Pediatr. Res. *16:* 65–68 (1982)

The Development of Cystathionase Activity During the First Year of Life

STANLEY H. ZLOTKIN AND G. HARVEY ANDERSON[16]

Departments of Nutrition and Food Science and Pediatrics, Hospital for Sick Children University of Toronto, Toronto, Ontario M5S 1A8 Canada

Summary

The development of hepatic cystathionase (EC 4.4.1.1) activity is dependent both upon the gestational age of the infant and the postnatal age. Full-term infants are born with greater hepatic cystathionase activity than pre-term infants, and the activity increases rapidly after birth reaching mature levels at about 3 months of age. Prematurely born infants have lower hepatic cystathionase activity at birth and like the full-term, the activity increases after birth. Cystathionase activity is not isolated to the liver. In both premature and full-term infants, it is present in the kidneys and adrenals, but of little significance in the pancreas. These *in vitro* measurements of cystathionase activity indicate that the premature infant is potentially capable of endogenous cysteine production if provided with adequate methionine.

Speculation

We postulate that, if given adequate methionine, the preterm infant may have sufficient cystathionase capacity to produce cysteine in amounts adequate to meet estimated needs.

The preparation for, and adaptation to extrauterine life by newborns requires maturation of biochemical and physiologic functions during fetal development. Thus, premature birth can be expected to result in the preterm infant having different nutrient requirements than term infants. One nutrient of current interest, which has been postulated to be required in the diet of preterm infants, is the amino acid cysteine. In the full-term infant and older human, it is well documented that cysteine is produced by gamma cleavage of cystathionine, with resulting transfer of the sulphur atom from cystathionine to the carbon skeleton of serine in the presence of the pyridoxal-phosphate dependent enzyme cystathionase (Fig. 1) (1). In the second trimester human fetus, enzymatic (4, 12) and immunological studies (9) have found reduced hepatic transsulfuration pathway enzyme activity. In particular, hepatic cystathionase activity was reported to be absent, although some renal cystathionase activity was found in early gestation (5). Thus, it is generally believed that the fetus is primarily dependent upon the maternal placental unit for its supply of cysteine. At birth, with the severing of the placental attachment, maternal substrate is no longer available and the newborn must either convert methionine, via the transsulphuraton pathway to cysteine, or receive cysteine in the diet. From the few assays of livers of premature infants it seems that, like other enzyme systems, the transsulfuration enzymes slowly increase their activity during the first few weeks of life. Supporting the observation of low fetal and premature hepatic cystathionase activity is the finding that the concentration of cystathionine, the substrate for cystathionase, is in high concentration in fetal liver but lower in the liver of older infants who also show increasing cystathionase activity (three infants) (4).

Although the enzyme data are derived from relatively few infants, these results have led to the general view that cysteine is an essential amino acid in the diet of the premature infant. However, premature infants on cysteine-free intravenous diets show excellent nitrogen retention and growth which was not improved by cysteine additions to the formulation (13). Therefore, the purpose of the present investigation was to quantify the development of cystathionase activity in premature and full-term infants during the first year of life and to identify factors affecting its maturation. By measuring cystathionase activity in organs capable of converting methionine to cysteine we suggest that the premature infant has a greater enzymatic capacity to produce cysteine than previously appreciated.

MATERIALS AND METHODS

Between February 1979 and March 1980, seventy-two samples of human liver tissue were obtained during post-mortem examination of infants who died prior to 1 year of age, premature and full term. In addition, during the latter months, samples of kidney, adrenal and pancreas were also obtained. The clinical diagnosis of the infants studied included: prematurity and respiratory distress syndrome (12 infants); prematurity and other complications (5); congenital heart disease (10); other congenital anomalies (8); sudden infant death syndrome (13); birth asphyxia (6); other causes (5). Samples from infants with congenital inborn errors of metabolism were excluded from the collection. The control group consisted of samples from children who died older than one year of age. Their cause of death was unrelated to primary hepatic, pancreatic, renal or adrenal pathology.

