Hepatic one-carbon metabolism in early folate deficiency in rats

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Glycine *N*-methyltransferase (GNMT) is inhibited by 5-methyltetrahydrofolate polyglutamate *in vitro*. It is believed to play a regulatory role in the synthesis *de novo* of methyl groups. We have used the amino-acid-defined diet of Walzem and Clifford [(1988) J. Nutr. **118**, 1089–1096] to determine whether folate deficiency *in vivo* would affect GNMT activity, as predicted by the studies *in vitro*. Weanling male rats were fed on the folate-deficient diet or a folate-supplemented diet pair-fed to the deficient group. A third group was fed on the folate-supplemented diet *ad libitum*. Development of folate deficiency rapidly resulted in decreased levels of *S*-adenosylmethionine (SAM) and elevation of *S*-adenosylhomocysteine (SAH). The ratios of SAM to SAH were 1.8, 2.7 and 1.5 in the deficient group for weeks 2, 3 and 4 of the experiment, and the values were 9.7, 7.1 and 8.9 for the pair-fed control group and 10.3, 8.8 and 8.0 for the control group *ad libitum* fed. The activity of GNMT was significantly higher in the deficient group than in either of the two control groups at each time period. This was not due to increased amounts of GNMT protein, but reflected an increase in specific enzyme activity. Levels of folate in both the cytosol and mitochondria were severely lowered after only 2 weeks on the diet. The distribution of folate coenzymes was also affected by the deficiency, which resulted in a marked increase in the percentage of tetrahydrofolate polyglutamates in both cytosol and mitochondria and a very large decrease in cytosolic 5-methyltetrahydrofolate. The increased GNMT activity is therefore consistent with decreased folate levels and decreased inhibition of enzyme activity.

INTRODUCTION

Glycine *N*-methyltransferase (GNMT) (EC 2.1.1.20) is a very abundant enzyme in liver, comprising about 1–3 % of the cystolic protein [1]. It catalyses the methylation of glycine by *S*-adenosylmethionine (SAM) to form sarcosine and *S*-adenosylhomocysteine (SAH). We have previously discovered that GNMT was identical with a folate binding protein of rat liver [2], in spite of the absence of the requirement for a folate cofactor in the enzymic reaction. It was subsequently learned that GNMT activity is inhibited by bound folate [3], and this observation was incorporated into a scheme for metabolic control of methyl-group metabolism [4]. The particular form of folate which is bound to GNMT *in vivo* is 5-CH_3-H_4PteGlu$_5$ [5], with polyglutamates being more inhibitory than monoglutamate forms [3]. Although inhibition of purified GNMT is easily demonstrated *in vitro*, it would be important to demonstrate that this is also the case *in vitro*. We have therefore used the amino-acid-defined folate-deficient diet developed by Walzem and Clifford [6] to determine whether GNMT activity is increased in folate deficiency. This diet rapidly produces the effects of folate deficiency, including loss of body weight, and decreased levels of both red and white blood cells.

Because we have shown that the folate pools of mitochondria and cytosol are not in rapid equilibrium when exposure to N_2O is used to deplete tissue methionine synthase levels and perturb the distribution of folate coenzymes [7], we have also examined the effect of early folate deficiency on the distribution of folates in the cytosolic and mitochondrial pools.

EXPERIMENTAL

Animals and diets

Three groups each consisting of 12 male weanling Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, U.S.A.) weighing about 47 g were housed in identical cages and were fed on the following diets.

1. The deficient group received an amino-acid-defined folate-deficient diet [6] containing 19.8 % sucrose, 39.7 % of dextrin, 10 % corn oil, 5 % cellulose, a complete amino acid mixture instead of protein, and minerals and vitamins essentially in amounts recommended by the AIN Ad Hoc Committee [8].
2. The '*ad libitum*' control group received the same diet, supplemented with 8 mg of folate/kg, *ad libitum*.
3. The pair-fed control group received the same diet as the '*ad libitum*' control group, but was individually pair-fed with the average daily food intake of the deficient group. Diets were obtained from Dyets Inc. (2508 Easton Avenue, Bethlehem, PA 18017, U.S.A.). All the animals had free access to drinking water. All the diets contained 1 % (w/w) succinylsulphathiazole, to repress the intestinal microfloral folate synthesis. Animals were weighed twice a week and were kept in an environmentally controlled animal laboratory with a 12 h-light/-dark cycle. The protocol for animal use was approved by the Vanderbilt University and VA Medical Center Animal Committees.

