ABSTRACT
Background: One-carbon metabolism involves both mitochondrial and cytosolic forms of folate-dependent enzymes in mammalian cells, but few in vivo data exist to characterize the biochemical processes involved.
Objective: We conducted a stable-isotopic investigation to determine the fates of exogenous serine and serine-derived one-carbon units in homocysteine remethylation in hepatic and whole-body metabolism.
Design: A healthy man aged 23 y was administered \([2,3,3-^{2}H_{3}]\)serine and \([5,5,5-^{2}H_{3}]\)leucine by intravenous primed, constant infusion. Serial plasma samples were analyzed to determine the isotopic enrichment of free glycine, serine, leucine, methionine, and cystathionine. VLDL apolipoprotein B-100 served as an index of liver free amino acid labeling.
Results: \([^{2}H_{1}]\)Methionine and \([^{2}H_{2}]\)methionine were labeled through homocysteine remethylation. We propose that \([^{2}H_{2}]\)methionine occurs by remethylation with \([^{2}H_{2}]\)methyl groups (as 5-methyltetrahydrofolate) formed only from cytosolic processing of \([^{2}H_{3}]\)serine, whereas \([^{2}H_{1}]\)methionine is formed with labeled one-carbon units from mitochondrial oxidation of C-3 serine to \([^{2}H_{1}]\)formate to yield cytosolic \([^{2}H_{1}]\)methyl groups. The labeling pattern of cystathionine formed from homocysteine and labeled serine suggests that cystathionine is derived mainly from a serine pool different from that used in apolipoprotein B-100 synthesis.
Conclusions: The appearance of both \([^{2}H_{1}]\)- and \([^{2}H_{2}]\)-methyl forms indicates that both cytosolic and mitochondrial metabolism of exogenous serine generates carbon units in vivo for methyl group production and homocysteine remethylation. This study also showed the utility of serine infusion and indicated functional roles of cytosolic and mitochondrial compartments in one-carbon metabolism. Am J Clin Nutr 2000;72:1535–41.

INTRODUCTION
Classic studies showed that the de novo synthesis of methyl groups accounts for a large proportion of the methyl balance in humans (1) and that remethylation of homocysteine is a critically important phase of methionine kinetics (2–4). Although the basic processes of mammalian one-carbon metabolism are understood, the functional roles of cytosolic and mitochondrial compartments in generating methyl groups and other one-carbon units remain uncertain (5). An understanding of the cellular and whole-body processing of one-carbon units from various donor molecules is essential to assessing the roles of nutritional and genetic variables.

Cellular one-carbon metabolism is compartmentalized (5, 6). Mitochondrial and cytosolic folate pools differ in their distribution of one-carbon substituents, susceptibility to nutritional deficiency, and response to inactivation of methionine synthase (or, 5-methyltetrahydrofolate-homocysteine S-methyltransferase, EC 2.1.1.13) (7). Serine has an integral role both as a direct and an indirect source of one-carbon units. Serine can be metabolized in the cytosol and mitochondria, both of which contain serine hydroxymethyltransferase (mSHMT and cSHMT, respectively), which reversibly converts serine and tetrahydrofolate to glycine and 5,10-methylenetetrahydrofolate (5, 6, 8, 9). The serine-derived one-carbon unit of mitochondrial 5,10-methylenetetrahydrofolate is ultimately converted to tetrahydrofolate and formate, and folate-dependent serine oxidation processes.
dation in mitochondria is an important source of cellular carbon units (9–12) (Figure 1). One-carbon metabolism appears to favor the formation of formate as the ultimate mitochondrial product from carbon in the 3-position of serine (12, 13), which suggests that cytosolic one-carbon units originate largely from mitochondrial production of formate. Formate ions entering the cytosol can be channeled through the sequential reactions catalyzed by 5,10-methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5); 5,10-methylenetetrahydrofolate cyclohydrolase (EC 3.5.4.9); and 10-formyltetrahydrofolate synthetase (or, formate-tetrahydrofolate ligase, EC 6.3.4.3) to yield 5,10-methylenetetrahydrofolate (5, 6). Cytosolic formation of 5,10-methylenetetrahydrofolate from serine and tetrahydrofolate may also occur through the action of cSHMT. Despite evidence that cSHMT acts on serine to yield one-carbon units in rats (14), much of the net flow of substrate through cytosolic SHMT appears to be in the direction of glycine to serine (5, 15).

