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Title: THE METABOLISM OF 5'-DEOXY-5'-METHYLTHIOADENOSINE IN SACCHAROMYCES CEREVISIAE

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Dr. A. J. Ferro

Cells of Saccharomyces cerevisiae are not normally permeable to the purine nucleoside 5'-deoxy-5'-methylthioadenosine (MTA). The exogenous supplementation of MTA to medium containing a cordycepin sensitive mutant of S. cerevisiae, however, satisfied both the purine and methionine auxotrophic requirements. Cordycepin sensitive strains accumulated MTA at a rate at least seven times that of an isogenic cordycepin resistant prototroph. The data indicate that the salvage of the purine moiety from S-adenosylmethionine may be accomplished by two independent mechanisms: one utilizing S-adenosylhomocysteine as an intermediate, the other MTA. The enzyme central to the purine salvage pathway from MTA, MTA phosphorylase, has been purified 135-fold from S. cerevisiae. A molecular weight of 90,000 D for the enzyme was estimated by gel filtration on Sephadex G-200. The enzyme catalyzed degradation of MTA yielded 5-methylthioribose-1-phosphate and adenine as products and had a

temperature optimum of 58°C. Michaelis constants (K_m) have been determined for MTA (18.7 μM) and phosphate (2.33 mM). Several analogs of MTA were tested as substrates for the purified enzyme. The 7-deaza analog, 5'-deoxy-5'-methylthiotubercidin, was inactive as substrate, whereas substitutions at the 5'-carbon showed varying degrees of reactivity; 5'-deoxy-5'-isobutylthioadenosine was the best substrate. MTA, as well as the 5'-substituted analogs, could serve as sole sources of purine in an adenine auxotrophic strain of S. cerevisiae.

The fate of the methylthioribose carbons was also studied. Evidence is presented for the recycling of the methylthio group and part of the ribose portion of MTA in a biosynthetic pathway which leads to the synthesis of methionine. The main pathway involves the phosphorylytic cleavage of MTA by MTA phosphorylase. Loss of the phosphate group of 5-methylthioribose-1-phosphate, concurrent with rearrangement of the ribose carbons leads to the synthesis of 2-keto-4-methylthiobutyric acid. In the final step of the sequence, 2-keto-4-methylthiobutyric acid is converted to methionine via transamination. Several compounds, not directly associated with the biosynthesis of methionine, were isolated. These compounds, arising through the degradation of intermediates in the pathway were: 5'-methylthioinosine, a deaminated catabolite of MTA; 5-methylthioribose, a result of the phosphorolysis of 5-methylthioribose-1-phosphate; and 3-methylthiopropionaldehyde, 3-methylthio-

propionic acid, and 2-hydroxy-4-methylthiobutyric acid, all arising from the catabolism of 2-keto-4-methylthiobutyric acid.

The Metabolism of 5'-deoxy-5'-Methylthioadenosine in *Saccharomyces cerevisiae*

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INTRODUCTION

The accumulation of S-adenosyl-L-methionine in the vacuoles of yeast cells is well documented (162). In *Saccharomyces cerevisiae*, a specific, high affinity permease for SAM transport has been described (163). Intracellular concentrations of SAM as great as 20 mM, resulting from the activity of the SAM permease, have been reported (164). Comparatively little, however, is known of the metabolic fate of exogenously supplied SAM and its metabolites in yeast cells. Yall, *et al.* (106) found that an adenine requiring strain of *S. cerevisiae* could utilize adenine or SAM as equivalent sources of purine for cell growth; S-adenosyl-L-homocysteine (SAH) and 5'-deoxy-5'-methylthioadenosine (MTA), however, would not support growth of this adenine auxotroph. Knudsen *et al.* (107) subsequently demonstrated that exogenously supplied SAH could serve as a source of purine, but only when an inoculum in the logarithmic phase of growth was utilized.

The proposed route by which SAM may be metabolized to serve as a source of purine consists of its demethylation to SAH, followed by the hydrolytic cleavage of SAH to adenosine and homocysteine; S-adenosyl-L-homocysteine hydrolase has been shown to be present in yeast extracts (54). However, the observation that adenine, as well as adenosine, labeled with ^{14}C was found to be produced from

exogenously supplied SAM (107) suggests an alternative purine salvage pathway from SAM.

A possible metabolic pathway by which adenine can be recovered from SAM involves the synthesis and degradation of MTA. Several routes for the synthesis of MTA from SAM have been described (157).

Both prokaryotic (39) and eukaryotic (38) cells, however, accumulate only trace amounts of this nucleoside. MTA is rapidly metabolized by one of two distinct reactions. In most microorganisms (87), and plants (44), MTA is degraded by a hydrolytic cleavage to 5-methylthioribose and adenine, whereas, in animal tissue (87), and the thermophilic bacterium Caldariella acidophila (51) a phosphorylytic cleavage results in the formation of 5-methylthioribose-1-phosphate and adenine. In Saccharomyces cerevisiae, the mechanism by which MTA is degraded has not been established.

The fate of the products of MTA has only recently been explained. In mammals the phosphorylase may account for the repeated demonstration of small quantities of adenine in the urine (165). This enzyme, therefore, may play an important role in the salvage and recycling of adenine.

Until recently, little was known of the fate of the methylthioribose moiety, except that the methiol group was recovered intact in methionine (122-126). Shapiro and Schlenk (128) have found that, in cultures of Candida albicans grown on [U-^{14}C-adenosine]MTA, the methiol group and part of the pentose ring was incorporated into the amino acid part of S-adenosylmethionine. A similar pathway may

exist in mammalian cells. Backlund and Smith (129) reported the primary function of MTA *in vitro*, in rat liver extracts, was the formation of methionine and that the pathway by which this takes place involves modifications in the ribose portion of the molecule.

Recently, Shapiro and Barrett (130) demonstrated that 5-methylthioribose-1-phosphate is an intermediate in the biosynthesis of methionine in *Enterobacter aerogenes* and that 2-keto-4-methylthiobutyric acid and 2-hydroxy-4-methylthiobutyric acid were somehow involved in this pathway. In rat liver and kidney (131), glutamine transaminase has been shown to catalyze the conversion of 2-keto-4-methylthiobutyric acid and glutamine to methionine and glutamate; the reverse reaction has also been demonstrated in fungi (34), rat liver (132), and bacteria (133). More recently, Backlund *et al.* (160) proved that 2-keto-4-methylthiobutyrate was an intermediate in the biosynthesis of methionine from MTA in rat liver. 2-keto-4-methylthiobutyric acid and 2-hydroxy-4-methylthiobutyric acid also have been isolated as products of dissimilation of methionine in fungi (34).

Unfortunately, the yeast cell membrane is impermeable to MTA as well as other nucleosides, rendering it impossible to follow the fate of exogenously supplied MTA.

In this study, however, we have utilized cordycepin sensitive mutants of *S. cerevisiae*, which transport adenosine (152), to demonstrate that MTA could fully support the requirement for adenine and methionine in several auxotrophic strains. Preliminary experiments

in our laboratory (108) demonstrated that MTA had no deleterious effects on the growth of these nucleoside permeable strains of yeast. Thus, S. cerevisiae was chosen for the study of the fate of the structural units of MTA both in vivo and in vitro. In addition, we report herein the purification and characterization of the MTA cleaving enzyme from S. cerevisiae.

LITERATURE REVIEW

The first observation of 5'-deoxy-5'-methylthioadenosine (MTA) was by Mandel and Dunham in 1912 (1) in extracts of a commercial yeast preparation. The compound, however, was erroneously identified as an adenine-hexose compound which lacked sulfur. The correct structure was elucidated in 1924 (2) (Fig. 1). MTA crystallized to a structure consisting of two independent, conformationally different molecules (A and B) per assymetric unit (3). The glycosidic torsion angle [O(1')-C(3')-N(9)-C(4)] was 163° in molecule A and -119° in molecule B and the ribose moiety was C(2')-exo, C(3')-exo in molecule A and C(2')-endo, C(3')-exo in molecule B. A feature common to both molecules was the disposition of the thiomethyl side group with respect to the ribose. In nucleotide nomenclature this is the trans,gauche conformation, rarely found in nucleotides. The molecules were linked by a 3-dimensional network of H-bonds between base-sugar and base-base residues (3).

Biosynthesis of MTA

MTA is enzymatically synthesized from S-adenosylmethionine (SAM) by at least seven independent pathways (Fig. 2). SAM may be directly cleaved into MTA and homoserine lactone by S-adenosylmethionine cyclotransferase (SAM lyase, SAM hydrolase, SAM cleaving enzyme). Enzyme activity has been reported in *Enterobacter aerogenes* (4), *Saccharomyces cerevisiae* (5), T3 infected *Escherichia coli* (6,7), *Vinca rosea* seedlings (8), porcine and rat liver (9),

Fig. 1. The structure of 5'-deoxy-5'-methylthioadenosine (MTA).

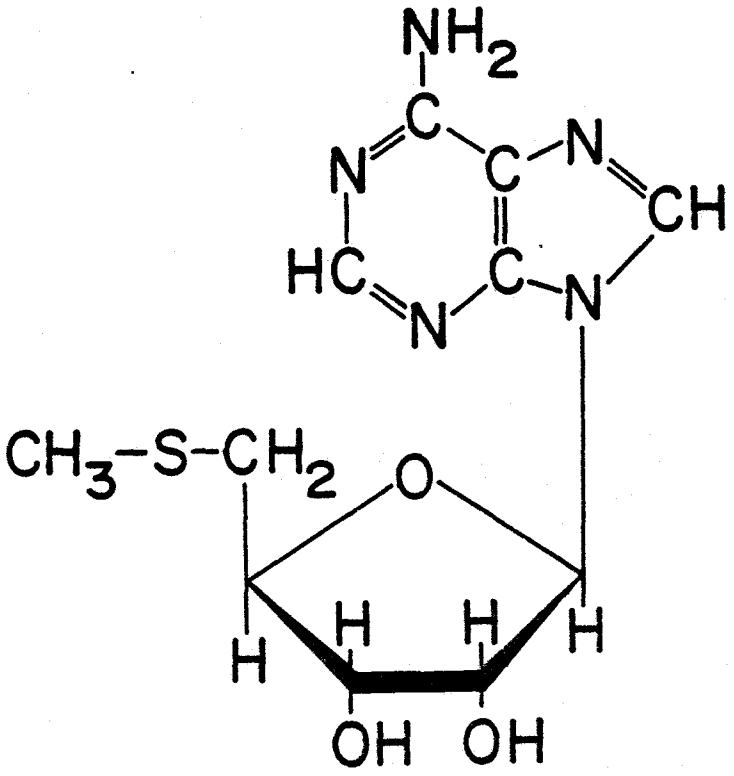

Fig. 1

and KB cells (9). Recently it was shown that the decarboxylated analogue of SAM, S-adenosyl-(5')-3-methylthiopropylamine, exerted a non-competitive inhibition of the enzyme activity (10) in liver.

A second metabolic pathway leading to the formation of MTA also leads to the synthesis of the polyamines spermine and spermidine (for review see refs. 11-13). Production of MTA takes place during the biosynthesis of spermine and spermidine. SAM is first decarboxylated by SAM decarboxylase, an enzyme purified extensively from E. coli (14), yeast (15), and mammalian cells (16). The propylamino group of the decarboxylated SAM is then transferred to either putrescine or spermidine by two distinct enzymes, spermidine synthase and spermine synthase, respectively. In each case MTA is also a product. MTA inhibits spermidine synthase in E. coli (157) and the spermine synthase reaction in several mammalian systems (17,18,93) and therefore may play a role in regulating its own biosynthesis. These first two routes of biosynthesis are common to most, if not all bacterial and animal cells tested.

The synthesis of the unusual nucleotides X (found in the extra-region of several E. coli tRNA's) and Y (yeast $tRNA^{phe}$) involves the transfer of the 3-amino-3-carboxypropyl group from SAM to guanosine (Y) and uridine (X). In each case MTA is also a product (19,20).

MTA is produced in E. coli during the synthesis of biotin (21). SAM is utilized to transaminate the biotin precursor 7-keto-8-amino-pelargonic acid to form 7,8-diaminopelargonic acid. The deaminated

SAM (S-adenosyl-2-oxo-4-methylthiobutyric acid) is degraded to form MTA and 2-oxo-3-butenoic acid.

More recently, Adams and Yang (22) have presented evidence showing MTA is derived from SAM during its conversion to ethylene in apple tissue. In this scheme of reactions, SAM is first converted to 1-aminocyclopropane-1-carboxylic acid (ACC) and MTA by ACC synthase, an enzyme whose presence has been demonstrated in tomato fruit tissue (23,24). In an oxygen dependent reaction, ACC is rapidly converted to ethylene (25). Ethylene has been observed in several organisms (for review see refs. 26,27), however, ACC synthase has been demonstrated only in plant tissue. The occurrence of ethylene in yeast (28), rat liver extracts (29), and polymorphonuclear leukocytes (30) is probably a result of the Haber-Weiss reaction (31) whereby 2-keto-4-methylthiobutyric acid or 3-methylthiopropionic acid (both formed from precursor methionine, ref. 32-34) is cooxidized in the xanthine oxidase reaction, with production of ethylene.

Finally, in Dictyostelium discoideum transfer of a 3-amino-3-carboxypropyl moiety from SAM to N^6-Δ^2-isopentenyl adenine results in the formation of discadenine (35) an inhibitor of spore germination (36). The removal of the 3-amino-3-carboxypropyl group from SAM would leave MTA as a second product, as in the ACC synthase reaction. The reaction has not been demonstrated elsewhere however N^6-Δ-isopentenyl adenine has been demonstrated to be present in yeast and mammalian tissue (37).

Cellular Levels of MTA

In spite of the multiple routes of biosynthesis, MTA has been shown to be in low concentrations in the few cells and tissues thus far investigated (38,39). Rhodes and Williams-Ashman (38) found that the concentration of MTA in rat ventral prostate was less than 0.2 μmole per gram of tissue; whereas both spermidine and spermine, polyamines produced in the formation of MTA, were relatively (5-7 μmole per gram) elevated in the same tissue. In E. coli, the spermine concentration is between 4.6 and 7.4 μmole per gram dry weight while the MTA level is only 0.38 μmole per gram dry weight (39). New methods have allowed for the determination of MTA levels in rat tissue as low as 0.2 nmole per gram wet weight (42). Levels in lung, liver, kidney, and heart ranged from 1.5 to 2.1 nmole per gram wet weight. Interestingly, the MTA concentrations observed in these tissues were similar to those of decarboxylated SAM as reported by Hibasami et al. (43). However, these levels are only minute when compared to the concentration of SAM in cells, examples are (158,159) rat liver, 70 to 84 nmole per gram and rat ventral prostate, 60 nmole per gram. The low levels of MTA may be attributed to the presence of an enzyme catalyzing a hydrolytic (44-46) or phosphorylytic (47-52) cleavage of the glycosidic bond.

Degradation of MTA

Shapiro and Mather (53) found enzymatic decomposition of S-adenosylmethionine in extracts of E. aerogenes but could isolate

only trace amounts of MTA; adenine and 5-methylthioribose were found in large quantities. They demonstrated that MTA was enzymatically degraded much more rapidly than SAM so that MTA was broken down as rapidly as it was formed in the crude extracts. In 1962, Duerre (46) purified a nucleosidase from E. coli capable of degrading MTA into 5-methylthioribose and adenine. The 160-fold purified enzyme also degraded S-adenosylhomocysteine (SAH) into adenine and ribosylhomocysteine. The enzyme had a pH optimum of 6.5 and a Km for MTA of 1.8 mM while the Km for S-adenosylhomocysteine was 3.0 mM. Nucleosidase activity has since been demonstrated in numerous other bacterial species (54). Ferro et al. (45) purified the MTA nucleosidase from E. coli 220-fold. The enzyme had a molecular weight of 31,000 D and a Km value of 3.1×10^{-7} M for MTA. The low Km indicated that the high affinity enzyme was alone capable of maintaining the low levels of MTA in E. coli cells. In addition to MTA, the analogues S-adenosylhomocysteine, 5'-ethylthioadenosine, and 5'-propylthioadenosine all served as substrates for the enzyme. Adams et al. (55) recently demonstrated that the levels of MTA nucleosidase in apple tissue culture rise and fall with the levels of ACC synthase.

Pegg and Williams-Ashman (47) purified a soluble MTA phosphorylase from rat prostate 30-fold. The reaction was completely dependent on the presence of inorganic phosphate in the assay medium. The Km for phosphate was 6×10^{-4} M while the Km for MTA was 3×10^{-4} M. The products of the reaction were not identified but it was suggested that MTA was degraded to adenine and 5-methylthioribose-1-phosphate

by a phosphorylytic cleavage. Purified purine nucleosidase from spleen and erythrocytes was unable to catalyze a phosphorylytic cleavage of MTA (47) indicating MTA phosphorylase was a unique enzyme.

Garbers (48) demonstrated the presence of MTA phosphorylase in various rat tissues; the highest activity was found in lung and liver. The activity found in kidney and heart extracts were, respectively, only 40 and 16% that found in the lung tissue. A detailed kinetic analysis suggested that rat lung MTA phosphorylase catalyzed an equilibrium-ordered reaction, and that MTA was the first substrate to bind while 5-methylthioribose-1-phosphate was the first product to be released.

MTA phosphorylase was purified 400-fold from human placenta (52) and 340-fold from human prostate (49). The placental enzyme had a molecular weight of 95,000 D leading the authors to suggest the possible association of 3 monomers of a molecular weight similar to the nucleosidase in E. coli (31,000 D). The enzymes isolated from human tissue require reducing agents for activity and are inhibited by thiol reagents. These data suggest the involvement of thiols in the catalytic process. MTA phosphorylase was purified 112-fold from rat liver by Ferro et al. (56). The demonstrated molecular weight of 90,000 D agreed well with the value for the human placental enzyme (52). The human prostate enzyme had a Km for MTA of 2.5×10^{-7} M while the rat liver MTA phosphorylase had a Km value of 4.1×10^{-7} M for MTA and a value of 2×10^{-4} M for phosphate.

MTA phosphorylase has also been demonstrated in Drosophilla melanogaster (50), Sarcoma 180 cells (57), human muscle (58), and in a thermophillic bacterium, Caldariella acidophila (51), the only prokaryote found to have an enzyme catalyzing a phosphate dependent cleavage of MTA. That the enzyme lacks rigid specificity for the 5' position of the substrate, in most systems tested, is indicated by its ability to catalyze the degradation of various 5'-substituted analogues such as 5'-deoxy-5'-n-isobutylthioadenosine (SIBA) and 5'-deoxy-5'-ethylthioadenosine (49,51,56). Recently, Savarese (57) showed that MTA phosphorylase from Sarcoma 180 cells catalyzed the degradation of 5'-deoxyadenosine with a Km of 23 μM as compared to a Km for MTA of 4 μM. He later tested the inhibitory ability of several compounds on MTA phosphorylase (60). Among those compounds tested, 5'-deoxy-5'-methylthioformycin was the best inhibitor, inhibiting the Sarcoma 180 cell MTA phosphorylase with a Ki= 1.4 μM.

Nicolette, et al. (59) has shown that castration of rats 34 days earlier resulted in a 95% reduction in ventral prostate MTA phosphorylase activity and orchiectomy 16 days earlier in a 67% reduction in uterine MTA phosphorylase activity. The effect was completely reversed by testosterone treatment (42,59).

Several cell lines which lack MTA phosphorylase have been isolated (61-63), all are of malignant origin. Kamatani and Carson (62) reported 30 to 40% of human leukemic cell lines are deficient in MTA phosphorylase activity. In addition, cells lacking MTA phosphorylase activity excreted MTA into the surrounding medium.

Thus, the endproducts of MTA degradation in animal cells are adenine and 5-methylthioribose-1-phosphate. The products in prokaryotes and plants, with one exception (51), are adenine and 5-methylthioribose. According to Schroeder et al. (64), 5-methylthioribose in E. coli represents a terminal product. Nevertheless, Ferro et al. (65,66) have purified 5-methylthioribose kinase from E. aerogenes; the enzyme catalyzed the ATP-dependent phosphorylation of 5-methylthioribose yielding ADP and 5-methylthioribose-1-phosphate as products. Enzyme activity has since been demonstrated in avocado and pear tissue (67).

Schlenk, et al. (68) have demonstrated that the adenosine deaminase from Aspergillus oryzae deaminates MTA to yield 5'-methylthioinosine. 5'-Methylthioinosine is resistant to, or slow in reacting with, MTA nucleosidases or nucleoside phosphorylases and does not show unusual inhibitory action in enzyme systems (157). Deamination of MTA has not been reported in other systems.

In studies on the metabolism of SIBA (5'-n-isobutylthioadenosine) however, 5'-n-isobutylthioinosine was among the products when the analogue of MTA was incubated in cell free extracts of rat and mouse liver (69). This is indirect evidence that a deamination might occur if the substrate specificity of the enzyme were not too strict. Deamination of SIBA has also been demonstrated in chick embryo fibroblasts (70) and in bacteria (71). It should also be noted that MTA phosphorylase from human placenta catalyzed the degradation of SIBA; adenine and 5-n-isobutylthioribose-1-phosphate were the products (72).

Effect of MTA on Cellular Systems

Williams-Ashman et al. (73) suggested that in certain situations the function of spermidine and spermine biosynthesis may be to produce MTA rather than polyamines, and that the MTA formed could act in some situations as a regulator of enzyme systems. Other investigators (11) however have suggested MTA may be produced as cellular "garbage". MTA has been shown to inhibit several enzyme and metabolic systems. Zappia et al. (74) demonstrated that MTA inhibited the methylation of histamine and acetylserotonin. Linn et al. (75) showed that methylation of DNA by E. coli B modification enzyme was dramatically reduced in the presence of micromolar concentrations of MTA, but not S-adenosylhomocysteine. In addition, the restriction endonuclease reaction (both nuclease and ATPase) were effectively inhibited by MTA. Cassellas and Jeanteur (76) found that MTA inhibited protein methylase I activity in Krebs II ascites cells with a Ki of 3.5×10^{-5} M. Galletti et al. (147) recently reported the inhibitory effect exerted by MTA on the carboxyl methyl esterification of human erythrocyte membrane proteins. The authors infered that in vivo, protein methylase II was directly inhibited by MTA. Recently, Oliva et al. (78) found that MTA inhibited S-adenosylmethionine:protein carboxyl methyltransferase from calf brain. Analogs such as SIBA and 5'-butylthioadenosine had no effect. SIBA however has been demonstrated to be an inhibitor of tRNA methylation (77) and thus is often labeled as an S-adenosylhomocysteine analogue.

Law et al. (79) established that MTA inhibited the incorporation of [^{3}H]uridine into RNA in the salivary glands of D. melanogaster. This inhibition was not due to inhibition of uptake. Experiments utilizing α amanitin suggested the synthesis of heterogenous RNA was completely inhibited. Law et al. (80) also examined the effect of MTA on specific transcriptional changes accompanying heat shock as visualized by autoradiography. MTA treatment resulted in a reduction of puff size at the heat shock loci which was correlated with decreased transcriptional activity. These results suggest that MTA plays a role in regulation of gene expression.

Drugs structurally related to MTA, such as SIBA and 3-deaza-SIBA, have been shown to have anti-viral (81,82) and anti-malarial activity (83), and, SIBA also was found to inhibit sugar transport into cells (84). It is possible that the pharmacological effects of such drugs may be due to an effect on the metabolism of MTA or may actually mimic the intracellular effect of MTA itself. Montgomery, et al. (148) have synthesized many such analogs, one of which, 5'-deoxy-5'-(ethylthio)-2-fluoroadenosine was cytotoxic.

MTA inhibited DNA and protein synthesis as well as cellular proliferation in human lymphocyte cultures stimulated with mitogens, antigens, or allogeneic cells (85). The effect of MTA was non-toxic, dose-dependent, and non-competitive with the stimulant. The MTA effect was completely reversible, with MTA phosphorylase playing a key role in the reversal (86,87).

MTA also inhibited the growth of murine lymphoid cell lines of both B- and T-cell origin in a reversible, non-toxic, dose-dependent fashion (88). MTA (250 μm) inhibited growth of these cells 50% when measured two days after its addition. Similarly, SIBA inhibited the growth of mouse mammary cells transformed by mouse mammary tumor virus (89), mitogen induced lymphocyte blastogenesis (90), and virus induced cell transformation (91). SIBA was found to modify the activity of reverse transcriptase associated with mouse mammary tumor virus particles (89). Whereas the effect of SIBA and MTA were reversible, the effects of the 7-deaza analogue of MTA, 5'-methylthiotubercidin were irreversible (85,88,92). 5'-methyl-thiotubercidin inhibited lymphocyte transformation with an I_{50} of 80 μM. The non-reversible effect has been attributed to the inability of MTA phosphorylase to degrade this analogue (Ki= 31 μM). Several 5'-substituted analogues of MTA were tested by these investigators (92); those analogues with the lower Km values as substrates for MTA phosphorylase were the least inhibitory to the lymphocyte transformation process.

Several cellular targets where MTA may exert its effects have been tested. As mentioned earlier, spermine synthase was inhibited by MTA in mammalian systems (17,18,93). Recently, Ferro et al. (94) demonstrated the irreversible inactivation of human erythrocyte S-adenosylhomocysteine (SAH) hydrolase by MTA (Ki= 34 μM). The authors suggested that the inactivation may explain some of the cell growth inhibitory properties of MTA. Similarly, Chiang et al. (95) reported

the 5'-substituted analogue of MTA, 5'-butylthioadenosine inhibited S-adenosylhomocysteine hydrolase from beef liver. Another related compound, 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-SIBA), inhibited S-adenosylhomocysteine hydrolase (149) and was shown to inhibit both phospholipid methylation and lymphocyte transformation (150).

MTA may also play a role in blood coagulation. The phenomenon of platelet aggregation may be induced by diverse chemical substances such as ADP, collagen, thrombin, epinephrine, arachidonic acid, etc. (96). Generally, it is believed that adenosine analogues that stimulate adenylate cyclase and raise cyclic AMP levels inhibit aggregation whereas those that block adenylate cyclase do not (97,98). Low concentrations of adenosine, AMP, ATP, or adenosine tetraphosphate strongly inhibit ADP-induced platelet aggregation. MTA which does not inhibit platelet adenylate cyclase, abolishes the inhibitory effects of adenosine and AMP (99,100). These investigators postulated that AMP inhibits platelet aggregation through conversion to adenosine by plasma phosphatases. It is also postulated that MTA competes with adenosine for membrane receptors, thereby preventing the inhibitory effects of adenosine (99).

An effect which may be closely related was recently observed by Zimmerman _et al_. (101). MTA, SIBA, and EHNA (an inhibitor of adenosine deaminase) were all inhibitors of lymphocyte-mediated cytolysis (LMC) _in vitro_ with 50% inhibitory concentrations of 375 μM for MTA, 250 μM for SIBA, and 160 μM for EHNA. Each of these

analogues was found to potentiate the lymphocyte cyclic AMP effect to various activators of adenylate cyclase (eg. prostaglandin E_1 and cholera toxin). The effect of MTA, SIBA, and EHNA on cyclic AMP metabolism was shown to be due to inhibition of the high-affinity phosphodiesterase present in lymphocyte homogenates (101,102). SIBA has been shown to cause an increase in cyclic AMP levels in Xenopus laevis oocytes (103), this was attributed, at least in part, to a change in the level of membrane bound adenylate cyclase. In addition, 3-deaza-SIBA was shown to cause an increase in the levels of cyclic AMP in mouse lymphocytes (104).

It is likely MTA exerts its effect at several loci. Pegg et al. (93) have suggested that the presence of MTA phosphorylase may be essential to prevent the inhibitory effects of MTA from becoming manifest. Savarese et al. (105) proposed that if MTA phosphorylase activity increases in rapidly growing tissues (eg. neoplasms), that this could be exploited chemotherapeutically. A potent inhibitor of MTA phosphorylase such as 5'-deoxy-5'-chloroformycin (52) or 5'-deoxy-5'-methylthioformycin (60) may cause major disruptions in the metabolism of neoplastic cells by preventing adenine salvage or interfering with polyamine synthesis and function. Upon reaction with MTA phosphorylase, MTA analogues may liberate cytotoxic products which may have anti-tumor activity, or, may act as inhibitors of the enzyme itself (60). Kamatani et al. (63) have demonstrated the selective killing of human malignant cells deficient in MTA phosphorylase. When cells were cultured in medium with methotrexate,

uridine, and thymidine, no cells grew. If MTA was added to the same medium, only enzyme positive cells grew; most of the MTA phosphorylase deficient cells were dead in three days. Thus, human malignant cell lines naturally deficient in MTA phosphorylase could be selectively killed when de novo purine synthesis was inhibited and MTA was the only exogenous source of purine (63). The same study indicated that seven out of 31 (23%) human malignant tumor cell lines had no detectable MTA phosphorylase activity. In a patient with a malignant tumor deficient in the enzyme, the infusion of MTA may protect normal tissues without affecting the toxicity of methotrexate for malignant cells.

The Role of MTA in Purine Salvage

The results of Kamatani et al. (63) proved that adenine can be salvaged from MTA and utilized as a source of purine in mammalian cells. The ability of MTA to serve as a purine source in other biological systems is not well documented. Yall, et al. (106) found that an adenine requiring strain of S. cerevisiae could utilize adenine or SAM as equivalent sources of the purine; S-adenosylhomocysteine and MTA, however, would not support the growth of the auxotroph. Knudsen, et al. (107) subsequently demonstrated that exogenously supplied S-adenosylhomocysteine could serve as a source of purine, but only when an inoculum in logarithmic phase of growth was utilized.

The proposed route by which SAM may be metabolized to serve as a source of purine consists of its demethylation to S-adenosylhomo-

cysteine, followed by hydrolytic cleavage of SAH to adenosine and homocysteine; SAH hydrolase has been found in yeast extracts (54). The observation that adenine as well as adenosine was produced from exogenously supplied SAM (107) suggested that an alternate purine salvage pathway may be operating. The degradation of SAM to MTA with the subsequent liberation of adenine is thus implicated. Unfortunately, the yeast cell membrane is impermeable to nucleosides such as MTA. Recently, it was demonstrated that a nucleoside permeable strain of S. cerevisiae could utilize exogenously supplied MTA as a source of purine (108). The cleavage of MTA by MTA phosphorylase would yield free adenine and the 5-methylthioribose moiety. In vivo, adenine may be converted by adenine phosphoribosyl transferase (APRT) to adenylate (Fig. 2) which is subsequently incorporated into nucleic acid (107,109). Alternatively, adenine may be deaminated to hypoxanthine which is converted to inosine monophosphate via hypoxanthine-guanine phosphoribosyltransferse (HGPRT, ref. 109).

