The metabolic role of 5-formyltetrahydrofolate is not known; however, it is an inhibitor of several folate-dependent enzymes including serine hydroxymethyltransferase. Methenyltetrahydrofolate synthetase (MTHFS) is the only enzyme known to metabolize 5-formyltetrahydrofolate and catalyzes the conversion of 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate. In order to address the function of 5-formyltetrahydrofolate in mammalian cells, intracellular 5-formyltetrahydrofolate levels were depleted in human 5Y neuroblastoma by overexpressing the human cDNA encoding MTHFS (5Y MTHFS cells). When cultured with 2 mM exogenous glycine, the intracellular serine and glycine concentrations in 5Y MTHFS cells are elevated approximately 3-fold relative to 5Y cells; 5Y MTHFS cells do not contain measurable levels of free methionine and display a 30–40% decrease in cell proliferation rates compared with 5Y cells. Medium supplemented with pharmacological levels of exogenous folinate or methionine ameliorated the glycine induced growth inhibition. Analysis of the folate derivatives demonstrated that 5-methylenetetrahydrofolate accounts for 30% of total cellular folate in 5Y cells when cultured with 2 mM exogenous glycine. 5Y MTHFS cells do not contain detectable levels of 5-methylenetetrahydrofolate under the same culture conditions. These results suggest that 5-formyltetrahydrofolate inhibits serine hydroxymethyltransferase activity in vivo and that serine synthesis and homocysteine remethylation compete for one-carbon units in the cytoplasm.

5-CHO-H$_4$PteGlu$^1$ normally accounts for 3–10% of total intracellular folate in mammalian cells; however, its metabolic function in cells has not been elucidated. 5-CHO-H$_4$PteGlu is synthesized from 5,10-CH$_2$-H$_4$PteGlu by both the mitochondrial and cytoplasmic isozymes of SHMT in vitro, and Escherichia coli lacking SHMT activity do not contain intracellular 5-CHO-H$_4$PteGlu, suggesting that SHMT catalyzes this reaction in vivo (1). The only enzyme known to metabolize 5-CHO-H$_4$PteGlu is MTHFS, an enzyme that catalyzes the ATP-dependent and irreversible conversion of 5-CHO-H$_4$PteGlu to 5,10-CH$_2$-H$_4$PteGlu. In rabbit liver, MTHFS is located in the cytoplasm (2), whereas human liver contains 85% of the MTHFS activity in the cytoplasm and 15% in the mitochondria (3). The combined enzymatic activities of MTHFS and SHMT constitute a futile cycle that may buffer cellular 5-CHO-H$_4$PteGlu concentrations and regulate SHMT activity (Scheme 1) (4).

Cytoplasmic one-carbon metabolism is responsible for the synthesis of purines, thymidylate, and methionine and numerous subsequent S-adenosylmethionine-dependent methylation reactions (Scheme 1). There is accumulating evidence that mitochondrial folate metabolism is primarily responsible for the generation of formate and that mitochondrial derived formate is the source of one-carbon units required for cytoplasmic folate-dependent anabolic reactions (5) (Scheme 1). The major source of one-carbon units in the form of formate are generated from serine in a reaction catalyzed by mSHMT, but formate can also be generated in the mitochondria from glycine in cells that contain a GCS. Recently, it has been demonstrated that glycine is the major source of one-carbon units in kidney proximal tubules, cells that also contain a GCS (6). The role of mitochondria in generating one-carbon units for cytoplasmic folate metabolism suggests that cSHMT may not play a major role in the generation of one-carbon units. In fact, there is accumulating evidence that cSHMT is not an efficient source of one-carbon units for cytoplasmic metabolism (Scheme 1). Recent studies of Chinese hamster ovary cells (CHO) that lack mSHMT activity have demonstrated that CHO cells deficient in mSHMT activity accumulate 15-fold increased intracellular serine concentrations over wild type CHO cells, and CHO cells lacking mSHMT activity are auxotrophic for glycine. Despite the accumulation of intracellular serine, the net metabolic flux through the cSHMT enzyme in CHO cells deficient in mSHMT activity is in the direction of serine synthesis (7). Therefore, the primary role of cSHMT may not be to generate glycine or one-carbon units but instead may have other metabolic functions including the synthesis of 5-CHO-H$_4$PteGlu.

