

Pharmacological and Biochemical Aspects of S-Adenosylhomocysteine and S-Adenosylhomocysteine Hydrolase*

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I. Introduction

THE function of methionine as a methyl donor in enzymatic transmethylation requires the presence of adenosine triphosphate (ATP) to form so-called active methionine (28, 33, 54). This compound was identified as

S-adenosyl-L-methionine (AdoMet)[†] by Cantoni in 1952 (34). AdoMet seems to be the most versatile methyl donor

[†] Abbreviations used are: AdoMet, S-Adosylmethionine; AdoHcy, S-adenosylhomocysteine; c³AdoHcy, S-3-deazaadenosylhomocysteine; SIBA, 5'-deoxy-5'-isobutyl thioadenosine; c⁷AdoHcy, S-tubercidinylhomocysteine; MTA, 5'-deoxy-5'-methylthioadenosine; Ado, adenosine; Hcy, L-homocysteine; ara-A, 9-β-D-arabinofuranosyladenine; dCF, 2'-deoxycoformycin; ara-AHcy, S-(5'-(9-arabinofuranosyladenyl))-L-homocysteine; c³Ado, 3-deazaadenosine.

* This review is dedicated to Professor Tolla B. Sirnes on the occasion of his 60th anniversary, October 17, 1982.

in mammalian systems, whereas 5-methyltetrahydrofolic acid (274), methylcobalamine (188), and betaine (204) function as methyl donors only in a few instances. Many low molecular weight compounds serve as substrates for AdoMet-dependent transmethylases (318). These include catechols (117), norepinephrine (219), histamine (72), serotonin (191) and tryptamine (239). Much interest has been devoted recently to methylation of membrane phospholipids, which has been assigned a role in various cellular processes related to membrane structure and function (143). Both tRNA and mRNA are methylated by specific transmethylases requiring AdoMet. Methylation of ribonucleic acids is probably involved in the regulation of protein synthesis at the ribosomal level (242, 261). DNA from different sources contains methylated bases (316), and enzymes catalyzing the incorporation of methyl groups into DNA have been described (71, 265). The role of methylation of eukaryotic DNA has not been established, but a relation to growth, differentiation (235), and carcinogenesis (27, 63) has been suggested. Incorporation of methyl groups from AdoMet into lysyl, arginyl, histidyl, aspartyl, glutamyl, and carboxyl groups of proteins is catalyzed by specific protein methyltransferases (173, 174, 213; for review, see ref. 214). Carboxymethylation of proteins seems to play a role in processes like chemotaxis and secretion (15, 207). Thus, methylation of cellular components may offer great utility in biological regulation, and has been compared with phosphorylation in this respect (15).

The biochemistry of AdoMet is the subject of recent reviews (241, 301, 319).

The formation of S-adenosyl-L-homocysteine (AdoHcy) from AdoMet upon transmethylation was demonstrated by Cantoni and Scarano in 1953 (37). Inhibition of AdoMet-dependent transmethylation by AdoHcy was first reported by Gibson et al. (107), who studied the enzymatic synthesis of phosphatidylcholine in rat liver. This observation was later confirmed and extended by others, showing that AdoHcy is a potent inhibitor of a number of AdoMet-dependent transmethylases (61, 64, 67, 114, 152a, 157, 159, 172, 192, 217, 228, 255, 320). The inhibitor constants for AdoHcy often equal or are lower than the corresponding K_m values for AdoMet; this indicates that the catalytic sites of most transmethylases have nearly the same or higher affinity for AdoHcy than AdoMet.

In the early papers on AdoHcy levels in tissues, the cellular content of this metabolite was probably overestimated (84, 147, 243) because of a rapid increase in the concentration of AdoHcy in tissues following death of the animal (152, 152a). Determination of AdoHcy content in tissues involving rapid processing of tissue samples indicates that the AdoHcy level is about 0.5 to 30 nmol/g in most organs of mammals, and that the AdoMet content is 5–10 times higher (103b, 152, 152a, 252, 253). This relationship between the cellular content of AdoHcy and AdoMet, and the reported K_i/K_m values of transmethylases, points to the possibility that AdoHcy is an

inhibitor of AdoMet-dependent transmethylation in vivo. It has been questioned whether AdoHcy exerts an inhibitory effect on most transmethylases under normal conditions (152, 154). However, under conditions of elevated cellular content of AdoHcy, inhibition of transmethylation reactions by AdoHcy has been demonstrated both in intact cells (164, 177, 180), isolated perfused liver (76, 154) and in whole animals (252, 253).

The inhibition of AdoMet-dependent transmethylation reactions by AdoHcy is relieved by the metabolic conversion of AdoHcy to adenosine and L-homocysteine. The reaction is catalyzed by the enzyme S-adenosylhomocysteine hydrolase (EC 3.3.1.1), which was first demonstrated in rat liver by de la Haba and Cantoni in 1959 (68). The activity of this enzyme may play a critical role in controlling tissue levels of AdoHcy and thereby influence methyl-transfer reactions, as suggested by Deguchi and Barchas (67) for the biogenic amines.

The involvement of transmethylation reactions in a wide variety of biological phenomena, points to the possibility that compounds inhibiting these reactions are pharmacologically active agents. The search for inhibitors of biological methylation has been carried out along three different lines. 1) Several analogues of AdoHcy have been synthesized, and some of these compounds are potent inhibitors of certain transmethylases; 2) inhibitors or inactivators of AdoHcy hydrolase may perturb biological methylation by blocking the degradation of AdoHcy; 3) biologically active AdoHcy analogues may be synthesized in the intact cell from synthetic adenosine analogues serving as substrates for AdoHcy hydrolase.

The first part of this article describes the biological and pharmacological properties of AdoHcy and its analogues, with emphasis on the analogues studied in most detail. This section is followed by a review of the literature on the properties and physiological role of AdoHcy hydrolase. This area of research has expanded during the last few years, and basic knowledge on the mechanism of action of AdoHcy hydrolase has stimulated the investigation into potent inhibitors of AdoHcy hydrolase. Some of these agents may be potential chemotherapeutic agents. The interaction of AdoHcy hydrolase with synthetic compounds and naturally occurring metabolites is described, together with a review on the pharmacology of adenosine analogues serving as inactivators or substrates for the enzyme. These data are discussed in light of the concept of AdoHcy hydrolase as a target for adenosine analogues with cytotoxic effects. Finally, data on the possible role of AdoHcy hydrolase in the molecular pathology of some diseases of impaired purine catabolism are presented.

II. Pharmacological Properties of S-Adenosylhomocysteine and Its Analogues

A. Introductory Data

Transmethylation reactions are involved in various biological phenomena related to the development of

pathological processes. Abnormal methylation patterns of tRNA have been described in various tumors and in cells transformed by oncogenic DNA and RNA viruses (8, 26, 190, 203). Viral and eukaryotic cellular mRNA contain methylated nucleosides (220, 261), and the terminal m⁷G residue is necessary for the efficient binding of mRNA to ribosomes (29). Methylation of the 5'-cap-structure in viral mRNA seems to be an essential reaction for the virus replication in the host cell (161). A decreased rate of AdoHcy metabolism has been observed in cells infected with Rous sarcoma virus (222). The data cited above suggest that inhibition of transmethylations may affect processes such as virus growth and replication, virus transformation of cells, and growth of malignant cells.

B. *S-Adenosylhomocysteine*

AdoHcy is a potent inhibitor of transmethylations in cell free systems (see section I). In contrast to analogues of AdoHcy, AdoHcy itself is a rather ineffective inhibitor of methylation of catecholamines in neuroblastoma cells (197), and tRNA methylation in phytohemagglutinin stimulated lymphocytes (45) and chick embryo fibroblasts (305). Furthermore, AdoHcy has no effect on oncogenic transformation of chick embryo fibroblasts (236). The inefficiency of exogenous AdoHcy in cellular systems has been explained by rapid metabolism of this compound by intact cells (66, 236). An additional explanation is offered by the fact that extracellular AdoHcy does not cross the cell membrane as an intact molecule. This statement is based on the following observations. Radioactive AdoHcy injected into rats is excreted in the urine as S-adenosyl- γ -thio- α -ketobutyrate, and only small amounts are incorporated into cellular proteins (78). Similar results were obtained when radioactive AdoHcy was injected intravenously into dogs. Only trace amounts of radioactivity could be detected in nucleic acids or proteins of tissues, and essentially all of the urinary radioactivity was associated with AdoHcy (309). These data show that AdoHcy is not taken up by cells and is not available for intracellular AdoHcy hydrolase. This conclusion is in accordance with the finding that AdoHcy is not taken up by isolated rat hepatocytes as an intact molecule. However, extracellular AdoHcy is hydrolyzed to adenosine and L-homocysteine by AdoHcy hydrolase leaking out of the hepatocytes. In addition, AdoHcy binds to acceptor(s) on the cell surface (1). Characterization of these acceptors of isolated hepatocytes and purified plasma membranes from rat liver has shown that AdoHcy specifically binds to a heterogeneous population of sites with an apparent K_d of 0.4 to 0.7 μ M (289). Phospholipid methyltransferase of the plasma membrane does not totally account for these acceptors for AdoHcy. This statement is based on competition studies with AdoHcy analogues and is supported by the observation that the binding capacity of the AdoHcy acceptors on the surface of rat hepatocytes (about 12 pmol/10⁶ cells) is high relative to the low specific activity

of phospholipid methyltransferase (289). The physiological role of membrane acceptors for AdoHcy and their relation to methyltransferases localized to the membrane are subjects for further investigation.

AdoHcy has sedative and anticonvulsive properties in the rabbit, rat, and cat (96, 99). This observation suggests an effect of AdoHcy on the central nervous system, and has been related to effects of AdoHcy on the metabolism of catecholamines (10, 93, 97, 98). However, the possibility exists that these effects of AdoHcy are mediated by AdoHcy metabolites rather than the parent compound. Recently, Fonlupt et al. (94) have demonstrated in vitro and in vivo binding of AdoHcy to membranes from rat cerebral cortex. Small variations were observed for the in vitro binding of AdoHcy to membranes from different regions of the rat brain, and the highest binding activity was found in the hippocampus. A similar regional distribution was observed for phospholipid methyltransferase (95a). It was suggested that the binding sites for AdoHcy may reside on phospholipid methyltransferase localized in the membrane (95, 95a).

The possibility of an anticonvulsant action of AdoHcy is supported by the data provided by Schatz and coworkers. The convulsant L-methionine *d,l*-sulfoximine (MSO) (194a) decreases the cerebral content of AdoHcy (as well as AdoMet) (249). Elevation of cerebral AdoHcy in vivo by administration of adenosine and homocysteine results in a decrease in methylation of histamine and phospholipid and protein carboxymethylation (252, 253) and partly prevents MSO-induced increase in methylation and protects against MSO seizures (254). Finally, the anticonvulsants, dilantin and phenobarbital, increase the cerebral content of AdoHcy, and upon administration of phenobarbital a slight reduction in protein carboxymethylation was observed (254).

C. *Analogues of S-Adenosylhomocysteine*

Many analogues of AdoHcy, modified in the sugar moiety, amino acid portion, or purine ring, have been synthesized (15, 17, 18, 22, 25, 46, 58, 60, 62, 139-141, 308). The purpose of these efforts is to obtain information on the structural requirements for binding of AdoHcy to various transmethylases, to provide analogues specific for certain methylases and compounds that resist metabolic degradation. Many AdoHcy analogues have been tested as inhibitors of catechol O-methyltransferase, phenylethanolamine N-methyltransferase, histamine N-methyltransferase, hydroxyindole O-methyltransferase (13, 15, 17, 18, 22-25, 58, 60, 62, 141) protein methylation, tRNA methyltransferase, and mRNA methyltransferase (21, 41, 89, 142, 185, 237, 238, 305). Some general conclusions have been drawn based on data from various small molecule methyltransferases and some macromolecule methyltransferases. Methyltransferases show strict structural requirements for AdoHcy. The following groups seem to be essential for the binding of AdoHcy to several methyltransferases: the terminal amino group, the sulfur atom in the thioether linkage, the 6-amino

group of adenine, and the ribose moiety. Most modifications in the ribose residue result in almost complete loss of activity, whereas modifications in the adenine ring have given compounds (S-3-deazaadenosylhomocysteine and S-tubercidinyl-homocysteine), which are potent inhibitors of methyltransferases (14). However, several exceptions to these general rules have been reported. For example, protein carboxymethyltransferase, virion mRNA (guanine-7-)-methyltransferase, and virion mRNA (nucleoside-2'-)-methyltransferase are very sensitive to sulfur modified analogues of AdoHcy (19, 227). Furthermore, various analogues modified in the adenine ring are weak inhibitors of these enzymes (19, 227). Thus, the structural requirements for the inhibition of transmethylases by AdoHcy analogues seem to be somewhat different from one enzyme to another. Continuing efforts are being made to synthesize new analogues of AdoHcy to obtain compounds that may allow for differential inhibition of methyltransferases (15, 154a, 308).

The effects of AdoHcy analogues in various biological systems have been studied to a certain extent. Some of these compounds have antiviral and growth inhibitory effects (15, 185, 237, 267). The biological properties of well characterized analogues will be reviewed in the following paragraphs. Their structural formulas are shown in figure 1.

1. *5'-Deoxy-5'-isobutyl thioadenosine*. *5'-Deoxy-5'-isobutyl thioadenosine* (SIBA) was synthesized by Hildeshcim and coworkers in 1971 (140). The compound is an antiviral agent in various systems. SIBA is an inhibitor of cell transformation by Rous sarcoma virus (236) and mouse sarcoma virus (237), and a particularly potent inhibitor of multiplication of mouse mammary tumor virus transformed cells (275). A single injection of 1 mg of SIBA prolongs (by 42%) the survival time of mice infected with Friends virus (237). Furthermore, SIBA also inhibits the multiplication of Herpes virus type I, and this effect is associated with reduction in viral protein synthesis and methylation of viral mRNA (161). Polyoma virus replication in mouse embryo fibroblasts and nucleic acid methylation in infected cells are inhibited at relatively low concentrations of SIBA (232). Finally, SIBA also exerts antiviral effects against Epstein-Barr virus and influenza virus (237).