Irrespective of the contributions of cytosolic and mitochondrial pathways in the formation of one-carbon units under fasting conditions, it remains unclear how exogenous serine is partitioned between the pathways involving cSHMT and mSHMT under postprandial conditions or during infusion of exogenous serine. The roles of mitochondrial and cytosolic compartments in one-carbon metabolism have been examined in various model systems (9, 12, 16–19), but quantitative aspects of human one-carbon metabolism are poorly understood.

We administered a primed, constant infusion of [3H]serine to a healthy man to test the hypothesis that both the mitochondrial and cytosolic compartments of one-carbon metabolism function in the metabolism of exogenous serine to yield one-carbon units for remethylation of homocysteine.

SUBJECTS AND METHODS

Methods

The stable-isotopic protocol was modeled on that reported by Schalinske and Steele (20) in which [3-14C]serine was infused into the duodenum of rats. Analyses were based on isotopic enrichment of free plasma amino acids and VLDL apolipoprotein (apo) B-100 as indicators of whole-body and hepatic metabolic processing, respectively. Serine was administered as [2,3,3-3H]serine, [5,5,5-3H]leucine was included in the infusion as a second tracer to assess overall protein turnover and short-term kinetics of apo B-100. These tracers were administered simultaneously as a primed, constant infusion (21). The study was conducted at the University of Florida General Clinical Research Center and was approved by the University of Florida Institutional Review Board. Informed consent was obtained.

The subject was a healthy Hispanic man aged 23 y who for 3 d before the infusion consumed meals at the General Clinical Research Center to avoid extremes of protein or vitamin intake. [2,3,3-3H]Serine and [5,5,5-3H]leucine (Cambridge Isotope Laboratories, Woburn, MA) were prepared in an isotonic saline solution and sterilized by filtration. After the subject fasted overnight, catheters were placed in a vein of each forearm for infusion and blood sampling, respectively. An intravenous priming dose of 4.63 µmol/kg (0.5 mg/kg) [3H]serine and 1.87 µmol/kg (0.225 mg/kg) [3H]leucine was administered as a 20-mL bolus over 5 min, which was equivalent to the amount administered per hour during the remaining continuous infusion. After the priming dose was administered, the infusion was maintained at 20 mL/h to provide =1.87 µmol [3H]leucine/kg·h⁻¹ and 4.63 µmol [3H]serine·kg⁻¹·h⁻¹. Venous blood samples (14 mL) were obtained immediately before the priming dose was administered (time 0) and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 5, and 9 h after infusion. Samples were immediately placed on ice. Plasma was separated by centrifugation (1000 x g, 20 min, 2–4°C) within ≈2 h of withdrawal and was frozen immediately at −80°C in 1-mL portions. To maintain a fed state during the infusion, the subject ingested portions of a liquid, fruit-flavored formula (5.4 kJ·kg⁻¹·h⁻¹) that contained adequate amounts of carbohydrate and fat but negligible amounts of vitamins and amino acids.

