Regulation of methionine metabolism: Effects of nitrous oxide and excess dietary methionine

Michael S. Frontiera, Sally P. Stabler, J. Fred Kolhouse, and Robert H. Allen

Department of Medicine and Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO USA

Methionine is an essential amino acid that is also converted to S-adenosylmethionine, which is used by methyltransferases that methylate DNA, RNA, protein, lipid, etc., and form S-adenosylhomocysteine that is hydrolyzed to adenosine and homocysteine. When methionine is present in excess, glycine N-methyltransferase and cystathionine beta-synthase are thought to play important regulatory roles, with the former using the excess CH_3- moiety to convert glycine to N-methylglycine, and the latter condensing homocysteine with serine to form cystathionine, which is cleaved by gamma-cystathionase to cysteine and alpha-ketobutyrate. When methionine is present in low amounts, the activities of the two regulatory enzymes are thought to decrease with the homocysteine being recycled to methionine by the cobalamin-dependent enzyme methionine synthase, which simultaneously converts 5-CH_3-tetrahydrofolate to tetrahydrofolate. To test this model, we fed a large dose of L-methionine to normal subjects. Using newly developed assays, we observed the following increases in serum levels: methionine, 25 fold; N-methylglycine, fourfold; homocysteine, threefold; cystathionine, 15 fold; and cysteine, unchanged. When leukemia patients were treated for 4 days with 35% nitrous oxide, which markedly inhibits methionine synthase, methionine decreased 80% by day 1 and then either stabilized or returned to normal during days 2 through 4. N-methylglycine fell 50 to 70%, and homocysteine increased 14 fold, but cystathionine increased twofold after an initial decrease or stabilization. Cysteine fell 50% by day 1 and then moved in parallel with methionine. Except for the latter increase in cystathionine, all the data support the current model of methionine regulation and demonstrate that methionine homeostasis is maintained or at least stabilized, even under conditions of extreme excess or deprivation. The unexpected increase in cystathionine levels during nitrous oxide administration is similar to what has been observed in cobalamin and folate deficiency, although the mechanism and physiologic importance remain to be determined. (J. Nutr. Biochem. 5:28–38, 1994.)

Keywords: methionine; homocysteine; cobalamin; folic acid; nitrous oxide

Introduction

Methionine is an essential amino acid that is converted into protein and *S*-adenosylmethionine. Once converted, it serves as the major CH_3 donor for a large number of methyltransferases that transfer CH_3 groups to DNA, RNA, protein, lipid, and other important molecules.[1] Methionine can be synthesized in humans by two different enzymatic reactions. The first is catalyzed by methionine synthase (EC 2.1.1.13), which requires CH_3-cobalamin (CH_3-Cbl)[1] and converts homocysteine and 5-CH_3-tetrahydrofolate (5-CH_3-THF)[1] to methionine and THF, respectively.[2] The second is catalyzed by betaine-homocysteine methyltransferase (EC 2.1.1.5), which converts homocysteine and betaine to methionine and N-N-dimethylglycine, respectively.[3,4] The latter reaction is not dependent on either Cbl or folate, although the N-N-dimethylglycine formed is subsequently metabolized to N-methylglycine (also known as sarcosine), then to glycine, and finally to CO_2 and NH_3 in a series of three enzymatic reactions, each of which results in

Supported by Department of Health and Human Services Research Grants (AG-09834, GM-26486, and DK-21365) from the National Institute on Aging, the National Institute of General Medical Sciences, and the National Institute of Diabetes and Digestive and Kidney Diseases.
Current address for Dr. Frontiera is the Dean Medical Center, Madison, WI USA.
Address reprint requests to Dr. Robert H. Allen at the Division of Hemetology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Campus Box B170, Denver, CO 80262 USA.
Received December 17, 1992; accepted June 29, 1993.

the transfer of a CH_3 group to THF to form 5,10-CH_2-THF.[5–7] The reactions that involve the synthesis of methionine from homocysteine are illustrated in *Figures 1 and 2*, together with related enzymatic pathways.

Mudd and coworkers[8,9] estimated that humans require approximately 0.35 mmole/kg of CH_3 groups every 24 hr with approximately 0.05 mmole/kg being obtained from dietary choline after it is converted to betaine by choline oxidase,[4] and then to methionine. The remaining 0.30 mmole/kg comes from both dietary methionine, which is usually insufficient, and from the recycling of homocysteine to form methionine via methionine synthase. The 5-CH_3-THF that methionine synthase uses as the source of CH_3 group is synthesized from 5,10-CH_2-THF by the enzyme 5,10-methylene-THF reductase (EC 1.1.99.15), which simultaneously converts NADPH to NADP.[10] Normal individuals can obtain essentially unlimited amounts of 5,10-CH_2-THF from a variety of sources that include the subsequent catabolism of N,N-dimethylglycine or from serine, which can be synthesized from glucose, as illustrated in *Figure 2*.

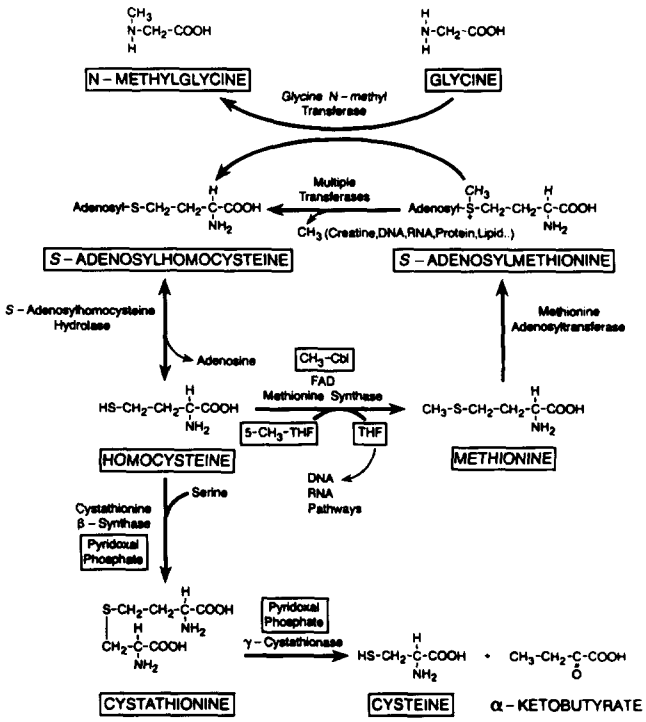

Figure 1 Enzymatic pathways centered on the CH_3-Cobalamin (CH_3-Cbl)-dependent enzyme, methionine synthase, which converts 5-CH_3-tetrahydrofolate (5-CH_3-THF) and homocysteine to THF and methionine, respectively. *S*-adenosylmethionine is formed from methionine via methionine adenosyltransferase and is the major CH_3 donor for a large number of methyltransferases that methylate DNA, RNA, protein, lipid, etc. with the resultant formation of *S*-adenosylhomocysteine, which is then hydrolyzed to adenosine and homocysteine by *S*-adenosylhomocysteine hydrolase. The latter can either be recycled to form methionine again or condensed with serine to form cystathionine by the pyridoxine-dependent enzyme, cystathionine beta-synthase. Cystathionine is then converted to cysteine and alpha-ketobutyrate by the pyridoxine-dependent enzyme, gamma-cystathionase. As documented in the Introduction, this overall pathway is thought to be tightly regulated by *S*-adenosylmethionine, which inhibits the formation of 5-CH_3-THF by 5,10-CH_2-THF reductase (reaction not shown) and stimulates cystathionine beta-synthase. Thus, when levels of *S*-adenosylmethionine are decreased, levels of 5-CH_3-THF and homocysteine should increase and lead to a compensatory increase in the synthesis of methionine via methionine synthase. When levels of *S*-adenosylmethionine are elevated, this should lead to decreases in levels of 5-CH_3-THF and homocysteine and a compensatory decrease in the synthesis of methionine via methionine synthase. 5-CH_3-THF also binds to glycine N-methyltransferase (see top of figure) and inhibits the activity of this enzyme. Thus, when levels of *S*-adenosylmethionine are elevated, decreased levels of 5-CH_3-THF should result in the stimulation of glycine N-methyltransferase, which would function to remove the CH_3 moiety of the methionine molecule from the cycle just as cystathionine beta-synthase removes the homocysteine moiety of the methionine molecule. 5,10-CH_2-THF reductase and all the enzymes shown are located in the cytoplasm.

