

# Decreased Rates of Methionine Synthesis by Methylene Tetrahydrofolate Reductase-deficient Fibroblasts and Lymphoblasts

GERRY R. BOSS and RICHARD W. ERBE, *Genetics Unit, Children's Service and Medical Services, Massachusetts General Hospital, Departments of Pediatrics and Medicine, Harvard Medical School, Boston, Massachusetts 02114*

**ABSTRACT** Methionine synthesis from homocysteine was measured in intact human fibroblasts and lymphoblasts using a [ $^{14}\text{C}$ ]formate label. Seven fibroblast lines and two lymphoblast lines derived from patients with 5,10-methylene tetrahydrofolate reductase deficiency had rates of methionine synthesis that were from 4 to 43% of normal. When the patients were divided by clinical status into mildly (two patients), moderately (two patients), and severely (three patients) affected, methionine biosynthesis expressed as a percent of control values was 43 and 33%, 11 and 10%, and 7, 6, and 4%, respectively, in fibroblasts. Similar data for the two lymphoblast lines were 36 and 26% for a mildly and moderately affected patient, respectively. These data are to be contrasted with the measurement of residual enzyme activity in cell extracts which agrees less precisely with the clinical status of the patients. In the presence of normal methionine synthetase activity, the rate of synthesis of methionine from homocysteine is a function of the activity of the enzyme 5,10-methylene tetrahydrofolate reductase, and measurement of the methionine biosynthetic capacity of cells deficient in this enzyme accurately reflects the clinical status of the patient from whom the cells were derived.

## INTRODUCTION

Deficiency of the enzyme 5,10-methylene tetrahydrofolate reductase (EC 1.1.1.68) leads clinically to neurologic dysfunction of variable type and severity, and biochemically to homocystinuria, homocystinemia, and

decreased plasma and intracellular concentrations of methionine (1). The enzyme catalyzes the essentially irreversible reduction of 5,10-methylenetetrahydrofolate ( $5,10\text{-CH}_2\text{-H}_4\text{PteGlu}$ ) to 5-methyltetrahydrofolate ( $5\text{-CH}_3\text{-H}_4\text{PteGlu}$ ) (2).  $5\text{-CH}_3\text{-H}_4\text{PteGlu}$  serves as the methyl donor in the conversion of homocysteine ( $\text{HC}$ )<sup>1</sup> to methionine ( $\text{Met}$ ) by the cobalamin-dependent enzyme, methionine synthetase.  $5,10\text{-CH}_2\text{-H}_4\text{PteGlu}$  reductase deficiency has been shown to result in reduced  $5\text{-CH}_3\text{-H}_4\text{PteGlu}$  levels in serum, erythrocytes, and brain (3), and a diminished proportion of total intracellular folates as  $5\text{-CH}_3\text{-H}_4\text{PteGlu}$  in fibroblasts cultured from these patients (4). Deficiency of  $5\text{-CH}_3\text{-H}_4\text{PteGlu}$  would be expected to result in reduced  $\text{Met}$  synthetase activity leading to diminished  $\text{Met}$  biosynthesis, but this has not yet been demonstrated directly although it is implied by two findings. First, skin fibroblasts from  $5,10\text{-CH}_2\text{-H}_4\text{PteGlu}$  reductase-deficient patients grow less rapidly (5) or, under more stringent conditions, not at all (1) when  $\text{HC}$  replaces  $\text{Met}$  in culture medium. Second, tissue amino acid analysis reported only for brain and liver of one patient indicated a reduced concentration of  $\text{Met}$  (6).

Activity of  $5,10\text{-CH}_2\text{-H}_4\text{PteGlu}$  reductase has been measured in crude extracts of skin fibroblasts or leukocytes using a coupled reaction that proceeds in the reverse direction from the physiologic reaction (2). The extent of  $5,10\text{-CH}_2\text{-H}_4\text{PteGlu}$  reductase deficiency measured in this way has correlated imperfectly with the severity of the clinical and metabolic abnormalities in the patients from whom the cells were derived. To test the hypothesis that the clinical and metabolic abnormalities would more closely parallel the degree of secondary disturbance of the  $\text{Met}$  synthetase reaction, we undertook to measure the  $\text{Met}$  biosynthetic capacity

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<sup>1</sup> Abbreviations used in this paper:  $\text{HC}$ , homocysteine;  $\text{PBS}$ , phosphate-buffered saline.

in a whole cell system of fibroblasts and lymphoblasts by means of a [ $^{14}\text{C}$ ]formate label. When applied to cells from seven patients with 5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$  deficiency, this method yielded results that correlate more precisely with the clinical status of the patients from whom the cells were derived than does the measurement of residual enzyme activity in cell extracts. Moreover, they constitute direct demonstration that Met synthesis is reduced by this inherited enzyme defect.

