The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine

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ABSTRACT A unified, biochemical hypothesis is proposed to explain the pathogenesis of homocysteinemia. This hypothesis is based on the existence of coordinate regulation by S-adenosylmethionine (SAM) of the partitioning of homocysteine between de novo methionine synthesis and catabolism through cystathionine synthesis. This coordination, which serves to modulate the cellular concentration of homocysteine based on the requirements for methionine, is impaired in homocysteinemia. This hypothesis is evaluated in the context of the conditions known to be associated with homocysteinemia, including enzymatic defects and vitamin deficiencies. The novelty of the hypothesis is the assertion that impairment of one homocysteine metabolic pathway must lead to the impairment of the other homocysteine metabolic pathway to cause homocysteinemia. This extends the simplistic view that a block of only one of the pathways is sufficient to cause homocysteinemia. Am J Clin Nutr 1992;55:131-8.

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Introduction

In recent years there has been renewed interest in homocysteine metabolism and the role it plays in the development of cardiovascular and cerebrovascular diseases (1, 2). This renewed interest has been spurred by the recognition that moderate homocysteinemia is widely prevalent and that it is associated with these vascular diseases. In a recent study Brattstrom et al (3) reported that 28–36% of patients with early onset cerebral and peripheral occlusive arterial diseases had homocysteinemia or evidence of impaired homocysteine metabolism. Genest et al (4) showed that patients with premature cardiovascular disease have a significantly higher concentration of homocysteine in plasma than that found in age-matched control subjects. No correlations were found between plasma homocysteine levels and other indices of cardiovascular disease, eg, serum cholesterol, high-density-lipoprotein cholesterol, very-low-density-lipoprotein cholesterol, or triglycerides, indicating that homocysteine is an independent risk factor (4). This essay provides a unifying hypothesis that explains the metabolic basis for observed increases in plasma homocysteine concentrations under various conditions. Understanding the basis for these abnormalities will provide better insight into the relationship between homocysteinemia and vascular diseases.

Cellular metabolism of homocysteine

Homocysteine is a sulfur-containing amino acid that stands at the intersection of two metabolic pathways: remethylation and transsulfuration (Fig 1). (A third pathway, that of homocysteine deamination, was shown to be of no significant importance in man and will not be discussed.) In remethylation, homocysteine acquires a methyl group from N-5-methyltetrahydrofolate or from betaine to form methionine. The reaction with N-5-methyltetrahydrofolate is catalyzed by the ubiquitous vitamin B-12-containing enzyme, N-5-methyltetrahydrofolate:homocysteine methyltransferase (EC 2.1.1.13). The methyl group of N-5-methyltetrahydrofolate is synthesized de novo when a carbon unit is transferred from a carbon source, such as serine or glycine, to tetrahydrofolate producing methylenetetrahydrofolate, which subsequently is reduced by methylenetetrahydrofolate reductase (EC 1.1.1.68). The reaction with betaine is B-12 independent and is catalyzed by betaine:homocysteine methyltransferase (EC 2.1.1.5). This reaction uses preformed methyl groups because betaine is derived from choline, which in part is supplemented in the diet and in part is synthesized through successive methylations of phosphatidylethanolamine. In rats, specifically in liver, the folate and betaine methylation reactions are equally important (5). In humans some evidence indicates that a significant amount of dietary choline can be utilized for betaine-dependent methyl transfer (6, 7), although the activity...
in liver of choline dehydrogenase (EC 1.1.99.1), involved in the synthesis of betaine from choline, was reported to be low (8).

A considerable proportion of methionine is activated by ATP to form S-adenosylmethionine (SAM). SAM serves primarily as a methyl donor to a variety of acceptors, including guanidinoacetate, nucleic acids, neurotransmitters, phospholipids, and hormones. S-Adenosylhomocysteine, the by-product of these methyl transfer reactions, is hydrolyzed, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer.

In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal-5'-phosphate (PLP)–containing enzyme, cystathionine β-synthase (EC 4.2.1.22). Cystathionine is hydrolyzed by a second PLP-containing enzyme, γ-cystathionase (EC 4.4.1.1), to form cysteine and α-ketobutyrate. Excess cysteine is oxidized to taurine and eventually to inorganic sulfates. Thus, in addition to the synthesis of cysteine, this transsulfuration pathway effectively catabolizes potentially toxic homocysteine, which is not required for methyl transfer.

