Measurements of S-Adenosylmethionine and L-Homocysteine Metabolism in Cultured Human Lymphoid Cells

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The intracellular content and turnover of S-adenosyl-L-methionine (AdoMet) were measured in cultured human lymphoid cells. AdoMet levels were found to be 59 nmol/ml cell volume in exponentially growing WI-L2 lymphoblasts, 3.3-8.1 nmol/ml cell volume in unstimulated peripheral blood mononuclear cells, and 25-33 nmol/ml cell volume 24-48 h after the latter were stimulated with phytohemagglutinin. Increases in the AdoMet content of stimulated cells occurred within 2 h after addition of lectin. First order, pool turnover rates were of the same order of magnitude (0.029-0.091/min) for all three types of cultured cells, but owing to the differences in AdoMet content, absolute utilization rates differed markedly and were 4.5, 0.12-0.40, and 1.41-1.57 nmol/min/ml cell volume in WI-L2, unstimulated peripheral mononuclear cells, and lectin-stimulated peripheral mononuclear cells, respectively.

Measurements of homocysteine accumulation in growth medium and of transsulfuration to cysteine indicate that a minimum of 82% of the AdoMet synthesized by WI-L2 is used for transmethylation. Remethylation of homocysteine by these cells could not be detected. AdoMet synthesis accounts for 20-23% of AdoMet synthesized by WI-L2. Judging from the accumulation of homocysteine in the medium of phytohemagglutinin-stimulated peripheral mononuclear cells, a minimum of 38% of AdoMet synthesized must be used for transmethylation.

Even though AdoMet utilization by unstimulated peripheral mononuclear cells is relatively small compared to that of stimulated cells and WI-L2, our data indicate that AdoMet turnover in such "resting" cells is three to five times that estimated for nonhepatic tissues. These findings may be relevant to the hypothesis that lymphoid cells are unusually sensitive to inhibition of transmethylation reactions.

AdoMet-1-mediated transmethylation reactions contribute to the metabolism of a number of different classes of compounds including proteins, nucleic acids, lipids, and small molecules (1, 2). Interest in transmethylation has been heightened in recent years by observations demonstrating or suggesting that such reactions are required for specialized biological phenomena such as chemotaxis (3-6), neurosecretion (7), mast cell degranulation (8), membrane-receptor interactions (9, 10), microbial DNA modification-restriction systems (11), DNA mismatch repair (12, 13), and cellular differentiation (14-17). Furthermore, there is evidence that interference with transmethylation may play a role in the pathogenesis of severe combined immunodeficiency disease found in children with heritable deficiency of adenosine deaminase (18-20).

Many of the insights concerning the involvement of transmethylation in cellular processes have been gained through the use of inhibitors such as AdoHcy and analogues of AdoHcy, whose specificities for transmethylyases are believed to be rather broad. The large number of transmethylation reactions in intact cells has discouraged efforts to discriminate between the effects of an inhibitor on one transmethylase or another, and in most instances it has been impossible to determine which single reaction or class of reactions is important to the process being observed. Progress in this field has been further impeded by a relative lack of information on general aspects of transmethylation such as absolute rates of AdoMet turnover, total methylation requirements of cells, and factors regulating AdoMet levels. In some short term experiments utilizing intact cells and exogenous methyl-labeled methionine to measure a particular transmethylation reaction, knowledge of the kinetics of intracellular AdoMet labeling is of some practical importance, yet such information is not generally available.

The recent development of HPLC techniques to measure AdoMet in small amounts of tissue now makes it feasible to study AdoMet turnover in intact cells. In order to establish a basis for interpretations of studies on the effects of various inhibitors of transmethylation, we report here our measurements of absolute rates of AdoMet utilization in cultured human lymphocytes.

EXPERIMENTAL PROCEDURES

Materials—RPMI 1640, Eagle’s minimal essential medium with Earle’s salts and nonessential amino acids (MEM), and fetal bovine serum were obtained from Gibco. The serum was heated at 56 °C for 30 min before use. PBS is 131 mM NaCl, 20 mM Na2HPO4, and 3.2 mM KH2PO4 at a final pH of 7.4. Lymphocyte separation medium is a product of Litton Bionetics and consists of 94 g/liter of sodium diatrizoate and 62 g/liter Ficoll at a density of 1.077-1.080 g/liter at 20 °C.