## METHODS

**Cell culture.** Fibroblast lines were obtained from the patients and control subjects after approval by Human Subjects Committee according to standard procedures and were grown in Eagle's minimum essential medium (Gibco Laboratories, Grand Island Biological Company, Grand Island, N. Y.) supplemented with nonessential amino acids and 10% fetal bovine serum (Gibco) in an atmosphere of 95% air and 5%  $\text{CO}_2$ . The lymphoblastoid cell lines were initiated by Epstein-Barr virus transformation of peripheral blood lymphocytes (7) and were grown in RPMI-1640 (Gibco) supplemented with 15% fetal bovine serum (Gibco) and 2 mM glutamine in 95% air and 5%  $\text{CO}_2$ . Fibroblasts were fed twice a week and lymphoblasts were maintained at a density between  $2\text{--}10 \times 10^5$  cells/ml. All cells were tested for mycoplasma by means of bioassay and DNA staining methods.

**5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$  reductase assay.** Fibroblasts were grown to confluency in either glass roller bottles or plastic petri dishes and maintained at confluency for at least 7 d before harvesting. The cells were released from the surface by trypsinization, washed in Dulbecco's phosphate-buffered saline (PBS) solution, and resuspended at  $10^8$  cells/ml in 0.25 M sucrose. The cells were disrupted by sonication and the crude extract centrifuged at 90,000 g for 1 h at  $4^\circ\text{C}$ . Lymphoblasts were kept in logarithmic growth before harvesting by centrifugation and then treated similarly to the fibroblasts.

Extracts were stored at  $-60^\circ\text{C}$  in small aliquots and thawed only once just before use. The standard 5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$  reductase assay was performed according to previously described procedures with the cells grown in either Met or Met-free HC-supplemented medium (8) in the direction of 5- $\text{CH}_3\text{-H}_4\text{PteGlu}$  to 5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$ .

**Methionine biosynthesis assay.** Fibroblasts were passaged 1:2 36 h before assay, and 12 h before assay were replated at a density of  $3 \times 10^5$  cells/35-mm plastic petri dish (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). 1 h before assay the standard medium was replaced by 0.6 ml of Met-free minimum essential medium that was supplemented with nonessential amino acids, 200  $\mu\text{M}$  DL-homocysteine-thiolactone-HCl, 100  $\mu\text{M}$  folic acid, 1.5  $\mu\text{M}$  hydroxocobalamin and 10% fetal bovine serum that had been dialyzed for at least 24 h against a 100-fold volume of 0.9% NaCl with two changes. The assay was initiated by the addition of sodium [ $^{14}\text{C}$ ]formate (final concentration of 0.61 mM, 54.7 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) and incubated at  $37^\circ\text{C}$  for 4–8 h. The assay was terminated by placing the dishes on ice and quickly washing the cells twice with 3 ml of ice-cold PBS, leaving an additional 0.5 ml of PBS in the dishes. Cells were removed from the surface by scraping with a teflon policeman and added to trichloroacetic acid (TCA) to yield a final concentration of 5%. The cells were extracted on ice for 30 min, then heated for 30 min at  $80^\circ\text{C}$  to solubilize the DNA and RNA (9). The tubes were cooled on ice for 15 min and the resulting protein precipitate, free of nucleic acids, was washed three times with 10% TCA. The