Analytic methods

Plasma pyridoxal 5-phosphate was determined by HPLC as the semicarbazone derivative (22). Total folate in plasma and erythrocytes was measured by microbiological assay with Lactobacillus casei (23). Free amino acids from 0.1 mL plasma were isolated and purified by cation exchange chromatography (24). VLDL was isolated from 0.5 mL plasma by ultracentrifugation at a density of 1.006 kg/L (24), after which the apo B-100 fraction was precipitated with iso-propanol (25) and hydrolyzed in 6 mol HCl/L at 110°C for 18 h (21). The resulting apo B-100 amino acids were purified by cation exchange chromatography (24). All amino acids were converted to their semicarbazone derivatives and isotopic enrichment was determined in electron capture negative ionization mode by gas chromatography–mass spectrometry (GC-MS) with a model 5890 gas chromatograph and model 5988A mass spectrometer (Hewlett-Packard Corp, Palo Alto, CA). Selected ion monitoring was conducted at a mass-to-charge ratio (m/z) of 293–294 for glycine, at m/z 519–522 for serine, at m/z 349–352 for leucine, at m/z 367–371 for methionine, and at m/z 678–682 for cystathionine.

![FIGURE 1. Compartmentation in one-carbon metabolism between cytosol and mitochondria. cSHMT, cytosolic serine hydroxymethyltransferase; mSHMT, mitochondrial serine hydroxymethyltransferase; THF, tetrahydrofolate; 5,10-CH2-THF, 5,10-methenyltetrahydrofolate; MTHFD, 5,10-methenyltetrahydrofolate dehydrogenase; MTHFR, 5,10-methenyltetrahydrofolate reductase; Met. synthase, methionine synthase.](https://academic.oup.com/ajcn/article-abstract/72/6/1535/4729578)
Isotopic enrichment of amino acids is expressed as a molar ratio of labeled to nonlabeled compounds, with corrections for natural abundance of stable isotopes (3).

**Preliminary study of possible isotopic exchange in peptidyl [2H3]serine during acid hydrolysis**

To test whether nonenzymatic exchange of deuterium atoms of free or peptidyl [2H3]serine with solvent protons could occur during the acid hydrolysis procedure used in the apo B-100 analysis, the following preliminary study was conducted. [2H3]Serine was attached randomly to both ends of a 16–amino acid synthetic peptide (H-CTHLFHDLPQALEDQG-OH) obtained previously for use in an unrelated project. This coupling was accomplished by incubating 20 mg [2H3]serine with 20 mg of the synthetic peptide with 20 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDC; Sigma Chemical Co, St Louis) in 2 mL of 0.1 mol NaHCO3, HCl buffer/L, pH 4.2, for 5 h at 20°C, following standard procedures (26). In addition, a poly-[2H3]serine peptide mixture was produced by incubating 20 mg [2H3]serine with 20 mg EDC under the same conditions. The resulting peptides in each preparation were recovered and low-molecular-weight constituents were removed by dialysis against two 1-L portions of distilled water at 2–4°C in cellulosic tubing with a nominal molecular weight cutoff of 1000 (Spectra/Por 7; Spectrum Laboratories Inc, Rancho Dominguez, CA). Duplicate portions of each preparation were subjected to treatment with 6 mol HCl/L at 110°C for 18 h and then analyzed for isotopic labeling as described above.

**Kinetic calculations and data analysis**

Isotopic enrichment data for each amino acid isotopomer were plotted versus infusion time, and the mean of the assumed plateau enrichments was determined for amino acids that reached apparent isotopic equilibrium. For plasma cystathionine, plasma methionine, and apo B-100 methionine, all of which were labeled with serine, the initial rate of labeling was determined as the slope of the linear component of each curve (27, 28). Fractional synthesis rates (FSRs) were calculated as:

\[
\text{FSR (h}^{-1}\text{)} = \frac{\text{initial rate (h}^{-1}\text{)}}{\text{plateau enrichment of precursor pool}}
\]

(1)

The FSR of apo B-100 was calculated by using data for both [2H3]serine and [2H3]leucine:

\[
\text{FSR (h}^{-1}\text{)} = \frac{\text{initial rate of apo B-100 labeling (h}^{-1}\text{)}}{\text{plateau enrichment of apo B-100}}
\]

(2)

The rate of appearance (Q) of serine and leucine was determined on the basis of plasma isotopic enrichment data:

\[
Q, (\mu\text{mol/h}) = \text{infusion rate (\mu mol/h)} \times \frac{\text{plateau enrichment of amino acid in plasma}}{1}\]

(3)


**RESULTS**

**Nutritional status of the subject**

Before the study, the subject had adequate folate (plasma folate: 15.2 nmol/L; erythrocyte folate: 1025 nmol/L) and vitamin B-6 (plasma pyridoxal 5-phosphate: 80 nmol/L) status.

