Development of Methyltransferase Activities of Human Fetal Tissues

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Extract

JV^5-Methyltetrahydrofolate-homocysteine methyltransferase specific activity was higher in fetal (2nd trimester) human liver and kidney (4.70 ± 0.20 and 7.25 ± 0.23 nmol/mg protein/hr) than in mature human liver and kidney (1.30 ± 0.16 and 0.76 ±0.18 nmol/mg protein/hr). During the same period, there was a significant correlation of decreasing specific activity of this enzyme in fetal brain with increasing crown-rump length *(r = —0.72; P < 0.005)*, reaching the specific enzymatic activity of adult brain (1.37 ± 0.26 nmol/mg protein/hr).

Betaine-homocysteine methyltransferase specific activity was lower in fetal liver and brain (1.82 ± 0.21 and 0.20 ± 0.05 nmol/mg protein/hr) than in mature liver and brain (7.78 ± 1.89 and 0.37 ± 0.07 nmol/mg protein/hr). During the same period, there was a significant correlation of increasing enzymatic activity in fetal kidney with increasing crown-rump length (r = 0.80; *P* < 0.005) toward the mean specific activity of mature kidney (22.6 ± 2.0 nmol/mg protein/hr).

Serine tetrahydrofolate 5,10-hydroxymethyltransferase specific activity showed no significant difference between fetal and mature liver and kidney; however, the fetal brain showed a significant correlation of decreasing specific activity of this enzyme with increasing crown-rump length *(r = —0.69; P < 0.005)*.

The specific activities of betaine-homocysteine methyltransferase and serine-tetrahydrofolate 5,10-hydroxymethyltransferase in the liver of the neonate was not different from that in the mature liver. iV^6-Methyltetrahydrofolate-homocysteine methyltransferase in neonatal liver attained a specific activity similar to that found in mature liver before cystathionase did.

Cystathionase in 2nd trimester human fetal kidney, in contrast to cystathionase in human fetal liver and brain, already has attained two-thirds of the mean specific activity of mature kidney.

Speculation

Although the measurements of these enzyme activities as assayed *in vitro* do not take into consideration substrate availability *in vivo,* the relative specific activities of these methyltransferases in human fetal liver and brain, considered together with the absence of cystathionase, suggests to us that perhaps, in these two organs, the trans-sulfuration pathway for the further metabolism of homocysteine is turned off in favor

of the N^6-methyltetrahydrofolate-B12remethylation pathway (Fig. 1). The latter pathway converts $7V^6$-methyl tetrahydrofolate, the major monoglutamic folate in liver and serum [1], to tetrahydrofolate. The latter form of folate reacts with the *f3* carbon of serine, on serine-tetrahydrofolate 5,10 hydroxymethyltransferase, to form $N^i \bullet {}^{10}$-methylenetetrahydrofolate, a 1-carbon precursor for the *de novo* synthesis of thymidylate, which is uniquely required for DNA but not for RNA. This suggests that the /3 carbon of serine is being shunted into DNA synthesis during periods of rapid cellular multiplication, rather than having the entire carbon skeleton accept the sulfur from homocysteine to form cysteine. The latter thus becomes an essential amino acid in human fetal liver and brain.

Introduction

In our previous studies of the transsulfuration pathway of methionine metabolism in the human fetus, cystathionase activity (EC. 4.2.1.15) was not measurable in the liver and brain [10, 19, 25], and trace amounts of hepatic cystathionase, at most, were demonstrable by radiochemical perfusion studies [10] or immunochemically [14]. Since the transsulfuration pathway was shut off, with accumulation of cystathionine but without the accumulation of homocyst(e)ine [10, 19], it was of interest to examine the enzymatic activities of some methyltransferases involved in the metabolism of homocysteine (Fig. 1). The two major alternative pathways for remethylation to methionine