Inhibition of mitogen-induced blastogenesis has been demonstrated in the presence of SIBA (12), and this effect may be related to the possible role of transmethyl-ation reactions in blast formation (260). Antiparasitic effects of SIBA have been demonstrated against *Plasmodium falciparum* in vitro (277). Potential oncostatic properties of SIBA are indicated by the finding that this compound inhibits the plating efficiency of cells transformed by methylcholanthrene (237).

A structural analogue of SIBA, 3-deaza-SIBA, is a noncompetitive inhibitor of AdoHcy hydrolase (49) and inhibits phospholipid methylation in intact cells (65). This compound has antiviral effects against Rous sarcoma virus, Gross murine leukemia virus (49), Herpes

virus, and the RNA type C virus (11). It also possesses antimalaria effects (278).

Data on the molecular effects of SIBA are accumulating, and it seems that this compound may induce various metabolic derangements in the intact cell. These effects are not limited to perturbation of transmethyl-ation reactions, but also include effects on cellular transport and on metabolism of methylthioadenosine and cyclic AMP.

SIBA is a relatively weak inhibitor of transmethylases in cell free systems (186, 232, 236, 305). The inhibitor constant of SIBA for protein methylase I is 100 to 600 μM (41, 186), and higher than 1000 μM for tRNA transmethylase (186, 280, 304), protein methylase II (209), protein methylase III (304), and DNA transmethylase (9). In contrast, inhibition of methylation can be demonstrated in intact cells (232, 306). For example, whereas SIBA does not inhibit phospholipid methyltransferase in purified rat liver plasma membranes and microsomes (245, 247), inhibition of phospholipid methylation in isolated rat hepatocytes is observed (247).

The synthesis of AdoHcy hydrolase is suppressed in cells cultivated in the presence of SIBA, and this compound is a weak inhibitor of isolated AdoHcy hydrolase (222). Only a slight or no increase in cellular content of AdoHcy in hepatocytes (247) or lymphocytes (326) exposed to SIBA could be demonstrated.

A remarkable property of SIBA (but not sinefungin) is its inhibitory effect on cellular uptake of nucleosides and sugar, compounds structurally unrelated to SIBA. A membrane factor interacting with SIBA has been postulated to explain the effect on cellular transport (223). SIBA exerts essentially no inhibitory effect on AdoHcy acceptors and phospholipid methyltransferase of rat liver plasma membranes (245, 289). Thus, there is no obvious reason to suggest that SIBA exerts its effect on cellular transport by a direct interaction with these membrane factors.

The possibility that SIBA influences membrane function is supported by the recent observation that SIBA increases the cyclic AMP content and stimulates adenylate cyclase in *Xenopus laevis* oocytes (256). No effect on cyclic AMP synthesis and adenylate cyclase in mouse lymphocytes could be demonstrated. However, in these cells, SIBA is an inhibitor of the low K_m cyclic AMP phosphodiesterase and thereby potentiates the cyclic AMP response of intact cells to several activators of adenylate cyclase (322). This effect was observed at physiologically active concentrations of the drug (322).

SIBA is a good substrate for the methylthioadenosine (MTA) cleavage enzyme from sarcoma 180 cells (244), and MTA phosphorylase from human placenta (39) and *Lupinus luteus* seeds (120), and MTA nucleosidase isolated from the bacterium *Calderiella acidophila* (40). These observations suggest that SIBA may interfere with the metabolism of MTA. In isolated rat hepatocytes, SIBA induces accumulation of MTA, which is associated with a pronounced export of this compound into the extracellular medium (246). The fact that SIBA is a

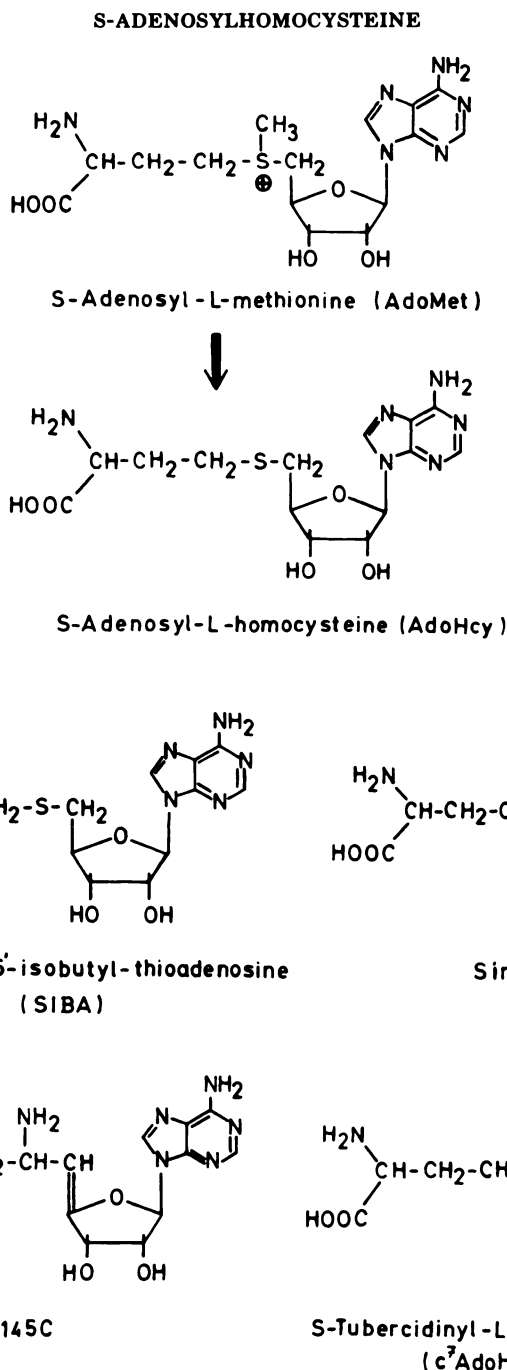


FIG. 1. Chemical structure of S-adenosyl-L-methionine, S-adenosylhomocysteine, and some S-adenosylhomocysteine analogues.

substrate and inhibitor of MTA phosphorylase suggests that SIBA may cause various metabolic derangements in the intact cells, including effects on polyamine biosynthesis and production of methylthio groups essential for cell division. It may also induce formation of large amounts of adenine (from SIBA) leading to cellular depletion of 5-phosphoribosyl-1-pyrophosphate (237).

An inhibitory effect of SIBA on the biosynthesis of polyamines is indicated by the finding that this compound decreases the spermidine content in transformed mouse fibroblasts. However, in a cell free system, SIBA is a more potent inhibitor of spermine synthase than of spermidine synthase. Cell growth cannot be restored to normal levels by the addition of spermidine, suggesting

that SIBA has other inhibitory actions toward cellular proliferation (218).

Some of the biological effects exerted by SIBA should perhaps be considered in the light of the finding that MTA is converted to methionine by intact cells and in biological extract (314a). Biosynthesis of methionine from MTA involves the conversion of MTA to 5'-methylthioribose-1-phosphate catalyzed by MTA phosphorylase. The conversion is followed by a sequence of reactions where the carbons of the ribose portion, the carbon and hydrogens of the methyl group, and the sulfur of MTA are all incorporated into methionine. This pathway may provide a significant synthesis of cellular methionine (314a). Inhibition of this salvage pathway for methionine

biosynthesis by blocking the MTA phosphorylase reaction in the presence of SIBA, may contribute to some biological effects of SIBA, including inhibition of biological methylation as well as inhibition of polyamine synthesis.

The uptake of SIBA into chick embryo fibroblasts has recently been studied by Enouf et al. (88). A mixed transport system composed of a high and a low affinity component could be demonstrated. The high affinity system is inhibited by SIBA analogues with an intact adenosine residue, modified at the 5'-S-side chain, and by S-adenosylmethionine. SIBA may enter cells by a carrier system transporting naturally occurring compounds.

SIBA is metabolized to 5'-deoxy-5'-isobutylthioribose and adenine in prokaryotes. In chick embryo fibroblasts, the compound is metabolized along two pathways, i.e. deamination to 5'-deoxy-5'-S-isobutylthioinosine or hydrolysis to 5'-deoxy-5'-S-isobutylthioribose and adenine (184). When SIBA is injected into rats or mice, the compound is rapidly and widely distributed to various organs. SIBA is extensively metabolized, and several metabolites in addition to those formed by chick embryo fibroblasts, can be demonstrated (183).

The role of various enzymes in the metabolism of SIBA has been evaluated by using human cell lines deficient in MTA phosphorylase, purine nucleoside phosphorylase, or adenosine deaminase. SIBA is not a substrate of adenosine deaminase. MTA phosphorylase catalyzes the conversion of SIBA to adenine and 5'-deoxy-5'-S-isobutylthioribose-1-phosphate. The latter compound serves as a substrate for purine nucleoside phosphorylase, which condenses it with hypoxanthine to form 5'-deoxy-5'-S-isobutylthioinosine (171a).

2. *Sinefungin*. Sinefungin is a "carba-analogue" of AdoHcy where the thio-ether is replaced by an amino-substituted methylene unit (fig. 1). This compound (A9145) was isolated from *Streptomyces griseolus* at Lilly Research Laboratories (121), and was shown to possess antifungal activity (115). Sinefungin and its metabolite, A9145C, are very potent inhibitors of transformation of chick embryo fibroblasts by Rous sarcoma virus, but A9145C is quite toxic at active concentrations (237, 304). Polyoma virus replication in mouse embryo fibroblasts is inhibited at nontoxic levels of sinefungin whereas cytotoxic concentrations are required for the inhibition of mouse sarcoma virus and mouse mammary tumor virus (237). Vaccinia virus plaque formation in mouse L-cells seems to be especially sensitive to sinefungin and A9145C, and is inhibited at low, nontoxic concentrations (10 μM) of these compounds (229).

Antiparasitic activity of sinefungin is demonstrated by the inhibition of *Plasmodium falciparum* grown in vitro at a low concentration of sinefungin (0.3 μM) (278), and inhibition of growth of *Trypanosoma cruzi* (237) and *Leshmania tropica* (185).

Preliminary experiments show that sinefungin inhibits tumor growth in vivo in mice, but the survival time of

the tumor-bearing animals is not increased (201). These data should stimulate investigation of the possible oncostatic properties of sinefungin and related compounds.

In contrast to SIBA, sinefungin and A9145C are very potent inhibitors of some transmethylases in vitro. Thus, it seems reasonable to suggest that these analogues of AdoHcy exert their biological effects by inhibiting cellular transmethylations reactions. Sinefungin is a more potent inhibitor of tRNA methyltransferase and protein methylase I and III from chick embryo fibroblasts than AdoHcy (304), and inhibits the tRNA methylation in whole cells (305). Both sinefungin and A9145C are extremely potent inhibitors of virion mRNA (guanine-7-)-methyltransferase with K_i values in the nanomolar range, as compared with K_i for AdoHcy of about 1 μM (227, 229). Sinefungin and A9145C are also inhibitors of various AdoMet-dependent transmethylases catalyzing the methylation of biogenic amines (103).

AdoMet:protein O-methyltransferase and phospholipid methyltransferase(s) have been assigned a role in cellular processes such as chemotaxis and secretion (15). The former enzyme is strongly inhibited by sinefungin and A9145C (16, 19, 201) whereas the latter enzyme is relatively insensitive to these AdoHcy analogues (245). Thus, these compounds may be valuable tools for studying the role of protein O-methyltransferase in chemotaxis and secretion (16).

Among the many synthetic analogues of AdoHcy, sinefungin is the most potent inhibitor of Δ^{24} -sterol-C-methyltransferase from *Saccharomyces cerevisiae*. Yeast grown in the presence of sinefungin revealed a dramatic increase in zymosterol, the substrate of this enzyme, and a concomitant decrease in the level of ergosterol, which is the product of this methyltransferase (194). These data suggest that inhibition of AdoMet-dependent transmethylations contributes to the antifungal properties of this agent (194).

Recent data provided by Pugh and Borchardt (227) suggest that sinefungin and A9145C may exert effects on cellular processes other than AdoMet-dependent transmethylations reactions. These compounds, but not AdoHcy and various synthetic AdoHcy analogues tested, stimulate viral guanylyl transferase activity and inhibit viral RNA synthesis. These effects may contribute to the antiviral properties of sinefungin and A9145C.

Cellular disposition of sinefungin and A9145C has not been studied in detail. Both agents are inactive as inhibitors of protein carboxymethyltransferase in intact synaptosomes, but are potent inhibitors of the enzyme in lysed synaptosomes and of isolated enzyme. Lack of effect could not be explained by extensive metabolism of these compounds (19). Likewise, sinefungin inhibits phospholipid methylation in rat liver plasma membranes (245), but is ineffective in intact rat hepatocytes (247). These findings might suggest that cellular transport of sinefungin and related compounds is an important factor determining their effectiveness in vivo (19).

The biological properties of sinefungin and its metab-

olite should stimulate further efforts to synthesize new carba-analogues of AdoHcy, and to evaluate their usefulness as chemotherapeutic agents (308).

3. *S-Tubercidinylhomocysteine*. S-Tubercidinylhomocysteine (c^7 AdoHcy) is the 7-deaza-analogue of AdoHcy, which was synthesized to obtain a transmethylase inhibitor resisting metabolic degradation (58, 59). The biological properties of this compound have been studied in some detail by Coward and coworkers (45, 58, 59, 165, 166, 197, 238). c^7 AdoHcy inhibits various transmethylases catalyzing the methylation of biogenic amines and histamine (18, 58), and is a potent inhibitor of protein carboxymethyltransferase (59) and tRNA methyltransferase (59, 281). c^7 AdoHcy is a potent inhibitor of Newcastle disease viron mRNA (guanine-7)-methyltransferase (228) but is a weak inhibitor of this enzyme from vaccinia virus (227). This finding suggests differences between the AdoHcy binding site on the vaccinia virus vs. the Newcastle disease virus (227). The vaccinia mRNA (nucleoside-2')-methyltransferase (227) and in vivo methylation of Novikoff cytoplasmic mRNA (165, 238) are also sensitive to c^7 AdoHcy. Among various AdoHcy analogues, c^7 AdoHcy is the most potent inhibitor of phospholipid methyltransferase(s) and the AdoHcy binding to plasma membrane from rat liver (245, 289). In general, the inhibitor constants of c^7 AdoHcy for AdoMet dependent transmethylases are often lower than the values obtained with AdoHcy for the same enzymes (59).

c^7 AdoHcy is also a potent inhibitor of methylation of biogenic amines and RNA in intact cells, and is more effective in this respect than its natural congener, AdoHcy (45, 165, 166, 197). This has been explained by the fact that c^7 AdoHcy is not metabolized by whole cells (66), and is not a substrate for AdoHcy hydrolase (52). Furthermore, whereas AdoHcy is not taken up by cells as an intact molecule (1, 78, 309), it has been suggested that some analogues of AdoHcy cross the cell membrane to reach their target enzyme (279). This general statement holds for c^7 AdoHcy, which nearly completely inhibits the phospholipid methylation in isolated rat hepatocytes (247). Radioactive c^7 AdoHcy has been prepared (66), and this compound may be useful for the investigation of cellular transport of AdoHcy analogues. Such investigation will probably not be obscured by extra- and intracellular metabolism of c^7 AdoHcy, but precaution should be taken to distinguish cellular uptake from binding of c^7 AdoHcy to acceptors on the cell surface.