The recycling of homocysteine to methionine via methionine synthase, versus its condensation with serine to form cystathionine via cystathionine beta-synthase (EC 4.2.1.22) (*Figure 1*) is thought to be highly regulated, as summarized in the legend to *Figure 1*. *S*-adenosylmethionine appears to play a critical role in this process because it directly stimulates cystathionine beta-synthase[13] and directly inhibits 5,10-CH_2-THF reductase.[14,15] It is also thought to indirectly regulate[5,9] the level of N-methylglycine methyltransferase (EC 1.5.99.1) (*Figure 1*), because the 5-CH_3-THF that is formed by 5,10-CH_2-THF reductase binds to N-methylglycine methyltransferase and inhibits its activity.[16] Thus, when methionine and *S*-adenosylmethionine are present in excess, the removal of the homocysteine and the CH_3 moieties of the methionine molecule would be accelerated by cystathionine beta-synthase and N-methylglycine methyltransferase, respectively. When methionine and *S*-adenosylmethionine are present in insufficient amounts, a higher proportion of homocysteine would be recycled to form methionine via methionine synthase, and a higher proportion of the CH_3 moiety of methionine would be conserved and available for the methyltransferases that methylate DNA, RNA, protein, lipid, etc. Mudd and coworkers[9] estimated that under normal dietary conditions approximately 50% of homocysteine is recycled to methionine, and that the turnover time of *S*-adenosylmethionine in human liver is very rapid at approximately 5 min.

Most of our knowledge about the regulation of methionine metabolism comes from animal studies or patients with inborn errors of metabolism.[1,8–12] Much less is known about methionine regulation in normal subjects, although it clearly plays an important role in individuals with acquired deficiencies of cobalamin or folate. In both of these conditions, serum total homocysteine levels are almost always elevated.[17,18] This elevation is thought to be due to both a primary decrease in the activity of methionine synthase, which requires both Cbl and folate for activity (*Figure 1*) and a secondary decrease in the activity of cystathionine beta-synthase.[11,12] The marked increase in homocysteine levels that results from this dual action is important and effective because it partially restores the recycling of homocysteine to methionine and results in the maintenance of normal levels of serum methionine in virtually all

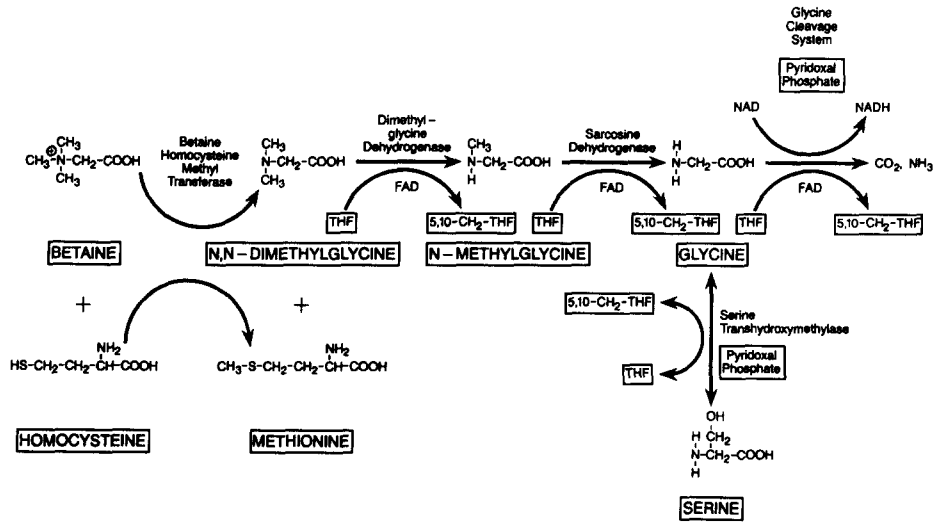

Figure 2 Enzymatic pathway for the conversion of betaine and homocysteine to N,N-dimethylglycine and methionine, respectively, by betaine-homocysteine methyltransferase, which requires neither cobalamin or folate. Betaine arises from choline via choline oxidase (reaction not shown). Methionine subsequently undergoes the reactions shown in *Figure 1*, and N,N-dimethylglycine is subsequently converted to N-methylglycine and then to glycine. The latter can be catabolized further to carbon dioxide and ammonia or converted to serine. Choline oxidase and all of the enzymes shown are located in the mitochondria except for betaine-homocysteine methyltransferase, which is located in the cytoplasm, and serine transhydroxymethylase, which is located in both mitochondria and the cytoplasm.

patients with Cbl or folate deficiency except those with the most severe life-threatening forms of deficiency.

We recently developed assays for cystathionine, betaine, N,N-dimethylglycine, and N-methylglycine, and have been able to detect and establish normal ranges for these four metabolites in normal human serum.[20,21] We used these assays, together with previously developed assays[22] for serum methionine, total homocysteine, and total cysteine that have been modified recently,[20] to obtain new information about the regulation of methionine homeostasis in vivo in humans. We studied patients with chronic myelogenous leukemia while they were treated with nitrous oxide because nitrous oxide causes a profound and immediate inhibition of methionine synthase.[2,14,23,24] We also studied normal subjects after they ingested large oral doses of methionine and betaine. The results obtained in these two situations, which are the equivalent of acute methionine insufficiency and excess, respectively, form the basis for this report.

Methods and materials

Assays for serum metabolites

Assays for serum methionine, total homocysteine, cystathionine, total cysteine, betaine, N,N-dimethylglycine, N-methylglycine, glycine, serine, methylmalonic acid, and total 2-methylcitric acid were performed as described elsewhere.[20-22,25] All the assays utilize capillary gas chromatography/mass spectrometry and are based on the stable isotope dilution principle that uses the ratio of unlabeled metabolite to labeled metabolite that contains stable isotopes. A labeled form of each metabolite was added to serum and urine samples, which were then partially purified using ion exchange chromatography followed by drying and derivatization with *N*-methyl-*N*[*tert*-butyldimethylsilyl] trifluoroacetamide to form *tert*-butyldimethylsilyl derivatives. Because betaine is a quaternary amine, it would retain a positive charge even after derivatization and would be essentially nonvolatile and thus incapable of being analyzed by our gas chromatography/mass spectrometry technique. Consequently, we isolated betaine free of other amino acids, treated it with added homocysteine and partially purified rat liver betaine-homocysteine methyltransferase to form unlabeled and labeled N,N-dimethylglycine and unlabeled and labeled methionine. These in turn were analyzed utilizing gas chromatography/mass spectrometry and used to calculate the amount of betaine present.[21] For all the assays, the mass spectrometer was operated in the selected ion monitoring mode, and specific ions were monitored for each endogenous unlabeled metabolite and each labeled metabolite. Because the labeled metabolites were added to samples in known amounts, it was not necessary to calculate recoveries, which can vary from sample to sample.

Administration of nitrous oxide

A mixture of 40 ± 10% N_2O and O_2 was obtained with an anesthesia machine with mounted N_2O and O_2 tanks and a calibrated rotameter. It was administered via a nasal cannula at a total gas flow of 4 to 5 liter/min., and the exhaled N_2O was scavenged by attaching a Hudson face tent to the central suction supply. Measurements of N_2O were made in the blood and urine of the patients[26,27] and gave average values of approximately 35% N_2O in both patients. The N_2O/O_2 was stopped for 40 min every 8 hr to allow the patients to eat and was well tolerated except for moderately severe headaches and mild nausea in both patients.

Both patients were in the chronic phase of Philadelphia chromosome positive chronic myelogenous leukemia and were

receiving intermittent and variable doses of hydroxyurea to control their peripheral white blood cell counts. Patient I was a 29-year-old Caucasian male diagnosed 3 years earlier, and Patient II was a 34-year-old Hispanic male diagnosed 2 years earlier. Both were in otherwise good general health, had stable and normal weights, and there was nothing unusual about their diets. The primary purpose of their involvement was to determine the effects of N_2O on their leukemia.

Informed consent was obtained and all studies were approved by the Institutional Review Board of the Clinical Research Center of the University of Colorado Health Sciences Center.

