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# Inactivation of S-Adenosylhomocysteine Hydrolase by 9-β-D-Arabinofuranosyladenine in Intact Cells[1]

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## ABSTRACT

The inactivation of S-adenosylhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1) in isolated rat hepatocytes by 9-β-D-arabinofuranosyladenine (ara-A) was associated with tight binding of ara-A to the enzyme and showed an initial phase obeying first-order kinetics characterized by $K_i$ (concentration of half-maximal rate of inactivation) of 12 μM for ara-A and a maximal rate of inactivation of 0.7 $min^{-1}$. Two to 3% of the enzyme in rat hepatocytes was not available for inactivation. Similar results were obtained with some cultured cells, including mouse plasmacytoma cells (MPC-11), mouse fibroblasts (L-929), and human chronic myelogenic leukemia cells (K-562). In a cellular medium devoid of adenosine deaminase, inhibitors of this enzyme did not affect the inactivation process in rat hepatocytes and only slightly enhanced this process in the cultured cells (at low concentrations of ara-A). Inactivation of AdoHcy hydrolase in rat hepatocytes was associated with a massive build-up of AdoHcy (from 75 to 5200 pmol/$10^6$ cells after 3 hr of incubation) and a moderate increase in cellular S-adenosylmethionine. The accumulation of AdoHcy in the cultured cells exposed to ara-A was less pronounced and no increase in cellular S-adenosylmethionine was observed. There was a quantitatively important export of AdoHcy from the rat hepatocytes and the cultured cells into the extracellular medium, whereas no leakage of S-adenosylmethionine was detected. The inactivation of AdoHcy hydrolase by ara-A in rat hepatocytes was inhibited in the presence of adenosine or homocysteine in the cellular medium. This effect of homocysteine correlated with increased cellular level of AdoHcy induced by this agent but was also associated with reduction in cellular uptake of ara-A.

## INTRODUCTION

The biological effects of ara-A,[3] an oncostatic and antiviral agent (4), are enhanced in the presence of adenosine deaminase inhibitors, which block the degradation of ara-A to its inactive metabolite, 9-β-D-arabinofuranosylhypoxanthine (6). The effects of ara-A have been attributed to its conversion to 9-β-D-arabinofuranosyladenine 5'-triphosphate, which is an inhibitor of various enzymes involved in the synthesis of DNA (4).

A nucleotide-independent mechanism of action of ara-A is suggested by the findings that ara-A is a potent inactivator of AdoHcy hydrolase obtained from various sources (8, 10, 13). These data suggest that ara-A exerts some of its biological effects by blocking the metabolic degradation of AdoHcy (21), a product from and a potent inhibitor of AdoMet-dependent transmethylation reactions (3). This suggestion has been reinforced by the recent observation that ara-A, among other adenosine derivatives, increases the AdoHcy level in intact lymphocytes (26).

The present work is devoted to the short-term effects of ara-A on AdoHcy hydrolase and the metabolism of AdoHcy and AdoMet in rat hepatocytes and some cultured cells. The kinetic parameters of the inactivation of intracellular AdoHcy hydrolase by ara-A are similar to those reported for this process in cell-free systems (8, 10). However, many features of the inactivation process and the metabolism of AdoHcy and AdoMet in cells exposed to ara-A could not be derived from data obtained with an isolated enzyme. These features include: (*a*) protection of a fraction of the intracellular enzyme by metabolites; (*b*) association of AdoHcy accumulation in cells exposed to ara-A with a pronounced egress of AdoHcy from the cells; and (*c*) effect of accumulation of AdoHcy on cellular disposition of ara-A.

## MATERIALS AND METHODS

**Chemicals.** AdoHcy, DL-homocysteine, adenosine, HEPES, collagenase (type I), albumin (Fraction V), and ara-A were from Sigma Chemical Co., St. Louis, Mo. dCF and erythro-9-(2-hydroxy-3-nonyl)adenine were gifts from the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, Md., and Burroughs Wellcome Co., Research Triangle Park, N. C., respectively. [8-$^{14}$C]Adenosine (0.59 Ci/mmol) and [2,8-$^{3}$H]adenine-β-D-arabinoside (22 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. [8-$^{14}$C]AdoHcy (0.59 Ci/mmol) was synthesized enzymatically from [8-$^{14}$C]adenosine and purified as described elsewhere (24).

**Purification of AdoHcy Hydrolase.** The enzyme was purified to apparent homogeneity from mouse liver according to a published procedure (22).

**Assay for AdoHcy Hydrolase.** This was performed by a radiochemical method described elsewhere (24). The assay mixture used for the measurement of the synthetic reaction contained 50 to 200 μM [8-$^{14}$C] adenosine and 3 mM DL-homocysteine. The temperature was 37°.

**Preparation of Isolated Rat Liver Hepatocytes.** Hepatocytes were prepared by a slight modification (18) of the collagenase perfusion method described by Berry and Friend (2). Cell viability (18) was higher than 95% at the start of the experiments.