Although little is known about the regulation of 5-CHO-H$_4$PteGlu, it has been reported that valproic acid treatment lowers the intracellular concentration of 5-CHO-H$_4$PteGlu in fetuses (8). In humans, valproic acid treatment is also associated with an increased incidence of neural tube defects, a disorder associated with folic acid status and elevated plasma homocysteine (9). 5-CHO-H$_4$PteGlu is a potent inhibitor of several folate-dependent enzymes including phosphoribosylaminomimidazole carboxamide formyltransferase and SHMT.
and may regulate folic acid-dependent metabolic pathways (4). 5-CHO-H₄PteGlu polyglutamates are slow tight binding inhibitors of SHMT and therefore may have a significant physiological relevance because slow binding inhibitors are generally more effective enzyme inhibitors in vivo (10). Inhibition of SHMT in vivo may be a mechanism whereby cells regulate not only folate-dependent glycine synthesis but also the supply of one-carbon units required for purine and thymidylate synthesis as well as homocysteine remethylation.

In order to determine the influence of 5-CHO-H₄PteGlu depletion on folic acid-mediated one-carbon metabolism and SHMT activity, we have overexpressed the human MTHFS cDNA in 5Y neuroblastoma and determined its effects on cell proliferation, intracellular serine, glycine and methionine concentrations, and the relative distribution of the folic acid one-carbon derivatives.

**Materials and Methods**

(6S)-[3H]Folinic acid (40 Ci/mmol) was obtained from New England Nuclear. 5-CHO-H₄PteGlu, MES, HEPES, and ATP were purchased from Sigma. Other chemicals were reagent grade. Fetal bovine serum, minimal essential medium (MEM), and its alpha modification (aMEM) lacking sodium bicarbonate, ribosides, ribotides, deoxyribosides, and deoxyribonucleotides were obtained from Hyclone Laboratories, Inc. Geneticin was obtained from Life Technologies, Inc.

**Vector Construction**—The full-length human MTHFS cDNA was subcloned into the XhoI/XbaI site of pcDNA3 (11). The pcDNA3 vector (Invitrogen) utilizes the human cytomegalovirus major intermediate early promoter/enhancer and the bovine growth hormone polyadenylation signal. A neomycin resistance marker expressed from the SV40 early promoter allows for selection of stable transformants in the presence of G418 sulfate.

**Cell Lines and Media**—The human SH-SY5Y neuroblastoma, a subline of the SK-N-SH neuroblastoma, were obtained from Dr. June Biedler (Fordham University) (12). The cells are nearly diploid, have biochemical and morphological properties of fetal noradrenergic neurons, and have been demonstrated to have a functional glycine cleavage system (13). The defined culture medium (aMEM) lacks glycine, serine, methionine, hypoxanthine, thymidine, and folate and allows variation of the relative distribution of nutrients with relevance to folate-dependent one-carbon metabolism. For all experiments, the fetal bovine serum was depleted of folic acid and other small molecules by dialysis at 4 °C over a 24-h period against a 12-fold excess of phosphate-buffered saline (PBS) with buffer changes every 4 h. The serum was then charcoal-treated to remove any remaining folic acid. G418 sulfate was supplemented into the medium for selection of stable cell colonies that integrated the MTHFS construction.

**Transfection by Electroporation**—The MTHFS construction (15 µg) was linearized with PvuII and transfected into 1 × 10⁶ 5Y neuroblastoma by electroporation (0.22 kV, 950 microfarad, Bio-Rad Gene Pulser II). Cells were cultured in aMEM with 10% dialyzed fetal bovine serum for 24–48 h prior to the addition of 500 µg/ml G418 sulfate for selection of stable integrants. The cells were incubated in a 5% CO₂ enriched, 37 °C incubator until single stable colonies formed. Over 20 colonies formed and exhibited resistance to G418 sulfate, and 6 were isolated and passaged until a stable line was generated.