III. S-Adenosylhomocysteine Hydrolase

A. Enzyme Catalysis—Interaction between Substrates and Enzyme

AdoHcy hydrolase catalyzes the reversible hydrolysis of AdoHcy to adenosine and L-homocysteine. When the concentration of the substrates is higher than in the micromolar range, the reaction favors synthesis of

AdoHcy (68). This can be more precisely expressed by the equilibrium constant of the reaction, K_{eq} , which is defined by the equation:

$$K_{eq} = \frac{[Ado] \times [Hcy]}{[AdoHcy]} = 10^{-6} \text{ M (68, 299).}$$

Both adenosine and L-homocysteine are potent inhibitors of the hydrolytic reaction, and the enzyme catalysis can be directed toward hydrolysis by removal of either adenosine or L-homocysteine (68).

Adenosine forms a stable complex with AdoHcy hydrolase from various tissues (50, 128, 168, 293). A fraction of adenosine tightly bound to the enzyme from liver is converted to adenine or a substance liberating adenine (50, 272, 297), and this reaction is reversible under certain conditions (272). Tightly bound adenosine cannot be released (128) or dissociates slowly (293) by incubation of the enzyme with excess unlabelled adenosine, but dissociates readily upon boiling or treatment of the enzyme with sodium dodecyl sulfate (128) or ethanol (2). Adenosine complexed with AdoHcy hydrolase is not available for deamination to inosine catalyzed by adenosine deaminase (272). This phenomenon has been termed sequestration of adenosine to indicate a possible physiological role of this process (272, 298). The conversion of adenosine to adenine and the sequestration of adenosine can also be demonstrated under conditions of enzyme catalysis, i.e. both during synthesis and hydrolysis of AdoHcy (294).

High concentrations of adenosine inactivate AdoHcy hydrolase from human lymphocytes, placenta (137), and rat liver (168). The kinetics of inactivation are consistent with a suicide mechanism (137, 168), which implies that the inactive complex is formed from a reversible adenosine-enzyme complex (310). The inactivation of the enzyme by adenosine and the sequestration of adenosine are probably related phenomena, which are demonstrated at low enzyme concentration and high enzyme concentration, respectively (294). Thus, enzyme forming a stable complex with adenosine is enzymatically inactive.

The kinetics of adenosine binding to and formation of adenine by AdoHcy hydrolase from plant has been studied in some detail by Jakubowski and Guranowski (163). The enzyme, which is a dimer, binds two molecules of adenosine. The binding of the first molecule is rapid whereas binding of the second molecule is a slow process. This suggests negative cooperativity among binding sites. The enzyme-adenosine complex reacts slowly to form adenine, ribose, and active enzyme. Thus, in contrast to mammalian AdoHcy hydrolase, adenosine does not inactivate the enzyme. The half-life of the newly formed complex is short, whereas the old complex dissociates slowly (163). Similar data have been obtained with mouse liver AdoHcy hydrolase (272), but comparable kinetic data for the mammalian enzyme are not available.

Kinetic studies and gel filtration experiments indicate

that AdoHcy forms a long-lived complex with AdoHcy hydrolase from mouse liver. This complex shows a half-life of 5 to 10 minutes (294). This finding should perhaps be related to the finding that AdoHcy inactivates purified hydrolase, but is less potent in this respect than adenosine (168).

B. Catalytic Mechanism

Data on the catalytic mechanism of AdoHcy hydrolase have been provided by Palmer and Abeles (215, 216). These workers used AdoHcy hydrolase purified to homogeneity from beef liver as the source of enzyme. The enzyme contains tightly bound NAD^+ , which participates in the catalytic cycle. A mechanism is suggested, which is based on oxidation/reduction at C-3' of the ribose residue of adenosine or AdoHcy, with a concomitant reduction/oxidation of NAD^+/NADH . The oxidation of the substrate activates the C-4' proton, and thus facilitates the elimination of C-5' substituent (OH-group of adenosine or homocysteinyl group of AdoHcy). Hydrolysis of AdoHcy thus involves oxidation of 3'-hydroxyl group of AdoHcy by enzyme bound NAD^+ . Following oxidation, L-homocysteine is eliminated to give 3'-keto,4'-5'-dehydroadenosine. This compound reacts with water to form 3'-ketoadenosine, which is then reduced to adenosine (fig. 2).

Oxidation of the nucleoside to 3'-ketonucleoside may offer an explanation for the formation of adenine (50, 272, 294). 3'-Ketonucleosides are known to spontaneously liberate the purine base (216).

C. Role in Intermediary Metabolism

AdoHcy hydrolase catalyzes the hydrolysis of AdoHcy to adenosine and L-homocysteine. Although the reaction favors synthesis of AdoHcy (see section III A), the metabolic flow is probably in the hydrolytic direction as adenosine and L-homocysteine are rapidly metabolized in the cell (57, 66, 68, 84).

The role of the AdoHcy hydrolase reaction as a cellular source of adenosine in various tissues has not been evaluated. It has been suggested that AdoHcy may be a site of production of adenosine in the mammalian heart (258).

Adenosine is metabolized along two pathways, i.e. deamination to inosine catalyzed by adenosine deaminase (EC 3.5.4.4.) or phosphorylation to AMP through the action of adenosine kinase (EC 2.7.1.20) (100).

The other product formed from AdoHcy is L-homocysteine. It should be noted that hydrolysis of AdoHcy is the only known metabolic pathway in vertebrates leading to L-homocysteine (36). L-Homocysteine is converted to L-methionine, and this reaction is catalyzed by two enzymes, i.e. 5-methyltetrahydrofolate:L-homocysteine methyltransferase (EC 2.1.1.13) or betaine:L-homocysteine S-methyltransferase (EC 2.1.1.5.). L-Homocysteine also reacts with serine to form cystathionine. This reaction is catalyzed by the enzyme cystathionine- β -synthase (EC 4.2.1.22) (205).

When adenosine or L-homocysteine or both are accumulating in the intact cell, the reaction catalyzed by AdoHcy hydrolase is directed toward synthesis of AdoHcy, and the enzymatic degradation of AdoHcy is

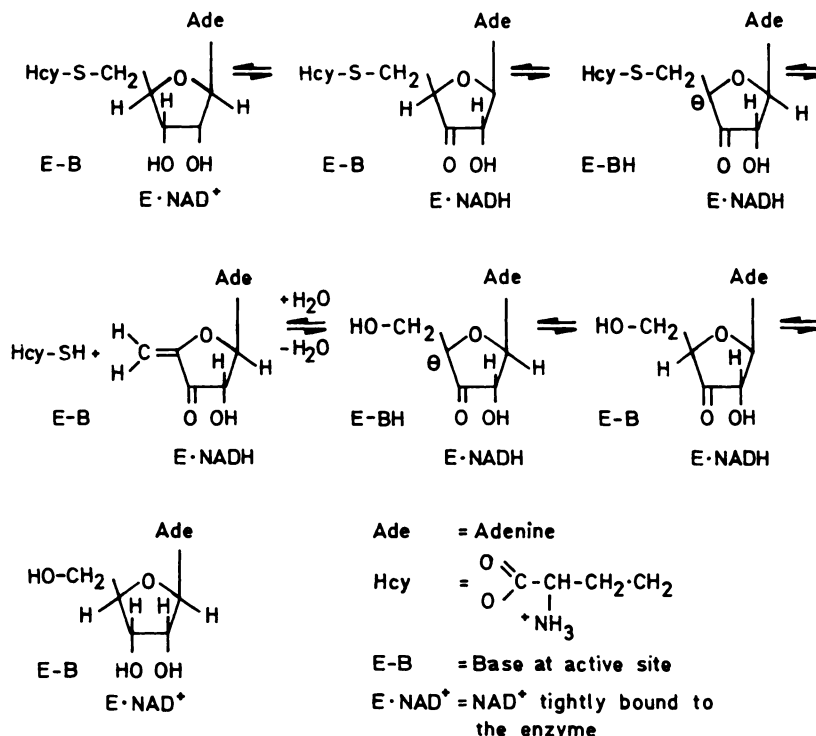


FIG. 2. Catalytic mechanism of S-adenosylhomocysteine (AdoHcy) hydrolase [modified from Palmer and Abeles (215)].

inhibited. High cellular level of AdoHcy can thus be obtained by supplementing exogenous adenosine and/or homocysteine, even without pharmacological inhibition of enzymes metabolizing adenosine. This has been demonstrated with cultured (164, 177, 180) and isolated (125) cells, isolated perfused liver and heart (76, 154), and in whole animals (252, 253).

In isolated liver of the rat, enzymatic synthesis of AdoHcy has been demonstrated even in the absence of exogenous homocysteine, under conditions of inhibited adenosine deaminase (75, 76). Explanations to these interesting findings are not readily apparent in light of the fact that the cellular level of homocysteine (179) is extremely low relative to the K_m for L-homocysteine of AdoHcy hydrolase (about 160 μM) (75). These data point to a role of AdoHcy hydrolase in the disposition of endogenous L-homocysteine. The existence of a cellular pool of bound L-homocysteine has been suggested (75).

The possibility exists that adenosine is compartmentalized in the intact cell through the interaction with AdoHcy hydrolase. This statement is based on the following observations: A fraction of adenosine added to concentrated crude extract from various mammalian tissues binds tightly to AdoHcy hydrolase and is not available for deamination and further degradation (298). Furthermore, the cellular level of AdoHcy hydrolase in mouse liver is about 10 μM (298), which is of the same order of magnitude as the tissue level of adenosine (6) and AdoHcy (152, 252, 253). The relevance of these data for the metabolism of adenosine in the intact cell is indicated by the findings that the sequestration of adenosine in the presence of purified AdoHcy hydrolase occurs both during synthesis and hydrolysis of AdoHcy (294). Recently adenosine has been found to form a stable complex with intracellular AdoHcy hydrolase in isolated hepatocytes of the rat. The formation of this complex is blocked by compounds interacting with the catalytic site of the enzyme (286, 295). In hepatocytes, the amount of endogenous adenosine bound to intracellular AdoHcy hydrolase is of the same order of magnitude as the amount of cellular adenosine that resists mobilization by high levels of extracellular adenosine deaminase (295). A similar conclusion has been reached from studies with hearts of the dog and the rat (210a). Thus, a substantial fraction of adenosine may be complexed with AdoHcy hydrolase in intact cells.

It should be noted that a fraction of endogenous adenosine bound to AdoHcy hydrolase readily liberates adenine. This may obscure the determination of total amount of cellular adenosine, at least in hepatocytes (295).

Adenosine transport by neuroblastoma cells deficient in adenosine kinase has been studied recently by Green (116). It was observed that the intracellular concentration of adenosine exceeds the extracellular concentration, and that this concentrative effect decreases as the con-

centration of adenosine increases. The author suggests that this may be explained by a saturable binding of adenosine to AdoHcy hydrolase and/or other intracellular components (116).

The physiological implication of the fact that adenosine forms a stable complex with AdoHcy hydrolase has been stressed by Fredholm and Sollevi (101) and by Schütz et al. (258). Adenosine is a regulator of lipolysis in adipose tissues, but most of the adenosine content in tissues is probably bound to intracellular proteins (101). Likewise, the vasodilatory effect of adenosine in the heart is attributed to the extracellular, free fraction of the nucleoside. A portion of intracellular adenosine may be bound to AdoHcy hydrolase or other proteins (258). In general, intracellular sequestration of adenosine should be taken into account when relating the pharmacological and physiological effects of adenosine (100) to the concentrations of this nucleoside in tissues.

A similar reasoning can be made for AdoHcy. AdoHcy complexed with AdoHcy hydrolase may not exert its inhibitory effect on AdoMet-dependent transmethyases (294). In addition, AdoHcy may be tightly bound to other cellular components (289). Cellular compartmentation of AdoHcy has in fact been suggested as an explanation of the finding that the tissue level of AdoHcy seems sufficiently high to entirely block some important transmethylation reactions (85).

Conversion of adenosine to adenine is a side-reaction catalyzed by AdoHcy hydrolase (294, 297). The reaction can also be demonstrated under conditions of enzymatic synthesis and hydrolysis of AdoHcy (294), but is rather inefficient *in vitro*. Furthermore, only small amounts of adenine are formed from adenosine in rat liver (76). It seems unlikely that this reaction is a quantitatively important source of cellular adenine. This statement is supported by the recent observation that, in human lymphoblastoid cells at least, adenine derives mainly from cleavage of methylthioadenosine, a product of polyamine synthesis (170).

The main points on the role of AdoHcy hydrolase in the metabolism of purines and sulfur compounds are summarized in figure 3.