**Cell Lines and Culture Conditions.** Mouse plasmacytoma cell line MPC-11 and mouse fibroblasts L-929 were grown in suspension culture in a mixture of an equal volume of RPMI 1640 and Dulbecco's modified Eagle's medium containing 13% heat-inactivated horse serum and 20 μg gentamicin per ml. The cultures were fed routinely every 24 hr by replacing one-half of the volume of the culture with an equal volume of fresh medium. The human chronic myelogenic leukemia K-562 cell line was grown in stationary suspension culture in RPMI 1640

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[3] The abbreviations used are: ara-A, 9-β-D-arabinofuranosyladenine; AdoHcy, S-adenosyl-L-homocysteine; AdoMet, S-adenosyl-L-methionine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; dCF, 2'-deoxycoformycin; RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640.

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supplemented with 25 mM HEPES, 10% fetal calf serum, 100 IU penicillin per ml, 100 μg streptomycin per ml, and 30 μg Fungizone per ml. The cells were subcultured twice a week at a cell density of $2 \times 10^5$ cells/ml.

**Incubation Conditions.** The isolated rat hepatocytes and the cultured cells were incubated in the same medium, when not otherwise indicated. The composition of the medium was as follows: 137 mM NaCl; 5.37 mM KCl; 0.81 mM $MgSO_4$; 4 mM $CaCl_2$; 0.34 mM $Na_2HPO_4$; 0.35 mM $KH_2PO_4$; 2% bovine serum albumin; 5 mM glucose; streptomycin (250 μg/ml); and penicillin (30 μg/ml) in 20 mM HEPES, pH 7.4 (18). In some experiments, the cultured cells, L-929 cells, were incubated in the culture medium, a mixture of an equal volume of RPMI 1640 and Dulbecco's modified Eagle's medium containing 13% heat-inactivated horse serum and 20 μg gentamicin per ml. The temperature was 37°, and the incubation was performed in 10- to 50-ml Erlenmeyer flasks placed in a shaking water bath. The cell suspensions were preincubated for 30 min before initiation of the experiments.

**Inactivation Experiments.** The incubations were started by the addition of 0.5 ml containing ara-A or other effectors to cell suspensions (2.0 ml), terminated by layering samples of 200 μl onto 700 μl of dinonyl phthalate:dibutyl phthalate (1:3) in 1.5-ml polyethylene microcentrifuge tubes, and centrifuged at 7000 rpm for 15 sec in a Beckman 152 microfuge. The supernatant and oil were immediately removed by suction, and the cell pellet was resuspended in ice-cold buffer. The composition of this buffer was 80 mM potassium phosphate, pH 7.0, containing 20% glycerol, 2 mM dithiothreitol, 3 mM DL-homocysteine, and 0.5% Triton X-100, when not otherwise indicated. The cellular extract was immediately frozen in liquid nitrogen and stored at −20° for 24 hr (overnight) before being assayed for AdoHcy synthase activity.

**Uptake Experiments.** The experimental design was the same as that described above except that [$^3$H]ara-A (2.2 Ci/mmol) and 0.4-ml polyethylene tubes containing 200 μl oil were used. Samples of 100 μl were removed from the incubation mixture at various times, and the cells were separated from the medium by centrifugation through the oil. The radioactivity associated with the cells was determined as described elsewhere (1).

**Determination of Cellular AdoHcy and AdoMet.** Samples of 1 ml of cell suspension (1.5 to $6 \times 10^6$ cells/ml) were centrifuged at 7000 rpm for 15 sec in a Beckman 152 microfuge, and the cell pellet was immediately homogenized in 150 μl ice-cold 5% sulfosalicylic acid. The cellular extracts were centrifuged for 5 min at 100,000 × *g* using a Beckman Air-fuge. The supernatants were stored at −80° and analyzed (20 μl) within 48 hr. AdoHcy and AdoMet were determined by high-pressure liquid chromatography using a Spectra Physics SP 8000B Liquid Chromatograph, a Partisil 10 SCX column, and a Model SP 8300 UV detector, recording the absorbance at 254 nm. The column was eluted isocratically at ambient temperature at a flow rate of 2 ml/min. The mobile phases were 100 mM ammonium phosphate, pH 2.3, and 500 mM ammonium phosphate, pH 2.6, for the determination of AdoHcy and AdoMet, respectively.

AdoHcy was also determined by analysis of sulfosalicylic acid extract, neutralized to pH 7.5 by the addition of 2 N Tris, on a 3 μM ODS Hypersil column (0.5 x 10 cm; 9,000 to 11,000 theoretical plates). The same liquid chromatograph and detector was used as above. The column was eluted isocratically at a flow rate of 1 ml/min in a constant-pressure mode (1840 psi) with 7.7% methanol in 9 mM potassium phosphate buffer, pH 6.0.

**Determination of Extracellular AdoHcy (and AdoMet).** Samples of 225 μl from the cellular medium from which the cells were removed by centrifugation were mixed with 25 μl of 50% sulfosalicylic acid. Precipitated protein was removed by centrifugation, and aliquots of 50 μl were analyzed by high-pressure liquid chromatography as described in the preceding paragraph.