Fig. 1. Interrelationships of methionine metabolism, folate metabolism, and DNA synthesis in the human fetus. *SAM:* S-Adenosylmethionine; *SAH:* S-adenosylhomocysteine; *dUMP:* deoxyuridinemonophosphate; *dTMP:* deoxythymidinemonophosphate; *ct-KB:* ketobutyrate; *SCH^FH^* $JV^{5,10}$-methyltetrahydrofolate; *FH₄:* tetrahydrofolate; *FH.,:* dihydrofolate; *5,10CH₂FHₜ:* $N^5-{}^{10}$-methylenetetrahydrofolate; *B^:* vitamin B₁₂; *B₆:* vitamin Bₑ; *M.THF:* methyltetrahydrofolate; *BH:* betaine-homocysteine.

were studied: N^5-methyltetrahydrofolate (MTHF)-homocysteine methyltransferase, the B₁₂-linked enzyme which generates tetrahydrofolate (THF) from MTHF, and betaine-homocysteine (BH) methyltransferase (EC. 2.1.2.5). L-Serine-tetrahydrofolate 5,10-hydroxymethyltransferase (STHF) (EC. 2.1.1.1) was also studied; it catalyzes the transfer of the /3 carbon of serine to THF, an important step in the *de novo* synthesis of thymidylate for incorporation into DNA, and has been reported to decrease in mouse brain during the neonatal period [2].

Methods

Included in this study were tissues from human fetuses (5.5-22.0 cm crown-rump length; approximately 2nd trimester) obtained at therapeutic abortion; livers from eight neonates, who died during the first 9 days of life and weighed from 680-4,000 g at birth; and tissues from mature humans with apparently normal organs at autopsy. Whole fetal brain was used, whereas the occipital lobe from mature brain was used. Control studies of rat liver, simulating conditions for postmortem autolysis, showed no significant changes in specific activity of the enzymes studied at 24 and 48 hr after death.

The tissues were homogenized with 4 volumes of 0.03 M potassium phosphate buffer, pH 6.9, and centrifuged at 15,000 x *g* for 30 min. The supernate was removed, recentrifuged, and assayed for cystathionase [9], MTHF-methyltransferase [7, 26], BH-methyltransferase [7, 8, 26], and STHF-methyltransferase [22]. In the latter assay, 0.33 ^mole 3-^{14}C-L-serine was used instead of the 0.1 yumole stated in the published method [22, 26]. Preliminary experiments with rat liver had shown that extracts prepared as indicated had specific activities of all three enzymes greater than or equal to extracts prepared by the original methods.
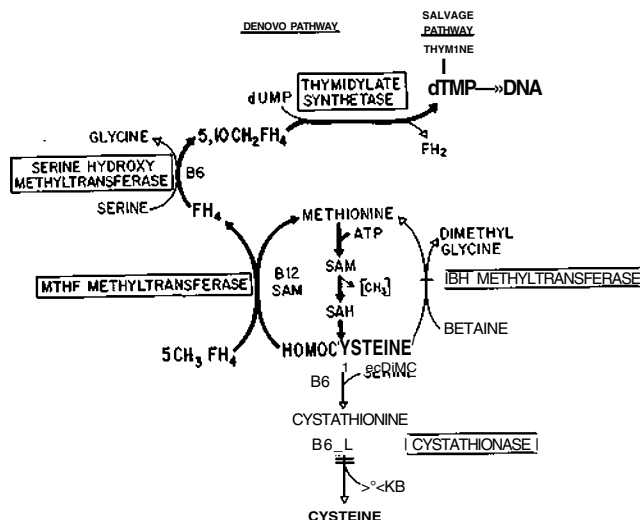
The assays for MTHF-methyltransferase and

STHF-methyltransferase are rate-limited with regard to one of their substrates, $2V^B$-methyltetrahydrofolate and serine, respectively. Therefore, control experiments were carried out in the monkey to be certain that the differences in specific activity were not due to differences in substrate concentration caused simply by different endogenous concentrations of these compounds. Monkey fetal organs showed the same relative specific activities of cystathionase and the methyltransferases as in the human fetus [12, 18]. Because we could obtain ample liver from the fetus and its own mother, unknown and uncontrollable variables such as nutrition and postmortem interval would be eliminated. The differences reported below for the human fetus were found in the monkey fetus even when saturating amounts of each substrate were employed. Furthermore, the difference in activity in all three methyltransferases between fetal and mature tissues was not due to differences in pH optima.

