Folate-deficiency-induced homocysteinaemia in rats: disruption of *S*-adenosylmethionine's co-ordinate regulation of homocysteine metabolism

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In a recent hypothesis [Selhub and Miller (1992) Am. J. Clin. Nutr. **55**, 131–138], we proposed that homocysteinaemia arises from an interruption in *S*-adenosylmethionine's (AdoMet) co-ordinate regulation of homocysteine metabolism. The present study was undertaken to test a prediction of this hypothesis, that homocysteinaemia due to folate deficiency results from impaired homocysteine remethylation due to the deficiency and impaired synthesis of AdoMet, with the consequent inability of this metabolite to function as an activator of homocysteine catabolism through cystathionine synthesis. Rats were made folate-deficient by feeding them with a folate-free amino-acid-defined diet supplemented with succinylsulphathiazole. After 4 weeks, the deficient rats exhibited a 9.8-fold higher mean plasma homocysteine concentration and a 3.2-fold lower mean hepatic AdoMet concentration compared with folate-replete controls. Subsequent supplementation for 3 weeks of the folate-deficient rats with increasing levels of folate in the diet resulted in graded decreases in plasma homocysteine levels, accompanied by graded increases in hepatic AdoMet levels. Thus plasma homocysteine and hepatic AdoMet concentrations were inversely correlated as folate status was modified. In a second experiment, the elevation of plasma homocysteine in the deficient rats was found to be reversible within 3 days by intraperitoneal injections of ethionine. This effect of ethionine is thought to be exerted through *S*-adenosylethionine, which is formed in the liver of these rats. Like AdoMet, *S*-adenosylethionine is an activator of cystathionine β-synthase and will effectively promote the catabolism of homocysteine through cystathionine synthesis. In crude liver homogenates of the rats treated with ethionine, cystathionine β-synthase activity was 3-fold higher than that measured in homogenates from vehicle-treated controls.

INTRODUCTION

Homocysteinaemia is a condition of disrupted homocysteine metabolism which has been shown to be an important health risk factor. Moderately elevated plasma homocyteine concentrations have been found to be highly prevalent in patients with coronary, peripheral and cerebrovascular diseases [1,2], as well as in patients who have suffered strokes [3]. In addition, a prospective investigation of participants in the Physicians' Health Study [4] showed that the risk of myocardial infarction within 5 years for individuals with no prior history of vascular disease was 3.4-fold greater for those with elevated plasma homocysteine concentrations than for those with normal plasma homocysteine levels [5].

Recently, we proposed a unifying hypothesis to explain biochemically the pathogenesis of homocysteinaemia under all conditions known to cause the disorder [6]. Normally, homocysteine is metabolized through two primary pathways, remethylation, in which homocysteine acquires a methyl group to form methionine, and trans-sulphuration, in which homocysteine is catabolized through cystathionine synthesis (Figure 1). Our hypothesis states that both of these pathways must be inhibited to precipitate homocysteinaemia. This hypothesis, which is based on original studies performed *in vitro* by Finkelstein et al. [7] and Kutzbach and Stokstad [8], centres on the fact that the two homocysteine pathways are co-ordinately regulated by the methionine metabolite *S*-adenosylmethionine (AdoMet). AdoMet is an activator of cystathionine synthesis [7,9] and an allosteric inhibitor of the synthesis of methyltetrahydrofolate [8,10,11], the methyl donor in homocysteine remethylation. Thus high tissue AdoMet concentrations promote homocysteine catabolism, and low tissue AdoMet concentrations promote remethylation. Conditions that cause homocysteinaemia do so by disrupting this co-ordinate regulation by AdoMet, leading to inhibition of both homocysteine metabolite pathways and the subsequent export of the unmetabolized homocysteine into the blood.

In conditions that directly affect remethylation, homocysteinaemia will develop when the block of remethylation is severe enough to inhibit synthesis of AdoMet. AdoMet is then at too low a concentration to activate cystathionine synthesis, and thus trans-sulphuration becomes depressed. In conditions that directly affect trans-sulphuration, homocysteinaemia will develop when the block of trans-sulphuration is severe enough to increase the synthesis of AdoMet through remethylation. In this case, the AdoMet will feed back and inhibit methylenetetrahydrofolate synthesis and thus block further remethylation.