**Determination of [$^3$H]ara-A Binding to Intracellular AdoHcy Hydrolase.** Isolated rat hepatocytes were incubated with [$^3$H]ara-A (2.2 Ci/mmol) for increasing periods of time (1 to 30 min), and the incubation was terminated by centrifugation of samples (200 μl) of cell suspension for 15 sec at 9000 × *g*. The cell pellet was immediately homogenized in ice-cold 15 mM HEPES, pH 7.4, containing 10 mM adenosine, 10 mM 2-mercaptoethanol, and 0.5% Triton X-100 and then centrifuged for 10 min at 100,000 × *g*. Samples (20 μl) from the supernatant were subjected to analysis on a I-250 protein column (Waters Associates, Inc., Milford, Mass.) eluted with 15 mM HEPES, pH 7.4, containing 150 mM KCl and 10 mM 2-mercaptoethanol at a flow rate of 0.8 ml/min. The same liquid chromatograph and detector were used as above, and the absorbance was recorded at 280 nm. A fraction collector was coupled in series after the detector, and fractions of 0.24 ml were collected. The radioactivity in the separate fractions was determined by liquid scintillation counting.

## RESULTS

**Extraction of AdoHcy Hydrolase from Intact Cells.** Inactivation of homogenous AdoHcy hydrolase by 100 μM ara-A at 37° proceeded at a rate of 0.43 $min^{-1}$ (8), whereas no inactivation of the enzyme by 100 μM ara-A was observed after 40 min of incubation at 0° (data not shown). Based on this observation, the inactivation of AdoHcy hydrolase by ara-A was terminated by separation of the cells from the medium (by centrifugation) in precooled tubes, and the cell pellet was immediately homogenized in ice-cold buffer.

The suitability of various buffers for the extraction of AdoHcy hydrolase from rat hepatocytes was evaluated by determining the recovery of endogenous activity in the extract and the recovery of purified enzyme (exogenous enzyme) added to the extract. For example, 15 mM HEPES buffer, pH 7.0, containing 10 mM mercaptoethanol, 20% glycerol, and 0.5% Triton gave low recovery (1 to 2%) of both endogenous and exogenous enzyme activity in extract from hepatocytes stored at −20° for 24 hr, whereas high recovery (about 70%) was obtained with 80 mM phosphate buffer, pH 7.0, containing 2 mM dithiothreitol, 3 mM DL-homocysteine, 20% glycerol, and 0.5% Triton X-100. The latter buffer, referred to as extraction buffer, was used routinely. Under these conditions, the progress curves for the inactivation of intracellular AdoHcy hydrolase by ara-A were not obscured by inactivation or inhibition of AdoHcy hydrolase by ara-A, its metabolites (8), or other cellular factors after cellular lysis. This statement is based on the observation (Chart 1*A*) that the recovery of exogenous enzyme added to the extraction buffer was independent of the time of cellular exposure to ara-A. The same results were obtained with cultured cells (data not shown).

**Characterization of the Inactivation of AdoHcy Hydrolase by ara-A in Intact Cells.** The inactivation of AdoHcy hydrolase in rat hepatocytes by ara-A showed an initial phase characterized by first-order kinetics and saturability with respect to ara-A. Half-maximal rate of inactivation was observed at 12.3 ± 2.2 (S.D., $n = 6$) μM ara-A. The maximal rate of inactivation was 0.70 ± 0.20 $min^{-1}$ (Chart 1*B*). In a cellular medium devoid of adenosine deaminase activity, there was no effect of 1 μM dCF (Chart 1*B*) or 10 μM erythro-9-(2-hydroxy-3-nonyl)adenine on the inactivation process (data not shown). Adenosine deaminase in the cellular medium (1.6 units/ml) almost completely blocked the inactivation of AdoHcy hydrolase in the hepatocytes by ara-A, but the effect of ara-A was restored by inclusion of 1 μM dCF in the medium (Chart 1*C*).

A fraction of AdoHcy hydrolase in rat hepatocytes (about 2.5%) was not available for inactivation by ara-A (Chart 1, *A*, *B*, and *C*). This residual activity was not decreased when the