**Folate Pool Analysis**—The relative distribution of the one-carbon forms of H₄PteGlu was performed by modification of previously reported procedures (14–16). Cells were passaged through three population doublings in aMEM without folate and supplemented with 200 µM glycine, 20 µM hypoxanthine, and 10 µM thymidine to deplete intracellular folate. Cells were labeled with [³H]folic acid by plating at mid to late log phase (4 × 10⁵ cells) on 100-mm dishes containing 3 ml of aMEM supplemented with 50 µM [³H]folic acid (20 Ci/mmol) for 24–36 h. Following incubation, cell monolayers were washed with PBS until the wash contained less than 500 cpm/ml. Cells were harvested in buffer containing 10 mM HEPES, 2 mM ascorbate, 10 mM 2-mercaptoethanol, pH 7.5. Cell extracts were boiled for 5 min, cooled to 4 °C, and centrifuged to remove cellular debris. Folate polyglutamates were converted to monoglutamates by incubation with rat serum at pH 7.0 for 3 h (15). H₄PteGlu, 10-CHO-H₄PteGlu, 5-CHO-H₄PteGlu, and 5-CH₃-H₄PteGlu standards were added to the sample prior to HPLC analysis.

The various folate derivatives were separated using a Shimadzu HPLC equipped with a diode-array spectrophotometric detector using a C₁₈ column (3.9 × 300 mm, 10 µm) and tetrabutylammonium phosphate/ethanol gradient as described previously (15), and 1-ml fractions were collected into scintillation vials. The fractions containing radioactivity were identified by comparison with the diode-array chromatogram and the absorbance spectra of the internal standards. The elution times were: p-aminobenzyglutamate, 9 min; 10-CHO-H₄PteGlu, 17 min; H₄PteGlu, 24 min; 5-CHO-H₄PteGlu, 36 min; and 5-CH₃-H₄PteGlu, 42 min.

**5,10-Methylenetetrahydrofolate Synthetase Assay**—The MTHFS enzyme activities were quantified by measuring the increase in absorbance at 360 nm due to the formation of 5,10-CH₃-H₄PteGlu. The assay was initiated by incubating 300 µl of cell extract with 700 µl of reaction buffer (100 mM MES, 0.5% Triton X-100, 14 mM 2-mercaptoethanol, 100 mM ATP, 150 mM MgCl₂, 0.2 mM (6S)-5-CHO-H₄PteGlu, pH 6.3). Protein concentrations were determined by the method of Lowry.

**Growth Studies**—Growth studies were performed to determine the effects of glycine, methionine, and folic acid on 5Y and SYMTHFS cell proliferation rates. Cells were grown in aMEM/dialyzed fetal bovine serum lacking folate and passaged for three population doublings to
deplete cells of intracellular folate. Following folate depletion, 1 × 10^6 cells were seeded into 15-mm wells at various glycine, methionine, and folate concentrations. Cells were harvested every 2 days and counted using a hemocytometer until at least three population doublings were observed. Cell viability was assessed by the ability of cells to exclude trypan blue.

**Amino Acid Analysis**—Free amino acids were isolated by a modification of a previously described procedure (17). The medium was removed, and the cell monolayers were washed three times with 10 ml of PBS. The cells were harvested from 100-mm plates using a cell scraper after the addition of 300 µl of 5% trichloroacetic acid. Cell extracts were transferred to microcentrifuge tubes and vortexed to ensure complete cell lysis. After centrifugation at 12,000 × g for 20 min, the supernatants were transferred to new microcentrifuge tubes, and the trichloroacetic acid was removed by extraction with water-saturated diethyl ether. The aqueous solution containing the free amino acids was vacuum dried overnight. The free amino acids were derivatized with o-phthaldialdehyde, and the amino acid concentrations were determined at the Cornell Biotechnology Analytical/Synthesis Facility. Amino acid concentrations were determined relative to cell number, protein concentration, and intracellular valine concentrations. All methods gave similar relative results.