From the end of week 2 of the experiment, four animals from each group were anaesthetized each week with intramuscular ketamine, 8 mg/100 g body wt. (Ketaset; Aveco Co., Fort Dodge, IA 50501, U.S.A.), and xylazine, 1.3 mg/100 g body wt. (Rompun; Mobay Corp., Shawnee, KS, U.S.A.). The abdominal cavity was opened, the liver removed, rinsed in cold saline, and a portion used immediately for determination of SAM and SAH, folate, and the rest of the tissue was kept at −70 °C, for further determinations.

Tissue folate assay

Liver (2 g) was homogenized in 6 ml of ice-cold H-medium [7],

Abbreviations used: GNMT, glycine *N*-methyltransferase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; H_4PteGlu, tetrahydropteroylglutamate; 5-CH_3-H_4PteGlu, 5-methyltetrahydropteroylglutamate; 5-HCO-H_4PteGlu, 5-formyltetrahydropteroylglutamate; 10-HCO-H_4PteGlu, 10-formyltetrahydropteroylglutamate.

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which contained 10 mM 2-mercaptoethanol and 10 mM sodium ascorbate using three up-and-down strokes of a Teflon/glass homogenizer. The suspension was centrifuged at 25000 *g* for 30 min, and the supernatant was removed (this is the cytosolic fraction). The pellet was resuspended in 6 ml of the H-medium with two up-and-down strokes of the homogenizer and again centrifuged at 25000 *g* for 30 min. The pellet was resuspended in 6 ml of the H-medium (this is the mitochondrial fraction).

Over 95% of the total folate of the rat liver is accounted for by the amounts present in the mitochondrial and cytosolic cell fractions [9]. We have previously shown that this rapid method of separating the mitochondrial and cellular compartments provides essentially the same values for folate distribution as more complex procedures for separating cellular organelles [10]. Therefore, in the present study, the post-mitochondrial supernatant was designated 'cytosol' and the pellet was designated the 'mitochondrial' fraction.

To the cytosolic and mitochondrial fractions 0.2 vol. of 5 × extraction buffer [100 g/l sodium ascorbate, 1 M 2-mercaptoethanol, 0.25 M Hepes, 0.25 M 2-(*N*-cyclohexylamino)-ethanesulphonic acid, pH 7.85] was added, and the tubes were mixed and heated in a boiling-water bath for 5 min to precipitate proteins. The tubes were cooled in ice and centrifuged in a Brinkman model 3200 microcentrifuge for 10 min. The supernatants were treated with rat serum conjugase, and folate coenzymes were separated by h.p.l.c. and quantified by microbiological assay as described previously [7].

Measurement of SAM and SAH

SAM and SAH were estimated by the method described by Cook et al. [11]. Briefly, the tissue was promptly homogenized in cold trichloroacetic acid (100 g/l), and centrifuged in the cold. The supernatant was washed with diethyl ether to remove the trichloroacetic acid, filtered, and SAM and SAH were separated and estimated by h.p.l.c.

Measurement of GNMT

GNMT was assayed in duplicate as described by Cook and Wagner [2]. The tissue was homogenized in 3 vol. of cold 0.25 M sucrose, containing 10 mM phosphate buffer, pH 7.0, 1 mM NaN_3 and 10 mM α-toluenesulphonyl fluoride (Eastman Kodak Co.). The homogenate was centrifuged at 20000 *g* for 30 min, and the supernatant was used for the assay. The assay mixture contained: 1.0 M Tris buffer, pH 9.0, 20 μl; 50 mM dithiothreitol (Sigma Chemical Co., St. Louis, MO, U.S.A.), 10 μl; 10 mM glycine (Bio-Rad Laboratories, Richmond, CA, U.S.A.), 20 μl; tissue extract, 20 μl; 1 mM *S*-adenosyl-L-[*methyl*-^{3}H]methionine (containing 90 μCi/mmol), 20 μl. This was incubated at 25° C for 15 min, after which the reaction was stopped by addition of 50 μl of trichloracetic acid (100 g/l) and 250 μl of charcoal suspension (38 mg/ml in 0.1 M acetic acid) was added to absorb SAM that had not reacted. The tubes were incubated at 0° C for 15 min, and centrifuged for 3 min in a Brinkman microfuge. Then 200 μl of the supernatant was added to 5 ml of aqueous counting scintillant (Bio-Safe II; Research Products International Corp., Mount Prospect, IL, U.S.A.) and assayed. Blank values obtained from duplicate incubations without glycine were subtracted to give the net counts. The enzyme activity was expressed as nmol of sarcosine formed/minute per mg of protein in the extract.