The significance of purine salvage is accentuated by the occurrence of metabolic disorders such as Gout, Lesch-Nyhan syndrome, and various immune deficiencies (110,111) in which one or more of the purine metabolic enzymes are deficient.

A deficiency in APRT in humans is associated with the urinary excretion of large quantities of adenine and its oxidation products 8-hydroxyadenine and 2,8-dihydroxyadenine (112). Previous in vitro metabolic studies had suggested the phosphorolysis of adenosine or 2'-deoxyadenosine by purine nucleoside phosphorylase was the

Fig. 2. Pathways of adenine production. Abbreviations are: SAM, S-adenosylmethionine; MTA, 5'-methylthioadenosine; PRPP, 5-phosphoribosyl-1-pyrophosphate. Enzymes are: 1, S-adenosylmethionine synthetase; 2, S-adenosylmethionine decarboxylase; 3, spermidine synthase; 4, spermine synthase; 5, 5'-methylthioadenosine phosphorylase; 6, adenine phosphoribosyltransferase; 7, adenosine kinase; 8, 5'-nucleotide or phosphatase(s).

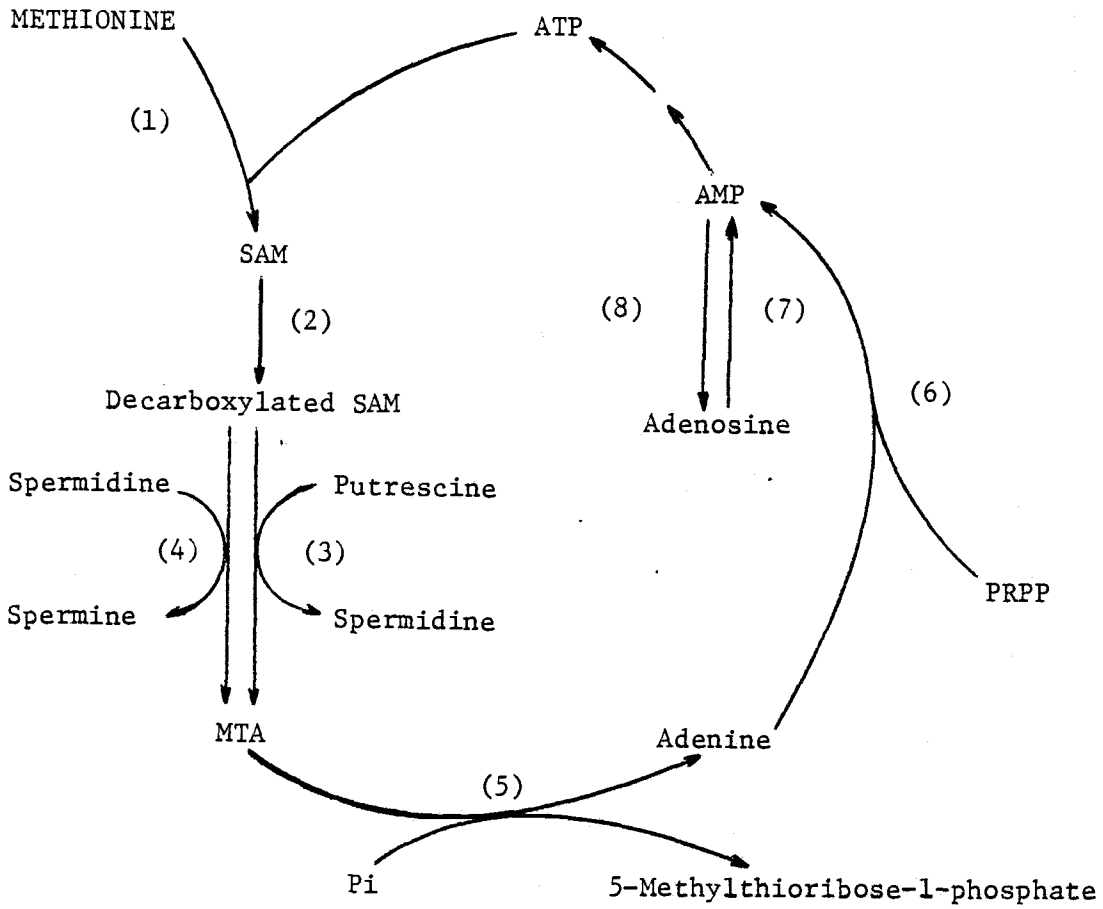
METHIONINE
ATP
(1)
AMP
SAM
(8)
(7)
(2)
Decarboxylated SAM
(6)
Adenosine
Spermidine
Putrescine
(4)
(3)
PRPP
Spermine
Spermidine
MTA
Adenine
(5)
Pi
5-Methylthioribose-1-phosphate

Fig. 2

major pathway by which free adenine may arise in animal cells (113). The concentrations of both adenosine and 2'-deoxyadenosine in tissues is low (114) and both compounds are inefficient substrates for mammalian purine nucleoside phosphorylase (115). Kamatani and Carson (116) recently demonstrated that dividing APRT deficient WI-L2 cells produced adenine at a rate of 0.27 nmole/mg protein/hour and that 85 to 97% of the adenine produced derives from the cleavage of MTA by MTA phosphorylase. Adenine has been shown to cause elevations of adenine nucleotides (117), to depress 5-phosphoribosyl-1-pyrophosphate (PRPP) accumulation (118), and inhibit de novo synthesis of purines (119). Likewise incubation of Sarcoma 180 cells with MTA resulted in elevations of adenine nucleotides and inhibition of PRPP accumulation (52). This effect may be due to the release of free adenine from MTA by the MTA phosphorylase catalyzed reaction. The presence of MTA intracellularly may thus play a role in the regulation of de novo purine synthesis via production of adenine.

Recycling of the 5-methylthioribose of MTA

Though the role of the MTA-derived adenine in purine salvage is becoming increasingly clear, the fate of the remaining 5-methylthioribose carbons is more obscure.

In 1953 Shapiro (120) demonstrated that MTA could support the growth of certain methionine auxotrophs of Aerobacter aerogenes. Later, Schwartz and Shapiro (121) suggested a new pathway of methionine biosynthesis may be operating and indicated that the methylthio group

of MTA was a precursor of the methylthio group in methionine. Experiments in yeast by Schlenk and Ehninger (122) established that the methylthio group of MTA was recovered in S-adenosylmethionine. These investigators originally suggested that the entire molecule may be incorporated into SAM, however, Schlenk et al. (123) later proved that degradation of the nucleoside was the first phase of the recycling process. In similar studies on protozoans (124), rat tissue (125), and apple tissue (22,126) it seemed possible that the methylthio group may be transferred as a unit to a four carbon acceptor. Toohey (23,127) reported that in extracts of certain cultured mammalian cells that the 5-methylthioribose-1-phosphate produced was further metabolized to an unknown product which he claimed was the product of a methylthio group cleavage reaction; he further postulated the existence of a "methylthiolase" which catalyzed the reaction (127).

The methylthio group transfer theory has recently been discredited. Shapiro and Schlenk (128) demonstrated the transfer of label from the pentose of MTA into the amino acid part of SAM. Backlund and Smith (129) have provided evidence that a similar pathway may exist in mammalian cells. Kinetics of product formation indicated that MTA was first rapidly converted to 5-methylthioribose-1-phosphate followed by its slower conversion to methionine. These investigators concluded that the pathway for methionine biosynthesis involves modifying the ribose part of MTA into the 2-aminobutyrate portion of methionine, with the methylthio group remaining intact.

Similarly, Shapiro and Barrett (130) demonstrated the enzymatic conversion of 5-methylthioribose to methionine and its deaminated derivatives, 2-keto-4-methylthiobutyric acid and 2-hydroxy-4-methiobutyric acid by cell free extracts of E. aerogenes. The first step in the recycling was the conversion of 5-methylthioribose to 5-methylthioribose-1-phosphate (65,66). It was not determined whether the deaminated derivatives isolated were products of methionine catabolism or intermediates in the synthetic reaction. Similar research by Trackman et al. (161) with rat liver homogenate has shown that carbon atom 1 of 5-methylthioribose-1-phosphate is released as formic acid.

Further investigations by Backlund et al. (160) have shown with the partially purified rat liver enzymes that 5-deoxy-5-methylthioribonic acid, its lactone, and 5-methylthioribose are not intermediates. In addition 2-keto-4-methylthiobutyric acid was identified as an intermediate, and a final step of transamination with glutamine or asparagine, leading to methionine was established (160).

Cooper and Meister (131) have purified glutamine transaminase from rat kidney and liver, an enzyme which was capable of catalyzing the conversion of 2-keto-4-methylthiobutyric acid plus glutamine to methionine plus glutamate or the reverse reaction. Thus, it is possible that this enzyme may play a role in the conversion of MTA to methionine utilizing 2-keto-4-methylthiobutyric acid as a precursor of methionine.

The deamination of methionine has been observed in several systems including fungi (34), rat liver (132) and in the bacterium, Achromobacter starkeyi (133). The deaminated product, 2-keto-4-methylthiobutyric acid has also been shown to be a precursor of ethylene in various systems (28-31,134). In humans, 2-keto-4-methylthiobutyric acid is excreted in the urine (135). In homocystinurics, the principal pathway of methionine metabolism is blocked and excretion of 2-keto-4-methiobutyric acid is strongly increased (135).

The other deaminated derivative of methionine isolated by Shapiro and Barrett (130) was 2-hydroxy-4-methylthiobutyric acid. This hydroxy analogue of methionine has been isolated as a product of methionine dissimilation in fungi (34). Dietary sulfur requirements have been met by the supplementation of 2-hydroxy-4-methylthiobutyric acid (136-138) and 2-keto-4-methylthiobutyric acid (137) in the diet of various animals.

Mitchell and Benevenga (139) have suggested transamination is an initial step in methionine catabolism and an alternate pathway that does not involve its activation to SAM (Fig. 3). Steele and Benevenga (33) have identified 3-methylthiopropionic acid as an intermediate of mammalian methionine metabolism in vitro. Further studies indicated that 2-keto-4-methiobutyric acid was the precursor of this compound (140) and that the decarboxylation of the keto acid occurred mainly in the liver mitochondria. Steele, et al. (141) reported a serious dysfunction of red cell hematopoiesis when rats

Fig. 3. Metabolic scheme for the catabolism of methionine via the transamination pathway and via the transulfuration pathway. TCA, tricarboxylic acid.

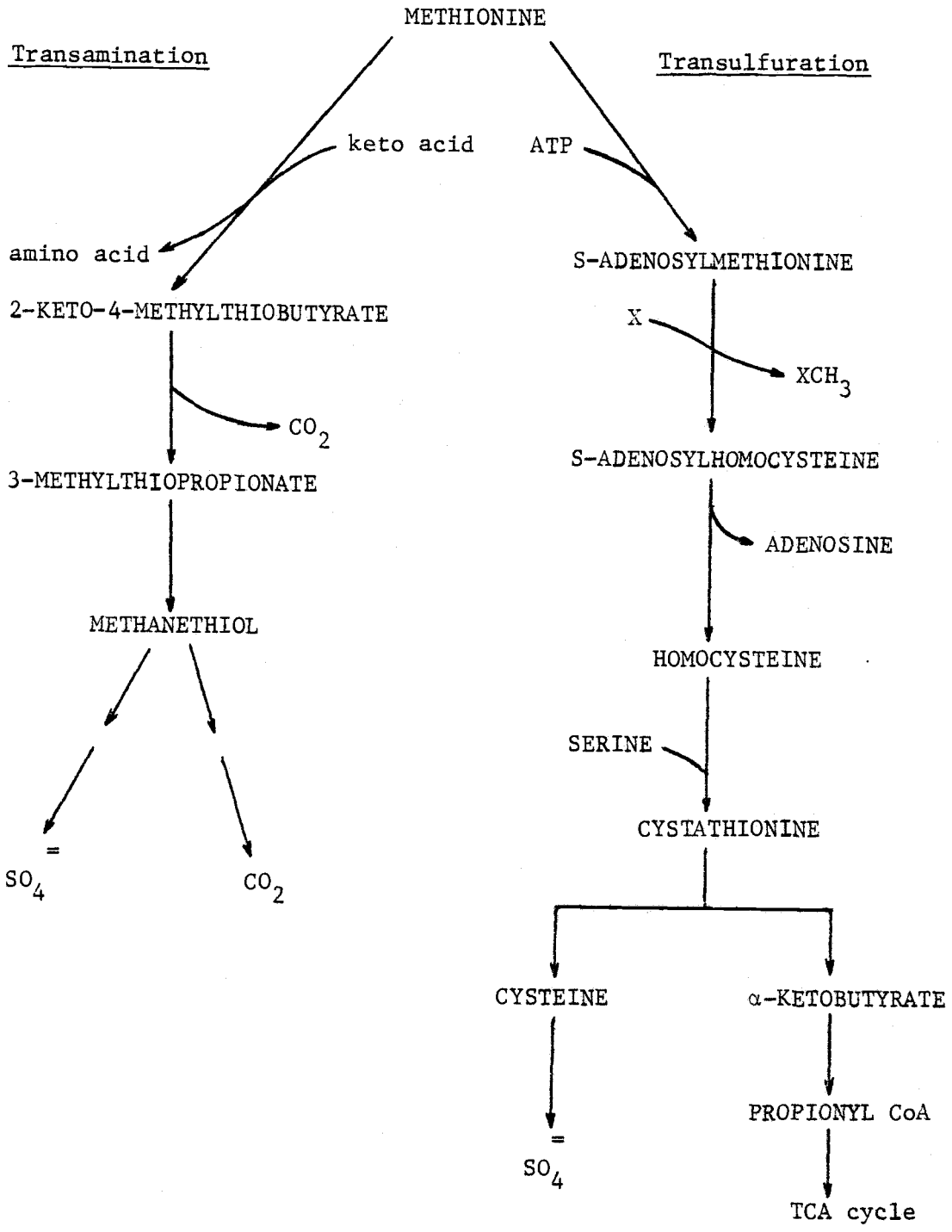
METHIONINE
Transamination
Transulfuration
keto acid
ATP
amino acid
S-ADENOSYLMETHIONINE
2-KETO-4-METHYLTHIOBUTYRATE
X
XCH3
CO2
S-ADENOSYLHOMOCYSTEINE
3-METHYLTHIOPROPIONATE
ADENOSINE
METHANETHIOL
HOMOCYSTEINE
SERINE
=
SO4
CO2
CYSTATHIONINE
CYSTEINE
α-KETOBUTYRATE
=
SO4
PROPIONYL CoA
TCA cycle

Fig. 3

were fed a basal diet supplemented with 3-methylthiopropionic acid (2.57%) for two weeks. Benevenga (142) suggested that toxicity of L-methionine is due to the metabolism of the methyl portion of the amino acid. The effects of 3-methylthiopropionic acid lend further support to his conclusion (141). It is interesting to note that Zappia et al. (151) has demonstrated that MTA is actively transported into isolated and perfused rat liver. They postulated the existence of a high-affinity permease specific for thioethers. These data suggest the liver may be of primary importance as the site of detoxification of methiol compounds such as MTA and 3-methylthiopropionic acid.

In rat liver homogenates 3-methylthiopropionic acid was further degraded to methanethiol and an unknown product (143). The methanethiol was ultimately degraded to H_2S and CO_2. Methanethiol and α-ketobutyric acid have also been isolated as degradation products of methionine in fungi (34) and bacteria (132). The probability that MTA metabolism may follow these pathways suggests a mechanism whereby MTA may supply the "methylthio" requirement of certain cells (61).

The data presented suggest that MTA sulfur may be ultimately released as H_2S gas, a disagreeable component of human flattus. Finally, an interesting result found by Matsui and Amaha (144,145) was the identification of 2-hydroxy-4-methylthiobutyric acid, 3-methylthiopropionic acid, and 3-methylthiopropionaldehyde in samples of beer, a fermentation product of S. carlsburgensis.

It has been demonstrated elsewhere (146) that yeast aldehyde dehydrogenase will catalyze the conversion of 3-methylthiopropionaldehyde to 3-methylthiopropionic acid, along with the reverse reaction. These data support the contention that similar metabolic pathways may exist in yeast.

MATERIALS AND METHODS

Organisms and culture conditions. The strains of Saccharomyces cerevisiae used in this study are listed in Table 1. All strains were cultured aerobically in a medium containing: Yeast Nitrogen Base, without amino acids and ammonium sulfate (Difco), 1.67 g/l; ammonium sulfate, 5 g/l; and glucose, 20 g/l. Appropriate amounts of auxotrophic requirements were added as needed (15). Cultures were grown at 30°C at 200 rpm on a rotary shaker (New Brunswick). Growth was monitored with a Klett-Summerson colorimeter (#60 filter). When the cultures were starved for adenine prior to growth experiments, cell suspensions (1.5×10^6 cells/ml) were incubated in adenine-free medium containing tetracycline (10 µg/ml) for three days. Following starvation, the cells were added directly to fresh medium. For enzyme purification, MCC was grown to late exponential phase at 28°C in a 10 l New Brunswick Microferm laboratory fermentor containing 8 l of medium consisting of tryptone (1%), yeast extract (0.5%), and ethanol (2%). For studies involving metabolism of [$^{14}CH_3$]MTA strain 2218A was grown in 800 ml YNB supplemented with 1 mM MTA as a source of both purine and methionine.

Measurement of uptake of radiolabeled compounds. Cells were grown to a density of 3×10^6 cells/ml on YNB minimal medium and collected on membrane filters (HAWP 25 mM, 0.45 µm pore size; Millipore Corp.) using a multifiltration apparatus (New Brunswick Scientific Corp.). The cells were washed with several volumes of distilled water and

Table 1. List of yeast strains.

Strain	Genotype	Source
2439-6C	a *ade* 1 *ade* 2 *ade* 5,7 *ura* 1 *ura* 4 *lys* 2 *trp* 1 *tyr* 1 *aro* 1D	Anderson and Roth (1)
50-3	a *ade* 1 *ade* 2 *ade* 5,7 *ura* 1 *ura* 4	Anderson and Roth (1)
S37	α *leu* 2-1 *met* 5	Yeast Genetic Stock Center
22-18A	α *ade*[a] *met* 5[s] *leu* 2-1 *lys* 2 cordycepin[s]	Segregant of 50-3 x S37
22-78C	α *leu* 2-1 *met* 5[s] *lys* 2 *tyr* 1 *ura*[a] cordycepin[s]	Segregant of 50-3 x S37
224A	a *ade*[a] *leu* 2-1 *tyr* 1,6 *trp* 1 *met* 5 *ura* 1,4	Segregant of 50-3 x S37
MCC	a/α wild type	Yeast Genetic Stock Center

[a]Only the auxotrophic requirement was determined.

transferred to fresh medium. Radiolabeled compounds were added to a final concentration of 0.25 mM. Duplicate samples (0.75 ml) were withdrawn at various times, collected on membrane filters, and washed with 10 ml of distilled water. The dried filters were placed into scintillation vials containing 10 ml of toluene-ethanol (1:1, v/v) containing 0.4% 2,5-diphenyloxazole. Radioactivity in the samples was determined using a Beckman LS8000 liquid scintillation spectrophotometer.

Measurement of incorporation of radiolabeled compounds. Cells were grown to a density of 3×10^6 cells/ml in YNB media, collected on membrane filters (HAWP 25 mm, 0.45 µm pore size; Millipore Corp.), washed with several volumes of distilled water, and transferred to fresh YNB media lacking adenine. Radioactively labeled compounds were added to a final concentration of 0.25 mM. Duplicate samples (0.75 ml) were withdrawn at various times and placed into an equal volume of cold 20% TCA. The samples were collected on Whatman GF/C glass fiber filters and washed twice with 20 ml of 20% TCA. The dried filters were placed directly into scintillation vials containing 10 ml of toluene:ethanol (1:1, v/v) with 0.4% 2,5-diphenyloxazole.

Extraction of whole cells. Cells cultured in the presence of radiolabeled compounds were centrifuged at 6,000 x g for 10 min and the supernatant discarded. The pellet was resuspended in 0.5 ml of 1.5 N perchloric acid. The suspension was stirred for 1.5 h and

centrifuged at 11,000 x g for 5 min. The supernatant was removed, neutralized with 1 N NaOH, and analyzed. Alternatively, the pelleted cells were resuspended in 1.0 ml of 95% ethanol and homogenized by hand in a dounce tissue homogenizer. The homogenate was centrifuged at 11,000 x g and the supernatant analyzed for products.

Preparation of cell extracts for MTA phosphorylase assay. Yeast cells were grown to a density of 2×10^7 cells/ml, collected by centrifugation in a Beckman J2-21 centrifuge (5,500 x g) and resuspended in buffer consisting of 0.05 M potassium phosphate (pH 7.3) and 3.0 mM dithiothreitol. The cells were homogenized with glass powder for 30 sec in a Braun MSK cell homogenizer. The resulting extract was centrifuged at 30,000 x g for 20 min to remove cellular debris. The supernatant fluid was dialyzed for 4 h at 4°C against the same buffer (three changes of buffer). Mammalian cell cultures were centrifuged at 5,000 x g for 15 min, washed, and resuspended in 0.05 M potassium phosphate (pH 7.2) containing 3.0 mM dithiothreitol. The cells were freeze thawed five times in liquid nitrogen, centrifuged at 22,000 x g for 20 min and the supernatant recovered and used as a source of enzyme in the MTA phosphorylase assays. Plant tissue was suspended in buffer (1 g/ml) consisting of 0.2 M potassium phosphate (pH 7.2), 1.0% polyvinylpyrrolidone, and 3.0 mM dithiothreitol and homogenized in a waring blender. The homogenate was passed through four layers of a cheesecloth and centrifuged at 20,000 x g for 20 min. Cultures of Enterobacter aerogenes were pelleted, washed, and resuspended in 3 volumes (ml/wet weight) of

0.05 M potassium phosphate (pH 7.3) with 5 mM 2-mercaptoethanol and passed through a French pressure cell two times at 20,000 p.s.i. Cellular debris was removed by centrifugation at 30,000 x g for 25 min.

5'-Methylthioadenosine phosphorylase assay. Enzyme activity was determined by measuring the conversion of 5'-[$^{14}CH_3$]MTA to 5-[$^{14}CH_3$] methylthioribose-1-phosphate (18). Unless stated otherwise, the standard reaction mixture contained in a total volume of 0.25 ml, 0.1 M sodium Hepes (pH 7.2), 10 mM potassium phosphate (pH 7.2), 74 μM 5'-[$^{14}CH_3$]MTA (7.9 x 10^6 cpm/μmol), and protein to allow from 5 to 20% total substrate consumption. The reaction mixture was incubated at 37°C (unless otherwise noted) for 30 min and the reaction terminated by the addition of 0.05 ml of 1.8 M trichloroacetic acid. The resulting precipitate was removed by centrifugation at 11,000 x g for 5 min and a 0.2 ml aliquot of the supernatant fluid was applied to a Dowex 50 H^+ X4 (100-200 mesh) column (0.5 X 3.0 cm) equilibrated with 3N H_2SO_4. Application of the sample was followed by elution with 3 ml of distilled water directly into scintillation vials. Under these conditions 5'-methylthioadenosine is retained on the column while the product, 5-methylthioribose-1-phosphate is detected in the eluate. A scintillation cocktail consisting of 0.4% diphenyloxazole in toluene/triton X-100 (2:1, v/v) was added to fill the vials and radioactivity determined in a Beckman LS 8000 scintillation spectrophotometer. One unit of enzyme activity is defined as the amount

of enzyme catalyzing the formation of 1 μmol of product in one min.

Preparation of cell-free extracts for enzyme purification. Cells were harvested by centrifugation for ten min at 7,000 x g, washed in distilled water, and resuspended in buffer containing 0.7 M sorbitol, 0.3 M mannitol, 0.1 M citrate, 10 mM K_2HPO_4, and 1 mM EDTA, at pH 5.8. Spheroplasts were formed by incubating the cells with 20% glusulase for 1 h at 28°C with gentle shaking. The spheroplasts were pelleted by centrifugation for 10 min at 7,000 x g, washed and resuspended in buffer containing 0.9 M sorbitol, 10 mM Tris, and 0.5 mM EDTA at pH 7.4. This suspension was passed through a French pressure cell at 1-2000 psi. Unbroken spheroplasts and cellular debris were removed by three centrifugations, each at 1,100 x g for 10 min. Mitochondria were removed by centrifugation at 12,000 x g for 20 min and utilized for other studies. Potassium phosphate (pH 7.2) was added to a final concentration of 0.05 M and the suspension centrifuged at 38,000 x g for 20 min to remove any remaining cellular debris.

Enzyme purification. The purification procedure described yields the highest specific activity enzyme of various procedures attempted. Following the first step, all enzyme preparations were stored in a standard buffer consisting of 0.05 M potassium phosphate, 3.0 mM dithiotreitol, 1.0 mM EDTA, 10% glycerol (v/v), 0.1 M sorbitol,

0.1 mM sodium azide, 1.0 mM phenylmethylsulfonylfluoride, and 0.5 mM p-chloromercuribenzoic acid (pH 7.2), at -80°C. All purification steps were performed at 4°C.

Step one: Streptomycin sulfate precipitation. A 36% streptomycin sulfate solution was prepared in standard buffer and added dropwise, with stirring, to the cell free extract at a flow rate of 0.07 ml/min, to give a final concentration of 6%. The solution was allowed to stir an additional 15 min, the precipitate removed by centrifugation at 38,000 x g for 20 min, and the remaining solution dialyzed for 90 min four times each in a 1:5 dilution of standard buffer. Following each dialysis step the solution was centrifuged at 38,000 x g for 20 min to remove any slowly precipitating material from the streptomycin sulfate treatment.

Step two: Hydroxylapatite chromatography. The dialyzed extract was adsorbed directly onto a 17 x 3 cm hydroxylapatite column equilibrated with a 1:5 dilution of standard buffer. Protein was eluted from the column by elution with 300 ml of a 1:2 dilution of standard buffer at a rate of 0.5 ml/min. 5'-Methylthioadenosine phosphorylase activity was then eluted with 300 ml of standard buffer at a rate of 0.5 ml/min in bulk.

Step three: DEAE cellulose chromatography. The hydroxylapatite eluate was applied directly to a 16 x 3 cm DEAE cellulose column that had been equilibrated with standard buffer. Following application, the column was washed with 150 ml of standard buffer followed by the elution of enzyme in a linear 0.05 M to 0.4 M potassium phosphate

gradient (500 ml) prepared from standard buffer. Fractions (7.5 ml) were collected at a rate of 0.5 ml/min. The fractions containing the highest enzyme activity were pooled and concentrated in an Amicon Ultrafiltration Cell, Model 52, under 50 psi nitrogen pressure through an Amicon XM50 filter (exclusion limit = 50,000 D). Further concentration to less than 2 ml was accomplished by vacuum dialysis in a Micro Pro-dicon negative pressure micro protein dialysis concentrator (Bio-Molecular Dynamics).

Step four: Sephadex G-200 chromatography. The concentrated solution (approximately 1.0 ml) was applied to a 1.5 x 85 cm Sephadex G-200 column previously equilibrated with standard buffer. Elution was accomplished with standard buffer at a flow rate of 0.3 ml/min. The most active fractions (2 ml) were pooled and concentrated as described above.

Estimation of molecular weight. The molecular weight of 5'-methylthioadenosine phosphorylase was estimated by gel filtration in Sephadex G-200 as described above. The column was calibrated with known molecular weight standards (blue dextran, aldolase, bovine serum albumin, ovalbumin, cytochrome c).

Protein determination. The protein concentration of the samples was determined by the method of Lowry et al. (19).

Enzyme purity. Analytical polyacrylamide gel electrophoresis as described by Brewer and Ashworth (20) was used as a criterion of enzyme purity.

High pressure liquid chromatography. Various analogs of 5'-methylthioadenosine were tested as substrates for the 5'-methylthioadenosine phosphorylase. Analogs were substituted in place of 5'-methylthioadenosine in the standard enzyme assay. The mixture was incubated at 47°C for 2 h and the reaction terminated with 0.05 ml of a 5% perchloric acid solution. Precipitated protein was removed by centrifugation at 11,000 x g for 5 min. Samples (25 µl) were injected into a Waters Assoc. model M-6000A high pressure chromatography system fitted with a Waters µ-bondapak G18 column. Products and unreacted compounds were eluted with solvent consisting of 10 mM potassium acetate, 5 mM Pic B-7 (Waters Assoc.), and 10% acetonitrile (pH 4.0) at a flow rate of 1 ml/min. Ultraviolet adsorbing compounds (254 nm) were detected with a Waters Assoc. model 440 absorbance detector. Authentic adenine was used as a standard.

Product identification. A reaction mixture consisting of 5 mM potassium phosphate (pH 7.2), 0.1 M sodium Hepes (pH 7.2), 2.0 mM 5'-[$^{14}CH_3$]MTA, and 2.5 µg of purified enzyme in a total volume of 0.05 ml was incubated for 2 h at 47°C. The reaction was terminated by the addition of 10 µl of 1.8 M trichloroacetic acid and any precipitate removed by centrifugation at 11,000 x g for 5 min. The total reaction mixture was spotted onto a silica gel thin layer chromatogram, developed in ethyl acetate:methanol (9:1, v/v), and compared against authentic standards.