**Retention of [2H3]serine labeling during acid hydrolysis**

The preliminary study regarding the stability of [2H3]serine labeling during acid hydrolysis yielded evidence of slight but significant exchange of solvent protons with deuterium during the acid hydrolysis. Both peptide preparations yielded similar molar distributions of serine isotopomers. Thus, data were pooled for the calculation of means observed in the analysis of the peptidyl forms of [2H3]serine and yielded the following values after correction for the natural abundance of the following stable isotopes: [1H]serine, 3.08%; [2H1]serine, 0.456%; [2H2]serine, 5.51%; and [2H3]serine, 91.4%. In contrast, free (nonpeptidyl) [2H3]serine underwent no significant change in labeling distribution during acid hydrolysis. In view of the small magnitude of this ex vivo appearance of the [2H1]- and [2H3]-forms of serine during acid hydrolysis, we conclude that the appearance of these serine isotopomers in VLDL apo B-100 during [2H3]serine infusion, as presented below, is almost completely of metabolic origin.

In vivo kinetics

Isotopic steady state of infused [2H3]serine and [2H3]leucine in plasma was achieved within 0.5–1 h (Figures 2 and 3). Plateau isotopic enrichment of [2H3]serine and [2H3]leucine in VLDL apo B-100 occurred in ~5 h. No labeling of glycine was detected in either plasma or apo B-100. Mean (±SD) plateau isotopic enrichments (molar ratio of tracer/tracee corrected for natural abundance) for [2H3]leucine and [2H3]serine in plasma were 0.016 ± 0.00043 and 0.032 ± 0.0042, respectively, and rates of appearance in the plasma compartment were 110 ± 30 and 140 ± 19 μmol·kg⁻¹·h⁻¹ for leucine and serine, respectively. This value for leucine is higher than that of 94 ± 3.6 μmol·kg⁻¹·h⁻¹, which was reported previously (28). The data for enrichment of [2H3]serine and total labeled serine in plasma suggest that the infusion may not have maintained a steady state of plasma serine (Figure 2).

Enrichment of [2H3]leucine in apo B-100 increased linearly at a rate of 0.00322 h⁻¹, then reached a plateau of 0.0130 ± 0.0011 after 5 h (Figure 2). This indicated an FSR of 0.248 h⁻¹ for apo B-100 based on [2H3]leucine enrichment (ie, 24.8% of the plasma VLDL apo B-100 pool was synthesized and secreted into plasma hourly).

Unexpected partial conversion of [2H3]serine to the M+1 and M+2 isotopomers ([2H4]serine and [2H5]serine) was apparent in both plasma and apo B-100. Plasma [2H3]serine and [2H3]serine reached a plateau after 3 h of infusion and accounted for ~22% and 24% of labeled serine, respectively, at steady state, and did not show the apparent decline seen for the precursor [2H3]serine (Figure 2). In contrast, the M+1 form of serine ([2H4]serine) was the predominant form of labeled serine in apo B-100, constituting ~54% of the total labeled serine species at its steady state, which was reached 6 h after infusion (Figure 2). This was evidence of a highly active state of hepatic serine undergoing SHMT-catalyzed interchange with glycine. The linearity of the apo B-100 [2H3]serine labeling curve was evidence of rapid and consistent hepatic enrichment of the hepatic precursor pool ([2H3]serine). Calculation of apo
B-100 kinetics on the basis of [3H]serine yielded an FSR of 0.223 h⁻¹ (22.3% of the plasma apo B-100 pool per hour) that was consistent with the leucine-derived value. The ratio of the total enrichment (sum of M+1, M+2, and M+3) of serine isotopomers in plasma to that in apo B-100 was ≈0.29 (0.0137/0.0469), whereas that of [3H]leucine was ≈0.79 (0.0130/0.0164).

