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Distribution of S-Adenosylhomocysteine Hydrolase, S-Adenosylhomocysteine Nucleosidase, S-Ribosylhomocysteine Cleavage Enzyme and Homoserine Dehydratase in Various Species

Robert D. Walker

DISTRIBUTION OF S-ADENOSYLHOMOCYSTEINE HYDROLASE, S-ADENOSYLHOMOCYSTEINE NUCLEOSIDASE, S-RIBOSYLHOMOCYSTEINE CLEAVAGE ENZYME AND HOMOSERINE DEHYDRATASE IN VARIOUS SPECIES

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This thesis submitted by Robert D. Walker in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota is hereby approved by the Faculty Advisory Committee under whom the work has been done.

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ABSTRACT

Microorganisms, plants and the major organs from cold and warm blooded vertebrates were assayed for the presence of S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase.

S-Adenosylhomocysteine hydrolase was found in all yeasts and plants assayed and in the livers of all cold blooded vertebrates assayed except the Polyodon spathula (Paddlefish). Liver extracts from all warm blooded vertebrates except the Odocoileus hemionus (Mule deer), Odocoileus virginianus (Whitetail deer) and Homo sapiens(human) were also found to possess S-adenosylhomocysteine hydrolase. This enzyme was also found in the spleens of the Ictalurus punctatus (Channel cat), Perca flavescens (Yellow perch) and Bos (steer) and in the kidneys of the Ictiobus bubalus (Buffalohead), Ictalurus punctatus (Channel cat), Thamnophis sirtalis (Garter snake), Anas platyrhynchos (Rouen duck), Totanus flavipes (Lesser Yellowleg), Rattus rattus (white rat), Oryctolagus cuniculus (New Zealand white rabbit) and Canis familiaris (dog). There was no hydrolase activity found in brains or hearts of any of the vertebrates assayed. S-Adenosylhomocysteine hydrolase was also lacking in all bacteria assayed.

S-Adenosylhomocysteine nucleosidase activity was not detectable in any yeasts, plants or vertebrate organs assayed. This enzyme was present in all bacteria assayed except Bacillus subtilis.

S-Ribosylhomocysteine cleavage enzyme was lacking in all extracts assayed except Citrobacter freundii, Enterobacter aerogenes, Escherichia coli and Proteus vulgaris.

Homoserine dehydratase was found in Bacillus subtilis, Bacillus cereus, Sarcina lutea and in all liver extracts except those of Ictiobus bubalus (Buffalohead), Galus galus (White Leghorn chicken), Pedioecetes phasianellus (Sharptail grouse), Cavia porcellus (Guinea pig), Oryctolagus cuniculus (New Zealand white rabbit) and Canis familiaris (dog). This enzyme was also found in brain extract of the Anas platyrhynchos (Rouen duck) and kidney extracts of Totanus flavipes (Lesser Yellowleg), Rattus rattus (white rat) and Odocoileus virginianus (Whitetail deer). Homoserine dehydratase was lacking in Gram negative bacteria, yeasts, plants and from hearts and spleens of all vertebrates assayed.

Intravenous injections of S-adenosylhomocysteine into an anesthetized dog resulted in a rapid excretion of this compound in the urine with minimal retention by any organ assayed. As the liver and kidney had been found to contain S-adenosylhomocysteine hydrolase these results suggested that this enzyme is inoperative against exogenous S-adenosylhomocysteine.

INTRODUCTION

S-Adenosyl-L-methionine is the primary source of methyl groups for transmethylation reactions. S-Adenosylmethionine is formed through the condensation of L-methionine and ATP which is catalyzed by L-methionine-S-adenosyltransferase. The methylsulfonium linkage of S-adenosylmethionine has high energy characteristics resulting in the methyl group being readily donated to innumerable methyl group acceptors in the presence of an appropriate enzyme. These reactions transect nearly every area of metabolism and are widespread throughout nature being found in biological systems from microorganisms to man.

The demethylated product found in all transmethylation reactions involving S-adenosylmethionine is S-adenosylhomocysteine. The metabolism of S-adenosylhomocysteine has been shown to involve at least two different enzymatic pathways. One pathway has been found in some mammalian livers and yeasts. In this pathway S-adenosylhomocysteine hydrolase cleaves S-adenosylhomocysteine to yield adenosine and homocysteine. This is a reversible reaction with synthesis being greatly favored.

The other pathway has been found in Gram negative

bacteria and involves two enzymes. One enzyme, S-adenosylhomocysteine nucleosidase, cleaves the glycosidic bond of S-adenosylhomocysteine to yield S-ribosylhomocysteine and adenine. The ribosylhomocysteine moiety is further metabolized by cleavage of the thioether linkage by the enzyme, S-ribosylhomocysteine cleavage enzyme, to yield a pentose and homocysteine.

Homocysteine, one of the end products of both pathways, may then be methylated to methionine or coupled with serine by the condensing action of cystathionine synthetase to form cystathionine. Cystathionine is further metabolized to cysteine and homoserine by homoserine dehydratase with homoserine being rapidly converted to α-ketobutyric acid.

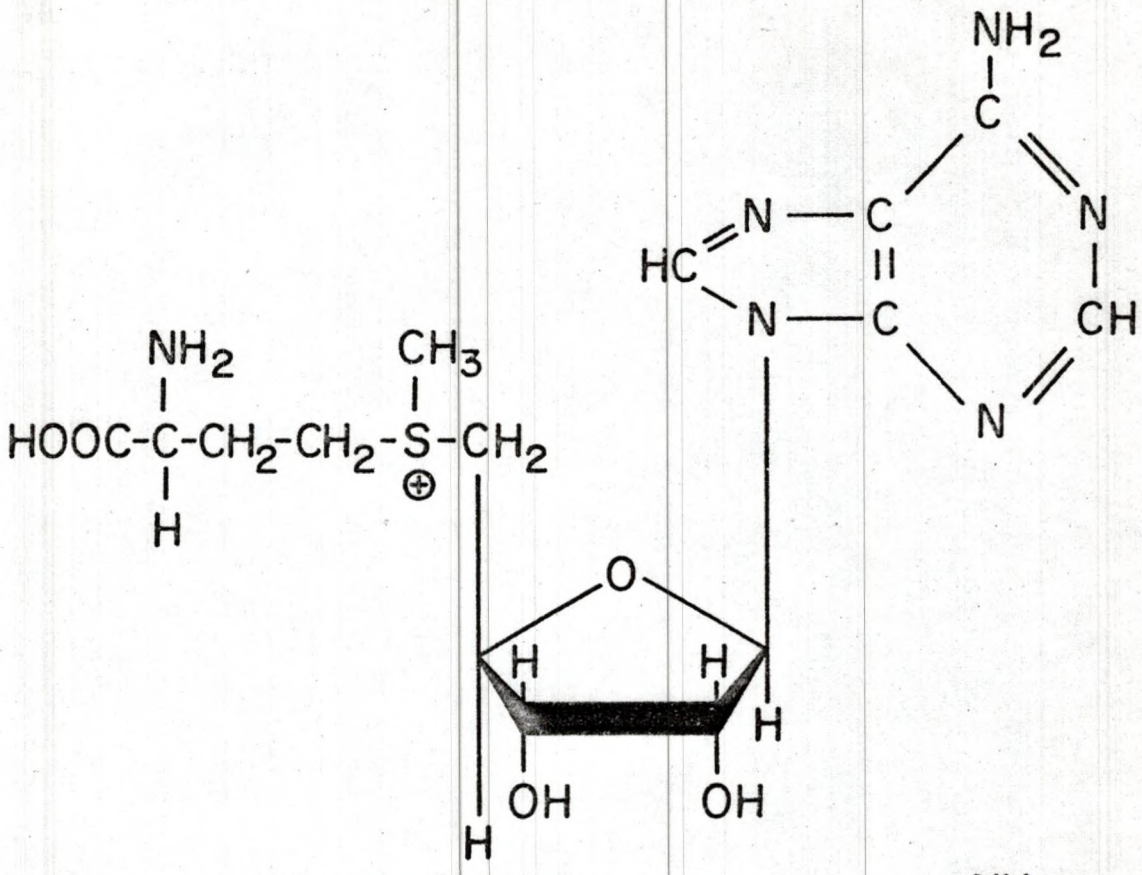
The purpose of this investigation was to determine the relative distribution of the enzymes, S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase in living systems.

HISTORICAL REVIEW

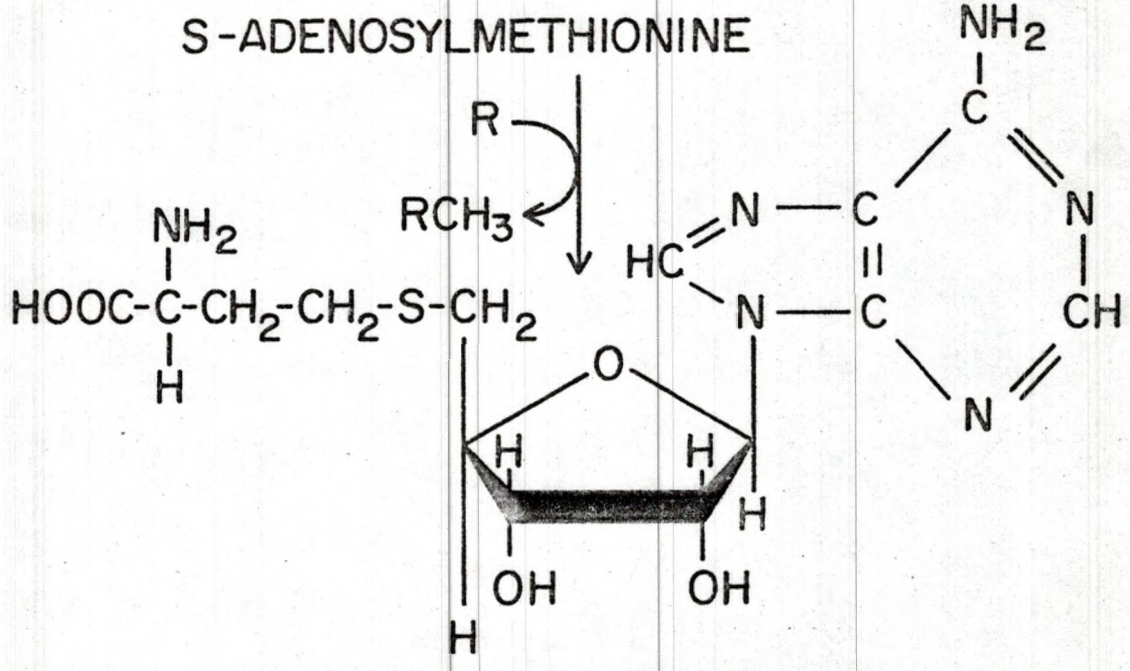
S-Adenosyl-L-homocysteine Formation

S-Adenosyl-L-homocysteine is the demethylated product of all transmethylation reactions involving S-adenosylmethionine. The generalized equation for this reaction is outlined under Reaction 1. The transfer of the methyl group from S-adenosylmethionine has been shown to be involved in innumerable reactions including methylation of nucleic acids, nicotinamide, nornicotine, guanidinoacetate, carnosine, histamine, creatine and choline (1,2,3). Du Vigneaud and Rachele (4) postulated that these transmethylation reactions transect nearly every area of metabolism being widespread throughout nature from microorganisms to man.

S-Adenosylhomocysteine was first characterized in 1954 by Cantoni and Scarano (5). They incubated S-[^{35}S] adenosylmethionine and guanidinoacetate with liver guanidinoacetate methyltransferase (S-adenosylmethionine guanidinoacetate N-methyltransferase, EC 2.1.1.2). After stopping the reaction unreacted S-[^{35}S]adenosylmethionine was removed by precipitation with ammonium reineckate. The resultant supernatant fluid was acidified and applied to a column of Norite A. After washing the column with water, aqueous pyridine was used to elute the radioactive material. Con-



S-ADENOSYLMETHIONINE



S-ADENOSYLHOMOCYSTEINE

REACTION I

taminating creatine, guanidinoacetate and pyridine were separated from the radioactive compound by paper chromatography. The resultant S-adenosyl-L-homocysteine was found to give a positive test in the quantitative ninhydrin reaction and the nitroprusside reaction. This compound also had an ultraviolet absorption spectrum characteristic of an adenine nucleoside with a maximum absorption at 260 mμ. Duerre (6) using various modifications of the procedures outlined by de la Haba and Cantoni (7) crystallized S-adenosyl-L-homocysteine. The structure was corroborated by total synthesis by Baddiley and Jamieson (8).

S-Adenosyl-L-homocysteine Hydrolase

The metabolic role of S-adenosylhomocysteine was first reported by Ericson, Williams and Elvehjem (9). In 1955 they incubated S-adenosylhomocysteine with rat liver homogenate and were able to isolate homocysteine from the reaction mixture. De la Haba and Cantoni (7) later identified an enzyme (S-adenosylhomocysteine hydrolase EC 3.3.1.1) in rat liver crude extracts which catalyzed the condensation of adenosine and L-homocysteine to yield S-adenosylhomocysteine. They proposed the following reaction:

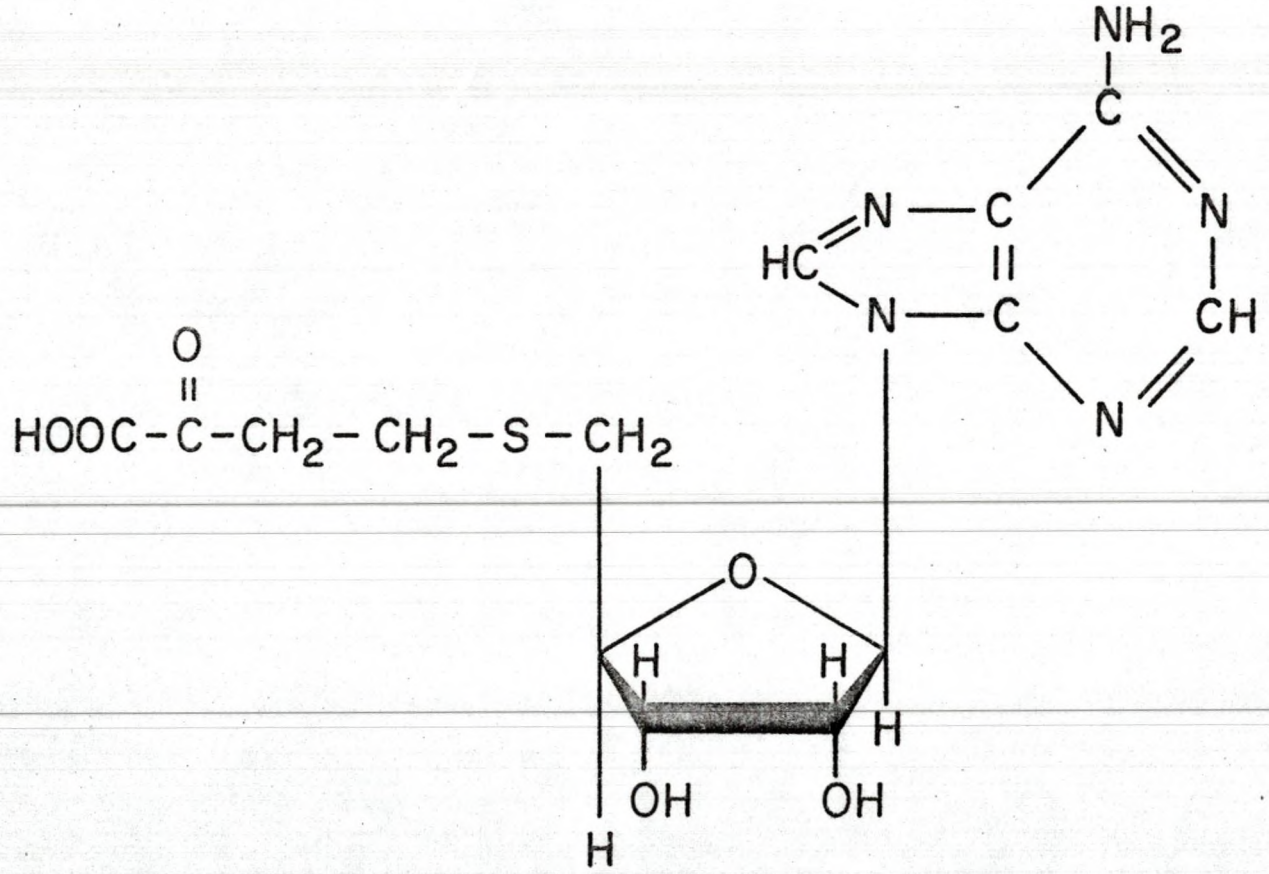
Adenosine + L-homocysteine $\rightleftharpoons$ S-Adenosyl-L-homocysteine

Reaction 2

They found that the reaction was reversible with synthesis being greatly favored.