Conditions that directly affect remethylation or trans-sulphuration, and therefore affect AdoMet's co-ordinate regulation of these pathways, include genetic enzyme defects and nutritional deficiencies of vitamins intimately involved in homocysteine metabolism, namely vitamins B-6, B-12 and folate (Figure 1) [12]. The present study was undertaken to test the hypothesis that in folate deficiency homocysteinaemia results from impaired homocysteine remethylation due to the deficiency and impaired synthesis of AdoMet, with the consequent inability of this metabolite to function as an activator of homocysteine catabolism through cystathionine synthesis. In this paper, the relationship between folate status, hepatic AdoMet concentration and plasma homocysteine concentration in rats is assessed. In addition, the effect of ethionine administration *in vivo* on plasma homocysteine concentration in the context of folate deficiency is assessed. Ethionine is known to be converted in liver into *S*-adenosylethionine, a metabolite which activates cystathionine synthesis in the same manner as AdoMet [7,9]. The purpose of the ethionine administration was to determine if the homo-

Abbreviations used: AdoMet, *S*-adenosylmethionine; AdoHCy, *S*-adenosylhomocysteine.

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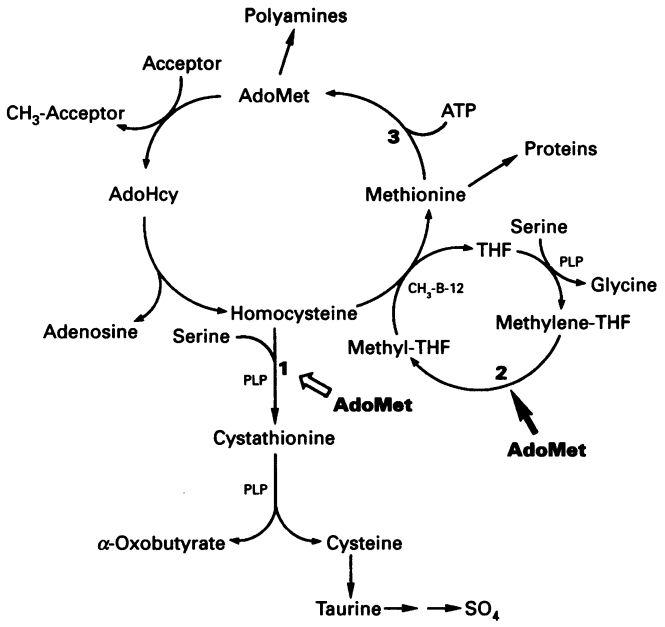

Figure 1 Co-ordinate regulation of homocysteine metabolism by AdoMet

Homocysteine can be methylated to form methionine (remethylation) or catabolized through cystathionine synthesis (trans-sulphuration). High tissue AdoMet concentrations activate trans-sulphuration (open arrow) and inhibit remethylation (closed arrow). Low tissue AdoMet concentrations promote remethylation. The B vitamins folate, B-12 and B-6 are required for normal homocysteine metabolism and normal regulation by AdoMet. Enzymes: 1, cystathionine β-synthase; 2, methylenetetrahydrofolate reductase; 3, AdoMet synthase. Abbreviations: PLP, pyridoxal 5′-phosphate (vitamin B-6); THF, tetrahydrofolate.

cysteinaemia could be reversed by activation of homocysteine trans-sulphuration, despite the block in homocysteine remethylation caused by the folate deficiency.

EXPERIMENTAL

Animals and diets

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Tufts University. Male Sprague–Dawley rats weighing 50–75 g were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) They were housed in stainless-steel cages at 22–24 °C in a light-controlled room (12 h on, 12 h off) with water available *ad libitum*. All rats were acclimated for 4 days, during which time they were fed on a folate-replete chow diet (Agway, Waltham, MA, U.S.A.) or a folate-replete amino-acid-defined diet [13] (Dyets, Bethlehem, PA, U.S.A.). Thereafter, the rats were fed on amino-acid-defined diets that were supplemented with 1% succinylsulphathiazole and contained either 8 mg of folate/kg of diet (control) or 0 mg of folate/kg of diet (folate-deficient).