Preparation of cell-free extracts for metabolic studies. For studies on yeast metabolism, strain 2218A was grown in 800 ml YNB supplemented with 1 mM MTA as a source of both adenine and methionine. Log phase cultures were harvested by centrifugation, washed, and sphaeroplasts prepared by resuspending the cells in buffer containing 0.5 M mercaptoethanol, 0.1 M Tris, pH 9.3 (2 ml/gm wet weight) for 5 min at 30°C with gentle shaking. The cells were washed two times in 1.1 M sorbitol, 10 mM potassium phosphate (pH 7.5), 0.4 M $CaCl_2$, then resuspended in 5 ml lyticase (154) and 0.3 ml glusulase per gram weight of cells. The suspension was incubated at 30°C with gentle shaking for 60 min. The sphaeroplasts were scored by microscopic observation and centrifuged for 5 min at 6,000 x g, washed once in 0.9 M sorbitol containing 0.5 mM EDTA and 10 mM Tris (pH 7.4). The sphaeroplasts were pelleted at 6,000 x g for 5 min and resuspended in 1 volume (ml/g wet weight) 0.05 M potassium phosphate (pH 7.2) containing 10% glycerol and 3 mM dithiothritol. The suspension was homogenized in a high speed tissue homogenizer for 8 min and centrifuged at 30,000 x g for 25 min. The supernatant was used immediately since a loss in activity was seen upon freezing.

Assay conditions. Standard assay conditions for studying the metabolism of MTA consisted of a reaction mixture containing 15 mM potassium phosphate (pH 7.2), 1.0 mM $MgCl_2$, 15 μM ^{14}C-labeled substrate and 0.5 mg protein from the 30,000 x g yeast sphaeroplast or mouse liver supernatant in a total volume of 0.05 ml. The

reaction mixture was incubated at 37°C for the specified times (up to 120 min) and the reaction was terminated by heating at 100°C for 1 min. Precipitated protein was pelleted at 11,000 x g and the supernatants applied to various chromatography systems.

Paper and thin layer chromatography. Identification of compounds and reaction products was accomplished by thin layer and paper chromatography whenever possible. The chromatographic systems used and respective Rf values of known compounds are presented in Table 2. Chromatography on the silica gel systems was sometimes modified by applying the sample, then spraying with a borate solution consisting of 0.2 M boric acid, 0.05 M NaCl, 0.05 M $Na_2B_4O_7$, (pH 8.0). This treatment binds cis-diols to the chromatogram preventing their migration and allows other compounds to migrate freely. Dried chromatograms were examined for ultraviolet absorbing substances with a short wave ultraviolet light source, and radioactively labeled compounds were detected with a Packard Radiochromatogram Scanner, model 7201. Oxidizable sulfur compounds were detected with platinic iodide reagent consisting of 0.67% $PtCl_6$ (w/v) dissolved in an equal volume of a 1.1% solution of KI.

Column chromatography. Charged compounds were separated by chromatography on Dowex 50W-X4 cation exchange resin (Bio-rad Laboratories). Columns (0.5 x 3 cm) were charged by passing 30 ml of 3 N H_2SO_4 through the column followed by 30 ml H_2O. Samples to be chromatographed were acidified (pH 3.0 to 5.0) with 1 N HCl and applied to

Table 2. Chromatography systems and Rf values.

Compound	Solvent System 1	2	3	4	5	6	7	8
5'-methylthioadenosine	0.74	0.81	0.27	0.75	0.46	0.56	0.75	0.67
5-methylthioribose	0.77	0.91	0.55	0.75	0.88	0.70	0.75	0.76
5'-methylthioinosine	0.57	0.80	0.09	0.61			0.61	
Adenine	0.53	0.61	0.20	0.62			0.60	
Hypoxanthine		0.62	0.10	0.43			0.43	
S-adenosylmethionine			0	0.09			0.09	
Adenosine			0.15	0.45			0.45	
5'-methylthioadenosine sulfoxide			0.3	0.34			0.34	
Adenosine monophosphate			0.06	0			0	
2-keto-4-methylthiobutyrate			0.04	0.73	0.48	0.79	0.73	0.76
Methionine			0	0.48	0.88	0.39	0	0.49
5-methylthioribose sulfoxide			0.12	0.60			0.12	
2-mercaptoethanol			0.70	0.92			0.70	
Dithiothreitol			0.74	0.99	0.57	0.81	0.74	
5-methylthioribose-1-phosphate			0	0.26	0.66	0	0	0.25
2-hydroxy-4-methylthiobutyrate			0.06		0.93	0.65	0.06	0.87
3-methylthiopropionaldehyde			0.86		0.65	0.82	0.86	0.93
3-methylthiopropionic acid			0.05		0.47	0.73	0.81	0.84
5-methylthioribose-1-phosphate sulfoxide						0		0.17
Time of development	12 h	12 h	1.5 h	6 h	1.5 h	2 h	3 h	8 h

Table 2. Continued

Solvent systems:

1. Butanol:acetic acid:H_2O (12:3:5) ascending, Whatman 1 M
2. Ethanol:acetic acid:H_2O (65:1:34) ascending, Whatman 1 M
3. Ethyl acetate:methanol (9:1) thin layer-silica gel
4. Butanol:acetic acid:H_2O (12:3:5) thin layer-cellulose
5. 1 M LiCl thin layer-cellulose
6. Methanol:chloroform (85:15) thin layer-silica gel
7. Butanol:acetone:acetic acid:H_2O (70:70:20:40) thin layer-cellulose
8. Butanol:acetone:acetic acid:H_2O (70:70:20:40) descending, Whatman 3 M

the column. Anionic and neutral compounds were washed from the column with 10 ml H_2O while cationic compounds were eluted with 6 ml of 2 N NH_4OH. Ribose (cis-diol) containing compounds were separated from non-ribose compounds by chromatography on Affi-gel 601 (Bio-rad Laboratories). Columns (0.5 x 3 cm) were washed with 15 ml formic acid followed by 15 ml 0.25 M sodium acetate buffer (pH 8.6). Samples to be chromatographed were brought to pH 9.0 with 1 N KOH and applied to the column. Non-cis-diol containing compounds were washed from the column with 8 ml of sodium acetate buffer while cis-diols were eluted with 8 ml 1 N formic acid. UV absorbing compounds (254 nm) were detected by high pressure liquid chromatography. Samples were acidified with one volume (v/v) of 1.5 N perchloric acid, centrifuged at 11,000 x g, and injected into the high pressure liquid chromatography system previously described.

Dansylation of compounds. Samples to be analyzed were dissolved in 0.15 ml 70% ethanol (v/v), mixed with 0.02 ml saturated sodium carbonate and 0.2 ml dansyl chloride solution (20 µg/ml in acetone) added to each. Samples were incubated at 37°C in the dark. After 2 h, 0.4 ml 20 µg/ml methionine was added and incubation continued at 25°C for 30 min in the dark. Dansylated compounds were extracted with 0.5 ml benzene.

Compounds. 5'-[$^{14}CH_3$]MTA was prepared from S-adenosyl-L-[$^{14}CH_3$] methionine (15) which was purchased from Amersham/Searle and purified by high pressure chromatography. Escherichia coli alkaline

phosphatase was obtained from Sigma Corp., while glusulase is a product of Endo Laboratories, Garden City, N.Y. $^{14}CH_3$ or non-labeled 5-Methylthioribose was prepared by hydrolyzing $^{14}CH_3$ or non-labeled 5'-methylthioadenosine in 0.1 N HCl for three hours at 100°C. 5'-Methylthiotubercidin was synthesized from tubercidin (Sigma Corp.) by treatment with $SOCl_2$ in hexamethylphosphoramide, isolation of the resulting 5'-chlorotubercidin by chromatography, and conversion into 5'-methylthiotubercidin by treatment with $NaSCH_3$ in liquid NH_3 (16,17). Similarly, 5'-Methylthioadenosine, 5'-ethylthioadenosine, 5'-n-propylthioadenosine, 5'-isopropylthioadenosine, 5'-n-butylthioadenosine, and 5'-isobutylthioadenosine were synthesized from adenosine with isolation of 5'-chloroadenosine and conversion to the analog with the appropriate thiol salt as described above for the tubercidin derivative. Adenine-[8-^{14}C-Adenine]Methylthioadenosine (2.75×10^5 cpm/μmol), [U-^{14}C-adenosine]MTA (2×10^6 cpm/μmol) and 5'-methylthioinosine were provided by Dr. Fritz Schlenk. 3-Methylthioproprionic acid was synthesized enzymatically (146) from 3-methylthioproprionaldehyde (Eastman) using yeast aldehyde dehydrogenase (Sigma). 2-Keto-4-[$^{14}CH_3$]methylthiobutyric acid was prepared enzymatically (155) from [$^{14}CH_3$]methionine (Amersham) using snake venom amino acid oxidase. [$^{14}CH_3$]Methylthioribose-1-phosphate was synthesized from [$^{14}CH_3$]MTA using purified S. cerevisiae MTA phosphorylase in a standard reaction. The products were separated by chromatography on system 6. Alternatively, [$^{14}CH_3$]methylthioribose-1-phosphate was synthesized from [$^{14}CH_3$]methylthioribose and

ATP using purified methylthioribose kinase (66). The products were separated on DEAE cellulose; 5-methylthioribose is washed from the column with 0.005 M potassium phosphate (pH 7.2) while 5-methylthioribose-1-phosphate is eluted with 0.03 M potassium phosphate. ATP is retained on the column under these conditions.

RESULTS

The Role of MTA in Purine Salvage in *Saccharomyces cerevisiae*

Transport of MTA. Strains of *Saccharomyces cerevisiae* do not transport nucleosides such as adenosine or MTA. A cordycepin sensitive strain (50-3), however, has been isolated which incorporated adenosine at a rate seven times greater than its parental prototroph (168). Because of the structural similarities between adenosine and MTA, we employed this strain (50-3) to examine whether MTA was also taken up by these cells. The ability of strains 50-3 and its cordycepin resistant parent, 2439-6C to accumulate exogenously supplemented adenosine and MTA is depicted in Fig. 4. Strain 2439-6C did not accumulate radioactivity from either [8-^{14}C-adenine]MTA or [$^{14}CH_3$]MTA to any significant extent in 100 min. Strain 50-3, however, accumulated the radioactivity from both labeled MTA and adenosine. By 100 min, 800 pmoles of radioactivity from adenosine and 620 pmoles radioactivity from MTA had accumulated within the cells.

To determine if MTA could be isolated from cells grown in the presence of MTA as its sole source of purine, cells were grown in the presence of [$^{14}CH_3$]MTA (1.1×10^7 cpm/μmole), harvested by centrifugation, the medium saved, and the pellet extracted. Chromatography of the medium shows MTA was the only compound present (Fig. 5A). A ^{14}C-containing compound extracted from whole cells migrated to the same Rf value (0.70) in solvent system 4 as authentic MTA

Fig. 4. The uptake of [8-^{14}C]adenosine and [8-^{14}C-adenine]MTA in S. cerevisiae. Cultures were grown in YNB medium supplemented with 20 µg/ml adenine to a density of 3 x 10^6 cells/ml, harvested by filtration, washed with distilled water, and reinoculated into fresh YNB-medium deficient in adenine. At time zero, radioactively labeled nucleosides were added to a final concentration of 0.25 mM. Symbols: (O), strain 2439-6C grown in the presence of [8-^{14}C]-adenosine; (●), 2439-6C grown in the presence of [8-^{14}C-adenine]MTA; (□), 50-3 in the presence of [8-^{14}C]-adenosine; (■), 50-3 in the presence of [8-^{14}C-adenine]MTA.

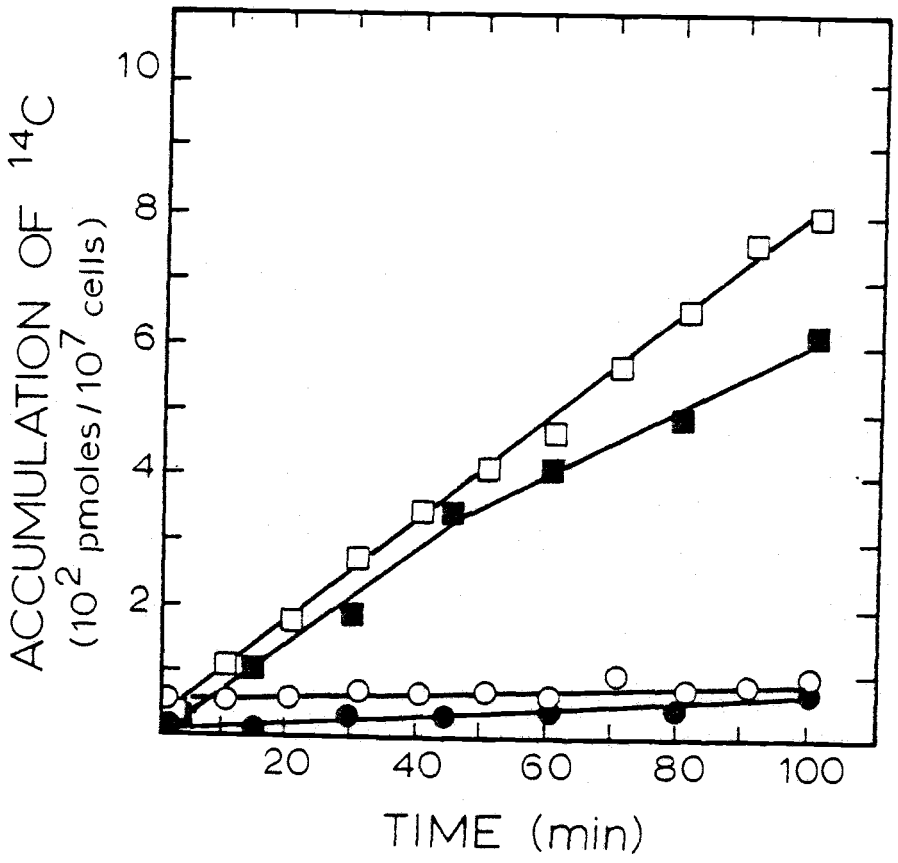
ACCUMULATION OF ^{14}C
(10^2 pmoles / 10^7 cells)
2
4
6
8
10
20
40
60
80
100
TIME (min)

Fig. 4

Fig. 5. Radioscans of radioactively labeled compounds separated by chromatography in system four. The scans are A, labeled compound found in the media and B, labeled compounds found in perchloric acid extracts of whole cells when strain 50-3 was cultured in the presence of $^{14}[CH_3]$MTA.

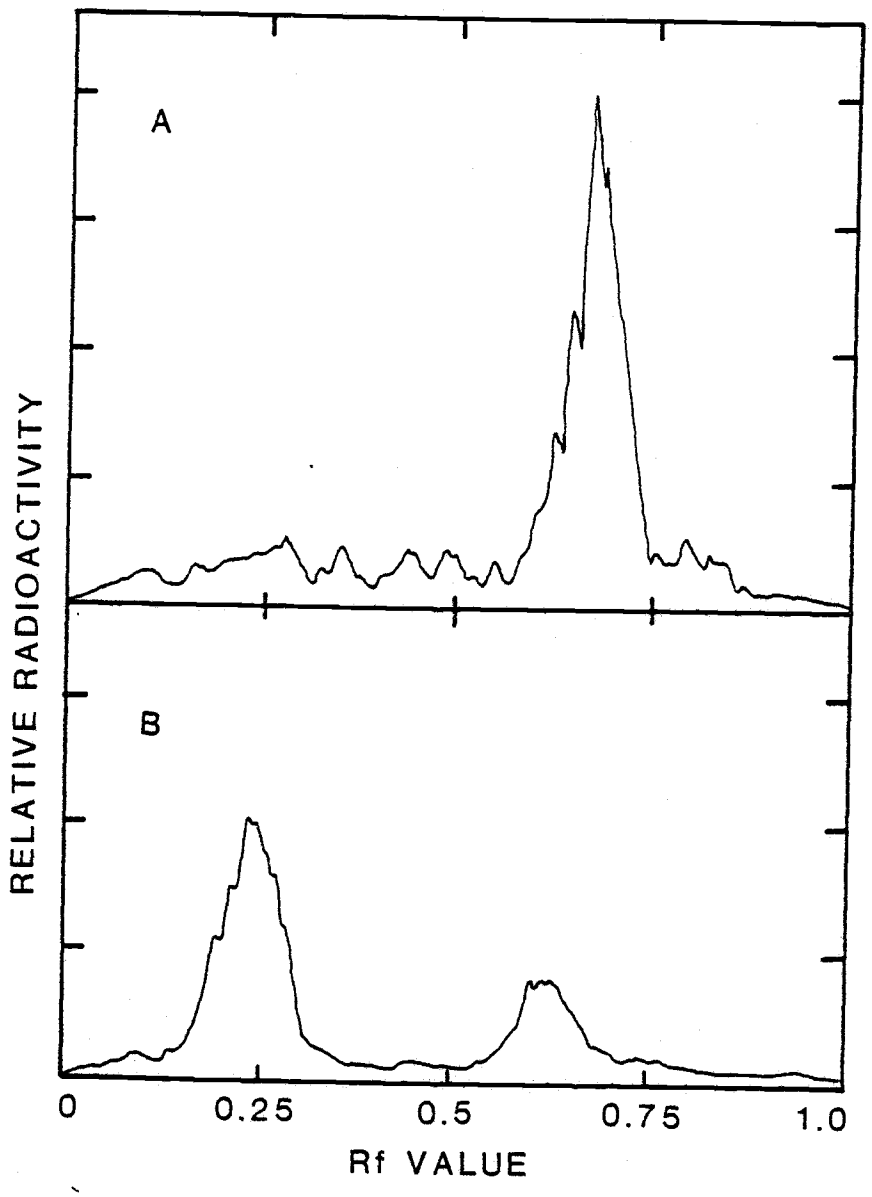

Fig. 5

(Fig. 5B). Most of the labeled material, however, migrated to an Rf 0.25. Data presented later will demonstrate this compound to be a product of MTA metabolism (5-methylthioribose-1-phosphate). No radioactive compounds could be detected from strain 2439-6C under the same conditions. The data indicate that MTA is transported into strain 50-3 and metabolized.

To determine if adenosine and MTA were taken up by a common mechanism, the effect of MTA on the accumulation of adenosine and the effect of adenosine on the accumulation of MTA were investigated (Table 3). Unlabeled adenosine (1 mM and 2.5 mM) inhibited the accumulation of 0.5 mM [8-^{14}C-adenine]MTA 29% and 47%, respectively. In the reciprocal experiment, 1 mM and 2.5 mM MTA inhibited the accumulation of 0.5 mM [8-^{14}C]-adenosine 28% and 52% respectively, approximately the same degree of inhibition. These results suggest that adenosine and MTA are taken up by a common mechanism in this strain.

Growth on purine containing substrates. Strains 2439-6C and 50-3 were starved for three days in adenine free medium to deplete the pool of free adenine, then resuspended in fresh medium supplemented with either adenine, adenosine (Fig. 6), or MTA (Fig. 7) as a source of purine. In the presence of adenine (20 µg/ml), both strains attained a similar cell density by 40 h, but the cordycepin sensitive mutant (strain 50-3) exhibited a much longer lag phase (Fig. 6). When adenosine was the source of purine, however, strain 2439-6C grew very slowly, and, after 39 h of growth, reached a cell density which was only 26% of that attained by strain 50-3 under

Table 3. Reciprocity of accumulation exclusion of MTA and adenosine in S. cerevisiae.

Supplement	% Inhibition of accumulation[a]	
	[8-^{14}C]adenosine	[adenosine-8-^{14}C]MTA
MTA		
1.0 mM	28	-
2.5 mM	52	-
Adenosine		
1.0 mM	-	29
2.5 mM	-	47

[a]Cells (3 x 10^6/ml) were incubated with labeled MTA or adenosine (0.5 mM) in the presence or absence of the other unlabeled substrate for 100 min. The radioactivity accumulated within the cells was determined as described in the text.

Fig. 6. Growth of strains 2439-6C and 50-3 in YNB media supplemented with adenosine or adenine. Cells were starved in adenine-free medium for three days and inoculated into fresh medium in the absence and presence of adenine (20 µg/ml) or adenosine (1.0 mM). Strain 2439-6C grown in the absence of adenine or adenosine, (●); in the presence of adenine, (○); or adenosine (□). Strain 50-3 grown in the presence of adenine, (▲); or adenosine, (△).

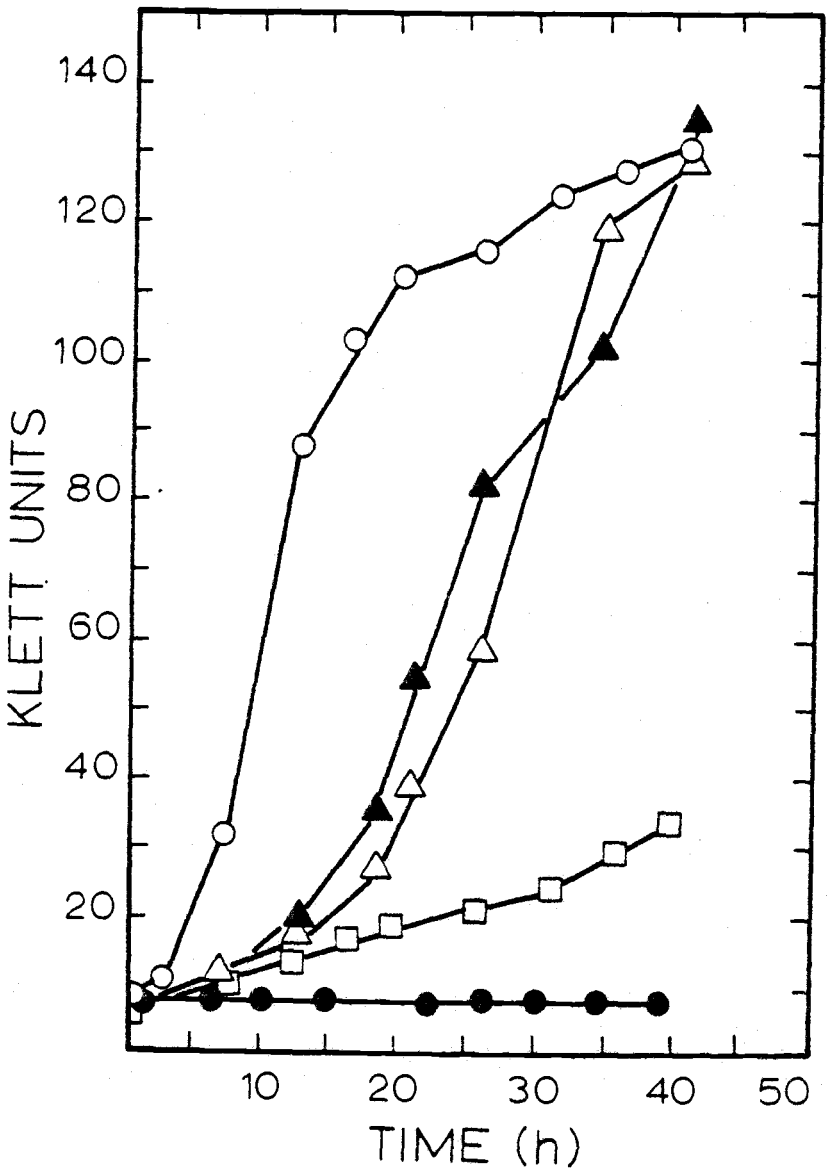

Fig. 6

identical conditions. When MTA was the source of purine (Fig. 7) similar results were observed; following a 10 h lag, strain 50-3 grew readily on 1.0 mM MTA but strain 2439-6C again reached a density only a fraction (less than 25%) of that achieved by the cordycepin sensitive strain. In the absence of a purine source both strains failed to grow in the time period examined.

The above experiments were extended to determine the dose-response of supplemented MTA on the growth of strain 50-3 (Fig. 8). Over a 40 h period, concentrations ranging from 225 μM to 740 μM MTA resulted in nearly the same final cell mass. MTA (100 μM) as the sole source of purine was sufficient for approximately 95% of maximum growth of strain 50-3. Half-maximal growth was reached at an MTA concentration of approximately 30 μM. The data indicate that low levels of MTA can satisfy the purine requirement of S. cerevisiae if the strain is sufficiently permeable to this nucleoside.

The growth of strains 2439-6C and 50-3 on several sources of purine is shown in Figures 9, 10, and Table 4. Growth of strain 2439-6C was minimal when supplied with adenosine or MTA as a purine source (Fig. 9). SAM and SAH supported growth of this strain to the same extent as did adenine. SAH was a better source of purine for strain 2439-6C than for strain 50-3, whereas the opposite was observed for cells grown on SAM (Fig. 10). All the purine containing compounds tested were able to support growth of the permeable strain, 50-3 (Table 4). That SAM and SAH can fulfill the purine requirement in a non-permeable strain reflects the presence of a mechanism by which

Fig. 7. Growth of strains 2439-6C and 50-3 in YNB medium supplemented with 1 mM MTA. Cells were incubated in the absence of adenine for three days and inoculated into fresh medium. Strain 50-3 was grown in the absence of adenine and MTA, (O); or in the presence of MTA, (●); strain 2439-6C was grown in the presence of MTA, (□).

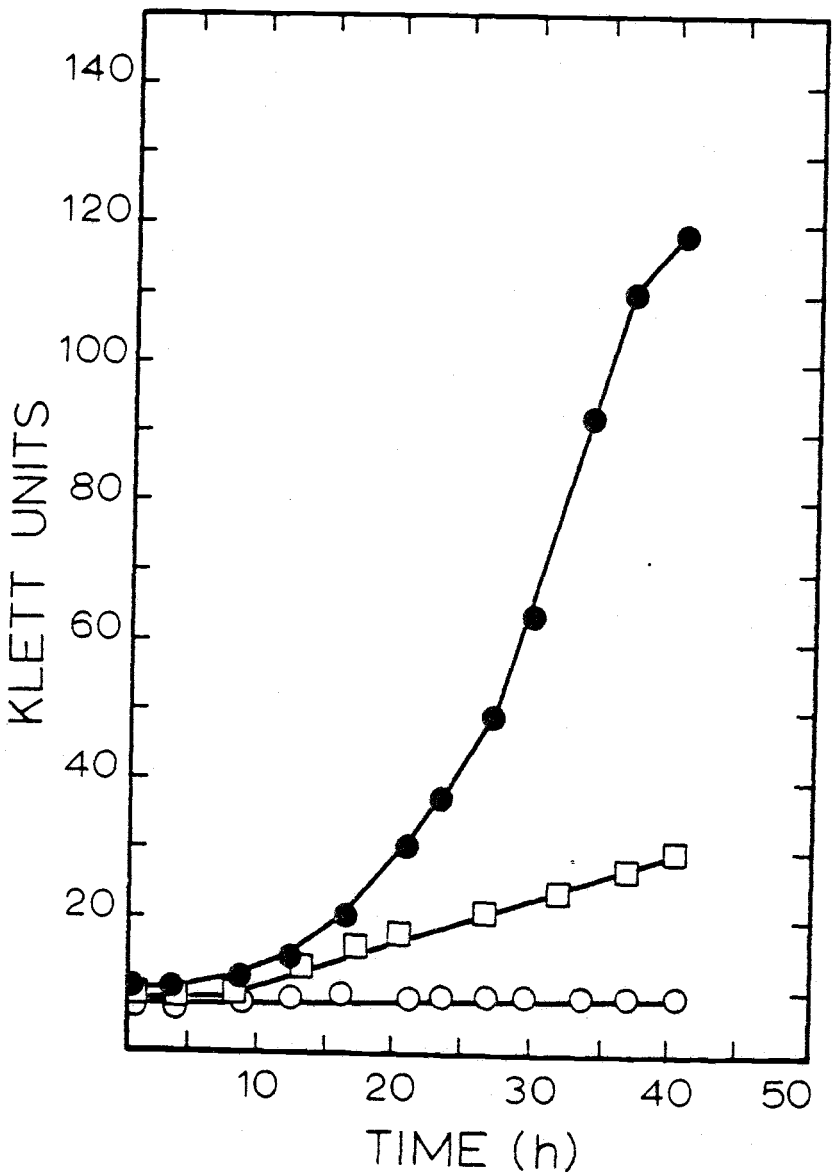

Fig. 7

Fig. 8. Growth of strain 50-3 on various concentrations of MTA. Adenine starved cells were inoculated into fresh adenine free YNB medium in the presence of various concentrations of MTA and allowed to grow for 39 h.

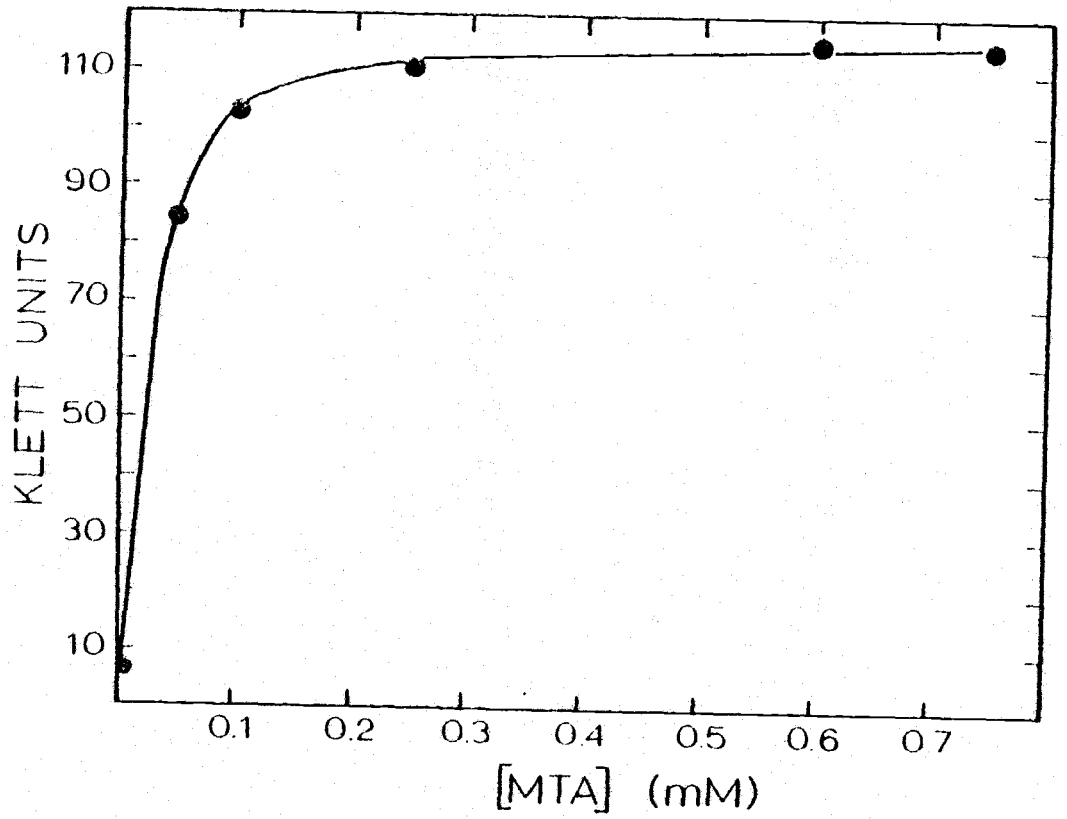

Fig. 8

Fig. 9. Growth of strain 2439-6C in YNB medium supplemented with various purine sources. Cells were adenine-starved for three days then inoculated into fresh medium in the absence of adenine (▲), presence of 20 µg/ml adenine (●), 1.0 mM SAM (o), 1.0 mM SAH (Δ), 1.0 mM adenosine (□), or 1.0 mM MTA (■).