**Impairment in homocysteine metabolism**

Homocysteine is present in plasma in its sulphydryl form and in mixed disulfide forms. Normally, the total homocysteine concentration in plasma ranges from 7–24 μmol/L (9–14). In urine the level of homocysteine is in the same range (13, 14). Abnormal elevations of homocysteine in plasma (homocysteinemia) and urine (homocystinuria) can be induced by several congenital and nutritional disorders that directly affect homocysteine metabolism. The classic congenital disorder is that of cystathionine β-synthase deficiency. This deficiency, which results from the homozygous carriage of the recessive gene, can lead to fasting plasma homocysteine concentrations as high as 200 μmol/L (1). It has a worldwide incidence of 1 in 200,000 and is associated with a marked increase in premature thromboembolic disease and atherosclerosis (1, 15). Other less-common cases of homocysteinemia are the result of congenital deficiencies of enzymes involved in de novo methionine synthesis. These include 5,10-methylenetetrahydrofolate reductase deficiency (16–20) and deficiencies of any of the enzymes leading to the synthesis of methyl-B12 (21–25).
Nutritional disorders that potentially lead to homocystinemia are deficiencies of vitamin B-12, folate, and vitamin B-6. As noted above, the de novo synthesis of methionine methyl groups requires both vitamin B-12 and folate coenzymes whereas the synthesis of cystathionine requires PLP (vitamin B-6). Studies from several laboratories conducted both in humans and laboratory animals demonstrated that deficiencies of B-12 and folate are associated with increased plasma concentrations of homocysteine (10, 26–34). The relationship of homocysteine concentrations to vitamin B-6 status, however, is less obvious. The data presented in Table 1 are from a recent survey that was conducted by us to assess the relationships between homocysteine concentrations and status of the above-mentioned three vitamins. As shown, the plasma homocysteine concentrations in sera from overnight-fasting volunteers bear a significant negative relationship to both folate and vitamin B-12 status but not to vitamin B-6 status. A related finding was reported by Brattstrom et al. (3, 37, 38), who showed that vitamin B-6 supplementation, unlike folinic acid, is ineffective in reducing plasma homocysteine concentrations. Several other studies also demonstrated this disparity (39–42).

This apparent lack of relationship between plasma homocysteine and vitamin B-6 status also was demonstrated in studies in which vitamin B-6 deficiency was induced by dietary manipulation. In one study that was conducted at the Human Nutrition Research Center (43), plasma homocysteine concentrations were measured in volunteers who were made vitamin B-6 deficient by consumption of a vitamin B-6-deplete diet. By the third week of the diet, all subjects were vitamin B-6 deficient as determined by several standard measures of vitamin B-6 status, yet 10 of the 11 volunteers measured had normal fasting plasma homocysteine concentrations. In another study, Park and Linkswiler (44) demonstrated that men who were made deficient in vitamin B-6 contained only traces of homocysteine but 5- to 14-fold increases in cystathionine concentrations in their urine, indicating that the vitamin B-6 deficiency caused cystathionuria but not homocystinuria. Similarly, when 23-mo-old rats were fed a vitamin B-6-deficient diet for 9 wk, fasting plasma homocysteine concentrations were not significantly different from those of normally fed controls (43). In younger (3-mo-old) rats, fasting plasma homocysteine concentrations were normal at 6 wk; moderate elevation of plasma homocysteine was seen in these younger rats when the vitamin B-6-deficient diet continued for 9 wk. These studies suggest that in vitamin B-6 deficiency, homocysteine metabolism is generally unaffected. However, the use of a dietary challenge, specifically a methionine load, suggests that in vitamin B-6 deficiency, homocysteine metabolism is somewhat impaired.

### Detecting impaired homocysteine metabolism by methionine loading

Methionine loading is a test that was originally used to identify heterozygotes for cystathionine β-synthase deficiency (45). As opposed to homoyogotes, these heterozygotes have normal or only slightly elevated concentrations of fasting plasma homocysteine. When given an oral load of L-methionine (100 mg/kg body wt), heterozygotes will develop a surge in plasma homocysteine that is higher and more sustained than in normal control subjects given the same methionine dose (45–50). Eventually, the homocysteine in plasma will return to its original (fasting) concentration. This characteristic of impaired homocysteine metabolism also has been observed in cases of induced vitamin B-6 deficiency. In the study by Park and Linkswiler described above (44), detectable amounts of homocysteine in urine of vitamin B-6-deficient male subjects were seen only after large oral doses of L-methionine. In a follow-up study, Shin and Linkswiler (51) demonstrated the same phenomenon in vitamin B-6-deficient female subjects. In vitamin B-6-deficient rats, plasma homocysteine concentrations increased almost sevenfold after fasting plasma concentrations after a challenge with a vitamin B-6–deficient diet that contained 0.6–1.85% L-methionine (52). Within hours the concentration of homocysteine in the plasma of these rats returned to normal. In control vitamin B-6–replete rats, homocysteine concentrations in plasma remained normal after the same dietary challenge.