Folate depletion and repletion

Rats were randomly assigned to the control diet or the folate-deficient diet and then subjected to the depletion/repletion protocol of Clifford et al. [14]. The rats were fed *ad libitum* for 4 weeks, at which time six rats from each dietary group were killed by CO_2 asphyxiation after an overnight fast. The remaining deficient rats then were divided equally among five amino-acid-defined resupplementation diets containing 125, 250, 500, 1000 and 8000 μg of folate/kg of diet respectively. The resupplementation diets were fed for 3 weeks and then each rat was killed by CO_2 asphyxiation after an overnight fast.

Effect of ethionine administration

Rats were fed *ad libitum* on the folate-deficient diet as described above for 4 weeks, at which time they entered the following ethionine-treatment protocol: after an overnight fast, a blood sample from each rat was collected via the tail vein into a vacutainer tube containing EDTA. The rats then were randomly assigned to receive intraperitoneal injections of ethionine (400 mg/kg body wt.) dissolved in 0.9% NaCl (20 mg of ethionine/ml) or an appropriate volume of saline without ethionine. Injections were administered on three successive mornings, one per morning. Rats were fed from day 1 to day 2 and then fasted from day 2 to day 3. Rats were killed by CO_2 asphyxiation approx. 1 h after the injection on day 3. In addition, some deficients rats were not subjected to tail bleeds or injections, were fasted overnight from day 2 to day 3, and were killed by CO_2 asphyxiation on day 3. These rats acted as controls for assessment of the effect of the stress of the tail bleeds and injections on the parameters to be measured.

Sample preparation

After CO_2 asphyxiation, blood was collected from the caudal vena cava and placed into vacutainer tubes containing EDTA. The blood was centrifuged at 500 *g* for 10 min at 4 °C and the plasma supernatant collected. Livers were excised and immediately frozen in liquid nitrogen. Both plasma and liver samples were stored at −70 °C until analysis.

Analytical methods

Folate concentration was determined in plasma and liver by a microtitre-plate assay using *Lactobacillus casei* [15]. Total plasma homocysteine concentration was determined by h.p.l.c. by the fluorimetric method of Araki and Sako [16]. Concentrations of AdoMet and *S*-adenosylhomocysteine (AdoHcy) in liver were determined by h.p.l.c. with u.v. detection by a modification of the method by Fell et al. [17]. Frozen liver samples were homogenized in 5 vol. of cold 0.4 M $HClO_4$ with a Polytron homogenizer (20 s, setting 7) (Brinkman Instruments, Westbury, NY, U.S.A.) and centrifuged at 1500 *g* for 10 min. A 1.0 ml sample of the supernatant was filtered through a 0.45 μm Gelman syringe filter (VWR Scientific). The filtered supernatants for each sample then were analysed by h.p.l.c. The column was 3 μm ODS Hypersil, 150 mm × 2 mm (Keystone Scientific, Bellafonte, PA, U.S.A.). The mobile phase was a linear gradient over 20 min from 100% solvent A (0.01 M ammonium formate/4 mM heptanesulphonic acid, pH 4.0) to 75% solvent A and 25% solvent B (50% mobile phase A + 50% acetonitrile, pH 4.0). The 75% A/25% B ratio was maintained from 20 to 25 min, and then the system was returned to 100% solvent A for a 15 min equilibration. The flow rate was 0.3 ml/min. AdoMet and AdoHcy peaks were detected by u.v. absorption at 254 nm. AdoMet and AdoHcy concentrations were determined by using external standards.