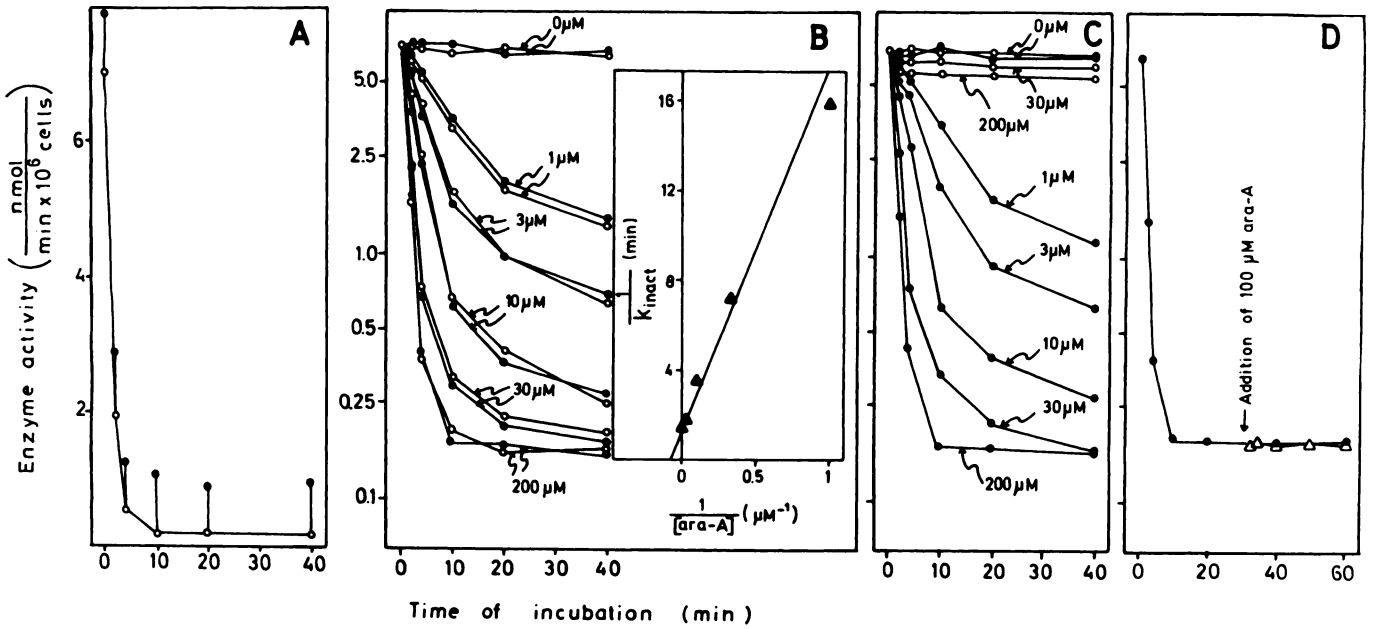


Chart 1. Time course for the inactivation of AdoHcy hydrolase by ara-A in rat hepatocytes. In *A*, rat hepatocytes ($4.8 \times 10^6$ cell/ml) were incubated for increasing periods of time with 100 μM ara-A (and 1 μM dCF). The cell suspensions were centrifuged, and the pellet was homogenized in extraction buffer (○) or extraction buffer supplemented with purified AdoHcy hydrolase (0.80 μg/ml) (●). In *B*, the progress curves for the inactivation of AdoHcy hydrolase were determined at various concentrations of ara-A in the absence (○) and presence (●) of dCF (1 μM). The cells were preincubated for 30 min with dCF before addition of ara-A. Note log scale. *Inset*, double-reciprocal plot for the rate constants of inactivation (initial velocity) *versus* the concentration of ara-A. In *C*, inactivation of intracellular AdoHcy hydrolase in rat hepatocytes suspended in a medium containing adenosine deaminase (1.6 units/ml) was determined in the absence (○) and presence (●) of 1 μM dCF. The experimental design was as described under *B*. In *D*, the inactivation of AdoHcy hydrolase in rat hepatocytes in the presence of 100 μM ara-A (and 1 μM dCF) was determined (●). A second addition of ara-A (100 μM) was made to a portion of the incubation mixture after 30 min of incubation (*arrow*). The enzyme activity of these cells are shown on the chart (△).

cell suspension was supplemented with 100 μM ara-A at the time (30 min) when the inactivation process had come to a halt (Chart 1*D*).

The kinetic parameters for the inactivation of AdoHcy hydrolase in MPC-11 cells, L-929 cells, and K-562 cells were the same but differed slightly from those for the inactivation of the enzyme in rat hepatocytes. Half-maximal rate of inactivation was observed at 25 ± 5 μM ara-A, and the maximal rate of inactivation was 0.53 ± 0.21 $min^{-1}$, when determined in the presence of 1 μM dCF. A fraction of AdoHcy hydrolase (0.6 to 2% of the enzyme activity in nontreated cells) was not available for inactivation by ara-A (data not shown).

The adenosine deaminase inhibitor, dCF (1 μM), increased slightly the rate and extent of inactivation of AdoHcy hydrolase in these cultured cells at low concentrations (<10 μM) of ara-A (data not shown).

**Binding of [$^3$H]ara-A to Intracellular AdoHcy Hydrolase.** Rat hepatocytes were incubated with [$^3$H]ara-A, and the binding of the nucleoside to intracellular AdoHcy hydrolase was determined by subjecting cellular extracts to analysis on a high-pressure liquid chromatographic protein column as described in "Materials and Methods." A radioactive peak was obtained with exactly the same retention time as homogeneous AdoHcy hydrolase (Chart 2). The height of this peak was not increased when purified AdoHcy hydrolase (100 μg/ml) was added to the cellular extract, suggesting that [$^3$H]ara-A did not bind to the enzyme after cellular disrupture.