D. Methods

The enzyme activity of AdoHcy hydrolase is assayed either in the synthetic or hydrolytic direction. The measurement of AdoHcy synthesis is performed by incubation of the enzyme preparation in the presence of adenosine and L-homocysteine (68). L-Homocysteine is present either as DL-homocysteine or is prepared from L-homocysteine thiolactone (77). This assay carries the advantage that the equilibrium of the enzyme catalysis favors synthesis of AdoHcy, and the substrates (adenosine and L-homocysteine) are available as radioactive compounds. Deamination of adenosine in crude tissue extract may be a source to erroneous data when the activity is measured

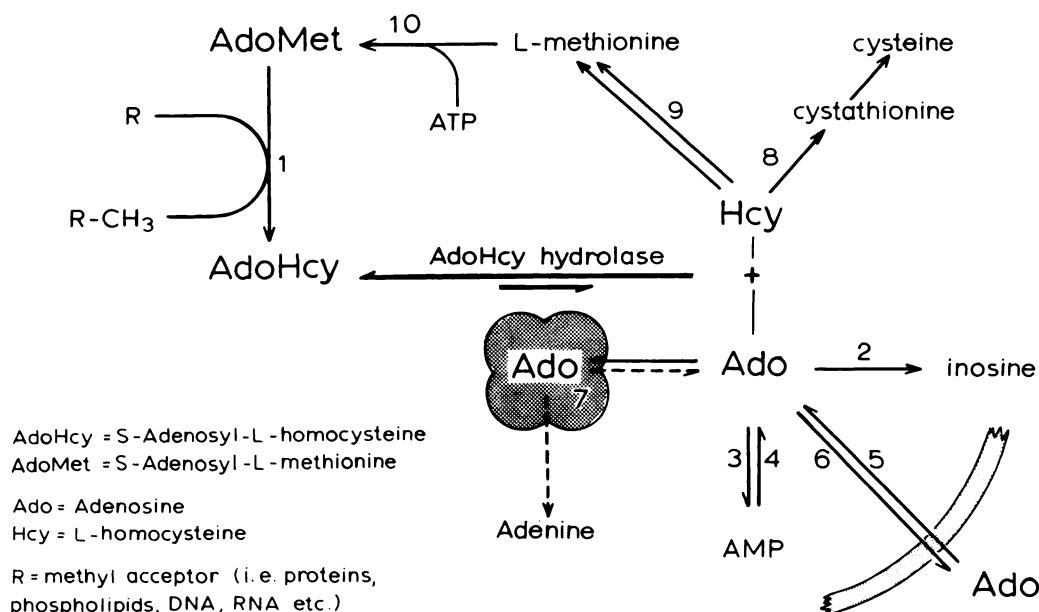


FIG. 3. Role of AdoHcy hydrolase in intermediary metabolism. AdoMet-dependent methyltransferases (1), adenosine aminohydrolase (adenosine deaminase) (2), adenosine kinase (3), adenosine 5'-monophosphate nucleotidase (4), adenosine uptake (5), adenosine release (6), adenosine tightly bound to AdoHcy hydrolase (7), cystathionine- β -synthase (8), 5-methyltetrahydrofolate:L-homocysteine methyltransferase and betaine:L-homocysteine S-methyltransferase (9), ATP:L-methionine S-adenosyltransferase (10).

in the synthetic direction (167). This problem can be circumvented by inclusion of adenosine deaminase inhibitors (110) in the incubation mixture (167).

The original method for the assay of AdoHcy synthesis, described by de la Haba and Cantoni (68), is based on measuring the disappearance of free sulfhydryl groups of L-homocysteine. This method is rapid, but L-homocysteine is oxidized when oxygen is present (68). The assay cannot be performed in the presence of reducing agents, which have been found to stabilize the enzyme (168, 288). The double isotope assay developed by Finkelstein and Harris (91) measures the formation of radioactive AdoHcy, which is isolated by column chromatography. Other radiochemical assays have been developed, which involve separation of adenosine from AdoHcy by paper chromatography (175), low-voltage electrophoresis (167), thin-layer chromatography (119, 134, 271), or column chromatography (234). The radioisotope techniques offer high sensitivity, but are rather laborious.

Measurement of the enzyme catalysis in the hydrolytic direction is performed by incubating the enzyme in the presence of AdoHcy. The products of the reaction, adenosine, and to a lesser degree L-homocysteine, are inhibitors of the hydrolytic reaction, and adenosine deaminase is therefore included in the assay mixture to trap adenosine formed (68). Hydrolysis of AdoHcy catalyzed by AdoHcy hydrolase is the only pathway for the degradation of AdoHcy in crude extracts from mammalian tissues (250), and the measurement of the hydrolysis of AdoHcy is therefore not obscured by metabolism of AdoHcy by other enzymes. This makes the assay suitable for the determination of enzyme activity in crude extract (282, 290).

AdoHcy labeled in the adenosyl-moiety is synthesized enzymatically from labeled adenosine with AdoHcy hydrolase as catalyst (68, 249, 299). AdoHcy is separated from inosine by column (234), paper (249), or thin-layer chromatography (299). The assay developed by Schatz et al. (250) is based on the separation of AdoHcy from its metabolites by high-pressure liquid chromatography. Trewyn and Kerr (280) have described a procedure for the synthesis of S-adenosyl-L-[2(n)- 3 H]homocysteine from S-adenosyl-L-[2(n)- 3 H]methionine. The reaction is catalyzed by glycine N-methyltransferase. Radioactive AdoHcy is used as a substrate for AdoHcy hydrolase, and unreacted AdoHcy is removed by adsorption to dextran-coated charcoal. This assay procedure combines high sensitivity and ease of operation (280).

A rapid spectrophotometric assay for the measurement of AdoHcy hydrolysis has been developed recently. The procedure is based on monitoring the formation of uric acid from AdoHcy in the presence of a coupled enzyme system, by recording the absorbance at 292 to 295 nm (257, 282). The formation of uric acid from AdoHcy is also the basis for activity staining of AdoHcy hydrolase in polyacrylamide gels (282).

The optimal conditions for the extraction of AdoHcy hydrolase from isolated rat hepatocytes have been worked out in the author's laboratory. The enzyme is rapidly inactivated by factors in the extract (probably adenosine or a derivative thereof), and this can be prevented by extraction of the hepatocytes in ice-cold buffer that contains homocysteine, dithiothreitol, and phosphate (125).

AdoHcy hydrolase has been purified to apparent homogeneity from mouse (291), bovine (215), calf (234), and

rat (102, 168) liver; human placenta (128); rat brain (251); bovine adrenal cortex (80, 81); and plant seeds (119). The purification procedures involve conventional methods (80, 81, 102, 168, 215, 234, 251, 291). Crystallization of the partially purified enzyme from calf liver has been done by Richards et al. (234) to obtain the enzyme in pure form. AdoHcy hydrolase has been purified to apparent homogeneity from rat liver by procedures that involve affinity chromatography on Sepharose-bound 6-mercaptopurine 9 D-ribose (43) and 8-(3-aminopropyl-amino)adenosine (168).

E. Distribution and Tissue Level

1. *Vertebrates.* The distribution of AdoHcy hydrolase in various tissues from the chicken, dog, rat, and rabbit was investigated by Walker and Duerre (309). The enzyme activity was highest in the liver, pancreas, and kidney; intermediate in spleen and testis; and low in brain and heart. These findings are largely in accordance with data on the occurrence of the enzyme in rat and mouse tissues published by others (84, 91, 250). In the rat, highest activity was in the liver and pancreas. Kidney and adrenal also contain high levels of AdoHcy hydrolase, i.e. the activities in these tissues are 20 to 40% of the values for liver and pancreas. Intermediate values for the enzyme activity are reported for brain, and low activities in heart, testes, muscle, prostate, spleen, and lung (84, 91, 250). AdoHcy hydrolase activity can also be demonstrated in the small intestine of the rat when precaution is taken to prevent deamination of adenosine catalyzed by the high level of adenosine deaminase in this tissue (92). Small variations in the enzyme activity are observed between different regions of the rat brain (30, 249).

There may be considerable species variations in AdoHcy hydrolase activity. The specific activity in homogenate of the rat liver is 5 times the activity found in human liver (290). Furthermore, the activity in the heart of the guinea pig is 20 times higher than the value determined for the heart of the dog (257).

The distribution of AdoHcy hydrolase in human tissues has not been systematically investigated. The enzyme has been demonstrated in human placenta (128), lymphoblasts (137), erythrocytes (136), fibroblasts (169), liver (290), and leukemia cells (125). These data, together with those cited above, suggest that AdoHcy hydrolase is ubiquitously distributed in animal tissues.

The tissue level of AdoHcy hydrolase can also be determined by a method based on the ability of the enzyme to form a stable complex with adenosine. The results obtained with this method are in accordance with those based on measurement of enzyme activity (298).

The specific activity of AdoHcy hydrolase purified to homogeneity from various tissues is of the same order of magnitude (81, 102, 216, 234, 299). Thus, the enzyme activity in crude extract may reflect the concentration of the enzyme in the tissue examined. The concentration of AdoHcy hydrolase in mouse liver has been calculated to

about 10 μM (298). Based on data on the specific activities in extract from various tissues (84, 91, 250, 298, 309), the concentration of this enzyme in most tissues can roughly be calculated to be in the range of 1 to 10 μM . This is in the same order of magnitude as the tissue level reported for adenosine (6) and AdoHcy (152, 252, 253). The metabolic implications of this relationship between AdoHcy hydrolase and its substrates (see section III C) probably apply to other tissues than mouse and rat liver. This suggestion should be related to the finding that the concentration of AdoHcy in tissues seems to parallel the tissue level of AdoHcy hydrolase (84).

AdoHcy hydrolase in rat liver seems to be under the influence of nutritional and hormonal factors. The specific activity of the enzyme increases in animals fed a high protein diet. Hydrocortisone and estradiol injections result in slightly increased enzyme activity, whereas thyroxine treatment is associated with a decrease in specific activity (91). In hormone-depleted rat prostate and testes after hypophysectomy, the AdoHcy hydrolase activity has been reported to decrease severely, but is restored after administration of testosterone (189). AdoHcy hydrolase activity in adrenals of the rat decreases after hypophysectomy, but dexamethazone does not restore the enzyme activity (315).

In rat liver, pyridoxine deficiency leads to a moderate increase in AdoHcy hydrolase activity, which seems to be associated with a block in the utilization of AdoHcy (86).

The level of the enzyme in the brain and liver of the rat does not fluctuate significantly during the life span (84, 152).

2. *Microorganisms and plants.* Knowledge on the occurrence of AdoHcy hydrolase in microorganisms and plants is mainly based on the work of Walker and Duerre (309). No bacteria possess AdoHcy hydrolase activity. The degradation of AdoHcy in bacteria is catalyzed by AdoHcy nucleosidase (73) and S-ribosylhomocysteine cleavage enzyme (198). The former enzyme has been purified from *Escherichia coli*, and has a specific activity which is about 1000 times greater than that of AdoHcy hydrolase (36, 73). This points to the importance of removal of AdoHcy in bacteria. It has been suggested that specific inhibitors of AdoHcy nucleosidase, not interacting with AdoHcy hydrolase, may be nontoxic antibacterial agents (36).

AdoHcy hydrolase has been found in various kinds of yeast (74, 79) and in plants (309), and has been purified from *Saccharomyces cerevisiae* (175) leaves of spinach beet (226), and to apparent homogeneity from yellow lupin seed (119).

3. *Subcellular.* Few data exist on the subcellular localization of AdoHcy hydrolase (100). Finkelstein and Harris (91) reported that the postmicrosomal supernatant of the rat liver contains almost all of the AdoHcy synthase activity, but a substantial amount is recovered in the nuclear fraction. AdoHcy hydrolase in human and rat liver is localized exclusively in the cytosol fraction. The activity recovered in the nuclear fraction could be ex-

plained by contamination of this fraction with soluble proteins (290). Likewise, AdoHcy hydrolase in the rat brain (30, 251) and the heart from various species (257) is a soluble protein. AdoHcy hydrolase can be demonstrated in the rat brain synaptosomes (30), which is in agreement with the fact that these structures contain cytoplasmic constituents (313).

F. Relation to Adenine Analogue Binding Proteins

A cyclic AMP binding protein not associated with protein kinase activity was demonstrated in rat liver by Chambaut et al. in 1971 (44). This report was followed by papers on similar proteins from other sources, including embryos of *Drosophila melanogaster* (284), rabbit and human erythrocytes (181, 317), mouse liver (291), rat and bovine tissues (268), bovine adrenal cortex (80), *Trypanosoma gambiense* (311), Jerusalem artichoke rhizome tissues (104), cow, dog (211, 212), and rat (87) heart, and bovine (312) and porcine (206) kidney. Most of these binding proteins have a molecular weight of about 200,000 and/or sediment as 9S (44, 80, 87, 104, 181, 268, 284, 291, 317). Adenosine binds to a site distinct from the cyclic AMP site (80, 156, 181, 268, 284, 291, 317). Sudgen and Corbin (268) suggested the name adenine-analogue binding protein for such proteins from rat and bovine tissues. The physiological role of this class of proteins remained unknown until Hershfield (128) and Hershfield and Kredich (134) demonstrated that adenosine binding proteins from human placenta, lymphoblasts, and spleen are associated with AdoHcy hydrolase activity. This finding has been confirmed for the cyclic AMP-adenosine binding protein from mouse liver (271) and bovine adrenal cortex (81). The question still remains whether adenine analogue binding proteins from other sources are identical to AdoHcy hydrolase. Near et al. (206) have described a 130,000 molecular weight protein from porcine kidney with binding characteristics that resemble those of adenine analogue binding proteins. The molecular weight of this protein is lower than the molecular weight of mammalian AdoHcy hydrolase (see section III G), and this protein may be another enzyme, for example glyceraldehyde 3-phosphate dehydrogenase (31, 32). The adenosine binding proteins from cow and dog heart described by Olsson et al. (211, 212) possess no AdoHcy synthase activity. Endrizzi et al. (87) have isolated a cyclic AMP-adenosine binding protein from rat heart. This protein has a molecular weight and subunit composition similar to those reported for AdoHcy hydrolase from other mammalian tissues. The binding protein can be separated from AdoHcy hydrolase by affinity chromatography (87). These data should perhaps be interpreted in light of the finding that AdoHcy hydrolase from different sources is inactivated in the presence of various naturally occurring purines, which include adenosine, 2'-deoxyadenosine (132, 137), AdoHcy (168), inosine (131), and adenine nucleotides (288, 299). Treatment of AdoHcy hydrolase from mouse liver with ATP does not affect the

molecular size of the enzyme, but changes the binding properties of the enzyme (288, 293). It is not unlikely that these changes are associated with altered behavior of the enzyme on an affinity column.