protein pellets were resuspended in 0.4 ml of ultra-pure 6 N HCl (Alfa Div., Ventron Corp., Danvers, Mass.) and hydrolyzed for 24 h at  $110^\circ\text{C}$ . The resulting hydrolysate was concentrated by vacuum-evaporation to dryness with a heat-lamp and resuspended in 50  $\mu\text{l}$  of  $\text{H}_2\text{O}$ . A 10- $\mu\text{l}$  sample was streaked on cellulose thin-layer chromatography plates with fluorescent indicator (Eastman Kodak Co., Rochester, N. Y.). Met (and its breakdown products Met sulfoxide and Met sulfone) were separated from serine and cysteine (and its products cystine and cysteic acid) in a phenol:ethanol: $\text{H}_2\text{O}$ : $\text{NH}_4\text{OH}$  (65:20:20:2) one-dimensional solvent system. Since serine condenses with HC to form cystathionine, which is converted to cysteine, any label that appears in cysteine actually originates from serine. The cold carrier amino acids were visualized using UV light after spraying the plates with fluorescamine (Kontes Co., Vineland, N. J.) and the spots cut out and quantified by liquid scintillation counting in Econofluor (New England Nuclear, Boston, Mass.) at 97% efficiency. To control unlabeled hydrolysates were added either [ $^{14}\text{C}$ ]methionine, [ $^{14}\text{C}$ ]serine, or [ $^{35}\text{S}$ ]cysteine; 98% of the Met label, 96% of the serine label, and 97% of the cysteine label cochromatographed with their respective unlabeled standards. The assay was linear with time in both the normal and the reductase-deficient cell lines to at least 8 h. Duplicate samples agreed within 10%. Leucine (Leu) incorporation into protein was measured in replicate plates by adding L-[3,4,5- $^3\text{H}$ (N)]leucine (120 Ci/mmol; New England Nuclear) at a final concentration of 14  $\mu\text{M}$ . After the TCA precipitation, the precipitate was collected on GF/A glass microfiber filters (Whatman, Inc., Clifton, N. J.) washed three times with 10% TCA and once with 0.1 N HCl, dried and counted in Econofluor at 35% efficiency. Protein was determined by the method of Lowry (10) in a third set of replicate plates. Amino acid analysis by ion exchange chromatography was performed on a Beckman amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.).

Lymphoblasts were maintained in logarithmic growth until the day of assay when they were recovered from the RPMI medium by centrifugation and resuspended at a density of  $1.5 \times 10^6$  cells/ml in the Met-free, HC-supplemented medium. 1 ml of the cells was placed in  $16 \times 100\text{-mm}$  glass tubes, flushed with 95% air-5%  $\text{CO}_2$ , capped, and allowed to equilibrate for 1 h. The cells were incubated for 4 h with the sodium [ $^{14}\text{C}$ ]formate at a final concentration of 0.18 mM and the [ $^3\text{H}$ ]leucine at 8.4  $\mu\text{M}$ . The reaction was terminated by plunging the tubes into ice water and adding 3 ml of ice-cold PBS. The cells were centrifuged at 500 g for 10 min and extracted in 1 ml of 5% TCA for 30 min. The remainder of the procedure was the same as for the fibroblasts.

**Origin of cell lines.** Four of the five control fibroblast lines were initiated in our laboratory and the fifth, MGF379, was obtained from the American Type Culture Collection (CLR1125), Rockville, Md. Three of these lines were derived from males and two from females, and the ages of these normal subjects approximated the ages of the 5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$  reductase-deficient patients. Passage number was controlled for between the normal and mutant cell lines. Three of the 5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$  reductase-deficient fibroblast lines, viz., MGF451, MGF452, and MGF548, were obtained from Dr. S. H. Mudd and Dr. W. B. Uhlendorf of the National Institutes of Health. Two mutant lines, MGF858 and MGF876, were obtained from the Canadian Repository for Mutant Cell Strains, Montreal, Quebec, Canada, with registry numbers WG735 and WG670, respectively. Cell line MGF735 was obtained from Dr. S. Cederbaum of the University of California, Los Angeles. Line MGF488 was initiated in our laboratory.

The two control lymphoblast lines, MGL8B2 and MGL10, originated in our laboratory. The 5,10- $\text{CH}_2\text{-H}_4\text{PteGlu}$  reduc-

tase-deficient lymphoblast line MGL28 was initiated in Dr. J. Edwin Seegmiller's laboratory at the University of California, San Diego, with log number 1082. Cell line MGL30 was started in our laboratory.

## RESULTS

Table I lists seven patients with 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase deficiency from six different families, and with a brief clinical summary, divides them by clinical status into mildly, moderately, and severely affected. Enzyme activity was assayed according to standard procedures (see Methods) in crude extracts of fibroblasts and lymphoblasts derived from the patients. For comparison the normal values for our laboratory are listed at the bottom of the table. The two mildly affected patients have only slightly more residual enzyme activity in their fibroblast extracts than the moderately af-

fected siblings (1.6 and 1.6 nmol HCOH/mg protein per h compared with 1.2 and 1.1 nmol HCOH/mg protein per h), the separation of these two groups of patients by residual enzyme activity thus being small. In fibroblasts from two of the severely affected patients (MGF858 and MGF876), enzyme activity is below the limits of detection by our assay (0.2 nmol HCOH/mg protein per h). The fibroblast extract of the third severely affected patient (MGF735) paradoxically has the highest residual enzyme activity (2.9 nmol HCOH/mg protein per h) of any of the patients. The two lymphoblast lines (MGL30 and MGL28) have more enzyme activity than in the corresponding fibroblast lines (MGF548 and MGF451) even after correction for the higher activity in the control lymphoblast lines compared with the control fibroblast lines.