The time course of the binding of [$^3$H]ara-A to intracellular AdoHcy hydrolase was determined at various concentrations of [$^3$H]ara-A (1 to 200 μM). Half-maximal binding of [$^3$H]ara-A to AdoHcy hydrolase was observed at times corresponding to the half-life of the enzyme activity at the same concentration of ara-A (data not shown). These data show a correlation between the binding of ara-A to and inactivation of AdoHcy hydrolase in intact hepatocytes.

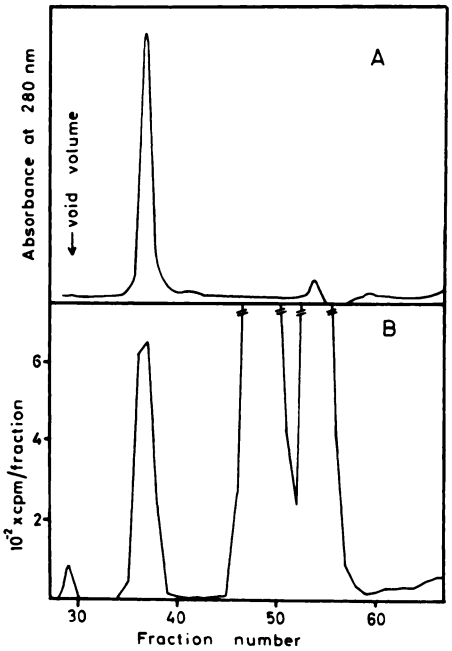


Chart 2. High-pressure liquid chromatogram of homogeneous AdoHcy hydrolase and cytosol from rat hepatocytes incubated with [$^3$H]ara-A. *A*, elution profile of homogeneous AdoHcy hydrolase (20 μl; 1 mg/ml) chromatographed on a high-pressure liquid chromatographic I-250 protein column as described in "Materials and Methods." *B*, distribution of radioactive material of cytosol from rat hepatocytes incubated with 20 μM [$^3$H]ara-A for 10 min. The same chromatographic system was used as above.

**Cellular Level of AdoHcy and AdoMet.** After a lag phase of 2 to 5 min, the amount of AdoHcy in rat hepatocytes exposed to 100 μM ara-A (and 1 μM dCF) increased almost linearly for at least 3 hr. The cellular content of AdoHcy in hepatocytes not exposed to ara-A and cells incubated with ara-A for 3 hr was about 75 and 5200 pmol/$10^6$ cells, respectively. Only a moderate increase in cellular AdoMet was observed (Chart 3). Accordingly, the [AdoMet]:[AdoHcy] ratio changed from about 6 to 0.2.

The metabolic response of the cultured cells used in this study to ara-A (100 μM) was somewhat different from the response of hepatocytes, in that the increase in cellular AdoHcy was less pronounced. Furthermore, the accumulation of AdoHcy leveled off after 1 to 2 hr of exposure to ara-A, and no increase in cellular AdoMet was observed (Chart 3). The [AdoMet]:[AdoHcy] ratio was in the range 0.3 to 0.5 after 3 hr of incubation with ara-A.

**Egress of AdoHcy from Intact Cells.** There was a time-dependent increase in the amount of AdoHcy in the extracellular medium of rat hepatocytes, MPC-11 cells, and L-929 cells accumulating AdoHcy during treatment with ara-A (100 μM) and dCF (1 μM). The progress curves for the accumulation of extracellular AdoHcy were characterized by a slow phase for the first hr, followed by a rapid increase in the amount of AdoHcy. After 3 hr of cellular exposure to ara-A, the amount of AdoHcy secreted into the extracellular medium was of the same order of magnitude as the amount retained within the cells. No leakage of AdoMet was observed (data not shown).