**Western Analysis—**5Y and 5YMTHFS cell pellets were suspended and lysed by sonication then incubated at 100 °C for 10 min in buffer containing 2% SDS, 100 mM dithiothreitol, 60 mM Tris, pH 6.8. SDS-polyacrylamide gel electrophoresis was carried out using a 5% stacking gel and a 8% separating gel in a slab gel apparatus (Hoefer, San Francisco, CA) with the discontinuous buffer system of Laemmli. Proteins were transferred overnight to a polyvinylidene difluoride membrane (Millipore) in a Bio-Rad Transblot apparatus at 30 V with a limit of 0.2 mA. The membrane was rinsed with 0.1% Tween 20 in PBS and blocked for 10 h with 1% nonfat dry milk, 0.1% Nonidet P-40 in PBS. Sheep anti-human MTHFS antiserum was generated from Chiron Mimetopes (San Diego, CA) using synthetic peptides representing amino acids 2-28 in the human MTHFS protein. The serum was diluted 1:10,000 in blocking buffer, and the membrane was incubated in the buffer for 15 h at 4 °C. The membrane was rinsed six times with 0.1% Tween 20 in PBS and incubated for 23 h with blocking buffer containing horseradish peroxidase-conjugated mouse anti-sheep IgG antibody in a 1:6000 dilution. The membrane was visualized using the SuperSignal Chemiluminescence substrate system from Pierce.

**RESULTS**

**Generation of 5YMTHFS Cells—**5-CHO-H_4PteGlu is the only derivative of H_4PteGlu that does not have a known metabolic function. In order to determine the role of 5-CHO-H_4PteGlu in mammalian metabolism and the metabolic consequences associated with its depletion in the cell, the human MTHFS cDNA was overexpressed in 5Y neuroblastoma. Over 20 neomycin-resistant colonies formed, and six colonies were isolated and passaged until stable cell populations were obtained. All transfectants have remained viable for over 1 year. The MTHFS enzyme activity was determined for each transformant. The MTHFS activity in the 5YMTHFS cell extracts ranged from 175 to 584 pmol 5,10-CH_2-H_4PteGlu formed/min/mg total protein, whereas the MTHFS activity was undetectable in 5Y cells (<5 pmol 5,10-CH_2-H_4PteGlu/min/mg) (Table I). All of the MTHFS activity was localized to the cytoplasmic fraction, suggesting that the previously reported MTHFS cDNA does not encode a mitochondrial MTHFS enzyme. However, we cannot rule out the possibility that mitochondrial MTHFS has been increased because we were not able to detect any MTHFS activity in isolated mitochondria from either 5Y or 5YMTHFS cells.

MTHFS overexpression was verified by Western blot analysis using the 5Y and 5YMTHFS cell lines (Fig. 1). While varying molecular masses have been reported for the MTHFS enzyme ranging from 19 to 32 kDa, the human cDNA is expected to express an enzyme with a molecular mass of 23 kDa (11). Although MTHFS protein was not detected in the 5Y cells consistent with the low levels of enzyme activity found in these cells, 5YMTHFS cell extracts contain a strong immunoreactive band present at 26 kDa, consistent with the expression of the MTHFS cDNA (11). A second uncharacterized 33-kDa immunoreactive band is present at equal intensity in both 5Y and 5YMTHFS cells.

**Amino Acid Concentrations—**Overexpression of the MTHFS cDNA in the cytoplasm would be expected to lower cytoplasmic 5-CHO-H_4PteGlu levels and perhaps activate cSHMT activity and influence intracellular serine and glycine metabolism. In order to determine if 5-CHO-H_4PteGlu depletion influenced cytoplasmic one-carbon metabolism, intracellular amino acid concentrations were determined for the 5Y and 5YMTHFS cells cultured in the presence and the absence of exogenous glycine (Table II). When cultured without glycine, serine and glycine concentrations were elevated approximately 60% in 5YMTHFS cells compared with 5Y cells; however, the glycine/serine ratio and intracellular methionine concentrations were unchanged. When cultured with exogenous glycine (2 mM), the intracellular glycine concentrations in 5YMTHFS cells were elevated nearly 3-fold compared with 5Y cells, whereas intracellular serine concentrations were elevated greater than 3-fold relative to 5Y cells. Additionally, no methionine was detected in the 5YMTHFS cells when cultured with 2 mM exogenous glycine. These results suggest that intracellular 5-CHO-H_4PteGlu influences intracellular serine, glycine, and methionine concentrations and that these changes are associated with changes in SHMT activity in the cytoplasm.