In vivo studies into the metabolic role of S-adenosylhomocysteine in the white rat were performed by Duerre, Miller and Reams (10). With intravenous injections of S-adenosyl-L-[^{3}H] homocysteine they found that less than 15% of the radioisotope was incorporated into protein methionine or excreted in the urine as α-ketobutyrate. The remaining isotope was found associated with a keto acid they identified as S-adenosyl-γ-thio-α-ketobutyrate (Formula I). These results indicated that S-adenosylhomocysteine undergoes direct deamination in vivo. Duerre et al (10) also performed perfusion studies that showed S-adenosylhomocysteine could not permeate liver cells. Additional perfusion studies using equimolar amounts of adenosine and L-homocysteine resulted in the formation of S-adenosyl-L-homocysteine indicating that liver cells were permeable to both adenosine and L-homocysteine. The formation of S-adenosylhomocysteine within liver cells supported de la Haba and Cantoni's findings of the condensing enzyme, S-adenosylhomocysteine hydrolase in rat liver cells.

An important finding in this study by Duerre et al (10) was that S-adenosylhomocysteine which was formed in other organs would not undergo hydrolysis in the liver. Instead it would be oxidatively deaminated to S-adenosyl-γ-thio-α-ketobutyrate in the kidney and eliminated in the urine. This would indicate that the utilization of homo-



S-ADENOSYL-γ-THIO-α-KETOBUTYRIC ACID

FORMULA I

cysteine from S-adenosyl-L-homocysteine in the mammalian system was not very efficient unless S-adenosylhomocysteine hydrolase could be found in other organs.

The metabolism of S-adenosyl-L-homocysteine in yeast was investigated by Duerre and Schlenk (11) who prepared cell free extracts of Candida utilis and Saccharomyces cerevisiae and incubated these extracts with adenosine and L-homocysteine. They found that S-adenosyl-L-homocysteine was formed in a manner similar to that found by de la Haba and Cantoni (7) in cell free extracts from rat liver. These results were further substantiated by Duerre (12) when he incorporated S-[$^{14}C_8$]adenosyl-L-homocysteine into the growth medium of Saccharomyces cerevisiae. He found that S-[$^{14}C_8$]adenosine accumulated in the cell and was utilized in the formation of ATP. When S-adenosyl-L-[^{3}H]homocysteine was incorporated into the media radioactive homocysteine, homocystine and methionine were isolated from spent media. He also found that cell free extracts catalyzed the formation of L-homocysteine from S-adenosylhomocysteine.

In 1972 Knudsen and Yall (13) partially purified S-adenosylhomocysteine hydrolase from yeast ghost cells. They found that S-adenosylhomocysteine could be readily synthesized from adenosine and homocysteine, but that only negligible amounts of S-adenosylhomocysteine were hydrolyzed. As a result of their study Knudsen and Yall suggested that S-adenosylhomocysteine could be hydrolyzed by S-adenosylhomocysteine hydrolase only when this enzyme acted in coordination with enzyme that converted homocysteine and

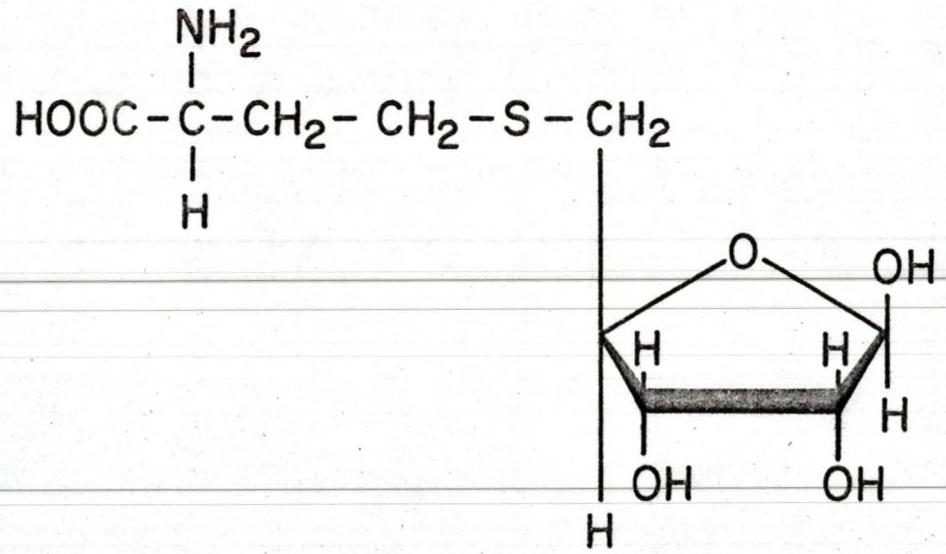
adenosine to other products. These results supported the results of Duerre and Schlenk (11) and Duerre (12) and clearly demonstrated the presence of the condensing and hydrolysing action of S-adenosylhomocysteine hydrolase in yeasts.

S-Adenosylhomocysteine Nucleosidase

Duerre (14) demonstrated the presence of an enzyme in Gram negative bacteria, that cleaved the glycosidic linkage of S-adenosylhomocysteine yielding adenine and a compound identified as ribosylhomocysteine (Formula II). In this study cell free extracts obtained from Escherichia coli, Enterobacter aerogenes and Salmonella typhimurium, treated with deoxyribonuclease and ribonuclease, were incubated with S-adenosylhomocysteine. Reaction products were identified by eluting the deproteinized reaction mixture through a Dowex 50-H^+ column and comparing the elution pattern with reference compounds obtained through acid hydrolysis of S-adenosylhomocysteine (6). This enzyme was also found to cleave 5'-methylthioadenosine. Shapiro and Mather (15) had previously reported that methylthio-adenosine was hydrolysed to methylthioribose and adenine in extracts from Enterobacter aerogenes. The mechanism of the reaction involved in the cleavage of the glycosidic bond of S-adenosylhomocysteine is as follows:

S-Adenosyl-L-homocysteine $\longrightarrow$ Adenosine + S-Ribosyl-L-homocysteine

Reaction 3



S-RIBOSYLHOMOCYSTEINE

FORMULA II

Duerre (14) found that this reaction was non-reversible. He based this conclusion on results he obtained when he incubated the purified enzyme with adenine and S-ribosylhomocysteine and found no measurable S-adenosylhomocysteine. He also failed to obtain labeled S-adenosylhomocysteine when he incubated S-[$^{14}C_8$]adenine with the reaction mixture and allowed the enzymatic hydrolysis of S-adenosylhomocysteine to proceed to 50% completion.

Shapiro (1) investigated the function of S-adenosylmethionine using methionine mutants of Enterobacter aerogenes and found that mutant 62 utilized S-adenosylhomocysteine for growth by cleaving S-adenosylhomocysteine to adenine and ribosylhomocysteine. Shapiro postulated that ribosylhomocysteine was further metabolized to homocysteine which was methylated to methionine. Further evidence to support this pathway was obtained by incubating cell free extract of this mutant with S-adenosylhomocysteine and S-[^{14}C]methyl methylmethionine. After deproteinizing the reaction mixture he was able to identify S-ribosylhomocysteine and radioactive methionine. Subsequent studies revealed that S-ribosylhomocysteine formation was dependent only on the presence of S-adenosylhomocysteine and the enzyme S-adenosylhomocysteine nucleosidase; whereas, formation of radioactive methionine required the presence of S-adenosylhomocysteine, S-[^{14}C]methyl methylmethionine and S-adenosylhomocysteine nucleosidase.

In 1964 Duerre and Bowden (16) investigated the growth response of a methionine requiring mutant of Salmonella

typhimurium M to L-methionine, L-homocysteine, ribosylhomocysteine and S-adenosylhomocysteine. S-Adenosylhomocysteine failed to support growth, while L-homocysteine and ribosyl-L-homocysteine supported growth. The response to these compounds was somewhat delayed compared to the growth obtained with methionine. From this study they concluded that this organism was impermeable to S-adenosylhomocysteine as it had previously been shown to have good nucleosidase activity.

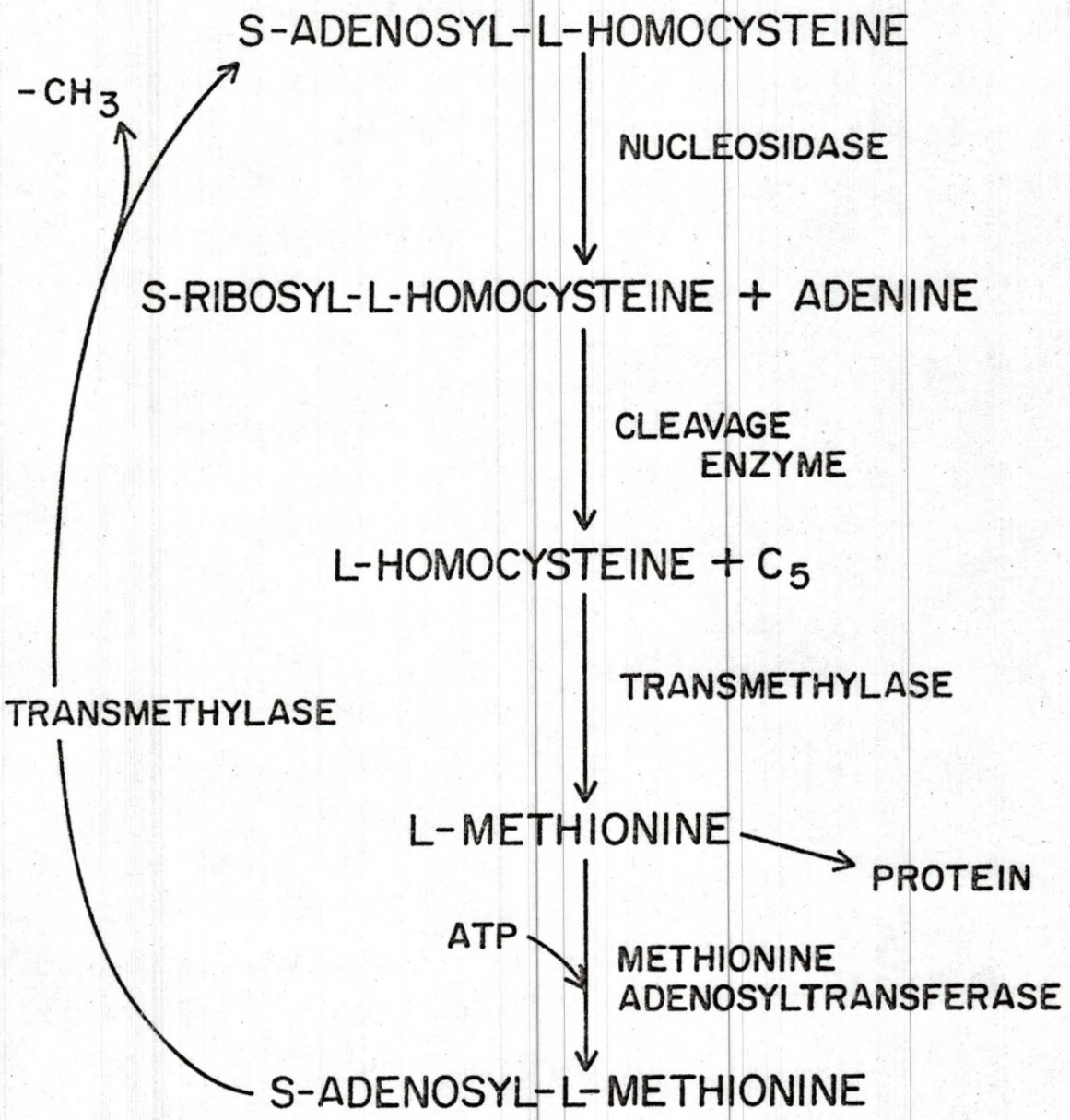
S-Ribosyl-L-homocysteine Cleavage Enzyme

The ability of ribosylhomocysteine to support growth of a methionine requiring Salmonella typhimurium M prompted further investigations into the role of this compound in metabolism. Initial studies involved supplementing growing cultures of various microorganisms with S-ribosylhomocysteine uniformly labeled with tritium in either the homocysteine or ribose moiety. It was found that Escherichia coli B and Escherichia coli W incorporated significantly higher amounts of tritium from S-ribosyl-L-[^{3}H]homocysteine than any other organism but that the amount of tritium incorporated from S-[^{3}H]ribosyl-L-homocysteine was limited (16). These findings indicated that ribosylhomocysteine was metabolized by Escherichia coli and that the homocysteine moiety was incorporated into protein methionine; whereas, the ribose moiety was not metabolized significantly.

Duerre and Miller (17) partially purified an enzyme from Escherichia coli which catalyzed the cleavage of the

thioether linkage of S-ribosylhomocysteine into two compounds L-homocysteine and a pentose. Homocysteine was identified as such by paper chromatography of the N-ethylmaleimide derivative. The other compound was a pentose whose exact structure has not yet been identified (18).

In reaction rates studies, using S-ribosylhomocysteine as a substrate with extracts from E. coli, it was found that free homocysteine was obtained at a linear rate during the first two hours of incubation. When S-adenosylhomocysteine was used as a substrate the rate of free homocysteine formation was markedly reduced. As a result of these studies it was concluded that cleavage of the glycosidic bond of S-adenosylhomocysteine was necessary prior to the cleavage of the thioether linkage of S-ribosylhomocysteine. Duerre and Miller (17) proposed the series of reactions shown in Scheme I for the incorporation of the homocysteine moiety of S-adenosylhomocysteine into protein methionine in E. coli. Based on the results of other investigators they postulated that homocysteine could be transmethylated by S-adenosylmethionine (19,20) or 5'-methyltetrahydrofolate (21-24) to yield methionine which could then be incorporated into protein or utilized in the formation of S-adenosylmethionine. Additional studies by Miller and Duerre (24) using purified S-adenosylhomocysteine nucleosidase and ribosylhomocysteine cleavage enzyme gave further support to the suggestion that two separate enzymatic reactions are responsible for the formation of homocysteine from S-adeno-


S-ADENOSYL-L-HOMOCYSTEINE
-CH3
NUCLEOSIDASE
S-RIBOSYL-L-HOMOCYSTEINE + ADENINE
CLEAVAGE ENZYME
L-HOMOCYSTEINE + C5
TRANSMETHYLASE
TRANSMETHYLASE
L-METHIONINE
PROTEIN
ATP
METHIONINE ADENOSYLTRANSFERASE
S-ADENOSYL-L-METHIONINE

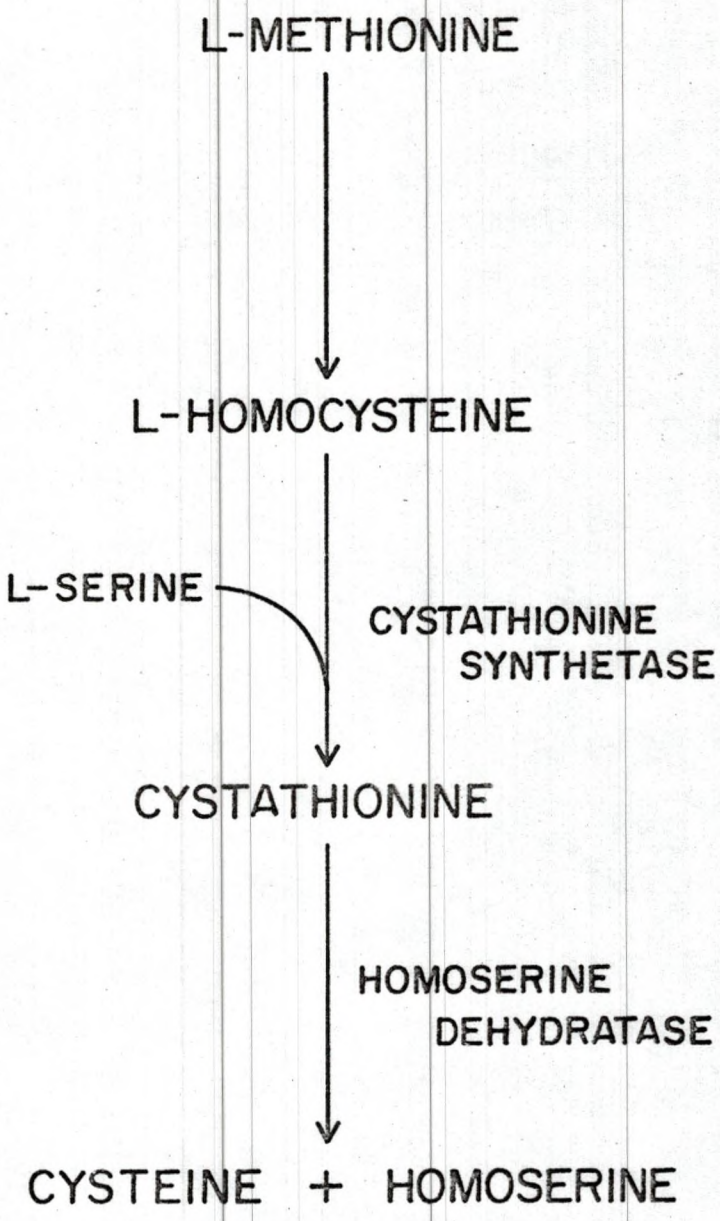
SCHEME I

sylhomocysteine in Escherichia coli. Experimental evidence by Duerre and coworkers (25) indicated this pathway to be present in several Gram negative bacteria and in the kidneys of chickens but not in mammals.