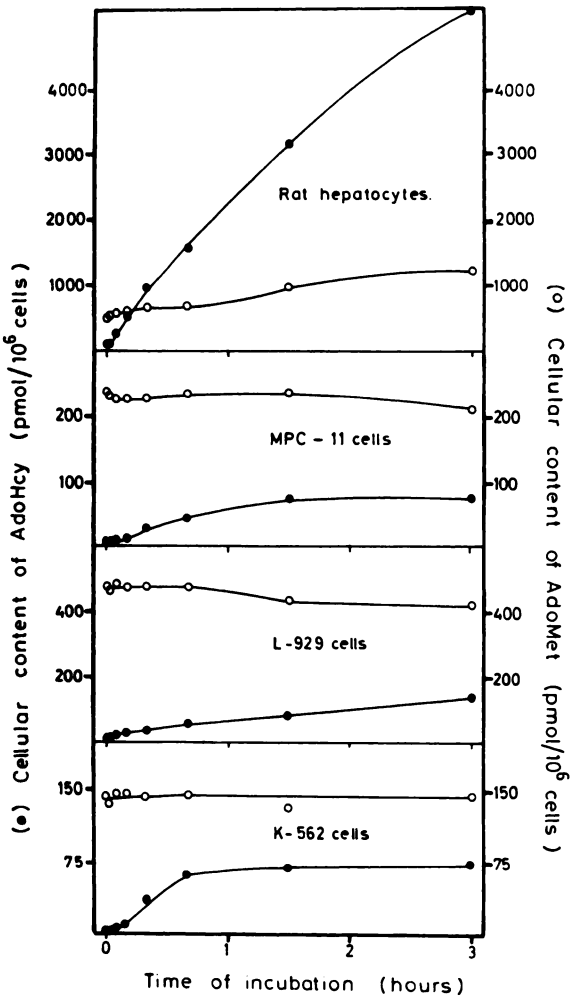


Chart 3. Effect of ara-A on cellular content of AdoHcy and AdoMet. Suspensions of rat hepatocytes (5 × $10^6$ cell/ml), MPC-11 cells (6 × $10^6$ cells/ml), L-929 cells (4.6 × $10^6$ cells/ml), and K-562 cells (1.5 × $10^6$ cells/ml) were preincubated for 30 min with 1 μM dCF and then incubated for increasing periods of time in the presence of 100 μM ara-A. The amount of cellular AdoHcy and AdoMet is plotted as a function of time of incubation of the cells with ara-A.

Hepatocytes were exposed to ara-A (plus dCF) for 30 min. At this time point, homocysteine (3 mM) was added to the cells, and the cell suspension was supplemented with this amount of homocysteine at 1-hr intervals. Both the AdoHcy and AdoMet content in hepatocytes so treated increased for about 1 hr to extremely high levels [13500 (AdoHcy) and 4800 pmol/$10^6$ cells (AdoMet)] and then remained nearly constant (for at least 4 hr). The amount of AdoHcy secreted by these cells increased in proportion to the increase in intracellular AdoHcy and equaled the intracellular amount after 3 to 4 hr, corresponding to an extracellular concentration of about 30 μM. Only trace amounts of AdoMet were found in the extracellular medium (data not shown).

The possibility existed that AdoHcy egress from cells was related to cell damage of the cultured cells caused by replacement of the culture medium with the incubation medium (see "Materials and Methods"). Therefore, cellular export of AdoHcy from L-929 cells exposed to ara-A was determined for cells in the culture medium. The same results were obtained under these conditions, except that the cellular content of both AdoMet and AdoHcy and the amount of AdoHcy secreted by the cells were about twice the corresponding values obtained with these cells suspended in the incubation medium. The results of this experiment are shown in Chart 4.

**Effect of Adenosine and Homocysteine on Inactivation of AdoHcy Hydrolase in Rat Hepatocytes.** When the suspension of hepatocytes was supplemented with 0.5 mM adenosine, the inactivation of AdoHcy hydrolase by 100 μM ara-A (and 1 μM dCF) was almost completely inhibited. The protection of the enzyme was not dependent on preincubation of the cells with adenosine. The cellular level of AdoHcy was increased to 1900 pmol/$10^6$ cells after 30 min in the presence of adenosine (0.5 mM).

In contrast, intracellular AdoHcy hydrolase was also protected against inactivation when the medium contained 3 mM DL-homocysteine, but the effect was dependent on the time of preincubation of the cells with homocysteine. The rate of inactivation of AdoHcy hydrolase by 100 μM ara-A (and 1 μM dCF) was 0.7, 0.34, and 0.10 $min^{-1}$ when the time of preincubation (with homocysteine) was 0, 5, and 30 min, respectively. The corresponding values for intracellular AdoHcy were 75 (0 min), 660 (5 min), and 3300 pmol/$10^6$ cells (30 min). Furthermore, both the inactivation process and cellular level of AdoHcy were essentially unaffected by 0.3 M DL-homocysteine (data not shown). These data suggest a relation between cellular build-up of AdoHcy and protection of AdoHcy hydrolase against inactivation.

Uptake of ara-A into rat hepatocytes was characterized by a rapid initial phase followed by slow uptake. Somewhat unexpectedly, it was observed that the second slow phase was inhibited by the presence of homocysteine (3 mM), and the inhibition was enhanced by preincubating the cells with homocysteine (Chart 5).