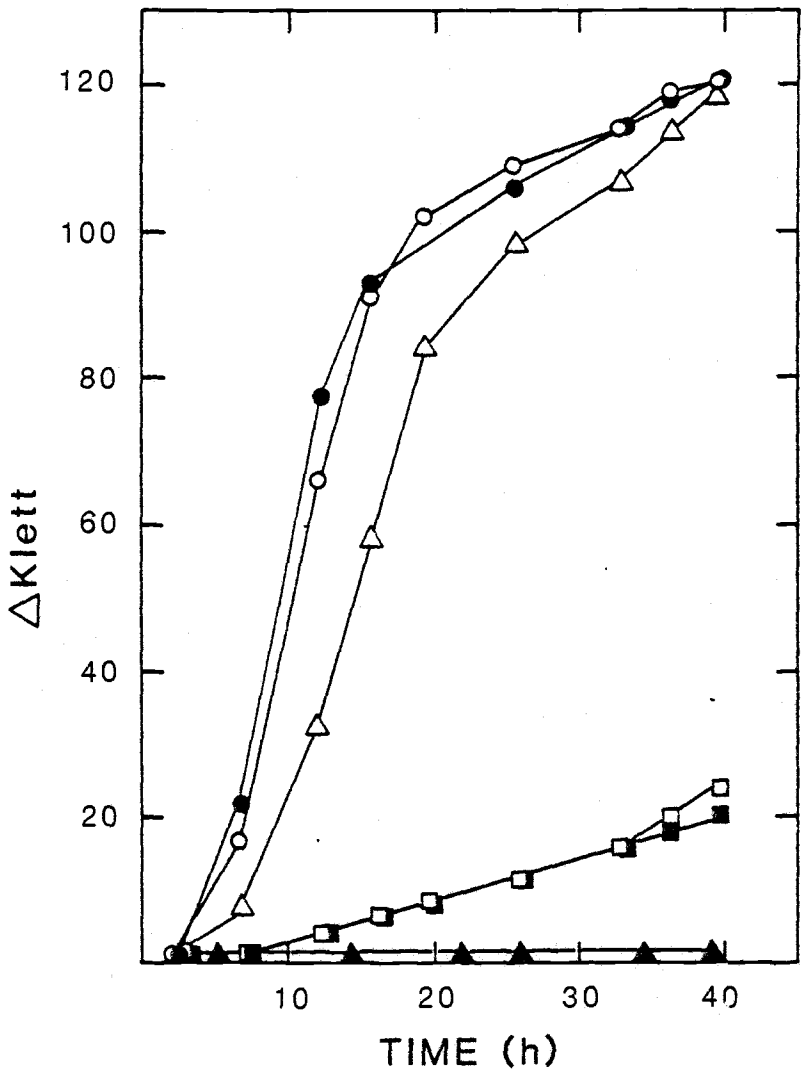

Fig. 9

Fig. 10. Growth of strain 50-3 in YNB medium supplemented with various purine sources. Cells were adenine-starved for three days then inoculated into fresh YNB medium supplemented with 20 µg/ml adenine (0), 1.0 mM adenosine (●), 1.0 mM SAH (Δ), or 1.0 mM SAM (▲).

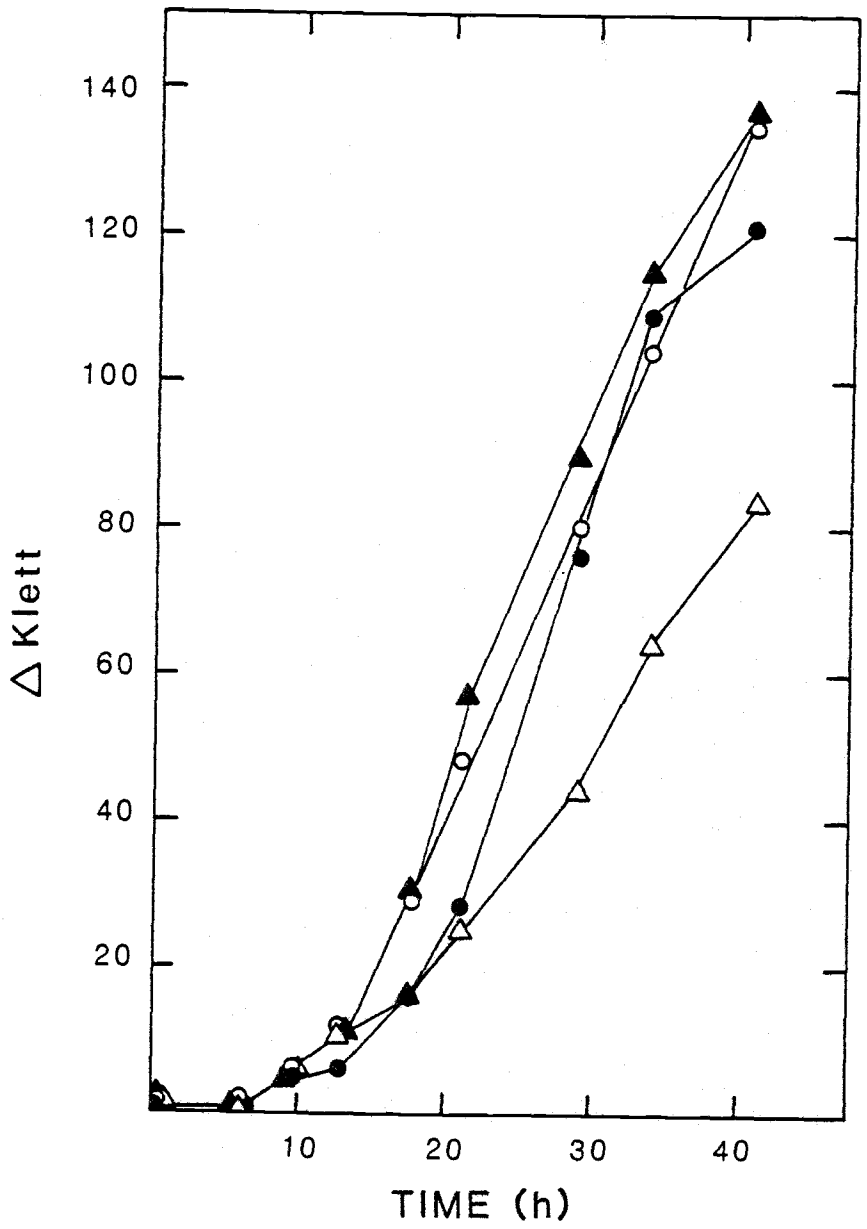

Fig. 10

Table 4. Ability of various substrates to fulfill an auxotrophic requirement for Adenine[a].

Substrate	Klett units attained by Strain 6-C	Strain 50-3
adenine	128	134
adenosine	33	130
MTA	30	116
SAM	128	147
SAH	128	94

[a]Growth after a 39 hour period.

these compounds can be transported (162). The ability of SAH to support growth in both strains indicates that S-adenosyl-L-homocysteine hydrolase cleaved SAH to yield adenosine and homocysteine (54).

SAM on the other hand, which also supports the growth of both strains therefore may follow two independent routes of degradation in order to supply the purine requirement. A transmethylation reaction may yield SAH with the subsequent degradation via SAH hydrolase, or, SAM may be degraded to MTA by any of several reported routes (87). The subsequent cleavage of MTA by MTA phosphorylase would thus liberate adenine which may enter the purine pool, and then be incorporated into nucleic acids. To demonstrate this possibility, cells were grown in the presence of [8-^{14}C-adenine)MTA and TCA precipitable material was isolated from the cells at specified intervals and assayed for radioactivity. Greater than 90% of the label from the adenine portion of MTA was incorporated into TCA precipitable material in strain 50-3. This result indicates the adenine moiety of MTA is metabolized and incorporated into TCA precipitable macromolecules, presumably nucleic acids.

MTA phosphorylase activity. To determine if free adenine was being liberated from MTA in crude extracts, [8-^{14}C-adenine]MTA was incubated with a crude cell-free extract from strain 50-3. A ^{14}C-product isolated from the reaction mixture did not migrate with authentic adenine. Instead, the product co-migrated with hypoxanthine in both TLC systems I and II suggesting that adenine deaminase was present in these extracts and it catalyzed the conversion of adenine to

hypoxanthine. An alternative possibility is that MTA was deaminated to yield 5'-methylthioinosine, which is then degraded to release hypoxanthine and the 5-methylthioribose moiety, though such an enzyme has not been demonstrated in S. cerevisiae.

Assays for MTA phosphorylase activity in crude extracts revealed that the enzyme was present in both strains (Table 5). The presence of MTA phosphorylase activity in strain 2439-6C eliminates the possibility that this strain does not grow on MTA due to a deficiency of this enzyme. In adenine grown cells, enzyme activity was present at approximately the same level in both strains (0.108 pmole/mg·min in strain 2439-6C vs 0.097 pmole/mg·min in strain 50-3). Growth of strain 50-3 on MTA, in lieu of adenine, caused an apparent enhancement of enzyme activity. The specific activity for MTA phosphorylase in crude extracts from cells grown in 0.25 mM and 0.5 mM MTA was 1.7 and 2.8-fold higher, respectively, than that of adenine grown cells. In another strain, 2218A, growth on 1 mM MTA resulted in an enhancement of MTA phosphorylase activity 40 fold over cells grown without MTA.

MTA, even with multiple pathways of biosynthesis, has not been found to accumulate in a number of different cells and tissues (38, 39,42). Accounting for the low intracellular levels of MTA is the rapid degradation to 5-methylthioribose and adenine in a variety of microorganisms (46) and to 5-methylthioribose-1-phosphate and adenine in mammalian tissue by the MTA phosphorylase (47-49,52). Such conclusions have important implications on those systems where MTA has been shown to be inhibitory (73). Furthermore, it appears that MTA

Table 5. Stimulation of MTA phosphorylase activity *in vivo* by MTA.

Purine Source	Specific activity of MTA phosphorylase (milliunits/mg)		
	Strain 6-C	Strain 50-3	Strain 2218A
adenine (20 µg/ml)	0.108	0.097	0.039*
0.25 mM MTA	NT	0.166	NT
0.5 mM MTA	NT	0.271	NT
1.0 mM MTA	NT	NT	1.585

*Methionine supplied to media at 20 µg/ml.

NT = not tested

nucleosidase or MTA phosphorylase is ubiquitous in a number of different systems tested, (Table 6) indicating its relevance in general cellular metabolism.

The most active source of enzyme was the nucleosidase from plant material catalyzing the conversion of 2304 pmole/mg·min, 1835 pmole/mg·min, and 1255 pmole/mg·min in avocado, pear, and apple tissue, respectively. Enterobacter aerogenes extracts, another source of nucleosidase (65), had only 20% to 40% the activity found in the plant tissues. Among the sources of MTA phosphorylase, a human melanoma cell line had activity 3-fold that found in lymphocytes. These levels of enzyme activity are comparable to those found in the yeast strains tested. In addition, enzyme activity was isolated from both the cytosolic and mitochondrial fractions of strain MCC. It was not determined whether these activities represented identical enzymes. Further studies involving the purification and characterization of the enzyme catalyzing the degradation of MTA should lead to further understanding of the role of MTA in the cell. For these studies strain MCC was chosen as it had the highest enzyme activity among those strains tested which did not require the exogenous supplementation of high concentrations of MTA.

Table 6. MTA phosphorylase (nucleosidase) activity in various extracts.

Source	Specific Activity pmole/mg·min
48 h PHA stimulated lymphocytes (human)	195
Human melanoma cells	687
Mouse liver	114
Musca Domestica ovary	128
Apple tissue (golden del)	1255
Pear tissue	1835
Avocado tissue	2304
Yeast strain 2218A (ade, met grown)	39
strain 2218A (1 mM MTA grown)	1585
strain MCC cytosolic	470
strain MCC mitochondrial	725
Enterobacter aerogenes	497

Purification and Characterization of MTA Phosphorylase from *Saccharomyces cerevisiae*

Enzyme purification. Early attempts at purifying 5'-methylthioadenosine phosphorylase proved to be futile since the enzyme was highly unstable in various buffers. Addition of glycerol, reducing agents, EDTA, and sorbitol resulted in a significant increase in stability during storage. The loss of enzyme activity from the action of proteases was also a hindrance throughout the isolation procedure, particularly during the latter stages of purification. This was alleviated by the addition of the protease inhibitors phenylmethylsulfonylfluoride and p-chloromercuribenzoic acid (169). EDTA may also have played a role in protecting the enzyme from digestion by proteases since it has been shown to inhibit protease α in *S. cerevisiae* (169). The enzyme was routinely stored at -80°C in standard buffer. Under these conditions a 20 to 30% loss in enzyme activity was found over a fifty day period.

The presence of excess nucleic acid rendered both hydroxylapatite and DEAE cellulose binding ineffective, thus, crude extracts were first brought to 6% saturation with streptomycin sulfate (Table 7). This treatment resulted in a change in the 280/260 adsorption ratio from 0.629 to 1.06; this represents a drop in nucleic acid content from 17% to 3% (170). Hydroxylapatite binding was effected at phosphate concentrations of less than 0.25 M, thus, it was possible to use standard buffer in a bulk elution step.

Table 7. Purification of 5'-methylthioadenosine phosphorylase.

Fraction	Volume	Total Protein	Total Units	Specific Activity munits/mg	Yield	Purification
Crude extract	25 ml	521 mg	0.244	0.47	100%	------
Streptomycin sulfate	53	278	0.319	1.15	130	2.45
Hydroxylapatite	245	51.5	0.193	3.76	79	8.00
DEAE cellulose	1.3	1.69	0.021	12.3	8.4	26.17
Sephadex G-200	1.9	0.114	0.007	63.4	3.0	134.9

The total bulk eluant was applied to DEAE cellulose column. Elution over a 0.05 M to 0.4 M linear phosphate gradient is illustrated in Fig. 11. Peak enzyme activity was found between 0.15 M and 0.2 M phosphate. Following elution from DEAE cellulose, the concentrated sample was applied to and eluted from a Sephadex G-200 column as described in Materials and Methods. Figure 12 reveals that the peak enzyme activity eluted between 95 and 120 ml. This corresponds to a molecular weight of approximately 90,000 D. Polyacrylamide gel electrophoresis revealed one major and two minor protein bands.

Product identification. Thin layer chromatographic analysis of the degradation products of 5'-[$^{14}CH_3$]methylthioadenosine incubated with purified enzyme yielded (solvent system 3) a single peak migrating to an R_f value of 0.03 relative to the solvent front (Fig. 13). Authentic 5'-methylthioadenosine migrates to an R_f value of 0.25, 5'-methylthioribose to 0.60, ribose-1-phosphate to 0.05, and 5-methylthioribose-1-phosphate remains at the origin. Exposing the product to acid or treatment with alkaline phosphatase resulted in the formation of a radioactively labeled compound which co-migrated with 5'-methylthioribose.

Utilization of [8-^{14}C-adenine]-5'-methylthioadenosine in the assay resulted in a single radioactively labeled peak which migrated to the same R_f as adenine (R_f = 0.1). Furthermore, when the product which migrated to R_f 0.03 was incubated with equimolar adenine under standard assay conditions (minus 5'-methylthioadenosine), small

Fig. 11. Elution of Saccharomyces cerevisiae 5'-methylthioadenosine phosphorylase from DEAE cellulose. An 8-fold purified enzyme preparation was applied to a 16 x 3 cm column equilibrated with standard buffer and fractions collected. The enzyme activity (0) was eluted with a 0.05 M to 0.4 M linear phosphate gradient (----).

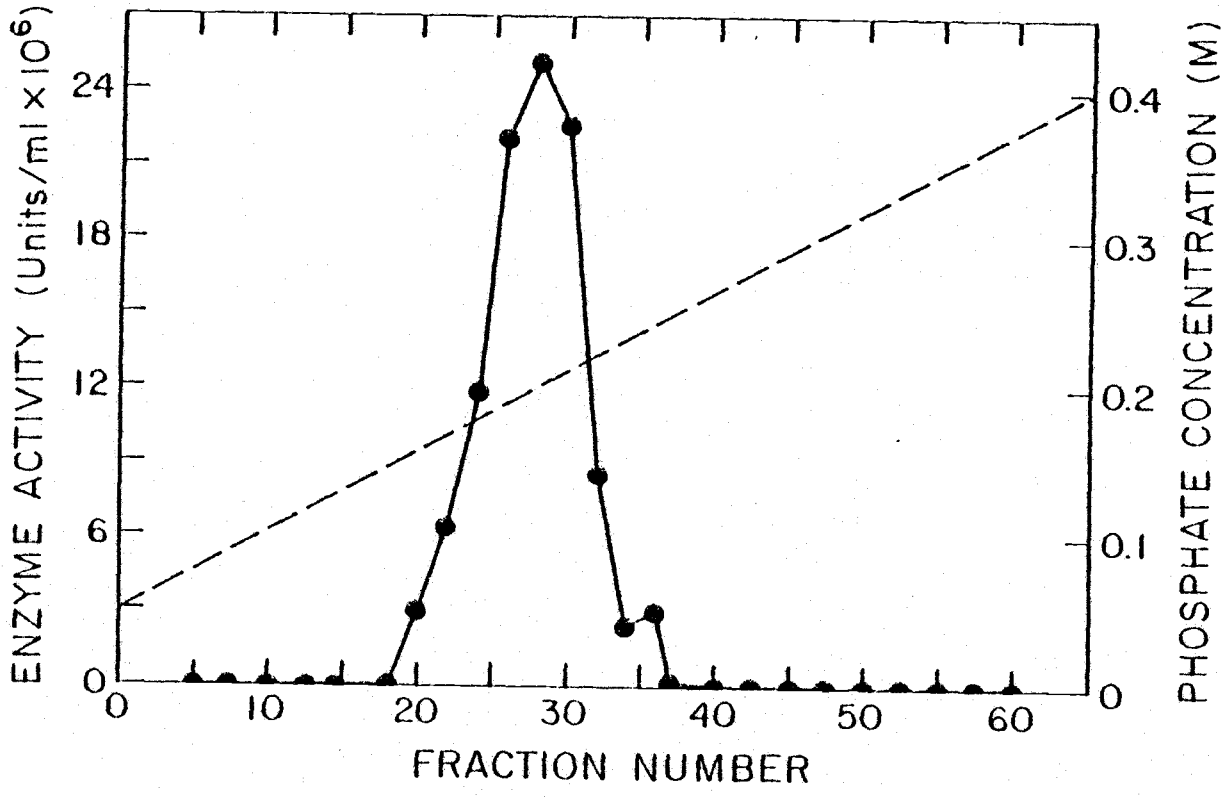
ENZYME ACTIVITY (Units/ml x 10⁶)
24
18
12
6
0
0
10
20
30
40
50
60
FRACTION NUMBER
0.4
0.3
0.2
0.1
0
PHOSPHATE CONCENTRATION (M)

Fig. 11

Fig. 12. Elution of Saccharomyces cerevisiae 5'-methylthioadenosine phosphorylase from a Sephadex G-200 column. An aliquot (1.3 ml) of a 26-fold purified extract was applied to the column and eluted with 200 ml of standard buffer.

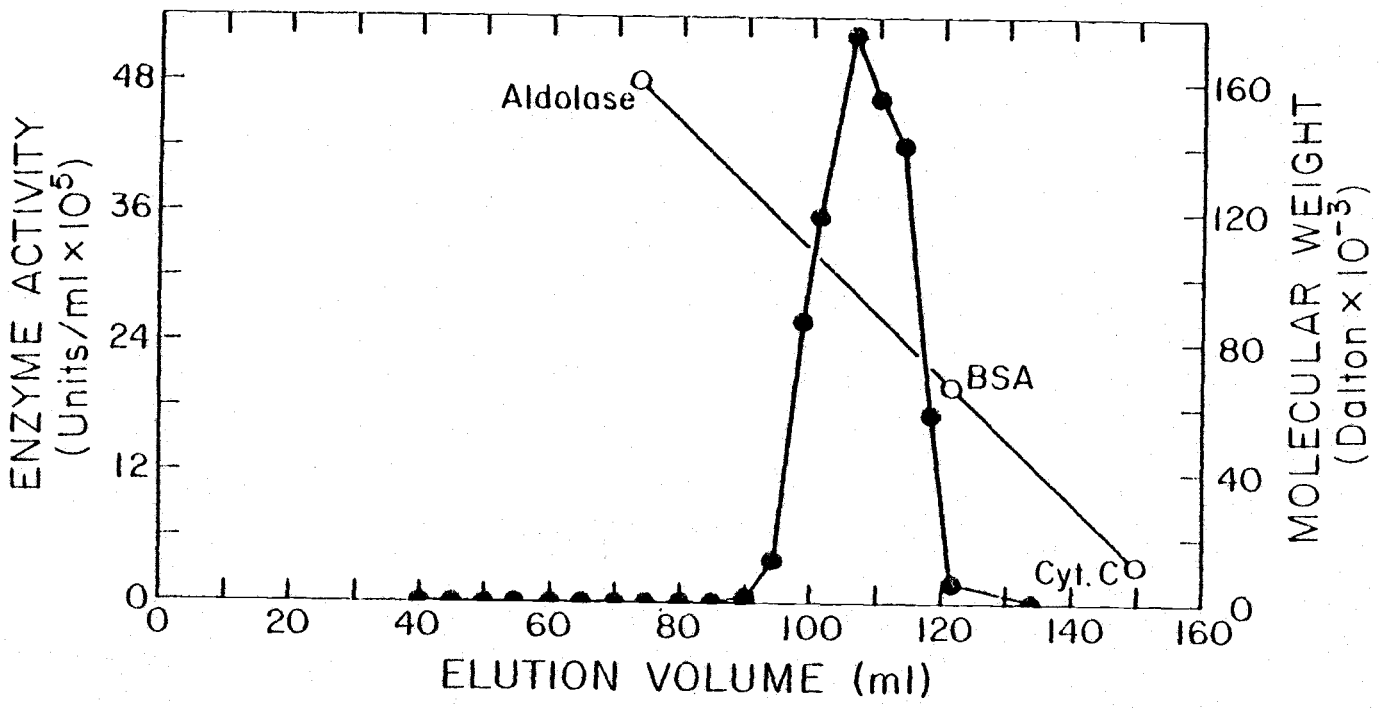
ENZYME ACTIVITY
(Units/ml x 10^5)
MOLECULAR WEIGHT
(Dalton x 10^{-3})
ELUTION VOLUME (ml)
Aldolase
BSA
Cyt. C
0
12
24
36
48
40
80
120
160
20
60
100
140

Fig. 12

Fig. 13. Radioscans of radioactively labeled compounds arising from the degradation of 5'-[Me-^{14}C]methylthioadenosine by 5'-methylthioadenosine phosphorylase. The radioscans are: A, 5'-[Me-^{14}C] methylthioadenosine (R_f = 0.25) incubated with 5'-methylthioadenosine phosphorylase; B, the radioactive reaction product in A (R_f 0.03) incubated with alkaline phosphatase. The radioactive product in A was scraped from the chromatogram (A) and incubated with alkaline phosphatase as described in Materials and Methods. The reaction mixtures were analyzed by thin layer chromatography in solvent system 3.

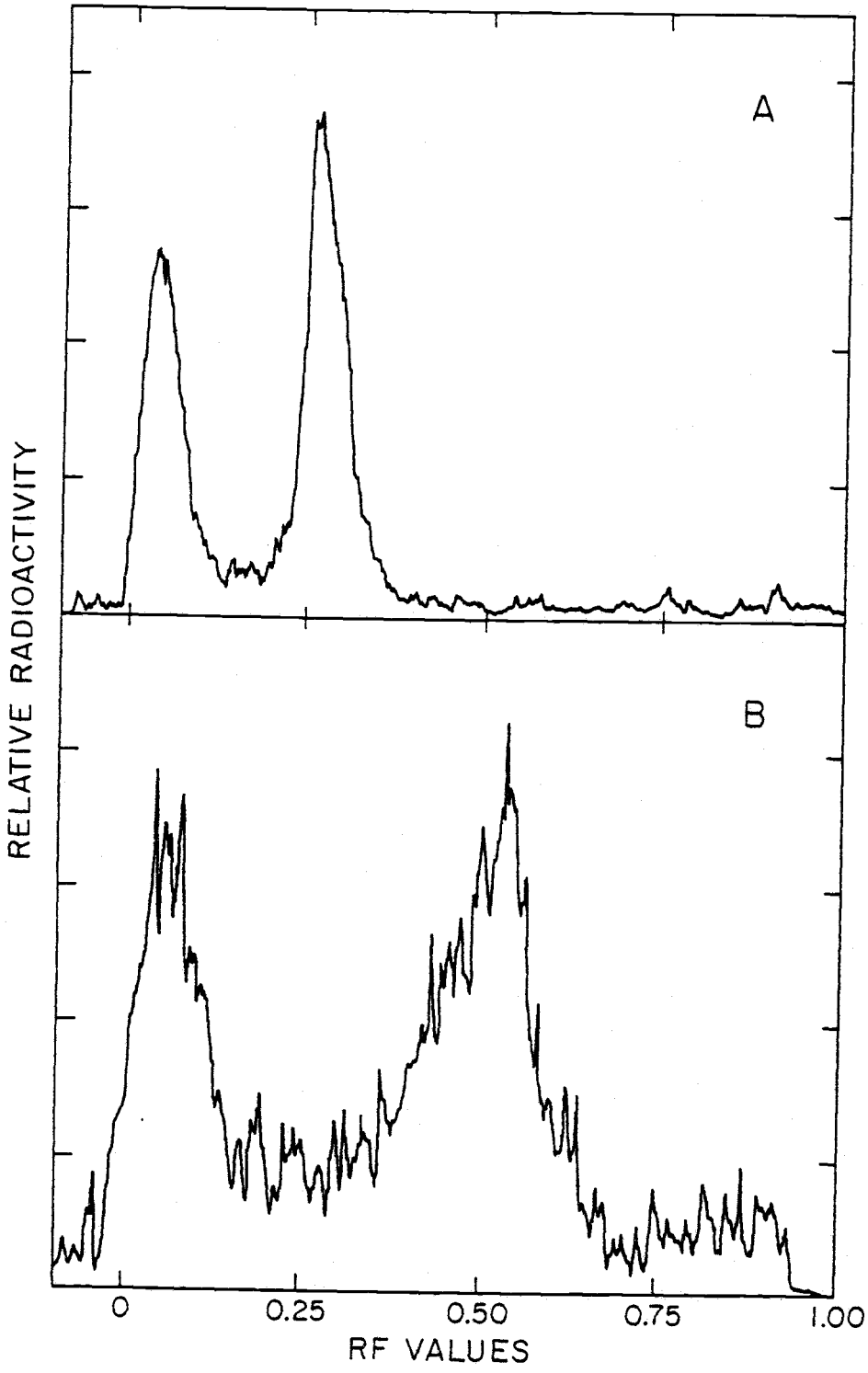

Fig. 13

amounts of a compound co-migrating with authentic 5'-methylthioadenosine were formed. Based on these data the products of the MTA phosphorylase reaction appear to be adenine and 5-methylthioribose-1-phosphate. In addition, the enzyme appeared to be capable of carrying out the reverse reaction, the synthesis of 5'-methylthioadenosine from adenine and 5-methylthioribose-1-phosphate.

Effect of temperature. The rate of 5'-methylthioadenosine degradation was greatly increased by temperature elevation. A maximum reaction rate was obtained at approximately 58°C with the rate rapidly falling between 65° and 70°C (Fig. 14). The stability of the enzyme was assayed by incubating the standard reaction mixture at various temperatures from 30° to 70°C for varied periods of time. Degradation over a 1 h period appeared to be linear at temperatures less than 47°C. Above 47°C an increasing degree of non-linearity with increasing temperature was found, presumably due to thermal denaturation of the enzyme.

Kinetic constants. Analysis of kinetic (Michaelis) parameters of 5'-methylthioadenosine phosphorylase at a phosphate concentration of 10 mM yielded an apparent Km value of 18.7 μM for 5'-methylthioadenosine with a Vmax of 77.5 nmole/mg·min as determined from the double reciprocal plot (Fig. 15). It should be noted the enzyme was sensitive to substrate inhibition at high MTA concentrations, thus creating an inflection of the curve in the double reciprocal plot.

Fig. 14. Activity of 5'-methylthioadenosine phosphorylase as a function of temperature. Reaction mixtures were incubated with a 134-fold purified enzyme preparation at the designated temperatures as described in Materials and Methods.