Methionine and betaine load studies

L-methionine and the monobasic form of betaine were administrated at a dose of 0.70 mmole/kg of body weight. This dose was chosen because that is what is utilized in the standard methionine load test in patients with vascular disease (see Discussion). Each was dissolved in 250 mL of chilled orange juice and swallowed over several minutes at approximately 8 a.m. The normal subjects ate their usual light breakfast approximately 1 hr earlier, which consisted of cereal and low fat milk in the case of Subject A and two pieces of toast with butter in the case of Subject B. They were not restricted in terms of subsequent meals. Blood samples were obtained just before the methionine or betaine was ingested and at 2, 4, 6, 8, 12, 24, and 48 hr later. Blood samples were allowed to clot at room temperature for 30 min and were then spun at 4° C at 1,500 g for 20 min, and the serum was removed and stored at −20° C. Two 24 hr urine samples were collected for each experiment. Urine samples were also stored at −20° C. A washout period of at least 1 week was used between each of the load and control studies.

The normal subjects consisted of a 54-year-old Caucasian male (A) and a 40-year-old Caucasian female (b), both of whom had stable and normal weights; were in good general health; and had normal levels of serum Cbl, folate, and each of the metabolites that were measured. Neither subject was ingesting any form of multivitamin supplement except those that are present in various foods such as breakfast cereals. Subject A weighed 79 kg and Subject B weighed 66 kg.

Results

Studies with nitrous oxide

When Patient I with chronic myelogenous leukemia was treated for 4 days with 35% N_2O, rapid and dramatic changes occurred in a number of metabolites that included methionine, total homocysteine, cystathionine, and N-methylglycine, as shown in *Figure 3*. Serum methionine (normal = 13–37 μM) fell from a level of 31 μM to 14 μM after 1 day, then to 5 to 6 μM, where it remained without falling further for the rest of the entire 4-day period, and subsequently returned to a normal level of 37 μM 2 days later. Serum total homocysteine (normal = 5–16 μM) increased from a baseline value of 12 μM to 54 μM at the end of day 1, rose further to 143 to 172 μM over the next 3 days, and rapidly returned toward normal at 34 μM 2 days after the N_2O was stopped. Serum cystathionine (normal = 0.04–0.34 μM) fell from 0.21 μM to 0.14 μM after 1 day of treatment and then began a steady increase up to 0.38 μM

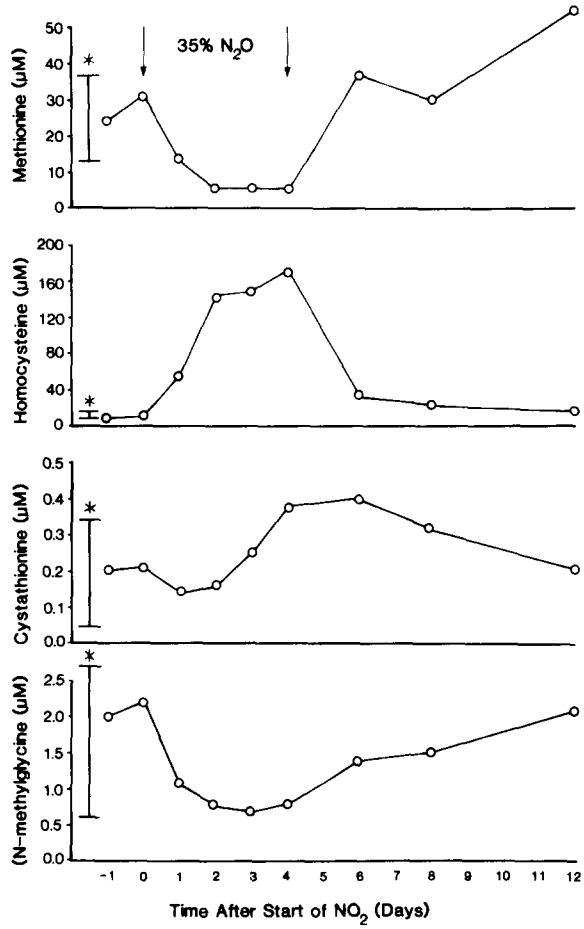

Figure 3 Levels of serum methionine, total homocysteine, cystathionine, and N-methylglycine before, during, and after 4 days of the administration of 35% N_2O to Patient I with chronic myelogenous leukemia. Levels of the four metabolites were assayed using capillary gas chromatography/mass spectrometry as described in Methods and materials. The bar under the asterisks at the left of each panel indicates the normal range for each metabolite calculated as the mean ± 2 SD for 50 normal blood donors after log normalization to correct for skewing toward higher values. The normal ranges were as follows: methionine, 13–37 μM; total homocysteine, 5–16 μM; cystathionine, 0.04–0.34 μM; and N-methylglycine, 0.6–2.7 μM.

on day 4, with a gradual decrease back to 0.21 μM over the next 8 days. Serum N-methylglycine (normal = 0.6–2.7 μM) fell from 2.2 μM to 1.1 μM after 1 day of N_2O treatment, fell further, and remained in the 0.7 to 0.8 μM range over the next 3 days, and gradually increased back to 2.1 μM over the next 8 days. The actual values for these four metabolites are presented in *Table 1* together with data from Patient II with chronic myelogenous leukemia, who was also treated with N_2O for 4 days. He showed very similar changes in each of the four metabolites, as noted above, except that after decreasing from 38 μM to 10 μM after 1 day of N_2O, his serum methionine then recovered spontaneously to the 43–58 μM range and remained there during the last 3 days of N_2O therapy.

Table 1 also contains data for serum total cysteine, betaine, N,N-dimethylglycine, and folate concentrations for both patients. Serum total cysteine (normal

Table 1 Serum levels of various metabolites and folate before, during, and after 35% N_2O was administered for 4 days to two patients with chronic myelogenous leukemia

Patient	Day (#)	Methionine µM	Methionine Versus Day 0	Total Homocysteine µM	Total Homocysteine Versus Day 0	Cystathionine µM	Cystathionine Versus Day 0	Total Cysteine µM	Total Cysteine Versus Day 0	N-Methylglycine µM	N-Methylglycine Versus Day 0	Betaine µM	Betaine Versus Day 0	N, N-Dimethylglycine µM	N, N-Dimethylglycine Versus Day 0	Folate nM*	Folate Versus Day 0
I																	
I	−7	38	1.2	9	0.8	0.28	1.3	220	1.0	1.9	0.9	35	0.8	3.5	1.1	32	1.1
I	−1	24	0.8	9	0.8	0.20	1.0	220	1.0	2.0	0.9	53	1.2	3.4	1.1	32	1.1
I	0†	31	(1.0)	12	(1.0)	0.21	(1.0)	220	(1.0)	2.2	(1.0)	45	(1.0)	3.2	(1.0)	29	(1.0)
I	1†	14	0.5	54	4.7	0.14	0.7	170	0.8	1.1	0.5	32	0.7	3.5	1.1	45	1.6
I	2†	6	0.2	143	12	0.16	0.8	130	0.6	0.8	0.4	18	0.4	3.0	0.9	59	2.0
I	3†	6	0.2	148	13	0.25	1.2	100	0.5	0.7	0.3	13	0.3	2.6	0.8	59	2.0
I	4†	5	0.2	172	15	0.38	1.8	120	0.6	0.8	0.4	19	0.4	3.4	1.0	66	2.3
I	6	37	1.2	34	2.9	0.40	1.9	220	1.0	1.4	0.6	29	0.6	3.0	0.9	34	1.2
I	8	30	1.0	23	2.0	0.32	1.5	260	1.2	1.5	0.7	35	0.8	3.4	1.1	29	1.0
I	12	54	1.8	16	1.4	0.21	1.0	250	1.1	2.1	0.9	41	0.9	3.1	1.0	29	1.0
II																	
II	0†	38	(1.0)	10	(1.0)	0.13	(1.0)	280	(1.0)	2.2	(1.0)	45	(1.0)	5.5	(1.0)	27	(1.0)
II	1†	10	0.3	37	3.7	0.17	1.3	210	0.8	1.5	0.7	38	0.8	4.9	0.9	52	1.9
II	2†	43	1.1	92	9.0	0.17	1.3	210	0.8	1.6	0.7	34	0.8	5.6	1.0	50	1.8
II	3†	40	1.0	132	13	0.29	2.2	150	0.5	1.1	0.5	23	0.5	4.6	0.8	52	1.9
II	4†	58	1.5	92	9.0	0.20	1.5	220	0.8	1.2	0.5	26	0.6	4.3	0.8	48	1.8
II	5	54	1.4	26	2.5	0.16	1.2	200	0.7	1.1	0.5	25	0.6	4.4	0.8	54	2.0
II	7	55	1.4	20	2.0	0.18	1.4	260	0.9	1.6	0.7	26	0.6	5.4	1.0	45	1.7
II	11	53	1.4	8	0.8	0.12	0.9	259	0.9	1.6	0.7	41	0.9	5.0	0.9	41	1.5
II	13	58	1.5	11	1.0	0.14	1.1	260	0.9	1.9	0.8	50	1.1	5.1	0.9	32	1.2
Normal Range		(13–37 µM)		(5–16 µM)		(0.04–0.34 µM)		(200–361 µM)		(0.6–2.7 µM)		(18–73 µM)		(1.4–5.3 µM)		(9–50 nM)	

*Expressed as equivalents of folic acid.
†35% N_2O was administered as described in Methods and materials.