The results of our whole-cell assay for Met biosyn-

TABLE I  
*Clinical Summary of Seven Patients with 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu Reductase Deficiency and Enzyme Activity in Fibroblast and Lymphoblast Extracts*

Patient	Fibroblasts		Lymphoblasts		Clinical summary
	Cell line number	5,10-CH <sub>2</sub> -H <sub>4</sub> PteGlu reductase activity	Cell line number	5,10-CH <sub>2</sub> -H <sub>4</sub> PteGlu reductase activity	
		nmol HCOH/mg protein/h*		nmol HCOH/mg protein/h*	
W.M.	MGF548	1.6±0.4	MGL30	2.9±0.1	Mildly affected: cerebellar ataxia with mild spastic quadraparesis; focal seizure disorder (1)
C.P.	MGF488	1.6±0.4	—	—	Mildly affected: abnormal gait with decreased muscle strength and focal seizure disorder (5, 11)
L.M.	MGF451	1.2±0.4	MGL28	2.1±0.3	Moderately affected: mental retardation with schizophrenia-like behavior; moderate muscle weakness with spasticity (5, 12, 13)
B.M.	MGF452	1.1±0.2	—	—	Moderately affected: sister of L.M., also mentally retarded with peripheral neuropathy (1, 5, 12, 13)
S.M.	MGF858	0.2	—	—	Severely affected: marked psychomotor retardation with hypotonia; apneic spells with hypothermia and coma age 7 mo; alive at 2 yr (14)
M.S.	MGF735	2.9±0.2	—	—	Severely affected: presentation in infancy; marked developmental delay; respiratory failure age 15 mo; alive at 4 yr†
S.S.	MGF876	0.2	—	—	Severely affected: severe psychomotor retardation; convulsions with coma at 6 months; death at 9 mo (14)
Normal subjects	Average of five lines	9.0±2.0	Average of two lines	11.7±1.2	

\* Values are the mean with SD of at least three assays performed in duplicate.

† Personal communication.

TABLE II  
Methionine Biosynthesis by Five Normal and Seven  
5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu Reductase-deficient  
Fibroblast Lines

Cell line designation	Methionine synthesis*			Clinical status
	pmol/mg protein/h	pmol/pmol Leu incorp.	Met	
			Total protein label† percent	
MGF287	903±205	172±52	58±11	Normal
MGF297	717±193	163±53	49±6	Normal
MGF316	513±96	117±6	42±8	Normal
MGF344	380±105	102±7	47±8	Normal
MGF379	897±224	197±66	48±4	Normal
Average of controls	682±232	163±53	49±6	
MGF548	273±53	69.5±11	46±11	Mildly affected
MGF488	253±60	53.8±1	23±6	Mildly affected
MGF451	53±16	17.3±6	17±5	Moderately affected
MGF452	50±26	16.3±5	26±11	Moderately affected
MGF858	42±18	11.8±3	5±1	Severely affected
MGF735	30±12	10.6±1	19±6	Severely affected
MGF876	21±15	6.5±4	8±2	Severely affected

\* Values are the mean with 1 SD of at least three separate assays performed in duplicate. Methionine synthesis includes radioactivity recovered in Met, Met sulfoxide, and Met sulfone.