Measurement of GNMT protein

For measurement of GNMT protein in the liver supernatant, the e.l.i.s.a. employed by Cook et al. [11] was used. Briefly, the samples and standards were incubated in 96-well polystyrene plates at 5 °C overnight. The samples were discarded, the wells were washed, the blocking solution containing BSA (10 g/l) and 1.0 mM NaN_3 in Tris buffer was added, incubated for 30 min at 37 °C, and the plates were washed again. Antiserum to GNMT prepared in rabbits was added to the plates, incubated for 30 min at 37 °C, and washed. Then biotinylated goat anti-rabbit antiserum (BioGenex Laboratories, Dublin, CA, U.S.A.) was added, incubated for 10 min, and washed. Alkaline phosphatase–streptavidin (BioGenex) solution was added, incubated for 10 min at room temperature, followed by *p*-nitrophenyl phosphate solution in 1 M diethanolamine/HCl buffer, pH 9.8. Plates were incubated for 15 min at room temperature, and the colour developed by addition of 25 μl of 2 M NaOH was read at 405 nm.

Measurement of protein

For this, 50 μl of the properly diluted extract (1/50–1/100) was added to 3.0 ml of Bradford reagent [12] and mixed. The A_{595} was measured against a blank (50 μl of distilled water added to 3.0 ml of the reagent), and the concentration of protein was obtained from a curve constructed with BSA.

Statistical methods

Results are expressed as means ± SEM, and statistical differences were determined by a two-factor ANOVA using the Superanova program (Abacus Concepts). *Post-hoc* analysis of significance was made by Fisher's protected least-squares difference test.

RESULTS

Food consumption and growth

The mean daily food consumption of the deficient (and pair-fed) groups was 7.2 ± 1.3 g, 10.6 ± 0.9 g, 12.7 ± 0.4 g and 10.8 ± 1.1 g for weeks 1–4 of the experiment. For the '*ad libitum*' control group the corresponding values were 7.5 ± 1.4 g, 12.0 ± 1.7 g, 16.3 ± 0.3 g and 17.8 ± 0.5 g. There was a difference in mean body weights after 4 weeks between the '*ad libitum*' control and the other two groups. The average weight gain for each group over the last 8 days of the experiment was different, being 1.06, 2.66 and 7.07 g per day for the deficient, pair-fed control and '*ad libitum*' control groups, respectively.

Folate levels

Table 1 shows the effects of folate deficiency on the levels of total folate in the cytosol and mitochondria after 2, 3 and 4 weeks. The

Table 1 Effect of folate deficiency on total liver folate

Values are expressed as nmol of total folate/g of liver, ± S.D. ($n = 3$). In each case $P < 0.05$ when the deficient group is compared with its pair-fed control.

Compartment	Treatment	Week 2	Week 3	Week 4
Cytosol	Pair-fed control	12.7 ± 0.09	17.9 ± 0.09	14.6 ± 1.10
	Deficient	1.5 ± 0.15	0.6 ± 0.02	0.4 ± 0.01
Mitochondria	Pair-fed control	11.9 ± 0.60	15.8 ± 0.30	15.7 ± 1.10
	Deficient	2.3 ± 0.20	1.8 ± 0.10	1.1 ± 0.03

Table 2 Distribution of folate coenzymes after 4 weeks of feeding on the folate-deficient diet

Values are expressed as percentages of total folate in the compartment. *Significantly different from corresponding control ($P < 0.05$).

Compartment	Treatment	10-HCO-H_4PteGlu	5-HCO-H_4PteGlu	H_4PteGlu	5-CH_3H_4PteGlu
Cytosol	Pair-fed control	14.7 ± 2.7	3.6 ± 0.7	11.7 ± 1.5	70.0 ± 5.0
	Deficient	14.4 ± 1.0	6.1 ± 0.8	59.0 ± 4.8*	18.7 ± 3.5*
Mitochondria	Pair-fed control	35.1 ± 0.4	10.8 ± 0.4	45.7 ± 0.7	8.3 ± 0.3
	Deficient	15.4 ± 1.2*	7.6 ± 0.8	65.8 ± 2.2*	10.9 ± 1.6

Table 3 Hepatic SAM, SAH and SAM/SAH ratio in folate-deficient, *ad libitum* and pair-fed control rats, measured by h.p.l.c. in trichloroacetic acid extracts of fresh tissue

Each value is the mean ± SEM of four rats after 2, 3 and 4 weeks on corresponding diets. The level of SAM is significantly lower in the deficient group ($P < 0.01$), except for week 3, where the difference between deficient and pair-fed control groups is not significant. In week 2, the pair-fed control group has a significantly higher SAM level ($P < 0.02$), otherwise there is no significant difference between the two control groups. The SAH values are significantly higher ($P < 0.005$) and the SAM/SAH ratio is significantly lower ($P < 0.003$) in the deficient group, and there is no significant difference in these values between the *ad libitum* and pair-fed control groups. Values of SAM or SAH containing the same superscript within each column are not significantly different from each other ($P > 0.05$).