**Growth Studies—**There is accumulating evidence that cells contain a greater number of folic acid-dependent enzymes than folic acid coenzymes and that folic acid metabolism is highly compartmentalized (5, 18). Evidence for channeling of the folic acid cofactor has also been suggested in a number of studies. This suggests that the purine, serine, and thymidylate synthetic, as well as homocysteine remethylation pathways are not saturated with folate and that these pathways compete for the limited supply of one-carbon units carried by folate acid cofactors. Initial studies of the 5YMTHFS cells suggested that all transfecants were growth inhibited in the presence of elevated exogenous glycine. In order to determine if overexpression of the MTHFS cDNA in 5Y cells disrupted cytoplasmic one-carbon metabolism, growth studies were performed to determine if the
5YMTHFS cells had additional nutrient requirements. Both 5Y and 5YMTHFS cells require exogenous glycine for maximum cell proliferation rates with growth rates reaching their optimum between 0.2 and 0.5 mM glycine (Fig. 2). When cultured in the presence of 1.0 μM (6R,6S)-5-CHO-H₄PteGlu, 5YMTHFS6 cells did not require exogenous thymidylate, purines, or methionine to maintain optimal growth rates. However, when cultured in the presence of 10 mM (6R,6S)-5-CHO-H₄PteGlu as described under “Materials and Methods,” the 5YMTHFS6 cells were growth inhibited relative to 5Y cells when exogenous glycine concentrations exceeded 1 mM (Fig. 2). 5YMTHFS6 cells display a 30–40% decrease in cell proliferation rates at glycine concentrations of 2 mM or greater relative to 5Y cell proliferation rates. The growth inhibition was ameliorated in the 5YMTHFS6 cells by the addition of 1.0 μM folic acid to the culture medium (Fig. 2).

In order to determine if the glycine-induced growth inhibition of 5YMTHFS6 cells resulted in the disruption of purine, thymidine, or methionine synthesis, the ability of methionine, hypoxanthine, and thymidylate to ameliorate the glycine-induced growth inhibition was examined (Fig. 3). The addition of exogenous hypoxanthine (500 μM) or thymidylate (500 μM) did not ameliorate the glycine-induced (5.0 mM) growth inhibition, whereas the addition of exogenous methionine at 1.0 mM completely ameliorated the glycine-induced growth inhibition in the 5YMTHFS6 cells. These results suggest that the growth inhibition exhibited by 5YMTHFS6 cells in the presence of glycine is due to depletion of intracellular methionine and that 5-CHO-H₄PteGlu depletion results in disruptions of homocysteine remethylation.

Analysis of the Folic Acid One-carbon Derivative Distribution—The individual distribution of folic acid one-carbon forms was determined for the 5Y and 5YMTHFS6 cells in the presence and absence of glycine (Table III). 5Y cells cultured with 0.0 or 0.2 mM exogenous glycine or 0.5 mM L-serine contain predominately 10-CHO-H₄PteGlu (63–70%) and H₄PteGlu (21–28%) with detectable levels of 5-CHO-H₄PteGlu (3–7%) and 5-CH₃-H₄PteGlu (3–4%). However, when cultured in the presence of 5 mM glycine, 10-CHO-H₄PteGlu (57%) and H₄PteGlu (8.5%) concentrations were decreased, whereas 5-CH₃-H₄PteGlu levels increased markedly (31%) without affecting 5-CHO-H₄PteGlu levels (3%). Cell fractionation studies suggested that all of the accumulated 5-CH₃-H₄PteGlu was localized to the cytoplasm. The increase in 5-CH₃-H₄PteGlu and decrease in H₄PteGlu levels associated with 5.0 mM exogenous glycine suggests that glycine can serve as a major source of one-carbon units for cytoplasmic folate metabolism via the GCS.