Erythro-9-(2-hydroxy-3-nonyl)adenine and purified PHA were obtained from Wellcome Research Laboratories, Research Triangle Park, NC. AdoMet, 4-14C-vinylpyridine, and salmon sperm DNA were purchased from Sigma. The latter was purified by the method of...
AdoMet was purified by HPLC (19) and standardized spectrophotometrically assuming an ε<sub>280</sub> of 14.6 x 10<sup>3</sup> M<sup>-1</sup> cm<sup>-1</sup> at pH 7. T-[<sup>35</sup>S]Methionine (1000 Ci/mmol) and [2,6-<sup>3</sup>H] ATP were obtained from New England Nuclear. Adenosylmethionine synthetase (ATP: L-methionine S-adenosyltransferase; EC 2.5.1.6) was purified from a mutant strain of Escherichia coli through the phenyl-Sepharose step described by Markham et al. (52). One unit of the enzyme gives 1 amol of product/min under the assay conditions described (22).

The WI-L2 human lymphoblast line (24, 25) was cultured in MEM containing 15% fetal bovine serum. All cells were incubated in plastic culture flasks at 37 °C with an atmosphere of 6% CO<sub>2</sub> in air. Cell numbers were estimated with a Coulter counter, and cell volumes were determined by the method of Tolbert et al. (26).

Model and Mathematical Calculations—Our calculations for AdoMet utilization in intact cells assume a three-compartment model consisting of extracellular free L-methionine, intracellular free L-methionine, and intracellular AdoMet (Scheme 1). Zero order rate constants (k<sub>0</sub>) describe the flow of compounds between compartments at steady state. The assumptions are: extracellular methionine is a very large pool whose mass (and radioactivity) do not vary appreciably during the time course of our experiments; intracellular methionine is derived from the extracellular pool at rate k<sub>1</sub>, and equilibration between these two compartments is so rapid that it is not appreciably affected by other sources of methionine such as peptide degradation and 5'-methylthioadenosine (27); methionine exits the extracellular pool by either efflux into the extracellular pool at rate k<sub>1</sub> or utilization for AdoMet synthesis at rate k<sub>3</sub> or utilization for all other intracellular processes such as protein synthesis at rate k<sub>3</sub>. AdoMet is utilized for transmethylation and other reactions at rate k<sub>4</sub> at steady state, the mass of each of the three compartments is constant, and therefore k<sub>1</sub> = k<sub>2</sub> + k<sub>3</sub> + k<sub>4</sub> and k<sub>5</sub> = k<sub>4</sub>. When at steady state a chemically minute amount of radiolabeled methionine is added to extracellular methionine at zero time, then entry of radiolabel into intracellular methionine can be described by

\[ \mu_2 = \mu_1(1 - e^{-k_1 t}) \]  
(1)

where \( \mu_1 \) is the specific radioactivity of extracellular methionine, \( \mu_2 \) and \( \mu_3 \) are the specific radioactivity and number of moles of intracellular methionine, and \( t \) is time. If we define the first order rate constant k<sub>1</sub> as k<sub>1</sub> = k<sub>1</sub>/M, then k<sub>1</sub> describes the turnover of intracellular methionine; substitution in Equation 1 gives

\[ \mu_2 = \mu_1(1 - e^{-k_1 t}) \]  
(2)

In a similar way, one can show that the relationship between radioactivity in extracellular methionine and AdoMet is given by

\[ \mu_2 = \mu_2(1 - e^{-k_2 t}) \]  
(3)

where \( \mu_3 \) is the specific radioactivity of AdoMet, and k<sub>2</sub> is the first order rate constant defining the turnover of the AdoMet compartment.

Values for k<sub>1</sub> were estimated by computer-assisted, iterative curve fitting of data points to Equation 2. Then k<sub>1</sub> was substituted in Equation 3, and data for the equilibration of AdoMet with extracellular methionine were fitted to Equation 3 in the same manner to solve for k<sub>2</sub>.

In some experiments, the specific activities of intracellular methionine and AdoMet appeared to plateau at levels less than that of the extracellular pool, suggesting the existence of an intracellular subcompartment of metabolically inert compound. In each case, the model approach by intracellular methionine, AdoMet was used as the value of \( \mu_2 \) rather than the experimentally determined specific radioactivity. This should give less than 10% error in our final calculations of AdoMet utilization, since this subcompartment represents only a minor pool (usually less than 20% of total AdoMet) that turns over either not at all or at a rate far less than that of the major pool.

