Baldwin, Monmouth, Oregon. The specimens were dissected from the snails and placed in 4°C saline.

### EXTRACTS OF TISSUE

Second generation rediae containing cercariae were collected by removal of the shell from eight infected snails with forceps and then dissecting out the rediae. Approximately 800 rediae containing cercariae were obtained. The redia were centrifuged five minutes and then stored frozen at -20°C. The frozen rediae containing cercariae were thawed and centrifuged five minutes in a Beckman microfuge II at 11,600 xg and the supernatant was replaced with 200 ml buffer (50 mM phosphate; 10% glycerol; 2 mM

dithiothreitol [DTT] pH = 7.2). The pellet was homogenized in a Dounce homogenizer on ice. The homogeneate was centrifuged 10 minutes in a Beckman microfuge II (11,600 xg). The supernatant was then frozen at -20°C for future enzyme assays.

A modification of the technique described by Barrett, et al., (1978) was used to prepare soluble extracts from the adult flukes. Approximately three to six flukes at a time were homogenized in a Dounce homogenizer on ice with buffer (25 mM sucrose, 0.01 mM glycine, 0.7 mM DTT, and 175 ml $H_2O$ for a total volume of 200 ml (pH = 9.11). The homogeneate was frozen for 10 minutes at -80°C, followed by rehomogenization. The homogeneate was centrifuged in a Beckman J-6B (1520 xg) for 10 minutes at 4°C, and the supernatant used for enzyme assays.

At the beginning of the study, the adult fluke supernatant was not dialyzed. After the first set of tests, the adult fluke supernatant was dialyzed for 18-26 hours at 4°C against a one liter dialysis solution consisting of 0.9% NaCl, 4.8% glycerol, and 1 mM DTT. The dialysis was done to remove exogenous phosphate that was believed to interfere with the MTA nucleosidase and MTA phosphorylase assay results. Late in the study, both dialyzed and nondialyzed adult fluke supernatant was employed.

METHYLTHIOADENOSINE PHOSPHORYLASE ASSAY

A modification of the technique described by White, et al., (1982) was used for the MTA phosphorylase assay. All assays included controls that were incubation mixtures run in the absence of added protein. For the redia containing cercariae each incubation mixture (250 ul) included: 25 ul of Hepes/$KH_2PO_4$ buffer, 0.5M/25 mM, pH 7.19; 50 ul [2,8,5'-$^3$H]5'-Chloroadenosine (ClAdo) ($6.2 \times 10^4$ cpm/umol, 0.35 mM); 75 ul of homogenized, pooled redia containing cercariae extract; and 100 ul $H_2O$. For the redia containing cercariae assay the reaction was allowed to proceed for one hour at 23°C. Only one assay was done due to the shortage of available extract and the difficulty in obtaining infected snails.

For the _Fasciola hepatica_ adult fluke assay, the incubation mixture (250 ul) contained: 25 ul Tris buffer 500 mM, pH 7.19; 50 ul [2,8,5'-$^3$H]ClAdo ($6.2 \times 10^4$ cpm/umol, 0.35 mM); 75 ul homogenized, pooled adult fluke extract; and 100 ul $H_2O$. For the adult fluke assays, the reaction was allowed to proceed for one hour at 22-24°C. Six assays were performed under these conditions.

Another seven adult fluke assays were run which involved changes in either the incubation mixture, time, temperature, or some combination of the three. Three assays involved changes in the incubation mixture that included: 25 ul Imidazole 500 mM, pH 7.2; 10 ul DTT 3 mM; 10 ul $KH_2PO_4$

1M, pH 7.2; 50 ul [2,8,5'-$^3$H]ClAdo, (6.2 x $10^4$ cpm/umol, 0.35 mM); 85 or 155 ul homogenized, pooled adult fluke extract; and 0 to 80 ul $H_2O$. The reaction was allowed to proceed for one hour at 23-24°C.

One assay involved a change in the incubation mixture that replaced the Imidazole 500 mM , pH 7.2 buffer with an Imidazole 500 mM, pH 9.0 buffer. The final three assays changed the temperature to 45°C, and increased incubation times to 4, 4.5 and 14.5 hours respectively. The incubation mixture changed to: 25 ul Imidazole 500 mM , pH 9.0 (for first assay) and pH 7.2 for last two assays; 10 ul DTT 3 mM; 50 ul [$^3$H-methyl]MTA (2 x $10^7$ cpm/umol, 0.37 mM); 10 ul $KH_2PO_4$, 1 M, pH 7.2; 155 ul homogenized, pooled adult fluke extract.

Reactions of rediae containing cercariae and adult flukes, except for the final two adult fluke reactions, were stopped by addition of 50 ul of 1.8 M trichloro acetic acid (TCA). The final two adult fluke reactions were stopped by the addition of 1 ml absolute ethanol.