When cultured in the absence of glycine, the 5YMTHFS6 cells do not contain any 5-CHO-H₄PteGlu, verifying that overexpression of MTHFS in the cytoplasm depletes intracellular 5-CHO-H₄PteGlu concentrations. In all culture conditions 10-CHO-H₄PteGlu (67–90%) is the predominant folate derivative in these cells, with the remaining folate present as H₄PteGlu (8–32%). 5-CH₃H₄PteGlu was not detected (<1%) in the culture conditions listed in Table III. These results suggest that

| Cell line | Medium Glycine mM | Glycine Serine mM | Serine Valine μM | Glycine Serine mM | Methionine Valine μM |
|-----------|------------------|------------------|------------------|------------------|---------------------|
| 5Y        | 0.0              | 1.40 ± 0.02      | 0.26 ± 0.02      | 5.38 ± 0.02      | 0.34 ± 0.01         |
| 5Y        | 2.0              | 2.32 ± 0.12      | 0.52 ± 0.03      | 4.67 ± 0.07      | 0.28 ± 0.04         |
| 5YMTHFS6  | 0.0              | 2.32 ± 0.07      | 0.42 ± 0.05      | 5.31 ± 0.06      | 0.22 ± 0.02         |
| 5YMTHFS6  | 2.0              | 6.10 ± 0.21      | 1.82 ± 0.08      | 3.35 ± 0.15      | <0.04               |

a Amino acid concentrations are represented as picomoles recovered relative to valine concentrations.

**DISCUSSION**

In the present study, the effects of 5-CHO-H₄PteGlu depletion on folate-dependent one-carbon metabolism were deter-
mained by overexpressing the MTHFS cDNA in the cytoplasm of human 5Y neuroblastoma. Previous studies of the 5Y neuroblastoma (13) have demonstrated that these cells contain both mitochondrial and cytoplasmic SHMT activities, as well as an active GCS. Analysis of the folic acid one-carbon pools demonstrated that overexpression of MTHFS in the cytoplasm depletes all cellular 5-CHO-H4PteGlu, suggesting that 5-CHO-H4PteGlu accumulates in the cell due to limiting MTHFS activity. However, although overexpression of the MTHFS cDNA resulted in accumulation of MTHFS enzyme in the cytoplasm, we cannot rule out the possibility that mitochondrial MTHFS activity was also increased because we were not able to detect any MTHFS activity in purified mitochondria from either 5Y or 5Y MTHFS cells. Analysis of the intracellular free amino acid pools suggests that depletion of 5-CHO-H4PteGlu increased both intracellular serine and glycine concentrations, suggesting that 5-CHO-H4PteGlu polyglutamates do inhibit cSHMT activity in vivo, consistent with previous in vitro studies (10).

In these studies, we have demonstrated that 5Y cells do not accumulate 5-CH3-H4PteGlu in the cytoplasm of the absence of exogenous glycine, whereas 5-CH3-H4PteGlu accounts for 31% of total folate in 5Y cells cultured with 5 mM exogenous glycine. This suggests that glycine is an effective one-carbon source for the synthesis of 5-CH3-H4PteGlu and subsequent homocysteine remethylation (Scheme 1). 5Y cells overexpressing MTHFS in the cytoplasm do not accumulate either free methionine or 5-CH3-H4PteGlu when cultured with elevated exogenous glycine, suggesting that glycine is no longer an effective source of one-carbon units for homocysteine remethylation. The observation that glycine is no longer an effective one-carbon source for homocysteine remethylation in 5Y MTHFS cells suggests that 5-CHO-H4PteGlu plays a critical role in regulating the activity of cSHMT and the relative distribution of the one-carbon forms of folate.