Measurements of Intracellular AdoMet and Methionine Turnover—Experiments were initiated by addition of T-[<sup>35</sup>S]methionine to give a final concentration of 1–3 μCi/ml of culture. Concentrated stock solutions of radiolabeled compound of high specific activity (>1 Ci/mmol) were used so that neither the volume nor the methionine concentration of the culture was appreciably changed (<5%) by such additions. At chosen time intervals, a volume containing approximately 10<sup>8</sup> cells (WI-L2) or 2 x 10<sup>9</sup> cells (peripheral blood lymphocytes) was removed, immediately poured over 0.7 volume of frozen (−20 °C) crushed PBS, and centrifuged at 4 °C for 2 min at 400 g. The supernatant medium was used for determination of extracellular methionine-specific activity; the cell pellet was resuspended in ice cold PBS containing bovine serum albumin at 1 mg/ml, and, following centrifugation at 4 °C for 1.5 min at 250 x g, was extracted with perchloric acid as described previously (18). The wash with PBS was omitted in experiments where the specific activity of intracellular methionine was not assayed. Neutralized perchloric acid extracts were used for AdoMet and intracellular methionine assays. In experiments with peripheral blood mononuclear cells, the acid-insoluble material was assayed for DNA by a modification (28) of the diphenylamine technique of Burton (29) using purified salmon sperm DNA as a standard.

Determination of AdoMet- and Methionine-specific Activities—Neutralized perchloric acid extracts were analyzed by HPLC as described previously (18, 19) using a Whatman Partisil-10 SCX column (0.46 x 25 cm) isocratically perfused at 1.5 ml/min with 0.2 M ammonium phosphate, pH 2.6, containing 10% acetonitrile. AdoMet was quantified by monitoring absorbance at 254 nm, and the eluate was collected for determination of radiolabel in a liquid scintillation counter.

The specific activity of L-methionine was determined by enzymatically converting it to AdoMet and then measuring the specific activity of the latter by HPLC. Medium was used directly in the assay, but pre-existing AdoMet in cell extracts necessitated prior treatment with 6–18 μg activated charcoal per 10<sup>9</sup> cells. Large quantities of charcoal removed excessive amounts of methionine. Complete removal of pre-existing AdoMet was verified by HPLC.

Enzymatic reaction mixtures contained 0.1 M Tris-HCl, pH 8.5, 0.1 M KCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 2.5 mM ATP, 3 x 10<sup>4</sup> unit adenosylmethionine synthetase; and 0.1 ml of sample in a final volume of 0.2 ml. After incubation for 60 min at 37 °C, the mixtures were extracted with perchloric acid, and the neutralized supernatants were analyzed by HPLC to determine the specific activity of the AdoMet formed from methionine.

Homocysteine and Methionine Assays—L-Homocysteine was assayed by an enzymatic technique in which samples were pretreated with a slightly alkaline solution of dithiothreitol to reduce homocysteine and mixed disulfides to free homocysteine (30). Medium was not deproteinized prior to assay and the values given include homocysteine bound to protein as the mixed disulfide. The concentration of methionine in medium was determined by a modification of a double
isotope dilution technique involving the enzymatic conversion of this amino acid to AdoMet using radiolabeled ATP (31). This assay was performed only with medium to which radiolabel had not been added previously. The 0.1-ml reaction mixture consisted of 0.1 M Tris-HCl, pH 8.5, 0.1 M KCl, 10 mM MgCl₂, 1 mM dithiothreitol, 0.2 μM L-[³⁵S]methionine, 10 Ci/mmol, 0.1 mM [³²P]ATP, 0.2 Cl/mmol, 0.001 unit of purified adenosylmethionine synthetase, and 0.05 ml of either the medium to be assayed or a standard solution of nonradiolabeled methionine at a concentration of 10 to 40 μM. After incubation for 1 h at 37 °C, 0.03-ml portions of the mixture were spotted onto squares (3 x 3 cm) of Whatman P-81 phosphocellulose paper that had been pretreated with sodium tripolyphosphate (22). These were washed three times with several liters of water, dried, and placed in a toluene-based scintillation fluid for determination of both ³H and ³²P radioactivity. An amount of perchloric acid extract equal to 4 nmol of methionine was assayed by HPLC as previously described (19). Glutathione and cysteine were converted to their S-(4-pyridylethyl) derivatives (32) after the addition of 0.1 ml of freshly prepared 25 mM 4-vinylpyridine and 24 ml and did not interfere with the assay. Approximately 1 μmol of the pyridylthiol derivative of glutathione was purified by HPLC and standardized by UV absorbance using the ε₅₀₀ of 5000 M⁻¹cm⁻¹ reported for S-(4-pyridylethyl)cysteine at pH 1 (33). The specific activities of glutathione and cysteine derivatives were determined by assaying fractions of the eluate in a scintillation counter.