Week	Group	SAM (nmol/g)	SAH (nmol/g)	SAM/SAH
2	Deficient	119.2 ± 5.1^{c}	65.0 ± 2.4^{b}	$1.8 \pm 0.06^{d,e,f}$
	'*ad libitum*' control	196.9 ± 9.3^{a}	19.8 ± 2.7^{a}	10.3 ± 1.1^{a}
	Pair-fed control	242.1 ± 10.2^{b}	25.1 ± 3.9^{a}	$9.7 \pm 1.4^{a,c}$
3	Deficient	138.5 ± 22.3^{c}	50.3 ± 5.3^{b}	2.7 ± 0.2^{d}
	'*ad libitum*' control	189.2 ± 12.3^{a}	21.4 ± 1.3^{a}	$8.9 \pm 0.7^{a,b,c}$
	Pair-fed control	162.0 ± 19.3^{a}	22.7 ± 0.2^{a}	7.1 ± 0.7^{c}
4	Deficient	122.6 ± 4.0^{c}	84.5 ± 11.0^{c}	$1.5 \pm 0.1^{e,f}$
	'*ad libitum*' control	200.5 ± 10.7^{a}	26.6 ± 4.5^{a}	$8.0 \pm 1.0^{b,c}$
	Pair-fed control	188.0 ± 14.8^{a}	21.0 ± 0.8^{a}	$8.9 \pm 0.7^{a,b}$

Table 4 Hepatic GNMT in *ad libitum* control, pair-fed control and deficient rats

Enzyme activity was measured by formation of sarcosine/mg of cytosol protein. Enzyme concentration was determined immunologically and expressed per mg of cytosol protein. Specific enzyme activity is 1000 × enzyme activity/enzyme concentration. Meaning of superscripts as in Table 3.

Week	Group	Enzyme activity (nmol sarcosine/ min per mg of cytosol protein)	Enzyme concn. (μg of GNMT/mg of cytosol protein)	Specific enzyme activity (nmol of sarcosine/ g of GNMT)
2	'*ad libitum*' control	$0.202 \pm 0.009^{a,b,c}$	$4.04 \pm 0.36^{a,c}$	51.4 ± 5.9^{a}
	Pair-fed control	$0.241 \pm 0.015^{b,c}$	2.40 ± 0.08^{b}	99.8 ± 8.4^{b}
	Deficient	0.443 ± 0.015^{e}	2.70 ± 0.23^{b}	171.1 ± 27.9^{c}
3	'*ad libitum*' control	0.142 ± 0.013^{a}	$4.74 \pm 0.22^{a,c}$	29.8 ± 2.3^{a}
	Pair-fed control	$0.170 \pm 0.007^{a,d}$	$3.92 \pm 1.05^{a,b,c}$	54.0 ± 14.0^{a}
	Deficient	0.406 ± 0.034^{e}	3.33 ± 0.34^{b}	128.2 ± 19.3^{b}
4	'*ad libitum*' control	0.146 ± 0.014^{a}	$4.41 \pm 0.47^{a,c}$	32.4 ± 2.2^{a}
	Pair-fed control	$0.222 \pm 0.018^{b,c,d}$	6.38 ± 0.67^{d}	35.7 ± 4.5^{a}
	Deficient	0.594 ± 0.026^{f}	4.93 ± 0.45^{c}	123.3 ± 7.2^{b}

data show that, even as early as 2 weeks on this diet, the levels of folate are greatly diminished in both the cytosolic and mitochondrial pools. There is a further slow decrease in subsequent weeks. Table 2 shows the distribution of the folate coenzymes after conversion into the respective monoglutamates in the two pools at the end of the experiment (4 weeks). The amount of each coenzyme form is presented as percentage of the total folate in the particular pool. The profound folate deficiency not only results in a decrease in the total folate, but also causes a redistribution of the relative amounts of the coenzymes, and this redistribution is different in the cytosolic and mitochondrial pools. In the cytosol, folate deficiency caused a large decrease in the percent of 5-CH_3-H_4PteGlu, whereas the percentages of H_4PteGlu and 5-HCO-H_4PteGlu were elevated. In the mitochondria, however, the percentage increase in H_4PteGlu was at the expense of 10-HCO-H_4PteGlu, whereas the percentage of 5-CH_3-H_4PteGlu was unchanged.