Results

Enzymatic Activities in Fetal Organs

The most striking finding (Table I) is that the specific activity of MTHF-methyltransferase was considerably higher in fetal liver, brain, and kidney than in the corresponding mature tissue: liver was fourfold higher; kidney was an order of magnitude higher; and, during the period studied, there was a significant correlation of decreasing specific activity of this enzyme in brain with increasing crown-rump length (r = —0.72; $P < 0.005$). This resulted in a threefold decrease to the specific activity of adult brain (Fig. 2).

In contrast, BH-methyltransferase was considerably lower in the same tissue; liver was fourfold lower; brain was twofold lower; and, during the period studied, there was a highly significant correlation of increasing specific activity in the kidney with increasing crown-rump length (r = 0.80; $P < 0.005$) toward, albeit not reaching the mean specific activity of mature kidney (Fig. 3).

There were no significant differences between the specific activities of STHF-methyltransferase in fetal and mature liver and kidney. The variance of the mean activities in these two tissues was great, somewhat more in the mature tissues than in the fetal tissues. The variance in mature brain was great as well, but fetal brain showed a significant correlation of decreasing specific activity of this enzyme with increasing crown-rump length (r = —0.69, $P < 0.005$) during the

Table I. Methyltransferase and cystathionase activities of human tissues[1]

	MTHF-methyl-transferase	BH-methyl-transferase	STHF-methyl-transferase	Cystathionase
Mature liver (17)	1.30 ± 0.16	7.78 ± 1.89	122 ± 20	126 ± 12[2]
Fetal liver (31)	4.70 ± 0.203	1.82 ± 0.21'	99 ± 9	0[2]
Neonatal liver				
680 g;1 day[4]	1.71	7.96	32	43
780 g; 1 day	5.09	5.55	69	0
1,650 g; 9 days	1.60	6.24	237	86
1,780 g; 3 days	1.33	5.99	52	40
2,250 g; 1 day	2.12	9.03	93	17
2,600 g; 6 days	1.99	9.33	192	43
2,950 g; 1 day	1.78	11.04	134	37
4,000 g;4 days	2.54	11.10	184	36
Mature brain (6)	1.37 ± 0.25	0.37 ± 0.07	7.38 ± 4.33	33 ± 6[5]
Fetal brain (28)	(cf. Fig.2)[6]	0.20 ± 0.053	(cf. Fig.4)[6]	0[2]
Mature kidney (6)	0.76 ± 0.18	22.6 ± 2.0	342 ± 166	32 ± 5[7]
Fetal kidney (18)	7.26 ± 0.233	(cf. Fig. 3)6	51.3 ± 5.7	21 ± 5[8]

[1] All values are nanomoles of product formed per milligram of soluble protein per hour ± SE. The numbers in parenthesis indicate numbers of individual samples. MTHF: Methyltetrahydrofolate; BH: betaine-homocysteine; STHF: L-serine-tetrahydrofolate 5,10-hydroxymethyltransferase.
[2] Values taken from References 10, 19.
[3] $P < 0.001$ by Student's unpaired t test when compared with the mature tissues.
[4] Birth weight of infant; age at death.
[5] Seven samples; includes one additional value for occipital lobe previously published [10, 19].
[6] Significant correlation.
[7] Eight samples; includes two additional values for mature kidney previously published, [20].
[8] Six samples; $0.01 < P < 0.05$ by Student's unpaired t test when compared with mature kidney.

period studied (Fig. 4). This resulted in a threefold decrease to the specific activity of mature brain by the end of this period.