Plasma free [3H]methionine and [3H]methionine isotopomers appeared continually throughout the infusion at approximately equivalent rates, whereas the plateau enrichment of [3H]methionine exceeded that of [3H]methionine in apo B-100 by several-fold (Figure 4). We propose that the labeling of methionine in this protocol occurs by remethylation of homocysteine, with methyl groups derived from [3H]serine, as either 5-[3H]methyltetrahydrofolate or 5-[3H]methyltetrahydrofolate (Figure 5).

The labeling of plasma cystathionine from coupling of homocysteine and serine occurred almost entirely as [3H]cystathionine (Figure 6). Small amounts of [3H]- and [3H]cystathionine forms were also detected, although these could not be quantified reliably because of limitations in the sensitivity of the GC-MS, which caused analytic imprecision for all cystathionine ions. [3H]Cystathionine enrichment in plasma increased rapidly and reached a mean (±SD) plateau of 0.038 ± 0.0060. This plateau enrichment did not differ significantly from that of plasma free [3H]serine but markedly exceeded that of apo B-100 [3H]serine.

**DISCUSSION**

The study was conducted as a pilot study to evaluate a primed, constant infusion of [3H]serine for investigating methyl group generation from serine for use in further studies of nutritional variables affecting one-carbon metabolism. On full examination of the isotopic data, it became apparent that the use of [3H]serine as a tracer permitted the differentiation of methyl group generation via serine processing in cytosol and mitochondria because of resulting differences in retention of deuterium atoms and provided novel evidence of active interchange between serine and glycine. On the basis of these data, we present a working hypothesis that both cytosolic and mitochondrial compartments yield one-carbon units needed for cellular processes such as homocysteine remethylation (Figure 5).

The results of this study showed that a primed, constant infusion of [3H]serine permitted a direct examination of major phases of one-carbon metabolism and transsulfuration because of serine’s multiple roles in cellular metabolism. The precision of certain kinetic measurements may be improved in future studies.

**FIGURE 2.** Isotopic enrichment of total [3H]serine (●), [3H]serine (■), [3H]serine (▲), and [3H]serine (▽) in plasma free amino acids and in VLDL apolipoprotein (apo) B-100 during a primed, constant infusion of [3H]serine.

**FIGURE 3.** Isotopic enrichment of [3H]leucine in plasma free amino acids (●) and VLDL apolipoprotein (apo) B-100 (■) during a primed, constant infusion of [3H]serine.
by optimizing the infusion rate of \[^{2}H_{3}\]serine. Concurrent infusion with \[^{2}H_{3}\]leucine, which is not synthesized in vivo, is an important control in this protocol because it allows assessment of any changes in whole-body protein turnover due to dietary treatments or other variables of interest in subsequent studies. Further applications of this protocol will include an independent methionine tracer to aid in interpretation of methionine kinetics.

Although analysis of only the plasma free amino acid pool would simplify this protocol, conclusions based only on plasma labeling kinetics would reflect whole-body metabolic events alone. The inclusion of apo B-100 in the analysis complements the plasma data by providing liver-specific data that, as shown here, may differ substantially from whole-body metabolism evidenced by plasma amino acid kinetics.

The findings of our study of the potential for ex vivo isotopic exchange during acid hydrolysis of peptidyl forms of \[^{2}H_{3}\]serine, which showed measurable but limited exchange, supports the validity of using apo B-100 in a study of this type. In our

**FIGURE 4.** Isotopic enrichment of \[^{2}H_{3}\]methionine isotopomers (methyl-labeled \(^{2}H_{2}\) and \(^{2}H_{1}\)) in plasma free amino acids and VLDL apolipoprotein (apo) B-100 during a primed, constant infusion of \[^{2}H_{3}\]serine.