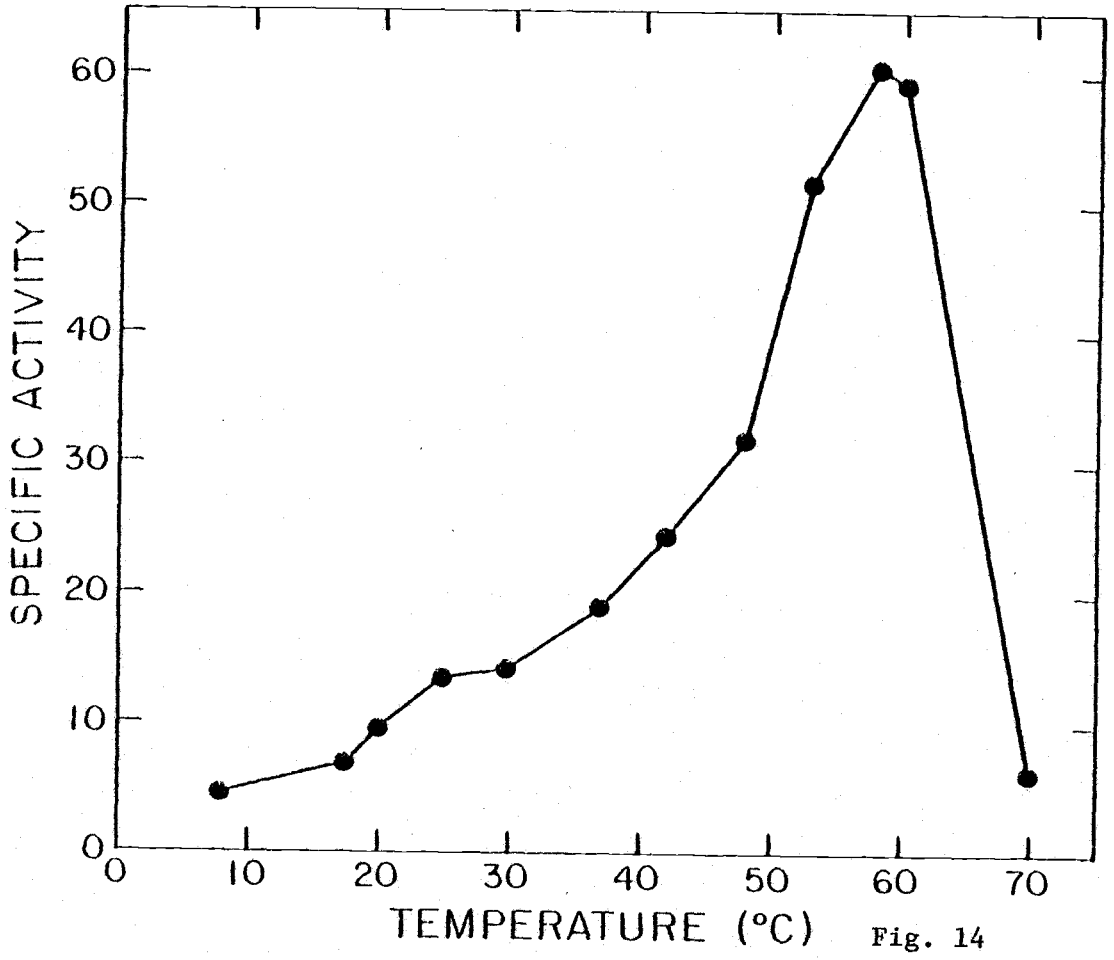
SPECIFIC ACTIVITY
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
TEMPERATURE (°C)

Fig. 14

Fig. 15. Reciprocal plot of velocity as a function of 5'-methylthioadenosine concentration. Enzyme assays were carried out at 47°C as described in Material and Methods except that the concentration of 5'-methylthioadenosine was varied as shown. The phosphate concentration was kept constant at 10 mM.

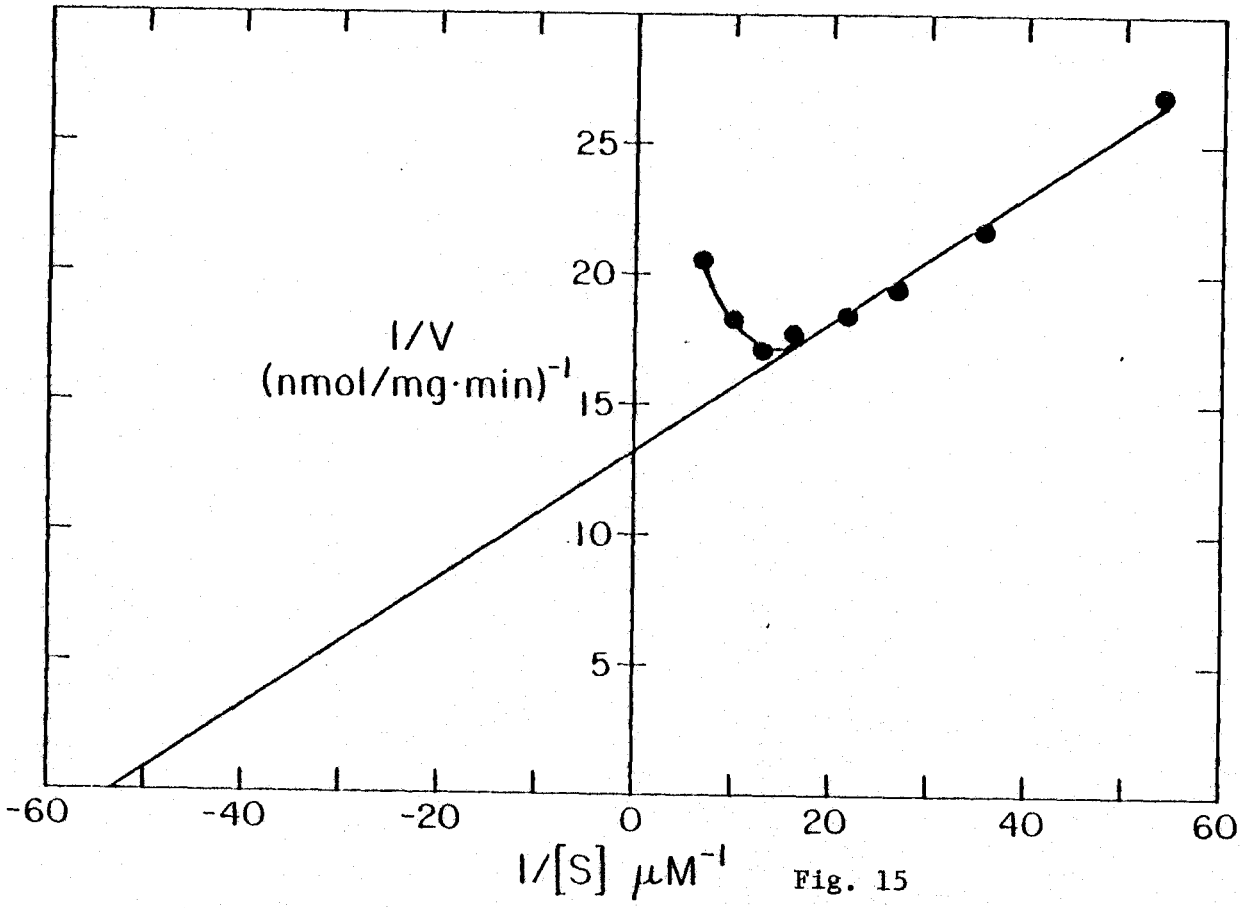

Fig. 15

The enzyme displayed a total dependence on phosphate as a second substrate, thus the kinetic constants varied according to concentration of substrate. The Michaelis constant for 5'-methylthioadenosine was between 13.3 μM and 23.2 μM for a phosphate concentration range of 5 to 15 mM. Above 15 mM there was no increase in velocity, whereas below 5 mM phosphate the velocity of the reaction decreased dramatically.

Phosphate also provided a stabilizing effect: dialysis against 0.05 M Hepes or Tris standard buffer (replacing phosphate in the standard buffer) for 6 h with four buffer changes results in a 90% loss in enzyme activity. A double reciprocal plot (1/[phosphate] vs. 1/velocity) is presented in Figure 16. The corresponding Km value is 2.33 mM for 74 μM 5'-methylthioadenosine concentration.

Substrate specificity. Various analogs of 5'-methylthioadenosine were tested for their ability to serve as substrates for the enzyme. Ultraviolet absorbing products were detected by high pressure liquid chromatography as described in Materials and Methods. Table 8 depicts the results of this study. The UV peak for each of the reacted adenosine-containing compounds increased in size when authentic adenine was added to the terminated reaction mixture, indicating adenine as a reaction product.

5'-Isopropylthioadenosine served equally well as 5'-methylthioadenosine as substrate in the reaction. In order of decreasing ability to serve as substrate, 5'-ethylthioadenosine, 5'-propylthio-

Fig. 16. Reciprocal plot of velocity as a function phosphate concentration. Enzyme assays were carried out at 47°C as described in Materials and Methods except that the concentration of phosphate was varied as shown. The concentration of 5'-methylthioadenosine was 74 μM (0).

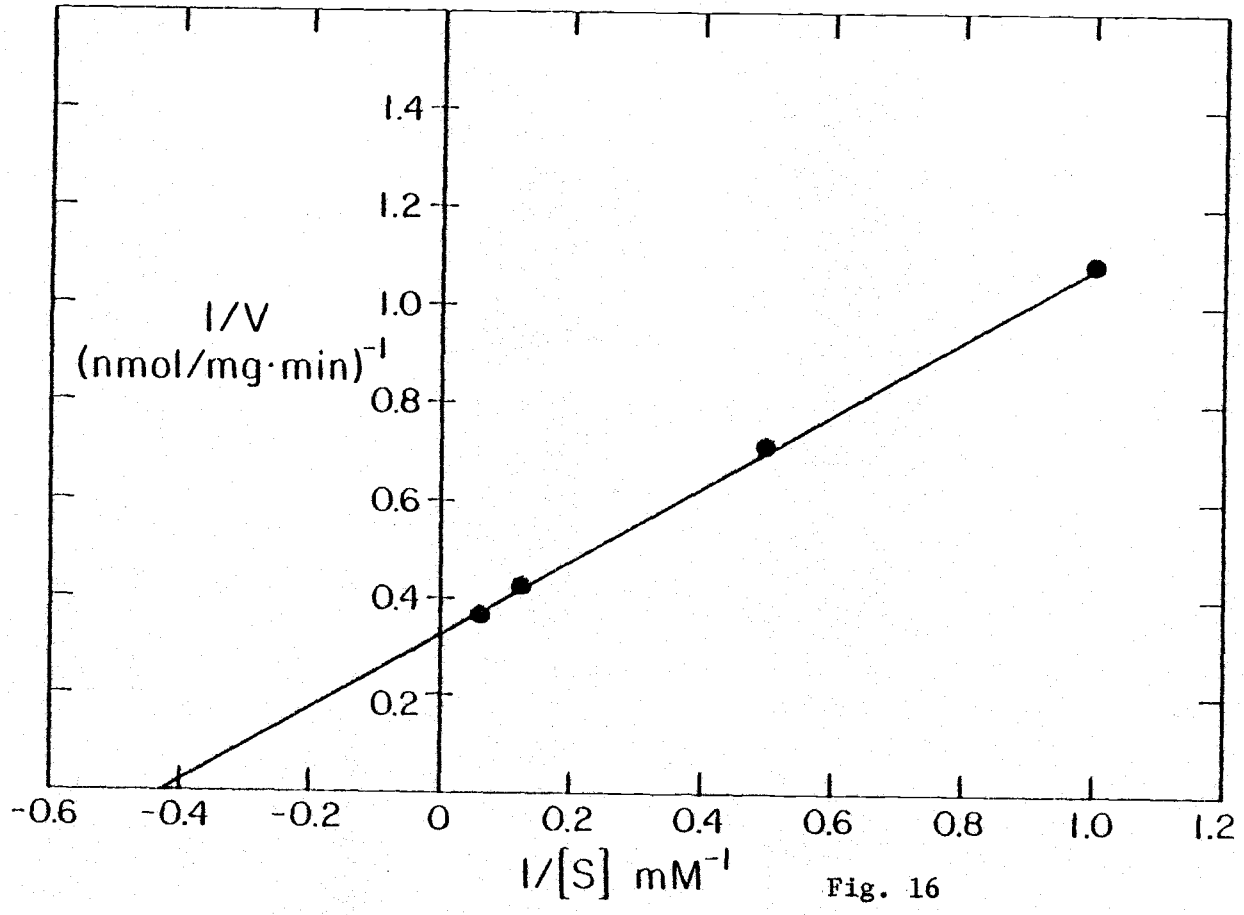
1.4
1.2
1.0
0.8
0.6
0.4
0.2
1/V
(nmol/mg·min)$^{-1}$
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1.0
1.2
1/[S] mM^{-1}

Fig. 16

Table 8. Substrate specificity of 5'-methylthioadenosine phosphorylase.

Compound Tested	pmoles product formed/2 h	% relative to 5'-methylthioadenosine
5'-methylthioadenosine	1495	100%
5'-methylthiotubercidin	<10	-
5'-ethylthioadenosine	1254	83.9
5'-propylthioadenosine	1084	72.5
5'-isopropylthioadenosine	1509	100.9
5'-butylthioadenosine	771	51.6
5'-isobutylthioadenosine	1041	69.6

adenosine, 5'-isobutylthioadenosine, and 5'-butylthioadenosine followed 5'-isopropylthioadenosine. 5'-Methylthiotubercidin, the 7-deaza analog of 5'-methylthioadenosine, yielded a very small UV absorbing peak, and exact concentration measurements were not possible.

That the yeast MTA phosphorylase lacks rigid specificity for the 5'-position of the substrate is indicated by the ability of the enzyme to cleave a variety of 5'-modified 5'-methylthioadenosine analogs. Some of these analogs have been found to be inhibitory to PHA-induced lymphocyte transformation and to growth of certain mammalian cell lines (86,92). Of special interest is 5'-isobutylthioadenosine, a powerful antiproliferative drug shown to inhibit the growth of transformed mouse mammary cells (89), cell transformation induced by oncogenic RNA or DNA viruses (81,82), mitogen-induced lymphocyte blastogenesis (90), and lymphocyte mediated cytolysis (101). This compound is degraded intracellularly in at least one system (69). With this consideration, we decided to investigate whether 5'-isobutylthioadenosine and the other 5'-substituted analogs tested in this paper would be useful as substrates in vivo and particularly, fulfill the cellular requirements for adenine in an adenine auxotroph, as does MTA. A nucleoside permeable derivative of strain 50-3, strain 2218A, was chosen for this investigation due to its less fastidious growth characteristics. The results of this study are presented in Figure 19. Each of these compounds served to some extent, as a source of purine in strain 2218A, reaching

Fig. 17. Growth of strain 2218A in YNB medium supplemented with various purine sources. Cells were taken from twenty-four hour culture and inoculated into fresh adenine free YNB medium supplemented with 1.0 mM 5'-ethylthioadenosine (Δ), 1.0 mM 5'-propylthioadenosine (■), 1.0 mM 5'-isopropylthioadenosine (□), 1.0 mM 5'-butylthioadenosine (O), 1.0 mM 5'-isobutylthioadenosine (⊖), 1.0 mM 5'-methylthioadenosine (●), or no supplement (▲).

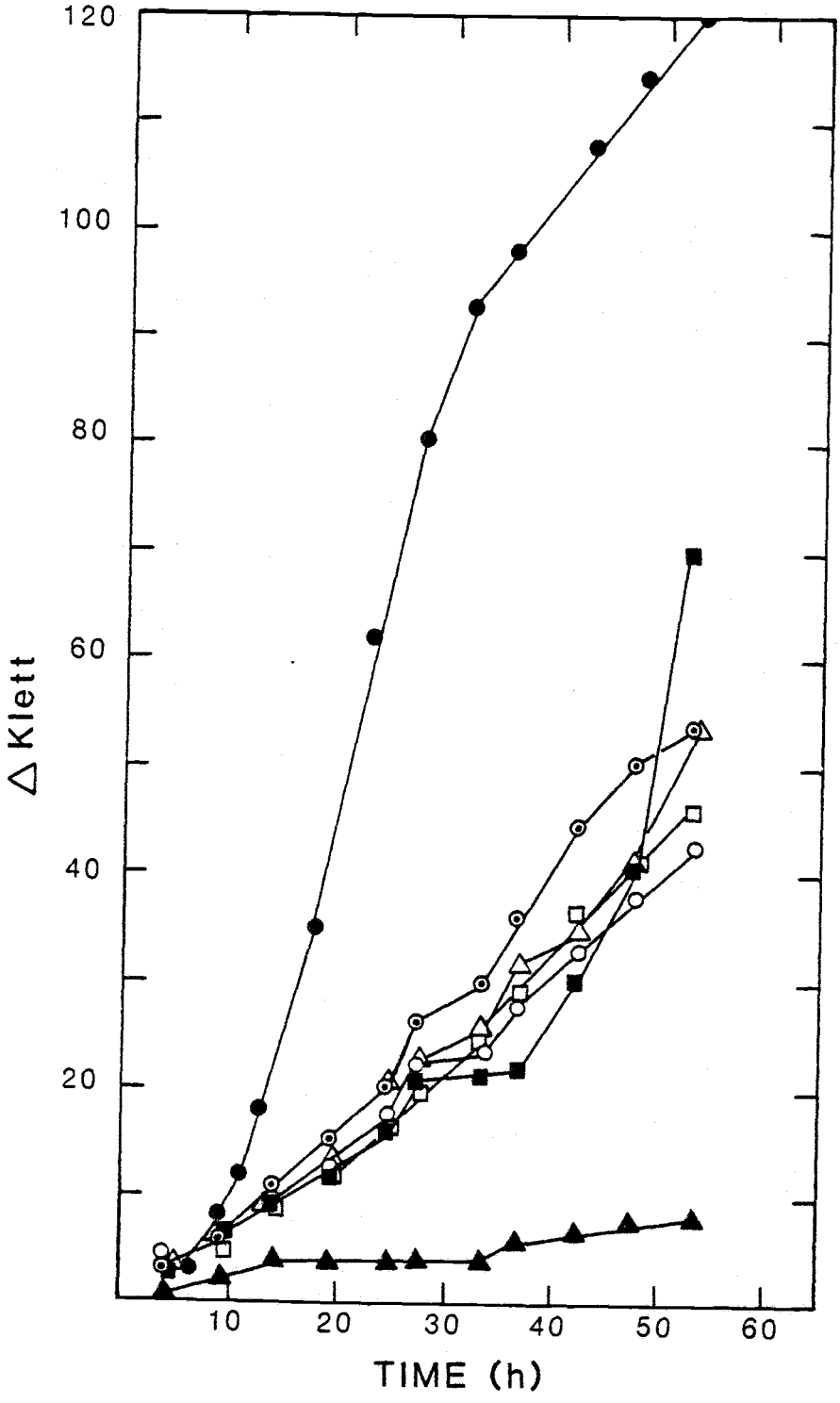

Fig. 17

between 35% and 58% the density of those cells grown on MTA. Cells grown on 5'-propylthioadenosine attained the highest cell density among the cells grown on the 5'-substituted analogs of MTA during the 53 hour period of growth. MTA clearly served as the best source of purine for the cells in these studies. The ability of these analogs of MTA to serve as a source of purine *in vivo* in an adenine requiring strain of yeast indicated that these compounds are taken up into the cell and degraded by the phosphorylase yielding adenine and the corresponding ribose analogue.

Inhibition studies. Various compounds were tested for their ability to act as inhibitors of the reaction utilizing 5'-methylthioadenosine as substrate (Table 9). It is interesting to note that one of the products, adenine, was 54.5% inhibitory; in a separate experiment the other product, 5-methylthioribose-1-phosphate, had either no effect or was slightly stimulatory at equimolar concentration with substrate. The polyamines spermine and spermidine, products during the synthesis of MTA, had little effect on the purified enzyme. S-Adenosylmethionine and 5-methylthioribose were 13.3% and 12.3% inhibitory, respectively, while the transmethylation inhibitor S-adenosylhomocysteine had virtually no effect.

The kinetics of inhibition by adenine were further analyzed (Fig. 18). Adenine was found to be a non-competitive inhibitor with a Ki value of 29.9 μM.

Table 9. Inhibition of 5'-methylthioadenosine phosphorylase.

Compound Tested	Specific Activity (milliunits/mg)	% Inhibition
5'-Methylthioadenosine	26.1	0
5'-Methylthiotubercidin	25.0	5.2
Adenosine	27.1	--
Adenine	11.9	54.4
Ribose-1-phosphate	26.2	--
5-Methylthioribose	22.9	12.3
Spermidine	24.9	5.6
Spermine	26.6	--
5-Methylthioribose-1-PO_4	30.5	--
S-Adenosylhomocysteine	25.2	3.3
S-Adenosylmethionine	22.6	13.3

Fig. 18. Non-competitive inhibition by adenine. Enzyme assays were carried out at 47°C as described in Materials and Methods except that adenine was added to a final concentration of 18.9 μM.

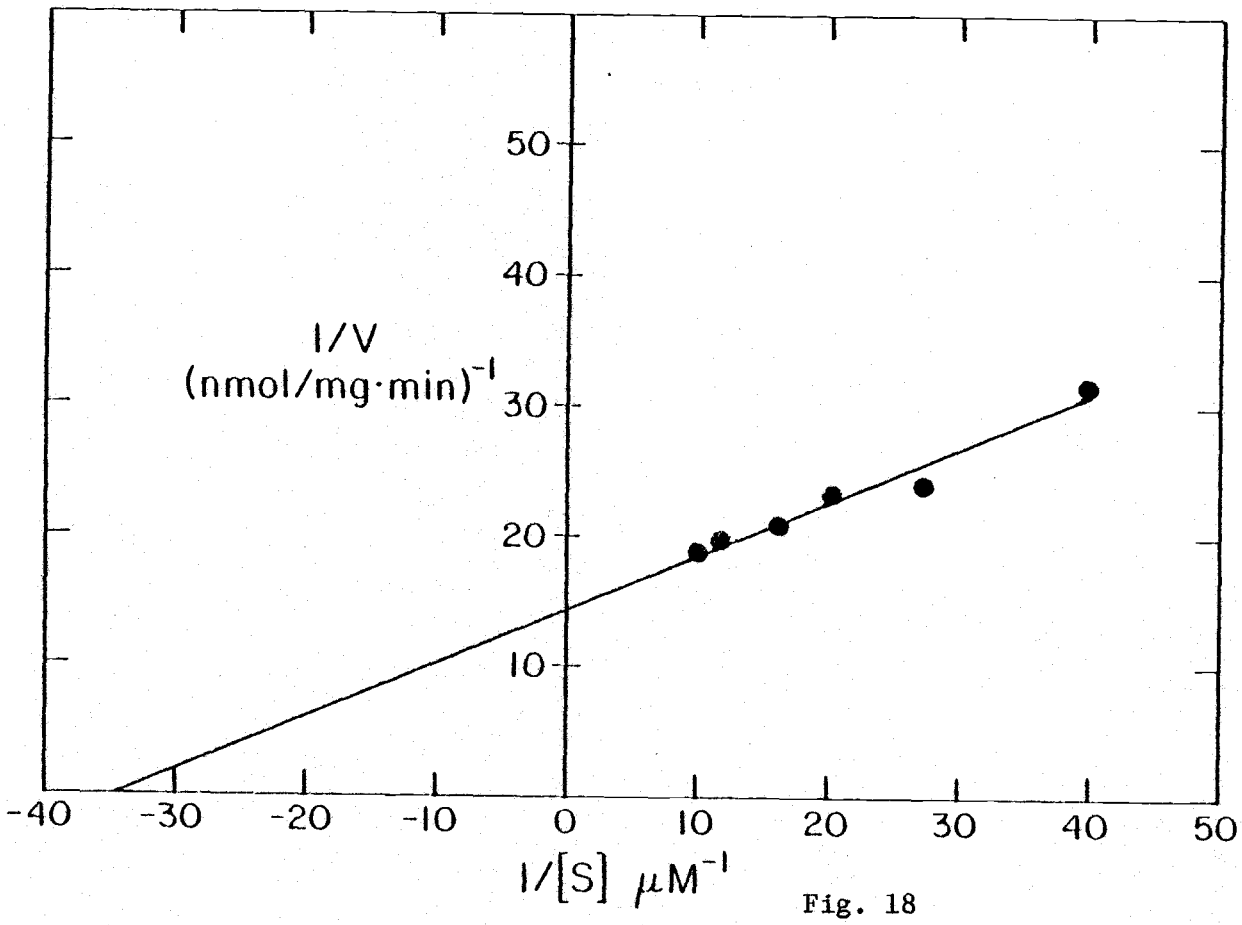

Fig. 18

The Role of MTA in Methionine Metabolism in *Saccharomyces cerevisiae*

MTA as a methionine source in vivo. MTA is degraded by MTA phosphorylase to yield adenine and 5-methylthioribose-1-phosphate in *S. cerevisiae* (Fig. 13). Although adenine is free to enter the purine nucleotide pool, the fate of 5-methylthioribose-1-phosphate is less certain. MTA has been shown to be an intermediate in methionine biosynthesis in several systems, presumably via 5-methylthioribose-1-phosphate (160). When used as a source of both adenine and methionine, MTA supported the growth of *S. cerevisiae* strain 2218A (Fig. 19). Growth of this strain was maximal with both methionine and adenine supplementation. MTA, in the presence of adenine and methionine, does not alter the growth rate (not shown). The addition of methionine to MTA supplemented cultures results in a faster growth rate than with MTA alone, however, cultures supplemented with adenine were not able to use MTA as a source of methionine.

Effect of methionine and adenine on MTA uptake. The inhibitory effect of adenine on MTA utilization in strain 2218A cannot be due solely to an effect on transport of the nucleoside.

Cells pregrown with adenine and methionine accumulated MTA at a rapid rate which was linear for 100 min (Fig. 20). In the presence of adenine, the rate of MTA uptake was initially as rapid as with MTA alone; however, by 40 min the rate of uptake of MTA in the

Fig. 19. Growth of strain 2218A in YNB medium supplemented with MTA as a methionine and purine source. Cells were inoculated from a twenty-four hour culture into fresh YNB medium with 20 µg/ml adenine containing either 1.0 mM MTA (▲), no supplements (■), 1.0 mM MTA, (O), 20 µg/ml methionine (●), 20 µg/ml methionine plus 1.0 mM MTA (Δ), or 20 µg/ml adenine and methionine (□).

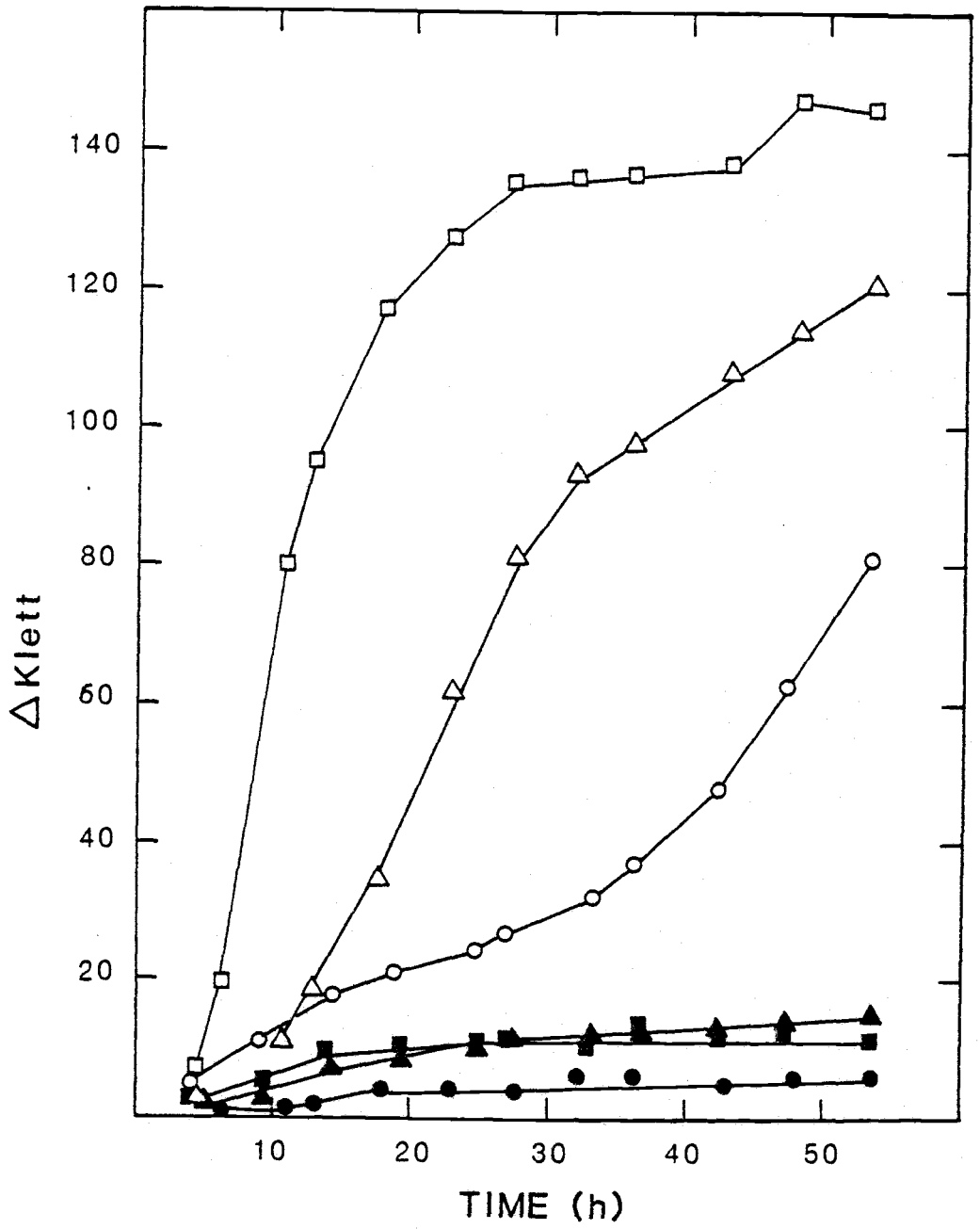

Fig. 19

Fig. 20. Accumulation of [$^{14}CH_3$]MTA by cells of S. cerevisiae 22-18A pregrown in medium containing adenine + methionine. Conditions of the experiment were as described in the text, except that the concentration of [$^{14}CH_3$]MTA (9.24×10^5 cpm/μmole) was 0.17 mM. The following supplements were added in addition to labeled MTA: none (●); 0.15 mM adenine (○); 0.13 mM methionine (▲); or 0.15 mM adenine + 0.13 mM methionine (△).