Homoserine Dehydratase

In addition to homocysteine being methylated to methionine as outlined in Scheme I it also undergoes transulfuration. In this reaction the homocysteine condenses with serine to form cystathionine. Cystathionine may then be degraded by the hydrolytic action of homoserine dehydratase (L-homoserine hydro-lyase (deaminating) EC 4.2.1.15) (formerly known as cystathionase) into cysteine and homoserine. This pathway was first suggested by Rachele, Reed, Kidwai, Ferger and du Vigneaud (26) who proposed the formation of cysteine from methionine. These conclusions were based on the results from studies in which radioactive methionine and cystathionine were fed to rats which resulted in the isolation of radioactive cysteine from rat hair. From these in vivo studies they proposed the series of reactions shown in Scheme II.

In vitro studies by Carroll, Stacy and du Vigneaud (27) working with cell free extracts from rat liver demonstrated the enzymatic cleavage of cystathionine into cysteine and homoserine. However, they also found that homoserine was deaminated by the same enzyme preparation to α-ketobutyric


L-METHIONINE
L-HOMOCYSTEINE
L-SERINE
CYSTATHIONINE SYNTHETASE
CYSTATHIONINE
HOMOSERINE DEHYDRATASE
CYSTEINE + HOMOSERINE

SCHEME II

acid which they identified by comparing its 2,4-dinitrophenylhydrazone derivative with an authentic sample of the 2,4-dinitrophenylhydrazone derivative of α-ketobutyric acid by mixed melting point determination. Binkley and Olson (28) partially purified an enzyme from rat liver that catalyzed the deamination of homoserine. However, in even their most purified preparations residual amounts of cystathionase activity persisted. Binkley (29) reported that his highly purified preparations of cystathionase also contained deaminase activity. By heat treating this enzyme preparation he was able to show that homoserine deaminase activity was more rapidly destroyed than the cystathionase activity. This led Binkley to suggest that two distinct and separable enzymes were involved in the cleavage of cystathionine and the deamination of homoserine.

Matsuo and Greenberg (30) later succeeded in isolating and crystallizing one enzyme from rat liver that was capable of catalyzing the cleavage of cystathionine into cysteine and homoserine and also the deamination of homoserine into ammonia and α-ketobutyric acid at approximately equal rates. Additional experiments by Matsuo and Greenberg (31) demonstrated that homoserine deaminase-cystathionase of rat liver contained tightly bound pyridoxal phosphate, which acted as a coenzyme. In working with this enzyme Matsuo and Greenberg found that rat livers had the highest enzyme activity of all mammals assayed but that the enzyme could also be found in beef, sheep, horse and hog livers. They

also reported much lower activity in the hearts and kidneys of these animals.

Delavier-Klutchko and Flavin (32) demonstrated the presence of cystathionase in fungi and bacteria. They found that this enzymatic reaction was reversible in higher fungi and is irreversible in bacteria. However, the enzymatic reaction in bacteria was of the opposite direction as that found in mammals with cystathionine being synthesized from the condensation of O-succinylhomoserine and cysteine.

EXPERIMENTAL PROCEDURE

Materials

Microorganisms

Cultures of Alcaligenes faecalis, Bacillus subtilis, Bacillus mycoides, Citrobacter freundii, Enterobacter aerogenes, Proteus vulgaris, Sarcina lutea and Staphylococcus aureus were typical class strains obtained from the Department of Microbiology at the University of North Dakota. These cultures were maintained on stock culture agar slants (Difco) at 4°C. Transfers were made every two months (Citrobacter freundii one month) to maintain viability. The cultures were periodically checked by Gram staining and growth on blood agar plates to determine culture purity. These cultures were grown overnight in one liter of Trypticase soy broth at 37°C on a rotational shaker. All bacterial cultures were harvested by centrifugation using a Sorvall RC-2 at 27,000 x g for fifteen minutes and washed once in 2 x distilled water. Part of the Alcaligenes faecalis, Bacillus subtilis, Bacillus mycoides, Citrobacter freundii, Enterobacter aerogenes, Proteus vulgaris, Sarcina lutea and Staphylococcus aureus cells were suspended in one volume 0.05M potassium phosphate buffer, pH 6.5 and the other part of the washed cells in 0.1M Tris-HCl buffer pH 7.5. The cells were ruptured with a Raytheon 10KC sonic oscillator for 10 minutes. The lysates were centrifuged

at 27,000 x g for 15 minutes to remove cellular debris.

Escherichia coli strain W. ATCC 9637 was obtained from American Type Culture Collection, Rockville, Maryland and was maintained at 4°C on Difco Stock Culture Agar. Cultures of E. coli were transferred every two months to maintain viability and periodically checked by Gram staining and streaking on blood agar plates to determine culture purity. Escherichia coli was grown in Anderson's M-9 media (33) (Appendix) at 37°C on a rotational shaker overnight. Escherichia coli was harvested by continuous flow centrifugation using a Sorvall RC-2 centrifuge at 27,000 x g. Part of the cells were suspended in one volume of 0.05M potassium phosphate buffer pH 6.5 and the remaining cells suspended in one volume 0.1M Tris-HCl buffer pH 7.5. The cells were ruptured using a nitrogen cooled pressure cell at 15,000 psi. The lysate was centrifuged at 27,000 x g for 15 minutes to remove cellular debris and remaining whole cells. All cell free bacterial extracts were dialysed overnight against their respective buffers with one change.

Candida albicans and Saccharomyces cerevisiae were typical class strains obtained from the Department of Microbiology, University of North Dakota. These organisms were maintained on Sabourauds plus dextrose agar slants (Difco) at 4°C. The cultures were transferred every two months to maintain cell viability. Culture purity was determined by growth on Sabourauds agar plates and Gram staining.

Yeast cultures were grown overnight in one liter of

Sabourauds plus dextrose broth at 37°C on a rotational shaker. These cells were harvested by centrifugation at 27,000 x g for 15 minutes and washed once in 2 x distilled water. Part of each yeast pellet was resuspended in 0.05M potassium phosphate buffer, pH 6.5 and the other part of each pellet in 0.1M Tris-HCl buffer, pH 7.5. Yeast cell free extracts were prepared by rupturing the yeast cells with a Raytheon 10KC sonic oscillator for 10 minutes in the presence of glass beads (Minnesota Mining beads). Cellular debris was removed by centrifuging the lysate at 27,000 x g for 15 minutes. The resultant cell free extracts were dialysed overnight against their respective buffer with one change.

Vertebrates

All fish used in this study were taken by net from Lake Sakakawea in North Dakota. Tissues were removed immediately after death and placed in plastic bags containing either 0.05M potassium phosphate buffer, pH 6.5, or 0.1M Tris-HCl buffer, pH 7.5 and stored on dry ice for transport back to the laboratory at the University of North Dakota. At the laboratory the tissue samples were kept frozen at -40°C until prepared for assaying.

Leopard frogs (Rana pipiens) were taken from a biology study area in the Turtle Mountains of North Dakota and maintained in the laboratory on worms for approximately three weeks.

Garter snakes (Thamnophis sirtalis) were also taken

from a biology study area in the Turtle Mountains of North Dakota and maintained in the laboratory on frogs for approximately three weeks.

Rats used were of the Sprague Dawley strain from White Bear Lake, Minnesota. The rabbits were New Zealand Whites from Gopher State Caviary, St. Paul, Minnesota. Guinea pigs were obtained from Mogal-Ed Biological Supply, Oshgosh, Wisconsin. White Leghorn chickens were obtained from Jack Frost Hatchery, St. Cloud, Minnesota. Ducks were of the Rouen variety obtained from Harold Schroder Duck Farm, Mekinock, North Dakota. The dogs were mongrels obtained from the Department of Physiology at the University of North Dakota. Frogs, snakes, rats, rabbits, guinea pigs, chickens and ducks were killed in the laboratory by decapitation. One dog was killed by an overdose of pentobarbital and the other by ether. Organs were removed immediately and placed in either 0.05M potassium phosphate buffer, pH 6.5, or 0.1M Tris-HCl buffer, pH 7.5. Mule deer (Odocoileus hemionus), Whitetail deer (Odocoileus virginianus) and Sharptail grouse (Pedioecetes phasianellus) were killed in the North Dakota Badlands. Their organs were removed within minutes after death and placed in plastic bags and stored on dry ice for transport back to the laboratory. The organs were stored at -40°C until used. Morning doves (Zenaidura macroura) and Lesser Yellowlegs (Totanus flavipes) were killed in Eastern North Dakota. Their organs were removed immediately and stored in plastic bags on ice for transport back to the laboratory.

Vertebrate tissue extracts were prepared by removing all fatty and fibrous material, mincing the tissues with scissors and then suspending the tissues in 1-2 volumes of either 0.05M potassium phosphate buffer, pH 6.5 or 0.1M Tris-HCl buffer, pH 7.5. The minced tissues were homogenized using a glass-teflon homogenizer with a Delta drill press. Cellular debris was removed by centrifugation at 27,000 x g for 30 minutes. The resultant extracts were dialysed against their respective buffers overnight with one change. All operations were conducted at 4°C unless otherwise specified.

Plants

Plant leaf extracts were obtained by washing fresh green bean and spinach leaves in 0.05M potassium phosphate buffer, pH 6.5, and pressing them at 15,000 psi with a nitrogen cooled press. The extracts were clarified by centrifugation at 27,000 x g for 30 minutes. The resultant supernatant fluids were concentrated ten fold by reverse dialysis. Plant pigments were removed from green bean extract by molecular sieve column chromatography employing a Sephadex G-25 (4 x 40 cm). The column was eluted with 0.05M potassium phosphate buffer, pH 6.5. The protein which passed directly through the column was reconstituted by reverse dialysis.

Plant sprouts from corn and barley were obtained by sprouting these seeds in moist sand beds in the absence of light. After seven days the sprouts were harvested and

washed in 0.05M potassium phosphate buffer, pH 6.5. The cells were ruptured by homogenizing the sprouts with a mortar and pestle. Cellular debris was removed by centrifugation at 27,000 x g for 30 minutes.

Chemicals

L-Alanine, D-ribose and L-homocysteine thiolactone were obtained from California Biochemical Corporation, Los Angeles, California. Alpha-ketobutyric acid and DL (+)-allo-cystathionine were obtained from Nutritional Biochemical Company, Cleveland, Ohio. Pyridoxal-5-phosphate monohydrate (Codecarboxylase) was obtained from Mann Research Laboratory, New York, New York. N-Ethylmaleimide, L-homoserine and adenosine were obtained from Sigma Chemical Company, St. Louis, Missouri. All other chemicals were obtained from Fisher Scientific Company, Fair Lawn, New Jersey.

Preparation of L-homocysteine from L-homocysteine Thiolactone

The method of Duerre and Miller (17) was used to cleave the thiolactone ring of L-homocysteine thiolactone to yield L-homocysteine free base. This method consisted of incubating 0.3 mmole of L-homocysteine thiolactone with 0.3 ml 3N NaOH for 5 minutes at room temperature. The solution was neutralized by the addition of 1.0M KH_2PO_4, diluted to 30 µmole/ml and stored under nitrogen to prevent oxidation of the free sulfhydryl group.

Preparation of S-Adenosyl-L-homocysteine

S-Adenosylhomocysteine hydrolase was prepared by a modification of the method of Cantoni and Scarano (5). Frozen rat livers were homogenized in 3 volumes of 0.01M acetic acid for one minute using a Waring blender operating at maximum speed. Clarification of the extracts was achieved by centrifugation at 10,000 x g for 30 minutes. Solid ammonium sulfate was added with constant stirring to the supernatant fluid to a concentration of 40% saturation. The resultant precipitate was removed by centrifugation at 10,000 x g for 15 minutes and discarded. Solid ammonium sulfate was added to the supernatant fluid with constant stirring to 50% saturation. The resultant precipitate was collected by centrifugation at 10,000 x g for 15 minutes, dissolved in 0.05M potassium phosphate buffer, pH 7.0 and dialysed. This served as the source of S-adenosyl-L-homocysteine hydrolase.

S-Adenosyl-L-homocysteine was prepared enzymatically by a modification of the method used by Duerre (6). Sixteen mmoles of adenosine, 32 mmoles of DL-homocysteine (free base) and 1.0 mmoles phosphate buffer (pH 6.5) were diluted to 1200 ml in distilled water in a 2 liter flask. The flask was flushed for 10 minutes with nitrogen to produce an anaerobic atmosphere. Approximately 290 units of S-adenosylhomocysteine hydrolase was added by a syringe and the reaction mixture was incubated for 90 minutes at 37°C. After incubation 0.025 ml of thiodiglycol per 100 ml was added to

maintain S-adenosylhomocysteine in the reduced state. The reaction mixture was deproteinized by heating in boiling water for 5 minutes. The reaction mixture was cooled in an ice bath and filtered at 4°C using Whatman No. 1 filter paper. S-Adenosyl-L-homocysteine was purified from the crude reaction material by column chromatography employing Amberlite CG-120 resin, 200-400 mesh. The column was washed with 6.0N H_2SO_4 and equilibrated with 1.0N H_2SO_4. A column 13 cm^2 in cross sectional area with a resin bed of 11 cm was used to purify 260 ml of the reaction mixture. Elution of S-adenosylhomocysteine was followed with u.v. absorption at 260 mμ. Sixteen hundred ml of 3.0N H_2SO_4 (containing 0.25 ml thiodiglycol per liter) was used to elute undesired nucleotides, nucleosides, bases and amino acids. After the removal of these impurities S-adenosyl-L-homocysteine was eluted with 6.0N H_2SO_4 containing 0.25 ml thiodiglycol. S-Adenosyl-L-homocysteine was precipitated with phosphotungstic acid and collected by decanting the liquid and centrifugation at 500 x g for 10 minutes. The precipitate was washed twice with 5 volumes of cold distilled water and dissolved in 5 volumes of acetone-water mixture (1:1). Phosphotungstate was removed by partition using 4 volumes of isoamyl alcohol-ethyl ether mixture (1:1). This procedure was repeated four times with the addition of water to maintain the volume. The pH of the resultant aqueous solution was adjusted to pH 4.5 with freshly prepared $BaCO_3$. The precipitate was removed by filtration and washed with a small

amount of water which was combined with the original filtrate. The filtrate was lyophilized, dissolved in water (50 μmoles/ml), frozen and stored at 4°C to accelerate crystallization. Crystals were harvested by filtration, washed with cold water and stored over silica gel under vacuum at 4°C. Samples of the purified compounds were chromatographed on Whatman No. 1 filter paper and found to be free of u.v. and ninhydrin-positive impurities.

