Fig 2B), we conclude that neither inter- nor intramolecular disulfides contribute to increased stability of elasmobranch tetramers.

The stability and the increased urea tolerance may be due to minor structural differences since studies with abnormal human hemoglobins have shown that single changes in primary sequence at key positions can greatly alter both the oxygen affinity and the equilibrium between dimers and tetramers (*13*). The sensitivity of elasmobranch hemoglobins to NaCl strengthens the possibility that increased electrostatic interactions between their subunits may provide the structural integrity to withstand the denaturing effect of urea. Experiments with human hemoglobin show this to be a real possibility. In its unliganded form, human hemoglobin possesses additional salt bridges between its subunits (*13*). These electrostatic interactions hold the unliganded hemoglobin in a conformation which is much more resistant to dilution-induced dissociation into subunits (*10, 13*). Our kinetic experiments show that the unliganded form is also more resistant to urea-induced dissociation. When the deoxy form of human hemoglobin is rapidly mixed with a solution containing CO, the time course of the reaction shows no evidence of quickly reacting material, even in the presence of 4*M* urea (*14*).

Urea has recently been proposed as a therapeutic agent for the treatment of sickle cell disease. Suggested dosages yield blood urea concentrations of 0.1*M* (*15*). As can be seen from Fig. 1, this concentration of urea has little direct effect on the oxygen affinity of HbA. Aqueous urea preparations, however, are known to form appreciable amounts of cyanate spontaneously. It has been proposed that the "urea effect" comes about by slow carbamylation of the $-NH_2$ termini of the chains by the cyanate (*16*). The ubiquitous presence of urea in blood and tissues of elasmobranchs suggests that their proteins might be highly carbamylated. If this is not the case, elasmobranchs may have evolved biochemical systems for the decarbamylation of amino groups or the removal of cyanate as it is formed.

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## Methionine Adenosyltransferase Deficiency: New Enzymatic Defect Associated with Hypermethioninemia

Abstract. *A specific deficiency of methionine adenosyltransferase has been demonstrated in the liver of an infant with hypermethioninemia. Since the enzymatic activity was below that in fetal liver and the metabolic abnormality has persisted (the infant now being 1 year of age), there is probably a genetic mutation. Mass screening for hypermethioninemia may uncover more such cases.*

Several enzymatic deficiencies on the pathway of metabolism of methionine (Fig. 1) have been described in humans; clinical and biochemical aspects of these deficiencies have been reviewed (*1*). The most well-studied and the most common is cystathionine

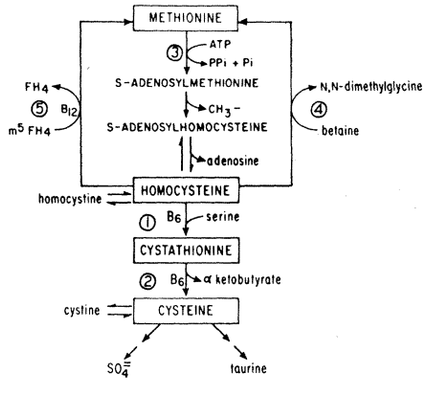


Fig. 1. Transsulfuration and remethylation pathways. (1) Cystathionine $\beta$-synthase; (2) cystathionase; (3) methionine adenosyltransferase; (4) betaine-homocysteine methyltransferase; and (5) $N^5$-methyltetrahydrofolate-homocysteine methyltransferase; $FH_4$, tetrahydrofolate; $m^5FH_4$, $N^5$-methyltetrahydrofolate. [Reproduced with permission of *Pediatric Research*]

$\beta$-synthase deficiency, which is associated with hypermethioninemia, homocystinemia, and hypocystinemia. So-called hereditary tyrosinemia and a variety of other diseases accompanied by cirrhoses of the liver have been associated secondarily with nonspecific deficiencies of both methionine adenosyltransferase and cystathionine $\beta$-synthase (*2*). Hypermethioninemia occurs in infants, especially in prematurely born infants and in infants fed formulas containing more than 5 percent protein, a concentration well above the 1 percent found in human milk (*3*). In these infants hypermethioninemia usually is accompanied by cystathioninuria (*4*) and is thought to result from a delay in the maturation of cystathionase. Cystathionase normally is absent from human fetal liver and reaches full activity some time after birth (*5*).