= 200–361 µM) fell from baseline values of 220 to 280 µM to levels as low as 100 to 150 µM over the 4 days of N_2O, and then returned to normal within 2 days after the N_2O was stopped. The serum betaine (normal = 18–73 µM) fell from baseline levels of 45 µM in both patients to lows of 13 µM and 23 µM, which occurred on day 3 of N_2O therapy for both patients, followed by steady increases back to baseline after the N_2O was stopped. Levels of serum N,N-dimethylglycine (normal = 1.4–5.2 µM) were essentially unchanged during and after N_2O therapy. Serum folate (normal = 3.8–22 ng/mL), which consists primarily of 5-CH_3-THF, rose from baseline values of 14 ng/mL and 12 ng/mL to values of 20 ng/mL and 23 ng/mL, respectively, remained in that range until the N_2O was stopped, and then fell back to baseline over the next few days.

Data showing the changes in the patients' peripheral white blood cell counts are presented in *Figure 4*. The pretreatment white blood cell counts were increasing in both patients. N_2O resulted in a mild decrease in Patient I and a marked decrease in Patient II. After the N_2O was stopped, Patient I's white blood cell count resumed its pretherapy rate of increase while that of Patient II continued to fall dramatically over the next several weeks.

Studies with excess methionine

Figure 5 shows that when normal Subject A ingested 0.70 mmole/kg of L-methionine, the serum methionine increased markedly from a baseline level of 24 µM to 680 µM at 2 hr and decreased steadily thereafter, reaching levels in the vicinity of baseline by 24 hr. Serum total homocysteine showed a modest increase from 11 µM to 29 µM at 2 hr, remained in the 30–35 µM range for the next 10 hr, and was back to a near baseline level of 16 µM at 24 hr. Levels of serum cystathionine showed a greater relative increase; going from a baseline of 0.21 µM to a range of 2.1–3.4 µM over the first 2 to 12 hr and also returned to a near baseline value of 0.47 µM by 24 hr. N-methylglycine rose from a baseline of 1.9 µM to a range of 4.0–5.7 µM over the first 12 hr, but did not approach baseline until it reached a level of 1.4 µM at 48 hr. Very similar changes were seen in each of these four metabolites with the second normal Subject B, as shown in *Table 2*. This table also contains values for serum total cysteine, betaine, and N,N-dimethylglycine, none of which showed any consistent change in either normal subject.

Values for all seven metabolites, obtained at the regular time intervals for both subjects during a control period during which nothing special was ingested except for 250 mL of orange juice, are shown in *Table 3*. Some variation in the serum levels of methionine and cystathionine were observed over the 48-hr period, and these may be due to fluctuations in dietary intake or diurnal variations. The levels of the other metabolites were much more constant. Serum folate levels fluctuated somewhat with both normal subjects (data not shown), and these changes appeared to be related to meals, with no consistent differences being observed between the methionine-ingestion periods and the control periods.

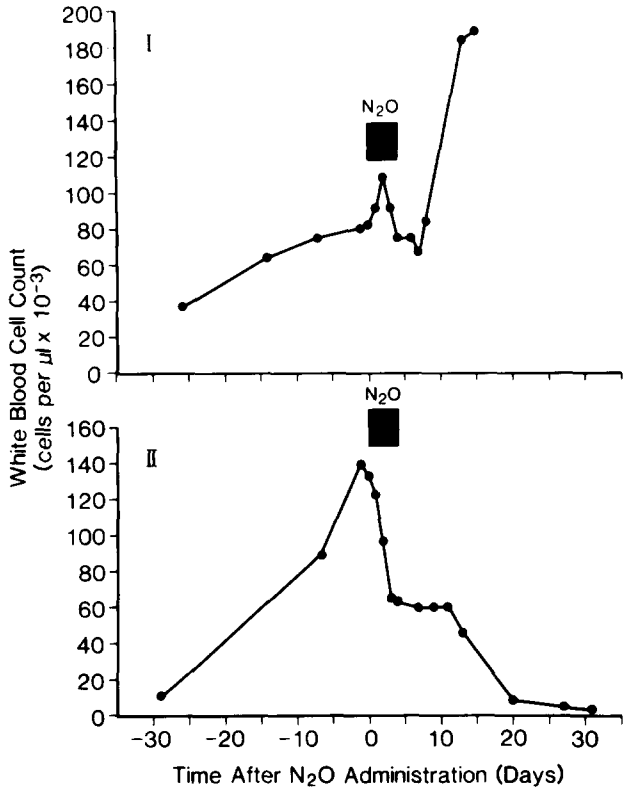

Figure 4 Serial white blood cell counts before, during, and after 4 days of the administration of 35% N_2O to Patients I (top) and II (bottom) with chronic myelogenous leukemia which was given as described in Methods and materials. The vertical arrows indicate the start and finish of the N_2O. Patient I was on oral hydroxyurea as follows: (1) day −30 to day −9, 1.25 g/day; (2) day −8 to day +13, none; (3) day +14 to day +15, 1.50 g/day. Patient II was on oral hydroxyurea as follows: (1) day −30 to day +19, 2.50 g/day; and (2) day +20 to day +31, 1.50 g/day.

Studies with excess betaine

Table 4 contains the data obtained with both normal subjects after they each ingested 0.70 mmole/kg of betaine. Serum betaine rose from pretreatment levels of 29 μM and 27 μM and peaked at 2 hr in both subjects, with values of 1,500 μM and 1,300 μM. These initial increases were 2 to 3 times those obtained for serum methionine when the same normal subjects ingested the same molar amount of L-methionine, although the time courses of the subsequent decreases in both metabolites were quite similar. After the excess betaine ingestion, levels of serum methionine and cystathionine showed little change in Subject A and appear to have increased at most only approximately two fold in Subject B. Serum levels of total homocysteine and total cysteine were unchanged in both subjects. Levels of N,N-dimethylglycine increased from baseline levels of 2.0 and 2.3 μM and reached peak levels of 12 μM and 16 μM at 8 hr, followed by very slow decreases toward baseline over the next 64 hr. Levels of N-methylglycine showed a similar time course, increasing from preingestion values of 1.6 μM and 1.7 μM to a peak of 6.7 μM and 7.4 μM at 24 hr and 12 hr, respectively, with baseline values not being approached or reached until 72 hr. Changes in serum folate levels were not different from those observed during the methionine ingestion and control periods (data not presented, see above).

Other analyses

Levels of serum glycine (normal = 147–422 μM) and serine (normal = 95–230 μM) decreased approximately 40 to 60% and reached their lowest levels at 6 to 12 hr after ingestion in the methionine-load studies with both normal subjects and were essentially unchanged in the other studies. Serum concentrations of methylmalonic acid (normal = 73–271 μM) and total 2-methylcitric acid (normal = 60–228 μM) were within the normal range throughout the N_2O studies performed with Patients I and II, and the control and ingestion studies performed with Subjects A and B, and no changes or trends were seen during any of the studies.