Preparation of S-Ribosyl-L-homocysteine

S-Adenosylhomocysteine nucleosidase was obtained from Escherichia coli. Escherichia coli was cultured in 20 liter carboy on Anderson's M-9 media (33). Four 20 liter carboys containing 15 liters of media were inoculated with a 1% inoculum grown in the same media. The cultures were incubated for 24 hours at 37°C under forced aeration. The cells were harvested with a Sorvall continuous flow centrifuge operated at 27,000 x g. The cells were washed once with 0.1M Tris-HCl buffer, pH 7.6, centrifuged and resuspended in 4 volumes of the same buffer. Cell free extracts were obtained by disrupting the cells in a nitrogen cooled pressure cell at 15,000 psi. Whole cells and cellular debris were removed by centrifugation at 27,000 x g for 20 minutes. The resultant cell free extracts were incubated at 35°C for 30 minutes with deoxyribonuclease and ribonuclease (0.8 mg each/100 ml) with 1 x 10^{-3}M $MgCl_2$. The material was cooled to 4°C and clarified by centrifugation. The

supernatant fluid was eluted through a Sephadex G-25 column with 0.05M Tris-HCl buffer, pH 7.6. A columm 12.56 cm^2 in cross sectional area with a 50 cm resin bed was used to purify 130 ml of extract. Elution of protein was followed using the Biuret (34) reaction (Appendix). Two liters of buffer was sufficient to elute all the protein. Saturated ammonium sulfate was added dropwise to the eluate to a concentration of 40% saturation. The precipitate was removed by centrifugation at 27,000 x g for 20 minutes and discarded. Additional saturated ammonium sulfate was added to the supernatant fluid to a concentration of 60% saturation. The precipitate was collected by centrifugation and dissolved in 0.05M potassium phosphate buffer, pH 7.6, containing 3×10^{-3}M 2-mercaptoethanol. The nucleosidase was further fractionated by gel filtration on a Sephadex G-150 column (4 x 42 cm) with a flow rate of 45 ml/hour. The column was eluted with 0.05M phosphate buffer, pH 7.6, containing 3×10^{-3}M 2-mercaptoethanol. Seventeen milliliter samples were collected with the aid of a fraction collector. S-Adenosylhomocysteine nucleosidase and protein concentrations were measured on selected tubes. Tubes containing enzyme activity were pooled and concentrated by reverse dialysis. The partially purified S-adenosylhomocysteine nucleosidase was stored at $-40^{\circ}C$ until used.

S-Ribosylhomocysteine was prepared enzymatically with this enzyme using a modification of the method of Duerre (14). One unit of enzyme was added per umoles of S-adenosyl-

L-homocysteine in the presence of 0.1M phosphate buffer, pH 6.5, and incubated for 2 hours at 37°C. The reaction mixture was deproteinized by heating in a boiling water bath for 5 minutes, cooled and clarified by centrifugation at 27,000 x g for 20 minutes. The supernatant fluid was placed on a Dowex-50 W column (2 x 15 cm) that had previously been washed with 6.0N HCl and equilibrated with 0.1N HCl. Phosphate buffer and 2-mercaptoethanol were eluted with approximately 50 ml of 0.1N HCl. S-Ribosylhomocysteine was eluted with 1.0N HCl with the elution profile being followed by the orcinol reaction. The eluate was adjusted to pH 4.0 with the addition of Dowex-2 resin (OH^- form). The resin was removed by filtration and washed with water until orcinol negative. The wash water was combined with the filtrate and evaporated to dryness by lyophilization. The dry S-ribosylhomocysteine was dissolved in water to the desired concentration as determined by the orcinol reaction. Compound purity was determined by chromatography on Whatman No. 1 filter paper. Traces of S-ribosylhomocysteine sulfoxide were detected.

Analytical Methods

Determination of Protein

The method of Lowry, Rosebrough, Farr and Randall (35) was used to determine the protein concentration of all crude extracts. Samples of the crude extract containing between 10 and 60 µg protein were diluted to a volume of 0.6 ml with water. Three ml of Reagent C (Appendix) were added. The solution was mixed and allowed to stand at room temperature for 10 minutes. For color development 0.3 ml of Folin-Ciocalteau Phenol reagent (1.0N with respect to sulfuric acid) was added with immediate and rapid mixing. After standing for 30 minutes at room temperature the samples were read in a Coleman Jr. spectrophotometer at 690 mµ against a reagent blank. A standard curve using Bovine serum albumin was found to be linear with protein concentration between 10 and 60 µg per ml. (Figure 1).

Protein samples lacking high nucleic acid concentrations such as those fractionated by gel filtration, were analyzed for protein concentration spectrophotometrically by the method of Warburg and Christian (36). Using this method an aliquot of each sample was diluted with water until an absorbancy could be recorded at 260 mµ and 280 mµ using a Beckman DB spectrophotometer. Water was used as the blank.

Fig. 1.--Reference curve for the determination of protein by the method of Lowry. Bovine serum albumin was used as the standard solution.

Absorbancy 690 mμ
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
μg of Protein

Protein concentrations in mg/ml were calculated using the following equation:

$$(1.55 \times O.D._{280}) - (0.76 \times O.D._{260}) \times \text{Dilution} = \text{mg protein/ml}$$

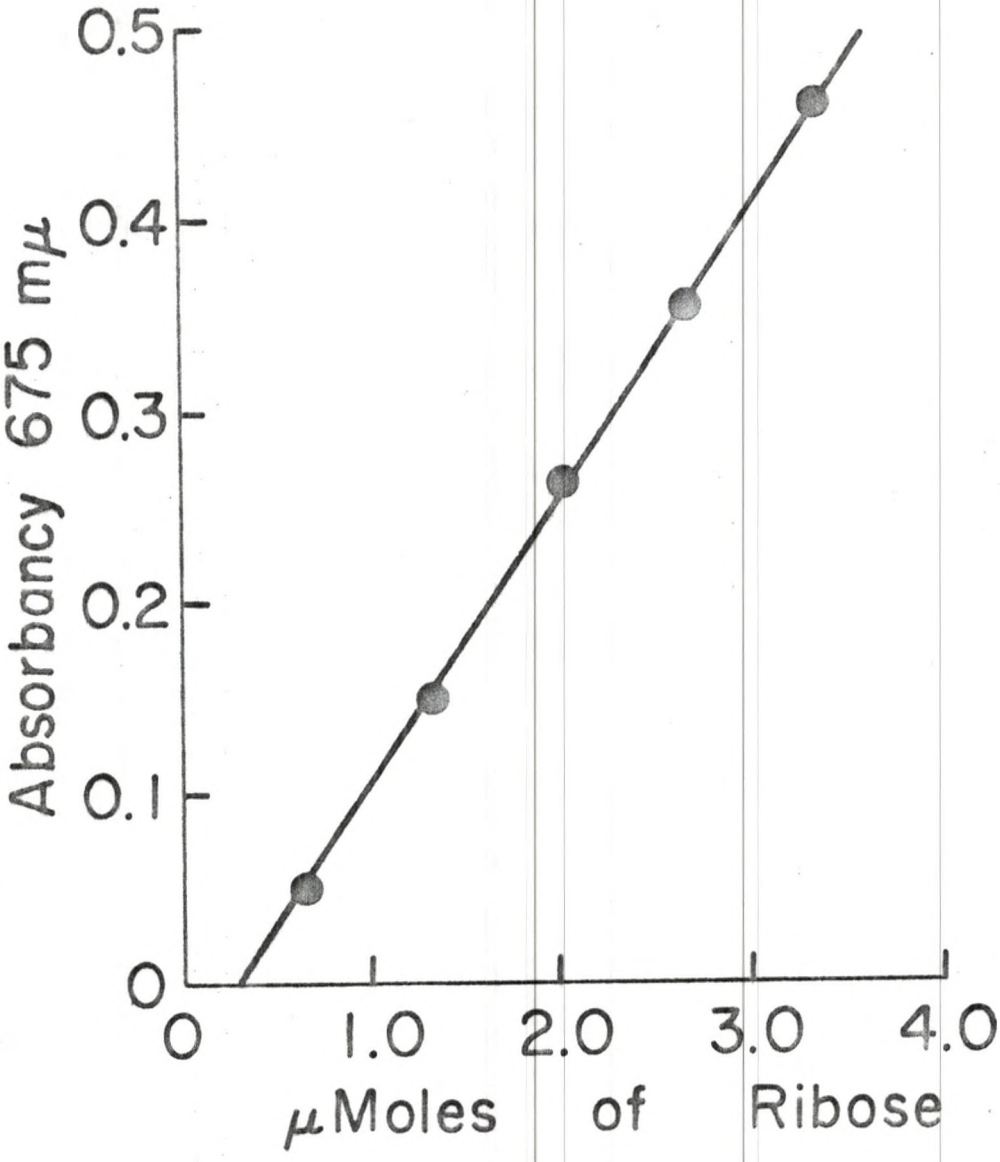
Determination of Pentoses

In enzymatic reactions involving the formation of reducing sugars the pentoses were measured quantitatively by the method of Benedict (37). Aliquots of deproteinized reaction mixtures were diluted to 1.0 ml with water. One ml of copper reagent (Appendix) containing 1% sodium bisulfite was added and the solutions placed in a boiling water bath for 6 minutes. The samples were cooled in an ice bath for 2 minutes after which time 1.0 ml of color reagent (Appendix) was added. After 1 minute the samples were diluted to 10 ml, mixed and allowed to stand at room temperature for 10 minutes before being read at 675 mμ on a Coleman Jr. spectrophotometer against a reagent blank. Ribose gave a linear response over a range of 10-30 μmoles (Figure 2).

S-Adenosylhomocysteine Hydrolase Assay

Reaction mixtures containing 12.0 μmoles of L-homocysteine, 200 μmoles of potassium phosphate buffer, pH 6.5, crude extract in a concentration of 1-2 mg of protein per ml and adenosine (16-24 μmoles) in a total volume of 2.0 ml were incubated at 37°C under a nitrogen atmosphere for 30 minutes. The reactions were stopped by placing the

Fig. 2.--Reference curve for the determination of pentose by the method of Benedict. Ribose was used as the standard.


0.5
0.4
0.3
0.2
0.1
0
Absorbancy 675 mμ
0
1.0
2.0
3.0
4.0
μMoles of Ribose

tubes in a boiling water bath for 3 minutes. The precipitated protein was removed by cooling the tubes in an ice bath and centrifuging them at 27,000 x g for 5 minutes. Zero time controls were included with all reaction tubes. These controls containing substrate, buffer and water were placed in a boiling water bath for approximately one minute before the addition of prescribed amounts of crude extract. After boiling for three minutes the tubes were cooled on ice and centrifuged to remove the precipitated protein. The amount of L-homocysteine utilized in the formation of S-adenosyl-L-homocysteine was measured by determining the amount of L-homocysteine in the incubated reaction mixture and in zero time controls. The N-ethylmaleimide method of detecting free sulfhydryl groups was used to measure L-homocysteine. One unit of activity was defined as the amount of enzyme which catalyzed the utilization of 1.0 μmole of L-homocysteine per 30 minutes under the described conditions.

S-Adenosylhomocysteine Nucleosidase Assay

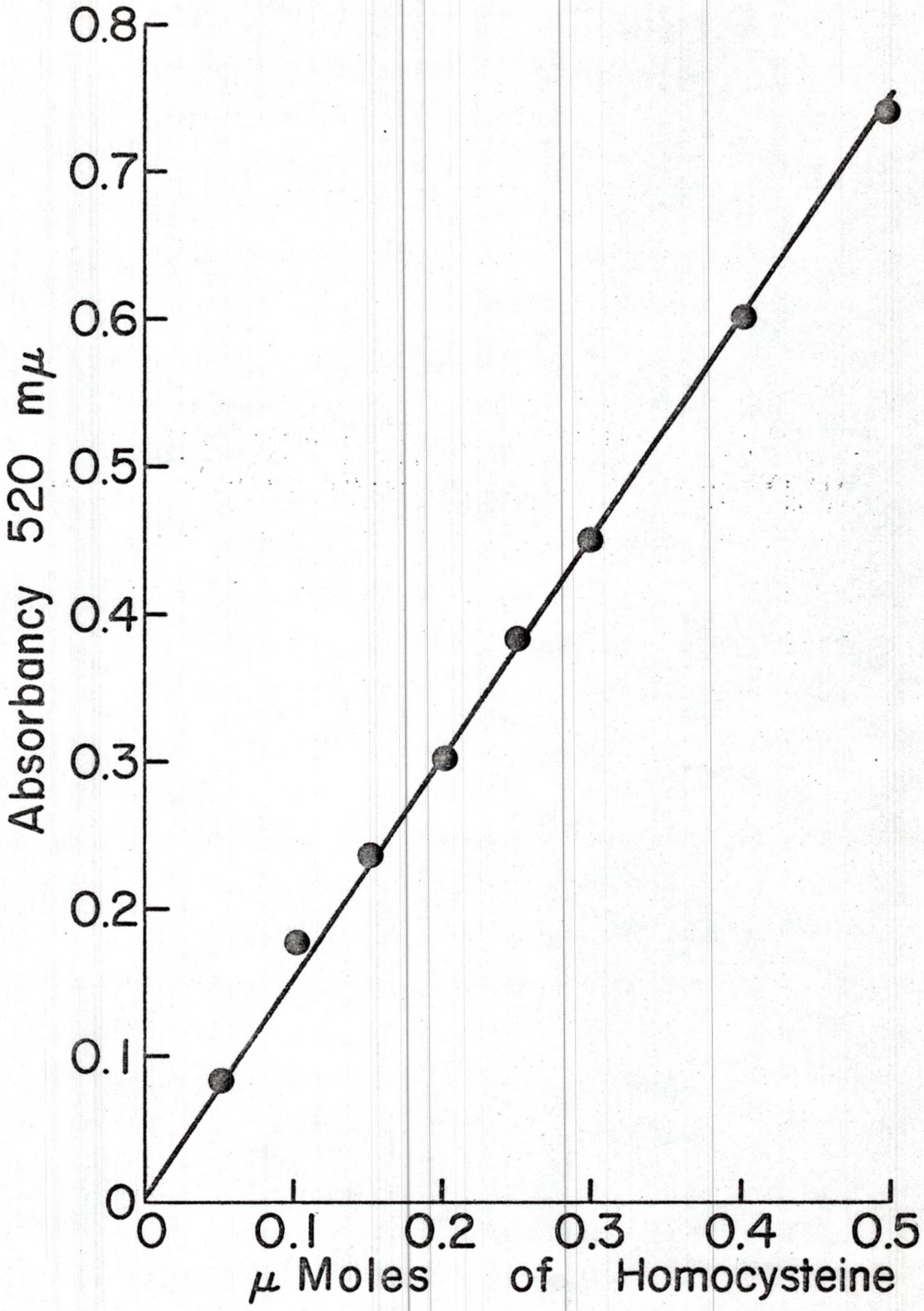
Reaction mixtures contained 30 μmoles of S-adenosyl-L-homocysteine, 100 μmoles potassium phosphate buffer, pH 6.5 and crude extract in a concentration of 1-2 mg of protein per ml. Reaction mixtures were incubated at 37°C for 30 minutes. The reactions were stopped by placing the tubes in a boiling water bath for 3 minutes. The tubes were cooled on ice and the precipitate removed by centrifugation at 27,000 x g for 5 minutes. The method of Benedict (37) was used to determine the amount of reducing compound

(S-ribosyl-L-homocysteine) present. One unit of activity was defined as that amount of enzyme protein catalyzing the liberation of 1.0 µmole of reducing compound per 30 minutes under the described conditions.