All reaction tubes were then centrifuged in a Beckman microfuge II (11,600 xg) for 4.5 minutes. A 200 ul aliquot of supernatant from each incubation tube was eluted over washed BioRad AG 50 columns, followed by 3 ml $H_2O$. These columns contain a cation exchange resin. The negatively charged radioactive adenine binds to the column while the [5-$^3$H]-5-Cl-Rib-1-P or [$C^3H_3$]MTA is eluted through and

available to be counted. Elution was directly into scintillation vials. To each vial containing the sample was added approximately 18 ml scintillation fluid (Beckman Ready Safe liquid scintillation cocktail). Each vial was vigorously shaken and then counted for [5-$^3$H]-5-Cl-Ribose-1-P or [C$^3$H$_3$]MTA in a Beckman LS 3801 scintillation counter.

The BioRad columns were recharged between each test by three 6 ml washes of 3.5 N $H_2SO_4$ followed by three 6 ml washes of $H_2O$.

## METHYLTHIOADENOSINE NUCLEOSIDASE ASSAY

The MTA nucleosidase assay was run similarly to the MTA phosphorylase assay. All assays included controls that were incubation mixtures run in the absence of added protein. For the redia containing cercariae each incubation mixture (250 ul) included: 25 ul Tris buffer 500 mM, pH 6.5; 50 ul [2,8,5'-$^3$H]ClAdo (6.2 x $10^4$ cpm/umol, 0.35 mM); 75 ul of homogenized, pooled redia containing cercariae extract; and 100 ul $H_2O$. For the redia containing cercariae assay the reaction was allowed to proceed for one hour at 23°C. Only one redia containing cercariae assay was performed due to the shortage of available extract and the difficulty in obtaining infected snails. For the homogenized, pooled adult fluke assay, the incubation mixture contained: 25 ul Tris buffer 500 mM, pH 6.43; 50 ul [2,8,5'-$^3$]ClAdo (6.2 x $10^4$

cpm/umol, 0.35 mM); 75 ul of homogenized, pooled adult fluke extract; and 100 ul $H_2O$. The reaction was allowed to proceed for one hour at 22-24°C. A total of seven assays were performed under these conditions.

Another seven adult fluke assays were run which involved changes in the incubation mixtures, times, temperatures, or some combination of the three. Four assays involved changes in the incubation mixture that included: 25 ul Imidazole buffer, 500 mM at a pH of 7.2, 7.6 or 9.0; 10 ul DTT 3mM; 50 ul [2,8,5'-$^3$H]ClAdo (6.2 x $10^4$ cpm/umol, 0.35 mM); and 165 ul of homogenized, pooled adult fluke extract. The reactions were allowed to proceed one hour at 23-24°C.

The last three assays involved changes in all three variables. The incubation mixture changed to: 50 ul Imidazole buffer, 500 mM at a pH of 9.0 or 7.2; 10 ul DTT 3 mM; 50 ul [$^3$H-methyl]MTA (2 x $10^7$ cpm/umol, 0.37 mM); and 165 ul homogenized, pooled adult fluke extract. Reactions were allowed to proceed at 45°C for 4, 4.5, and 14.5 hours, respectively. Reactions of redia containing cercariae and adult fluke were stopped by addition of 50 ul of 1.8 M TCA, except the final two adult fluke reactions which were stopped by 1 ml absolute ethanol. The reaction tubes were then centrifuged in a Beckman microfuge II (11,600 xg) for 4.5 minutes. A 100 ul aliquot of supernatant from each incubation tube was eluted over washed BioRad AG 50 columns,

followed by 3 ml $H_2O$. Elution was directly into scintillation vials. To each vial containing the sample was added approximately 18 ml scintillation fluid (Beckman Ready Safe liquid scintillation cocktail). Each vial was vigorously shaken and then counted for [5-$^3$H]-5-Cl-Ribose-1-P or [C$^3$H$_3$]MTA in a Beckman LS 3801 scintillation counter.

The BioRad columns were recharged between each test by three 6 ml washes of 3.5 N $H_2SO_4$ followed by three 6 ml washes of $H_2O$.

## METHYLTHIORIBOSE KINASE ASSAY

Slight modifications of the techniques described by Ferro et al., (1978) were utilized for MTR kinase assay. All assays included controls that were incubation mixtures run in the the absence of added protein. Many assays included a positive control, _Klebsiella pneumoniae_ an organism known to have MTR kinase. The incubation mixture (250 ul) was similar for both redia containing cercariae and adult flukes. It contained: 25 ul of Tris 500 mM or glycine 1M buffer, pH varied from 6.43 to 9.50; 25 ul DTT, 3 mM; 25 ul adenosine tri-phosphate (ATP); 10 ul [$^3$H-methyl]MTR (1.1 x $10^7$ cpm/umol, 2.1 mM, pH 7.2); extract, amount varied from 25 ul to 165 ul, and $H_2O$. Various incubation times (0-240 minutes) and temperatures (1-56°C) were tested. Six tests were run where the pH was

variable, five tests were done with variable temperatures, and seven tests were done with variable times.

The reactions were halted by the addition of 1 ml of absolute ethanol. The reaction tubes were frozen for a minimum of 5 minutes at -20°C, then thawed and centrifuged for 5 minutes in a Beckman microfuge II (11,600 xg). A 200 ul aliquot of supernatant from each incubation tube was eluted over washed BioRad AG-X8-formate (100-200 mesh) columns. These are an anion exchange resin column. The positively charged [$C^3H_3$]MTR-1-P molecule binds to the column at first and is then released by the 0.75 N sodium formate, and available to be counted.