For the analysis of cellular protein, a pellet of approximately 10⁸ cells was suspended in 0.7 ml of 0.05 M Tris-HCl, pH 7.6, 1 mM dithiothreitol, and 0.5 mM Na₂EDTA and freeze-thawed several times. After centrifugation for 10 min at 10,000 × g, the supernatant was dialyzed for several hours against this same buffer, and then made 8 M in urea and 50 mM in 4-vinylpyridine by addition of these reagents. A 2-ml sample of dialyzed medium was treated in the same way with urea and 4-vinylpyridine. After incubation for 2 h at 25 °C, these protein samples were dialyzed overnight against 10 mM acetic acid, lyophilized, and hydrolyzed in 6 N HCİ at 110 °C for 16 h in vacuo. HCl was removed with a stream of N₂, and the residue was analyzed for S-(4-pyridylethyl)cysteine by HPLC.

RESULTS

Turnover of Methionine and AdoMet in WI-L2—The time course of radiolabel equilibration between extracellular methionine and intracellular AdoMet in exponentially growing WI-L2 is shown in Fig. 1. Data obtained from separate experiments were virtually identical and showed that the specific activity of AdoMet reaches a value 50% that of extracellular methionine in about 10 min and then approaches an asymptote equal to the specific activity of extracellular methionine. The short lag period observed in the first 1–2 min can be accounted for by the time required to equilibrate the intracellular methionine pool that is precursor to intracellular AdoMet. Direct measurements of intracellular methionine-specific activities showed rapid and complete equilibration of the pool with extracellular methionine (Fig. 1) with a rate constant of 0.8/min. Using this value for k₁, the data for AdoMet equilibration are best fitted to Equation 3 by a k₂ value of 0.076/min.

Exponentially growing WI-L2 cells were found to contain 98 nmol of AdoMet/10⁶ cells. The cell volume was calculated to be 1.65 ml/10⁹ cells giving an AdoMet content of 59 nmol/ml cell volume. Using a k₁ value of 0.076/min, one can calculate that, in WI-L2, AdoMet is utilized at a rate of 7.4 nmol/min/10⁹ cells or 4.5 nmol/min/ml cell volume (Table I). The growth rate of exponentially growing WI-L2 cells was 6.3 × 10⁻⁴/min in these experiments, and comparison of this value with the rate of AdoMet utilization indicates that 11.7 μmol AdoMet are utilized during an increase in mass of 10⁶ cells.

Homocysteine Metabolism in WI-L2—Since hydrolysis of
AdoHcy (34) is the only known metabolic source of homocysteine in mammalian cells, measurements of the production of the latter should provide an estimate of overall transmethylation. WI-L2 cells were inoculated at 5 x 10^6 cells/ml, and samples of medium were analyzed for homocysteine and methionine at various times until the culture reached its maximum cell density of 1.0 x 10^9 cells/ml. Homocysteine was found to accumulate in amounts directly proportional to the cell density and finally reached a concentration in the medium of 12.4 μM (Fig. 2). During the exponential phase of growth (2-6 x 10^6 cells/ml), homocysteine accumulation was first order with a rate constant of 6.1 x 10^{-4}/min. At the cell densities and growth rate measured in this experiment, this was calculated to be equivalent to 9.2 μmol per increase in mass of 10^9 cells, or 79% of AdoMet utilization.

Loss of methionine from the medium was also measured and correlated well with cell density (Fig. 2). Comparison of these data with homocysteine accumulation showed that 20-23% of the methionine removed by cells from the medium was recovered as homocysteine. Therefore, at least this fraction of the methionine requirement is used for transmethylation reactions in WI-L2 cells.