S-Ribosylhomocysteine Cleavage Enzyme Assay

Reaction mixtures contained 3.0 µmoles of ribosyl-L-homocysteine, 100 µmoles of Tris-HCl buffer, pH 7.5, and crude extract in a concentration of 1-2 mg of protein per ml. Reaction mixtures were incubated under a nitrogen atmosphere for 30 minutes at 37°C. The reactions were stopped by placing the tubes in a boiling water bath for 3 minutes. The tubes were then cooled on ice and the precipitate removed by centrifugation at 27,000 x g for 5 minutes. To 0.3 ml of the deproteinized reaction mixtures an equal volume of 0.375M N-ethylmaleimide was added. After incubating for 10 minutes at room temperature 0.12 ml of 1.0N NaOH was added. The formation of an N-ethyl-maleimide derivative of homocysteine was determined by the appearance of a pink color upon the addition of the alkali. Since this color had a tendency to fade all tubes were read 30 seconds after the addition of the alkali in a micro-curvette at 520 mµ using a Beckman DU spectrophotometer. Control reactions contained water in place of the substrate. Free homocysteine when incubated with N-ethylmaleimide at room temperature gave a linear response over a range of 0.05 to 0.5 µmoles (Figure 3).

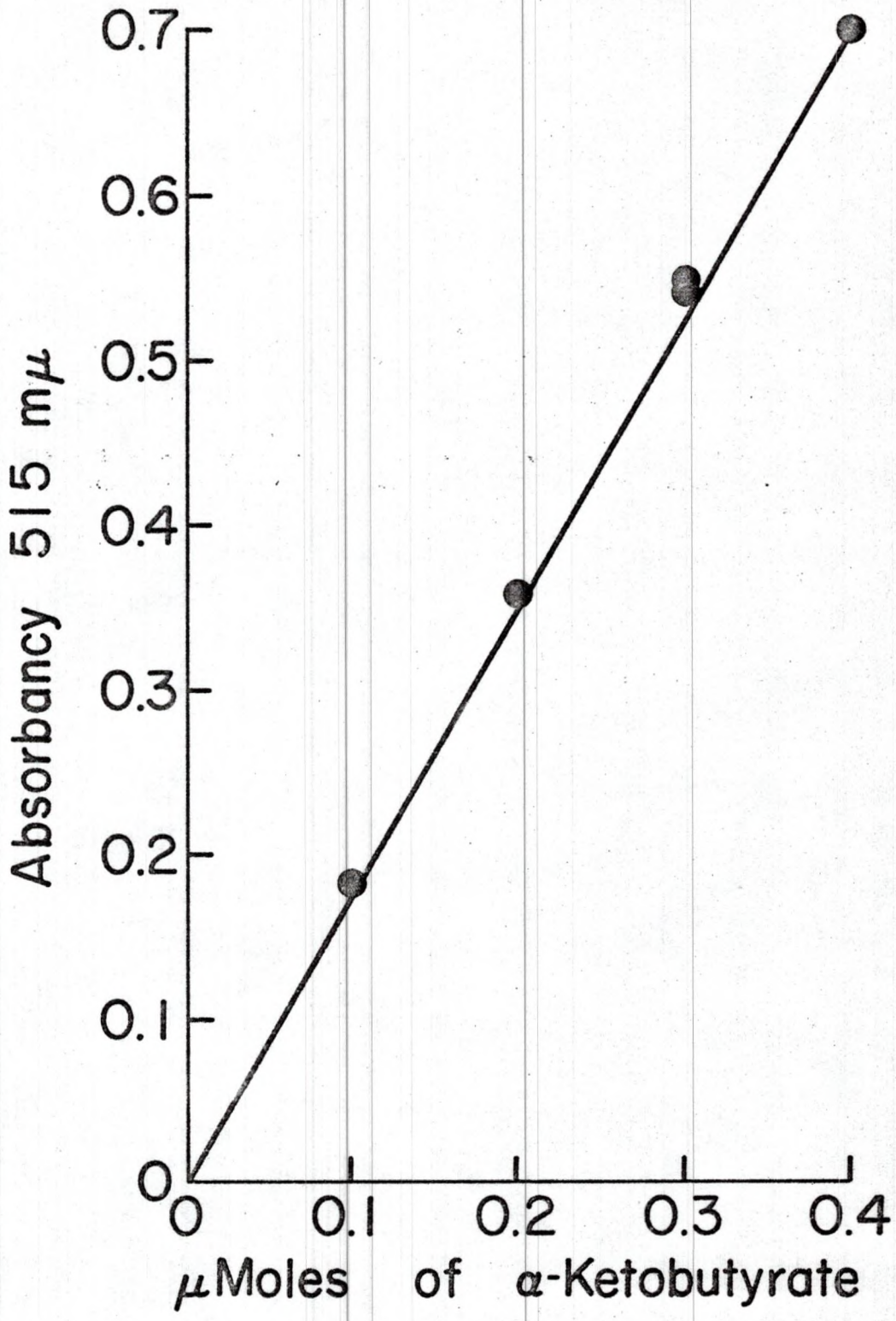
Fig. 3.--Reference curve for the determination of free sulfhydryl groups of L-homocysteine by the N-ethylmaleimide method. The L-homocysteine standard was prepared as described in the text.


Absorbancy 520 mμ
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
μ Moles of Homocysteine

Homoserine Dehydratase Assay

Reaction mixtures contained 16 µmoles of L-homoserine, 50 µmoles Tris-HCl buffer, pH 7.5, 25 µmoles citrate NaOH buffer, pH 7.5, 0.08 µmoles pyridoxal phosphate and crude extract in a concentration of 1-2 mg of protein per ml. Reaction mixtures were incubated at 37°C for 30 minutes. The reactions were stopped by the addition of 0.17 ml of 100% trichloroacetic acid. The precipitate was removed by centrifugation at 15,000 x g for 5 minutes. Reaction controls contained water in place of L-homoserine. One ml samples of the reaction mixtures were assayed for the presence of keto acids by the 2,4-dinitrophenylhydrazine method of Freidmann and Haugen (38). This method involved adding 0.05 ml of 100% trichloroacetic acid to one ml samples of the deproteinized reaction mixture. To this solution 0.25 ml of a 0.1% solution of 2,4-dinitrophenylhydrazine in 20% HCl was added. The tubes were incubated for 5 minutes at room temperature after which time 2 ml of 4.0N NaOH was added with immediate mixing. After the tubes were incubated at room temperature for 10 minutes the absorbancy of the samples was determined at 515 mµ with a Coleman Jr. spectrophotometer against a reagent blank. Alpha-keto-butyrate standard gave a linear response between 0.1 and 0.4 µmoles (Figure 4). A unit of activity was defined as that amount catalyzing the liberation of 100 mµmoles of keto acid per 30 minutes under the described conditions.

Fig. 4.--Reference curve for the determination of ∝-ketobutyrate by the 2,4-dinitrophenylhydrazine method.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbancy 515 mμ
0
0.1
0.2
0.3
0.4
μMoles of α-Ketobutyrate

RESULTS

Microorganisms

Microorganisms were assayed for the presence of S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase. None of the bacterial extracts assayed exhibited detectable hydrolase activity. However, the yeasts Saccharomyces cerevisiae and Candida albicans did possess hydrolase activity. (Table 1). The greatest hydrolase activity for these microorganisms was found at pH 6.3 for Saccharomyces cerevisiae and pH 7.3 for Candida albicans.

All of the Gram negative bacteria assayed possessed S-adenosylhomocysteine nucleosidase activity (Table 2). Previous extensive studies of purified S-adenosylhomocysteine nucleosidase from Escherichia coli had shown that the optimum pH was between pH 5.5-7.0. Similar results were found with Alcaligenes faecalis, Citrobacter freundii, Proteus vulgaris and Enterobacter aerogenes. However, there was no enzyme activity detected for Enterobacter aerogenes at pH 5.5.

Of the Gram positive bacteria assayed Bacillus cereus variety mycoides, Sarcina lutea and Staphylococcus aureus were found to possess S-adenosylhomocysteine nucleosidase

TABLE 1

ASSAY FOR S-ADENOSYLHOMOCYSTEINE HYDROLASE AND HOMOSERINE DEHYDRATASE IN MICROORGANISMS

MICROORGANISMS	S-ADENOSYLHOMOCYSTEINE HYDROLASE	HOMOSERINE DEHYDRATASE
Alcaligenes faecalis	NDA[a]	NDA
Citrobacter freundii	NDA	NDA
Enterobacter aerogenes	NDA	NDA
Escherichia coli	NDA	NDA
Proteus vulgaris	NDA	NDA
Bacillus cereus	NDA	0.13
Bacillus subtilis	NDA	0.03
Sarcina lutea	NDA	0.03

TABLE 1--Continued

MICROORGANISMS	S-ADENOSYLHOMOCYSTEINE HYDROLASE	HOMOSERINE DEHYDRATASE
Staphylococcus aureus	NDA	NDA
Saccharomyces cerevisiae	0.45 (pH 6.3)	NDA
Candida albicans	0.29 (pH 7.3)	NDA

Reaction mixtures for S-adenosylhomocysteine hydrolase contained 10-12 µmoles of adenosine, 6 µmoles of L-homocysteine, 1-2 mg of protein and 200 µmoles of either sodium acetate buffer, pH 5.5, potassium phosphate buffer, pH 6.5, or Tris-HCl buffer, pH 7.5. Reaction mixtures for homoserine dehydratase (cystathionase) contained 16 µmoles of L-homoserine, 1-2 mg of protein, 50 µmoles Tris-HCl buffer, pH 7.5, 25 µmoles citrate NaOH buffer, pH 7.5, and 0.08 µmoles pyridoxal phosphate. Activity expressed in µmoles of substrate utilized/mg of protein/30 min.

[a]NDA = No detectable activity.

[b]pH at which greatest enzyme activity was recorded.

TABLE 2

ASSAY FOR S-ADENOSYLHOMOCYSTEINE NUCLEOSIDASE AND S-RIBOSYLHOMOCYSTEINE CLEAVAGE ENZYME

MICROORGANISMS	S-ADENOSYLHOMOCYSTEINE NUCLEOSIDASE			S-RIBOSYLHOMOCYSTEINE CLEAVAGE ENZYME
	AH	MTA	MTA/AH	
Alcaligenes faecalis	0.29 (pH 5.5)[a]	NDA[b]	-[c]	NDA
Citrobacter freundii	0.26 (pH 5.5)	0.41 (pH 5.5)	1.60	0.02 (pH 7.5)
Enterobacter aerogenes	0.28 (pH 6.7)	0.45 (pH 6.7)	1.61	0.15 (pH 7.5)
Escherichia coli	0.69 (pH 5.5)	1.12 (pH 5.5)	1.62	0.14 (pH 7.5)
Proteus vulgaris	0.18 (pH 5.5)	0.29 (pH 5.5)	1.60	0.08 (pH 7.5)
Bacillus cereus	0.25 (pH 6.7)	0.35 (pH 6.7)	1.40	NDA
Bacillus subtilis	NDA	NDA	-	NDA
Sarcina lutea	0.14 (pH 7.0)	NDA	-	NDA

TABLE 2--Continued

MICROORGANISMS	S-ADENOSYLHOMOCYSTEINE NUCLEOSIDASE			S-RIBOSYLHOMOCYSTEINE CLEAVAGE ENZYME
	AH	MTA	MTA/AH	
Staphylococcus aureus	0.19 (pH 5.5)	0.25 (pH 5.5)	1.33	NDA
Saccharomyces cerevisiae	NDA	-	-	NDA
Candida albicans	NDA	-	-	NDA

Reaction mixtures for S-adenosylhomocysteine nucleosidase contained 3.0 µmoles of S-adenosylhomocysteine (AH) or 3.0 µmoles of methylthioadenosine (MTA), 1-2 mg protein and 100 µmoles of either sodium acetate buffer, pH 5.5, potassium phosphate buffer, pH 6.5, or Tris-HCl buffer, pH 7.5. Reaction mixtures for S-ribosylhomocysteine cleavage enzyme contained 3.0 µmoles of S-ribosylhomocysteine, 1-2 mg protein and 100 µmoles of either sodium acetate buffer, pH 5.5, Tris-HCl buffer, pH 7.5, or Tris-HCl buffer, pH 8.5. Activity expressed in µmoles of substrate utilized/mg of protein/30 min.

[a]pH at which greatest enzyme activity was recorded.

[b]NDA = No detectable activity.

[c]Not done.

activity. (Table 2). It had previously been shown that this enzyme would also cleave the glycosidic linkage of methylthioadenosine (14). Cell free extracts from all bacteria exhibiting S-adenosylhomocysteine nucleosidase activity were therefore incubated with methylthioadenosine. It was found that cell free extracts from all these microorganisms, except Alcaligenes faecalis and Sarcina lutea exhibited enzyme activity toward this substrate. The ratio of enzymatic activity toward methylthioadenosine compared to S-adenosylhomocysteine was relatively constant with Gram negative bacteria suggesting that the enzyme from all of these sources was active toward both substrates. Bacillus cereus and Staphylococcus aureus both exhibited enzyme activity toward methylthioadenosine; however, the ratio of methylthioadenosine to S-adenosylhomocysteine was less than that found with Gram negative activity.

All of the Gram negative bacteria having S-adenosyl-homocysteine nucleosidase activity were found to have S-ribosylhomocysteine cleavage enzyme activity except Alcaligenes faecalis (Table 2). All other microorganisms assayed lacked detectable S-ribosylhomocysteine cleavage enzyme activity.

Of the eleven microorganisms assayed only three exhibited homoserine dehydratase activity. (Table 1). These bacteria were Bacillus cereus, Bacillus subtilis, both Gram positive rods and Sarcina lutea a Gram positive cocci.

Plants

Four plants were assayed for the presence of S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase. All these plants were found to possess S-adenosylhomocysteine hydrolase activity (Table 3). Corn sprouts had the highest activity under the assay conditions employed. There was no S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme or homoserine dehydratase activity detected in any extracts assayed.

Cold Blooded Vertebrates

Brains, hearts, livers, spleens and kidneys of seven cold blooded vertebrates were assayed for S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase (Table 4). S-Adenosylhomocysteine hydrolase was found in the livers of all vertebrates tested except the Paddlefish. S-Adenosylhomocysteine hydrolase was also found in kidney extracts of the Buffalohead, Channel cat and Carter snake; and, in the spleen extracts of Channel cat and Yellow perch. There was no S-adenosylhomocysteine hydrolase activity detected in the brain or heart extracts of any of these cold blooded vertebrates. S-Adenosylhomocysteine nucleosidase and S-ribosylhomocysteine cleavage enzyme activity was not detectable in any of the organs

TABLE 3

ASSAY FOR S-ADENOSYLHOMOCYSTEINE HYDROLASE IN GREEN PLANTS

PLANTS	S-ADENOSYLHOMOCYSTEINE HYDROLASE
Phaseolus vulgaris (green bean)	0.38
Spinaces oleraces (spinach)	0.08
Zea mays (field corn)	2.12
Hordeum distichon (barley)	0.65

Reaction mixtures contained 10-12 µmoles of adenosine, 6 µmoles of L-homocysteine, 1-2 mg of protein and 200 µmoles of potassium phosphate buffer, pH 6.5. Enzyme activity expressed in µmoles of substrate utilized/mg of protein/30 min.

TABLE 4

ASSAY FOR S-ADENOSYLHOMOCYSTEINE HYDROLASE IN COLD BLOODED VERTEBRATES

VERTEBRATES	ORGANS		
	LIVER	SPLEEN	KIDNEY
Ictiobus bubalus (Buffalohead)	0.43	NDA[a]	0.26
Ictalurus punctatus (Channel cat)	0.39	0.30	0.25
Polyodon spathula (Paddlefish)	NDA	NDA	NDA
Salmo gairdneri (Rainbow trout)	0.65	NDA	NDA
Perca flavescens (Yellow perch)	0.79	0.30	NDA
Rana pipiens (Leopard frog)	0.97	NDA	NDA
Thamnophis sirtalis (Garter snake)	0.52	-[b]	0.37

Reaction mixtures for S-adenosylhomocysteine hydrolase contained 10-12 µmoles of adenosine, 6 µmoles of L-homocysteine, 1-2 mg of protein and 200 µmoles of potassium phosphate buffer, pH 6.5. Activity expressed in µmoles of substrate utilized/mg of protein/30 min.