In conclusion, adenine analogue binding proteins, which show physicochemical properties resembling those of AdoHcy hydrolase, are probably identical to this enzyme. Enzymatically inactive binding proteins with such properties may represent AdoHcy hydrolase converted to an inactive form either in the intact cell or during the isolation procedure. However, the existence of enzymatically inactive adenosine binding proteins, which are phylogenetically closely related to AdoHcy hydrolase, or proenzymes devoid of AdoHcy hydrolase activity should be considered. Binding proteins for adenosine and its derivatives, with physicochemical properties which differ markedly from those of AdoHcy hydrolase, may represent various other enzymes involved in purine metabolism.

Binding proteins for cyclic AMP in mammalian tissues is the subject of a recent review article (82).

G. Properties

1. Physicochemical properties. Most data on the physicochemical properties of AdoHcy hydrolase are rather consistent. The enzyme purified to homogeneity from various mammalian tissues has a molecular weight of 180,000 to 190,000, a sedimentation coefficient of about 9S, and is composed of four subunits of 45,000 to 48,000 daltons (81, 102, 128, 215, 216, 234, 251, 268, 291, 296). In contrast, the adenine analogue binding protein from rabbit erythrocytes ($M_r = 240,000$ and subunit M_r of 48,000) (317) and AdoHcy hydrolase from calf liver ($M_r = 237,000$ and subunit M_r of 50,000–60,000) (234) seem to be proteins of slightly higher molecular weight and different subunit composition.

AdoHcy hydrolase, purified to apparent homogeneity from mouse liver, bovine liver, and bovine adrenal cortex in the same laboratory, has been subjected to gel filtration on a HPLC gel permeation column. These three enzymes eluted with exactly the same retention time (81). This method is characterized by high resolution and reproducibility (233). Thus, AdoHcy hydrolase from these sources has exactly the same Stokes radius. When the enzymes are analyzed by discontinuous polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate under conditions favoring high resolution, two bands of equal density can be distinguished (81). These data are consistent with a molecular composition of AdoHcy hydrolase given by the symbol $\alpha_2\beta_2$.

AdoHcy hydrolase contains tightly bound NAD^+ which participates in the catalytic cycle (see section III B). Enzyme bound NAD^+ was first demonstrated by Palmer and Abeles (215, 216) for the enzyme from beef liver, and has later been confirmed by others for the enzymes from calf liver (234), human placenta (128), and rat (102) and mouse (288) liver. About 1 mol of NAD^+ is bound per mol of enzyme subunit (102, 128, 216, 234,

288). The isoelectric point of AdoHcy hydrolase from mouse (291), rat (102, 168), calf (234) and bovine (81) liver, bovine adrenal cortex (81), and rat brain (251) is in the range from pH 5.35 to pH 6.0.

The enzyme purified to homogeneity from yellow lupin seeds (119) shows physicochemical properties that differ markedly from those of mammalian AdoHcy hydrolase. The plant enzyme has a molecular weight of 110,000, and is composed of two identical subunits of 55,000 daltons. The isoelectric point of this enzyme is 4.9, which is lower than that of mammalian AdoHcy hydrolase.

The data reviewed above, suggest that mammalian AdoHcy hydrolase from different sources shows nearly no variations with respect to molecular size, subunit composition, and charge. However, two isoelectric focusing variants have been observed in calf liver with pI values of 5.8 and 6.0 (234). Two enzymes can be separated in extract from bovine adrenal cortex by DEAE-cellulose chromatography. The predominating form focuses at pH 5.35, which is the same isoelectric point as that observed for the enzyme from bovine liver (81). The mouse liver enzyme focuses at pH 5.7 (81). The bovine enzymes can also be separated from the mouse liver enzyme by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (81). Thus, AdoHcy hydrolase may exist as isoenzyme forms, or the enzyme is modified to various degrees during extraction and purification. Comparative enzymology on AdoHcy hydrolase should be carried out with the view to explain different pharmacological responses of tissues to inhibitors of AdoHcy hydrolase (35, 36).

2. Kinetic properties. The kinetic properties of AdoHcy hydrolase are a matter of some controversy. Walker and Duerre (309) reported that K_m for adenosine of the rat liver enzyme was 1.5 mM. This value has later been corrected to 0.66 mM by the same authors (152). These data are somewhat in variance with those obtained by Finkelstein and Harris (91), who reported a K_m value of 0.25 mM for adenosine of the rat liver enzyme. Kajander et al. (167) using the same assay procedure and source of enzyme as Walker and Duerre, could not reproduce the

results of these authors. An apparent K_m value of 0.7 μM for adenosine was obtained (167), and this value has later been confirmed with homogenous enzyme for rat liver (168). Rather inconsistent data on the K_m values for AdoHcy hydrolase purified to homogeneity from mammalian tissues have been reported, i.e. K_m values for adenosine from 0.2 μM to 420 μM , and for AdoHcy from 0.75 μM to 60 μM (102, 216, 234, 251, 271, 299). The kinetic properties of AdoHcy hydrolase purified to homogeneity from mouse liver, bovine adrenal cortex, and bovine liver have been compared in the same laboratory. Exactly the same K_m values for adenosine (0.2 μM) and AdoHcy (0.75 μM) were obtained for these enzymes (81). Thus, different kinetic properties reported for AdoHcy hydrolase may, partly at least, be explained by modification of the enzyme during isolation. Furthermore, the presence of some factor(s) may be critical for the kinetic behavior of the enzyme in vitro.

Few data exist on the kinetic properties of AdoHcy hydrolase from sources other than mammalian tissues. The K_m values of the homogenous enzyme purified from yellow lupin seeds for adenosine and AdoHcy are 2.3 μM and 12 μM , respectively (119). The corresponding values for the enzyme from spinach beet are 13 μM and 41 μM (226).

The physicochemical and kinetic properties of AdoHcy hydrolase from various sources are summarized in table 1.

3. Binding properties. The adenosine-cyclic AMP binding factor from rabbit erythrocytes binds both adenosine ($K_d = 0.1 \mu\text{M}$) and cyclic AMP ($K_d = 0.3 \mu\text{M}$). These ligands seem to bind to different sites on the protein (317). Based on inhibition studies with 77 analogues of adenosine, Olsson (210) concluded that the adenosine site shows strict structural requirements, whereas the cyclic AMP site seems to be less restrictive. The binding of adenosine and cyclic AMP to AdoHcy hydrolase from mouse liver shows characteristics that are almost identical to those reported for the erythrocyte protein (291, 293). AdoHcy hydrolase from mouse liver (287) and adenine analogue binding proteins from rat

TABLE 1
Physicochemical and kinetic properties of S-adenosylhomocysteine hydrolase purified to homogeneity from various sources

Source	M_r	Subunits		pI	K_m for			References
		No.	M_r		Ado μM	AdoHcy μM	Hcy mM	
Rabbit erythrocytes	240,000	5	48,000					(317)
Beef liver	192,000	4	48,000		45	10.5		(215, 216)
Mouse liver	185,000	4	45,000–46,500	5.7	0.2	0.75	0.15	(81, 271, 291, 296, 299)
Yellow lupin seeds	110,000	2	55,000	4.9	2.3	12.0	4.6	(119)
Calf liver	237,000	4	50,000–60,000	5.8 6.0	420	63		(234)
Human placenta	190,000	4			0.5			(128)
Rat brain	180,000	4	48,000	5.6		36.6		(251)
Rat liver	220,000	5	47,000	6.6	0.6	0.90	0.06	(168)
Rat liver	188,000	4	47,000	5.7	1.05	15.2	0.155	(102)
Bovine adrenal cortex	185,000	4	45,000–46,000	5.35	0.2	0.75		(80, 81)
Bovine liver	185,000	4	45,000–46,000	5.35	0.2	0.75		(81)

and bovine tissue (268) also bind adenine nucleotides other than cyclic AMP. Whereas adenine interacts with the adenosine site only, AMP, ADP, and ATP bind to both the cyclic AMP and the adenosine site (287). The binding capacity of the cyclic AMP site is increased by a process termed activation, induced by adenine nucleotides (287, 292). This process is not associated with dissociation or polymerization of the enzyme (288, 291) or dissociation of enzyme bound NAD^+ (288), but conformational changes in the protein structure occurs as judged by altered reactivity of SH-groups (296). The observation that the so-called activation of the cyclic AMP site is associated with loss of catalytic activity (288) points to the possibility that this process is related to partial denaturation of the enzyme.

Adenine and adenine nucleotides competitively inhibit the synthesis and hydrolysis of AdoHcy catalyzed by the mouse liver enzyme (299). The effects of these compounds on enzyme catalysis parallels their inhibitory effect on adenosine binding (287), thus indicating that the adenosine binding site may be identical to the catalytic site (299). This possibility is supported by the finding that AdoHcy inhibits the adenosine binding to AdoHcy hydrolase from human placenta (128).

H. Gene Localization

Hershfield and Francke (133a), using human-Chinese hamster cell hybrids and monoclonal antibodies to human AdoHcy hydrolase, have localized the gene for AdoHcy hydrolase to chromosome 20. The gene for adenosine deaminase has also been mapped to this chromosome (303). It is suggested that the occurrence of the genes coding for these two enzymes on the same chromosome may have evolutionary significance. Adenosine deaminase catalyzes the catabolism of adenosine and 2'-deoxyadenosine and thereby creates conditions favoring AdoHcy hydrolase activity (see sections III A and IV B 1). Furthermore, adenosine deaminase occurs both in pro- and eukaryotes, whereas AdoHcy hydrolase is an eukaryotic enzyme. Evolution of AdoHcy hydrolase may have occurred after adenosine deaminase. The fact that AdoHcy hydrolase forms a stable complex with adenosine, binds adenine nucleotides, and contains tightly bound NAD^+ (see sections III A, III G 3 and III G 1) points to the possibility that evolution of the AdoHcy hydrolase gene involves recombination of part of the adenosine deaminase gene with a DNA sequence coding for NAD^+ and adenine nucleotide binding domain(s) (133a).

IV. Substrates, Inhibitors, and Inactivators of S-Adenosylhomocysteine Hydrolase

A. Introductory Data

Adenine toxicity to mammalian cells was first demonstrated by Aranow in 1961 (5). This discovery was followed by the observation that several adenine nucleosides are toxic to cells and are effective antitumor and/or

antiviral agents. These compounds include 3'-deoxyadenosine (cordycepin) (162), 2',3'-dideoxyadenosine (69), 9- β -D-arabinofuranosyladenine (ara-A) (162), 9- β -D-xylofuranosyladenine (83, 122), formycin (108), carbocyclic analogue of adenosine (\pm aristeromycin), N^6 -methyladenosine, nebularin (purine ribonucleoside), pyrazomycin (269), and tubercidin (3). Some of these compounds are naturally occurring nucleosides found in various organisms (269).

It has been suggested that adenosine toxicity requires phosphorylation of the nucleoside. This conclusion is based on studies with mouse fibroblasts (160) and Chinese hamster cells (193) with low levels of adenosine kinase and resistant to growth inhibition by adenosine. The observation by Ishii and Green (160) that adenosine toxicity is prevented by uridine suggests that adenosine interferes with pyrimidine synthesis. However, cells made resistant to adenosine-induced pyrimidine starvation by addition of exogenous uridine (180) or mutual loss of adenosine kinase, are sensitive to high concentrations of adenosine (138). Likewise, adenine is toxic to cells deficient in adenine phosphoribosyltransferase (138). Adenosine toxicity is enhanced by L-homocysteine and is associated with increased cellular levels of AdoHcy and inhibition of cellular methylation (111, 180). These data point to AdoHcy hydrolase as a target for adenosine toxicity (134).

The role of AdoHcy hydrolase in adenosine toxicity has been evaluated by Kamatani et al. (171) with mutant murine lymphoid cells partially deficient in AdoHcy hydrolase. Under conditions where the AdoHcy hydrolase reaction is directed toward synthesis of AdoHcy, i.e. in the presence of exogenous homocysteine and high concentration of adenosine, the mutant cells accumulate less AdoHcy and are less sensitive to the toxic effect of the drug combination than the parent cells. In contrast, a low concentration of adenosine alone, which is expected to exert only an inhibitory effect on AdoHcy hydrolase leading to an accumulation of AdoHcy derived from AdoMet, induces higher levels of AdoHcy in the mutant cells compared to the parent cells. Both cell lines are equally sensitive to the growth inhibitory effect of adenosine. Thus, in the murine lymphoblasts at least, only the toxic effect of high concentrations of adenosine in combination with homocysteine seems to be mediated by AdoHcy (171).

2'-Deoxyadenosine is a cytotoxic adenosine analogue (182), which seems to be particularly effective against lymphocytes (39). 2'-Deoxyadenosine (but not adenosine) inhibits [^3H]leucine incorporation during the first day of blastogenesis in phytohemagglutinin-stimulated lymphocytes (285). It has been suggested that the enzyme 2'-deoxyadenosine kinase catalyzing the phosphorylation of the nucleoside, is required for producing cytotoxicity (300). Incubation of stimulated lymphocytes with 2'-deoxyadenosine causes a dramatic elevation of intracellular levels of dATP that may inhibit the synthesis of DNA (273).

The antibiotic, ara-A, has oncostatic and antiviral properties, and has been evaluated as a chemotherapeutic agent in humans (42). The biological effects of ara-A have been attributed to its conversion to ara-ATP, which is an inhibitor of ribonucleotide reductase and some DNA polymerases (42), and the incorporation into DNA may inhibit the replication of DNA (283). A nucleotide independent mechanism of action of ara-A was first suggested by Trewyn and Kerr (281), showing that ara-A is a potent inhibitor of AdoHcy hydrolase from rat liver. These workers proposed that ara-A may block the degradation of AdoHcy and lead to inhibition of biological methylation (281).

Inhibition of biological methylation by adenosine analogues has been demonstrated. Adenosine and various adenosine analogues, including cordycepin, xylofuranosyladenine, tubercidine, 8-azaadenosine, and formycin are inhibitors of nuclear RNA methylation in intact cells (111-113, 266). 2'-Deoxyadenosine is without effect (111).