† Total protein label includes radioactivity recovered in Met (and Met sulfoxide and Met sulfone), serine, and cysteine (and cystine and cysteic acid).

thesis in five normal fibroblast lines are given in Table II. The data are expressed as picomoles of [<sup>14</sup>C]formate incorporated into Met either per milligram of cell protein or, to control for differences among the cell lines in rates of protein synthesis during the incubation period, per picomole of Leu incorporated into cell protein. Because we analyzed only labeled Met that becomes incorporated into protein (and not the free amino acid pool), the latter method of analysis may be more appropriate because the incorporation of the labeled Leu may be subject to the same factors, e.g., dilution. In control experiments we incubated cells with L-[methyl-<sup>14</sup>C]methionine and found that ~65% of the total cellular radioactivity appeared in the protein fraction and thus this method is representative of the Met synthesized by the cells. The percent column demonstrates that, of the formate label that enters the 5,10-

CH<sub>2</sub>-H<sub>4</sub>PteGlu pool and then becomes incorporated into Met, about half is in Met and half is in serine-cysteine. Complete amino acid analysis of a labeled hydrolysate by ion exchange chromatography and simultaneous determination of radioactivity demonstrated that >95% of the label in protein was identifiable as either Met or serine-cysteine. Of the remaining 5%, ~1% appeared to be in methyl-histidine and the remaining label did not co-elute with any known amino acid.

The bottom half of Table II shows the Met synthetic data of the seven 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient fibroblast lines. All lines are clearly deficient in Met synthesis when compared with the normal fibroblasts. The cells derived from the two mildly affected patients (MGF548 and MGF488) are easily distinguished from the two moderately affected siblings (MGF451 and MGF452) through the use of this assay of Met synthesis. The moderately affected patients, L.M. and B.M. (from whom cell lines MGF451 and MGF452 were derived, respectively), are sisters and show the expected intrafamily similarities in their rates of Met synthesis. The fibroblasts from the three severely affected patients (MGF858, MGF735, and MGF876) all show marked reductions in Met synthetic capacity. The percentage of the label that enters Met (label in Met/label in Met plus serine-cysteine × 100%) shows greater variability than do the absolute rates of Met synthesis. With the exception of line MGF548, however, a considerably smaller proportion of the formate label enters Met in the reductase-deficient cells than in the control cells.

Cell lines MGF548 and MGF488 derived from patients with mild disease have 43 and 33% of control Met synthesis when expressed as Met synthesized per picomole of Leu incorporated into protein. Both of these values are considerably higher than the residual enzyme activity (18% of control cells) measured in cell extracts. Cell lines MGF451 and MGF452 derived from the moderately affected patients have 11 and 10%, respectively, of control values for Met synthesis, and these numbers compare favorably with residual enzyme activity (13 and 12% of normal, respectively). The cell lines derived from the three severely affected patients (MGF858, MGF735, and MGF876) all exhibit marked reductions in their Met synthetic capacity (7, 6, and 4% of controls, respectively) even though cell line MGF735 has 32% of normal enzyme activity in the cell extract.

In Table III the two 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient lymphoblast lines are compared with two control lines. Cell line MGL30 has 36% of the Met synthetic capacity of the control lines, and this corresponds closely to the fibroblast line, which has 43% of the control value and is derived from the same patient (MGF548). However, MGL28 has 26% of the Met synthetic capacity of the control lymphoblast lines, and this

TABLE III  
Methionine Biosynthesis by Two Normal and Two  
5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu Reductase-deficient  
Lymphoblast Lines

Cell line designation	Methionine synthesis*			Clinical status
	pmol/mg protein/h	pmol/pmol Leu incorp.	Met	
			Total protein label† percent	
MGL8B2	328±130	271±47	43±2	Normal
MGL10	294±96	210±42	46±1	Normal
MGL30	55±11	86±16	30±1	Mildly affected
MGL28	61±2.3	64±12	21±3	Moderately affected

\* Values are the mean with 1 SD of at least three separate assays performed in duplicate. Met synthesis includes radioactivity recovered in Met, Met sulfoxide, and Met sulfone.

† Total protein label includes radioactivity recovered in Met (and Met sulfoxide and Met sulfone), serine, and cysteine (and cystine and cysteic acid).

does not correspond well to the fibroblast line derived from the same patient (MGF451) who has 11% of control values.

This same assay was performed in two controls and three of the 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient fibroblast lines at low concentrations of Met (1, 3, 10, and 30  $\mu$ M). Although the apparent rates of Met synthesis were lower owing to the dilutional effect of the unlabeled Met, the differences between the normal and the deficient lines remain unchanged. Since the activity of 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase is known to be higher in confluent cells than in logarithmically growing cells (15), the assay was also performed once in three normal and in two deficient fibroblast lines after 7 d at confluency. Although one of the normal lines doubled its rate of Met biosynthesis compared with the usual assay conditions at late log growth, the other two normal lines showed no significant change. The two reductase-deficient lines showed small but insignificant increases in rates of Met biosynthesis.