Each column was washed with 10 ml of 0.01 N sodium formate, followed by a second wash of the same. Glass test tubes were then placed beneath the columns, followed by elution with 6 ml of 0.75 N sodium formate. A 2 ml aliquot of the eluate was placed into scintillation vials with approximately 18 ml scintillation fluid (Beckman Ready Safe liquid scintillation cocktail). Each vial was vigorously shaken and then counted for [$C^3H_3$]MTR-1-P for 5 minutes in a Beckman LS 3801 scintillation counter.

The BioRad columns were recharged between each test by two washes with 10 ml 1 N NaOH, two washes with 10 ml $H_2O$, two washes with 10 ml 1 N formic acid, and two washes with 10 ml $H_2O$.

## THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) was performed to determine what products were produced in the positive MTR kinase assays for both redia containing cercariae and adult fluke. The TLC utilized silica gel II, with butanol:acetic acid:water (13:5:2/v/v/v). The tested extracts included positive samples from previous MTR kinase assays for both rediae containing cercariae and adult fluke; controls run without added protein, and standards including MTR, methionine, and alpha 2-keto-4-methylthiobutyrate.

TLC preparation included complete evaporation of ethanol from the MTR kinase assay tubes, followed by addition of 10 ul $H_2O$ to each tube. For quantification of radioactivity, one $cm^2$ samples were scraped from the chromatogram in 1 cm increments, placed in scintillation vials with approximately 18 ml scintillation fluid (Beckman Ready Safe liquid scintillation cocktail), and counted in a Beckman LS 3801 scintillation counter.

## PAPER CHROMATOGRAPHY

Descending paper chromatography was used to determine what products were produced in the positive MTR kinase assay for adult fluke. The test was performed utilizing #1 Whatman paper with butanol:glacial acetic acid:water (12:3:5/v/v/v). The tested extracts included a positive adult fluke MTR kinase assay, control run without added

protein, positive control *Klebseilla pneumonia*, and the standards MTR, methionine, and alpha 2-keto-4-methylthiobutyrate. For quantification of radioactivity, the chromatogram was cut into 1 cm x 3 cm sections which were placed in scintillation vials with approximately 18 ml scintillation fluid (Beckman Ready Safe liquid scintillation cocktail) and counted in a Beckman LS 3801 scintillation counter.

## PROTEIN CONCENTRATION

Protein concentration for the enzyme extracts were calculated according to the method established by Bradford (1976).

# RESULTS

## METHYLTHIOADENOSINE PHOSPHORYLASE ASSAY

The redia containing cercariae of Fasciola hepatica which were incubated with [2,8,5'-$^{3}$H]ClAdo for one hour at 23°C showed no detectable enzyme labelled radioactivity over the control with no added protein. This assay was unrepeated due to the shortage of available redia containing cercariae collected from infected snails.

The initial undialyzed pooled adult fluke extract incubated with [2,8,5'-$^{3}$H]ClAdo for one hour at 23°C showed detectable enzyme labelled radioactivity over the control with no added protein. The molecule that is being counted in the scintillation counter is [5-$^{3}$H]-5-Cl-Ribose-1-P, where the phosphorylase attaches to the labelled compound at the site where the adenine is cleaved. This assay was done a total of nine times with only two reactions showing detectable enzyme labelled radioactivity over the control with no added protein (Table II). Both dialyzed and undialyzed pooled adult fluke extract were used in these assays, with a positive reaction being obtained from both a dialyzed and undialyzed extract.

TABLE II

MTA PHOSPHORYLASE ACTIVITY IN CELL FREE EXTRACTS OF *FASCIOLA HEPATICA*

| EXTRACT SOURCE | DIALYZED/ UNDIALYZED | pH | TEMP. (°C) | TIME (MIN) | LABEL | EXTRACT AMOUNT USED (ul) | ACTIVITY* MICRO MOLES/ MIN/MG |
|---|---|---|---|---|---|---|---|
| POOLED ADULT FLUKE | UNDIALYZED | 7.19 | 23° | 60' | ClAdo | 75 ul | $1.4 \times 10^4$ |
| POOLED ADULT FLUKE | DIALYZED | 7.20 | 23° | 60' | ClAdo | 75 ul | $1.9 \times 10^3$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.00 | 24° | 60' | ClAdo | 155 ul | $1.4 \times 10^3$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.00 | 45° | 240' | MTA | 155 ul | $4.9 \times 10^6$ |
| POOLED ADULT FLUKE | UNDIALYZED | 7.20 | 45° | 270' | MTA | 155 ul | $2.5 \times 10^6$ |
| POOLED ADULT FLUKE | DIALYZED | 7.20 | 45° | 870' | MTA | 155 ul | $2.6 \times 10^6$ |

*Protein concentration of adult fluke was 7.1 mg/ml per Tower.

The MTA phosphorylase and MTA nucleosidase assays were run side by side, so the results of both assays when compared together determined what steps needed to be taken next. We were working under the assumption that adult Fasciola hepatica would show MTA nucleosidase.

