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# Differences in Liver Folate Enzyme Patterns in Premature and Full Term Infants

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## Summary

**The specific activities of four folate enzymes have been measured in livers from preterm infants (Group 1), full-term infants (Group 2), and from control subjects (Group 3). The four enzymes studied were methylenetetrahydrofolate reductase (EC 1.1.1.68), methionine synthetase (EC 2.1.1.13), methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5), and glutamate formiminotransferase (EC 2.1.2.5). The specific activities for methylenetetrahydrofolate reductase were 6.62 ± 0.51, 4.42 ± 0.31, and 2.60 ± 0.40 (nmoles formaldehyde/mg protein/h, mean ± S.E.) for groups 1, 2 and 3, respectively.**

**The specific activities for the three groups for methionine synthetase were 0.99 ± 0.11, 0.64 ± 0.06, and 0.42 ± 0.05 (nmoles methionine/mg protein/h, mean ± S.E.). The specific activities for the three groups for glutamate formiminotransferase were 84.1 ± 10.7, 108.6 ± 14.6, and 104.3 ± 17.8 (nmoles methenyltetrahydrofolate/mg protein/min, mean ± S.E.). The specific activities for the three groups for methylenetetrahydrofolate dehydrogenase were 0.16 ± 0.03, 0.39 ± 0.07, and 0.92 ± 0.16 (nmoles methenyltetrahydrofolate/mg protein/min, mean ± S.E.). During development, the specific activities of methylenetetrahydrofolate reductase and methionine synthetase decreased whereas the specific activity of methylenetetrahydrofolate dehydrogenase increased and that of glutamate formiminotransferase remained constant. In addition, the activities of methylenetetrahydrofolate reductase, methionine synthetase, and methylenetetrahydrofolate dehydrogenase were significantly influenced by postnatal age.**

## Speculation

**Deficiency of two of the folate enzymes studied, methylenetetrahydrofolate reductase and glutamate formiminotransferase, are associated with genetic disorders. Knowledge of the developmental patterns of the folate enzymes in human liver may have important implications in the diagnosis and early treatment of these inborn errors of metabolism.**

Reduced folates act as carriers of one-carbon units, which may be derived from serine, glycine, histidine, or from formate (6). These one-carbon units can be used in the synthesis of purines, pyrimidines and methionine. To date, deficiencies of five folate enzymes have been linked to inborn errors of metabolism. The enzymes are dihydrofolate reductase (EC 1.5.1.3), methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9), methylenetetrahydrofolate reductase (EC 1.1.1.68), methionine synthetase (EC 2.1.1.13), and glutamate formiminotransferase (EC 2.1.2.5) (2). At present, only two of these five enzymes have been demonstrated nonequivocally to be associated with inborn errors of folate metabolism: methylenetetrahydrofolate reductase and glutamate formiminotransferase (2).

Although at least one patient with severe methylenetetrahydrofolate reductase deficiency has responded to combined vitamin and methionine therapy (4), success with therapy of severely affected patients has not been universal (9); thus, the possibility exists that prenatal diagnosis of a deficiency of this enzyme may be important.

The present study attempts to examine the development of activity in preterm and full-term infants during the first year of life. The four folate enzymes studied are methylenetetrahydrofolate reductase, methionine synthetase, glutamate formiminotransferase, and methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5).

## MATERIALS AND METHODS

Liver samples were obtained during postmortem examination of infants free of liver disease, as previously described (15). Consent for each postmortem examination was received before autopsy. Samples from autopsies completed within 48 h of death were collected and immediately frozen. Samples were prepared and analyzed for cystathionase activity as previously reported (15). The remaining aliquots of liver were shipped frozen to Montreal where they were stored at −70°C until preparation for

Table 1. *Characteristics of each group of subjects*

| | Methylenetetrahydrofolate reductase and methionine synthetase | | | Cystathionase | | | Methylenetetrahydrofolate dehydrogenase and glutamate formiminotransferase | | |
|---|---|---|---|---|---|---|---|---|---|
| Group number | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Number of subjects | 22 | 36 | 10 | 24 | 36 | 13 | 16 | 19 | 8 |
| Gestational age (wk) | | | | | | | | | |
| Range | 23–35 | 37–41 | | 23–36 | 37–41 | | 26–35 | 37–41 | |
| Mean | 29.6 ± 0.7[1] | 39.5 ± 0.2 | | 29.9 ± 0.7 | 39.5 ± 0.2 | | 29.9 ± 0.7 | 39.3 ± 0.2 | |
| Postnatal age (days) | | | | | | | | | |
| Range | 0.2–66 | 0.2–300 | 420–3468 | 0.2–66 | 0.2–300 | 420–6570 | 0.4–66 | 0.5–240 | 420–3468 |
| Mean | 6.8 ± 3.1 | 55.9 ± 12.1 | 2170 ± 370 | 6.7 ± 2.8 | 51.4 ± 11.8 | 2620 ± 450 | 6.6 ± 4.0 | 53.5 ± 15.2 | 1900 ± 410 |

examination of the activity of the four folate enzymes. Cystathionase values for these samples have been previously reported (15) and serve for comparison with the four enzymes in this study.