The effects of methionine loading provide an explanation for several other studies in which elevated plasma homocysteine concentrations were observed in rats (53–55) and pigs (56) fed vitamin B-6–deficient diets. Of these studies, one indicates that plasma samples were collected from nonstarved animals (53) whereas the others do not specify the prandial state of the animals at blood draw. We make the assumption that plasma homocysteine concentrations in these latter studies also were determined from animals that were not starved and conclude that the observed elevations in homocysteine concentration were induced by the methionine consumed by the animals in their respective diets. Therefore, it seems clear that in vitamin B-6 deficiency, as well as in heterozygotes for cystathionine β-synthase deficiency, homocysteine metabolism is impaired but that this impairment is manifested as homocysteinemia only after ingestion of methionine, either alone or as a constituent of the diet.

### The pathogenesis of homocysteinemia

As stated earlier, homocysteine is potentially toxic to cells (57). Therefore, removal of homocysteine from a cell is paramount. Besides catabolism through cystathionine synthesis and remethylation to form methionine, there are at least two additional ways by which low cellular homocysteine concentrations are maintained. One mechanism is related to the conversion of S-adenosylhomocysteine to homocysteine which is catalyzed by...
S-adenosylhomocysteine hydrolase (EC 3.3.1.1). The equilibrium of this reaction actually favors the formation of S-adenosylhomocysteine and is driven forward only by the removal of products (1). A second mechanism is that of homocysteine export from the cell (1, 58, 59). It can be predicted, therefore, that if impaired homocysteine metabolism persists, a threshold concentration of intracellular S-adenosylhomocysteine is reached at which hydrolysis of S-adenosylhomocysteine becomes significant and homocysteine begins to accumulate in the cell. When intracellular accumulation does occur, the cell’s homocysteine export mechanism leads to the disposition of homocysteine into the blood and urine. This export maintains low intracellular homocysteine concentrations and prevents toxicity to the cell but causes clinical homocysteinemia and homocystinuria.

The simplest hypothesis to explain homocysteinemia, then, is that impairment of either cystathionine synthesis or methionine synthesis leads to intracellular accumulation of homocysteine and its subsequent export into the blood. This hypothesis, however, is inconsistent with two important observations. The first is that vitamin B-6 deficiency, unlike folate and vitamin B-12 deficiencies, is not associated with elevated fasting plasma concentrations of homocysteine. The second is that experimental evidence indicates that the activities of remethylation and transulfuration are coordinated. According to Finkelstein and Martin (5), these two pathways can be considered to be competing for available homocysteine; reduction of activity in one pathway will lead to the more effective use of homocysteine by the second pathway. Studies by Mudd and Poole (6) and Mudd et al (7) demonstrated that when the intake of labile methyl groups (ie, methionine and choline) is curtailed, the de novo synthesis of methionine methyl groups is enhanced. When a basal methionine-containing diet was fed, homocysteine moieties cycled through the transmethylation pathway ~ 1.5–2.0 times before being catabolized to cystathionine. When dietary methionine was halved, the number of cycles per homocysteine moiety increased twofold. Conversely, when excess dietary methionine was fed, homocysteine cycling decreased below basal concentrations. Similar adaptations to changing amounts of dietary methionine were observed by Eloranta et al (60) in rats. These results are consistent with the previously cited studies in which normal humans and laboratory animals were shown to be capable of handling large doses of methionine without significant, prolonged increases in plasma homocysteine concentrations.

These studies reveal the capacity of the body to adapt to varying amounts of methionine in the diet and suggest that homocysteine, which is derived solely from methionine, should not accumulate when only one of its metabolic pathways is impaired. The fact that it does accumulate points to the existence of conditions whereby a defect in one pathway will lead to the impairment of the other. Therefore, the hypothesis that homocysteinemia is the result solely of inhibited synthesis of methionine methyl groups and/or cystathionine is not sufficient to explain all the observations that have been made.

The alternate view proposed in this essay is based on the observations that SAM is both an allosteric inhibitor of methylenetetrahydrofolate reductase (61–63) and an activator of cystathionine ß-synthase (64, 65). [SAM also has been reported to inactive betaine-homocysteine methyltransferase under in vitro conditions (66). However, the physiological significance of this observation is unclear because the inactivation could not be reproduced in vivo (66). Therefore, SAM’s role as an effector of this enzyme is not considered here.] The ability of SAM to act as an enzymatic effector provides a means by which remethylation and transulfuration can be coordinated. When cellular SAM concentration is low, the synthesis of 5-methyltetrahydrofolate will proceed uninhibited whereas cystathionine synthesis will be suppressed. This will result in the conservation of homocysteine for methionine synthesis. Conversely, when SAM concentration is high, inhibition of 5-methyltetrahydrofolate synthesis is accompanied by the diversion of homocysteine through the transsulfuration pathway because of stimulated cystathionine synthesis.