A method based on that of Mudd et al. [18] for determining cystathionine β-synthase (EC 4.2.1.22) activity in crude liver homogenates was developed which required no radioactive substrates. Frozen liver samples were homogenized in 10 vol. of 0.05 M potassium phosphate buffer, pH 7.2, with a Polytron homogenizer (20 s, setting 7). The homogenates were then centrifuged at 14000 *g* for 10 min and the supernatants stored on

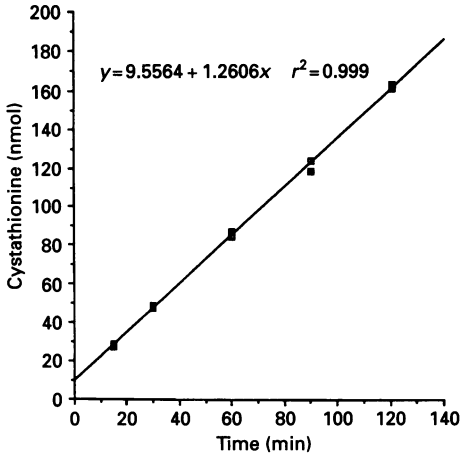

Figure 2 Linearity of the cystathionine β-synthase assay over time

Cystathionine β-synthase activity in crude liver homogenates was determined as described in the Experimental section. Assays at each time point (15, 30, 60, 90 and 120 min) were performed in duplicate by using 0.42 mg of total protein per assay. In the present study, the 60 min time point was used.

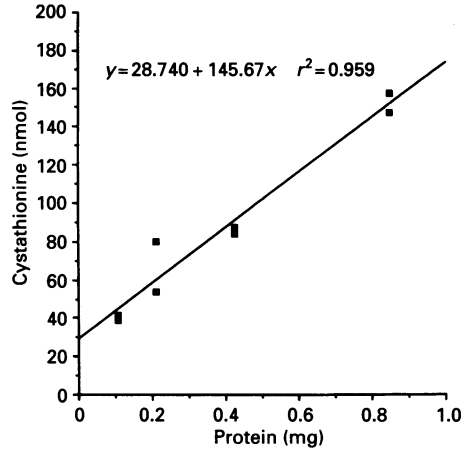

Figure 3 Linearity of the cystathionine β-synthase assay with amount of protein

Cystathionine β-synthase activity in crude liver homogenates was determined as described in the Experimental section. Assays using different amounts of protein (0.10, 0.21, 0.42 and 0.84 mg) were performed in duplicate with each assay stopped after 60 min of incubation. In the present study, the amount of protein used per assay ranged from 0.20 to 0.40 mg.

ice until assayed. Incubations in duplicate consisted of 0.02 ml of reaction mixture (0.1 M serine, 5 mM EDTA and 2.5 mM propargylglycine in 1 M Tris buffer, pH 8.4), 0.02 ml of 2.5 mM pyridoxal 5′-phosphate, 0.02 ml of liver protein supernatant, 0.1 ml of water and 0.02 ml of homocysteine reagent. The homocysteine reagent was made by dissolving 0.0154 g of homocysteine thiolactone in 0.4 ml of 2.5 M KOH. After being left at room temperature for 5 min, the solution was then neutralized by adding 0.6 ml of a mixture of 2.57 ml of 4.5 M HCl and 4.43 ml of 1 M Tris buffer, pH 8.4. The final pH of the homocysteine reagent was ~7.5. Incubations were for 1 h at 37 °C and were stopped by addition of 0.1 ml of 40 % (w/v) trichloroacetic acid. Each incubation then was thoroughly vortex-mixed and centrifuged at 10000 *g* for 5 min. Cystathionine concentrations in the supernatants were determined by h.p.l.c. with u.v. detection by the method of Einarsson et al. [19]. Protein was determined by the method of Lowry et al. [20]. Figures 2 and 3 demonstrate that this assay is linear with time and over a range of protein concentrations that encompasses the protein concentrations used in this study.

Statistical significance of the data obtained was evaluated by using analyses of variance followed by Tukey's honestly significant difference (HSD) test and standard Student *t* tests [21]. Because the magnitude of variability for homocysteine measurements increases in direct proportion with homocysteine concentration, homocysteine values were logged before statistical analysis. Statistics were performed by using 'Systat 5 for the MacIntosh' (Systat, Evanston, IL, U.S.A.).