The two major routes of homocysteine metabolism in human cells are remethylation to methionine and transsulfuration to cystine (35). To measure the latter, cells were grown in L-[35S]methionine during a 24-fold increase in cell number in order to achieve nearly complete equilibrium of the radiolabel with all compartments. Cells and medium were then analyzed for the incorporation of 35S into AdoMet, free cysteine, and cystine, and into the cysteine moieties of glutathione and protein. In this experiment, [35S]cysteine could be detected only in intracellular glutathione and cellular protein, both of which had specific activities that were about 5% that of AdoMet (Table I). The total amount of radiolabel found in these two compartments was that expected from the transsulfuration of 0.44 μmol homocysteine per increase in mass of 10^9 cells. Addition of this amount to that found for homocysteine accumulation in medium gives a total of 9.6 μmol per increase in mass of 10^9 cells. Comparing this value with the 11.7 μmol AdoMet utilized per increase of 10^9 cells, we conclude that transmethylation accounts for at least 82% of AdoMet consumption in exponentially growing WI-L2 cells.

In a separate experiment designed to measure the remethylation of homocysteine to methionine, L-[35S]homocysteine thiolactone (0.15 μCi/ml; 250 Ci/nmol) was added exponentially growing WI-L2, which were harvested after 3 or 21 h and assayed for radiolabeled AdoMet and glutathione. In order to estimate the entry of the radiolabeled thiolactone into the intracellular pool of homocysteine, 50 μM adenosine and 5 μM adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine were added to a portion of both cultures 1 h prior to harvest to cause accumulation of measurable amounts of AdoHcy (18). The specific activity of AdoHcy was easily determined by HPLC and was assumed to reflect accurately the specific activity of intracellular homocysteine. Radiolabel could not be detected in AdoMet at a level of sensitivity indicating that less than 0.5% of its methionine moiety was derived from the remethylation of homocysteine. The specific activity of intracellular glutathione in these experiments was found to be similar to that in cells grown to near equilibrium with L-[35S]methionine, i.e. about 5% of that of intracellular homocysteine. It seems likely that the appreciable amount of homocysteine is remethylated to methionine in WI-L2 grown under these culture conditions, and that only a small portion (less than 5%) of the homocysteine produced by these cells is converted to cysteine by transsulfuration.

**Turnover of Methionine and AdoMet in Human Peripheral Mononuclear Cells—** Following separation from peripheral blood, cells from a single donor were incubated without PHA for 1 to 48 h prior to addition of L-[35S]methionine to the medium. Measurements of AdoMet turnover showed that the specific activity of intracellular AdoMet reaches a value 50% that of extracellular methionine in 12-24 min and then approaches an asymptote that varied in different experiments between 70 and 90% that of extracellular methionine. Data from one such experiment are shown in Fig. 3. The initial lag period noted in WI-L2 was not readily apparent, and measurements of intracellular methionine equilibration gave a value of approximately 2.5/min for k1 with an asymptote of 85-95% (data not shown). From this value for k1 and an asymptote of 85% noted for AdoMet equilibration, the data in Fig. 3 for unstimulated cells are best fitted to Equation 3 by a k2 value of 0.048/min. This rate varied between 0.029 and 0.060/min using unstimulated cells obtained either from different donors or from the same donor weeks or months apart.

Unstimulated human mononuclear cells were found to contain 1.4 and 3.4 nmol of AdoMet/10^9 cells and had a cell volume of 0.42 ml/10^9 cells. The AdoMet content of 3.3 to 8.1 nmol/ml cell volume calculated from these data is only 6-14% that of WI-L2. From cultures in which both k1 and AdoMet content were measured, utilization was calculated to be 0.05-0.17 nmol/min/10^9 cells or 0.12-0.40 nmol/min/ml cell volume (Table I).
In experiments with PHA-stimulated cells, AdoMet turnover and content were measured at various times after addition of lectin. In each of three experiments, $k_2$ values were found to increase by as much as 60% at 3 to 5 h and to return to nearly the zero time level by 8 h. Changes in $k_2$ after 24 to 48 h of stimulation varied from one experiment to another, and ranged from no difference at all to an 80% increase over the zero time value, which was 0.040-0.051/min in these particular studies. In control experiments with unstimulated cells, the elevations in $k_2$ that occurred at 3 to 5 h in PHA-stimulated cultures were not observed, but at 24 to 48 h, $k_2$ values were increased by as much as 50%