Human fetal kidney had not previously been examined for specific activity of cystathionase, but 3rd trimester fetal monkey kidney showed a mean specific activity of cystathionase equal to or greater than that of mature monkey kidney [12, 18]. Furthermore, human fetal kidney resembled human fetal liver and brain in
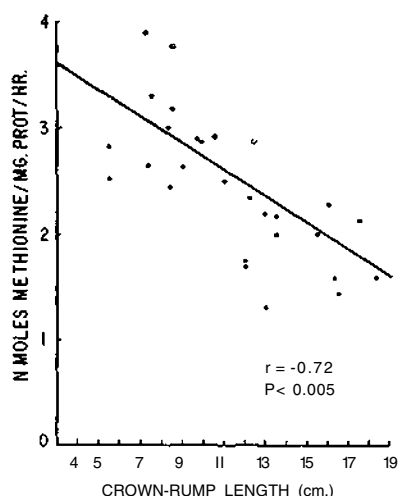
Fig. 2. iV^B-methyHetrahydrofolate-homocysteine methyltransferase activity in human fetal brain. *PROT:* protein.
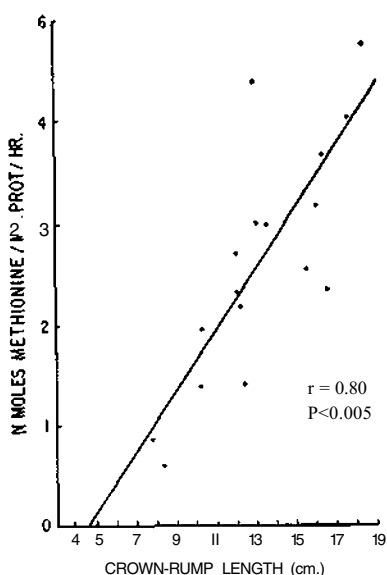

Fig. 3. Betaine-homocysteine methyltransferase activity in human fetal kidney. *PROT:* protein.

that the mean specific activity of fetal kidney MTHF-methyltransferase was higher than that of mature kidney (Table I). It differed from fetal liver and brain in that the specific activity of BH-methyltransferase was already increasing toward that of mature kidney (Fig. 3). It is of considerable interest, therefore, that 2nd trimester human fetal kidney also differs from 2nd trimester human fetal liver and brain in having a mean specific activity of cystathionase that is two-thirds that of mature kidney (Table I).

Enzymatic Activities in Neonatal Liver

Our previous work indicated that the emergence of cystathionase was a postnatal phenomenon [10, 19]. Thus, liver extracts from newly born infants were examined for the specific activity of these three methyltransferases, as well as for cystathionase (Table I). Liver extracts from all of the neonates, but one, had easily measurable activities of cystathionase, although only one of these approached the mean activity found in mature liver. The specific activities of MTHF-methyltransferase in all of the neonatal liver extracts, but one, ranged from twofold higher to the mean activity of mature liver. The liver extract with the highest specific activity of cystathionase had the lowest specific activity of MTHF-methyltransferase. The one neonatal liver extract that had no measurable cystathionase activity had the highest specific activity of MTHF-methyltransferase, a specific activity similar to that found in fetal liver.

Extracts of liver from all of the neonates had specific activities of BH-methyltransferase which were in the range of those of mature liver. Extracts of liver from only two infants had specific activities of STHF-methyltransferase which were low. Thus, it appears that adult values of activity of BH- and STHF-methyltransferases are attained before MTHF-methyltransferase activity in human liver, most likely during the last trimester of gestation, but possibly during the 1st postnatal day. The MTHF-methyltransferase appears to attain adult values of activity in the immediate postnatal period, but before cystathionase. These results do not eliminate the possibility that adult values for the
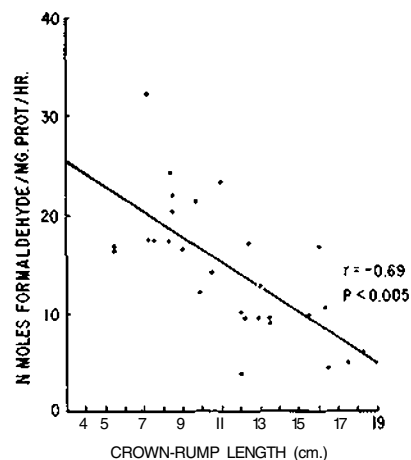

Fig. 4. Serine-tetrahydrofolate 5,10-hydroxymethyltransferase activity in human fetal brain. *PROT:* protein.

specific activity of MTHF-methyltransferase may be attained during the 3rd trimester of gestation.