Urine samples were collected for two consecutive 24-hr intervals during the ingestion studies and analyzed for the various metabolites. The data are presented in *Table 5* and showed that after the betaine load, large amounts

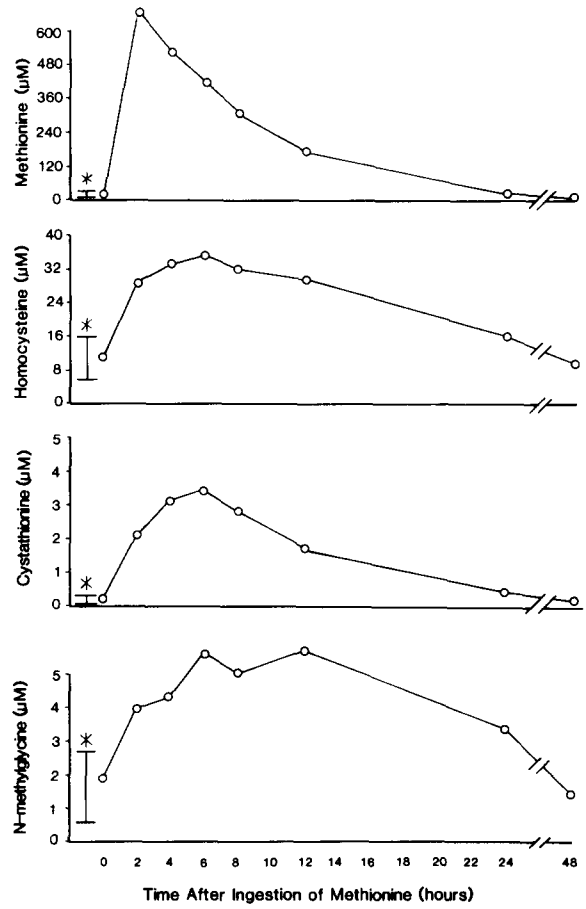

Figure 5 Serum levels of methionine, total homocysteine, cystathionine, and N-methylglycine after the ingestion of 0.7 mmol/kg of L-methionine by normal Subject A. All four metabolites were assayed using capillary gas chromatography/mass spectrometry as described in Methods and materials. The bars below the asterisks at the left of each panel indicate the normal range for each metabolite and were obtained and the values were as described in the legend to *Figure 3*.

Table 2 Serum levels of various metabolites before and after the oral ingestion of L-methionine at a dose of 0.70 mmoles/kg of body weight in two normal subjects

		Methionine		Total Homocysteine		Cystathionine		Total Cysteine		N-Methyl-glycine		Betaine		N,N-Di-methylglycine	
Subject	Hour	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0
A															
A	0	24	(1.0)	11	(1.0)	0.21	(1.0)	290	(1.0)	1.9	(1.0)	34	(1.0)	3.1	(1.0)
A	2	680	29	29	2.7	2.1	10	280	1.0	4.0	2.1	35	1.0	3.5	1.1
A	4	530	23	33	3.1	3.1	15	280	1.0	4.3	2.2	31	0.9	3.2	1.1
A	6	420	18	35	3.2	3.4	16	280	1.0	5.6	2.9	32	0.9	3.5	1.1
A	8	310	13	32	2.9	2.8	8.3	280	1.0	5.0	2.6	30	0.9	3.4	1.1
A	12	180	7.7	30	2.7	1.7	8.3	250	0.9	5.7	2.9	30	0.9	3.0	1.0
A	24	33	1.4	16	1.5	0.47	2.2	300	1.0	3.4	1.8	36	1.1	3.3	1.1
A	48	17	0.7	10	0.9	0.21	1.0	300	1.0	1.4	0.7	34	1.0	2.5	0.8
B															
B	0	23	(1.0)	9.0	(1.0)	0.09	(1.0)	250	(1.0)	1.7	(1.0)	29	(1.0)	3.2	(1.0)
B	2	530	24	25	(2.8)	1.7	19	250	1.0	5.9	3.6	33	1.2	3.1	1.0
B	4	460	20	29	3.3	1.6	18	240	1.0	6.3	3.8	28	1.0	3.6	1.1
B	6	330	15	27	3.1	1.8	20	240	1.0	9.0	5.4	39	1.4	3.5	1.1
B	8	180	7.8	22	2.5	1.2	14	210	0.8	6.8	4.1	25	0.9	3.4	1.1
B	12	62	2.7	19	2.1	0.64	7.2	230	0.9	6.6	4.0	30	1.1	3.1	1.0
B	24	27	1.2	9.2	1.0	0.14	1.6	230	0.9	3.1	1.9	30	1.0	3.3	1.1
B	48	17	0.8	6.2	0.7	0.09	1.0	190	0.8	1.2	0.7	21	0.8	2.1	0.7
Normal Range		(13–37 μM)		(5–16 μM)		(0.04–0.34 μM)		(200–361 μM)		(0.6–2.7 μM)		(18–73 μM)		(1.4–5.3 μM)	

Table 3 Serum levels of various metabolites in two normal subjects over a 48-hour control period

		Methionine		Total Homocysteine		Cystathionine		Total Cysteine		N-methyl-glycine		Betaine		N,N-di-methylglycine	
Subject	Hour	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0
A															
A	0	26	(1.0)	11	(1.0)	0.24	(1.0)	300	(1.0)	1.6	(1.0)	38	(1.0)	2.0	(1.0)
A	2	18	0.7	11	1.0	0.22	0.9	310	1.0	1.4	0.9	39	1.0	2.2	1.1
A	4	16	0.6	10	0.9	0.16	0.7	300	1.0	1.1	0.7	31	0.8	1.9	1.0
A	6	23	0.9	10	0.9	0.13	0.5	300	1.0	1.2	0.8	31	0.8	2.1	1.1
A	8	18	0.7	10	0.9	0.20	0.8	310	1.0	1.1	0.7	36	0.9	2.3	1.2
A	12	22	0.8	10	0.9	0.14	0.6	290	1.0	1.7	1.1	33	0.9	2.3	1.2
A	24	26	1.0	10	0.9	0.17	0.7	300	1.0	1.4	0.9	36	0.9	2.1	1.1
A	48	27	1.0	10	0.9	0.16	0.7	300	1.0	1.4	0.9	35	0.9	2.1	1.1
B															
B	0	26	(1.0)	8.1	(1.0)	0.10	(1.0)	240	(1.0)	1.8	(1.0)	31	(1.0)	2.5	(1.0)
B	2	20	0.8	7.8	1.0	0.11	1.1	220	0.9	1.9	1.1	40	1.3	2.6	1.0
B	4	23	0.9	8.7	1.1	0.10	1.0	250	1.0	1.7	0.9	35	1.1	2.7	1.1
B	6	29	1.1	8.6	1.1	0.14	1.4	240	1.0	1.9	1.1	34	1.1	2.4	1.0
B	8	20	0.8	8.6	1.1	0.12	1.2	240	1.0	1.5	0.8	33	1.1	2.6	1.0
B	12	41	1.6	7.6	0.9	0.39	3.9	190	0.8	2.7	1.5	27	0.9	2.4	1.0
B	24	22	0.8	8.2	1.0	0.08	0.8	240	1.0	1.6	0.9	29	0.9	2.3	0.9
B	48	17	0.7	7.4	0.9	0.08	0.8	210	0.9	1.4	0.8	28	0.9	2.1	0.8
Normal Range		(13–37 μM)		(5–16 μM)		(0.04–0.34 μM)		(200–361 μM)		(0.6–2.7 μM)		(18–73 μM)		(1.4–5.3 μM)	

of betaine in the range of 16–18% of the oral dose were excreted in the initial 24-hr urine samples of both normal subjects. Much smaller but significant increases were also observed in the excretion of methionine, total homocysteine, and cystathionine after the methionine load, and in N,N-dimethylglycine and N-methylglycine after the betaine load. Values for glycine, serine, methylmalonic acid, and total 2-methylcitric acid were essentially constant and ranged from 910 to 3,400 total μmoles, 89 to 200 total μmoles, 10 to 19 total μmoles, and 16 to 30 total μmoles, respectively, in the urine samples collected over the twelve 24-hr periods for the two normal subjects.