Investigation of the biological effects and mechanisms of action of adenosine analogues has been stimulated by the recent development of potent inhibitors of adenosine deaminase such as the tight binding inhibitor, 2'-deoxycoformycin (dCF), and the aliphatic alcohol analogue of adenine, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (109). Adenosine deaminase inhibitors potentiate the biological effects of adenosine analogues that are deaminated by this enzyme. For example, adenosine deaminase inhibitors block the conversion of ara-A to the inactive metabolite, 9- β -D-arabinofuranosylhypoxanthine, and have been shown to enhance the effects of ara-A in several cell systems and in vivo (42). Likewise, these inhibitors also block the degradation of adenosine, 2'-deoxyadenosine, cordycepin and xylofuranosyladenine, and they potentiate the biological effects of these agents (109). Potent adenosine deaminase inhibitors have renewed the interest in adenosine analogues as useful chemotherapeutic agents.

B. Adenosine Analogues

1. *2'-Deoxyadenosine.* Irreversible inactivation of AdoHcy hydrolase by 2'-deoxyadenosine was first demonstrated by Hershfield (132) for the enzyme from human lymphoblasts. This observation has later been confirmed for AdoHcy hydrolase from other sources (2, 50, 137). 2'-Deoxyadenosine binds tightly to the enzyme. The inactivation shows first-order kinetics and saturability. The enzyme is protected against inactivation by adenosine and AdoHcy, but not by homocysteine (132). These data suggest a suicide or K_{cat} mechanism of inactivation of AdoHcy hydrolase by 2-deoxyadenosine (310). A mechanism of action of 2'-deoxyadenosine based on knowledge of the catalytic mechanism of the enzyme has been provided by Abeles et al. (2). The inactivation of homogeneous AdoHcy hydrolase from bovine liver is accompanied by conversion of 2'-deoxyadenosine to adenine and irreversible reduction of enzyme bound NAD^+ . The authors suggest that 2'-deoxyadenosine is oxidized to 3'

keto-2'-deoxyadenosine by enzyme bound NAD^+ . The 3'-keto derivative eliminates adenine, and the enzyme bound NAD^+ is irreversibly reduced to NADH. The fate of the sugar moiety of 2'-deoxyadenosine is unknown (2).

2'-Deoxyadenosine has been shown to inactivate AdoHcy hydrolase in intact human erythrocytes (136) and human lymphoblasts in culture (135, 137). The kinetics of the inactivation process in cells shows similarities with the kinetics of this process in a cell-free system. A small fraction of the intracellular enzyme is not inactivated, probably because of protection of the enzyme by AdoHcy accumulating in the cells in the presence of 2'-deoxyadenosine (135). 2'-Deoxyadenosine also causes cellular build-up of dATP that inhibits ribonucleotide reductase and leads to depletion of deoxynucleotide substrates required for DNA synthesis. A human lymphoblastoid cell line deficient in adenosine kinase accumulates less dATP, but it is also resistant to inactivation of AdoHcy hydrolase. This is explained by accumulation of adenosine in adenosine kinase deficient cells, leading to protection of AdoHcy hydrolase. The role of dATP accumulation and AdoHcy hydrolase inactivation in cellular toxicity was evaluated by supplementing the cellular medium with deoxynucleosides. It was concluded that inactivation of AdoHcy hydrolase as well as dATP accumulation contribute to the toxicity of 2'-deoxyadenosine (135).

Mitchell et al. (202) reported on a 13-year-old boy with refractory acute T-cell lymphoblastic leukemia who was treated with the potent adenosine deaminase inhibitor, 2'-deoxycoformycin. Inhibition of adenosine deaminase gives increased plasma levels of 2'-deoxyadenosine, but not adenosine, accumulation of dATP in lymphoblasts and nearly total inhibition of lymphoblast AdoHcy hydrolase. The patient died 3 days after the therapy was discontinued, and postmortem examination revealed complete absence of leukemic cells in all organs. It was suggested that lysis of T lymphoblasts is mediated by 2'-deoxyadenosine (202). Inactivation of AdoHcy hydrolase resulting in accumulation of AdoHcy may contribute to this effect. This case report should stimulate the investigation of the possible use of compounds interfering with AdoHcy metabolism as chemotherapeutic agents in leukemia.

2. *9- β -D-Arabinofuranosyladenine.* Hershfield (132) showed that ara-A is even a more potent inactivator of AdoHcy hydrolase than 2'-deoxyadenosine. The kinetic characteristics of the inactivation process are similar for ara-A and 2'-deoxyadenosine. ara-A is a suicide inactivator of AdoHcy hydrolase (132). The mouse liver enzyme is protected against inactivation by ara-A in the presence of its substrates, adenosine and AdoHcy, but also by other purines interacting with the catalytic site of the enzyme, such as adenine, AMP, and ADP (123).

A mechanism of inactivation of AdoHcy hydrolase has been proposed, which resembles the mechanism of action of 2'-deoxyadenosine. The irreversible inactivation of the enzyme is associated with tight binding of ara-A (123),

conversion of bound ara-A to adenine, and reduction of enzyme bound NAD^+ to NADH (124, 130, 133). The formation of adenine is kinetically closely related to reduction of NAD^+ (124). The mechanism suggested involves enzymatic oxidation of ara-A to its 3'-keto-derivative by NAD^+ , as described for 2'-deoxyadenosine in the preceding paragraph. However, in the presence of L-homocysteine, S-(5'-(9-arabinofuranosyladenyl)-L-homocysteine (ara-AHcy) is formed at a rate that is about one half of the rate of inactivation, and the velocity of the inactivation process is reduced accordingly. It is concluded that reduction of NAD^+ and formation of adenine result from an abortive catalytic cycle, and the enzyme entering a complete cycle leading to formation of ara-AHcy, is not inactivated (124).

Inactivation of AdoHcy hydrolase by ara-A in intact cells, such as human lymphoblasts (137), human erythrocytes (136), mouse plasmacytoma cells, mouse L-cells, human chronic leukemia cells, and isolated cells of rat liver (125) has been demonstrated. The inactivation process shows kinetic characteristics resembling those observed in cell-free systems, i.e. first order kinetics, saturability, and half-maximal rate of inactivation at nearly the same level of ara-A. It is concluded that ara-A readily gains access to intracellular AdoHcy hydrolase. However, in contrast to cell-free systems the inactivation of intracellular AdoHcy hydrolase does not proceed to completion, in that 2% to 3% of the AdoHcy hydrolase activity in rat hepatocytes and some cultured cells is not inactivated. This is explained by protection of the intracellular enzyme by AdoHcy or possibly other metabolites. Furthermore, the observation that adenosine deaminase inhibitors exert essentially no effect on the kinetics of inactivation suggests that deamination of ara-A is not a major factor limiting the short-term effect of ara-A on AdoHcy hydrolase. The inactivation of the enzyme leads to a massive build-up of cellular AdoHcy, which is particularly pronounced in the hepatocytes, and the accumulation is associated with export of AdoHcy into the extracellular medium (125).

A process leading to reactivation of AdoHcy hydrolase in intact cells exposed to ara-A has been described (126). The reactivation is associated with a pronounced fall in the cellular content of AdoHcy. Reactivation of AdoHcy hydrolase in intact hepatocytes is stimulated by supplementing the extracellular medium with adenosine deaminase, which induces a decrease in the amount of adenosine and ara-A in the hepatocytes. On the other hand, the reactivation is markedly inhibited by the adenosine deaminase inhibitor, dCF, c^3Ado , and, to a lesser degree, by homocysteine. Inhibitor of protein synthesis and inosine were without effect. These data form the basis of the hypothesis that the reactivation mechanism is enhanced under cellular conditions favoring hydrolysis of AdoHcy. Alternatively, the ara-A-AdoHcy hydrolase complex is continuously reactivated and the reactivated enzyme is trapped by forming an inactive complex with

adenosine or adenosine analogues, which are substrates of adenosine deaminase (126).

The observation that both dCF and c^3Ado inhibit the reactivation process suggests that these compounds may potentiate the effect of ara-A on biological methylation *in vivo* (126).

The existence of a cellular process reactivating intracellular AdoHcy hydrolase inactivated by ara-A should probably be considered in light of the fact that naturally occurring nucleosides, such as adenosine, AdoHcy (137, 168), and 2'-deoxyadenosine (132) are inactivators of AdoHcy hydrolase. This process may protect the enzyme against metabolites converting the enzyme to an inactive form. It seems that a relation between tight binding of adenosine to AdoHcy hydrolase (section III C), and the reactivation process may exist. This possibility should be investigated.

AdoHcy hydrolase in various organs of mice treated by injection with ara-A is rapidly inactivated, and AdoHcy accumulates in these tissues. Especially high concentrations are obtained in the liver and kidney. In the absence of an adenosine deaminase inhibitor the enzyme activity gradually recovers and is nearly fully restored in most tissues within 8 to 10 hours. The increase in enzyme activity is not affected by cycloheximide and is partly inhibited by dCF. Inhibition of the reactivation process by dCF correlates with a massive accumulation of AdoHcy in organs of mice treated by injection with ara-A plus dCF, whereas only a slight increase was observed after injection with ara-A alone. These data support those obtained with isolated cells in suspension. Thus, dCF enhances the effect of ara-A on AdoHcy metabolism by blocking the reactivation of AdoHcy hydrolase *in vivo*. In addition, increased tissue levels of adenosine induced by dCF may also contribute to this effect (127).

An essential question related to the duration of the inhibition of AdoHcy catabolism in the presence of ara-A is the turnover of intracellular AdoHcy hydrolase. No investigation has been devoted to this subject. Preliminary studies in the author's laboratory indicate that the half-life of the enzyme is more than 12 hours in tissues of mice. However, precaution should be taken when measuring the half-life of AdoHcy hydrolase in the presence of toxic inhibitors of protein synthesis. These compounds may cause the accumulation of metabolites inactivating AdoHcy hydrolase.

AdoHcy hydrolase inactivation and AdoHcy accumulation have been demonstrated in erythrocytes from patients receiving ara-A treatment for virus infection (129, 240). The enzyme activity is partly restored 9 days after cessation of the treatment (240). As erythrocytes are incapable of protein synthesis, this may be explained by reactivation of the intracellular enzyme. Suppression of enzyme activity was also observed in mononuclear cells, but there are marked fluctuations with partial return of enzyme activity during therapy in these cells (240).

AdoHcy accumulation leading to inhibition of cellular methylation may account for the antiviral properties (129, 240) as well as some side effects of ara-A.

Recent studies with the ara-A analogue, 9- β -D-arabino-furanosyl-2-fluoroadenine (2-F-ara-A) have shed some doubt on the role of AdoHcy hydrolase as a mediator of the cytotoxic effects of these compounds. 2-F-ara-A, a nucleoside that is resistant to deamination, has cytotoxic effects against cultured cells comparable to that of ara-A plus dCF. The nucleoside triphosphate derivatives of both nucleosides are potent inhibitors of DNA polymerase and ribonucleotide reductase, but 2-F-ara-A is a weak inactivator of AdoHcy hydrolase. Furthermore, cells that have lost their capacity to phosphorylate these compounds are resistant to them. These data indicate that the cytotoxic effects of ara-A and 2-F-ara-A are related to inhibition of DNA synthesis (312a).

3. 3-Deazaadenosine. Chiang et al. (52) tested 43 analogues of AdoHcy with modifications in the purine, sugar, 5'-thioether linkage, and amino acid portion as substrates and inhibitors of AdoHcy hydrolase. It was found that S-3-deazaadenosyl-L-homocysteine (c^3 AdoHcy) is a good substrate for the enzyme (52). c^3 AdoHcy is hydrolyzed to L-homocysteine and c^3 Ado. c^3 Ado serves as a good substrate for the enzyme in the synthetic direction. The K_m value for c^3 Ado is about the same as the K_m for adenosine, and V_{max} is about 1.26 times higher than V_{max} for adenosine (234). c^3 Ado induces a drastic increase in the content of AdoHcy in isolated rat hepatocytes, and c^3 AdoHcy is formed in these cells (52). These data form the basis for the investigation of the biological and pharmacological properties of c^3 Ado (see section IV C).

4. Various nucleoside analogues. Biosynthesis of S- N^6 -methyladenosylhomocysteine from N^6 -methyladenosine and homocysteine was demonstrated by Hoffman (148) in crude extract from mouse liver. This observation has been confirmed by others by using intact cells (325) and homogenous enzyme (118). S- N^6 -Methyladenosylhomocysteine is formed in the liver of mice treated with injections of S- N^6 -methyladenosine (148). N^6 -Methyladenosylhomocysteine is a potent inhibitor of viral mRNA methylation (228) and tRNA methylation (185a, 279); this suggests that administration of N^6 -methyladenosine may block RNA methylation in vivo (148).

Periodate-oxidized nucleosides structurally resemble the proposed intermediate in the catalytic cycle of AdoHcy hydrolase, i.e. 3'-keto-adenosine (see section III B, fig. 2). These compounds were therefore tested as inhibitors of the enzyme (150). The periodate-oxidized derivatives of nonsubstrate nucleosides are relatively weak inhibitors, whereas derivatives of nucleosides known to function as substrates for the enzyme, adenosine, c^3 Ado, and N^6 -methyladenosine, are potent competitive inhibitors of the enzyme with K_i values three orders of magnitude less than K_m (150). The inactivation of AdoHcy hydrolase by adenosine dialdehyde and re-

lated compounds shows kinetic characteristics of affinity-labeling reagents, and are directed toward the active site of the enzyme. Adenosine dialdehyde binds tightly to the enzyme, and both the binding and the inactivation are prevented in the presence of adenosine and AdoHcy. Furthermore, the time-dependent inactivation shows saturability and seems to occur via a unimolecular reaction. Whereas the inactivation induced by ara-A and 2'-deoxyadenosine is an irreversible process in vitro (see section IV B, 1 and 2), reversibility of the adenosine-dialdehyde-induced inactivation can be demonstrated by dialysis of the enzyme against Tris-buffer (but not phosphate buffer). It is suggested that an amino acid residue at the catalytic site, possibly a lysinyl residue, forms a Schiff base adduct with the adenosine dialdehyde (20).

Injection of periodate-oxidized derivatives of adenosine into mice causes an increase in the AdoHcy content of the liver (151). Furthermore, the AdoHcy level in Friend erythroleukemic mouse cells increases markedly by supplementing the cellular medium with these adenosine derivatives. In both the intact liver and in the cells, the AdoMet-level increases by 50%; thus indicating inhibition of AdoMet-dependent transmethylations (149). Adenosine dialdehyde inactivates AdoHcy hydrolase and inhibits phospholipid methylation and carboxymethylation in mouse L-cells (20).