Another set of four MTA phosphorylase assays were conducted later in the study. For the first of these assays, the pH was raised to 9.0. This pH change was done to determine if the crude extract would demonstrate MTA phosphorylase at a pH where other enzymes are less active. The reaction for the undialyzed adult fluke produced detectable enzyme labelled radioactivity over the control with no added protein (Table II). The detectable enzyme labelled radioactivity was not as high as for the original undialyzed fluke. Another assay was done at a pH of 9.0 with the label changed to [$^{3}$H-methyl]MTA and the incubation time increased to 4 hours at 45°C. The label was changed as it was thought that 5'-ClAdo might not be a substrate for this particular enzyme. The temperature was raised to 45°C based on positive MTA kinase results we were seeing with this particular organism. The time was increased to insure that what we were seeing was not a heat-induced variation. The assay showed significant increases in detectable enzyme labelled radioactivity over the control without added protein (Table II).

The assay was repeated twice more with the [$^3$H-methyl]MTA label, with the pH back at 7.2 and the incubation times increased to 4.5 hours and 14.5 hours. Both assays used dialyzed flukes and both showed detectable enzyme labelled radioactivity over the controls without added protein.

METHYLTHIOADENOSINE NUCLEOSIDASE ASSAY

The redia containing cercariae incubated with [2,8,5'-$^3$H]ClAdo for one hour at 23°C showed an increase in detectable enzyme labelled radioactivity over the control without added protein (Table III). The molecule that is being counted in the scintillation counter is [5-$^3$H]-5-Cl-Ribose. This assay was only done once due to the available redia containing cercariae collected from infected snails.

The initial undialyzed pooled adult fluke extract incubated with [2,8,5'-$^3$H]ClAdo for one hour at 23°C showed detectable enzyme labelled radioactivity over the control without added protein. This assay was done a total of ten times utilizing both dialyzed and undialyzed pooled adult flukes. Three reactions, two dialyzed and one undialyzed, showed varying amounts of enzyme labelled radioactivity over controls without added protein (Table III).

However, these results combined with the first nine MTA phosphorylase results did not enable us to determine if the

TABLE III

MTA NUCLEOSIDASE ACTIVITY IN CELL FREE EXTRACTS OF *FASCIOLA HEPATICA*

| EXTRACT SOURCE | DIALYZED/ UNDIALYZED | pH | TEMP. (°C) | TIME (MIN) | LABEL | EXTRACT AMOUNT USED (ul) | ACTIVITY* MICRO MOLES/ MIN/MG |
|---|---|---|---|---|---|---|---|
| POOLED REDIA CONTAINING CERCARIAE | UNDIALYZED | 6.50 | 23° | 60' | ClAdo | 75 ul | $2.9 \times 10^{6}$ |
| POOLED ADULT FLUKE | UNDIALYZED | 6.43 | 23° | 60' | ClAdo | 75 ul | $2.4 \times 10^{5}$ |
| POOLED ADULT FLUKE | DIALYZED | 6.69 | 24° | 60' | ClAdo | 75 ul | $1.4 \times 10^{6}$ |
| POOLED ADULT FLUKE | DIALYZED | 7.20 | 23° | 60' | ClAdo | 75 ul | $2.3 \times 10^{5}$ |

*Protein concentration of redia containing cercariae was 0.3 mg/ml and for adult fluke 7.1 mg/ml per Tower.

adult fluke of *Fasciola hepatica* had MTA phosphorylase or MTA nucleosidase action. The pH of the next assay was raised to 9.0, this showed no detectable enzyme labelled radioactivity over controls without added protein. Another assay was done where the label was changed to [$^3$H-methyl]MTA and the time and temperature increased to 4 hours at 45°C. This produced a detectable enzyme labelled radioactivity for undialyzed adult flukes over the control without added protein (Table III).

The final two assays were done with the pH back at 7.2 and the temperature at 45°C with incubation times of 4.5 and 14.5 hours. Both of these assays showed no detectable enzyme labelled radioactivity over the controls without added protein.

## METHYLTHORIBOSE KINASE ASSAY

All MTR kinase assays involved three variables that were varied: incubation time, temperature, and buffer pH. The first assays were run at physiological temperatures, one hour incubations, and neutral pH's. However, these three variables were altered if no positive results were obtained at physiological values. The particular enzyme activity we were seeking could be less apparent should another enzyme be showing more activity at that particular variable combination.

For redia containing cercariae a total of five assays were performed with each including a control that lacked added protein. An assay that varied pH showed a detectable enzyme labelled radioactive level over the control at a pH of 9.17 when incubated one hour at 23°C (Table IV). Further assays that varied temperatures showed detectable enzyme labelled radioactivity at a pH of 9.17 at incubation times of one hour at temperatures of 22°C and 57°C (Table IV). Additional assays for redia containing cercariae were impossible to perform due to a shortage of available extract.

For the pooled adult fluke, the MTR kinase assays were done also utilizing the changeable variables: incubation time, temperature, and pH. A total of eighteen assays were conducted, including six for pH, five for temperature, and seven for time. All assays were run with controls that lacked added protein, and many but not all assays were run with a known MTR kinase positive control, <u>Klebsiella pneumonia</u>.