RESULTS

Folate status and homocysteinaemia: relationship to hepatic AdoMet concentration

Table 1 shows the effect of folate depletion and repletion on hepatic folate, plasma homocysteine and hepatic AdoMet concentrations. After 4 weeks, the rats fed on the folate-deficient diet had a 16-fold lower mean hepatic folate concentration and a 9.8-fold greater mean plasma homocysteine concentration as compared with the folate-replete controls. The mean liver AdoMet concentration in the folate-deficient rats was 3.2-fold lower than in controls.

After 3 weeks' resupplementation of the folate-deficient rats, mean hepatic folate concentrations were positively correlated with the level of folate in the diet. Rats receiving 125 μg of folate/kg of diet had the lowest mean hepatic folate concentration, and those receiving 8000 μg of folate/kg had the highest mean hepatic folate concentration. In addition, only at a level of 1000 μg of folate/kg of diet or above did the mean hepatic folate concentration reach that observed in the folate-replete rats after 4 weeks. Mean plasma folate concentrations after the 3-week repletion period (results not shown) mirrored the pattern of hepatic folate concentrations presented in Table 1.

Table 1 Effect of folate depletion and repletion on hepatic folate, plasma homocysteine and hepatic AdoMet concentrations

Baseline groups were fed on a folate-deficient or folate control diet for 4 weeks. Resupplemented groups were fed on a folate-deficient diet for 4 weeks and then were fed on diets supplemented with various levels of folate as listed for 3 further weeks. After 4 weeks, hepatic folate and AdoMet concentrations in the folate-deficient rats were significantly lower than in controls, whereas plasma homocysteine was significantly elevated. Subsequent resupplementation of the folate-deficient rats caused increases in hepatic folate and AdoMet concentrations, and decreases in plasma homocysteine concentrations, in a dose-dependent manner. Values are means ± S.D.: *significantly different from the control value ($P \leqslant 0.025$).

Rat	*n*	Folate (μg/g)	Homocysteine (nmol/mg)	AdoMet (nmol/g)
4-Week baseline groups				
Folate controls	6	3.71 ± 1.89	6.4 ± 2.3	35.0 ± 7.6
Folate-deficient	6	0.23 ± 0.08*	62.4 ± 18.3*	10.9 ± 4.8*
Resupplemented groups (μg of folate/kg of diet)				
125	6	0.78 ± 0.40*	71.1 ± 52.0*	8.1 ± 1.9*
250	6	0.89 ± 0.26*	17.3 ± 5.5*	17.5 ± 5.8*
500	6	1.71 ± 0.41*	8.0 ± 2.5	20.3 ± 4.8*
1000	6	3.84 ± 0.65	4.9 ± 0.6	35.0 ± 9.4
8000	5	6.40 ± 1.33*	4.2 ± 1.3	38.4 ± 9.2

Table 2 Effect of ethionine administration on total plasma homocysteine concentration in folate-deficient rats

Folate-deficient rats received intraperitoneal injections of ethionine, 0.9% NaCl or nothing on three successive mornings and then were killed 1 h after the third injection. Deficient rats treated with ethionine had a significantly lower plasma homocysteine concentration than those treated with saline or nothing. Pre-treatment homocysteine values were not significantly different for the treatment groups. Values are means ± S.D.: *significantly different from saline and non-injected values ($P \leq 0.002$).

		Homocysteine (nmol/ml)	
Treatment	*n*	Pre-treatment	Post-treatment
Saline	7	59.5 ± 26.6	96.9 ± 48.4
Ethionine	8	58.9 ± 24.9	23.6 ± 2.0*
No injection	3	–	117.4 ± 20.4

Table 3 Effect of ethionine administration on hepatic cystathionine β-synthase activity and hepatic AdoMet and AdoHcy concentrations in folate-deficient rats

Folate-deficient rats were treated as described in Table 2. Measurement of cystathionine β-synthase (CβS) in crude liver extracts showed a more than 3-fold higher mean enzyme activity in the livers from ethionine-treated rats than in those livers from saline-treated controls. Hepatic AdoMet concentration showed no significant difference between rats treated with ethionine and those not treated with ethionine. Hepatic AdoHcy concentration in the ethionine-treated rats was significantly lower than in the rats not treated with ethionine. Values are means ± S.D.: *significantly different from saline and non-injected values ($P \leq 0.003$).