The antiviral agent, 9-((S)-2,3-dihydroxypropyl)-adenine ((S)-DHPA) is an aliphatic nucleoside analogue, which was found by Votruba and Holy (307) to be an inhibitor of isolated AdoHcy hydrolase from rat liver. The same workers have studied the interaction of aliphatic adenine derivatives containing hydroxyl groups in the aliphatic chain, so-called eritadenines, with rat liver AdoHcy hydrolase. Among these compounds, D-eritadenine is a potent inhibitor and inactivator of the enzyme (307a). It was proposed that some of the biological effects exerted by these agents may be mediated by inhibition of AdoHcy catabolism (307, 307a).

Numerous nucleoside analogues have been tested as substrates, inhibitors, and inactivators of AdoHcy hydrolase (50, 118, 137, 307, 325). In addition to adenosine and c^3 Ado, 2-aza-3-deazaadenosine, nebularine, and, to a lesser degree, formycin are good substrates for the isolated enzyme (118), and formation of the corresponding AdoHcy analogues in intact cells has been demonstrated (118, 325). Carbocyclic adenosine is a particularly potent competitive inhibitor of the purified enzyme, with K_i in the nanomolar range, whereas c^3 Ado and 8-aminoadenosine are less effective (118). Carbocyclic adenosine induces a marked elevation of AdoHcy in intact cells (118). 2-Chloro-adenosine has been reported to be a more potent inactivator of purified AdoHcy hydrolase than ara-A, which is followed by carbocyclic adenosine (\pm aristeromycin), pyrazomycin, 2-amino-6-chloro-purine riboside, nebularin, 2-chloro-ara-A, and 2'-deoxyadenosine in the order mentioned (50). These analogues inactivate the enzyme according to first order kinetics, and are classified

as type A analogues, which are distinguished from class B analogues (as for example adenosine-5'-carboxamide) that show a curvilinear inactivation (50).

Cordycepin (3'-deoxyadenosine) is an inactivator of isolated AdoHcy hydrolase (50, 137). This observation shows that inactivation of AdoHcy hydrolase by adenosine analogues does not in all instances involve oxidation of the 3'-hydroxyl group of the ribose residue. Inactivation of AdoHcy hydrolase by some analogues is not associated with reduction or loss of enzyme bound NAD⁺ (130).

The observation that cordycepin is an inactivator of AdoHcy hydrolase raises the possibility that interference with RNA metabolism and the cytotoxic effects observed in the presence of this compound stem from blocking the degradation of AdoHcy. However, cordycepin is not a potent inactivator of AdoHcy hydrolase (137), and this compound does not induce an accumulation of AdoHcy in WI-L2 cells (111). Experimental data provided by Glazer and Hartman (111) and Kredich (176) indicate that the inhibitory effect of cordycepin on the methylation of nuclear RNA is not mediated by AdoHcy hydrolase. Cordycepin is metabolized to the 3'-deoxyanalogue of AdoMet in the intact cell (176, 321), which may in turn be responsible for the inhibition of nucleic acid methylation.

Similar results are obtained with 9- β -D-xylofuranosyladenine. This adenosine analogue inhibits RNA methylation in murine erythroleukemia cells, and the inhibition is associated with accumulation of xylofuranosyl analogues of AdoMet and AdoHcy. 9- β -D-Xylofuranosyladenine is not a substrate of AdoHcy hydrolase, and adenosine kinase deficient cells accumulate small amounts of the xylofuranosyl analogue of AdoHcy. These data indicate that this adenosine analogue is phosphorylated to the corresponding triphosphate, which is further metabolized to its AdoMet and AdoHcy analogues. The latter two compounds may interfere with methyltransfer reactions (103a).

Several analogues of adenosine were tested by Zimmerman et al. (325) with respect to their ability to increase the AdoHcy level or be metabolized to their corresponding S-nucleosidylhomocysteine derivatives in mouse lymphocytes. It was concluded that base modified adenosine analogues such as c³Ado, 2-aminoadenosine, N⁶-methyladenosine, formycin A, N⁶-hydroxy-adenosine, purine ribonucleoside, and 8-aza-adenosine are condensed with L-homocysteine to form their corresponding AdoHcy analogue. Ribo-modified adenosine analogues (ara-A, 2'-deoxyadenosine, 5'-deoxyadenosine, and adenine) function as inhibitors of AdoHcy hydrolase, and detectable amounts of the corresponding AdoHcy analogues are not formed (325).

The concept of AdoHcy hydrolase as a pharmacological target for the development of chemotherapeutic agents (52, 132) has been exploited recently by Montgomery and coworkers (203a, b). With the view that some of

the properties of ara-A, c³Ado, and carbocyclic adenosine (see sections IV B, 2, 3, and 4 and IV C) might be combined, ara-3-deazaadenine (203a) and the carbocyclic analogue of c³Ado, 3-deaza-C-Ado (203b), were synthesized. Ara-3-deazaadenine is a mediocre inhibitor of AdoHcy hydrolase and reveals only moderate antiviral properties against herpes simplex type 1 virus (203a). In contrast, the results obtained with 3-deaza-C-Ado are more promising. This compound is not subjected to deamination or phosphorylation, is an effective competitive inhibitor of AdoHcy hydrolase, and induces AdoHcy accumulation in intact cells. The compound has antiviral properties at noncytotoxic concentrations (203b). The results obtained with 3-deaza-C-Ado suggest that knowledge on the structure-activity relationship of the interaction between adenosine analogues and AdoHcy hydrolase may be of great value for the synthesis of biologically active purines.

In conclusion, many adenosine analogues are inhibitors or substrates for isolated AdoHcy hydrolase. Some of these induce high cellular levels of AdoHcy and condense with L-homocysteine to form the corresponding S-nucleosidylhomocysteine derivatives. Thus, biological effects of some adenosine analogues may be related to inhibition of transmethylation reactions. The functions of adenosine analogues as substrates, inhibitors, and inactivators of AdoHcy hydrolase are summarized in table 2.

C. Biological and Pharmacological Properties of 3-Deazaadenosine

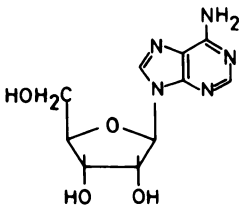
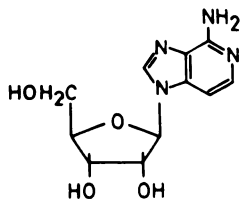
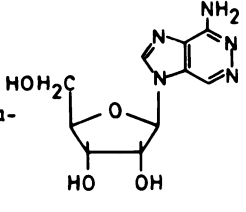
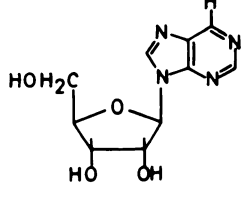
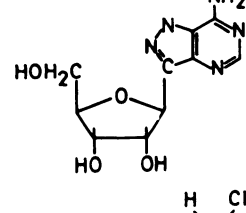
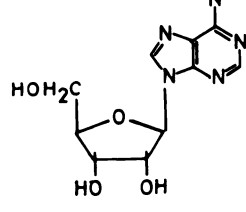
C³Ado is both a substrate and an inhibitor of isolated AdoHcy hydrolase. When injected into rats, c³Ado increases the tissue level of AdoHcy and AdoMet and causes a marked appearance of c³AdoHcy. These biochemical changes are associated with a decrease in the AdoMet/AdoHcy ratio and perturbation of various transmethylation reactions, i.e. reduction in the level of creatinine in the liver, decreased urinary excretion of 3-methoxy-4-hydroxymandelic acid and reduction in methylation of lipids (48).

Some tissue differences in the response to c³Ado have been observed. There is a 10-fold increase in the AdoHcy level in rat spleen and no alteration in the AdoMet level. In the heart, lung, and kidney of the rat, c³Ado induces no increase in the cellular content of AdoHcy and AdoMet, and only trace amounts of c³AdoHcy are formed. The tissue level of AdoHcy, AdoMet, and c³AdoHcy is high in the rat liver, whereas in the liver of hamster there is no increase in AdoHcy content, but the concentration of AdoMet increases and c³AdoHcy is formed (179). The differential response of various tissues to c³Ado has been related to different K_m/K_i of AdoHcy hydrolase from various sources (35).

Evaluation of the use of c³Ado as a pharmacological tool for the investigation of biological methylation reactions requires knowledge of effects and reactions of this

TABLE 2

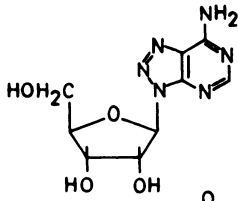
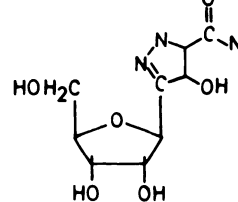
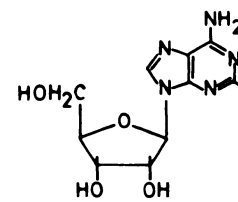
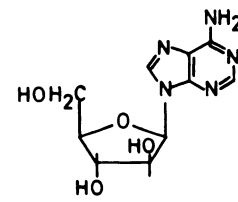
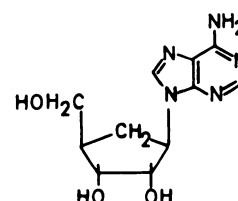
Adenosine analogues as substrates, inhibitors or inactivators of S-adenosylhomocysteine hydrolase

Name	Chemical structure	Substrate	Inhibitor	Inactivator	References
Adenosine		+++	+++	(+)	(50, 118, 117, 168, 325)
Base-modified adenosine analogues					
3-Deaza-adenosine		+++	+++	(+)	(50, 52, 118, 234, 325)
2-Aza-3-deaza-adenosine		+++	+		(118)
Nebularin		++	(+)	++	(50, 118, 325)
Formycin		+	+	(+)	(50, 118, 325)
N ⁶ -Methyl-adenosine		+	+	(+)	(50, 118, 148, 325)

compound in systems not related to the AdoHcy hydrolyase reaction. c^3 Ado is neither a substrate nor an inhibitor of adenosine deaminase (158) or adenosine kinase (199). No effect on DNA and protein synthesis and only a slight effect on RNA synthesis can be demonstrated in cells exposed to 0.1 mM c^3 Ado. Radioactive c^3 Ado is not

incorporated into nucleic acids (7). c^3 Ado is a weak inhibitor of adenylyl cyclase from human fibroblasts and neuroblastoma cells (48), and increases only slightly the cyclic AMP level in human and rabbit neutrophils (327). However, Zimmerman et al. (323) have reported recently that lymphocytes incubated with micromolar

TABLE 2—Continued

Name	Chemical structure	Substrate	Inhibitor	Inactivator	References
8-Aza-adenosine		+	+	(+)	(50, 118, 325)
Pyrazomycin		(+)	(+)	++	(50, 118)
2-Chloro-adenosine				+++	(50, 325)
Sugar-modified adenosine analogues					
Adenine-araboside		(+)	++	+++	(50, 118, 123, 124, 125, 130, 132, 133, 136, 137, 281, 325)
(±) Aristero-mycin		(+)	++++	+++	(50, 118)

concentrations of c^3 Ado (or adenosine) in a medium supplemented with homocysteine accumulate supramillimolar concentrations of c^3 AdoHcy (or AdoHcy). These cells exhibit an increased (5- to 40-fold) cyclic AMP response to prostaglandin E, adenosine, 2-chloroadenosine, isoproterenol, and cholera toxin. These effects have been explained by inhibition of cyclic AMP phosphodiesterase and amplification of adenylate cyclase activity (323). Likewise, c^3 Ado, alone or in combination with homocysteine, increases the cyclic AMP level in rat hepatocytes exposed to hormones. The effect could be explained by inhibition of cyclic AMP degradation by high level of c^3 AdoHcy and AdoHcy accumulating in these cells (248).

The enzymatic synthesis of phosphatidylcholine from

phosphatidylethanolamine (143) is particularly sensitive toward inhibition by c^3 Ado (48, 154). The conversion involves three stepwise methyl transfer reactions that seem to be catalyzed by two AdoMet-dependent methyltransferases (143). Various biological phenomena related to membrane function and structure, such as coupling of β -adrenergic receptors to adenylate cyclase, the number of available β -adrenergic binding sites, binding of benzodiazepines to its receptors, ATPase activity, leukocyte chemotaxis, histamine release from mast cells, and lymphocyte mitogenesis seem to depend on phospholipid methylation (143, 145). Inhibition of phospholipid methylation by c^3 Ado is associated with inhibition of histamine release induced by concanavalin A in rat mastcells (144) and immunoglobulin E mediated hista-

TABLE 2—Continued

Name	Chemical structure	Substrate	Inhibitor	Inactivator	References	
2'-Deoxy - adenosine				+	(2, 50, 118, 132, 135, 136, 137, 325)	
9-(S)-(2,3-dihydroxy - propyl) adenine				++	(307)	
D-Eritadenine				+++	+++	(307a)
Adenosine dialdehyde				+++	+++	(20, 149, 150, 151)
Base and sugar-modified analogues						
ara-3-deaza-adenine				+	(203a)	
Carbocyclic 3-dezaadenosine		(+)		+++	(203b)	

mine release, arachidonic acid release in rat leukemic basophils (64a, 65), and chemotactic response of human monocytes (225). Furthermore, c^3 Ado also decreases the number of β -adrenergic receptors on HeLa cells (143).

Phospholipid methylation and the biological processes are inhibited by concentrations of c^3 Ado at which nucleic acid and protein methylation are not affected (143).

The effect of c^3 Ado on phospholipid biosynthesis in

the liver from rat and hamster was investigated by Chiang et al. (51). The methylation of phospholipids or phosphatidylethanolamine in the liver is drastically reduced when c^3 Ado is injected into rat and hamster. The inhibition is associated with increased incorporation of choline into phosphatidylcholine, and the total amount of phospholipids in the liver remains constant (51). Similar findings have been made in vitro with cultured hepatocytes (53) and macrophages and neutrophils (224). It seems that phosphatidylcholine synthesis via CDP-choline, the so-called Kennedy pathway, and N-methylation of phosphatidylethanolamine are mutually regulated. The biosynthesis of phosphatidylcholine via CDP-choline may become de-repressed upon perturbation of phospholipid methylation by c^3 Ado (51, 53).