[a]NDA = No detectable activity.

[b]Tissue not available.

tested from cold blooded vertebrates.

The Paddlefish was the only vertebrate lacking detectable S-adenosylhomocysteine hydrolase in any of its organs. The extract prepared from the liver contained large amounts of pigmented material that might inhibit the hydrolase enzyme. Therefore fresh liver extracts were chromatographed on Sephadex G-25 column (2.5 x 50 cm) to remove pigments or possible inhibitors. Protein elution was followed by the Biuret reaction and the protein fraction was concentrated by reverse dialysis. The concentrated Paddlefish liver preparation was assayed for the presence of S-adenosylhomocysteine hydrolase and S-adenosylhomocysteine nucleosidase. The extract so prepared lacked detectable hydrolase and nucleosidase activity.

Warm Blooded Vertebrates

There were five birds included in this study (Table 5). They were the White Leghorn chicken, the Sharptail grouse, the Rouen duck, the Morning dove and the Lesser Yellowleg. Direct chemical analysis for S-adenosylhomocysteine hydrolase indicated that White Leghorn chicken, Rouen duck and Lesser Yellowleg possessed measurable quantities of this enzyme in liver extracts. The Rouen duck and Lesser Yellowleg also exhibited traces of hydrolase activity in kidney extracts.

There was no detectable hydrolase activity in extracts prepared from any of the organs from the Sharptail grouse or the Morning dove as measured by direct chemical assay.

TABLE 5

ASSAY FOR S-ADENOSYLHOMOCYSTEINE HYDROLASE IN WARM BLOODED VERTEBRATES

VERTEBRATES	ORGANS		
	LIVER	SPLEEN	KIDNEY
Galus galus (White Leghorn chicken)	0.67	NDA[a]	NDA
Pedioecetes phasianellus (Sharptail grouse)	0.01[c]	NDA	NDA
Anas platyrhynchos (Rouen duck)	1.02	NDA	Trace
Zenaidura macronra (Morning dove)	0.06[c]	-[b]	NDA
Totanus flavipes (Lesser Yellowleg)	1.76	-	Trace
Cavia porcellus (Guinea pig)	1.50	NDA	NDA
Rattus rattus (white rat)	2.11	NDA	0.76
Oryctolagus cuniculus (New Zealand white rabbit)	0.95	NDA	0.32
Canis familiaris (dog)	1.20	-	0.45

TABLE 5--Continued

VERTEBRATES	ORGANS		
	LIVER	SPLEEN	KIDNEY
Odocoileus hemionus (Mule deer)	NDA	NDA	NDA
Odocoileus virginianus (Whitetail deer)	NDA	NDA	NDA
Bos (steer)	2.46	0.02	-
Homo sapiens (human)	NDA	-	-

Reaction mixtures for S-adenosylhomocysteine hydrolase contained 10-12 µmoles of adenosine, 6 µmoles of L-homocysteine, 1-2 mg of protein and 200 µmoles of potassium phosphate buffer, pH 6.5. Activity expressed in µmoles of substrate utilized/mg of protein/30 min.

[a]NDA = No detectable activity.

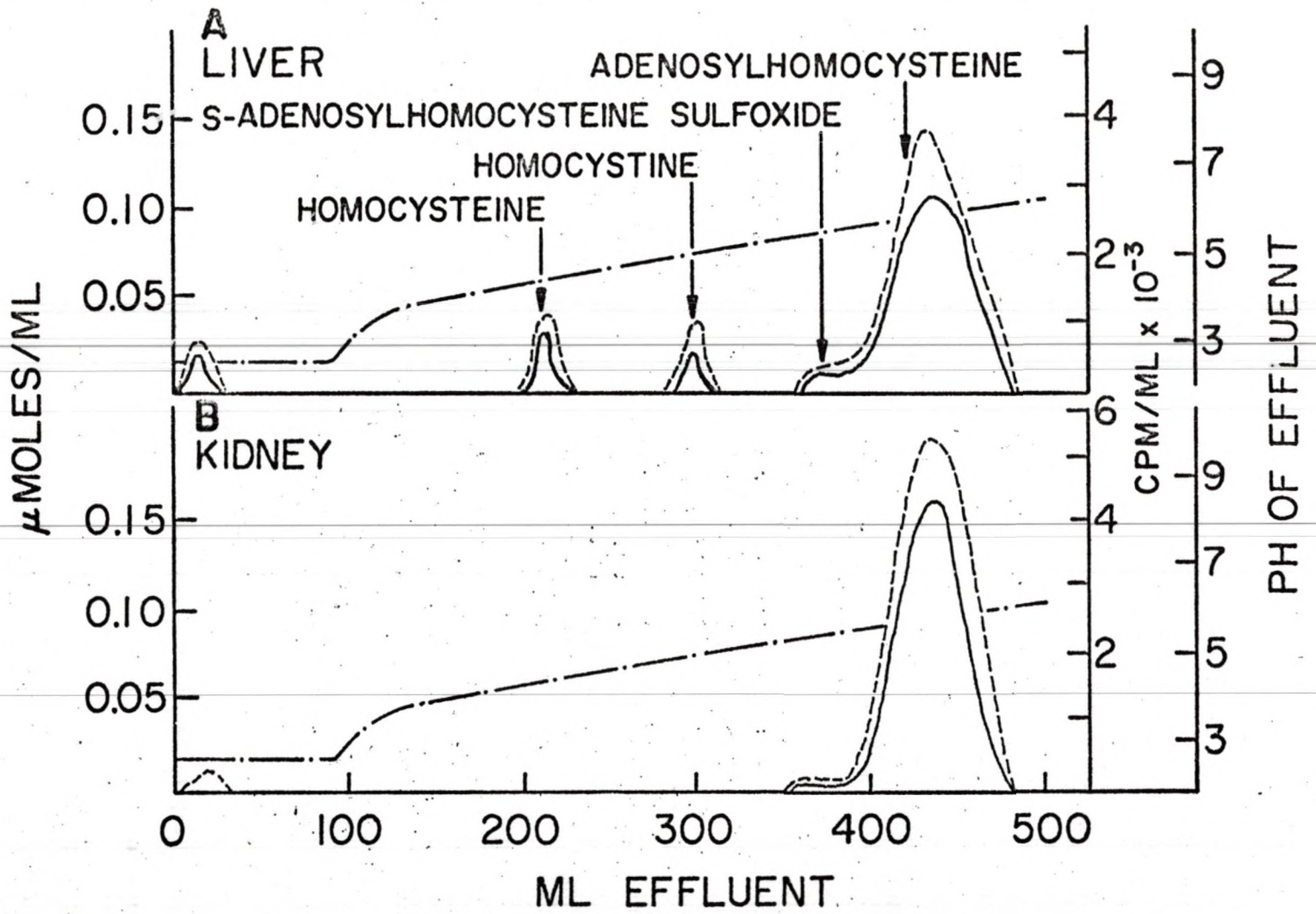
[b]Tissue not available.

[c]Determined by radiological assay.

Consequently, these and other tissues were reassayed for both hydrolase and nucleosidase activity employing a ~~very~~ more sensitive, radiological assay. Reaction mixtures contained 12 µmoles of S-adenosyl-L-[$^{3}_{H}$] homocysteine, 200 µmoles of potassium phosphate buffer pH 6.5 and 1-2 mg of protein. After incubation at 37°C for 1 hour the deproteinized reaction mixtures were chromatographed on a 1 x 20 cm column of Amberlite CG-120, (200-400 mesh) resin. Ten ml samples were collected and analyzed with the aid of a Technicon automatic amino acid analyzer. The elution patterns obtained with chicken livers and kidneys are shown in Figure 5. The radioactivity of each sample was determined by using a Packard Tri-Carb scintillation spectrometer. Five tenths ml of each sample was suspended in 10 ml of Bray's (39) counting solution (Appendix).

Tritium was found only in those fractions eluting in the same region as homocysteine and homocystine. These results indicated that chicken liver extracts did contain S-adenosylhomocysteine hydrolase activity as previously indicated by chemical analysis of the deproteinized reaction mixture. Reaction mixtures from chicken kidney extracts which had been found by chemical analysis to contain slightly more reducing sugars than the reaction control when assayed radiologically were found to lack S-adenosylhomocysteine hydrolase and S-adenosylhomocysteine nucleosidase activity (Figure 5). Reaction mixtures of Sharptail grouse and Morning dove liver extracts when chromatographed were also

Fig. 5.--Chromatographic separation of reaction mixtures produced by incubating S-adenosyl-L- ^{3}H homocysteine with crude dialyzed tissue extracts. The reaction mixtures containing 6.0 µmoles of S-adenosyl-L-[^{3}H] homocysteine, 200 µmoles of potassium phosphate buffer, pH 6.5, and between 1-2 mg of protein were incubated 30 minutes at 37°C. After deproteinizing, one ml of the reaction mixture was placed on an Amberlite CG-120, 200-400 mesh resin (1 x 20 cm). Elution was by a pH gradient obtained by allowing 250 ml of 0.2N sodium citrate solution to flow into a mixing chamber containing an equal volume of 0.2N citrate buffer, pH 2.9. Both solutions contained 0.1 ml of pentachlorophenol and 100 mg Brij-35 per liter. The flow rate was 0.7 ml per minute and the fractions were collected in 10 ml volumes. A, chromatograph of a reaction mixture in which chicken liver extract was used. B, chromatograph of a reaction mixture in which chicken kidney extract was used. (._____.) pH gradient; (----) radioactivity; (_____) amino acids as determined by a Technicon amino acid analyzer. These compounds were identified by comparison of their chromatographic properties to those of the authentic compounds previously published from this laboratory (10).


A
LIVER
S-ADENOSYLHOMOCYSTEINE SULFOXIDE
ADENOSYLHOMOCYSTEINE
HOMOCYSTINE
HOMOCYSTEINE
B
KIDNEY
µMOLES/ML
0.15
0.10
0.05
CPM/ML x 10⁻³
PH OF EFFLUENT
0
100
200
300
400
500
ML EFFLUENT

found to contain hydrolase activity. The specific activity of these systems was found to be 0.01 and 0.06 both of which were below the sensitivity of the chemical assay.

There were eight mammals assayed for S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, ribosylhomocysteine cleavage enzyme and homoserine dehydratase (Table 5). These were the white rat, the New Zealand white rabbit, the Guinea pig, Mule and Whitetail deer, the dog, steer and humans.

The white rat was found to contain hydrolase activity in the liver and kidney extracts with liver activity being the greatest. Another rodent assayed was the New Zealand white rabbit. This organism was also found to possess hydrolase activity in the liver and kidney extracts. The Guinea pig was found to possess S-adenosylhomocysteine hydrolase only in its liver. There was no hydrolase activity found in brain, heart or spleen preparations of these animals.

Herbivorous systems assayed were the Mule deer and Whitetail deer, both browsers and a year old steer, a grazer. Chemical analysis of both deer systems indicated that there was no hydrolase activity in the brain, heart, livers, spleens and kidneys of these animals.

To corroborate these results radiological assays were run as described under Figure 5. With liver extracts there was no measurable radioactivity found in regions where ribosylhomocysteine (S-adenosylhomocysteine nucleosidase) or homocysteine (S-adenosylhomocysteine hydrolase) eluted

from the column. These results indicated that neither sulfur metabolizing enzyme was present in either the Mule deer or the Whitetail deer.

Duerre et al (10) had found that S-adenosylhomocysteine was oxidatively deaminated in rats to adenosyl-γ-thio-α-ketobutyrate. They had found that S-adenosyl-γ-thio-α-ketobutyrate was eluted in the 200-230 ml fractions from an Amberlite CG-120, 200-400 mesh, resin (1 x 20 cm column) set up as previously described. Therefore, each ten ml fraction of the reaction mixtures from the deer liver extracts were analyzed for u.v. absorption at 260 mμ using a Beckman DB spectrophotometer. It was found that there was a slight increase in optical density in the fraction eluted between 190 and 240 ml. However there was no corresponding increase in radioactivity in these fractions and chemical analysis using the 2,4-dinitrophenolhydrazone reaction did not indicate the presence of any keto acids. As a result of these assays it was felt that there was no detectable L-amino acid oxidase acting on S-adenosylhomocysteine in deer liver extracts.

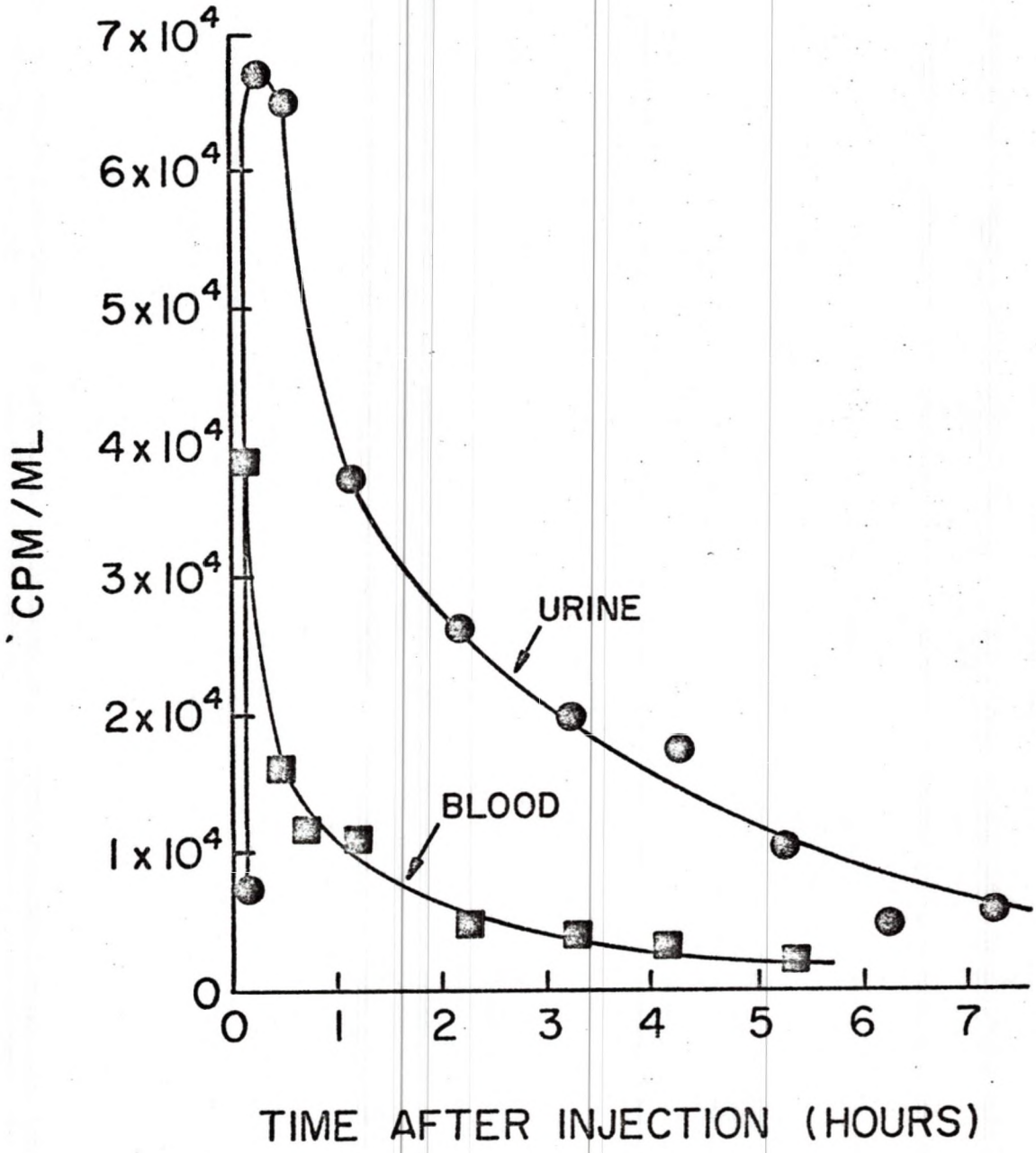
Chemical analysis of the steer liver and spleen indicated that S-adenosylhomocysteine hydrolase activities were present in both systems. The nucleosidase assay of beef liver extract indicated that this system might also contain traces of reducing sugars. A deproteinized reaction mixture of beef liver extract was therefore chromatographed and assayed radiologically in a manner similar to that used for

birds and deer extracts (Figure 5). A radioactive ninhydrin positive material eluting in the same position as homocysteine indicated that only S-adenosylhomocysteine hydrolase was present.