Discussion

The fact that homocyst(e)ine does not accumulate in fetal tissues in the absence of cystathionase is one of the reasons we investigated the related methyltransferases. There are four enzymes competing for homocysteine (Fig. 1): cystathionine synthase, MTHF-methyltransferase, BH-methyltransferase, and 5-adenosylhomocysteine hydrolase (EC. 3.3.1.1). The last is readily reversible, although the equilibrium is in favor of synthesis of S-adenosylhomocysteine [4]. This is probably one reason why homocysteine is not measurable in tissues, even in liver extracts from two patients with cystathionine synthase deficiency [12]. The K_m for cystathionine synthase is 1.2×10^{-3}, whereas that of MTHF-methyltransferase is 6×10^{-8}, and that of BH-methyltransferase is 4×10^{-5} [5]. Thus, the methyltransferases would be likely to compete for the limited free homocysteine against cystathionine synthase rather effectively on the basis of their low K_m values, and S-adenosylhomocysteine hydrolase would be readily pulled in the direction of cleavage rather than of synthesis.

Consideration of relative K_m values and competition for homocysteine would offer an explanation of why homocysteine does not accumulate when cystathionase is absent. It alone does not provide explanation of our earlier striking finding that there is less cystathionine in fetal human brain than in mature human brain [10, 19], despite the fact that cystathionase, the first enzyme for its further degradation, is virtually absent from fetal brain. A lower concentration of cystathionase in fetal brain than in adult brain has also been reported for Rhesus monkey [18]. Cystathionine synthase has been considered essentially unidirectional, and with cystathionase absent one might expect more, rather than less, cystathionine in fetal brain. However, at least one assay for cystathionine synthase involves the use of cystathionine as substrate and measures the production of homocysteine trapped as its 5,5'-dithiobis[(l-nitrobenzoic)]acid derivative [3]. Thus, it is reversible, at least when homocysteine is constantly removed. Under the circumstances in which cystathionase is absent, cystathionine synthase is at least partially reversible, and homocysteine is constantly removed, the concentration of cystathionine in fetal brain would be less than in adult brain; *i.e.,* the concentrations of cystathionine in brain is a function of the activity of

MTHF-methyltransferase, as well as of the activity of cystathionase.

The control experiments with increasing concentrations of substrate which were carried out in extracts of liver from monkey established that the differences in specific activity of MTHF-methyltransferase between fetal and maternal liver were not spurious differences in activity due to the use of an enzymatic assay rate-limited with regard to substrate. They also showed that there was a difference in the concentration of substrate required for saturation [21]. The MTHF-methyltransferase from fetal liver required more MTHF for full saturation; however, at any particular concentration of MTHF, the fetal extract showed a higher specific activity. This interesting difference will be explored further on purified extracts.

Since the specific activity of BH methyltransferase is lower in fetal than in mature tissues, the enzymatic potential is thereby provided for serine to be shunted in the direction of the folate-B_{12} remethylation pathway with eventual incorporation of its *f3* carbon into thymidylate and production of glycine by the residuum. Further support for this hypothesis comes from fragmentary evidence that two other enzymes important in the *de novo* synthesis of thymidylate, dihydrofolate reductase [15] and thymidylate synthase [16], are higher in fetal liver than in mature liver. However, proof of this hypothesis must await direct evidence of the metabolic flux.