Of the pH assays run, four out of the six assays showed detectable enzyme labelled radioactivity. The pH's that showed positive were at 9.0 and 8.8. Both showed detectable enzyme labelled radioactivity on at least two assays (Table V). The incubation times for these assays were one hour at temperatures of either 40°C or 45°C. Positive results came from both dialyzed and undialyzed extracts.

TABLE IV

MTR KINASE ACTIVITY IN CELL FREE REDIA CONTAINING CERCARIAE EXTRACTS OF _FASCIOLA HEPATICA_

| EXTRACT SOURCE | DIALYZED/ UNDIALYZED | pH | TEMP. (°C) | TIME (MIN) | LABEL | EXTRACT AMOUNT USED ( ul) | ACTIVITY* MICRO MOLES/ MIN/MG |
|---|---|---|---|---|---|---|---|
| POOLED REDIA CONTAINING CERCARIAE | UNDIALYZED | 9.17 | 23° | 60' | MTR | 50 ul | $3.6 \times 10^{7}$ |
| POOLED REDIA CONTAINING CERCARIAE | UNDIALYZED | 9.17 | 57° | 60' | MTR | 50 ul | $5.1 \times 10^{7}$ |
| POOLED REDIA CONTAINING CERCARIAE | UNDIALYZED | 9.17 | 22° | 60' | MTR | 50 ul | $2.2 \times 10^{7}$ |

*Protein concentration of redia containing cercariae was 0.3 mg/min per Tower.

TABLE V

MTR KINASE ACTIVITY IN CELL FREE ADULT FLUKE EXTRACTS OF *FASCIOLA HEPATICA* INCUBATED WITH VARIABLE PH'S

| EXTRACT SOURCE | DIALYZED/ UNDIALYZED | pH | TEMP. (°C) | TIME (MIN) | LABEL | EXTRACT AMOUNT USED (ul) | ACTIVITY* MICRO MOLES/ MIN/MG |
|---|---|---|---|---|---|---|---|
| POOLED ADULT FLUKE | DIALYZED | 8.89 | 45° | 60' | MTR | 25 ul | $3.0 \times 10^5$ |
| POOLED ADULT FLUKE | DIALYZED | 9.00 | 40° | 60' | MTR | 25 ul | $4.2 \times 10^5$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.00 | 45° | 60' | MTR | 165 ul | $1.8 \times 10^5$ |
| POOLED ADULT FLUKE | UNDIALYZED | 8.86 | 45° | 60' | MTR | 165 ul | $1.8 \times 10^5$ |

*Protein concentration of adult fluke was 7.1 mg/min per Tower.

For the temperature assays, three out of the five assays showed detectable enzyme labelled radioactivity over controls without added protein. The favored temperature was 45°C on all assays at an incubation time of one hour and a pH of either 9.0 or 9.14 (Table VI). Again, both dialyzed and undialyzed extracts showed positive results.

Of the incubation time assays, six out of the seven assays showed detectable enzyme labelled radioactivity over controls without added protein. The favored times for the adult fluke extracts were 20 minutes on three assays, and 60 minutes on five assays (Table VII). The pH was either 9.0 or 9.14 at a temperature of 45°C. Both dialyzed and undialyzed extracts showed detectable enzyme labelled radioactivity for 20 and 60 minutes.

## THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was used to determine [5-$^3$H]-5-Cl-Ribose-1-P was catalyzed in the positive MTR kinase assays incubated with redia containing cercariae and adult <u>Fasciola</u> <u>hepatica</u>. With the redia containing cercariae the [$^3$H-methyl]MTA was converted to a radioactive compound which remained near the origin and had an $R_f$ value of 0.12. In the utilized system AdoMet has an $R_f$ value of 0.09. A control of incubation mixture with no added protein, was also plated, giving an $R_f$ value of 0.71. In the utilized system MTR had a $R_f$ value of 0.75. The

TABLE VI

MTR KINASE ACTIVITY IN CELL FREE ADULT FLUKE EXTRACTS OF _FASCIOLA HEPATICA_ INCUBATED WITH VARIABLE TEMPERATURES

| EXTRACT SOURCE | DIALYZED/ UNDIALYZED | pH | TEMP. (°C) | TIME (MIN) | LABEL | EXTRACT AMOUNT USED (ul) | ACTIVITY* MICRO MOLES/ MIN/MG |
|---|---|---|---|---|---|---|---|
| POOLED ADULT FLUKE | UNDIALYZED | 9.14 | 45° | 60' | MTR | 100 ul | $1.4 \times 10^6$ |
| POOLED ADULT FLUKE | DIALYZED | 9.00 | 45° | 60' | MTR | 25 ul | $2.8 \times 10^6$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.00 | 45° | 60' | MTR | 165 ul | $1.2 \times 10^6$ |

*Protein concentraction of adult fluke was 7.1 mg/min per Tower.