Discussion

Studies in rats,[4,23,24,28,29] monkeys, [30,31] and pigs[4,32] demonstrated that N_2O causes a number of deleterious effects on Cbl metabolism. Many of these occur within minutes of the administration of N_2O and are centered on methionine synthase. These changes in rats include

Table 4 Serum levels of various metabolites before and after the oral ingestion of betaine at a dose of 0.70 mmoles/kg of body weight in two normal subjects

		Methionine		Total Homocysteine		Cystathionine		Total Cysteine		N-methylglycine		Betaine		N,N-Di-methylglycine	
Subject	Hour	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0	μM	Versus Hour 0
A															
A	0	26	(1.0)	11	(1.0)	0.21	(1.0)	300	(1.0)	1.6	(1.0)	29	(1.0)	2.0	(1.0)
A	2	20	0.8	10	0.9	0.22	1.1	320	1.1	2.5	1.6	1500	52	5.1	2.6
A	4	21	0.8	10	0.9	0.18	0.9	310	1.0	2.2	1.4	520	18	6.7	3.4
A	6	36	1.4	10	0.9	0.28	1.4	310	1.0	4.0	2.5	340	12	12	6.0
A	8	25	1.0	10	0.9	0.24	1.2	300	1.0	3.1	1.9	230	7.9	12	6.0
A	12	33	1.3	10	0.9	0.21	1.1	310	1.0	5.3	3.3	190	6.6	11	5.5
A	24	38	1.5	10	0.9	0.24	1.2	300	1.0	6.7	4.2	150	5.2	11	5.5
A	48	24	1.3	12	1.1	0.63	3.2	290	1.0	4.8	3.0	52	1.8	5.8	2.9
A	72	18	0.7	11	1.0	0.28	1.4	320	1.1	1.9	1.2	47	1.6	3.2	1.6
B															
B	0	19	(1.0)	8.6	(1.0)	0.08	(1.0)	230	(1.0)	1.7	(1.0)	27	(1.0)	2.3	(1.0)
B	2	20	1.1	6.8	0.8	0.08	1.0	220	1.0	2.7	1.6	1300	48	4.0	1.7
B	4	32	1.7	7.7	0.9	0.10	1.3	230	1.0	4.1	2.4	360	13	10	4.3
B	6	43	2.3	8.4	1.0	0.15	1.9	230	1.0	6.0	3.5	220	8.1	15	6.5
B	8	35	1.8	9.6	1.1	0.15	1.9	230	1.0	6.0	3.5	180	6.7	16	7.0
B	12	35	1.8	8.3	1.0	0.13	1.6	210	0.9	7.4	4.4	140	5.2	11	4.8
B	24	23	1.2	6.8	0.8	0.10	1.3	220	1.0	5.1	3.0	80	3.0	5.6	2.4
B	48	25	1.3	6.9	0.8	0.09	1.1	220	1.0	2.8	1.6	46	1.7	3.5	1.5
B	72	16	0.8	8.0	0.9	0.11	1.4	220	1.0	1.7	1.0	37	1.4	2.5	1.1
Normal Range		(13–37 μM)		(5–16 μM)		(0.04–0.34 μM)		(200–361 μM)		(0.6–2.7 μM)		(18–73 μM)		(1.4–5.3 μM)	

Table 5 Total amounts of various metabolites excreted in 24-hour urine samples during a control period and after the oral ingestion of L-methionine and betaine at a dose of 0.70 mmoles/kg of body weight in two normal subjects

Subject	Time period (hr)	Load	Methionine total μmoles	Total homocysteine total μmoles	Cystathionine total μmoles	Total cysteine total μmoles	N-methyl-glycine total μmoles	Betaine total μmoles	N,N-dimethyl-glycine total μmoles
A	0–24	none	10	6	9	260	9	270	50
A	24–48	none	11	11	9	280	9	250	51
A	0–24	methionine*	108	21	45	260	8	280	56
A	24–48	methionine	108	6	10	220	8	210	47
A	0–24	betaine*	14	4	6	220	37	9070	260
A	24–48	betaine	14	7	13	240	23	230	190
B	0–24	none	8	4	6	200	6	140	45
B	24–48	none	12	5	5	240	8	150	57
B	0–24	methionine†	65	12	32	220	7	160	52
B	24–48	methionine	10	4	5	180	8	170	51
B	0–24	betaine†	15	5	3	210	25	8650	260
B	24–48	betaine	10	4	4	190	8	80	88

*The amount ingested was 55,300 μmoles.
†The amount ingested was 46,200 μmoles.

the formation of Cbl analogues from Cbl,[24] the displacement of Cbl from methionine synthase,[24] a decrease in the level of CH_3-Cbl,[24] and an 80 to 90% decrease in the activity of methionine synthase.[23,24] The recovery of methionine synthase activity parallels the reappearance of Cbl bound to the enzyme,[24] with both occurring slowly and requiring approximately 1 week to fully reach baseline levels.[23,24] N_2O does not have any immediate effect on the second mammalian Cbl-dependent enzyme, L-methylmalonyl-CoA mutase (EC 5.4.99.2), which utilizes adenosyl-Cbl and catalyzes the conversion of L-methylmalonyl-CoA to succinyl-CoA.[24,28] The conversion of Cbl to Cbl analogues caused by N_2O continues to occur, however, and the Cbl analogues are preferentially excreted in the urine.[24] After several weeks of exposure to N_2O, tissue levels of Cbl and L-methylmalonyl-CoA mutase activity begin to decrease, and this is accompanied by a further decrease in the activity of methionine synthase.[24] The difference in the time course of loss of activity between the two Cbl-dependent enzymes has also been documented in vivo by studies demonstrating that levels of serum total homocysteine

increase immediately after N_2O administration, while levels of serum methylmalonic acid remain normal initially and then increase dramatically after several weeks of N_2O exposure.

Studies in humans have demonstrated that N_2O causes a number of clinical abnormalities. These include the development of megaloblastic changes in the bone marrow within 6 hr of N_2O administration[33] and the development of severe and sometimes fatal pancytopenia and megaloblastic anemia in patients with tetanus who were treated with N_2O for several weeks.[34] Intermittent exposure to N_2O over many months can cause neurologic abnormalities.[35–38] Ermans et al.[39] recently described several metabolic changes that occurred in 42 patients who were given anesthesia with 70% N_2O for a median duration of 5 hr, with two patients receiving it for 24 hr spread over 3 days. The mean baseline levels of serum folate and serum total homocysteine increased approximately 50 and 80%, respectively, at the end of anesthesia. In the two patients who received 24 hr of N_2O, serum total homocysteine values increased five to ten fold and folate levels increased 2 fold. The mean methionine level decreased significantly but the absolute changes were very slight in most patients and the actual numerical decrease was not given. Serum levels of methylmalonic acid and 2-methylcitric acid were not measured in these patients. We studied a dentist who abused N_2O over many years, and developed severe ataxia and a peripheral neuropathy, together with mild cerebral dysfunction and minimal hematologic abnormalities.[37] He had markedly elevated levels for serum total homocysteine, 81 μM (normal = 5–16 μM); methylmalonic acid, 12,300 nM (normal = 73–271 nM); and total 2-methylcitric acid, 1,050 nM (normal = 60–228 nM). Cerebrospinal fluid levels of methylmalonic acid, 166,000 nM (normal = 168–593 nM), and total 2-methylcitric acid, 5,750 nM (normal = 323–1,070 nM) were also markedly elevated.

The studies reported here with two patients with chronic myelogenous leukemia who received 35% N_2O for 4 days are in agreement with, and supplement those of, Ermans et al.[39] We observed more dramatic decreases in the level of serum methionine together with marked increases in serum total homocysteine of 10 to 15 fold and a two-fold increase in serum folate. The serum methionine stabilized at a moderately low level in one patient and returned to mid-normal levels in the second patient despite the continuation of 35% N_2O over an additional 3 days. These observations support the concept that the methionine levels are tightly regulated. We also observed an initial mild decrease or stabilization of serum cystathionine levels together with an increase in serum total homocysteine and a decrease in N-methylglycine, indicating that cystathionine beta-synthase and glycine N-methyltransferase play important roles in the regulatory process. Taken together, these changes are all in accord with and provide additional support for the current model of methionine regulation as outlined in the Introduction and the legend of *Figure 1*. Serum betaine levels fell 30 to 50% and this may have been due to the elevated levels of homocysteine, which is one of the substrates for betaine-homocysteine methyltransferase, or to some other regulatory change.[4] We also observed a delayed increase in serum levels of cystathionine, which are also elevated in the majority of patients with Cbl or folate deficiency[20] or inborn errors of Cbl and folate metabolism.[20] We have suggested that this may be due to a decrease in the activity of gamma-cystathionase (EC 4.4.1.1) (*Figure 1*), which converts cystathionine to cysteine and alpha-ketobutyrate, although the mechanism and possible importance are unknown.[20]