Both human liver samples were found to lack detectable S-adenosylhomocysteine hydrolase and S-adenosylhomocysteine nucleosidase activity when assayed chemically. Radiological studies using S-adenosyl-L-[^{3}H] homocysteine as previously employed (Figure 5) also failed to yield detectable hydrolase or nucleosidase activity.

The only other system assayed was the dog, a carnivore. S-Adenosylhomocysteine hydrolase activity was found in both liver and kidney extracts. As the dog was readily accessible it was decided to inject S-[^{3}H]adenosyl-L-homocysteine (adenosine labeled) intravenously and measure the uptake and/or excretion of this compound <u>in vivo</u>. Four hundred µmoles (1.93×10^{6} DPM/µmole) of radioactive S-adenosylhomocysteine was injected intravenously into a 5.6 kg mongrel dog. The dog was anesthetized with pentobarbital throughout the experiment. Urine collection was done by catheterization. The dog was also bled periodically to determine the amount of radioactivity remaining in the blood. It was found that radioactivity in the blood decreased quite rapidly in the first hour (Figure 6). Radioactivity in the urine increased markedly in the first half hour and then began to decrease. After one hour there was a very rapid decrease in radioactivity in the urine. These results indicate that S-adenosylhomocysteine was

Fig. 6.--Clearance of S-[^{3}H]adenosyl-L-homocysteine from blood via urine following an intravenous injection of four hundred umoles (1.93×10^6 DPM/umoles) of S-[^{3}H]adenosylhomocysteine into a 5.6 kg dog. Urine samples were collected by catheritization. Two tenth ml of each urine (●——●) and blood (■——■) samples were diluted to 10 ml with Bray's (39) counting solution and counted on a Packard Tri-Carb scintillation spectrometer.


7x10^4
6x10^4
5x10^4
4x10^4
3x10^4
2x10^4
1x10^4
0
CPM/ML
URINE
BLOOD
0
1
2
3
4
5
6
7
TIME AFTER INJECTION (HOURS)

cleared quite rapidly from the blood and was excreted in the urine. After seven hours the animal was sacrificed by an overdose of pentobarbital. Heart, liver, spleen, kidney, muscle, lung and small intestine were removed, weighed and homogenized in demineralized water. Two tenths ml of each homogenate was diluted to ten ml with Bray's (39) counting solution and assayed for radioactivity using a Packard Tri-Carb scintillation spectrometer (Table 6). An additional two ml of each tissue homogenate was extracted with equal volumes of 10% cold TCA followed by two more extractions with cold 5% TCA. Nucleic acids were then solubilized with 5% hot TCA. Four tenths of a ml of each nucleic acid fraction was assayed radiologically. The precipitate constituted the protein fraction which was found devoid of radioactivity.

Considering the specific activity of the S-adenosylhomocysteine injected none of the tissues assayed contained a very substantial amount of isotope. Furthermore, essentially all of the isotope was soluble in cold TCA indicating that the dog was unable to metabolize exogenous S-adenosylhomocysteine to any measurable degree.

Dog urine, from the 1-2 hour sample, was chromatographed on Amberlite CG-150 as described for Figure 5. Essentially all of the radioactivity found in the urine was associated with S-adenosylhomocysteine.

All vertebrate tissues assayed for S-adenosylhomocysteine hydrolase and S-adenosylhomocysteine nucleosidase were also assayed for S-ribosylhomocysteine cleavage enzyme. All

TABLE 6

MEASUREMENT OF RADIOACTIVITY FROM INTRAVENOUS INJECTIONS OF S-[^{3}H]ADENOSYL-L-HOMOCYSTEINE (ADENOSINE LABELED) IN DOG ORGAN CRUDE EXTRACTS AND NUCLEIC ACIDS

TISSUE	CPM/g TISSUE WHOLE HOMOGENATE	CPM/g TISSUE NUCLEIC ACID
Heart	6,807	0
Liver	40,072	1,539
Spleen	23,580	2,516
Kidney	23,870	1,775
Muscle	3,548	97
Lung	5,298	514
Small Intestine	15,756	784

Four hundred μmoles (1.93×10^6 DPM/μmole) of S-[^{3}H]adenosyl-L-homocysteine (adenosine labeled) was administered intravenously to a 5.6 kg dog. The dog was sacrificed seven and a half hours after administration. All organs were removed immediately and homogenized in distilled water. Two tenths ml of each homogenate was added to 9.8 ml of Bray's (39) counting solution. An additional two ml was extracted three times with cold 5% TCA. The precipitate from this extraction was extracted twice with hot 5% TCA (80-85°C) for 30 minutes. Four tenths ml of this sample (nucleic acids) was added to 9.6 ml of Bray's counting solution.

cold and warm blooded vertebrates assayed were found to lack detectable ribosylhomocysteine cleavage enzyme activity.

Effects of Age on S-Adenosylhomocysteine Hydrolase Activity

To determine if there was an increase or decrease in S-adenosylhomocysteine hydrolase activity with age, four age groups of rats were assayed (Table 7). Livers were removed from newborn, one month old, twelve month old and thirty month old rats. It was found that liver extracts from year old rats had the greatest activity; whereas, liver extracts from newborns exhibited the lowest activity.

Homoserine Dehydratase

All fish assayed except the Buffalohead exhibited homoserine dehydratase activity in their liver extracts (Table 8). In addition to detectable enzyme activity in their liver extracts the Channel cat and the Yellow perch were found to possess homoserine dehydratase activity in heart and kidney extracts.

The Leopard frog and Garter snake also exhibited homoserine dehydratase activity in their liver extracts. The frog lacked detectable enzyme activity in all other organs assayed. In addition to homoserine dehydratase activity in the Garter snake liver this enzyme was also found in snake kidney, but lacking in the brain or heart.

Of the five birds assayed only three, the Rouen duck, Morning dove and Lesser Yellowleg exhibited detectable homoserine dehydratase activity in liver extracts (Table 9).

TABLE 7

EFFECTS OF AGE ON S-ADENOSYLHOMOCYSTEINE HYDROLASE ACTIVITY IN WHITE RATS

AGE OF RATS IN MONTHS	SPECIFIC ACTIVITY
Newborns	1.16
One	1.72
Twelve	2.11
Thirty	1.47

Reaction mixtures for S-adenosylhomocysteine hydrolase contained 10-12 µmoles adenosine, 6 µmoles L-homocysteine, 1-2 mg of protein and 200 µmoles of potassium phosphate buffer, pH 6.5. Specific activity expressed in µmoles of substrate utilized/mg of protein/30 min.

TABLE 8

ASSAY FOR HOMOSERINE DEHYDRATASE IN COLD BLOODED VERTEBRATES

VERTEBRATES	ORGANS		
	HEART	LIVER	KIDNEY
Ictiobus bubalus (Buffalohead)	NDA[a]	NDA	NDA
Ictalurus punctatus (Channel cat)	0.01	0.06	0.03
Polyodon spathula (Paddlefish)	NDA	0.03	NDA
Salmo gairdneri (Rainbow trout)	NDA	0.08	-[b]
Perca flavescens (Yellow perch)	0.03	0.06	0.05
Rana pipiens (Leopard frog)	NDA	0.03	NDA
Thamnophis sirtalis (Garter snake)	NDA	0.05	0.03

Reaction mixtures for homoserine dehydratase (cystathionase) contained 16 µmoles of L-homoserine, 1-2 mg of protein, 50 µmoles Tris-HCl buffer, pH 7.5, 25 µmoles citrate NaOH buffer, pH 7.5, and 0.08 µmoles pyridoxal phosphate. Activity expressed in µmoles of substrate utilized/mg of protein/30 min.

[a]NDA = No detectable activity.

[b]Tissue not available.

TABLE 9

ASSAY FOR HOMOSERINE DEHYDRATASE IN WARM BLOODED VERTEBRATES

VERTEBRATES	ORGANS		
	BRAIN	LIVER	KIDNEY
Galus galus (White Leghorn chicken)	NDA[a]	NDA	0.03
Pedioecetes phasianellus (Sharptail grouse)	NDA	NDA	NDA
Anas platyrhynchos (Rouen duck)	0.01	0.09	0.02
Zenaidura macronra (Morning dove)	NDA	0.02	NDA
Totanus flavipes (Lesser Yellowleg)	NDA	0.12	0.24
Cavia porcellus (Guinea pig)	NDA	NDA	NDA
Rattus rattus (white rat)	NDA	0.13	0.06
Oryctolagus cuniculus (New Zealand white rabbit)	NDA	NDA	NDA

TABLE 9--Continued

VERTEBRATES	ORGANS		
	BRAIN	LIVER	KIDNEY
Canis familiaris (dog)	NDA	NDA	NDA
Odocoileus hemionus (Mule deer)	NDA	0.04	NDA
Odocoileus virginianus (Whitetail deer)	NDA	0.07	0.01
Bos (steer)	- [b]	0.02	-
Homo sapiens (human)	-	0.04	-

Reaction mixtures for homoserine dehydratase (cystathionase) contained 16 µmoles of L-homoserine, 1-2 mg of protein, 50 µmoles Tris-HCl buffer, pH 7.5, 25 µmoles citrate NaOH buffer, pH 7.5, and 0.08 µmoles pyridoxal phosphate. Activity expressed in µmoles of substrate utilized/mg of protein/30 min.

[a]NDA = No detectable activity.

[b]Tissue not available.

Three birds also exhibited homoserine dehydratase activity in kidney extracts. These were the White Leghorn chicken, Rouen duck and Lesser Yellowleg. The Sharptail grouse lacked any detectable enzyme activity in all five organs assayed. The Rouen duck was the only vertebrate assayed that was found to possess homoserine dehydratase activity in brain extracts.

Of the three rodents assayed the white rat was the only one to exhibit homoserine dehydratase activity. This enzyme was found in both the liver and kidney extracts.

Of the remaining five mammals assayed for the presence of homoserine dehydratase activity only the dog lacked enzyme activity in liver extracts. The Whitetail deer also had detectable activity in its kidney extracts while the dog lacked detectable homoserine dehydratase activity in all organs assayed.

DISCUSSION

S-Adenosylmethionine serves as the principle methyl donor in living systems from microorganisms to man (4). The demethylated product in all transmethylation reactions involving S-adenosylmethionine is S-adenosylhomocysteine. The metabolism of S-adenosylhomocysteine has been investigated by a number of workers (7, 9-15).

This study corroborates and expands upon the information gathered by these researchers. In this study bacteria, yeasts, plants and organs from cold blooded and warm blooded vertebrates were assayed for the presence of enzymes involved in the metabolism of S-adenosylhomocysteine and its derivatives. The most widely distributed enzyme found in the metabolism of S-adenosylhomocysteine was S-adenosylhomocysteine hydrolase.

In addition to the two yeasts previously assayed, Candida utilis and Saccharomyces cerevisiae (11-13) a third yeast Candida albicans was assayed. This yeast, like Candida utilis and Saccharomyces cerevisiae was found to possess S-adenosylhomocysteine hydrolase.

Extracts from plants, which had not been assayed prior to this study, were also found to possess S-adenosylhomocysteine hydrolase activity. Some of the differences seen in

specific activity between the various plant extracts was probably due to the way in which the extracts were prepared. Spinach extracts were prepared by lysing the cells in a nitrogen cooled press, centrifuging the lysate and dialyzing the resultant supernate overnight. Green bean extracts were also prepared this way, but lacked any detectable hydrolase activity. As there was a large amount of pigment in these samples it was possible that the pigment might have served as an inhibitor, or interfered with the assay. After the cell free extracts from green beans were eluted through a G-25 (4 x 50 cm) molecular sieve column to remove pigments S-adenosylhomocysteine hydrolase activity was detectable.

Sprout extracts of corn and barley, grown in the absence of light, lacked pigmentation and were therefore assayed directly. With these extracts there was less time elapsed from cell lysis to assaying and less handling which could have resulted in higher specific activities for these extracts.

Prior to this study the only published information concerning the metabolism of S-adenosylhomocysteine in vertebrate systems involved the metabolism of S-adenosyl-homocysteine in the white rat (4,5,7,9,10). In this study all vertebrates assayed were found to possess S-adenosyl-homocysteine hydrolase activity in crude liver extracts except the Paddlefish, Mule deer, Whitetail deer and human.

The possibility exists that the human livers could have lost considerable activity prior to collection. Both human liver samples obtained were from autopsy material in

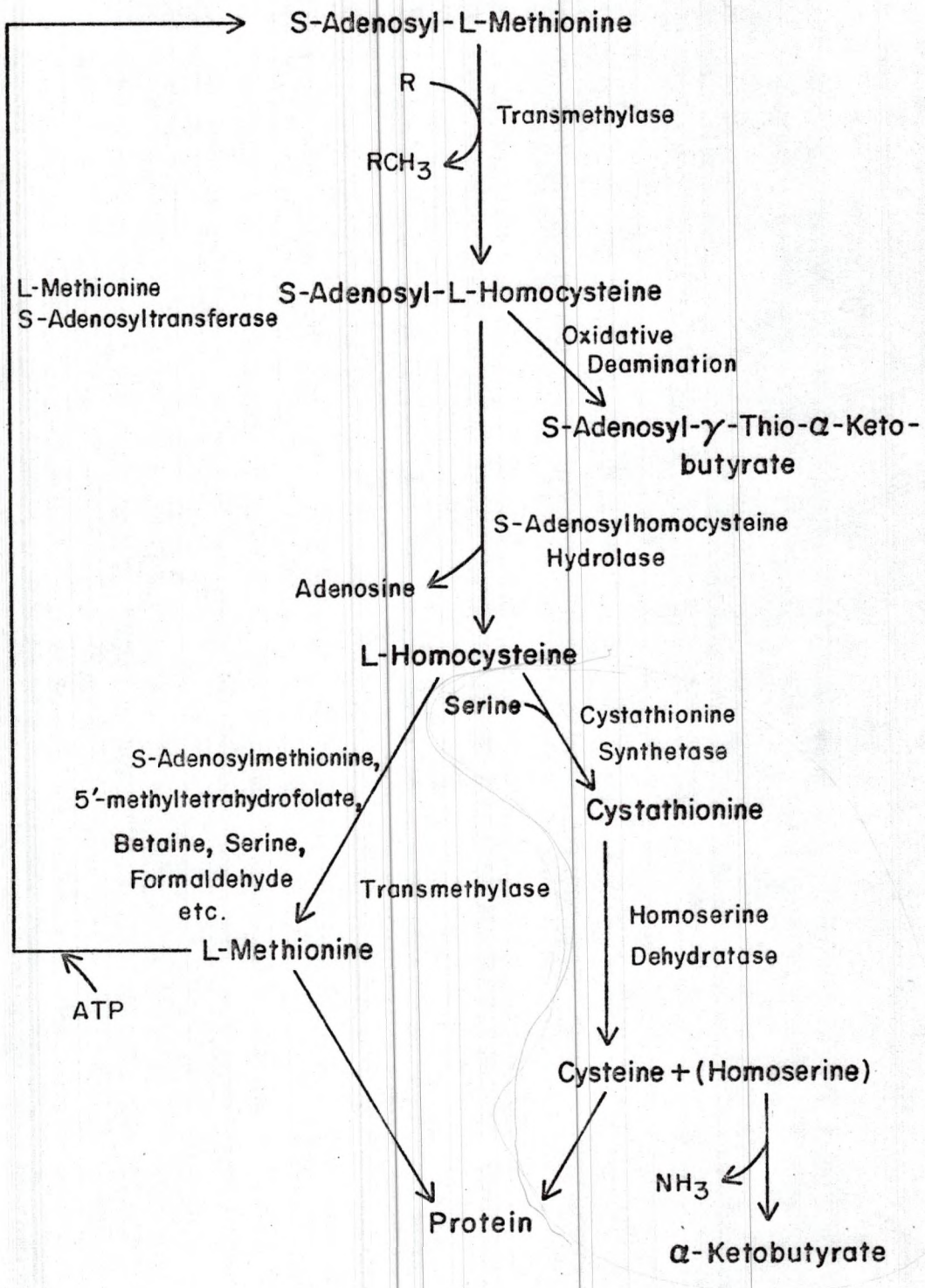
which the victims had been dead a minimum of four hours. Attempts to obtain fresh liver biopsy material from normal subjects have been unsuccessful.