**FIGURE 5.** Proposed metabolic pathways for generation of \[^{2}H_{3}\]methionine and \[^{2}H_{3}\]methionine isotopomers involving cytosolic and mitochondrial serine hydroxymethyltransferase and associated compartmentation of folate-dependent enzymes. D, deuterium; cSHMT, cytosolic serine hydroxymethyltransferase; mSHMT, mitochondrial serine hydroxymethyltransferase; THF, tetrahydrofolate; 5,10-CD\(_{2}\)-THF, \[^{2}H_{3}\]5,10-methylene-tetrahydrofolate; 5,10-CD = THF, 5,10-\[^{2}H_{3}\]methenyltetrahydrofolate; 10-CDO-THF, 10-\[^{2}H_{1}\]formyltetrahydrofolate; 5-CDO\(_{2}\)-THF, 5-\[^{2}H_{2}\]methyltetrahydrofolate; MTHFD, 5,10-methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase.
Cytosolic processing of the infused $[2,3,3-\text{H}_3]$serine would yield insight regarding the intracellular source of the methyl groups. Regardless of its subcellular compartment of origin.

Advantage of $[3-\text{13C}]$serine as an alternative tracer because methionine labeling of methyl groups may also be considered a disadvantage. A useful property of $[2,3,3-\text{H}_3]$serine is its ability to detect the formation of other serine isotopomers (eg, $[\text{M}+1]$ and $[\text{M}+2]$) isomerization of $[\text{M}+0]$, $[\text{M}+1]$, and $[\text{M}+2]$ serine isotopomers during apo B-100 hydrolysis would likely be less than that observed in this preliminary study of peptide analysis.

The FSR values based on leucine and serine tracers for apo B-100 (0.248 and 0.223 h$^{-1}$, respectively) indicate turnover of apo B-100 in this single subject under the conditions of this protocol that is similar to that observed under fasting conditions by others. For example, Lichtenstein et al (24) reported a mean FSR of 0.13 h$^{-1}$, whereas Cryer et al (29) reported a range in 5 normal subjects of 0.246–0.479 h$^{-1}$ ($\bar{x} \pm \text{SD: 0.383} \pm 0.100$ h$^{-1}$).

The use of serine as a tracer is necessary to directly quantify the generation of methyl groups for the remethylation of homocysteine and the formation of cystathionine in homocysteine catabolism. The use of $[2,3,3-\text{H}_3]$serine has both advantages and disadvantages. A useful property of $[2,3,3-\text{H}_3]$serine is its ability to detect the formation of other serine isotopomers (eg, $[\text{M}+1]$ and $[\text{M}+2]$), indicating rapid serine-glycine interconversion, and the formation of both the $[\text{M}+1]$ and $[\text{M}+2]$ forms of methionine primarily through pathways involving mitochondrial and cytosolic forms of SHMT, respectively. The fact that $[\text{H}]$serine yields both singly and doubly labeled methyl groups may also be considered a disadvantage of this tracer because kinetic interpretation is more complex than with a $^{13}$C-labeled serine tracer. Quantitative simplicity is a distinct advantage of $[3-\text{13C}]$serine as an alternative tracer because methionine labeling would occur only as $[\text{methyl-}^{13}\text{C}]$methionine regardless of its subcellular compartment of origin.