A full discussion of the chemotherapeutic potential of N_2O is beyond the scope of the current study and was recently reviewed.[40,41] Nevertheless, the effects on the white blood cell count noted in our two patients with chronic myelogenous leukemia were similar to what others have observed previously.[42,43] It was not possible to perform these studies on normal subjects because the decrease in white blood cell counts might have been dangerous, as when patients with tetanus were treated with N_2O in the past.[44]

Our studies with excess dietary methionine also resulted in a number of metabolic changes that provide new support for the current model of methionine regulation. After the ingestion of 0.7 mmole/kg of L-methionine, which provides twice the estimated total 24 hr requirement for CH_3 groups,[9] serum methionine increased approximately 30 fold together with a modest three-fold increase in total homocysteine, a 10- to 20-fold increase in serum cystathionine and a three-fold increase in N-methylglycine. These changes, and the 50% decreases in serine and glycine levels, support the concept that cystathionine beta-synthase and glycine N-methyltransferase are very active during periods of methionine excess and play important regulatory roles by removing the homocysteine and CH_3 moieties of the methionine molecule, respectively. The fact that total cysteine levels did not increase during methionine excess indicates that the enzymes that catabolize cysteine are also under some form of regulatory control. The fact that levels of serum betaine did not change during the period of excess methionine suggests that betaine-homocysteine methyltransferase is not tightly regulated, and that the decrease in serum betaine levels observed in the patients exposed to N_2O may have been due mostly to the marked increase in homocysteine levels rather than regulation of levels of enzyme activity itself, as has been observed in rats and pigs treated with N_2O.[4]

Our studies involving the ingestion of a large amount of betaine, equivalent to that used in the methionine ingestion studies, indicate that betaine-homocysteine methyltransferase does not play a prominent role in the regulation or maintenance of methionine homeostasis. Although serum levels of betaine became markedly increased (1,300 to 1,500 μM) 2 hr after ingestion, serum levels of methionine and cystathionine increased at most by twofold, and no change was observed in serum total homocysteine or total cysteine levels. These very modest changes are consistent with our recent observations that oral betaine given to patients with genetic defects in the ability to synthesize CH_3-Cbl is relatively ineffec-

tive in lowering serum total homocysteine levels or restoring serum methionine levels, even though very high serum betaine levels in the 350–3,900 μM range are achieved.[21] Levels of serum N-methylglycine did increase approximately fourfold, but this does not necessarily indicate an increase in the activity of glycine N-methyltransferase because N-methylglycine is also formed from N,N-dimethylglycine, as shown in *Figure 2*. The fact that N,N-dimethylglycine levels increased six to seven fold after the betaine load supports the concept that the increase in N-methylglycine is mostly, or even entirely, due to an increase in the conversion of betaine to N,N-dimethylglycine and then to N-methylglycine rather than to an increase in the activity of glycine N-methyltransferase. It is interesting that the urinary excretion of N-methylglycine increased only after the betaine load and not after the methionine load (see Results and *Table 5*), even though serum levels of N-methylglycine rose somewhat higher in the latter than in the former (*Tables 2 and 4*). This difference may be due to the fact that betaine-homocysteine methyltransferase is present only in the kidney and the liver,[3,4] while glycine N-methyltransferase is more widely distributed (Conrad Wagner, Vanderbilt University, unpublished observations, 1992)[45,46] and provides additional support for the concept that the increase in serum N-methylglycine levels arose from different enzymes after the betaine and methionine loads.

The new metabolite assays[20,21] that we used in the current studies may also prove to be useful in developing improved techniques for identifying patients who are heterozygotes for cystathionine beta-synthase deficiency. Identification of such patients may be important because such heterozygotes occur with a frequency of about 1% in the general population, and several studies suggest that they may be at increased risk for the development of all major forms of vascular disease, including cerebrovascular, coronary artery, and peripheral vascular disease.[1,47–50] Previous studies have shown that approximately 80% of obligate heterozygotes for cystathionine beta-synthase deficiency have greater increases in serum homocystine, homocysteine-cysteine mixed disulfide, and total homocysteine after the ingestion of 0.70 mmole of L-methionine (the same dose of methionine used in our studies) than do normal subjects.[47–49] The ability to measure changes in levels of cystathionine, N-methylglycine, glycine, and serine; and ratios of metabolites, e.g., total homocysteine:cystathionine may improve our ability to recognize these heterozygotes and other causes of abnormal homocysteine metabolism. Studies designed to explore this hypothesis are in progress.

Acknowledgments

The authors wish to thank Linda B. Farb, Paul D. Marcell, and Beverly K. Raab for expert technical assistance, and Valarie L. Ruebush and Colleen Z. Nevergall for expert assistance in preparing the manuscript. We also thank Dr. Mark Hilberman and Dr. Deborah Mitchell for assistance in administering the N_2O, Dr. William A. Robinson (University of Colorado Health Sciences Center) for the opportunity to study his patients, and the staff of the Clinical Research Center where these studies were performed.

References

1 Mudd, S.H., Levy, H.L., and Skovby, F. (1989). Disorders of transsulfuration. In *Metabolic Basis of Inherited Disease, 6th Edition,* (C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds.), p. 693–734, McGraw-Hill, New York, NY USA

2 Banerjee, R.V. and Matthews, R.G. (1990). Cobalamin-dependent methionine synthase. *FASEB J.* **4,** 1450–1459

3 Ericson, L.E. (1960). Betaine-homocysteine-methyl-transferases. I. Distribution in nature. *Acta. Chem. Scand.* **14,** 2102–2112

4 McKeever, M.P., Weir, D.G., Molloy, A., and Scott, J.M. (1991). Betaine-homocysteine methyltransferase; organ distribution in man, pig and rat and subcellular distribution in the rat. *Clin. Science* **81,** 551–556

5 Wagner, C. (1986). Proteins binding pterins and folates. In *Folates and Pterins, Vol. 3,* (R.L. Blakley and V.M. Whitehead, eds.), p. 251–295, John Wiley & Sons, New York, NY USA

6 Scott, C.R. (1989). Sarcosinemia. In *Metabolic Basis of Inherited Disease, 6th Edition,* (C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds.), p. 735–741, McGraw-Hill, New York, NY USA

7 Nyhan, W.L. (1989). Nonketotic hyperglycinemia. In *Metabolic Basis of Inherited Disease, 6th Edition,* (C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds.), p. 743–753, McGraw-Hill, New York, NY USA

8 Mudd, S.H. and Poole, J.R. (1975). Labile methyl balances for normal humans on various dietary regimens. *Metabolism* **24,** 721–735

9 Mudd, S.H., Ebert, M.H., and Scriver, C.R. (1980). Labile methyl group balances in the human: The role of sarcosine. *Metabolism* **29,** 707–720

10 Rosenblatt, D.S. (1989). Inherited disorders of folate transport and metabolism. In *Metabolic Basis of Inherited Disease, 6th Edition,* (C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds.), p. 2049–2064, McGraw-Hill, New York, NY USA

11 Finkelstein, J.D. (1990). Methionine metabolism in mammals. *J. Nutr. Biochem.* **1,** 228–237

12 Selhub, J. and Miller, J.W. (1992). The pathogenesis of homocysteinemia; interruption of the coordinate regulation by *S*-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Amer. J. Clin. Nutr.* **55,** 131–138

13 Finkelstein, J.D., Kyle, W.E., Martin, J.J., and Pick, A. (1975). Activation of cystathionine synthase by adenosylmethionine and adenosylmethionine. *Biochem. Biophys. Res. Comm.* **66,** 81–87

14 Shane, B. and Stokstad, E.L.R. (1985). Vitamin B_{12}-folate interrelationships. *Ann. Rev. Nutr.* **5,** 115–141

15 Jencks, D.A. and Matthews, R.G. (1987). Allosteric inhibition of methylenetetrahydrofolate reductase by adenosylmethionine: Effect of adenosylmethionine and NADPH on the equilibrium between active and inactive forms of the enzyme and on the kinetics of approach to equilibrium. *J. Biol. Chem.* **262,** 2485–2493