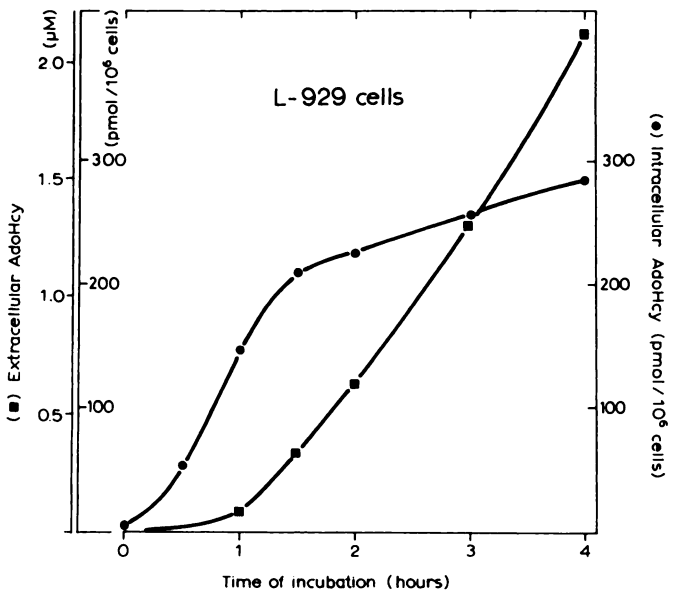


Chart 4. Egress of AdoHcy from L-929 cells exposed to ara-A. L-929 cells ($5.3 \times 10^6$ cells/ml), incubated in the culture medium, were preincubated for 30 min with 1 μM dCF. At this time point, the cell suspension was made 100 μM in ara-A, and the amount of intracellular (●) and extracellular (■) AdoHcy was determined as a function of time of cellular exposure to ara-A.

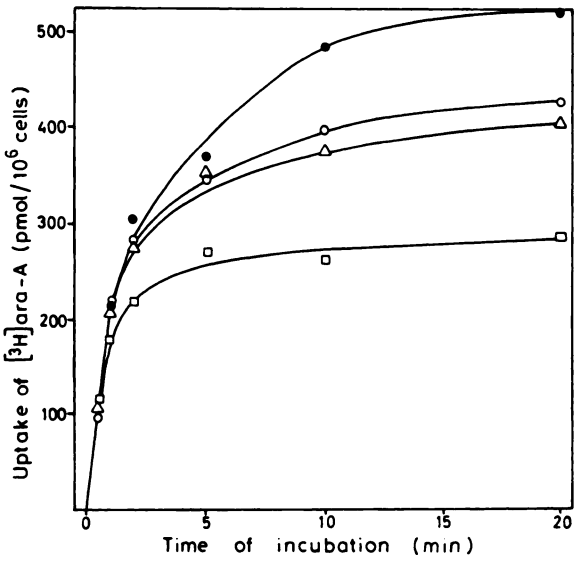


Chart 5. Uptake of [$^3$H]ara-A by rat hepatocytes preincubated with homocysteine. Rat hepatocytes ($2.8 \times 10^6$ cells/ml) were preincubated for 0 (○), 5 (△), and 30 min (□) with 3 mM DL-homocysteine. At these time points, the cell suspension was made 20 μM in [$^3$H]ara-A. The uptake of [$^3$H]ara-A was determined and compared with the uptake curve obtained in the absence of homocysteine (●).

## DISCUSSION

The kinetic parameters of the inactivation of AdoHcy hydrolase by ara-A in rat hepatocytes differ somewhat from those of the inactivation process in the cultured cells used in this study. This could be explained by different properties of AdoHcy hydrolase from these cells or by cellular factors affecting the inactivation. Nevertheless, the kinetic characteristics of ara-A-induced inactivation of the enzyme in intact cells resemble those of the inactivation by ara-A of the purified enzyme from various sources (8, 10, 13). Similar data have been obtained for the inactivation of AdoHcy hydrolase by 2′-deoxyadenosine in cell-free systems and in intact lymphoblasts (12). These observations suggest that 2′-deoxyadenosine (12) and ara-A gain access readily to intracellular AdoHcy hydrolase. Furthermore, the finding that adenosine deaminase inhibitors do not affect (Chart 1*B*) or only slightly enhance the inactivation of intracellular AdoHcy hydrolase by ara-A indicates that intracellular deamination of ara-A is not a major factor limiting the short-term effects of ara-A related to inactivation of AdoHcy hydrolase. In contrast, when the extracellular medium contains high level of adenosine deaminase, the presence of inhibitors of this enzyme is required for the interaction of ara-A with intracellular AdoHcy hydrolase (Chart 1*C*). This observation should perhaps be related to the finding that adenosine deaminase inhibitors enhance the effect of ara-A on AdoHcy hydrolase in intact animals.[4]

The inactivation of AdoHcy hydrolase by ara-A in cell-free systems proceeds to completion (8, 10, 13) whereas a fraction of the intracellular enzyme is not available for inactivation in intact cells (Chart 1). Likewise, complete inactivation of AdoHcy hydrolase by 2′-deoxyadenosine in intact lymphoblasts was not obtained (12). In the case of ara-A, this residual enzyme activity could not be explained by metabolic consumption of inactivator, as demonstrated by no further inactivation of AdoHcy hydrolase after a second addition of ara-A to the cell suspension (Chart 1*D*). There are several possible explanations why a fraction of intracellular AdoHcy hydrolase resists inactivation. Intracellular AdoHcy hydrolase may be protected by AdoHcy accumulating in cells exposed to ara-A (Chart 3) or 2′-deoxyadenosine (12). This suggestion is in accordance with the protection of the enzyme by AdoHcy in cell-free systems (8, 10). Furthermore, AdoHcy hydrolase has been shown to form a stable complex with adenosine (9, 11, 23) which protects the purified enzyme against inactivation by ara-A and 2′-deoxyadenosine (8, 10). Adenine and adenine nucleotides also interact with AdoHcy hydrolase (24) and partly protect the enzyme against inactivation by ara-A, at least *in vitro* (8).