TABLE VII

MTR KINASE ACTIVITY IN CELL FREE ADULT FLUKE EXTRACTS OF *FASCIOLA HEPATICA* INCUBATED AT VARIABLE TIMES

| EXTRACT SOURCE | DIALYZED/ UNDIALYZED | pH | TEMP. (°C) | TIME (MIN) | LABEL | EXTRACT AMOUNT USED (ul) | ACTIVITY* MICRO MOLES/ MIN/MG |
|---|---|---|---|---|---|---|---|
| POOLED ADULT FLUKE | DIALYZED | 9.14 | 37° | 20' | MTR | 25 ul | $1.5 \times 10^{7}$ |
| POOLED ADULT FLUKE | DIALYZED | 9.14 | 37° | 60' | MTR | 25 ul | $4.5 \times 10^{6}$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.14 | 37° | 20' | MTR | 25 ul | $1.5 \times 10^{6}$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.14 | 45° | 20' | MTR | 25 ul | $7.5 \times 10^{6}$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.14 | 45° | 60' | MTR | 25 ul | $1.2 \times 10^{7}$ |
| POOLED ADULT FLUKE | DIALYZED | 9.14 | 45° | 60' | MTR | 25 ul | $2.2 \times 10^{6}$ |
| POOLED ADULT FLUKE | DIALYZED | 9.14 | 45° | 60' | MTR | 25 ul | $1.4 \times 10^{7}$ |
| POOLED ADULT FLUKE | UNDIALYZED | 9.00 | 45° | 60' | MTR | 165 ul | $2.0 \times 10^{6}$ |

*Protein concentraction of adult fluke was 7.1 mg/min per Tower.

standards MTR, methionine and 2-keto-4-methylthiobutyrate were also all plated.

The pooled adult fluke extract converted to two radioactive compounds having $R_f$ values of 0.65 and 0.71. These two peaks were near the expected MTR $R_f$ value of 0.75 and at the control without added extract $R_f$ value of 0.71.

## PAPER CHROMATOGRAPHY

Paper chromatography was used to obtain a more reliable separation of compounds than was seen with the pooled adult fluke on thin layer chromatography. An adult fluke extract that showed a positive MTR kinase result was subjected to descending paper chromatography. In addition, control without added protein, Klebsiella pneumonia, a positive control, and the standards MTR, methionine and 2-keto-4-methylthiobutyrate were also all tested.

The adult fluke positive MTR kinase incubation mixture converted to one radioactive compound at an $R_f$ value of 0.65. The MTR standard had a peak of 0.68 which was close to the expected $R_f$ value of 0.67 for the utilized system. The Klebsiella pneumonia extract had a $R_f$ value at 0.69 and second $R_f$ value at 0.81.

## DISCUSSION

The metabolism of methionine by Fasciola hepatica adult flukes and the larval stages, rediae containing cercariae were of interest since these parasites exist in an environment where methionine is in short supply. Methionine is a sulfur-containing amino acid that plays an important role in a variety of cellular functions including protein synthesis, transmethylation, and polyamine biosynthesis. The ability of an organism to recycle this compound allows cellular functions to continue even when environmental methionine is low.

The methionine cycle includes MTA as a co-product of polyamine synthesis. MTA does not accumulate in cells but is rapidly degraded by one of two different pathways dependent upon the metabolism of the organism. Mammalian cells and several microorganisms use MTA phosphorylase while in other microbes, MTA nucleosidase and MTR kinase are utilized.

Fasciola hepatica as a digenetic trematode posed an interesting question as to which MTA degradation pathway it would follow during its two distinct lifecycles, the same or different metabolic pathways. No published reports could be found concerning methionine metabolism in trematodes, cestodes, and nematodes. Since Fasciola hepatica has two

distinct life forms, redia containing cercariae and adult, a postulation was put forward that it could be conceivable but not probable that at different times in the flukes' life cycle there could be utilization of the two different degradation pathways.

Redia containing cercariae of _Fasciola_ _hepatica_ were tested first for both MTA phosphorylase and MTA nucleosidase. The MTA phosphorylase assay incubated with [2,8,5'-$^{3}$H]ClAdo produced no increase in radioactive counts, while the MTA nucleosidase assay incubated with the same label produced significant increases in radioactive counts.

Methylthioribose kinase assay was then performed upon the redia containing cercariae extract, based on the positive assay at a pH of 9.14 incubated at one hour at 24°C, further assays were taken over a temperature range. Significant results for the assay were obtained at a temperature of 56°C.

Thin layer chromatography was done on positive redia containing cercariae cell free extract kinase assays to determine if MTR had gone on to MTR-1-P or beyond indicating a definite MTA nucleosidase/MTR kinase pathway. Good separation on the plate showed that MTA in the redia containing cercariae extract was indeed recycled back to AdoMet via MTA nucleosidase/MTR kinase mechanism. This result was consistent with those results as reported by Sugimoto, et al., (1976) for _O_. _malhamensis_, Fitchen, et

al., (1987) for P. falciparum, and Kushad, et al., (1983) for P. americana and L. esculentum.

Dialyzed and undialyzed adult fluke extract were also assayed for both MTA phosphorylase and MTA nucleosidase. Initial results showed radioactivity for both assays which is an indication of MTA nucleosidase. For it to be MTA phosphorylase it must have an absolute zero background in the absence of added phosphate for a dialyzed extract. These initial results tended to support the idea that Fasciola hepatica in all its life stages utilized the MTA nucleosidase/MTR kinase mechanism for recycling MTA.