The small and transient increases in $k_2$ after PHA stimulation were accompanied by more dramatic changes in AdoMet levels. Since clumping of PHA-stimulated cells interfered with counting, cell quantities were estimated from DNA analyses of the insoluble pellet obtained after perchloric acid extraction. From the value of 5.56 pg of DNA/cell, which we obtained with unstimulated cells, AdoMet levels could then be calculated as nanomoles/10⁶ cells. Data from three different experiments are shown in Fig. 4. AdoMet levels were noted to increase as early as 2-3 h after PHA stimulation and after 7 h had risen approximately 4-fold over starting values. After 24-48 h, increases of 10- to 16-fold had occurred to levels of 17-23 nmol/10⁶ cells. AdoMet content rose less than 2-fold after 48 h in unstimulated cells. In order to estimate the extent to which increases in the AdoMet content of PHA-stimulated cells might be due to changes in cell volume, we measured the DNA content and volume of cell pellets from cultures at various times after the addition of lectin. Increases in the ratio of cell volume/DNA were observed in most instances, but were no greater than 47% at 7 h, 38% at 24 h, and 65% at 48 h. The largest change noted gave a volume for stimulated cells of 0.69 ml/10⁶ cells. From this value, we estimate the AdoMet content of PHA-stimulated cells at 25-33 nmol/ml cell volume, which is 42-56% that of WI-L2 cells.

Rates of AdoMet turnover were calculated from values of $k_2$ and AdoMet content obtained at various times after PHA stimulation and in unstimulated, control cultures. In the experiment depicted in Fig. 5, the increases observed in PHA-stimulated cells during the first 2 to 3 h were primarily due to changes in $k_2$, whereas subsequent increases were the result of higher AdoMet levels. From a zero time rate of 0.06 nmol/

![Fig. 4 (left). Changes in AdoMet content of human peripheral mononuclear cells after stimulation with PHA. The lectin was added to cell suspensions at zero time, and samples were withdrawn for analysis at times up to 48 h. In one experiment, cells from the same donor were incubated with PHA (O) and without PHA (●). In two other experiments, only PHA-stimulated cells were used (△, ○). Cell numbers were calculated from the DNA content of acid-insoluble pellets using an experimentally derived value of 5.56 pg of DNA/cell.

Fig. 5 (right). AdoMet utilization as a function of incubation time in human peripheral mononuclear cells. Cells from the same donor were incubated with (O) or without PHA (●) from 0 to 46 h. Values for both $k_2$ and AdoMet content were determined for each point shown, and were used to calculate AdoMet utilization.

**DISCUSSION**

The availability of HPLC methods that measure subnanomole quantities of AdoMet has allowed us to use radiolabel equilibration techniques to estimate intracellular turnover of this methyl donor in relatively small samples of cultured lymphoid cells. The values obtained represent only upper limits for the rate of transmethylation since AdoMet serves not only as a methyl donor but also as a substrate for polymerization synthesis and for several other reactions as well (1, 2). On the other hand, measurements of homocysteine accumulation and metabolism by cultured cells offer minimum estimates of transmethylation, because hydrolysis of the transmethylation product AdoHcy by AdoHcy hydrolase (EC 3.3.1.1) (34) is the only known source of homocysteine in higher organisms. The data presented in this study give a relatively narrow range between these upper and lower limits for rates of transmethylation. For WI-L2 cells, the AdoMet turnover rate of 7.4 nmol/min/10⁶ cells is such that during exponential growth 11.7 μmol are consumed for an increase in mass of 10⁶ cells. This rate of AdoMet utilization is accompanied by the accumulation of 9.2 μmol of homocysteine in the medium and by another 0.44 μmol of cysteine derived from homocysteine via transsulfuration. The apparent lack of in vivo remethylation of homocysteine to methionine in WI-L2 may be due to our culture conditions, because methionine synthetase (5-methyltetrahydropteroyl-L-glutamate:S-methyltransferase; EC 2.1.1.13) requires methylcobalamin as a cofactor (36), and no cobalamin was present in our medium other than that provided from fetal bovine serum. Furthermore, efficient entry of cobalamin into human cells requires the serum binding protein transcobalamin II, the bovine equivalent of which is relatively ineffective with human cells (37). From the 9.6 μmol of homocysteine accounted for by accumulation in the medium and transsulfuration to cysteine, we estimate that a minimum of 82% of the AdoMet synthesized by WI-L2 is used for transmethylation. This value does not include homocysteine that might be present in our cultures as cystathionine, which was not measured in these experiments. Presumably, AdoMet not utilized for transmethylation is required for polyamine synthesis and other reactions.