Although a small fraction of AdoHcy hydrolase in both rat hepatocytes and cultured cells is not inactivated by ara-A, this residual enzyme activity does not provide sufficient metabolic capacity to prevent a massive cellular accumulation of AdoHcy (Chart 3). The increase in the amount of AdoHcy is particularly pronounced in rat hepatocytes (Chart 3). The progressive, almost linear accumulation of AdoHcy in these cells may result from transmethylation reactions not sensitive to AdoHcy inhibition. A similar conclusion has been made by Hoffman (15) investigating the metabolism of AdoHcy in the liver of mice given injections of periodate-oxidized adenosine. Accumulation of AdoMet in the hepatocytes indicates reduced consumption of the methyl donor caused by inhibition of AdoMet-dependent transmethylations. However, the increase in the level of AdoMet in the hepatocytes was moderate, and no accumulation of AdoMet in the cultured cells was observed (Chart 3). These observations suggest that AdoMet has a negative feedback effect on its own synthesis (16).

It has been reported recently from our laboratory that AdoHcy is not taken up by rat hepatocytes (1), and this con-

[4] S. Helland and P. M. Ueland, unpublished observation.

clusion is in accordance with data provided by others (5, 25). Thus, the finding that AdoHcy is released into the extracellular medium suggests a vectorial transport of AdoHcy from the intracellular compartment into the extracellular medium. This suggestion is in agreement with the finding that AdoHcy is released into the perfusate of isolated perfused rat liver (14). Isolated rat hepatocytes leak cellular constituents into the extracellular medium (7), pointing to the possibility that release of AdoHcy into the medium is related to cell damage. The fact that essentially no extracellular AdoMet could be demonstrated under conditions of high cellular level of this compound does not support this possibility. However, failure to demonstrate leakage of AdoMet could be explained by uptake of this compound by the hepatocytes. Cellular uptake of AdoMet by cells, including rat hepatocytes, has been reported by some workers (20) but has been questioned by others (14).

AdoHcy egress could also be demonstrated with cultured cells, placed in the medium used for the hepatocytes or incubated in the culture medium (Chart 4). Several features of the secretion process, *i.e.*, shape of the progress curves for the accumulation of extracellular AdoHcy and the relationship between intra- and extracellular amounts of AdoHcy, showed similarities from one cell type to another. Thus, efflux of AdoHcy may be a cellular mechanism decreasing the AdoHcy content in cells when the metabolic degradation of this metabolite is blocked. This hypothesis is supported by our recent finding of high level of AdoHcy in serum of mice given injections of ara-A.[4]

Addition of adenosine or a high concentration (3 mM) of homocysteine to the cellular medium inhibits the inactivation of intracellular AdoHcy hydrolase by ara-A, as reported previously for inactivation of the enzyme in lymphoblasts by 2′-deoxyadenosine (12). Adenosine may exert its effect by one or a combination of several mechanisms, which include competitive inhibition of cellular transport of ara-A (4), increase in cellular content of AdoHcy (12), and a direct protective effect on AdoHcy hydrolase, as reported for the enzyme in cell-free systems (10). The mechanism of action of homocysteine, which only slightly decreases the rate of inactivation of purified AdoHcy hydrolase (8), is probably related to the accumulation of AdoHcy, which in turn prevents the inactivation of the enzyme (8, 10). The inhibition of ara-A uptake by homocysteine also seems to be related to increased concentration of AdoHcy in the cells (Chart 5). Intracellular AdoHcy may inhibit either transport or metabolism of ara-A. The possibility that AdoHcy inhibits the metabolic component of the uptake process is supported by the observation that only the second slow phase of the uptake curve is suppressed (Chart 5). Furthermore, it has been reported recently that AdoHcy is an inhibitor of adenosine kinase (19), and inhibition of this enzyme may block the conversion of ara-A to its nucleotides (17). However, further studies are required to determine the effect of AdoHcy on the disposition of ara-A in intact cells.

In conclusion, ara-A inactivates AdoHcy hydrolase from various sources and induces an increase in cellular content of AdoHcy. The inactivation of AdoHcy hydrolase in intact cells shows striking similarities to this process in cell-free systems. However, the response of the intracellular AdoHcy hydrolase to ara-A and the cellular accumulation of AdoHcy may be modulated by phenomena such as protection of AdoHcy hydrolase by metabolites, efflux of AdoHcy from the cells, and interference with cellular disposition of ara-A. Long-term studies with cells and *in vivo* may reveal further characteristics of the effect of ara-A on AdoHcy hydrolase and the metabolism of AdoHcy in the intact cell.

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