As a corollary, when cystathionase activity appears, the entire carbon skeleton of serine could be converted to cysteine. Cystathionine is cleaved to cysteine and a-ketobutyrate. Since the latter is readily metabolized, cystathionase is pulled in the direction of cysteine. Furthermore, cystathionase is reversed only with great difficulty; it requires amounts of homoserine likely to be available only under conditions of homoserine loading, and it will not reverse at all with «-ketobutyrate as cosubstrate with cysteine [11].

In the fetal kidney there is easily measurable activity of cystathionase, and it is possible that during fetal development, the kidney partly subserves the transsulfuration function of the liver and brain in postnatal life. However, even in the adult, the activity of cystathionase in kidney reaches only one-quarter that in liver (Table I). Studies with ^{35}S-methionine in the pregnant Rhesus monkey [18] do not lend support to the idea that the kidney can subserve the transsulfuration function of the liver in the fetus. It is of interest that the kidney, when mature, is an organ which incor-

porates relatively large amounts of ^{35}S from ^{35}S-methionine and ^{3s}S-cystine into cystine [17, 18].

Comparison of these studies in the human fetus with our studies in the monkey fetus [18], with similar studies in the immature rat [7], and with studies in the immature mouse [2] further highlights the differences between species and the importance of studying the tissues of the previable human fetus. Some differences between species may be due to variations in maturity at birth: STHF-methyltransferase decreases during the postnatal period in the brain of the mouse [2] and during the 2nd trimester in the brain of the human fetus (Fig. 4), which would be corresponding periods of rapid growth in each [6]. The specific activity of MTHF-methyltransferase in rat liver decreases gradually during the postnatal period [7], whereas most of the human neonatal livers studied by us had already attained the specific activity found in adult liver (Table I). Other differences may be due to intrinsic variations between species: BH-methyltransferase in the liver of the rat decreases slightly as the animal matures [7], whereas there is a striking increase in the human (Table I). There is considerably more BH-methyltransferase in the mature human kidney than in the mature human liver (Table I), which is in striking contrast to the rat, which has very little activity in the kidney [7, 23].

The high activities of the B_{12}-linked MTHF methyltransferase in human fetal brain suggests that, in the inherited deficiency of MTHF-methyltransferase because of failure of activation of vitamin B_{12} to its coenzyme form [12], severe impairment of brain development may well occur early because of a slow rate of synthesis of DNA during periods of rapid neuronal multiplication. Indeed, one wonders how this defect is compatible with survival, unless there is an MTHF-methyltransferase pathway in human fetal brain which is not B_{12} dependent, such as may exist in mitochondria from rat liver [24]. Alternatively, despite the fact that MTHF is the major folate in maternal blood, there may be sufficient placental transfer of THF and its other derivatives from the mother to circumvent the block in synthesis of THF by the affected fetus until after birth.

Summary

Activity of $2V^5$-methyltetrahydrofolate-homocysteine methyltransferase was higher in liver, brain, and kidney of the human fetus (2nd trimester) than in the same organs from the mature human. The reverse was true for the activity of betaine-homocysteine methyltransferase. Activity of serine tetrahydrofolate 5,10-hydroxymethyltransferase in liver and kidney at this period of fetal development was not different from that in the mature human; however, this activity was higher in fetal brain and decreased to the mean activity in the brain of the mature human during the 2nd trimester. These methyltransferases appear to reach mature activity by the early neonatal period and somewhat earlier than cystathionase.

The development of cystathionase in human fetal kidney is different from its development in human fetal liver and brain in that it is not absent and has attained two-thirds of the mean specific activity found in adult kidney.

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25. Cystathionase has been used rather than L-homoserine hydrolyase (deaminating) EC. 4.2.1.15, since it is the transsulfuration function we are studying.

26. 5-(Methyl-ᵘC)-tetrahydrofolate and 3-ᴹC-L-serine were obtained from Amersham/Searle Corporation, Arlington Heights, 1ll.

27. (Methyl-"C)-betaine was obtained from New England Nuclear, Boston, Mass.