Although the primary effect of this coordinate control is the regulation of cellular SAM concentrations, another important outcome is the maintenance of a concentration of homocysteine that is compatible with the need for de novo methyl groups. The model that we propose predicts that homocysteinemia stems from conditions in which the cell is no longer capable of coordination between the two pathways. Impairment of the remethylation pathway will thwart the induction of transulfuration and prevent catabolism of excess homocysteine. Similarly, an impairment of the transsulfuration pathway will prevent the induction of remethylation and inhibit disposal of homocysteine through its conversion to methionine. What follows is a discussion of this model as it relates to the various causes of homocysteinemia.

In folate deficiency, or in individuals with defective methylenetetrahydrofolate reductase, a decreased availability of 5-methyltetrahydrofolate will result in decreased de novo synthesis of methionine. A decrease in de novo methionine synthesis will lead to a greater reliance on dietary sources (ie, methionine and choline) to meet the demands for methyl groups. Normal dietary supplies of methionine and choline, however, are not sufficient to meet these demands (6, 7) and therefore an impairment in de novo methionine synthesis will result in a marked decrease in cellular SAM concentration. The result of cellular SAM depletion is an unstimulated cystathionine ß-synthase enzyme and, consequently, homocysteine accumulation. Similarly, under conditions of vitamin B-12 deficiency or enzymatic defects in any of the steps that lead to the synthesis of methyl–B-12, depression in the synthesis of methionine, and consequently SAM, will lead to homocysteine accumulation because of unstimulated cystathionine ß-synthase.

In a case study, a patient with a congenital disorder of partially defective SAM synthesis also had abnormal homocysteine conservation leading to excessive methionine synthesis and hypermethioninemia (67). The authors predicted that this high homocysteine conservation probably resulted from a concentration of SAM insufficient to inhibit methylenetetrahydrofolate reductase activity and insufficient to stimulate cystathionine ß-synthase to rid the cell of excess homocysteine through the transsulfuration pathway. This is consistent with our hypothesis.

When cystathionine ß-synthase is completely inactive, the resulting homocysteinemia can be considered to be the result of 1) the inability of SAM to activate the fully inactive cystathionine ß-synthase and 2) the SAM-induced incapacity of the remethylation pathway to completely dispose of excess homocysteine. In this condition, the homocysteine, which ordinarily would be transsulfurated, will initially induce accelerated de novo methionine synthesis until the concentration of methionine is increased enough to cause the accumulation of SAM. Then, the high concentration of SAM will inhibit synthesis of 5-methyltetrahydro-
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Folate and thus inhibit disposal of homocysteine through its conversion to methionine. Subsequently, SAM will be metabolized and its concentration will decrease, leading to renewed methionine synthesis from homocysteine. Eventually, a state will be reached at which some homocysteine is converted to methionine, but the rest cannot be converted because of SAM’s inhibitory effect on methylenetetrahydrofolate reductase. This interpretation is consistent with the observation that patients with homocysteinemia due to homozgyous cystathionine β-synthase deficiency also tend to have hypermethioninemia (2). Furthermore, an effective treatment of homocystinuria due to homozygous cystathionine β-synthase deficiency is betaine supplementation (68–71). Betaine effectively converts homocysteine to methionine, thus compensating for the inhibited folate-dependent remethylation.

Our proposed model also predicts that, in cases in which cystathionine β-synthase is only partially active, a normal to slightly elevated synthesis of methionine will provide a concentration of SAM capable of stimulating residual synthase activity. This stimulated activity is sufficient to dispose of homocysteine when the methionine burden is low, eg, after fasting. When the methionine burden is high, however, homocysteine will temporarily accumulate because the residual enzyme activity cannot immediately dispose of the newly synthesized homocysteine, even with SAM stimulation. Partially active cystathionine β-synthase occurs in heterozygotes for deficiency of this enzyme (72) and in animals which are made vitamin B-6 deficient (73). As was described earlier, these cases exhibit normal concentrations of fasting plasma homocysteine, which rise after a methionine load.