All the 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient cell lines had normal Met synthetase (5-CH<sub>3</sub>-H<sub>4</sub>PteGlu: homocysteine cobalamin methyltransferase) activity (16). To be certain that fibroblast line MGF735 might not represent a deficiency of some other enzyme of the folate pathway, the cellular extract was also assayed for activity of 5,10-CH=H<sub>4</sub>PteGlu cyclohydrolase (17), 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu dehydrogenase (17), serine hydroxymethyltransferase (18), and 10-CHO-H<sub>4</sub>PteGlu synthetase (17). In each instance the activity was similar to control values (data not shown). In addition,

line MGF735 exhibited the same poor growth pattern in Met-free, HC-supplemented medium as did other 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient fibroblast lines. The detailed description of patient M.S. is in preparation by Dr. S. Cederbaum.<sup>2</sup>

## DISCUSSION

The studies we report here clearly demonstrate that fibroblasts and lymphoblasts from 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient patients exhibit decreased capacity to synthesize Met from HC. The magnitude of the defect in Met synthesis accurately reflects the clinical status of the patients from whom the cells were derived. Met synthesis from HC in human fibroblasts is completely dependent on 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase activity since there is no detectable betaine homocysteine methyltransferase activity (19).

The disparity between the measured Met synthesis (average, 38% of normal) and the measured residual enzyme activity in the extracts (18% of normal) in the cells derived from the mildly affected patients is striking and of interest. Met synthesis from HC is directly under the control of the enzyme Met synthetase. The product of the 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase reaction is 5-CH<sub>3</sub>-H<sub>4</sub>PteGlu, a substrate along with HC in the Met synthetase reaction. It appears that 18% of normal reductase activity can generate enough 5-CH<sub>3</sub>-H<sub>4</sub>PteGlu to support Met synthesis at a rate ~40% of normal. Perhaps only 30–40% of 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase activity is required to maintain Met synthesis at near normal rates and hence such a mutation would not be clinically apparent. This is consistent with our observation that fibroblasts from heterozygous parents of reductase-deficient patients grow at normal rates in medium in which HC replaces Met (unpublished data).

As has been widely believed and elegantly demonstrated in the case of Yoshida's studies (20) on glucose-6-phosphate dehydrogenase deficiency, the residual enzyme activity measured in an in vitro extract under potentially nonphysiologic conditions may correlate poorly with the severity of the phenotypic disturbance. He showed that a measurement of the physiologic activity of the enzyme may correlate better with the clinical status of the patient. Although patient M.S. in our studies is severely affected clinically, her fibroblast line (MGF735) has 32% of normal 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase activity as measured in a crude cell extract. The Met synthetic capacity of her fibroblast line, however, is only 6.5% of normal values and very similar to the other two lines derived from severely affected children.

Patient S.S. died at 9 mo of age. Her fibroblast line (MGF876) synthesized Met at 4% of normal values, the lowest rate of any line studied. The data suggest that

<sup>2</sup> Personal communication.

>4% of normal Met synthetic capacity as measured in our whole cell system is necessary for survival.

The poor correlation of Met synthetic rates in lymphoblast line MGL28, 26% of normal, with the Met synthetic function in the fibroblast line derived from the same patient, MGF451, 11% of normal, is unexplained. It is known that the transformation of human fibroblasts by simian virus 40 may also be associated with altered rates of Met biosynthesis and Met requirements (21). Perhaps Epstein-Barr virus transformation of human lymphocytes likewise is associated with changes in rates of Met biosynthesis. Alternatively, this discrepancy might be due to tissue specific differences in expression of the enzyme defect caused by factors such as those identified by Yoshida.

Patients with 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase deficiency present clinically with neurologic dysfunction of variable degree. The etiology of their neurologic disturbance is unclear but may relate to altered metabolism of neurotransmitters (13). Many of the neurotransmitters undergo methylation in their synthesis and the main intracellular donor of methyl groups is S-adenosylmethionine (AdoMet). Methionine and adenosine-triphosphate are the precursors of AdoMet. The brain, like any other organ, meets its requirement for Met either by uptake from the blood or endogenous synthesis (22). We have demonstrated decreased Met synthetic capacity in 5,10-CH<sub>2</sub>-H<sub>4</sub>PteGlu reductase-deficient fibroblasts and lymphoblasts and postulate a similar decrease in the brain cells of these patients, leading to inadequate AdoMet generation for normal neurotransmitter synthesis.

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