For assay of the folate enzymes, liver samples (about 0.5 g) were homogenized with 3 volumes of 0.25 M sucrose and the homogenate centrifuged at 1085 × *g* for 10 min. The supernatant was next sonicated for 90 sec (6 × 15 sec) on ice with an ultrasonic disintegrator (16) and then centrifuged for 1 h at 27,000 × *g*. The supernatant was removed and frozen in aliquots at −20°C.

Methylenetetrahydrofolate dehydrogenase was assayed according to the method of Tan *et al.* (14). Glutamate formiminotransferase was assayed according to the method of Drury and MacKenzie (1). Methionine synthetase was assayed according to the method of Kamely *et al.* (5). Methylenetetrahydrofolate reductase was assayed as previously described (13). Protein concentrations were determined by the method of Lowry *et al.* (7) using human serum albumin as a standard.

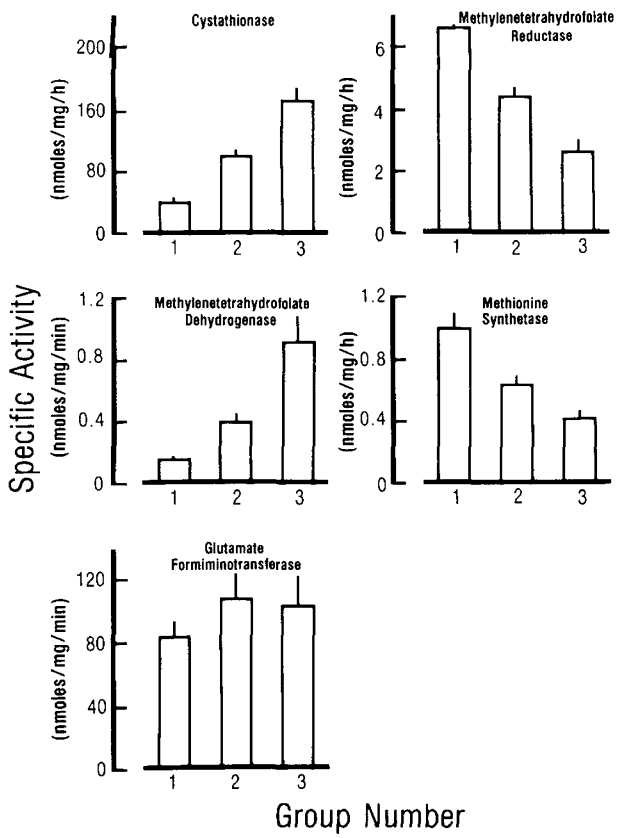


Fig. 1. Changes in liver folate enzyme activity during development. Group 1 consists of preterm infants, group 2 full-term infants, and group 3 individuals older than 1 year of age (as described in Table 1). Mean values are represented by the height of the bars and vertical lines indicate the mean ± S.E.

## RESULTS

The specific activities of four folate enzymes, methylenetetrahydrofolate reductase, methionine synthetase, methylenetetrahydrofolate dehydrogenase, and glutamate formiminotransferase, were measured in human liver. Cystathionase activity (15) was used as a control for comparison.

The liver samples were divided into three groups: preterm infants (Group 1), full-term infants (Group 2), and control subjects greater than 1 year of age (Group 3). The characteristics of each group are presented in Table 1. Not all enzymes were assayed in each liver sample since not enough material was available.

Group means (Fig. 1) were determined for each of the four enzyme activities. A one-way analysis of variance followed by Duncan's new multiple-range test were used to test the significance of the difference between the means for each enzyme activity. There was a significant decrease (Table 2) in methylenetetrahydrofolate reductase activity with development whereas methionine synthetase activity decreased significantly only in full-term as compared to preterm infants. Methylenetetrahydrofolate dehydrogenase activity was greater in tissues from full-term than preterm infants and greater still in samples from the control group. The activity of glutamate formiminotransferase did not differ significantly between groups.

The data was analyzed by analysis of variance to determine whether the observed differences could be attributed to lability of the enzymes. The effect of postmortem delay, length of time that the liver tissue had been kept frozen at −70°C, and duration of freezing of the extract at −20°C on group means for each of the four folate enzymes was determined. None of these factors appeared to significantly influence the results ($P > 0.05$).