When cultured with exogenous glycine, 5Y MTHFS6 cells appear to accumulate serine, whereas methionine pools are depleted. These results suggest that there are two reactions competing for one-carbon units in the form of 5,10-CH2-H4PteGlu: serine synthesis catalyzed by cSHMT and 5-CH3-H4PteGlu synthesis catalyzed by the enzyme methylenetetrahydrofolate reductase (Scheme 1). The combined activation of cSHMT resulting from 5-CHO-H4PteGlu depletion and the availability of exogenously supplied glycine inhibits the flow of one-carbon units from 10-CHO-H4PteGlu to 5-CH3-H4PteGlu due to an increase in serine synthesis by cSHMT. In the absence of exogenous glycine, depletion of 5-CHO-H4PteGlu does not affect cell proliferation rates in 5Y MTHFS cells. However, when cultured with 10 mM folic acid, exogenous glycine (2 mM) inhibits 5Y MTHFS cell proliferation 30–40% relative to 5Y cell proliferation rates. This suggests that both cSHMT activation and a source of exogenous glycine is required to deplete 5-CHO-H4PteGlu and thereby inhibit homocysteine remethylation.

We have also attempted to overexpress the human MTHFS cDNA in human MCF-7 cells and CHO cells.2 These cell lines do not contain a GCS and require serine as the major source of one-carbon units. We were not able to obtain stable cell lines that overexpressed MTHFS in the CHO cells, suggesting that MTHFS overexpression may be lethal in these cells. We were only able to obtain two stable MCF-7 cell transfecants that overexpressed the MTHFS cDNA. In the MCF-7 transfecants, MTHFS activity was elevated less than 6-fold, suggesting that increases in MTHFS activity in MCF-7 cells may also be lethal.

The low levels of 5-CHO-H4PteGlu in the cell do play a role in folate-dependent one-carbon metabolism. 5-CHO-H4PteGlu is required to keep cytoplasmic folate metabolism in homeostasis by mediating the flow of one-carbon units in the cytoplasm through either the homocysteine remethylation or serine synthesis pathways. The need for folate-dependent serine synthesis in the cytoplasm is not clearly understood because serine synthesis from glycolytic intermediates also occurs in the cytoplasm. However, these studies suggest that the slow tight binding inhibitor 5-CHO-H4PteGlu regulates cSHMT activity and folate-dependent serine synthesis. This may be a mechanism used by cells to prevent accumulation of intracellular folate as 5-CH3-H4PteGlu and thereby regenerate H4PteGlu for purine biosynthesis. This notion is supported by previous in vitro kinetic studies that have demonstrated substrate channelling of THF polyglutamate cofactor from cSHMT to 10-formyltetrahydrofolate synthetase during the cSHMT catalyzed conversion of glycine to serine (19).

Finally, it is also of interest that increased availability of either methionine or folic acid can ameliorate the glycine-induced growth inhibition of 5Y MTHFS cells. It has been well established that disruptions in folate-dependent homocysteine remethylation can result in incomplete closure of the neural tube during development (9). The ability of 5-CHO-H4PteGlu to influence 5-CH3-H4PteGlu concentrations and homocysteine remethylation suggests that regulation of the 5-CHO-H4PteGlu futile cycle may be critical to maintain one-carbon homeostasis especially during rapid proliferative stages in development.

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J. R. Suh and P. Stover, unpublished data.

| Cell line | Glycine | Serine | 10-CHO-H4PteGlu | H4PteGlu | 5-CHO-H4PteGlu | 5-CH3-H4PteGlu |
|-----------|--------|-------|----------------|----------|----------------|---------------|
| 5Y        | 0      | 0     | 72 ± 3         | 21 ± 3   | 3 ± 1          | 4 ± 1         |
| 5Y        | 0.25   | 0     | 65 ± 2         | 28 ± 2   | 4 ± 1          | 3 ± 1         |
| 5Y        | 5.0    | 0     | 57 ± 3         | 9 ± 3    | 3 ± 3          | 31 ± 3        |
| 5Y        | 0      | 0.5   | 61 ± 1         | 27 ± 2   | 7 ± 2          | 4 ± 1         |
| 5YMTHFS6  | 0.25   | 0     | 67 ± 4         | 32 ± 4   | <1             | <1            |
| 5YMTHFS6  | 5.0    | 0     | 76 ± 3         | 23 ± 3   | <1             | <1            |
| 5YMTHFS6  | 0      | 0     | 70 ± 3         | 29 ± 3   | <1             | <1            |
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