In hamster liver administration of c^3 Ado induces the appearance of c^3 AdoHcy and the tissue level of AdoHcy remains essentially unaffected (48); this suggests that c^3 AdoHcy and AdoHcy are about equally effective as inhibitors of phospholipid methylation in vivo (51). This suggestion is supported by in vitro data showing that c^3 AdoHcy is an effective competitive inhibitor of phospholipid methylation in cell-free systems (53, 245).

c^3 Ado is an inhibitor of phospholipid methylation in human platelets (155, 263). Stimulation of the platelets with agonists such as epinephrine, ADP, or thrombin decrease phospholipid methylation (262). The interpretation of these data from experiments with platelets is complicated by the finding that 3-deaza(\pm)aristeromycin, which inhibits phospholipid methylation in platelets, does not affect aggregation or secretion induced by stimulatory agents (53).

Differentiation of fibroblasts to fat cells is stimulated by c^3 Ado. This nucleoside may be a tool for studying the possible role of phospholipid methylation and other methyl transfer reactions in cellular obesity (47).

c^3 Ado inhibits, rapidly and reversibly, the synaptic response between retinal neurons and muscle fibers in culture (276). A role of biological methylation in neurotransmission is also indicated by the finding that low concentrations of c^3 Ado substantially enhance depolarization-dependent neurotransmitter release from cultured pheochromocytoma cells, and that the effect is potentiated by the simultaneous addition of homocysteine (230). However, no strict correlation between inhibition of phospholipid methylation or protein carboxymethylation and enhancement of transmitter release could be demonstrated. The small increase in cyclic AMP levels induced by c^3 Ado in these cells does not account for the effect on cellular exocytosis (231). These data from pheochromocytoma cells add a cautionary note to the use of c^3 Ado as a specific transmethylation inhibitor.

Inhibition of chemotaxis of rabbit neutrophils by c^3 Ado was demonstrated by O'Dea et al. (208). Inhibition of chemotaxis by c^3 Ado in a mouse macrophage cell line seems to be mediated by c^3 AdoHcy, and not AdoHcy. This conclusion is based on the observation that another

macrophage cell line, which is not sensitive to the inhibitory effect of c^3 Ado on chemotaxis, accumulates AdoHcy but not c^3 AdoHcy in response to c^3 Ado. Furthermore, c^3 AdoHcy is not formed in sonicates from these cells. In contrast, both AdoHcy and c^3 AdoHcy accumulate in intact macrophages sensitive to c^3 Ado, and c^3 AdoHcy is synthesized in broken cell preparation from responsive cells (3a). Phagocytosis by mouse macrophages is also inhibited by c^3 Ado, whereas the phagocytic function of human blood monocytes is not affected (187, 270).

Zimmerman et al. (324) suggested that c^3 Ado may be a valuable tool for studying the relationship between methylation reactions and leukocyte functions. c^3 Ado is a potent inhibitor of neutrophil chemotaxis and lectin-dependent neutrophil-mediated cytotoxicity. The inhibition is associated with cellular buildup of AdoHcy, c^3 AdoHcy, and AdoMet and is enhanced by the presence of L-homocysteine. Comparison of the dose response curves for these effects suggests that increased cellular levels of AdoHcy rather than c^3 AdoHcy is responsible for inhibition of neutrophil functions (327). The inhibition of lymphocyte-mediated cytotoxicity by c^3 Ado is associated with metabolic events similar to those described for the neutrophils, and the inhibition is more pronounced in the presence of homocysteine (324). Whereas inhibition of neutrophil functions roughly correlates with inhibition of carboxymethylation (327), the disparity between the dose response curves of c^3 Ado for inhibition of lymphocyte-mediated cytotoxicity and inhibition of carboxymethylation suggests the involvement of other methylation reactions in cytotoxicity (327). An elevated level of cyclic AMP is inhibitory to lymphocyte-mediated cytotoxicity. Thus, the increase in cellular level of cyclic AMP induced by c^3 Ado may contribute to its effect on lymphocyte function (326).

c^3 Ado (plus homocysteine thiolactone) inhibits the cytotoxicity of natural killer (NK) cells at concentrations where methylation of phospholipids, but not of protein and nucleic acid, is inhibited (153). The authors suggest that phospholipid methylation (and phospholipase A_2 activation) plays a role in the recognition function of NK cells (153).

Investigation into the antiviral properties of c^3 Ado was stimulated by the observation that analogues of AdoHcy inhibit virus replication (see section II C). Bader et al. (7) reported that c^3 Ado inhibits the reproduction of Rous sarcoma virus and malignant transformation of chick embryo cells by this virus. The reproduction of Sindbis virus, Newcastle disease virus, vesicular stomatitis virus (7), and Gross murine leukemia virus (49) in chick embryo cells is inhibited by c^3 Ado. Furthermore, c^3 Ado exhibits antiviral activity against Herpes simplex type 1 virus, simian virus 40, and RNA type C virus isolated from cultured human acute myelogenous leukemia cells (11). The oncogenic transformation induced by simian virus 40 and RNA type C virus is also inhibited by c^3 Ado (11). Recently, Trager et al. (278) have shown that c^3 Ado has antimalarial activity against *Plasmodium falciparum*.

parum in culture. These findings point to c^3 Ado as a useful chemotherapeutic agent in the future.

In conclusion, c^3 Ado seems to be a metabolically stable adenosine analogue that acts via the AdoHcy hydrolase reaction as an inhibitor of biological methylation. c^3 Ado may be a useful tool for studying the regulation and physiological role of methylation reactions. However, among the many effects exerted by c^3 Ado, some may not be related to inhibition of methylation, and the specificity of this compound as a transmethylase inhibitor should be questioned. The possibility exists that some effects of c^3 Ado are mediated by hitherto unrecognized metabolic products of this compound. Nevertheless, the wide variety of biological effects observed with c^3 Ado should stimulate further investigation of this compound as a drug for treatment of human diseases.

D. Metabolites

In addition to the substrates of AdoHcy hydrolase, various naturally occurring purines and other metabolites affect AdoHcy hydrolase, at least in vitro.

5'-Deoxy-5'-methylthioadenosine (MTA), a product formed from AdoMet during synthesis of the polyamines, spermidine, and spermine (314), is an inactivator of AdoHcy hydrolase from human placenta, lymphoblasts (137), and erythrocytes (90). The inactivation of the enzyme by MTA obeys first order kinetics, shows saturability with respect to MTA, and is irreversible. Furthermore, the enzyme is protected against inactivation by adenosine and AdoHcy (90). These data suggest an active-site-directed mechanism of action of MTA, as reported for other adenosine analogues (137). However, MTA is a less potent inactivator than 2'-deoxyadenosine (90, 137), and it remains to be established whether cellular effects of MTA (218, 302) are related to inactivation of AdoHcy hydrolase.

Adenine is a competitive inhibitor of AdoHcy hydrolase from mouse liver (299) and an inhibitor of the enzyme from human lymphoblasts (134) and rat brain (251). In addition, adenine induces a time dependent, reversible inactivation of the mouse liver enzyme (288, 299). The inactivation is partly inhibited by the presence of inorganic phosphate (288). The effect of adenine on AdoHcy hydrolase is in agreement with the finding that adenine increases the cellular level of AdoHcy in human lymphoblasts (178) and mouse lymphocytes (325). However, high concentrations of adenine are required to elevate the cellular level of AdoHcy. This may be explained by rapid metabolism of adenine by cells or by insensitivity of the intracellular enzyme toward adenine. Thus, the physiological implication of the effect of adenine on AdoHcy hydrolase remains to be defined.

Adenine nucleotides (AMP, ADP, and ATP) bind to AdoHcy hydrolase from mouse liver, and also competitively inhibit the enzyme catalysis (see section III G). These metabolites also induce an irreversible inactivation of the purified enzyme in vitro (288, 299). Both the

inhibition and inactivation of the enzyme are counteracted by inorganic phosphate (288), which points to the possibility that the effect of the nucleotides are an in vitro phenomenon.

The effect of inorganic phosphate on the response of purified AdoHcy hydrolase to adenine and adenine nucleotides is observed at low concentrations of phosphate, with half-maximal effect at 2 to 5 mM (288). Thus the possibility of phosphate as a modulator of AdoHcy hydrolase activity in vivo should be considered. Furthermore, the presence of phosphate should be taken into account when comparing in vitro data on AdoHcy hydrolase (288).

Data on the effect of AdoMet on AdoHcy hydrolase seem somewhat inconsistent. The finding of no inhibitory effect of AdoMet on the enzyme from calf liver (52) is not in agreement with the observation that AdoMet is a competitive inhibitor of the rat brain enzyme (251). Furthermore, it has been reported that AdoHcy hydrolase from rat liver is inhibited by AdoMet, and the inhibitor constant K_i for AdoMet equals K_m for AdoHcy. Based on this observation, a possible role of AdoMet in the regulation of AdoHcy hydrolase activity was suggested (281).

Inosine induces a reversible, phosphate dependent inactivation of AdoHcy hydrolase isolated from human placenta. Moderate inhibition of the enzyme in intact human erythrocytes and lymphoblastoid cells (131) has been demonstrated. In addition, inosine is a substrate for AdoHcy hydrolase purified from beef liver, plants (36), and human placenta (131). This reaction is very inefficient in that K_m for inosine is about 1000 times higher than for adenosine and V_{max} is 40 times smaller (36, 130). The product of the reaction, S-inosylhomocysteine, has been demonstrated to be an inhibitor of methyl transfer reactions, but this compound is a relatively weak inhibitor compared to AdoHcy and several other AdoHcy analogues (14, 58, 60, 320). Zimmerman et al. (325) have reported that S-inosylhomocysteine is formed in intact mouse lymphocytes exposed to exogenous inosine. The possible effects of S-inosylhomocysteine formation in cellular functions under normal and pathological conditions deserve further attention.

V. Inborn Errors of Purine Metabolism

Some defects of purine metabolism are associated with immunodeficiency disease. Severe combined immunodeficiency disease is a uniformly fatal inherited disorder characterized by failure of development of specific cellular and humoral immunity. Giblett and coworkers (105, 195) demonstrated in 1972 adenosine deaminase deficiency in certain individuals with this disease. Another inherited immunodeficiency disease with defects in cellular immunity and relatively normal humoral immunity has later been shown by Giblett and coworkers (106) to be linked to nucleoside phosphorylase deficiency. Some patients with the neurological disease, Lesch-Nyhan syn-

drome, have subtle abnormalities of B-lymphocyte function (4). The disease is associated with lack of purine salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase. Furthermore, an abnormally high level of adenine phosphoribosyltransferase activity has been found in erythrocytes from patients afflicted with this disease (259). The discovery of these abnormalities has encouraged the investigation of the role of purine metabolism in immunofunction. Immunodeficiency disease and defects of purine metabolism are subjects of recent reviews (146, 196, 303).

An increased level of adenosine in plasma has been reported in patients with adenosine deaminase deficiency (136, 200), and 2'-deoxyadenosine was demonstrated in the urine (264). The concentration of ATP in the erythrocytes is increased (200), but deoxyadenosine triphosphate and deoxyadenosine 5'-diphosphate seem to be the most abundant nucleotides in the erythrocytes from these patients (55, 56, 70). The abnormal deoxynucleotide metabolism (38) indicates interference with DNA synthesis (55). However, Simmonds et al. (264) suggested that 2'-deoxyadenosine may be a metabolite toxic to proliferating cells of lymphoid origin.

Data cited in section IV point to a nucleotide-independent mechanism for the toxic effect of adenosine and 2-deoxyadenosine. AdoHcy hydrolase seems to be a target enzyme for these metabolites (132). Hershfield et al. (136) demonstrated deficiency of AdoHcy hydrolase in the erythrocytes of three adenosine deaminase-deficient patients, thereby indicating a secondary inactivation of AdoHcy hydrolase. This observation was confirmed by Kaminska and Fox (169), who also found low AdoHcy hydrolase activity in erythrocytes from patients with purine nucleoside phosphorylase deficiency, and from patients lacking hypoxanthine-guanine phosphoribosyltransferase. Besides 2'-deoxyadenosine, no compounds known to accumulate in these metabolic disorders were found by these workers to inactivate AdoHcy hydrolase in extracts from erythrocytes. Furthermore, the enzyme shows a normal half-life in Lesch-Nyhan syndrome (169). However, inosine inactivates AdoHcy hydrolase in the presence of phosphate. This finding may offer an explanation for the AdoHcy hydrolase deficiency in purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase-deficient patients (131).

A lymphoblastoid cell line has been established from a patient with adenosine deaminase deficiency disease. These cells lack adenosine deaminase (ADA-cells), and contain about 60% of the AdoHcy hydrolase activity in adenosine deaminase positive lymphoblastoid cells (ADA+ cells). Cells from relatives of the patient are partially deficient in adenosine deaminase, but have normal AdoHcy hydrolase activity. The ADA-cells are more sensitive to the inhibitory effect of exogenous 2'-deoxyadenosine on AdoHcy hydrolase, cell growth, and immunoglobulin production than the ADA+ cells (283a). These data favor a role of AdoHcy hydrolase in the

pathogenesis of adenosine deaminase deficiency disease.

As a consequence of a secondary inactivation of AdoHcy hydrolase in certain immunodeficiency diseases, the concentration of AdoHcy in biological material, e.g. urine, should be determined in patients with these disorders. Preliminary data on this subject has recently been published (199a), but further data should be provided to clarify the diagnostic value of urinary AdoHcy level in immunodeficiencies.

VI. Summary and Conclusion

The product of the AdoMet-dependent transmethylation reactions, AdoHcy, is a potent negative feedback inhibitor of a number of transmethylases *in vitro*. Some synthetic analogues of AdoHcy are effective inhibitors of certain methylation reactions, and these compounds may exhibit antiviral and oncostatic properties. Inhibition of biological methylation is also observed in the presence of adenosine analogues, which may serve as inhibitors or substrates for AdoHcy hydrolase. This observation forms the basis of the concept of AdoHcy hydrolase as a target for adenosine analogues with cytotoxic effects. Perturbation of biological methylation may contribute to the antiviral and oncostatic properties of some adenosine analogues. AdoHcy hydrolase may be a useful enzyme for the design of chemotherapeutic agents in the future.

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