Treatment	*n*	CβS activity (nmol of cystathionine synthesized/h per mg of protein)	AdoMet (nmol/g)	AdoHcy (nmol/g)
Saline	6 or 7	173.0 ± 32.0	6.6 ± 2.3	38.8 ± 8.5
Ethionine	8	501.3 ± 135.6*	9.9 ± 3.9	16.9 ± 2.7*
No injection	3	233.8 ± 32.6	9.8 ± 3.4	38.1 ± 1.4

After the resupplementation period, plasma homocysteine concentrations were inversely correlated with the level of folate in the diet. Rats receiving 125 μg of folate/kg of diet had the highest mean plasma homocysteine concentration and rats receiving 8000 μg of folate/kg had the lowest. In addition, only at the level of 500 μg of folate/kg of diet or above did the mean plasma homocysteine concentration reach that observed in the folate-replete rats after 4 weeks.

After 3 weeks' resupplementation, the hepatic AdoMet concentrations, like hepatic folate, were positively correlated with the level of folate in the diet. Rats receiving 125 mg of folate/kg of diet had the lowest mean hepatic AdoMet concentration and those receiving 8000 μg of folate/kg had the highest. Similarly to hepatic folate, only at the level of 1000 μg of folate/kg of diet or above did the mean hepatic AdoMet concentration reach that observed in the folate-replete rats after 4 weeks.

Effect of ethionine administration on plasma homocysteine concentration in folate deficiency

After three intraperitoneal injections of ethionine on successive days, the mean plasma homocysteine concentration in rats fed on the folate-deficient diet was 4-fold lower than that of folate-deficient rats who received three intraperitoneal injections of physiological saline (Table 2). These results are contrasted with the pre-injection plasma homocysteine concentrations for these same groups of rats, which were not significantly different. The rats which were not subjected to tail bleeds or injections were found to have a mean plasma homocysteine concentration that was not significantly different from the value measured for the group which received saline injections.

Cystathionine β-synthase activity in crude liver extracts was approx. 3-fold greater for the rats treated with ethionine than for the rats treated with saline (Table 3); the activity in liver extracts from the rats not subjected to tail bleeds or injections was not significantly different from that measured for the saline-treated rats.

Table 3 also summarizes the effect of the ethionine treatments on hepatic AdoMet and AdoHcy concentrations. No difference in mean hepatic AdoMet concentration was observed between the rats treated with ethionine and those treated with saline. The mean hepatic AdoHcy concentration, however, was 2.3-fold lower in the ethionine-treated rats than in those treated with saline. In addition, all chromatograms generated to measure hepatic AdoMet and AdoHcy for the ethionine-treated rats exhibited a substantial peak immediately after the peak for AdoMet that was attributable to *S*-adenosylethionine. Although the size of this peak was variable between ethionine-treated rats, it represented a hepatic concentration of *S*-adenosylethionine that was at least an order of magnitude higher than the AdoMet or AdoHcy concentrations. This peak was not observed in chromatograms generated for any of the rats not treated with ethionine. In the rats not subjected to tail bleeds or injections, hepatic AdoMet and AdoHcy concentrations were not significantly different from those measured in the saline-treated rats.

DISCUSSION

Our hypothesis states that homocysteinaemia develops when the co-ordinate regulation of homocysteine metabolism by AdoMet is disrupted and both pathways of homocysteine metabolism become inhibited [6]. In folate deficiency, it is predicted that a primary block of homocysteine remethylation due to the deficiency will lead to a decrease in AdoMet synthesis. The ensuing decrease in tissue AdoMet concentration will result in a lack of activation of cystathionine β-synthase, thus producing a secondary block of homocysteine trans-sulphuration.