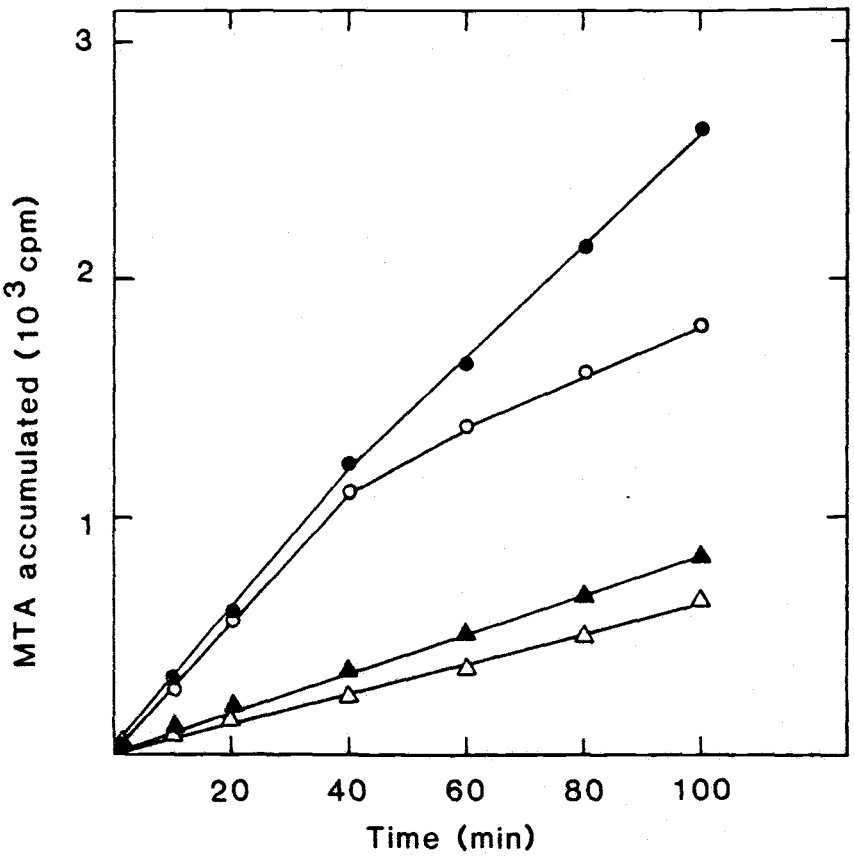

Fig. 20

presence of adenine had begun to decrease. Incubation of the cells with methionine inhibited the uptake of MTA to an even greater extent; however, the uptake of MTA by the cells incubated with methionine proceeded at a linear rate during the experiment. The combination of adenine + methionine was slightly more inhibitory than methionine alone. The data indicate that both methionine and adenine inhibit the uptake of MTA by S. cerevisiae cells pregrown in adenine + methionine.

Cells pregrown in 0.15 mM MTA accumulated less MTA than the cells pregrown in adenine + methionine (Fig. 21). The initial rate of MTA accumulation by the MTA-grown cells was slightly faster than the rate at which cells grown in adenine and methionine accumulated MTA, this rate decreased greatly after the first 20 min. The presence of adenine had no effect on MTA accumulation by the cells pregrown in MTA, whereas the inhibition of MTA accumulation by methionine was similar to that observed in the cells grown in adenine and methionine. If the inhibitory effect exerted by adenine on MTA utilization were mediated solely by inhibition of uptake, an even greater effect would have been expected by methionine since methionine inhibited the uptake of MTA to a much greater extent than did adenine.

Effect of adenine, methionine, and adenosine on MTA phosphorylase activity. Another possible explanation for the inability of MTA to serve as a methionine source when adenine was also present is the

Fig. 21. Accumulation of [$^{14}CH_3$]MTA by cells of S. cerevisiae 22-18A pregrown in medium containing MTA. Conditions for the experiment were as described for Fig. 7. The following supplements were added in addition to labeled MTA: none (●); 0.15 mM adenine (○); 0.13 mM methionine (▲); or 0.15 mM adenine + 0.13 mM methionine (△).

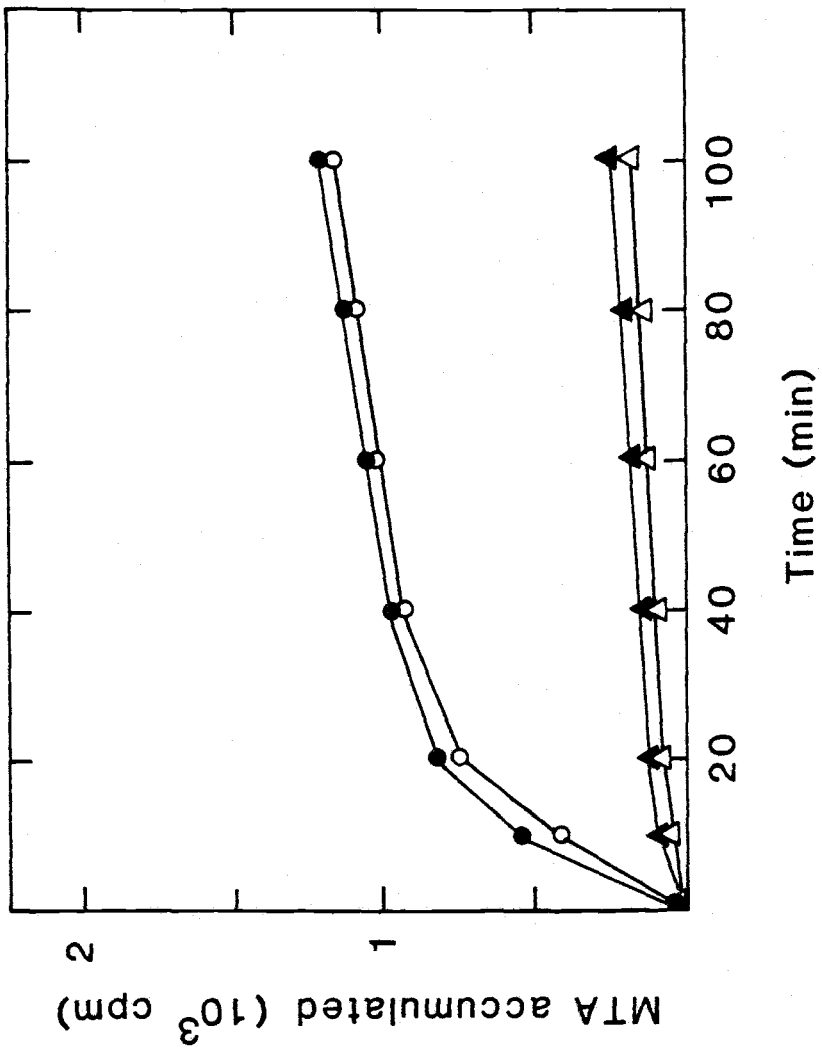

Fig. 21

inhibition of MTA phosphorylase activity by adenine, a product of the reaction catalyzed by this enzyme. In crude cell extracts of strain 22-18A the activity of MTA phosphorylase was determined and found not to be inhibited by equimolar adenine, methionine, or adenosine (Table 11). This, however, is not necessarily an accurate picture of what happens in vivo. We have previously demonstrated the purified MTA phosphorylase from strain MCC was inhibited by adenine (Ki = 29.9 μM, Fig. 18); it is possible that in vivo conditions mimic the enzyme in its purified state rather than the crude mixture of proteins assayed here. The data implies the inhibitory effect adenine has on MTA utilization may be due to a combination of effects; adenine may also exert an inhibitory effect on another intermediate step in methionine biosynthesis from MTA.

Metabolism of [$^{14}CH_3$] MTA in vivo. Earlier studies demonstrated that the methylthiol group of MTA was somehow recycled back to S-adenosylmethionine (122). Toohey (23,127) recently reported that in mammalian cells 5-methylthioribose-1-phosphate was metabolized to an unknown product, which was suggested to be the product of a methylthiol-group cleavage reaction. A methylthiol group transfer may liberate free methanethiol or require the presence of an acceptor molecule in order to form methionine or its precursor. Incubation of [$^{14}CH_3$]MTA with α-ketobutyrate or α-aminobutyrate, as acceptor molecules, with cell-free extracts of strain 2278C resulted in no change in the profile of radioactive material on thin layer

Table 10. Effect of methionine, adenine, and adenosine on MTA phosphorylase activity in S. cerevisiae 22-18A.

Additions[a]	Specific Activity (pmole/mg·min)
None	56.9
Adenine	64.3
Methionine	67.0
Adenosine	73.8

[a]The assay system contained the added substance and MTA at equimolar concentration (74 μM).

chromatograms relative to controls without acceptor molecules. Furthermore another methionine auxotroph, strain S37 was not able to utilize methanethiol sodium salt as its sole source of methionine (data not shown). It is likely that the mechanism by which MTA is recycled to methionine is far more complex.

Several strains of S. cerevisiae with improved ability to utilize MTA as the sole source of methionine were selected. One of these, strain 224A, was grown to log phase in 0.35 mM MTA then transferred to fresh media with 0.35 mM [$^{14}CH_3$]MTA followed by incubation for 9 h. Perchloric acid extraction and subsequent chromatography indicated labeled material had accumulated within the cell (Fig. 22). The peaks correspond to compounds which migrate to the same Rf values as standard MTR-1-PO_4, methionine, MTA or 2-keto-4-methylthiobutyric acid, and 3-methylthiopropionic acid or 3-methylthiopropionaldehyde. Development of this same extract on silica gel in solvent system 3 resulted in the appearance of labeled peaks corresponding to the same Rf values as MTA and 3-methylthiopropionaldehyde. Methionine, 3-methylthiopropionic acid and 5-methylthioribose-1-phosphate do not move off the origin in this system and thus were indistinguishable. Therefore, the products from the in vivo metabolism of MTA appear to include 5-methylthioribose-1-phosphate, methionine, and 3-methylthiopropionaldehyde. To determine which compounds were intermediates in the biosynthesis of methionine from MTA, or catabolites of methionine, the metabolism of MTA was also studied in vitro.

Fig. 22. Radioscan of radioactively labeled products of [$^{14}CH_3$]MTA metabolism. Strain 224A was grown in 0.35 mM [$^{14}CH_3$]MTA (specific activity = 5 x 10^6 cpm/μmole) for 9 h and homogenized in ethanol. The sample was applied to a cellulose thin layer plate and the chromatogram was developed in system four.

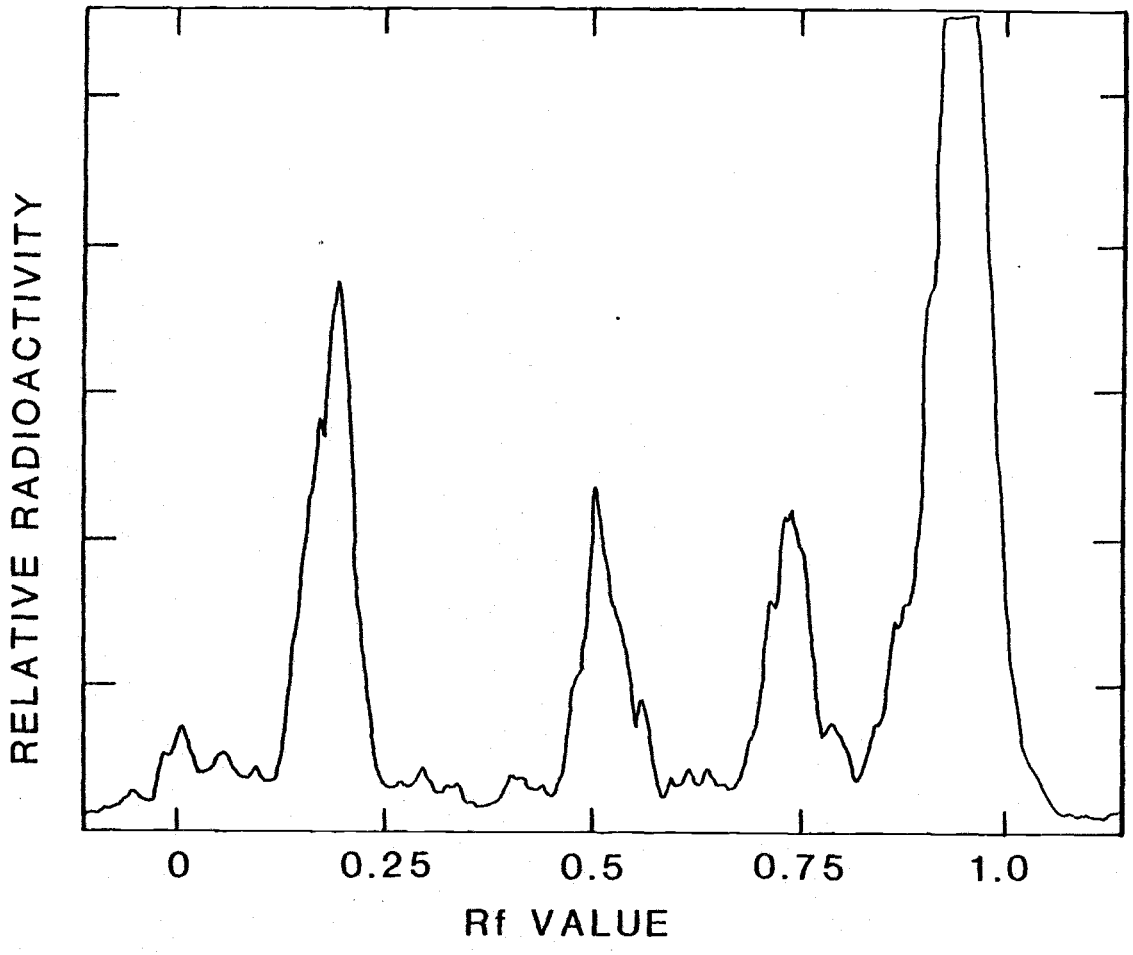

Fig. 22

Metabolism of [^{14}C]MTA in cell-free extracts. [U-^{14}C-adenosine]MTA was synthesized to determine if the ribose carbons could become incorporated into the 2-aminobutyrate portion of methionine. A double labeling experiment was performed using [C$^{3}H_3$]MTA and [U-^{14}C-adenosine]MTA as substrate ($^{3}H/^{14}C$ ratio = 0.925). Methionine was isolated from the reaction mixture by elution from a Dowex 50W-X4 column with 2 N NH_4OH followed by chromatography on paper in solvent system 8. The methionine eluted from the paper had a $^{3}H/^{14}C$ ratio of 2.08, corresponding to approximately 4.5 carbons of the ribose recycled into methionine. It appears, therefore, that the formation of methionine from MTA in S. cerevisiae apparently involves modification of the ribose portion of MTA to form the carbon chain of the amino acid.

To study the intermediate steps involved in the synthesis of methionine, [$^{14}CH_3$]MTA was incubated with cell-free extracts of strain 2218A grown in 1.0 mM MTA as a source of both methionine and adenine. Labeled products were separated by paper chromatography in solvent system 8 (Fig. 23). Figure 23A shows the migration of substrate [$^{14}CH_3$]MTA after 0 min of incubation. After 120 min of incubation (Fig. 23B), paper chromatographic separation revealed six peaks. For convenience, these have been designated peaks 1 thru 6 in order of increasing Rf value.

Fig. 23. Separation of the products from the enzymatic reaction of [$^{14}CH_3$]MTA with cell-free extracts by paper chromatography. The assay mixture described in Materials and Methods was incubated with an extract of strain 2218A at 37°C for A, 0 min, or B, 120 min. The reaction was stopped by heating at 100°C for 1 min. Each sample was applied to the paper and the chromatogram was developed in solvent system 8.

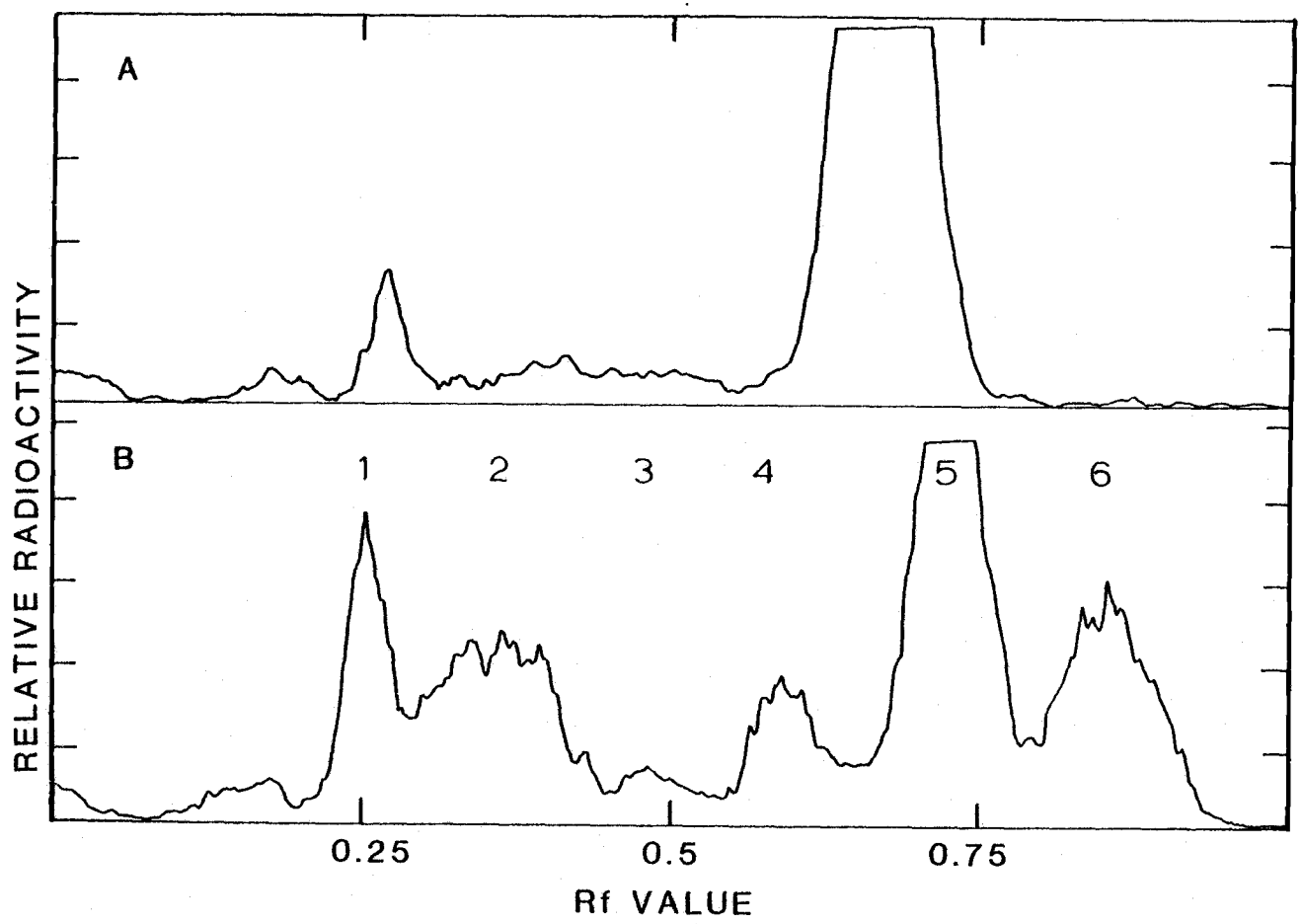

Fig. 23

Peak 1 co-migrated with standard 5-methylthioribose-1-phosphate. The labeled material from peak 1 was eluted from the chromatogram and incubated with alkaline phosphatase (see Materials and Methods). Subsequent chromatography of the reaction mixture resulted in the appearance of a product which co-migrated with standard 5-methylthioribose, the expected product if a phosphate group had been removed from 5-methylthioribose-1-phosphate by alkaline phosphatase.

Peak 2 could not be identified, none of the standard compounds tested co-migrated with this labeled product. In some reaction mixtures, a double peak appeared in the region of peak 2. This observation, along with the broadness of peak 2 in Figure 23, suggested that peak 2 may have been composed of two compounds (compounds 2A and 2B).

Product 3 was identified as methionine by chromatography on thin layer silica gel plates developed in solvent system 6. Furthermore, dansylation of product 3 followed by high pressure liquid chromatography resulted in the radioactive peak co-migrating with reference dansylated methionine.

Product 4 migrated to the same Rf value as standard 5'-methylthioinosine. Deamination of MTA has only been reported in Aspergillus oryzae (68). To further demonstrate that deamination of MTA may be possible in S. cerevisiae, product 4 was eluted from the paper and reisolated by high pressure liquid chromatography. The

radioactive product co-migrated with authentic 5'-methylthioinosine (Fig. 24), demonstrating that deamination of MTA had occurred.

Peak 5 was composed of 2-keto-4-methylthiobutyric acid, 5-methylthioribose, and an unidentified product, designated compound 5A. The products were isolated by development in chromatography systems 3 and 7 following separation of ribose from non-ribose containing compounds in the reaction mixture (Fig. 23) on a boronate column designed to bind cis-diol compounds. Only trace amounts of MTA remained after 120 min of incubation as determined by high pressure liquid chromatography.

The components of peak 6 were eluted from the chromatogram (Fig. 23) and re-chromatographed in systems 3 and 6. Three compounds which co-migrated with the following compounds were identified: 2-hydroxy-4-methylthiobutyric acid, 3-methylthiopropionaldehyde and 3-methylthiopropionic acid.

In all, eleven products of MTA metabolism were isolated. The labeled products were: 5-methylthioribose-1-phosphate, methionine, 5'-methylthioinosine, 2-keto-4-methylthiobutyric acid, 2-hydroxy-4-methylthiobutyric acid, 5-methylthioribose, 3-methylthiopropionaldehyde, 3-methylthiopropionic acid, and three unidentified products designated compounds 2A, 2B, and 5A. It was therefore, necessary to break down the individual components of MTA metabolism and study their respective products of metabolism in order to define a reaction sequence.

Fig. 24. Profile of UV absorbing (254 nm) compounds eluted from reverse phase high pressure liquid chromatography column. The reaction product was isolated by development in chromatography system 8 (peak 4, see Fig. 23), eluted with H_2O, and an aliquot analyzed by high pressure liquid chromatography. An aliquot was applied to the column A, alone or B, spiked with standard 5'-methylthioinosine.

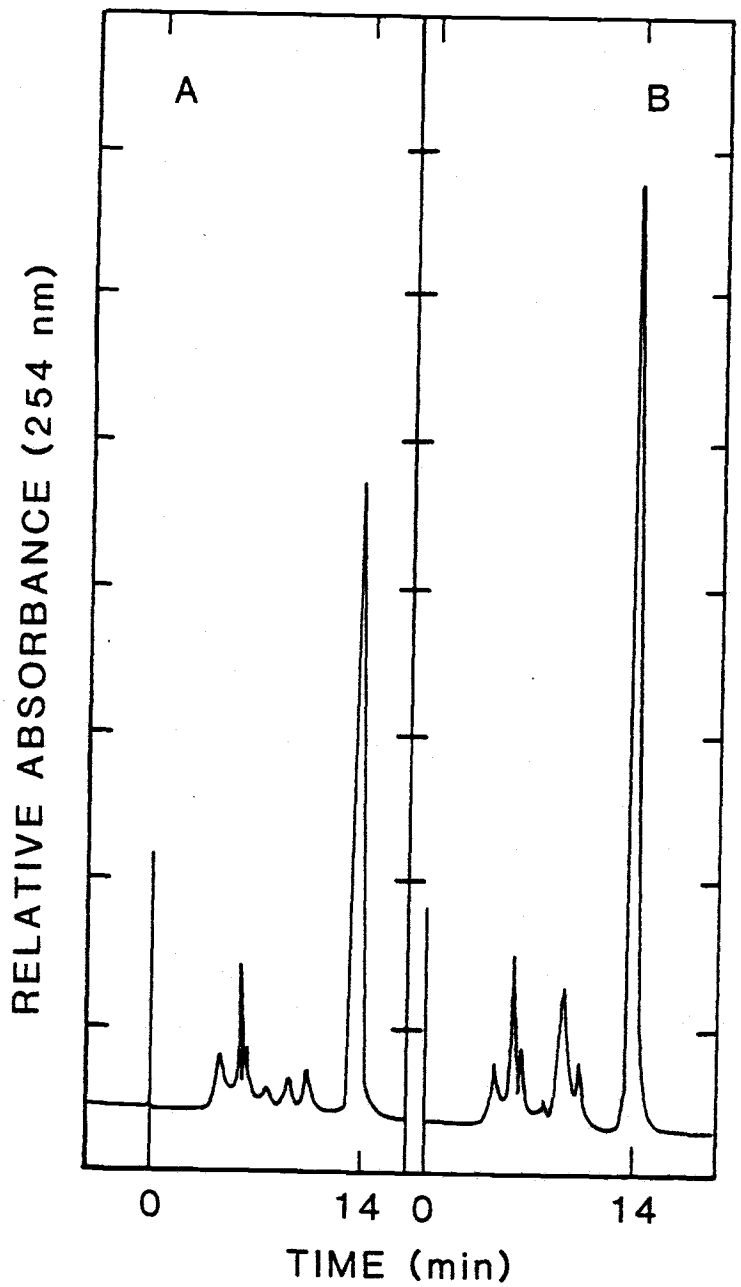

Fig. 24

Metabolism of 2-keto-4-[$^{14}CH_3$]methylthiobutyric acid in cell-free extracts. To investigate the nature of these reactions, 2-keto-4-[$^{14}CH_3$]methylthiobutyric acid was synthesized and incubated with the reaction mixture described above. Paper and thin layer chromatography of the reaction mixture (systems 3, 6, and 8) resulted in the identification of methionine, 2-hydroxy-4-methylthiobutyric acid, 3-methylthioproprionic acid, and 3-methylthioproprionaldehyde, as products (Fig. 25). Incubation of [$^{14}CH_3$]methionine with cell extracts indicated that the reverse reaction was not operative *in vitro*; no degradation was seen. The data indicate that 2-keto-4-methylthiobutyric acid is a precursor of methionine and that the reverse reaction is inoperative *in vitro*. Incubation of 2-hydroxy-4-methylthiobutyric acid, 3-methylthiopropionic acid, or 3-methylthiopropionaldehyde with extracts from strain 2218A resulted in little or no production of methionine suggesting that these three compounds probably resulted from the degradation of 2-keto-4-methylthiobutyric acid and were not intermediates in the pathway from 2-keto-4-methylthiobutyric acid to methionine.

Metabolism of [$^{14}CH_3$]methylthioribose-1-phosphate in cell-free extracts. That 5-methylthioribose-1-phosphate is an intermediate in the biosynthesis of methionine has been demonstrated in *Enterobacter aerogenes* (130) and in rat liver (160). To determine if this was the case for *S. cerevisiae*, extracts of strain 2218A were prepared as for our earlier studies with [$^{14}CH_3$]MTA. Enzymatically synthesized [$^{14}CH_3$]methylthioribose-1-phosphate was isolated and incubated with

Fig. 25. Separation of the products from the enzymatic reaction of 2-keto-4-[$^{14}CH_3$]methylthiobutyrate with cell-free extracts by paper chromatography. The assay mixture as described in Materials and Methods was incubated with an extract of strain 2218A at 37°C for A, 0 min or B, 120 min before stopping the reaction by heating at 100°C for 1 min. Each sample was applied to Whatman 3 M paper and developed in solvent system 8. Numerals refer to authentic compounds which co-migrated with the labeled compounds represented by each peak. The compounds are: 1, methionine; 2, 2-keto-4-methylthiobutyric acid; 3, 3-methylthiopropionic acid; 4, 2-hydroxy-4-methylthiobutyric acid; and 5, 3-methylthiopropionaldehyde.

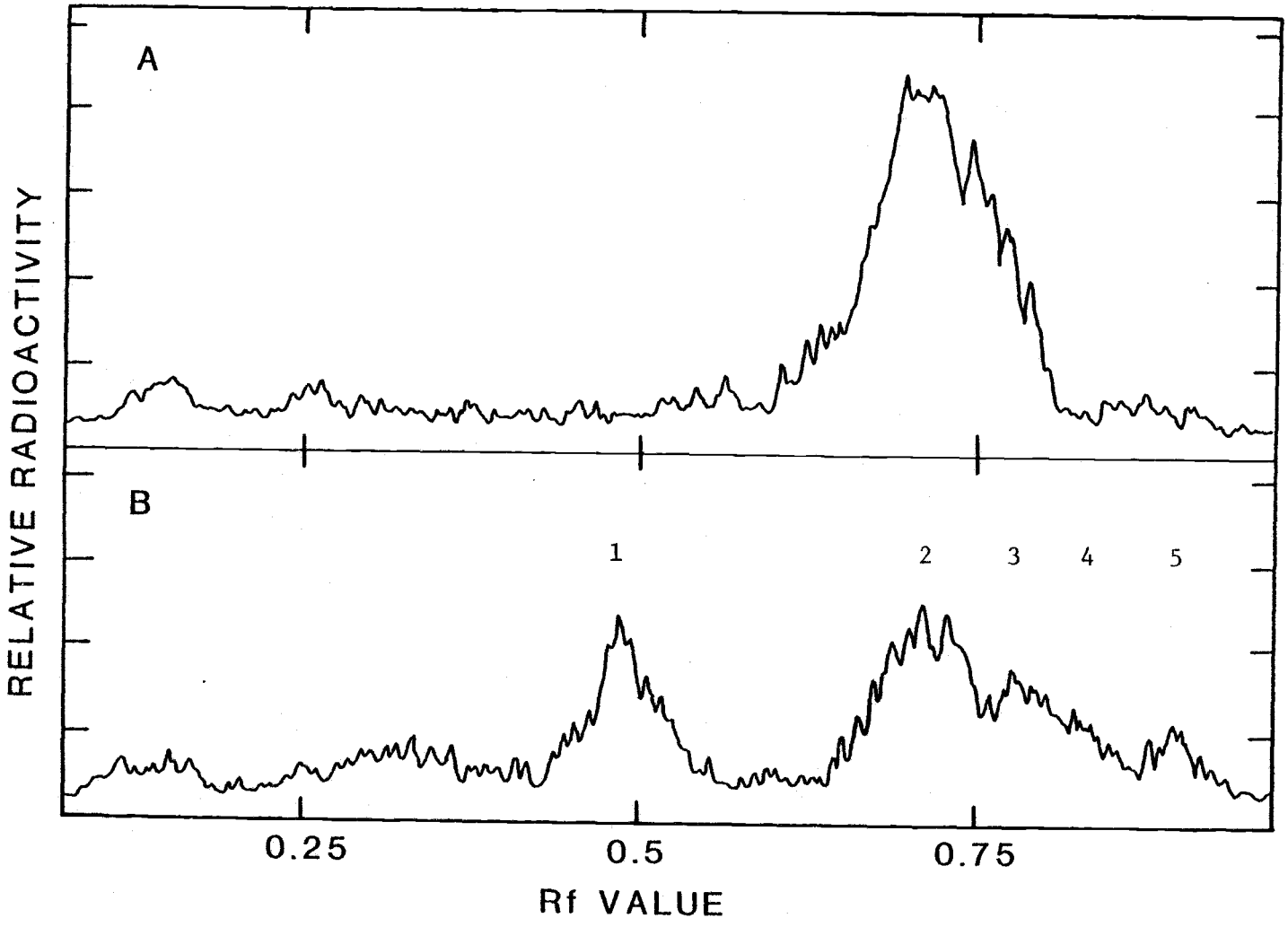

Fig. 25

the extracts and the products separated by paper and thin layer chromatography in solvent systems 6 and 8. After 120 min of incubation, the labeled products identifiable were compounds 2A and 2B, 5-methylthioribose, 2-keto-4-methylthiobutyric acid, 2-hydroxy-4-methylthiobutyric acid, 3-methylthiopropionic acid, 3-methylthiopropionaldehyde and a small amount of methionine. Product 2 and 5-methylthioribose were the first to appear in reaction mixtures (by 15 minutes). A significant amount of 2-keto-4-methiolbutyric acid did not appear until 60 minutes into the reaction (Fig. 26). Further evidence that 5-methylthioribose and 2-keto-4-methylthiobutyric acid were products in this reaction was verified by the following procedure: a reaction mixture was applied to a silica gel thin layer plate and sprayed with a borate spray designed to bind cis-diols to the plate, preventing their migration. Development in system six resulted in a greater than 95% reduction in the amount of label in the 5-methylthioribose peak migrating to the same Rf value that 5-methylthioribose had prior to treatment. The 2-keto-4-methylthiobutyric acid peak remained the same as did the peak corresponding to compounds 2A and 2B. This also suggested that compound 2 was not a cis-diol containing compound.