All of the vertebrates assayed except the Buffalohead, Sharptail grouse, Guinea pig, rabbit and dog possessed detectable homoserine dehydratase in one or more organs. The duck was the only vertebrate assayed that exhibited enzyme activity in brain extracts while the Channel cat and Yellow perch were the only vertebrates assayed that were found to have detectable enzyme activity in heart extracts. There was no detectable homoserine dehydratase activity detected in any spleen extracts assayed.

From this study it is concluded that the metabolism of S-adenosylhomocysteine in higher organisms such as yeast, plants, cold and warm blooded vertebrates, involves the hydrolysis of S-adenosylhomocysteine yielding adenosine and homocysteine. The homocysteine moiety may then be converted to methionine via transmethylation (19,20,22,23,40,41) or undergo transulfuration. In transulfuration reactions the sulfur atom of homocysteine may appear in the urine as inorganic sulfate, in cysteine or in taurine via cysteine (29,30) with the carbon skeleton being excreted as α-keto-butyrate. This pathway is illustrated in Scheme III.

Duerre, Miller and Reams (10) also found that S-adenosylhomocysteine may undergo oxidative deamination in the rat yielding S-adenosyl-γ-thio-α-ketobutyrate, which is excreted in the urine.

METABOLISM OF S-ADENOSYLHOMOCYSTEINE IN EUCARYOTIC ORGANISMS



SCHEME III

In the dog, which does not possess L-amino acid oxidase most of the S-adenosylhomocysteine administered intravenously was excreted in the urine unaltered. Only trace amounts of the radioactive adenine moiety was detectable in nucleic acid. Essentially no radioactive purines were excreted in the urine of the dog. Since the dog contains a very active S-adenosylhomocysteine hydrolase in the kidney and the liver it is concluded that both kidney and liver cells are impermeable to S-adenosylhomocysteine or the enzyme is bound in such a manner as to be inactive against this compound.

Duerre et al (10) had previously found that the rat failed to hydrolyze significant quantities of S-adenosylhomocysteine administered in vivo. They concluded that since the S-adenosylhomocysteine was excreted as adenosyl-γ-thio-α-ketobutyrate that S-adenosylhomocysteine was oxidatively deaminated in the kidneys. In this study it was found that kidney cells also contain S-adenosylhomocysteine hydrolase. Consequently this enzyme must be compartmentalized or bound in such a manner as to be inoperative against exogenous S-adenosylhomocysteine.

None of the bacteria assayed in this study contained measurable S-adenosylhomocysteine hydrolase activity. Of the nine bacteria assayed all except Bacillus subtilis were found to possess S-adenosylhomocysteine nucleosidase activity. Three Gram negative bacteria, including Escherichia coli, Enterobacter aerogenes and Salmonella typhimurium had

previously been found to possess this enzyme (14). However, this is the first report of S-adenosylhomocysteine nucleosidase being found in Gram positive bacteria.

Previous studies by Duerre (14) had suggested that the S-adenosylhomocysteine nucleosidase enzyme would also cleave methylthioadenosine. In this study extracts prepared from all Gram negative bacteria except Alcaligenes faecalis, yielded a ratio of methylthioadenosine to S-adenosylhomocysteine of approximately the same value. Extracts prepared from Bacillus cereus and Staphylococcus aureus were also quite active toward methylthioadenosine yielding a similar methylthioadenosine to S-adenosylhomocysteine ratio that was slightly lower than that found in Gram negative bacteria. Alcaligenes faecalis and Sarcina lutea were the only bacteria that exhibited activity toward S-adenosylhomocysteine but failed to exhibit activity toward methylthioadenosine.

All of the Gram negative bacteria tested except Alcaligenes faecalis possessed measurable levels of ribosylhomocysteine cleavage enzyme. Since all the Gram positive organisms lacked detectable ribosylhomocysteine cleavage activity it is presently not understood how these microorganisms might utilize ribosylhomocysteine. The ribosylhomocysteine formed might be excreted directly into the media or cleavage of this compound could occur in a manner that was not detectable under these assay conditions.

No homoserine dehydratase was detectable in any of the Gram negative bacteria. These results essentially confirmed those of Delavier-Klutchko and Flavin (32) in so far as they

found that extracts from Escherichia coli and Salmonella typhimurium lacked homoserine dehydratase catalyzing either γ or β elimination (Reactions 4 and 5).

Cystathionine + H_2O ——— Cysteine + γ-ketobutyrate + NH_3

Reaction 4 (γ-elimination)

Cystathionine + H_2O ——— Homocysteine + Pyruvate + NH_3

Reaction 5 (β-elimination)

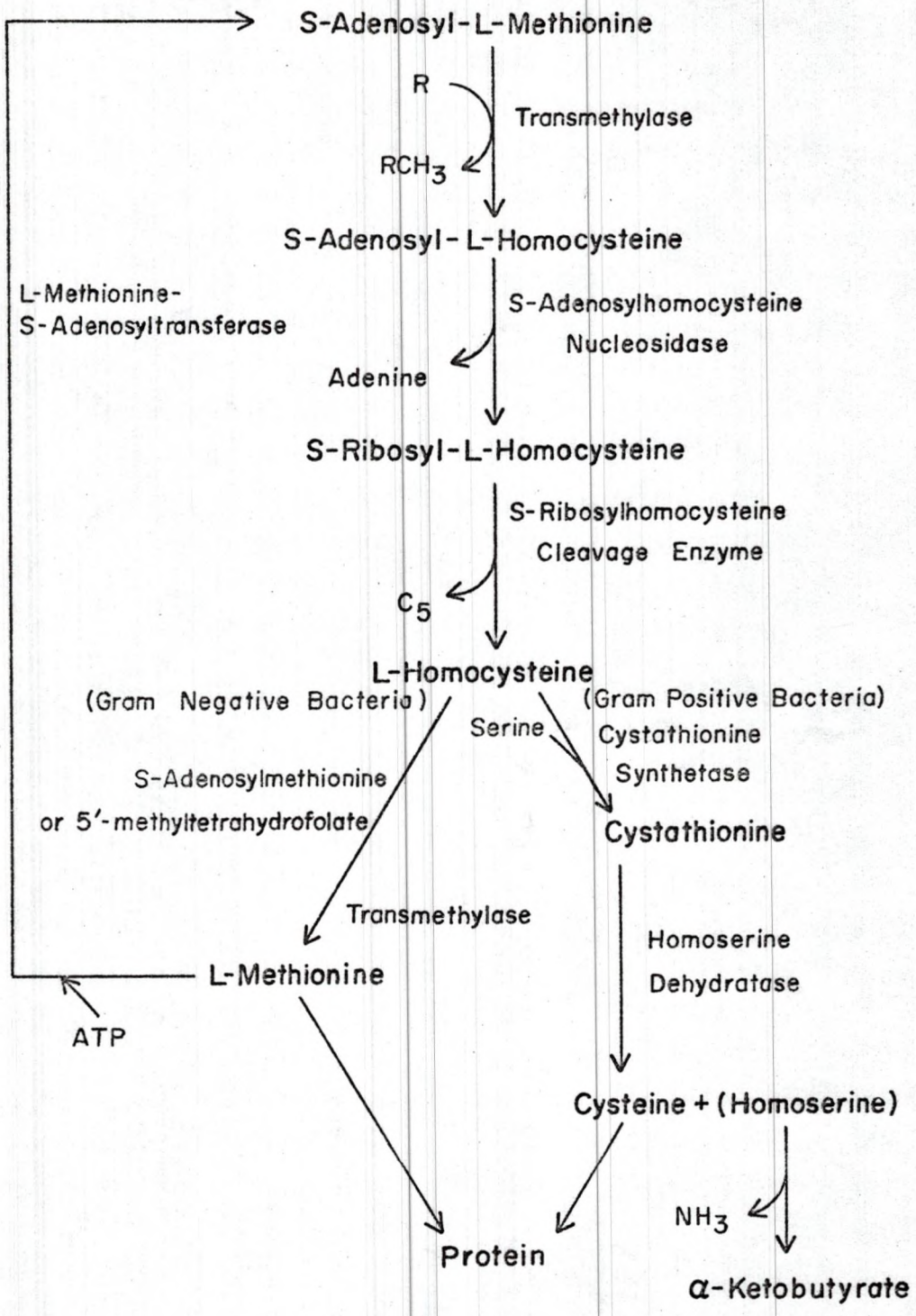
Delavier-Klutchko and Flavin have shown that these microorganisms contain an enzyme that catalyzes the condensation of O-succinylhomoserine + cysteine to yield cystathionine (γ-replacement). In contracts to the Gram negative bacteria the Gram positive bacteria were found to catalyze γ-elimination of cystathionine (Reaction 4). The assay we employed in this study did not allow for the measurement of β-elimination.

The three yeasts employed in this study lacked homoserine dehydratase activity as measured under these assay conditions. Delavier-Klutchko and Flavin (32) had previously shown that Saccharomyces cerevisiae, one of the microorganisms employed in this study, catalyzed both γ and β elimination of cystathionine. Since we employed homoserine as substrate it may have been possible that the yeast would not catalyze the

deamination of L-homoserine to ∝-ketobutyrate. Similarly the method we employed in preparation of yeast cell free extracts were such that they may have inactivated homoserine dehydratase. Another possible accounting for this difference is that the strain we employed lacked this enzyme.

As a result of this study it is suggested that in bacteria S-adenosylhomocysteine is metabolized by cleaving the glycosidic bond yielding adenine and ribosylhomocysteine. Ribosylhomocysteine is then further metabolized by cleavage of the thio-ether linkage yielding a pentose and homocysteine. The homocysteine moiety may then methylate to methionine as found in Gram negative bacteria (32), or may be involved in a transulfuration reaction and contribute to the formation of cysteine as found in Gram positive bacteria. This pathway is illustrated in Scheme IV.

METABOLISM OF S-ADENOSYLHOMOCYSTEINE IN PROCARYOTIC ORGANISMS



SCHEME IV

SUMMARY

Eleven microorganisms, four plants and the major organs from fish, frogs, snakes, birds and mammals were assayed for the presence of S-adenosylhomocysteine hydrolase, S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase.

All Gram negative bacteria assayed were found to possess S-adenosylhomocysteine nucleosidase. This enzyme was also found in all Gram positive bacteria except Bacillus subtilis.

S-Ribosylhomocysteine cleavage enzyme was found in all Gram negative bacteria except Alcaligenes faecalis. This enzyme was lacking in all Gram positive bacteria. Homoserine dehydratase was lacking in all bacteria assayed except Bacillus subtilis, Bacillus cereus and Sarcina lutea.

S-Adenosylhomocysteine hydrolase was not detected in any bacteria but was found in Saccharomyces cerevisiae, Candida albicans and all plants assayed. Yeasts and plants lacked detectable S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and homoserine dehydratase.

All cold blooded vertebrates assayed except the

Paddlefish were found to possess S-adenosylhomocysteine hydrolase in their liver extracts. This enzyme was also found in the spleens of Channel cat and Yellow perch and in the kidneys of the Buffalohead, Channel cat and Garter snake, but was not detected in any brains or hearts. S-Adenosylhomocysteine nucleosidase and S-ribosylhomocysteine cleavage enzyme were not detected in any cold blooded vertebrate organs.

Homoserine dehydratase was found in the livers of all cold blooded vertebrates except the Buffalohead. This enzyme was also found in the hearts of the Channel cat and Yellow perch and in the kidneys of Channel cat, Yellow perch and Garter snake. However, there was no homoserine dehydratase detected in the brains or spleens of any cold blooded vertebrates assayed.

Thirteen warm blooded vertebrates were tested for the above mentioned enzymes. S-Adenosylhomocysteine hydrolase was found in the livers of all warm blooded vertebrates tested except the Mule deer, Whitetail deer and human. This enzyme was also found in the spleen of the steer and in the kidneys of the Rouen duck, Lesser Yellowleg, white rat, New Zealand white rabbit and dog. However there was no detectable hydrolase in the brains or hearts of these vertebrates.

S-Adenosylhomocysteine nucleosidase and S-ribosyl-homocysteine cleavage enzyme were not detected in any vertebrate organ.

Homoserine dehydratase was found in the livers of all warm blooded vertebrates except the White Leghorn chicken, Sharptail grouse, Guinea pig, New Zealand white rabbit and dog. This enzyme was also found in the brains of the Rouen duck and in the kidneys of the White Leghorn chicken, Rouen duck, Lesser Yellowleg, white rat and Whitetail deer. There was no detectable homoserine dehydratase in any warm blooded vertebrate hearts or spleens.

In vivo studies involving intravenous injections of S-[^{3}H]adenosyl-L-homocysteine (adenosine labeled) into an anesthetized dog indicated that exogenous sources of S-adenosylhomocysteine are not metabolized by the major organs of the dog but are excreted as S-adenosylhomocysteine.

APPENDIX

PREPARATIONS OF REAGENT SOLUTIONS

M-9 Medium (Anderson)

Compound	Amount per liter
KH_2PO_4	3 g
K_2HPO_4	6 g
NaCl	3 g
$(NH_4)_2SO_4$	2 g
$MgSO_4$	0.1 g
Glucose (autoclaved separately)	5 g

Salt Solution Added to M-9 Medium

Compound	Amount per liter
$CaCl_2$	0.25 g
$ZnSO_4$	1 mg
$MnCl_2$	1 mg
$FeCl_3$	0.5 mg
$CuSO_4$	0.1 mg
KI	0.1 mg

Add 1.0 ml of this solution to every 15 liters of M-9 medium.

Orcinol Determination of Pentose

Bial's reagent

Orcinol	0.2 g
HCl (conc.)	60 ml
$FeCl_3$	0.2 ml

Determination of Protein (Lowry)

Reagent A

2.0% Na_2CO_3 in 0.1 N NaOH

Reagent B

0.5% $CuSO_4 \cdot 5H_2O$ in water

Reagent C

1.0% Na or K tartrate in water

Reagent D

Reagent A	50	ml
Reagent B	0.5	ml
Reagent C	0.5	ml

Determination of Pentose (Benedict)

Copper reagent

A. Na_2CO_3 (anhydrous)	15	g
Alanine	3	g
Rochelle salts	2	g
Water	250	g
B. $CuSO_4 \cdot 5H_2O$	3	g
Water	100	ml

C. Mix A and B. dilute to 500 ml with water, store at 4°C

Bisulfite solution

1% $NaSO_3$ in water

Copper reagent containing bisulfite

Bisulfite solution	1	ml

Copper reagent..... 20 ml

Do not use after second day

Color Reagent

Molybdic acid.....150 ml

Na_2CO_3 (anhydrous) ..... 75 ml

Water.....500 ml

Heat to boiling; filter; wash residue from filter until filtrave plus washings equal 600 ml.

H_3PO_4.....300 ml

Dilute with water to 1 liter.

Ninhydrin Reagent for the Determination of Amino Acids

Ninhydrin Reagent

A. Methyl cellosolve.....750 ml

B. 4N Acetate buffer, pH 5.5.....250 ml

C. Ninhydrin - added under nitrogen atmosphere 10 g

Clealand's Reagent

A. Dithiothreitol ..... 60 mg

B. 3 x Distilled-Deionized Water .....500 ml

C. Methyl cellosolve .....500 ml

Bray's Counting Solution

100% Ethanol	200 ml
Toluene	800 ml
PPO 2,5 Diphenyloxazole	6 g/liter
POPOP 1,4-bis-[2-(5-phenyloxazolyl)]-Benzene	100 mg/liter

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