Using the data for the full-term infants (Group 2), each of the folate enzyme activities was plotted as a function of postnatal age (Fig. 2). Methylenetetrahydrofolate reductase and methionine synthetase activities decreased with postnatal age. Methylenetetrahydrofolate dehydrogenase activity increased with postnatal age whereas glutamate formiminotransferase activity did not vary significantly with postnatal age.

## DISCUSSION

We have thus measured the levels of four folate enzymes in liver tissue from preterm and full-term infants and from a control group. It appeared that during development the level of both methylenetetrahydrofolate reductase and methionine synthetase decreased whereas the activity of methylenetetrahydrofolate de-

Table 2. *Comparison of group means for each enzyme studied*

| | Specific activity | | | | |
|---|---|---|---|---|---|
| | Methylenetetrahydrofolate reductase (nmoles formaldehyde/mg protein/h) | Methionine synthetase (nmoles methionine/mg protein/h) | Cystathionase (nmoles cysteine/mg protein/h) | Glutamate formiminotrasferase (nmoles methenyltetrahydrofolate/mg protein/min) | Methylenetetrahydrofolate dehydrogenase (nmoles methenyltetrahydrofolate/mg protein/min) |
| Group 1 | 6.62 ± 0.51[1] | 0.99 ± 0.11 | 39.6 ± 5.1 | 84.1 ± 10.7 | 0.16 ± 0.03 |
| Group 2 | 4.42 ± 0.31 | 0.64 ± 0.06 | 99.3 ± 8.2 | 108.6 ± 14.6 | 0.39 ± 0.07 |
| Group 3 | 2.60 ± 0.40 | 0.42 ± 0.05 | 171.5 ± 14.4 | 104.3 ± 17.8 | 0.92 ± 0.16 |
| | | | *P* value[2] | | |
| Group 1 *vs.* 2 | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | not significant | |
| Group 2 *vs* 3 | $P < 0.01$ | not significant | $P < 0.01$ | not significant | $P > 0.01$ |
| Group 1 *vs* 3 | $P < 0.01$ | $P < 0.01$ | not significant | $P < 0.01$ | $P < 0.01$ |

[1] Mean ± S.E.

[2] The significance of the differences between group means was tested using a one-way analysis of variance followed by Duncan's new multiple-range test.