5-Methylthioribose does not appear to be an intermediate in the biosynthesis of methionine. [$^{14}CH_3$]methylthioribose was incubated with extracts from strain 2218A under these same conditions. Chromatography in systems 3 and 8 revealed only one product of 5-methylthioribose metabolism. This product is believed to be the

Fig. 26. Separation of the products from the enzymatic reaction of [$^{14}CH_3$]Methylthioribose-1-phosphate with cell-free extracts by paper chromatography. The assay mixture as described in Materials and Methods was incubated with an extract from strain 2218A at 37°C for A, 0 min or B, 120 min before stopping the reaction by heating at 100°C for 1 min. Each sample was then applied to silica gel thin layer plates and developed in solvent system 6. Numerals refer to authentic compounds which co-migrated with the labeled compounds represented by each peak. The compounds are: 1, 5-methylthioribose-1-phosphate; 2, compounds 2A and 2B; 3, 5-methylthioribose; and 4, 2-keto-4-methylthiobutyric acid.

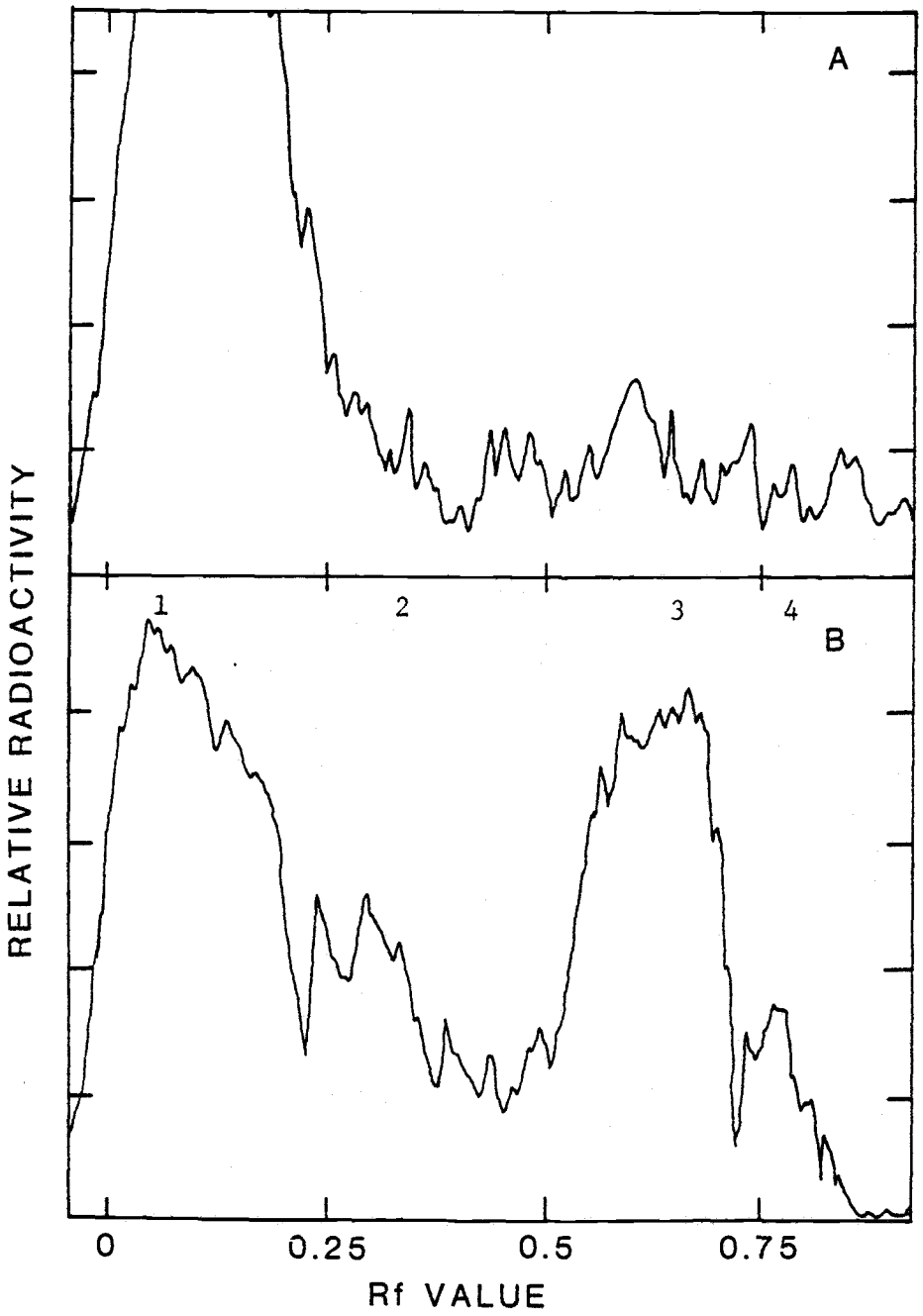

Fig. 26

same as product 5A described earlier. 5-Methylthioribose is apparently metabolized via another pathway, different from that involved in methionine biosynthesis. Furthermore, 5-methylthioribose was unable to fulfill the requirement for methionine in cultures of strain 2218A. However, strain 2218A also requires adenine for growth, a condition which prevented utilization of MTA as a sole source of methionine in this strain (Fig. 27). In order to circumvent the possibility that adenine may be exerting an inhibitory effect on the ability of 5-methylthioribose to be utilized as a source of methionine, a strain which was not auxotrophic for adenine was used. Growth of this strain, 2278C, was minimal (less than 20% as compared to cells grown on MTA or methionine) when supplied with exogenous 5-methylthioribose (1 mM) as a source of methionine (not shown). Both strain 2218A (Fig. 27) and strain 2278C (not shown) grew nearly as well with 2-keto-4-methylthiobutyric acid and 2-hydroxy-4-methylthiobutyric acid, as a source of methionine, as they did with methionine itself (Fig. 27). Whereas cells of strain 2218A incubated in the presence of 3-methylthioproprionaldehyde did not grow. This observation is consistent with the evidence suggesting that 3-methylthiopropionaldehyde is a degradation product of 2-keto-4-methylthiobutyric acid and is not involved in methionine synthesis for MTA.

The data indicate that the central pathway of methionine biosynthesis in S. cerevisiae consists of the conversion of MTA to

Fig. 27. Growth of strain 2218A in YNB medium supplemented with adenine and various sources of methionine. A 24 h culture was inoculated into fresh YNB plus 20 µg/ml adenine containing either 1.0 mM 2-keto-4-methylthiobutyrate (▲), 1.0 mM 2-hydroxy-4-methylthiobutyrate (●), 1.0 mM 3-methylthiopropionaldehyde (□), 20 µg/ml methionine (O), 1 mM 5-methylthioribose (Δ), or no supplement (■).

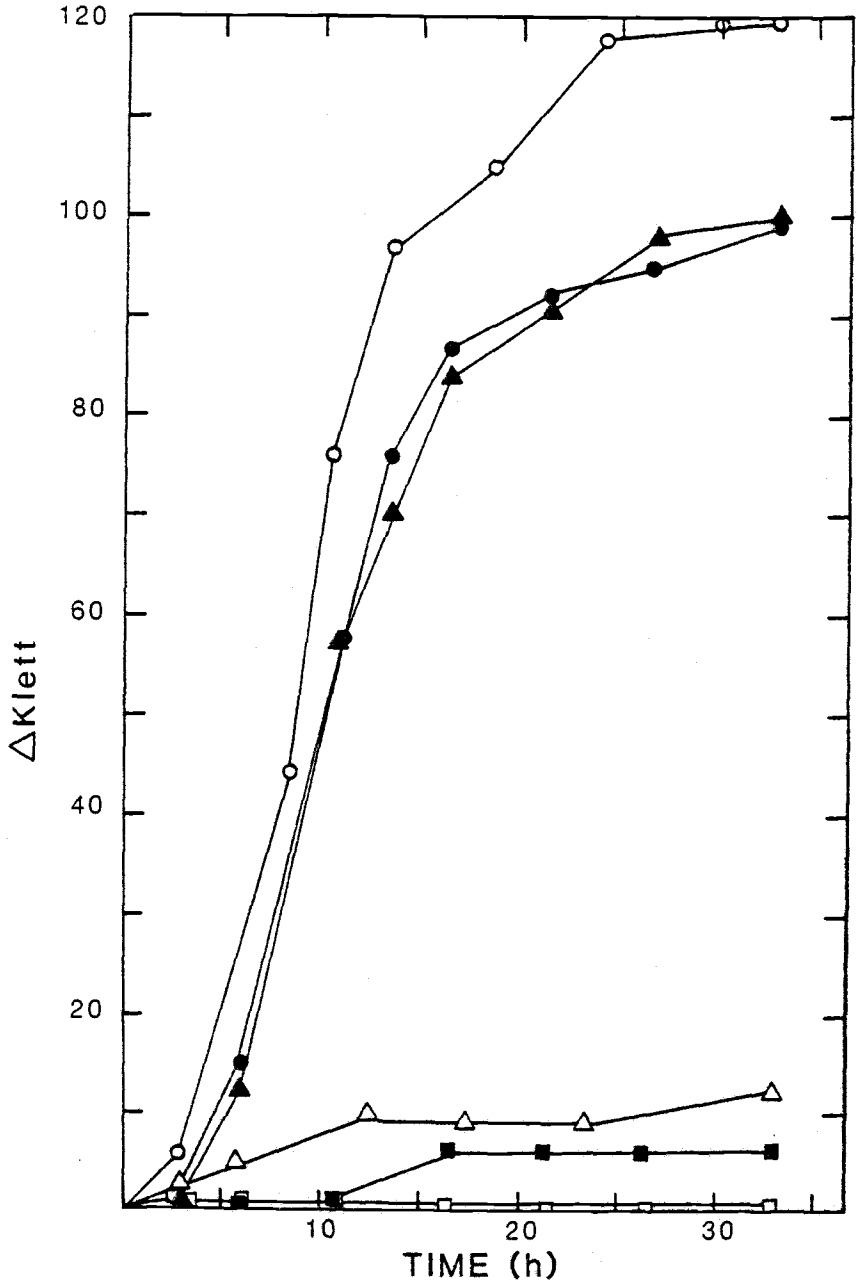

Fig. 27

5-methylthioribose-1-phosphate and subsequent conversion to 2-keto-4-methylthiobutyric acid followed by its conversion to methionine. The remaining compounds identified here appear to be the result of the degradation of the intermediates in this pathway.

DISCUSSION

Cordycepin (3'-deoxyadenosine) is an analog of adenosine that inhibits nucleic acid biosynthesis in some microorganisms (166). Saccharomyces cerevisiae is not only resistant to this antibiotic, but also cannot utilize adenosine as a purine source (167). In contrast, cordycepin sensitive mutants of S. cerevisiae have been isolated and found to grow well in medium containing adenosine as its sole source of purine (168). Yeast are normally also impermeable to MTA. We have shown, however, that the exogenous supplementation of MTA to the medium containing a cordycepin-sensitive adenine auxotroph will support the growth of this strain. MTA is transported into these cells, apparently by the same or interacting mechanism by which adenosine is transported, and half-maximal growth is achieved at an MTA concentration of approximately 30 μM.

That both MTA and SAH, as well as SAM, support the growth of the cordycepin sensitive adenine auxotroph implies that two mechanisms of purine salvage from SAM are present; one yielding adenosine, the second, adenine. Transmethylation reactions which give rise to SAH are ubiquitous and the degradation of SAH yields adenosine and homocysteine in eukaryotes (54). MTA, on the other hand, is synthesized from SAM by several different mechanisms in yeast: 1) SAM may be directly cleaved to MTA and homoserine by a SAM cleaving enzyme (SAM lyase, SAM hydrolase, or SAM splitting enzyme) (4-9); 2) one mole of MTA is produced per mole of spermidine and

two moles of MTA produced per mole of spermine (11-13); and 3) the synthesis of the unusual nucleoside Y (of yeast tRNA) also results in the production of MTA (19). The first two routes of MTA biosynthesis probably account for the majority of the MTA synthesized. The cleavage of MTA by MTA phosphorylase would thus yield free adenine and the methylthioribose moiety. In vivo, adenine may be converted by adenine phosphoribosyltransferase (APRT) to adenylate which is subsequently incorporated into nucleic acid (106). Alternatively, adenine may be deaminated to hypoxanthine which is converted to IMP via hypoxanthine-guanine phosphoribosyltransferase (109). In the present study, a compound migrating to the same Rf value as hypoxanthine has been detected as a product of the metabolism of MTA in cell free extracts.

We have projected a role for MTA phosphorylase in the purine salvage system of S. cerevisiae. The apparent stimulation of MTA phosphorylase activity by exogenously supplied MTA or its inhibition by adenine further support the contention that the enzyme plays a key role in purine recycling from SAM. The data presented demonstrate the occurrence in S. cerevisiae of an enzyme catalyzing the cleavage of MTA. Unlike the Escherichia coli enzyme (95) and like the enzyme purified from mammalian sources (47-49,52), the enzyme is activated by phosphate ions. Based on several criteria, 5-methylthioribose-1-phosphate and adenine were identified as the products of the enzyme reaction. That the yeast MTA phosphorylase more closely resembles the mammalian enzyme in contrast to the E. coli nucleosidase is

also exemplified by the enzyme's molecular weight. In this study, the yeast enzyme has been calculated to possess a molecular weight of approximately 90,000 daltons; a value similar to the values obtained from human tissue (49,52), rat tissue (48), and the thermophilic bacterium Caldariella acidophila (51). Ferro et al. (45) reported a molecular weight of 31,000 daltons for the nucleosidase purified from E. coli (45) and Cacciapuoti et al. (52) have suggested that the association of three monomers of such molecular weight can be postulated for the human placental enzyme. Interestingly, Guranowski, et al. (44) recently found the MTA nucleosidase from Lupinus luteus seeds consists of two polypeptide chains of identical molecular weight (31,000 D). Even in the absence of detailed studies, it is tempting to note that these similarities appear to suggest a phylogenetic relationship between these enzymes from various biological sources.

The apparent Km for MTA from the yeast enzyme (18.7 μM) also differs from the E. coli enzyme (0.31 μM, ref. 45), and is again, more similar to the Km values reported for MTA from higher organisms (4-60 μM, refs. 47-49,52). A maximum reaction velocity for the yeast enzyme was obtained at 58°C, yet the assay for enzyme activity was routinely performed at 47°C. At temperatures above 47°C the enzyme was very labile, a characteristic similar to that observed for the human placental enzyme (52).

That the yeast MTA phosphorylase lacks rigid specificity for the 5'-position of the substrate is indicated by the ability of the

enzyme to cleave a variety of 5'-modified 5'-methylthioadenosine analogs. Of special interest is 5'-isobutylthioadenosine, a powerful antiproliferative drug shown to inhibit the growth of transformed mouse mammary cells (89), cell transformation induced by oncogenic RNA or DNA viruses (81,82), mitogen-induced lymphocyte blastogenesis (90), and the capping of herpes virus mRNA (171). 5'-Isobutylthioadenosine (SIBA) was originally synthesized as an analog of S-adenosylhomocysteine (165). Zappia and co-workers (72) however, have suggested that SIBA more closely resembles MTA and found that human placenta 5'-methylthioadenosine phosphorylase utilized SIBA as a substrate. Our data is consistent with the contention that SIBA is an analog of MTA.

Catabolism of MTA by MTA phosphorylase appears to be the major, if not sole, source of free adenine in S. cerevisiae; the adenine formed enters the purine nucleotide pool (110). Less is known of the fate of 5-methylthioribose-1-phosphate. Evidence is presented for the effective salvage and recycling of the 5-methylthioribose moiety of MTA in the biosynthesis of methionine. The primary step in this pathway is the phosphorylytic cleavage of MTA to yield 5-methylthioribose-1-phosphate and adenine. The final step in the biosynthesis of methionine is the conversion of 2-keto-4-methylthiobutyric acid to methionine by a transamination reaction. The data suggest at least one intermediate step is involved in the conversion of 5-methylthioribose-1-phosphate to 2-keto-4-methylthiobutyric acid; a non-ribose containing compound was isolated as a catabolic product

of 5-methylthioribose-1-phosphate but has not, as yet, been identified. A low yield of methionine produced from MTA *in vitro* may be attributed to the rapid dissimilation of the reactive intermediates by branch pathways not associated with the synthesis of methionine which do, however, have important implications for sulfur metabolism in *S. cerevisiae*.

Several products of MTA metabolism not directly associated with the methionine biosynthetic pathway were identified (Fig. 28). MTA may be deaminated to form 5'methylthioinosine; the fate of which has not been determined here. The identification of 5'-methylthioinosine represents the first observation of this compound in *S. cerevisiae*; it had previously been isolated only from *Aspergillus* (68). The data of Lawrence and co-workers (69-71) and Carteni-Farena *et al.* (72) showed that deamination of an analog of MTA, 5'-isobutylthioadenosine (SIBA) does occur in rat and chick extracts, respectively. This suggests that MTA may also be deaminated in these systems.

The catabolism of 5-methylthioribose-1-phosphate was also studied. The major product was 5-methylthioribose. 5-Methylthioribose however was not isolated as a product of MTA metabolism *in vivo*. Furthermore, incubation of 5-methylthioribose with cell-free extracts did not result in the production of methionine or any known precursors of methionine. A metabolite of 5-methylthioribose was isolated but not identified. It is possible 5-methylthioribose arises *in vitro* through the action of phosphatases that normally

Fig. 28. Proposed metabolic pathway for the catabolism of 5'-methylthioadenosine in S. cerevisiae.

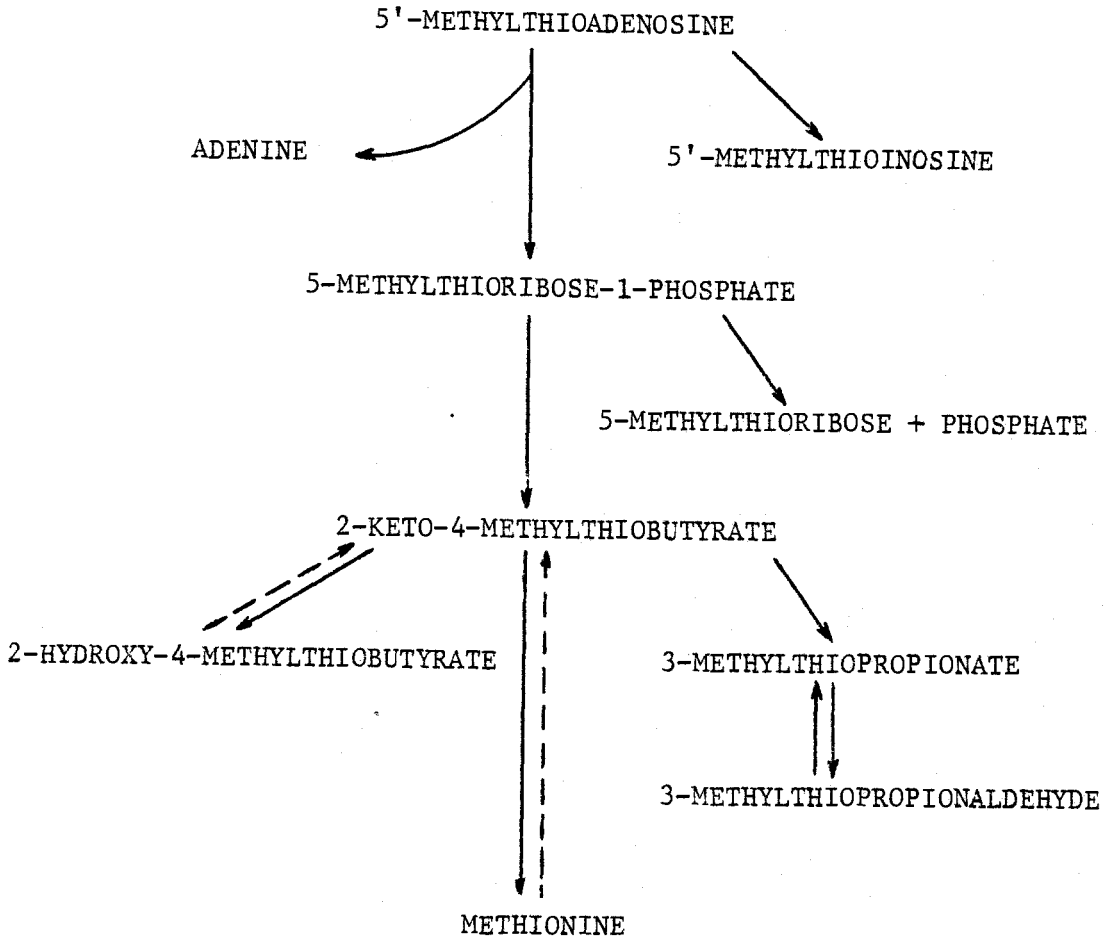
5'-METHYLTHIOADENOSINE
ADENINE
5'-METHYLTHIOINOSINE
5-METHYLTHIORIBOSE-1-PHOSPHATE
5-METHYLTHIORIBOSE + PHOSPHATE
2-KETO-4-METHYLTHIOBUTYRATE
2-HYDROXY-4-METHYLTHIOBUTYRATE
3-METHYLTHIOPROPIONATE
3-METHYLTHIOPROPIONALDEHYDE
METHIONINE

Fig. 28

are not available to act on 5-methylthioribose-1-phosphate *in vivo*.

Several catabolites of 2-keto-4-methylthiobutyric acid were isolated. Among these, the hydroxy analog of methionine, 2-hydroxy-4-methylthiobutyric acid, was the only one capable of satisfying the methionine auxotrophic requirement in these cells. This result is in accord with the data presented by Robinson *et al.* (138) and Harter, *et al.* (137) who demonstrated that the keto and hydroxy analogs of methionine can fulfill the needs for dietary methionine in fingerling channel catfish and chicks, respectively.

2-Keto-4-methylthiobutyric acid is decarboxylated in rat liver extracts to form 3-methylthiopropionic acid (140), which is further degraded to form methanethiol and ultimately H_2S and CO_2. A similar mechanism may be operating in *S. cerevisiae*, although 3-methylthioproprionaldehyde may be an intermediate in the conversion of 2-keto-4-methylthiobutyric acid to 3-methylthioproprionic acid; both compounds have been isolated in this study. Aldehyde dehydrogenase, the enzyme most likely to catalyze this reaction, has been isolated from yeast in high yield (146) and does catalyze this reaction *in vitro*. It should also be noted that 3-methylthiopropionic acid, 3-methylthioproprionaldehyde, and 2-hydroxy-4-methylthiobutyric acid also have been isolated from lager beer (144,145), a fermentation product of *S. carlsburgensis*.

The pathways described represent mechanisms by which S. cerevisiae can salvage and recycle the carbons of MTA to methionine and also illustrates alternative routes of catabolism for the intermediates in this pathway.

BIBLIOGRAPHY

1. Mandel, J. A. and Dunham, E. K. Preliminary note on a purine-hexose compound. J. Biol. Chem. 11:85-86 (1912).
2. Suzuki, V., Odake, S. and Mori, T. Biochem Z. 154:278.
3. Borkakoti, N. and Palmer, R. A. Crystal structure and conformational variations of 5'-methylthioadenosine. Acta Cryst. B34:867-874 (1978).
4. Shapiro, S. K. and Mather, A. N. The enzymatic decomposition of S-adenosylmethionine. J. Biol. Chem. 233:631 (1958).
5. Mudd, S. H. Enzymatic cleavage of S-adenosylmethionine. J. Biol. Chem. 234:87 (1954).
6. Gold, M., Havsman, R. L., Maifra, V. and Hurowitz, J. The enzymatic methylation of RNA and DNA. VIII. Effects of bacteriophage infection on the activity of the methylating enzymes. Proc. Natl. Acad. Sci. 52:292 (1964).
7. Spoerel, N. and Herrlich, N. Colivirus-T3-coded S-adenosyl-methionine hydrolase. Eur. J. Bioch. 95:227-233 (1979).
8. Baxter, C. and Coscia, C. J. In vitro synthesis of spermidine in the higher plant Vinca rosea. Biochem. Biophys. Res. Commun. 54:147 (1973).
9. Swiatek, K. R., Simon, L. N. and Chao, K. L. Nicotinamide methyltransferase and S-adenosylmethionine: 5'-methylthio-adenosine hydrolase. Control of transfer ribonucleic acid methylation. Biochemistry 12(23):4670 (1977).

10. Porcelli, M., Della Ragione, F., Cacciapuoti, G. and Carteni-Farina, M. S-Adenosyl-(5')-3-methylthiopropylamine as substrate and inhibitor of S-adenosylmethionine lyase from rat liver. Boll. Soc. Ital. Biol. Sper. 56(3):245-249 (1980).

11. Janne, J., Poso, H. and Raina, A. Polyamines in rapid growth and cancer. Biochem. Biophys. Acta 473:241 (1977).

12. Tabor, C. W. and Tabor, H. 1,4-Diaminobutane (putrescine), spermidine, and spermine. Ann. Rev. of Biochem. 45:285-306 (1976).

13. Williams-Ashman, H. G. and Cannelakis, Z. N. Polyamines in mammalian biology and medicine. Perspectives in Biology and Medicine 22:421 (1979).

14. Wickner, R. B., Tabor, C. W. and Tabor H. Purification of adenosylmethionine decarboxylase from Escherichia coli w: evidence for covalently bound pyruvate. J. Biol. Chem. 245: 2132-2139

15. Cohn, M. S., Tabor, C. W. and Tabor, H. Identification of a pyruvoyl residue in S-adenosylmethionine decarboxylase from Saccharomyces cerevisiae. J. Biol. Chem. 252:8212-8216 (1977).

16. Pegg, A. E. Purification of rat liver S-adenosyl-L-methionine decarboxylase. Biochem. J. 141:581-583

17. Hibasami, H. and Pegg, A. Differential inhibition of mammalian aminopropyltransferase activities. Biochem. Biophys. Res. Commun. 81(4):1398-1405 (1978).

18. Pajula, R. L. and Raina, A. Methylthioadenosine, a potent inhibitor of spermine synthase from bovine brain. FEBS Letters 99(2):343-345 (1979).

19. Lacharme, J., Segle-Murandi, F. and Steiman, R. Metabolisme du nucleoside Y chez une souche mutante auxotrophe a la guanine (gua 2 su^{+}) de Saccharomyces cerevisiae. Comptes Rendes des Seances de la Societe de Biologie. Extract du tane 171(4):814 (1977).

20. Nishimura, S., Taya, Y., Kuchino, Y. and Ohasi, Z. Enzymatic synthesis of 3-(3-amino-3-carboxypropyl) uridine in Escherichia coli phenylalanine transfer RNA: Transfer of 3-amino-3-carboxypropyl group from S-adenosylmethionine. Biochem. Biophys. Res. Commun. 57:702 (1974).

21. Stoner, G. L. and Eisenberg, M. A. Purification and properties of 7,8-diaminopelargonic acid aminotransferase. J. Biol. Chem. 250:4029 (1975).

22. Adams, D. O. and Yang, S. F. Methionine metabolism in apple tissue. Plant Physiol. 60:892-896 (1977).

23. Boller, T., Herner, R. C. and Kerde, H. Assay for enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145:293-303 (1979).

24. Yu, Y. B., Adams, D. O. and Yang, S. F. 1-Aminocyclopropane carboxylate synthase, a key enzyme in ethylene biosynthesis. Arch. Bioch. Biophys. 198:280-286 (1979).

25. Adams, D. O. and Yang, S. F. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. 76:170-174 (1979).

26. Lieberman, M. Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. 30:533-591 (1979).

27. Yang, S. F. Regulation of ethylene biosynthesis. Hort Science 15(3):238 (1980).

28. Thomas, K. C. and Spencer, M. L-Methionine as an ethylene precursor in Saccharomyces cerevisiae. Can. J. Microbiol. 23:1669-1674 (1977).

29. Fu, P. C., Zic, V. and Ozimy, K. Studies of ethylene-forming systems in rat liver extract. Biochimica Biophysica Acta 585: 427-434 (1979).