SAM and SAH levels

These are presented in Table 3; SAM values are decreased and the SAH values are increased after only 2 weeks of folate

deficiency, whereas there was little difference between the levels of the two control groups. There is evidence that many methyltransferases may be regulated by the ratio of SAM to SAH, rather than their absolute levels in the cell [13]. Table 3 shows that folate deficiency caused a marked decrease in the SAM/SAH ratio even after 2 weeks.

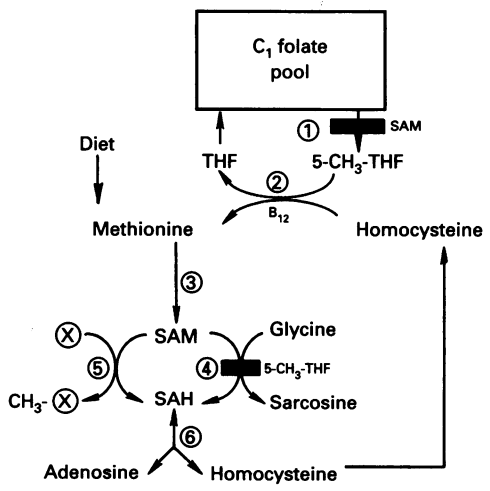
Activity of GNMT

When GNMT activity was measured in the unfractionated liver cytosol of the deficient rats, it was much higher than in either control group at every time point (Table 4). In order to determine whether this was due to an increase in the specific activity of the enzyme (i.e. decreased inhibition), or whether it was due to an actual increase in the amount of GNMT protein, we used an e.l.i.s.a. method to quantify the amount of GNMT immunologically. Table 4 also shows that the amount of immunologically measured GNMT was lower in both the pair-fed control group as well as the deficient group after week 2. At later times, the amount of GNMT protein increased in both the pair-fed and the deficient groups, indicating a biphasic effect of food restriction on the amount of GNMT. When the enzyme activity was expressed per mg of enzyme protein, however, the values for the deficient group were clearly greater than for the other two groups.

DISCUSSION

The amino-acid-defined diet containing 1% succinylsulphathiazole developed by Walzem and Clifford [6] provides a valuable model for examining the metabolic effects of folate deficiency in the rat. Not only do these animals lose weight and have depleted tissue stores of folate, but they develop anaemia and leukopenia after 4–5 weeks. We have adopted this diet to study the early effects of folate deficiency on C_1 metabolism.

As shown in Table 1, the folate level is greatly decreased after just 2 weeks of feeding with the folate-deficient diet. Folate is depleted in both the cytosol and the mitochondria. Measurement of the various folate coenzymes (Table 2) in folate deficiency provides another example of the 'compartmentation' of folate between cytosol and mitochondria. This was first discovered when exposure of rats to N_2O in order to deplete the B_{12}-dependent methionine synthase had a differential effect on the distribution of folate coenzymes in the cytosol and mitochondria [7]. The percentage of 5-CH_3-H_4PteGlu is markedly decreased in the cytosol, but remains unchanged in the mitochondria. These results also show that the amount of 5-CH_3-H_4PteGlu in the cytosol at week 4 has fallen from 10.2 to 0.07 nmol/g of liver. The control folate levels reported here are somewhat higher than those we reported previously [7] which is probably due to the higher levels of dietary folate (8 mg/kg of diet) in this study, compared with 2–3 mg/kg in the diet in the previous study.



Scheme 1 Regulation of methyl-group metabolism

The enzymes and coenzymes of the C_1 folate pool are enclosed in the box. Abbreviation: THF, tetrahydrofolate (H_4PteGlu in text). The circled numbers refer to the following enzymes: 1, 5,10-methylene-THF reductase; 2, methionine synthase; 3, methionine adenosyltransferase; 4, GNMT; 5, a group of methyltransferase enzymes; 6, SAH hydrolase. Synthesis of 5-CH_3-THF is inhibited by SAM, and the utilization of SAM via GNMT is inhibited by 5-CH_3-THF. GNMT provides an alternate path for conversion of SAM into SAH. The circled X refers to a variety of methyl acceptors.