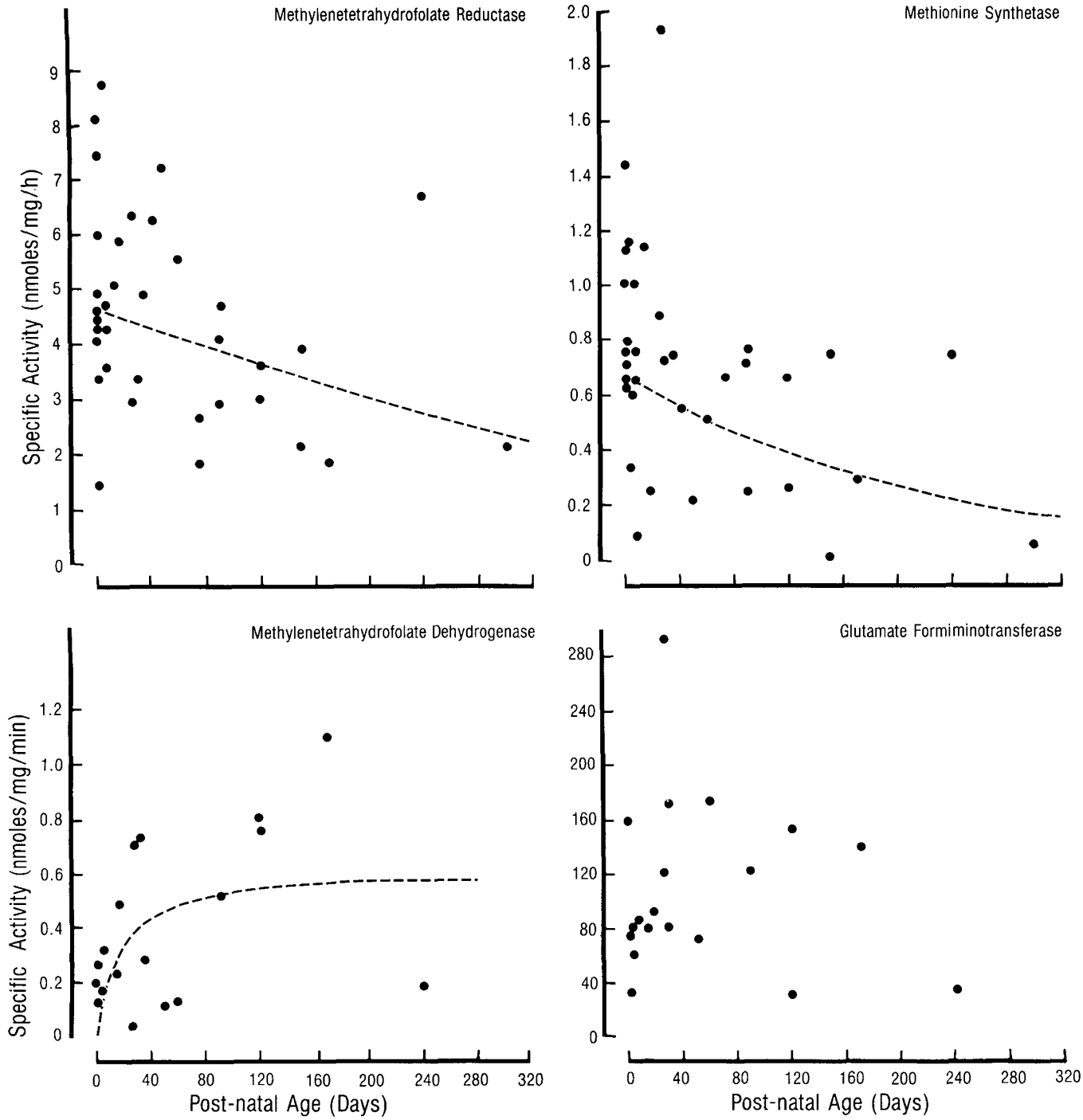


Fig. 2. Influence of postnatal age on liver folate enzyme levels in full-term infants. Each point represents an individual measurement. Where possible, a line has been fitted to the points to relate each enzyme activity to postnatal age. The correlation coefficients for the lines drawn are as follows: methylenetetrahydrofolate reductase, $r = 0.33$, $P < 0.05$; methionine synthetase, $r = 0.43$, $P < 0.01$; and methylenetetrahydrofolate dehydrogenase, $r = 0.51$, $P < 0.05$. There is no correlation ($P > 0.05$) between glutamate formiminotransferase activity and postnatal age.

hydrogenase increased and that of glutamate formiminotransferase remained constant. The differences in activities in the three groups could not be accounted for by postmortem delay or storage time of the livers or of the extracts.

Methylenetetrahydrofolate dehydrogenase has been described in porcine liver as being associated with both methylenetetrahydrofolate cyclohydrolase and formyltetrahydrofolate synthetase in a single polypeptide chain (14). Methylenetetrahydrofolate cyclohydrolase deficiency has been putatively linked to an inborn error of metabolism. Because this enzyme is difficult to measure in extracts of cultured human fibroblasts (2) we chose to measure the activity of the methylenetetrahydrofolate dehydrogenase in our liver extracts. We found the activity of this enzyme, like that of cystathionase (15), to increase during development.

Gaull *et al.* (3) have measured the levels of methionine synthetase in liver and brain from human fetuses. Similar to our results, the enzyme activity was found to be higher in second trimester fetal liver and brain than in mature liver and brain.

More recently, it has been found that the activities of two folate-dependent enzymes involved in thymidine synthesis, dihydrofolate reductase, and thymidylate synthetase, was greatest in the cerebellum of the rat after birth and then decreased (8). A similar trend was observed for methylenetetrahydrofolate reductase activity.

Serine hydroxymethyltransferase activity, also important for *de novo* DNA synthesis, has been found to be increased in fetal brain as compared to mature brain. It does not appear to change in the liver with development (3).

Of the four folate enzymes examined, methylenetetrahydrofolate reductase and glutamate formiminotransferase are associated with known autosomal recessive inborn errors of metabolism (2, 10). Glutamate formiminotransferase deficiency has been described in several families with variable clinical presentation ranging from severe neurologic abnormalities to virtually asymptomatic individuals (2). This enzyme is not present in cultured human fibroblasts making confirmation of the deficiency more difficult (2). Our studies show that glutamate formiminotransferase activity does not vary significantly with development. Methylenetetrahydrofolate reductase deficiency is characterized by homocystinuria and variable neurologic manifestations depending on the severity of the enzyme deficiency (11). At least one patient with severe deficiency has been shown to respond to dietary therapy with folate, vitamins $B_{12}$ and $B_6$, and methionine (4). At least three partially treated patients did not respond to vitamin therapy (12), but methionine supplementation was not attempted (9). The present study demonstrates that the level of methylenetetrahydrofolate reductase along with that of methionine synthetase is relatively high, at least in liver, in premature infants and falls after term. Because of the high activity of the methylenetetrahydrofolate reductase during early development it is possible that early diagnosis and treatment of this disorder is important. As yet, not enough children have been diagnosed early and treated to comment on the long term benefits of dietary and vitamin therapy.

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16. MSE 100 watt Ultrasonic Disintegrator.

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