16 Nyhan, W.L. (1989). Nonketotic hyperglycinemia. In *Metabolic Basis of Inherited Disease, 6th Edition,* (C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds.), p. 743–753, McGraw-Hill, New York, NY USA

17 Stabler, S.P., Marcell, P.D., Podell, E.R., Allen, R.H., Savage, D.G., and Lindenbaum, J. (1988). Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. *J. Clin. Invest.* **81,** 466–474

18 Allen, R.H. (1991). Megaloblastic anemias. In *Cecil Textbook of Medicine, 19th Edition,* (J.B. Wyngaarden, L.H. Smith, Jr., J.C. Bennett, and F. Plum, eds.), p. 846–854, W.B. Saunders Co., Orlando, FL USA

19 Higginbottom, M.C., Sweetman, L., and Nyhan, W.L. (1978).

A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia: Neurologic abnormalities in a vitamin B_{12}-deficient breast-fed infant of a strict vegetarian. *N. Engl. J. Med.* **299,** 317–323

20 Stabler, S.P., Lindenbaum, J., Savage, D.G., and Allen, R.H. (1993). Elevation of serum cystathionine levels in patients with cobalamin or folate deficiency. *Blood* **81,** 3404–3413

21 Allen, R.H., Stabler, S.P., Savage, D.G., and Lindenbaum, J. (1993). Serum betaine, N,N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. *Metabolism* in press

22 Stabler, S.P., Marcell, P.D., Podell, E.R., Allen, R.H., Savage, D.G., and Lindenbaum, J. (1987). Quantitation of total homocysteine, total cysteine, and methionine in normal serum using capillary gas chromatography-mass spectrometry. *Analytical Biochem.* **162,** 185–196

23 Deacon, R., Lumb, M., Perry, J., Chanarin, I., Minty, B., Halsey, M.J., and Nunn, J.F. (1978). Selective inactivation of vitamin B_{12} in rats by nitrous oxide. *Lancet* **II,** 1023–1024

24 Kondo, H., Osborne, M.L., Kolhouse, J.F., Binder, M.J., Podell, E.R., Utley, C.S., Abrams, R.S., and Allen, R.H. (1981). Nitrous oxide has multiple deleterious effects on cobalamin metabolism and causes decreases in activities of both mammalian cobalamin-dependent enzymes in rats. *J. Clin. Invest.* **64,** 1270–1283

25 Allen, R.H., Stabler, S.P., Savage, D.G., and Lindenbaum, J. (1993). Elevation of 2-methylcitric acid I and II in the serum, urine, and cerebrospinal fluid of patients with cobalamin deficiency. *Metabolism* **42,** 978–988

26 Soloojee, Y. and Cole, P. (1978). Estimation of nitrous oxide in blood. *Anaesthesia* **33,** 779–783

27 Sonander, H., Stenqvist, O., and Nilsson, K. (1983). Exposure to trace amounts of nitrous oxide. *Br. J. Anaesth.* **55,** 1225

28 Stabler, S.P., Brass, E.P., Marcell, P.D., and Allen, R.H. (1991). Inhibition of cobalamin-dependent enzymes by cobalamin analogues in rats. *J. Clin. Invest.* **87,** 1422–1430

29 Horne, D.W., Patterson, D., and Cook, R.J. (1989). Effect of nitrous oxide inactivation of vitamin B_{12}-dependent methionine synthetase on the subcellular distribution of folate coenzymes in rat liver. *Arch. Biochem. Biophys.* **270,** 729–733

30 Scott, J.M. and Weir, D.G. (1981). The methyl trap hypothesis. A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic acid-induced exacerbation of subacute combined degeneration in pernicous anemia. *Lancet* **II,** 337–340

31 Scott, J.M., Dinn, J.J., Wilson, P., and Weir, D.G. (1981). Pathogenesis of subacute combined degeneration: A result of methyl group deficiency. *Lancet* **II,** 334–337

32 Weir, D.G., Keating, S., Molloy, A., McPartlin, J., Kennedy, S., Blanchflower, J., Kennedy, D.G., Rice, D., and Scott, J.M. (1988). Methylation deficiency causes vitamin B_{12}-associated neuropathy in the pig. *J. Neurochem.* **51,** 1949–1952

33 Amess, J.A.L., Burman, J.F., Rees, G.M., Nancekievill, D.G., and Mollin, D.L. (1978). Megaloblastic haemopoiesis in patients receiving nitrous oxide. *Lancet* **II,** 339–342

34 Lassen, H.C.A., Henriksen, E., Neukirch, F., and Kristensen, H.S. (1956). Treatment of tetanus. Severe bone-marrow depression after prolonged nitrous-oxide anaesthesia. *Lancet* **I,** 527–530

35 Layzer, R.G., Fishman, R.A., and Schafer, J.A. (1978). Neuropathy following abuse of nitrous oxide. *Neurology* **28,** 504–506

36 Layzer, R.B. (1978). Myeloneuropathy after prolonged exposure to nitrous oxide. *Lancet* **II,** 1227–1230

37 Stabler, S.P., Allen, R.H., Savage, D.G., and Lindenbaum, J. (1991). Cerebrospinal fluid methylmalonic acid levels in normal subjects and patients with cobalamin deficiency. *Neurology* **41,** 1627–1632

38 Vishnubhakat, S.M. and Beresford, H.R. (1991). Reversible myeloneuropathy of nitrous oxide abuse: Serial electrophysiological studies. *Muscle Nerve* **14.,** 22–26

39 Ermens, A.A.M., Refsum, H., Rupreht, J., Spijkers, L.J.M., Guttormsen, A.B., Lindemans, J., Ueland, P.M., and Abels, J. (1990). Monitoring cobalamin inactivation during nitrous oxide anesthesia by determination of homocysteine and folate in plasma and urine. *Clin. Pharmacol. Ther.* **49,** 385–393

40 Abels, J., Kroes, A.C., Ermens, A.A., and van Kapel, J. (1990). Anti-leukemic potential of methyl-cobalamin inactivation by nitrous oxide. *Am. J. Hematol.* **34,** 128–131

41 Koblin, D.D. (1990). Nitrous oxide: A cause of cancer or chemotherapeutic adjuvant? *Semin. Surg. Oncol.* **6,** 141–147

42 Lassen, H.C.A. and Kristensen, H.S. (1959). Remission in chronic myeloid leucaemia following prolonged nitrous oxide inhalation. *Danish Medical Bulletin* **6,** 252–255

43 Eastwood, D.W., Green, C.D., Lambdin, M.A., and Gardner, R. (1963). Effect of nitrous oxide on the white cell count in leukemia. *New Engl. J. Med.* **268,** 297–299

44 Lassen, H.C.A., Henriksen, E., Neukirch, F., and Kristensen, H.S. (1956). Treatment of tetanus. Severe bone marrow depression after prolonged nitrous-oxide anaesthesia. *Lancet* **I,** 527–530

45 Kerr, S.J. (1972). Competing methyltransferase systems. *J. Biol. Chem.* **247,** 4248–4252

46 Kerr, S.J. and Heady, J.E. (1973). Modulation of tRNA methyltransferase activity by competing enzyme systems. In *Advances in Enzyme Regulation, Vol. 12,* (G. Weber, ed.), p. 103–117, Pergamon Press, New York, NY USA

47 Boers, G.H.J., Smals, A.G.H., Trijbels, F.J.M., Fowler, B., Bakkeren, J.A.J.M., Schoonderwaldt, H.C., Kleiger, W.J., and Kloppenborg, P.W.C. (1985). Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *New Engl. J. Med.* **313,** 709–715

48 Clarke, R., Daly, L., Robinson, K., Naughte, E., Cahalane, S., Fowler, B., and Graham, I. (1991). Hyperhomocysteinemia: An independent risk factor for vascular disease. *New Engl. J. Med.* **324,** 1149–1155

49 Ueland, P.M. and Refsum, H. (1992). Plasma homocysteine and cardiovascular disease. In *Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function,* (R.B. Francis, Jr., ed.), p. 183–236, Marcel Dekker, Inc., New York, NY USA

50 Stampfer, M.J., Malinow, M.R., Willett, W.C., Newcomer, L.M., Upson, B.M., Ullman, D., Rishler, P.V., and Hennekens, C.H. (1992). A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in U.S. physicians. *JAMA* **268,** 877–881