The results described in Table 4 clearly show that the specific enzyme activity of GNMT was increased in the folate-deficient group. This is based on the results of immunologically measured enzyme. These data indicate that the regulation of GNMT activity, which we have postulated on the basis of studies of enzyme inhibition *in vitro* [3], also operates *in vivo*. GNMT is a cytosolic enzyme [5], and there is a 100-fold decrease in the amount of 5-CH_3-H_4PteGlu in the cytosol after 4 weeks of deficiency.

It is also noteworthy that there appears to be an effect of food deprivation on the amount of immunologically measured GNMT. After the first 2 weeks, the amount of GNMT of the deficient and pair-fed groups was about half that of the '*ad libitum*' fed control group. Values for the latter did not change during the course of the experiment. On the other hand, values for the pair-fed control and deficient groups both increased until week 4. It is not clear why food restriction should have a differential effect on the amount of GNMT present in the cell. However, Ogawa and Fujioka [14] showed that GNMT was induced by giving a casein-based diet, containing 3% methionine in place of the normal 0.5%. Although animals lost weight on this diet, there was a 2.7-fold increase in the amount of immunoprecipitable GNMT.

Another possible consequence of food restriction is the induction of gluconeogenesis. Xue and Snoswell [15] have shown that in alloxan-diabetic sheep liver GNMT activity was increased 65-fold. We have observed (C. Wagner, unpublished work) a 2-fold increase in GNMT activity of rat liver brought about by fasting or by alloxan-diabetes. Phosphorylation of purified rat liver GNMT by cyclic-AMP-dependent protein kinase also resulted in an increase in GNMT activity [16]. Phosphorylation of GNMT may therefore be responsible for the significant increase in specific enzyme activity in the pair-fed control group (Table 4) as compared with the '*ad libitum*' control group at the end of week 2. It would be interesting to measure the extent of phosphorylation of GNMT in these groups. Such measurements require the isolation of sufficient amounts of chromatographically pure protein to permit the chemical determination of covalently bound phosphate [16]. Much larger amounts of tissue would be needed than were available from these experiments.

The abundance of GNMT in the liver [1], plus the fact that sarcosine, the product of glycine methylation, has no physiological role, gave rise to the suggestion that the function of GNMT is to regulate the ratio of SAM to SAH. As described in Scheme 1, this is an alternative route from SAM to SAH which does not require the methylation of any metabolically important intermediate (designated as X) by a variety of methyltransferase enzymes. Our discovery that GNMT is inhibited by 5-CH_3-H_4PteGlu *in vitro* [3], together with the well-known inhibition of

5-CH_3-H_4PteGlu synthesis by SAM [17], led to the realization of the reciprocal relation between these two metabolites, i.e. that utilization of SAM is inhibited by 5-CH_3-H_4PteGlu, and synthesis of 5-CH_3-H_4PteGlu is inhibited by SAM. This serves to link the synthesis of methyl groups *de novo* from the C_1 folate pool to the availability of pre-formed dietary methyl groups in the form of methionine.

This regulatory mechanism is sensitive to changes in the relative amounts of 5-CH_3-H_4PteGlu. If the amount of dietary methionine is limited, the amount of SAM will be decreased. This will decrease the inhibition of 5,10-methylene-H_4PteGlu reductase and more 5-CH_3-H_4PteGlu will be formed. The elevated 5-CH_3-H_4PteGlu will inhibit GNMT to conserve the decreased amounts of SAM for the physiologically important methylation of the compounds designated as X.

In the experiments described here, there is a marked decrease in the total folate pool as well as in the percentage of 5-CH_3-H_4PteGlu. The decrease in 5-CH_3-H_4PteGlu results in an increase in the specific enzyme activity of GNMT (Table 4). This is reflected in the decreased concentration of SAM in the folate-deficient group (Table 3). Because the total folate pool is decreased, the decreased concentration of SAM cannot regenerate 5-CH_3-H_4PteGlu from the C_1 folate pool, however, as would be the case in folate-adequate animals. In folate deficiency, there should therefore be a decreased activity of methionine synthase and accumulation of homocysteine. The equilibrium of the SAH hydrolase favours SAH synthesis [18] and an elevation of SAH levels is seen in the deficient group (Table 3). The decreased SAM and increased SAH result in a lowered SAM/SAH ratio, i.e. the so-called 'methylation ratio' [13]. Because this ratio is thought to have a regulatory effect on a wide variety of methylation reactions [19], folate deficiency may result in a broad range of secondary effects.

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