30. Klebanoff, S. J. and Rosen, H. Ethylene formation by polymorphonuclear leukocytes: role of myeloperoxidase. J. Exp. Med. 148(2):490-506 (1978).

31. Diguiseppi, J. and Fridovich, I. Ethylene from 2-keto-4-thiomethylbutyric acid: the Haber-Weiss Reaction. Arch. Bioch. and Biophys. 205:323-329 (1980).

32. Tsan, M. F. and Chen, J. W. Oxidation of methionine by human polymorphonuclear leukocytes. J. Clinical Inves. 65(5):1041-1050 (1980).

33. Steele, R. D. and Benevenga, N. J. Identification of 3-methylthioproprionic acid as an intermediate in methionine metabolism in vitro. J. Biol. Chem. 253:7844-7850 (1978).

34. Ruiz-Herrera, J. and Starkey, R. L. Dissimilation of methionine by fungi. J. Bacteriol. 99:544-551 (1969).

35. Taya, Y., Tanaka, Y. and Noshimura, S. Cell-free biosynthesis of discadenine, a spore germination inhibitor of Dictyostelium discoideum. FEBS Letters 89:326 (1978).

36. Ihara, M., Taya, Y., Nishimura, S. Developmental regulation of cytokinin, spore germination inhibitor discadenine and related enzymes in Dictyostelium discoideum. Exp. Cell. Res. 126:273-278 (1980).

37. Robins, M. J., Hall, R. M. and Thedford, R. N^6-(Δ^2-isopentenyl) adenosine. A component of the transfer ribonucleic acid of yeast and of mammalian tissue. Methods of isolation, and characterization. Biochemistry 6:1837 (1967).

38. Rhodes, J. B. and Williams-Ashman, H. G. Med. Exp. 10:281 (1969).

39. Chu, T. M., Mallette, M. F. and Mumma, R. O. Isolation and characterization of 5'-S-methyl-5'-thioadenosine from Escherichia coli. Biochem. 7:1394-1406 (1968).

40. Dubin, D. T. and Rosenthal, S. M. The acetylation of polyamines in Escherichia coli. J. Biol. Chem. 235:776-782 (1960).

41. Ferroluzzi-Ames, G. and Ames, B. N. The multiplication of T4rII phage in E. coli K12 (λ) in the presence of polyamines. Bioch. Biophys. Res. Commun. 18:639-647 (1965).

42. Seidenfeld, J., Wilson, J. and Williams-Ashman, H. G. Ardrogenic regulation of 5'-deoxy-5'-methylthioadenosine phosphorylase activity in relation to polyamine metabolism of rat prostate. Biochem. Biophys. Res. Commun. 95(4):1861-1868 (1980).

43. Hibasami, H., Hoffman, L. and Pegg, A. E. Decarboxylated S-adenosylmethionine in mammalian cells. J. Biol. Chem. 255: 6675-6678 (1980).

44. Guranowski, A. B., Chiang, P. K. and Cantoni, G. L. 5'-Methylthioadenosine nucleosidase purification and characterization of the enzyme from Lupinus luteus seeds. Eur. J. Bioch. 114:293-299 (1981).

45. Ferro, A. J., Barret, A. and Shapiro, S. K. Kinetic properties and the effect of substrate analogues on 5'-methylthioadenosine nucleosidase from Escherichia coli. Biochim. Biophys. Acta 438:487-494 (1976).

46. Duerre, J. A. A hydrolytic nucleosidase acting on S-adenosyl-homocysteine and on 5'-methylthioadenosine. J. Biol. Chem. 237:3737-3741 (1962).

47. Pegg, A. E. and Williams-Ashman, H. G. Phosphate-stimulated breakdown of 5'-methylthioadenosine by rat ventral prostate. Biochem. J. 115:241-247 (1969).

48. Garbers, David L. Demonstration of 5'-methylthioadenosine phosphorylase activity in various rat tissues. Biochim. Biophys. Acta 523:82-93 (1978).

49. Zappia, V., Oliva, A., Cacciapuoti, G., Galletti, P., Mignucci, G. and Carteni-Farina, M. Substrate specificity of 5'-methylthioadenosine phosphorylase from human prostrate. Biochem. J. 177:77-88 (1978).

50. Shugart, L., Tancer, M. and Moore, J. Methylthioadenosine nucleoside activity in drosophilla melanogaster. Int. J. Biochem. 10:901-904 (1980).

51. Carteni-Farina, M., Oliva, A., Romeo, G., Napolitano, G., DeRosa, M., Gambacorta, A. and Zappia, V. 5'-Methylthioadenosine phosphorylase from Caldariella acidophila. Eur. J. Biochem. 101:317-324 (1979).

52. Cacciapuoti, G., Oliva, A. and Zappia, V. Studies on phosphate-activated 5'-methylthioadenosine nucleosidase from human placenta. Int. J. Biochem. 9:35-41 (1978).

53. Shapiro, S. K. and Mather, A. N. The enzymatic cleavage and decomposition of S-adenosylmethionine. J. Biol. Chem. 233: 631-633 (1958).

54. Walker, R. D. and Duerre, J. A. S-Adenosylhomocysteine metabolism in various species. Can. J. Biochem. 53:312-319 (1975).

55. Adams, D. O., Wang, S. H. and Lieberman, M. Control of ethylene production in apple cell suspension cultures. Plant Physiol. 65(6 sup):41 (1980).

56. Ferro, A. J., Wrobel, N. C. and Nicolette, J. A. 5-Methylthioribose-1-phosphate: A product of partially purified rat liver 5'-methylthioadenosine phosphorylase activity. Biochim. Biophys. Acta 570:65-73 (1979).

57. Savarese, T. M., Crabtree, G. W. and Parks, R. E. 5'-Methylthioadenosine phosphorylase-I substrate activity of 5'-deoxyadenosine with the enzyme from sarcoma 180 cells. Bioch. Pharm. 30:189-199 (1981).

58. Kar, N. C. and Pearson, C. M. Methylthioadenosine nucleosidase in normal and distrophic human muscle. Clinica Chimica Acta 108:465-468 (1980).

59. Nicolette, J. A., Wrobel, N. C. and Ferro, A. J. The stimulatory effect of testosterone proprionate an 17β estradiol on 5'-methylthioadenosine phosphorylase activity in rat target tissues. Bioch. Biophys. Acta 627:190-198 (1980).

60. Savarese, T. M., Chu, M. Y., Chu, S. H., Abrams, H. and Parks, R. E. Methylthioadenosine phosphorylase as a chemotherapeutic target enzyme. Proc. Am. Assoc. Cancer Res. 21 (1980).

61. Toohey, J. F. Methylthioadenosine nucleoside phosphorylase deficiency in methylthio-dependent cancer cells. Biochem. Biophys. Res. Commun. 83(1):27-35 (1978).

62. Kamatani, N. and Carson, D. A. Abnormal regulation of methylthioadenosine and polyamine metabolism in methylthioadenosine phosphorylase-deficient human leukemic cell lines. Cancer Res. 40:4178-4182 (1980).

63. Kamatani, N., Nelson-Rees, W. A. and Carson, D. A. Selective killing of human malignant cell lines deficient in methylthioadenosine phosphorylase, a purine metabolic enzyme. Proc. Natl. Acad. Sci. 78(2):1219-1223 (1981).

64. Schroeder, H. R., Barnes, C. J., Rohnski, R. C. and Mallette, M. F. Biological production of 5-methylthioribose. Can. J. Microbiol. 19:1347-1354 (1973).

65. Ferro, A. J., Barrett, A. and Shapiro, S. K. 5-Methylthioribose kinase - A new enzyme involved in the formation of methionine from 5'-methylthioadenosine. J. Biol. Chem. 253:6021-6025 (1978).

66. Ferro, A. J. and Marchitto, K. M. Methods in Enzymol.. 82 (1982).

67. Kushad, M. M., Marchitto, K. S. and Ferro, A. J. The role of 5-methylthioribose kinase in methionine metabolism in avocado and pear tissue. In preparation.

68. Schlenk, F., Zydek-Cwick, C. R. and Hutson, N. K. Enzymatic deamination of adenosine sulfur compounds. Arch. Bioch. Biophys. 142:144-149 (1971).

69. Lawrence, F., Chermann, J. C. and Robert-Gero, M. Metabolism of 5'-deoxy-5'[^{35}S]-iso-butylthio-adenosine (SIBA) in rats and mice. Biochemical Pharmacology 29:1963-1965 (1980).

70. Lawrence, F., Richou, M. and Robert-Gero, M. Simultaneous decrease in deamination of 5'-adenylic acid and 5'-deoxy-5'-S-isobutylthioadenosine in chick-embryo fibroblasts infected by Rous Sarcoma virus. Eur. J. Biochem. 107:467-473 (1980).

71. Lawrence, F., Richou, M., Vedel, M., Farrugia, G., Blanchard, P. and Robert-Gero, M. Identification of some metabolic products of 5'-deoxy-5'-S-isobutylthioadenosine, an inhibitor of virus-induced cell transformation. Eur. J. Biochem. 87:257-263 (1978).

72. Carteni-Farina, M., Della Rayione, F., Ragostar, G., Oliva, A. and Zappia, V. Studies on the metabolism of 5'-isobutyl-thioadenosine (SIBA). FEBS Letters 104(2):266-270 (1979).

73. Williams-Ashman, H. G. Trends in the biochemical pharmacology of 5'-Deoxy-5'-methylthioadenosine. Biochem. Pharm. 31(3):277-288 (1982).

74. Zappia, V., Zydek-Cwick, R. and Schlenk, F. Specificity of S-adenosylmethionine derivatives in methyl transfer reactions. J. Biol. Chem. 244:4499 (1969).

75. Linn, S., Eskin, B., Lautenberger, J. A., Lackey, D. and Kimball, M. Host-controlled modification and restriction enzymes of Escherichia coli B and the role of adenosylmethionine in the biochemistry of S-adenosylmethionine (Borek, Zappia, Salvatore, Williams-Ashman, Schlenk, eds.) pp 521-535 (1977) Columbia Univ. Press.

76. Cassellas, P. and Jeanteur, P. Protein methylation in animal cells. II. Inhibition of S-adenosyl-L-methionine: protein (arginine) N-methyltransferase by analogs of S-adenosyl-L-homocysteine. Biochim. Biophys. Acta 519:255-268 (1978).

77. Vedel, M., Robert-Gero, M., Legraverend, M., Lawrence, R. and Lederer, E. Inhibition of tRNA methylation in vitro and in whole cells by an oncostatic S-adenosyl-homocysteine (SAH) analogue: 5'-deoxy-5'-S-isobutyladenosine (SIBA). Nucl. Acids Res. 5(8):2979-2989 (1978).

78. Oliva, A., Galletti, P., Zappia, V., Paik, W. K. and Kim, S. Studies on substrate specificity of S-adenosyl-methionine: protein-carboxyl methyltransferase from calf brain. Eur. J. Biochem. 104(2):595-602 (1980).

79. Law, R. E., Sinibaldi, R. M., Cummings, M. R. and Ferro, A. J. Inhibition of RNA synthesis in salivary glands of Drosophilla melanogaster by 5'-methylthioadenosine. Biochem. Biophys. Res. Commun. 73(3):600-606 (1976).

80. Law, R. E., Sinibaldi, R. M., Ferro, A. J. and Cummings, M. R. Effect of 5'-methylthioadenosine on gene action during heat shock in Drosophilla melanogaster. FEBS Letters 99(2):247-250 (1979).

81. Bodner, A. J., Cantoni, G. L. and Chiang, P. K. Anti-viral activity of 3-Deazaadenosine and 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-siba). Bioch. Biophys. Res. Commun. 98(2):476-481 (1981).

82. Raies, A., Lawrence, F. and Robert-Gero, M. Effect of 5'-deoxy-5'-S-isobutyl-adenosine on polyoma virus replication. FEBS Letters 72(1):48-52 (1976).

83. Trager, W., Robert-Gero, M. and Lederer, E. Antimalarial activity of S-isobutyl adenosine against Plasmodium falciparum in culture. FEBS Letters 85(2):264-266 (1978).

84. Pierre, A. and Robert-Gero, M. Inhibition of nucleoside and sugar transport into cells by an oncostatic methylase inhibitor 5'-deoxy-5'-S-isobutylthioadenosine (SIBA). FEBS Letters 101(2):233-238.

85. Vandenbark, A. A., Ferro, A. J. and Barney, C. L. Inhibition of lymphocyte transformation by a naturally occurring metabolite: 5'-methylthioadenosine. Cell. Immunol. 49:26-33 (1980).

86. Ferro, A. J., Vandenbark, A. A. and Marchitto, K. S. The role of 5'-methylthioadenosine phosphorylase in 5'-methylthio-adenosine-mediated inhibition of lymphocyte transformation. Biochim. Biophys. Acta 588:294-301 (1979).

87. Ferro, A. J. Function and metabolism of 5'-methylthioadenosine. in Transmethylation, Usdin, Borchardt, Crevelin, eds. Elsevier N. Holland (1979).

88. Wolford, R. W., MacDonald, M. R., Zehfus, B., Rogers, T. J. and Ferro, A. J. Effect of 5'-methylthioadenosine and its analogs on murine lymphoid cell proliferation. Cancer Res. 41:3035-3039 (1981).

89. Terrioux, C., Crepin, M., Gros, F., Robert-Gero, M. and Lederer, E. Effect of 5'-deoxy-5'-S-isobutyladenosine (SIBA) on mouse mammary tumor cells and on the expression of mouse mammary tumor virus. Biochem. Biophys. Res. Commun. (1978).

90. Bona, C., Robert-Gero, M., Lederer, E. Inhibition of mitogen induced blastogenesis by 5'-deoxy-5'-S-iso-butyl adenosine. Biochem. Biophys. Res. Commun. 70(2):622 (1976).

91. Robert-Gero, M., Lawrence, F., Farrugia, G., Berneman, A., Blanchard, P., Vigier, P. and Lederer, E. Inhibition of virus-induced cell transformation by synthetic analogues of S-adenosylhomocysteine. Biochem. Biophys. Res. Commun. 65: 1242-1249 (1975).

92. White, M. W., Vandenbark, A. A., Barney, C. L. and Ferro, A. J. Structural analogs of 5'-methylthioadenosine as substrates and inhibitors of 5'-methylthioadenosine phosphorylase and as inhibitors of human lymphocyte transformation. Biochem. Pharm. 31(4):503-508 (1982).

93. Pegg, A. E., Borchardt, R. T. and Coward, J. K. Effects of inhibitors of spermidine and spermine synthesis on pilyamine concentration and growth of transformed mouse fibroblasts. Biochem. J. 194(1):79-90 (1981).

94. Ferro, A. J., Vandenbark, A. A. and MacDonald, M. R. Inactivation of S-adenosylhomocysteine hydrolase by 5'-deoxy-5'-methylthioadenosine. Bioch. Biophys. Res. Commun. 100(2):523-531 (1981).

95. Chiang, P. K., Richards, H. H. and Cantoni, G. L. S-Adenosyl-L-homocysteine hydrolase: Analogues of S-adenosyl-L-homocysteine as potential inhibitors. Molecular Pharmacology 13:939-947 (1977).

96. Mills, D. C. B. and Macfarlane, D. E. in Platelets in Biology and Pathology (Ed. J. I. Gordon) p. 159 North-Holland, Amsterdam, 1975.

97. Smith, J. B. and Macfarlane, D. E. in The Prostaglandins vol. 2 (Ed. P. W. Ramwell) p. 293 Plenum Press, New York, 1974.

98. Salzman, E. W. Cyclic AMP and platelet function. New England J. Med. 286:358 (1972).

99. Agarwahl, K. C., Logreco, R. G. and Parks, R. E. Blockade of AMP effect on human platelet aggregation by a natural nucleoside, 5'-deoxy-5'-methylthioadenosine. Fed. Proceeds. 40(6):1729 (1981).

100. Agarwahl, K. C. and Parks, R. E. 5'-methylthioadenosine and 2',5'-dideoxyadenosine blockade of the inhibitory effects of adenosine on ADP-induced platelet aggregation of different mechanisms. Bioch. Pharm. 29:2529-2532 (1980).

101. Zimmerman, P., Schmitges, C. J., Wolberg, G., Duncan, G. S. and Deeprose, R. D. Immunosuppressive effects of 5'-deoxy-5'-S-methylthioadenosine (MTA), 5'-deoxy-5'-S-isobutyl-thioadenosine (SIBA) and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA): Inhibition of cyclic AMP phosphodiesterase (PDE). Proc. Am. Assoc. Cancer Res. 22 (1981).

102. Zimmerman, T. P., Schmitges, C. J., Wolberg, G. Deeprose, R. D. and Duncan, G. S. Inhibition of cyclic AMP phosphodiesterase by 5'-deoxy-5'-isobutylthioadenosine at biologically active concentrations of the drug. Life Sciences 28:647-652 (1981).

103. Schorderel-Slatkine, S., Hepicard, J., Hanoune, J. and Baulieu, E. Effect of a methyltransferase inhibitor, 5'-deoxy-5'-S-isobutylthioadenosine (SIBA) on cAMP level and progesterone induced meiosis reinitiation in Xenopus laevis docytes. Bioch. Biophys. Res. Commun. 100(2):544-550 ( ).

104. Zimmerman, T. P., Schmitges, C. J., Wolberg, G., Duncan, G. S., Cuatrecesas, P. and Elion, G. B. Modulation of cyclic AMP metabolism by S-adenosylhomocysteine and S-3-deazaadenosyl-homocysteine in mouse lymphocytes. Proc. Natl. Acad. Sci. 77:5639-5645 (1980).

105. Savarese, T. M., Crabtree, G. W. and Parks, R. E. Reaction of 5'-deoxyadenosine and related analogs with the 5'-methylthioadenosine cleaving enzyme of sarcoma 180 cells. A possible chemotherapeutic target enzyme. Biochemical Pharmacology 28:2227-2230 (1980).

106. Yall, I., Norrell, S. A., Joseph, R. and Knudsen, R. C. Effect of L-methionine and S-adenosylmethionine on growth of an adenine mutant of Saccharomyces cerevisiae. J. Bact. 93:1551-1558 (1977).

107. Knudsen, R. C., Moore, K. and Yall, I. Uptake and utilization of S-adenosyl-L-methionine and S-adenosyl-L homocysteine in an adenine mutant of Saccharomyces cerevisiae. J. Bact. 98:629-636 (1969).

108. Cone, M. C., Marchitto, K., Zehfus, B. and Ferro, A. J. Role of 5'-methylthioadenosine in purine salvage in Saccharomyces cerevisiae. J. Bact. in press (1982).

109. Burridge, P. W., Woods, R. A. and Henderson, J. F. Purine metabolism in Saccharomyces cerevisiae. Can. J. Biochem. 55:935-941 (1977).

110. Murray, A. W. The biological significance of purine salvage. Ann. Rev. Biochem. 40:811-826 (1971).

111. Hirschorn, R. Adenosine deaminase deficiency and immunodeficiencies. Fed. Proceed. 36:2166 (1977).

112. VanAcker, K. J., Simmonds, H. A., Potter, C. and Cameron, J. S. Complete deficiency of adenine phosphoribosyltransferase. New Engl. J. Med. 247:127-132 (1977).

113. Snyder, F. F. and Henderson, J. F. Alternate pathways of deoxyadenosine and adenosine metabolism. J. Biol. Chem. 248:5899-5904 (1973).

114. Fox, I. H. and Kelley, W. N. The role of adenosine and 2'-deoxyadenosine in mammalian cells. Ann. Rev. Biochem. 47:655-686 (1978).

115. Parks, R. E. and Agarwal, R. P. in The Enzymes, 2nd Ed. vol. 7 (Boyer, P. D., ed.), p. 483-514, Academic Press, New York.

116. Kamatani, N. and Carson, D. A. Dependence of adenine production upon polyamine synthesis in cultured human lymphoblasts. Bioch. Biophys. Acta 675:344-350 (1981).

117. Bagnara, A. S., Letter, A. A. and Henderson, J. F. Multiple mechanisms of regulation of purine biosynthesis de novo in intact tumor cells. Biochem. Biophys. Acta 374:259 (1974).

118. Henderson, J. F. and Khoo, M. K. Y. Availability of 5-phosphoribosyl-1-pyrophosphate for ribonucleotide synthesis in erkch ascites tumor cells in vitro. J. Biol. Chem. 240: 2358 (1965).

119. Henderson, J. F. Feedback inhibition of purine biosynthesis in ascites tumor cells. J. Biol. Chem. 237:2631 (1962).

120. Shapiro, S. K. Response of Aerobacter aerogenes methionine auxotrophs to adenine thiomethyl compounds. J. Bact. 65: 310-312 (1953).

121. Schwartz, M. and Shapiro, S. K. The mechanism of utilization of thiomethyladenosine in the biosynthesis of methionine. J. Bact. 67:98-102 (1953).

122. Schlenk, F. and Ehninger, D. J. Observations on the metabolism of 5'-methylthioadenosine. Arch. Bioch. and Biophys. 106: 95-100 (1964).

123. Schlenk, F., Zydek-Cwick, and Dainko, J. N. 5'-Methylthioadenosine and related compounds as precursors of S-adenosylmethionine in yeast. Bioch. Biophys. Acta 320:357-362 (1973).

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

125. Edwards, C. H., Wade, W. B., Freeburne, M. M., Jones, E. G., Stacey, R. F., Sherman, L., Seo, C. and Edwards, G. A. Formation of methionine from α-amino-n-butyric acid and 5'-methylthioadenosine in the rat. J. Nutrition 107:1927 (1977).

126. Murr, D. P. and Yang, S. F. Conversion of 5'-methylthioadenosine to methonine by apple tissue. Phytochemistry 14: 1291-1292 (1975).

127. Toohey, J. I. Methylthio group cleavage from methylthioadenosine, description of an enzyme and its relationship to the methylthio requirement of certain cells in culture. Biochem. Biophys. Res. Commun. 78(4):1273-1280 (1977).

128. Shapiro, S. K. and Schlenk, F. Conversion of 5'-methylthioadenosine into S-adenosylmethionine by yeast cells. Biochem. Biophys. Acta 633:176-180 (1980).

129. Backlund, P. S. and Smith, R. A. Methionine synthesis from 5'-methylthioadenosine in rat liver. J. Biol. Chem. 256: 1533-1535 (1981).

130. Shapiro, S. K. and Barrett, A. 5-methylthioribose as a precursor of the carbon chain of methionine. Biochem. Biophys. Res. Commun. 102:302-307 (1981).

131. Cooper, A. J. L. and Meister, A. Isolation and properties of a new glutamine transaminase from rat kidney. J. Biol. Chem. 249(8):2554-2561 (1974).

132. Everett, G. B., Mitchell, A. D. and Benevenga, N. J. Methionine transamination and catabolism in vitamin B-6 deficient rats. J. Nutrition 109:597 (1979).

133. Ruiz-Herrera, J. and Starkey, R. L. Dissimilation of methionine by Achromobacter starkeyi. J. Bacteriol. 104:1286-1293 (1970).

134. Primrose, S. B. Evaluation of the role of methional, 2-keto-4-thiomethylbutyric acid and peroxidase in ethylene formation by Escherichia coli. J. Gen. Microbiol. 98(2):519-528 (1977).

135. Favier, A. and Caillat, D. Determination of urinary α-keto-γ-methiolbutyric acid in hypermethioninemia by use of gas chromatography and flame photometry. Clin. Chem. Acta 79(2):419-424 (1977).

136. Brommelsick, W. A., Shirley, R. L. and Palmer, A. Z. Effect of methionine hydroxy analog on energy utilization of a bird-resistant sorghum grain diet fed steers. J. Animal Sci. 48(6):1475-1482 (1979).

137. Harter, J. M. and Baker, D. H. Sulfur amino acid activity of glutathione, DL-α-hydroxy methionine, and α-keto-methionine in chicks. Proc. Soc. Exptl. Biol. and Med. 156:201-204 (1977).

138. Robinson, E. H., Allen, O. W., Poe, W. E. and Wilson, R. P. Utilization of dietary sulfur compounds by fingerling channel catfish: L-methionine, DL-methionine, methionine hydroxy analogue, taurine, and inorganic sulfate. J. Nutr. 108(12): 1932-1936 (1978).

139. Mitchell, A. D. and Beneverga, N. J. The role of trans-amination in methionine oxidation in the rat. J. Nutrition 108:69 (1978).

140. Dixon, J. L. and Beneverga, N. J. The decarboxylation of α-keto-γ-methiolbutyrate in rat liver mitochondria. Biochem. Biophys. Res. Commun. 97(3):939-946 (1980).

141. Steele, R. D., Barber, T. A., Lalich, J. and Beneverga, N. J. Effects of dietary 3-methylthioproprionate in metabolism, growth, and hematopoiesis in the rat. J. Nutr. 109:1739-1751 (1979).

142. Benevenga, N. J. Toxicities of methionine and other amino acids. Agricultural and Food Chemistry 22:2 (1974).

143. Steele, R. D. and Benevenga, N. J. Metabolism of 3-methylthio-propionate in rat liver homogenates. J. Biol. Chem. 254: 8885-8890 (1979).

144. Matsui, S. and Amaha, M. Gas chromatography analysis of medium and high-boiling sulfur volatiles in beer. Nippon Nogeikagaku Kaishi 54(8):629-636 (1980).

145. Matsui, S., Eto, M. and Anaha, M. Determination of methyl and ethy mercaptans in lager beer. Nippon Nogeikagaku Kaishi 54(8):621-628.

146. Steele, R. D. and Beneverga, N. J. The synthesis of radioactive 3-methylthioproprionate and other alkylthio fatty acids. Anal. Bioch. 98:281-286 (1979).

147. Galletti, P., Oliva, A., Manna, C., Della-Ragione, F. and Carteni-Farina, M. Effect of 5'-methylthioadenosine on *in vivo* methyl esterification of human erythrocytic membrane proteins. FEBS Letters 126(2):236-240 (1981).

148. Montgomery, J. A., Shortnacy, A. T. and Thomas, H. J. Analogs of 5'-deoxy-5'-(methylthio)-adenosine. J. Medicinal Chemistry 17(11):1197-1207 (1979).

149. Chiang, P. K., Cantoni, G. L., Bader, I. P., Shannon, W. M., Thomas, H. I. and Montgomery, J. A. Adenosylhomocysteine hydrolase inhibitors: synthesis of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine and its effect on rous sarcoma virus and gross murine leukemia virus. Biochem. Biophys. Res. Commun. 82:417-422 (1978).

150. Hirata, F., Toxorshima, S., Axelrod, J. and Waxdal, M. J. β-Adrenergic receptor agonists increase phospholipid methylation, membrane fluidity, and B-adrenergic receptor-adenylate cyclase coupling. Proc. Natl. Acad. Sci. 76:368-372 (1978).

151. Zappia, V., Gallenti, P. and Porcelli, M. Uptake of adenosylmethionine and related sulfur compounds by isolated rat liver. FEBS Letters 90:331 (1978).

152. Anderson, J. M. and Roth, R. M. Cordycepin sensitivity in adenosine utilizing mutants of S. cerevisiae. Biochem. Biophys. Acta 335:285-289 (1974).

153. Anderson, J. M. and Roth, R. Adenosine utilization in cordycepin-sensitive mutants of Saccharomyces cerevisiae. J. Bacteriol. :689-691 (1976).

154. Scott, J. H. and Schekman, R. Lyticase: endoglucanase and protease activities that act together in cell lysis. J. Bact. 142:414-427 (1980).

155. Colowick, S. P. and Kaplan, N. C. Procedure for the preparation of α-keto acids using L-amino acid oxidase. Methods in Enzymology 3:406-409 (1957).

156. Schlenk, F. and Zydek-Cwick, C. Stability of the glycosidic bond of S-adenosyl sulfonium compounds towards acid. Arch. Biochem. Biophys. 134:414-422 (1969).

157. Schlenk, F. 5'-Methylthioadenosine. Review submitted Biochem. Biophys. Acta (1982).

158. Eloranta, T. O. Tissue distribution of S-adenosylmethionine and S-adenosylhomocysteine in the rat. Biochem. J. 166: 521-529 (1977).

159. Eloranta, T. O. and Raina, A. M. S-Adenosylmethionine metabolism and its relation to polyamine synthesis in rat liver. Biochem. J. 168:179-185 (1977).

160. Backlund, P. J., Chang, C. P. and Smith, R. A. J. Biol. Chem. In press (1982).

161. Trackman, P. C. and Abeles, R. H. Biochem. Biophys. Res. Commun. 103:1238-1244 (1981).

162. Nakamura, K. D. and Schlenk, F. Active transport of exogenous S-adenosylmethionine and related compounds into cells and vacuoles of Saccharomyces cerevisiae. J. Bacteriol. 120: 482-487 (1974).

163. Spence, K. D. Mutation of Saccharomyces cerevisiae preventing uptake of S-adenosylmethionine. J. Bacteriol. 106:325-330 (1971).

164. Schlenk, F., Zydek, C. R., Ehninger, P. J. and Dainke, J. L. The production of S-adenosyl-L-methionine and S-adenosyl-L-ethionine in yeast. Enzymologia 24:283-298 (1965).

165. Hildesheim, J., Hildesheim, R. and Lederer, E. Synthesis of inhibitors of methyltransferases: Analogs of S-adenosylhomocysteine. Biochemie 53:1067-1071 (1971).

166. Guarino, A. J. Cordycepin. In Antibiotics: Mechanism of Action, vol. I (D. Gottlieb and P. D. Shaw, eds.), Springer-Verlag, New York (1967).

167. Roy-Burman, P. Analogues of nucleic acid components. p. 77-78, Springer-Verlag, New York (1970).

168. Anderson, J. M. and Roth, R. Adenosine utilization in cordycepin-sensitive mutants of Saccharomyces cerevisiae. J. Bacteriol. 128:689-691 (1974).

169. Pringle, J. R. in Methods in Cell Biology, vol. XII, Yeast Cells (D. M Prescott, Ed.) p 149-184, Academic Press, New York (1975).

170. Warburg, O. and Christian, W. Biochem. Z. 310:384-421 (1941).

171. Zappia, V., Della Ragioni, F., Cacciapuoti, G., Porcelli, M. and Carteni-Farina, M. J. Biol. Chem. In Press (1982).