This concept of coordinately regulated homocysteine metabolism is not confined to mammalian systems. In a scheme proposed 20 years ago (74), coordinate regulation between remethylation and transulfuration was elucidated in the mold Neurospora crassa. In N crassa, the transulfuration pathway functions in the opposite direction. Cysteine, which in animals is derived from homocysteine catabolism, serves in N crassa as a precursor for homocysteine synthesis (Fig 2). In this synthesis, cysteine condenses with (acetyl)homoserine in a reaction that is catalyzed by cystathionine γ-synthase (EC 4.2.99.9). The product of this condensation, cystathionine, is hydrolyzed by β-cystathionase (EC 4.4.1.8) to form homocysteine, which then is remethylated by 5-methyltetrahydrofolate (polyglutamates) to methionine, a reaction which is catalyzed by vitamin B-12 independent) 5-methyltetrahydrofolate (polyglutamates):homocysteine methyltransferase (EC 2.1.1.14).

As is the case for animals, N crassa has a methylenetetrahydrofolate reductase enzyme that is inhibited by SAM. Also, SAM is an allosteric inhibitor of cystathionine γ-synthase. This latter
enzyme (whose $\beta$-synthase counterpart in animals is activated by SAM) is activated by 5-methyltetrahydrofolate (polyglutamates). Therefore, when SAM concentration is low and there is a need for methionine methyl groups, the synthesis of methyltetrahydrofolate activates cystathionine synthesis and, consequently, increases the availability of homocysteine for remethylation. When SAM concentration is high and the methionine requirement is diminished, the syntheses of cystathionine and methyltetrahydrofolate are suppressed, thus inhibiting further homocysteine synthesis and remethylation.

These regulatory mechanisms in N. crassa, like those in animals, serve to limit homocysteine synthesis to that needed for production of methionine methyl groups. The fact that the metabolic situations in N. crassa and in mammalian cells are so different yet seem to be similarly regulated for the common purpose of controlling homocysteine metabolism provides strong support for the legitimacy of the proposed hypothesis explaining the pathogenesis of homocysteinemia.

Concluding remarks

The hypothesis proposed in this essay is the first to explain all known causes of homocysteinemia by a single, biochemical principle. Like all hypotheses, certain issues remain to be resolved to establish its validity. Foremost of these issues is the demonstration that modulating SAM concentrations in vivo is indeed effective in regulating cystathionine $\beta$-synthase activity. Such a demonstration would be important, for example, in interpreting the data by Sturman et al. (76), which indicated that cystathionase activity was 10-fold lower in liver extracts from vitamin B-6-deficient rats than in extracts from vitamin B-6-supplemented rats whereas the activity of cystathionine $\beta$-synthase was only 18% lower. One explanation for these results is that cystathionine $\beta$-synthase has a much greater affinity for PLP than for cystathionase. An alternate explanation is that endogenous SAM was responsible for the relatively high cystathionine $\beta$-synthase activity observed in the vitamin B-6-deficient extracts. This second explanation, if shown to be correct, would support the present hypothesis.

A second issue is that the intracellular events proposed to precede the onset of homocysteinemia are somewhat speculative. Very little is known about the effect of enzymatic and vitamin deficiencies on intracellular $S$-adenosylhomocysteine and homocysteine concentrations, particularly before homocysteinemia develops. Some evidence exists to suggest that cellular concentrations of these compounds do increase before the onset of homocysteinemia (77-80) but this remains to be proven for all causes of this condition. Further studies also are required to understand the nature of the homocysteine export mechanism. Thus far, all studies on cellular homocysteine export were conducted under in vitro conditions (58, 59, 78, 79). Although these studies have provided important information, there nevertheless is a need to understand the processes that lead to cellular export of homocysteine in vivo.

The validation of this hypothesis will be important for understanding the relationship between homocysteinemia and vascular diseases. The basis for this relationship is at present unknown. It is unclear whether vascular diseases result from the exposure of vascular cells to homocysteine or from separate pathological processes that coincidentally are associated with homocysteinemia as well. This hypothesis provides the theoretical basis on which these alternatives can be tested. This hypothesis also will be important for understanding the usefulness of measuring blood homocysteine concentrations. As stated, the conditions under which homocysteine is measured (ie, fasting or nonfasting) can determine whether or not an elevated homocysteine concentration is observed. Also, knowledge of the possible causes of an elevated homocysteine concentration is necessary for understanding how to treat the condition. Finally, this hypothesis provides a theoretical basis for the development of intervention strategies to reduce plasma homocysteine concentrations and determine if such interventions are effective in reducing the risk of vascular diseases. Such interventions might include prophylactic supplements of vitamin B-12, folic acid, and/or betaine.

References