Based on the results of the MTA phosphorylase and MTA nucleosidase assays and our expectations, MTR kinase assays were undertaken. Initial MTR kinase assay results were not consistently positive until the variables; temperature, pH, and finally incubation times were changed. Increased radioactivity was noted at a pH range of 8.8 to 9.29. At this pH range the Tris buffer used previously was not dependably accurate, so an Imidazole buffer was substituted. Temperature ranges using this pH revealed increased radioactivity a 45°C. The temperatures were changed beyond physiological boundaries due to the possibility that other enzymes might be more active at physiological temperatures than the enzyme we were investigating. Also, we only wanted to determine the presence or absence of the enzyme and not define its optional working range. Finally, time ranges

showed peak activity at one hour incubation in our assays, beyond that time activity levels dropped off considerably.

Thin layer chromatography and descending paper chromatography were both performed on cell free extracts of positive adult flukes kinase assays to determine if MTR was metabolized. Separation by TLC and paper chromatography showed very little or no movement of the MTR in the cell free extract from the expected MTR $R_f$ value. Thus, definite MTR kinase activity could not be proved for the adult stage of Fasciola hepatica.

This inability to obtain a definite MTR kinase activity on a chromatograph lead to reevaluation of the results of the MTA phosphorylase and MTA nucleosidase assays. With the dialyzed adult extract, no radioactive activity could be detected for either assay utilizing the previous assay parameters. Changes of the radioactive label to [$^3$H-methyl]MTA and increase in the incubation time to 4 hours produced a positive MTA phosphorylase assay. This positive result for MTA phosphorylase is consistent with results for human serum (Russo, et al., 1987), and some eubacteria as described by Shimizu, et al., (1988).

This positive MTA phosphorylase assay result was surprising and not expected. Further incubation times of 4.5 hours and 14.5 hours at a lower pH with the MTA label also showed positive MTA phosphorylase assays but at a lower level from the 4 hour incubation. MTA nucleosidase assay

still continued to show moderate activity at 4 hours with the MTA level, but this disappeared at the increased incubation times of 4.5 and 14.5 hours.

Reevaluation of the results from the TLC and paper chromatography supported the conclusion that adult *Fasciola hepatica* does not convert MTR to MTR-1-P. This lack of conversion of MTR is an expected result of an organism that utilizes the MTA phosphorylase pathway of methionine recycling. These results would support the idea that not all life stages of *Fasciola hepatica* utilize the same mechanism for recycling methionine.

## LITERATURE CITED


Abeles, F.B. 1973. Ethylene in Plant Biology. p 302. Academic, New York.

Adams, D.O., & S.F. Yang. 1977. Methionine metabolism in apple tissue: Implication of S-adenosylmethionine as an intermediate in the conversion of methionine to ethylene. Plant Physiol. 60:892-896.

Barrett, J., Coles, G.C., & K.G. Simpkin. 1978. Pathways of acetate and propionate production in adult Fasciola hepatica. Int. J. Parasit. 8:117.

Boray, J.C. 1969. Experimental fascioliasis in Australia. Adv. Parasit. 7:96-210.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Cacciapuoti, G., Oliva, H., & V. Zappia. 1978. Studies on phosphate-activated 5'-methythioadenosine nucleosidase from human placenta. Int. J. Biochem. 9:35-41.

Chu, T.M., Mallette, M.F., & R.O. Mumman. 1968. Isolation and characterization of 5'-S'-methyl-5'-thioadenosine from Escherichia coli. Biochem. 7:1399-1406.

Cooper, A.J.L. 1983. Biochemistry of sulfur-containing amino acids. Annal. Rev. Biochem. 53:187-222.

Duerre, J.A., Miller, C.H., & G.C. Reams. 1969. Metabolism of S-adenosyl-L-homocysteine in vivo by the rat. J. Biol. Chem. 237:3737-3741.

Ferro, A.J., Barrett, A., & S.K. Shapiro. 1978. 5-Methylthioribose kinase: A new enzyme involved in the formation of methionine from 5-methylthioribose. J. Biol. Chem. 253:6021-6025.

Fitchen, J.H., Riscoe, M.K., & A.J. Ferro. 1987. Exploitation of methylthioribose kinase in the development of antiprotozoal drugs. pp. 199-209. In: Progress in Polyamine Research, Plenum Press, New York.

Garbers, D.L. 1978. Demonstration of 5'-methylthioadenosine phosphorylase activity in various rat tissues. Some properties of the enzyme from rat lung. Biochem. Biophys. Acta. 523:82-93.

Ghoda, L.Y., Savarese, T.M., Northup, C.H., Parks, R.E., Garofalo, J., Katz, L., Ellenbogen, B.B., & C.J. Bacchi. 1988. Substrate specificities of 5' deoxy-5'-methyl-thioadenosine phosphorylase from Trypanosoma Brucei brucei and mammalian cells. Mol. Biochem. Parasit. 27:109-118.

Gianotti, A.J., Tower, P.A., Sheley, J.A., Conte, P.A., Spiro, C., Ferro, A.J., Fitchen, J.H. & M.K. Riscoe. 1990. Selective killing of Klebsiella pneumoniae by 5-trifluoromethylthioribose: Chemotherapeutic exploitation of the enzyme 5-methylthioribose kinase. J. Biol. Chem. in press.