To test this prediction, we first sought to correlate changes in hepatic AdoMet concentration with plasma homocysteine concentration as folate status is modified. Dietary folate deprivation for 4 weeks produced an almost 10-fold increase in plasma homocysteine concentration that was accompanied by a 3.2-fold decrease in hepatic AdoMet concentration. Subsequent resupplementation for 3 weeks of the folate-deficient animals led to increases in hepatic AdoMet concentrations and decreases in plasma homocysteine concentrations that were correlated with the level of folate in the diet. Thus, as folate status was modified, plasma homocysteine concentration was inversely related to hepatic AdoMet concentration. Furthermore, it was at the level of 500 μg of folate/kg of diet that plasma homocysteine was lowered to within 'normal' limits as defined by the 4-week control rats. If tissue AdoMet concentration is the critical factor in whether or not plasma homocysteine becomes elevated, due to its ability to activate homocysteine catabolism, then the minimum hepatic AdoMet concentration necessary to prevent homocysteinaemia in this species of rat is approx. 20 nmol/g, i.e. the mean hepatic AdoMet concentration in the rats resupplemented at the level of 500 μg of folate/kg of diet (Table 1).

Our second strategy for testing our prediction was to show that folate-deficiency-induced homocysteinaemia could be

reversed by activating homocysteine catabolism, despite the folate deficiency and despite the decrease in tissue AdoMet concentration that folate deficiency produces. Our approach was to activate homocysteine catabolism by administration of ethionine. Ethionine is converted in the liver into *S*-adenosylethionine by the same enzyme that converts methionine into AdoMet, AdoMet synthetase (EC 2.5.1.6) [22]. Unlike AdoMet, however, *S*-adenosylethionine is not metabolized at an appreciable rate and will accumulate in the liver [22]. On the other hand, *S*-adenosylethionine does activate cystathionine β-synthase, presumably by a mechanism similar to that of AdoMet [7,9]. Therefore it was predicted that the effect of ethionine administration would be to activate homocysteine catabolism without the confounding factor of increasing homocysteine synthesis that might be expected from methionine administration.

The effect of ethionine administration in our folate-deficient rats was to lower significantly the plasma homocysteine concentration as compared with deficient animals that were administered vehicle alone. To support the interpretation that this effect of ethionine administration was through its conversion into *S*-adenosylethionine and the consequent activation of homocysteine catabolism, we found that, when we measured hepatic AdoMet and AdoHcy concentrations in the ethionine-treated rats, a large peak in our chromatographs attributable to *S*-adenosylethionine was observed which indicated that this metabolite did accumulate in the liver. Furthermore, the mean cystathionine β-synthase activity in crude liver extracts from those rats treated with ethionine was found to be 300% higher than that measured in extracts from the rats treated with vehicle alone. Apparently, the accumulated hepatic *S*-adenosylethionine was at a high enough concentration to activate cystathionine β-synthase, even after the expected dilution of this metabolite that would be expected to occur in our cystathionine β-synthase assay procedure.

A possible confounding factor to our interpretation of these results does exist, however. Ethionine competes with methionine for AdoMet synthetase [22]. It therefore could be postulated that the effect of the ethionine administration was to inhibit homocysteine synthesis rather than to activate homocysteine catabolism. In the folate-deficient rats treated with ethionine, the mean hepatic AdoMet concentration was not different from that determined for the folate-deficient rats treated with vehicle alone. This suggests that in fact AdoMet synthesis and consequently homocysteine synthesis was not inhibited. In addition, hepatic AdoHcy concentrations were significantly decreased by the ethionine administration. Since the equilibrium of the interconversion between AdoHcy and homocysteine favours AdoHcy synthesis and is only driven toward homocysteine by product removal, this decrease in AdoHcy observed as a result of ethionine treatment can be attributed to *S*-adenosylethionine's activation of homocysteine catabolism.

The data presented in this paper support the contention that the development of homocysteinaemia in folate deficiency is due to (1) impaired homocysteine remethylation because of the folate deficiency, and (2) impaired trans-sulphuration because the folate deficiency induces a decrease in tissue AdoMet to a concentration which is insufficient to activate homocysteine catabolism. Ethionine treatment effectively opens the trans-sulphuration pathway by activating cystathionine β-synthase, thus reversing the homocysteinaemia in the face of the folate deficiency because only one homocysteine metabolic pathway is left blocked. Because of the increasing evidence supporting a connection between elevated plasma homocysteine and vascular diseases, this work illustrates the significant influence of nutritional factors, such as folate, on homocysteine metabolism and suggests an important connection between nutritional deficiencies and vascular disease.

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