A key to the interpretation of methionine labeling is the inference regarding the intracellular source of the methyl groups. Cytosolic processing of the infused $[2,3,3-\text{H}_3]$serine would yield a doubly labeled one-carbon unit as $5,10-[\text{H}]$methylene tetrahydrofolate that would eventually yield doubly labeled methionine on homocysteine remethylation, as illustrated in our proposed metabolic model (Figure 5). It is likely that most of the $[\text{H}]$serine detected in apo B-100 (ie, formed in liver) is labeled with a deuterium atom at the C-2 position. This is because the 2 deuterium atoms at the C-3 position would be lost during passage through cSHMT to yield $5,10-[\text{H}]$methyltetrahydrofolate and $[2-\text{H}]$glycine. On passage of this $[\text{H}]$glycine back through cSHMT with incorporation of an unlabeled methylene group, the product would be $[2-\text{H}]$serine. This C-2-labeled form of $[2-\text{H}]$serine probably would not yield direct labeling of methionine unless any of the label is retained in passage through a mitochondrial glycine cleavage pathway. We propose that most of the singly labeled methyl groups forming $[\text{H}]$methionine are of mitochondrial origin from oxidation of the C-3 of $[\text{H}]$serine to $[\text{H}]$formate (Figure 5), as shown previously in yeast (9, 10).

To our knowledge, the preliminary results of this study provide the first in vivo evidence in a human subject of the mitochondrial generation of one-carbon units derived from exogenous serine for eventual remethylation of homocysteine. The results also indicate that exogenous serine yields methyl groups through cSHMT as well as via mSHMT, which is consistent with the data of an analogous study in which rats were given $[\text{H}]$serine (14). These findings are contrary to other research, which suggests that net flux through cSHMT typically occurs in the glycine to serine direction (5, 9–11, 15, 31), which suggests that cSHMT is readily reversible in vivo. Studies with cultured mammalian cells (11, 16) also indicated that mSHMT serves a critical function in cellular metabolism by forming one-carbon units and glycine for cellular needs.

The infusion of exogenous serine in this study may provide a driving force for a net cytosolic generation of one-carbon units through cSHMT in the serine to glycine direction. The postprandial direction of net flow of substrate through cSHMT is probably affected by the ratio of glycine to serine in dietary sources as well as the ratio of tetrahydrofolate to 5,10-methylene tetrahydrofolate. Tissues having the glycine cleavage system also use glycine as a source of one-carbon units (5, 18). It is likely that protein intake may influence the partitioning of serine among subcellular compartments and rates of these reactions. It is unclear why we observed significant accumulation of $[\text{H}]$serine but not of $[\text{H}]$glycine. The absence of measurable labeling of glycine in this study may indicate that the enrichment of $[\text{H}]$glycine, when formed, is below the detection limits of GC-MS (ie, molar enrichment $< \approx 0.001$) because of extensive endogenous production of unlabeled glycine. Another potential mechanism is the SHMT-catalyzed exchange of glycine pro-2S protons with solvent protons (32).
An additional noteworthy aspect of the present study was the measurement of the rate of homocysteine catabolism via the transsulfuration pathway, because the cystathionine $\beta$-synthase-mediated coupling of $[{}^{3}H]_{2}$serine and homocysteine yields $[{}^{1}H,{}^{3}H]_{2}$cystathionine. The fact that cystathionine labeling paralleled and was comparable with that of plasma $[{}^{3}H]_{2}$serine and greatly exceeded the enrichment of apo B-100 $[{}^{3}H]_{2}$serine strongly suggests that the serine pool responsible for the primary short-term formation of cystathionine is distinctly different from the serine pool involved in hepatic apo B-100 synthesis. In agreement with our observations of $M+3$ labeling of cystathionine in the present study, we saw in rats given a flooding dose of $[{}^{3}H]_{2}$serine that labeling of cystathionine is mainly as the $M+3$ form ($[{}^{3}H,{}^{3}H]_{2}$cystathionine), with small amounts of the $M+1$ and $M+2$ forms (14). In conclusion, the results of this study indicate that infusion of isotopically labeled serine can be used to investigate in detail human one-carbon metabolism in vivo and the influence of nutritional and genetic factors thereon.

The helpful discussions of this protocol with Waldo R Fisher and Jacob Sellhub are gratefully acknowledged.

REFERENCES