Grembergen, G. van. 1949. Le metabolsme respiratoire du trematode Fasciola hepatica Linn. Enzymologia 13:241-257.

Guranowski, A. 1983. Plant 5-methythioribose kinase. Plant Physiol. 71:932-935.

Isseroff, H., Sawma, J.T., & K. Reino. 1977. Fascioliasis: Role of proline in bile duct hyperplasia. Science 198:1157-1159.

Kurelec, B. & M. Rijavec. 1966. Amino acid pool of the liver fluke (Fasciola hepatica L.) Comp. Biochem. Physiol. 19:529.

Kushad, M.M., Richardson, D.G., & A.J. Ferro. 1982. 5-Methylthioribose kinase activity in plants. Biochem. Biophys. Res. Commun. 108:167.

Kushad, M.M., Richardson, D.G., & A.J. Ferro. 1983. Intermediates in the recycling of 5-methylthioribose to methionine in fruits. Plant Physiol. 73:257.

Kushad, M.M., Richardson, D.G., & A.J. Ferro. 1985 5'-methylthioadenosine nucleosidase and 5-methylthioribose kinase activities and ethylene production during tomato fruit development and ripening. Plant Physiol. 79:525-529.

Lahoud, H., Prichard, R.K., McManus, W.R., & P.J. Schfield. 1971. Volatile fatty acid production by the adult liver fluke Fasciola hepatica. Comp. Biochem. Physiol. 38B:379-391.

Lloyd, G.M. 1986. Energy metabolism and its regulation in the adult liver fluke Fasciola hepatica. Parasit. 93:217-248.

Mansour, T.E. 1959. Studies on the carbohydrate metabolism of the liver fluke Fasciola hepatica. Biochem. Biophys. Acta. 34:456-464.

Miller, R.L. & D.P. Toorchen. 1988. MTA phosphorylase in protozoa: A potential target for chemotherapeutic attack. pp211-218. In: Progress in Polyamine Research. Plenum Press, New York.

Moss, G.D. 1970. The excretory metabolism of the endo-parasitic digenean Fasciola hepatica and its relationship to its respiratory metabolism. Parasit. 60:13.

Pantelouris, E.M. 1965. The common liver fluke, Fasciola hepatica. chapter 11, Pergamen Press, New York.

Pegg, E., & H.G. Williams-Ashman. 1969. Phosphate-stimulated breakdown of 5-methylthioadenosine by rat ventral prostate. J. Biol. Chem. 115:241-247.

Rhodes, J.B., & H.G. Williams-Ashman. 1964. Enzyme of the citric acid cycle. Med. Exp. 54:281-285.

Russo, G.L., Ragione, F.S., Utili, R., Andreana, A., Ruggiero, G., & V. Zappia. 1987. Studies on human serum 5'-deoxy-5'-methylthioadenosine phosphorylase: Molecular properties and clinical perspectives. pp229-238. In: Progress in Polyamine Research, Plenum Press, New York.

Sakayu, S., Takaharu, A.,Y. Hideaki. 1988. Distribution of methylthioadenosine phosphorylase in eubacteria. FEMS Micro. Letters. 51:117-180.

Schlenk, F. 1983. Methioadenosine. p 195. In: Advances in Enzymology and Related Area of Molecular Biology. A. Meister, Ed., John Wiley & Sons, New York.

Shapiro, S.K., & A. Barrett. 1981. 5-methylthioribose as a precurosor of the carbon chain of methionine. Biochem. Biophys. Res. Commun. 102:302-307.

Shimizu, S., Abe, T., & H. Yamada. 1988. Distribution of methylthioadenosine phosphorylase in eubacteria. FEMS Micro. Letters. 51:177-180.

Sinclair, K.B. 1967. Pathogenesis of fasciola and other liver flukes. Helmin. Abst. 36-2:122.

Sugimoto, Y., Toraya, T., & S. Fukui. 1976. Studies on metabolic role of 5'-methylthioadenosine in Ochromonas malhamensis and other microorganisms. Arch. Micro. 108:175-182.

Von Brand, T. 1966. Biochemistry of Parasites. p. 116 Academic Press, New York.

Wang, S.Y., Adams, D.O., & M. Leiberman. 1982. Recycling of 5'-methylthioadenosine-ribose carbon atoms into methionine in tomato tissue in relation to ethylene production. Plant Physiol. 70:117-121.

White, M.W., Vandenbark, A.A., Barney, C.L., & A.J. Ferro. 1982. Structural analogs of 5'-methylthioadenosine phosphorylase and as inhibitors of human lymphocyte transformation. Biochem Pharmacol. 31:503-507.

Yang, S.F. 1974. In: The Chemistry and Biochemistry of Plant Hormones. pp 131-164. V.C. Runeckles, E. Sondheimer, & D.C. Walton, Eds., Academic, New York.

Yung, K.H. & S.F. Yang. 1982. Methionine synthesis from 5-methylthioribose in apple tissue. Biochem. Biophys. Res. Commun. 104:771-777

Zappia, V., Oliva, A., Cacciapuoti, G., Galletti, P., Mignucci, G., & M. Carteni-Farina. 1978. Substrate specificity of 5'-methylthioadenosine phosphorylase from human prostate. Biochem. J. 175:1043-1050.