In a mass screening survey, a newly born infant with hypermethioninemia was identified. Methionine in the plasma was 128 $\mu$mole/100 ml ($>30$ times the concentration in normal plasma) and was unaccompanied by homocystinemia, hypocystinemia, or cystathioninuria. Hyperthioninemia was still

Table 1. Enzymatic activities in the liver of a child with persistent hypermethioninemia; comparison with activities in fetal and mature control human liver. Enzymatic assays were performed essentially as described in (*7*) (methionine adenosyltransferase, cystathionine $\beta$-synthase, and cystathionase) and (*8*) (methyltetrahydrofolate-homocysteine methyltransferase). Activities are expressed as nanomoles of product formed per milligram of soluble protein per hour ± standard deviation. The numbers in parentheses represent the number of separate determinations.

| Source | Methionine adenosyltransferase | Cystathionine $\beta$-synthase | Cystathionase | Methyltetrahydrofolate-homocysteine methyltransferase |
|---|---|---|---|---|
| Patient | 5.0; 10.0 | 118.2 | 310 | 2.5 |
| Fetus | 26 ± 15 (24)* | 21 ± 20 (24)* | 0 (24)* | 4.7 ± 1.1 (31)† |
| Mature | 86 ± 48 (9)* | 98 ± 57 (9)* | 126 ± 36 (9)* | 1.3 ± 0.67 (17)† |

* Values taken from (*5*). † Values taken from (*11*).

present when the infant was 6 months of age, at which time two successive percutaneous needle biopsies of the liver were performed 2 weeks apart. At this age, the infant appeared generally healthy, and "liver function" tests and serological tests for hepatogenic infectious agents were normal. However, electron microscopy of hepatocytes showed breaks in the outer membranes of the mitochondria and hyperplasia of the smooth endoplasmic reticulum. These abnormal findings differ from those seen in the hepatocytes of patients with homocystinuria due to deficiency of cystathionine synthase: abnormally shaped mitochondria with occasional breaks in the outer membrane, hyperplasia of the smooth endoplasmic reticulum, and increased numbers of lysosomes in younger patients (*6*).

Extracts of liver were assayed, by minor modifications of previously described methods, for activity of methionine adenosyltransferase (ATP:L-methionine *S*-adenosyltransferase, E.C. 2.5.1.6) (*7*); cystathionine $\beta$-synthase [L-serine hydrolyase (adding homocysteine), E.C. 4.2.1.22] (*7*); cystathionase [L-cystathionine cysteine-lyase (deaminating), E.C. 4.4.1.1] (*7*); and $N^5$-methyltetrahydrofolate - homocysteine methyltransferase (5-methyltetrahydropteroyl-L-homocysteine *S*-methyltransferase, E.C. 2.1.1.13) (*8*).

Activity of methionine adenosyltransferase in extracts from the two separate biopsies was severely deficient as compared to both adult and fetal human hepatic controls (Table 1). Activities of other enzymes of the transsulfuration and remethylation pathways (Fig. 1) were not deficient. When the infant was 1 year of age, despite reduction in dietary methionine intake, the plasma concentration of methionine remained ten times normal, and serum folate was high (> 30 ng/ml; the normal was 4 to 18 ng/ml).

The persistence of the metabolic abnormality (the child is now 1 year of age) and the presence of an apparently specific deficiency of hepatic methionine adenosyltransferase activity, which is in a range well below that found in the second trimester fetus, suggest that this is a genetic mutation rather than either a delay in enzymatic maturation or an acquired disease. Genetic and tissue culture studies of the family are in progress and should help to establish the mode of inheritance.

Although the infant's physical and mental development apparently is normal, there are definite morphological abnormalities in the hepatocytes. Furthermore, abnormalities in appearance and development in some inborn errors of amino acid metabolism, such as homocystinuria due to cystathionine synthase deficiency, may not become clinically apparent until the sixth year or later. Because of the key role that methionine adenosyltransferase plays in biosynthesis and metabolism, it seems surprising that an organism with a deficiency of this enzyme can survive. Presumably the 6 to 12 percent of mean adult control activity is sufficient. It is also possible that the quantitative role of $N^5$-methyltetrahydrofolate as a methyl donor for a variety of methyltransferases (*9*) is even greater than anticipated. Perhaps $N^5$-methyltetrahydrofolate can substitute for *S*-adenosylmethionine in the event of a diminished supply of the latter. Nevertheless, because of the key role methionine adenosyltransferase plays in drug detoxification, it is conceivable that deficient patients might have difficulties in handling, for example, large excesses of catecholamines. The high concentration of total serum folates suggests that activity of $N^5$methyltetrahydrofolate-homocysteine methyltransferase, in vivo, may be decreased because of a lack of *S*-adenosylmethionine (which is a cofactor for $N^5$-methyltetrahydrofolate-homocysteine methyltransferase and the product of methionine adenosyltransferase) or because of negative feedback caused by the accumulation of methionine (the product of $N^5$-methyltetrahydrofolate - homocysteine methyltransferase). These findings emphasize the metabolic importance of the cycle of demethylation and remethylation of methionine (*8, 10*).

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