Our data do not allow us to calculate AdoMet consumption in peripheral blood lymphocytes as a function of an increase in cell mass. However, when expressed as a rate per cell
volume, the range of 0.12–0.40 nmol/min/ml cell volume in unstimulated cells is only 3–9% that determined for exponentially growing WI-L2 cells (Table I). The greater consumption in WI-L2 is due partly to a slightly higher pool turnover rate (k), but for the most part it is the result of a 15-fold higher AdoMet concentration (Table I). Following PHA stimulation, AdoMet utilization increases as much as 10- to 15-fold over unstimulated values, and when calculated as a rate per cell volume it is about one-third that found in WI-L2.

From the accumulation of homocysteine in the medium, we calculate that at least 38% of the AdoMet synthesized by stimulated, peripheral blood mononuclear cells must have been used for transmethylation. Although not directly measured in these experiments, homocysteine metabolism may have accounted for a significant fraction of the total production of this amino acid, since such cells are known to be capable of transsulfuration (38) and should contain sufficient cobalamin for methionine synthetase activity. It is possible, therefore, that the portion of AdoMet utilized by these cells for transmethylation was appreciably greater than our minimum estimate of 38%.

WI-L2 and peripheral blood mononuclear cells differed in several ways with respect to methionine and AdoMet metabolism. The pool turnover rate for intracellular methionine (k) in peripheral blood cells was approximately three times that of WI-L2; and, whereas virtually complete equilibrium of radiolabel between intracellular and extracellular methionine occurred in WI-L2, it approached only 70 to 90% completion in peripheral blood cells. The latter difference may be due to the fact that, unlike essentially homogeneous WI-L2 cultures, peripheral blood mononuclear cell cultures consist of a mixture of different classes of lymphocytes as well as monocytes. Furthermore, even within the same class of cells, some may be metabolically inactive owing to injury or death incurred during separation and culture. Such cells might retain pools of methionine and AdoMet that would not equilibrate with extracellular radiolabel. The higher level of equilibration noted after PHA stimulation may be due to an expansion of the pools that are metabolically active. The wide range of values for AdoMet turnover noted in peripheral blood mononuclear cell cultures is probably due to differences in endogenous lymphocyte "activity" from one donor to another and in the same person on different days.

The nature of our studies with WI-L2 does not allow discrimination between AdoMet utilized for cell growth (or increase in mass) and that required simply for maintenance of cellular integrity. However, AdoMet turnover in unstimulated, peripheral mononuclear cells is approximately 10% that of PHA-stimulated cultures, suggesting that there exists a relatively small but significant requirement for AdoMet, and presumably transmethylation, in nondividing, "resting" lymphoid cells.

An interesting perspective is provided by comparing our data with estimates of total body methyl group requirements. The metabolic balance studies of Mudl and Poole (39) indicate that a normal human subject turns over approximately 12 to 18 mmol of AdoMet/day. However, most of this is needed for the hepatic synthesis of creatine, and that amount used for other methylation reactions and polyamine synthesis is estimated to be only 2.1–3.7 mmol/day. For a 70-kg subject with an intracellular volume of 28 liters (40), the latter value is equivalent to an AdoMet turnover rate of 0.052–0.092 nmol/ min/ml cell volume, which is only 1–2% that found in WI-L2 and 21–37% the average value of 0.25 nmol/min/ml cell volume observed in unstimulated, resting peripheral mononuclear cells. We cannot rule out the possibility that AdoMet turnover is increased in such cells as a result of stimulation that occurs from our separation procedure or culture conditions. However, if the values obtained from our measurements are an accurate reflection of in vivo conditions, then resting lymphocytes seem relatively active with respect to AdoMet metabolism, and consume AdoMet at a rate three to five times the average for nonhepatic tissues. This finding is significant in view of speculation that the immune defect in heritable adenosine deaminase deficiency may be, at least in part, due to an unusual sensitivity of lymphocytes to inhibition of transmethylation reactions by AdoHcy (18–20).

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