Organ samples (200 mg) from autopsies completed within 48 h of death (mean time=25 h) were collected and immediately frozen at −20°C. Within 4 h of collection, samples were added in a 10:1 ratio (ml/g wet weight) to cold 0.03 M potassium phosphate buffer, pH 6.9, and immediately homogenized in a glass grinding chamber using a teflon pestile and a Tri-Rotir-R (model K43) stirring apparatus (Tri-R Instruments, Rockville Center, NY). The homogenates were centrifuged at 5000 × *g* for 7 min and the supernatant fluid either immediately assayed or stored at −70°C. All steps were performed below 4°C. The method of Gaull *et al.* (3) was followed for the measurement of cystathionase activity on duplicate samples. The assay is based on the measurement of cysteine formed from cystathionine under optimal conditions for enzyme activity. The amount of cysteine formed was quantified by the method of Gaitonde (2), but the amount of ninhydrin reagent was doubled in order to increase the intensity of the colour reaction. Recovery of cysteine remained at greater than 95% of control values while doubling the reagent allowed quantitation of results at values as low as 250 μg cysteine/ml. Protein was determined by the method of Lowry *et al.* (8), with human serum albumin as standard. All samples were analyzed for cystathionase activity within 6 months of collection (3).

STATISTICS

Group means were compared using Student's *t* test. In addition, multiple regression analysis was used to determine the relationship

between the predictor variables postnatal age and gestational age, and the dependent variable cystathionase activity (10). The suitability of the linear equation determined by this method was checked using residual analysis, quadratic transformation of the predictor variable and logarithmic transformation of the dependent variable. By implementing the procedures described above, it was determined that a nonlinear model was most appropriate to mathematically describe developing cystathionase activity. Differences in results with a $P < 0.01$ were considered to be statistically significant.

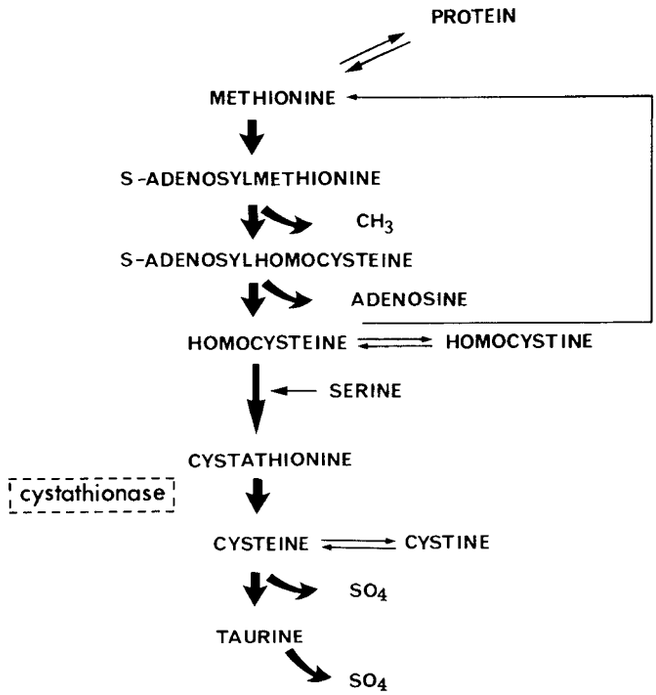

Fig. 1. The transsulfuration pathway

Table 1. *Clinical data*

Number of subjects	59
Gestational age (wks)	36 ± 1[1]
Postnatal age (days)	36 ± 8
Birth Weight (g)	2375 ± 177
Death Weight (g)	3228 ± 270
Sex	
male	41
female	18
Feeding	
iv glucose only	29
some oral	8
well fed	22
Hepatic cystathionase activity (HCA) (nmoles cysteine/mg protein/h)	80.3 ± 6.7
Control HCA[2] (n = 13) (nmoles cysteine/mg protein/h)	171.5 ± 14.5

[1] Mean ± S.E.

[2] All control subjects died from accidents and were older than 1 year of age.

RESULTS

Gestational age, postnatal age, birth and death weight, sex, feeding history and hepatic cystathionase activity (HCA) are presented in Table 1. Cystathionase activity was detected in all but one of the liver samples analyzed. The one liver with zero activity was neither the youngest gestational age nor postnatal age infant (gestational age = 35 wk, postnatal age = 9 days). The sample with the greatest activity (209 nmoles cysteine/mg protein/h) was from a previously well, full-term female child who died at 310 days of age (our oldest study sample). The sex of the infants in the study group were not evenly distributed since, for unknown reasons, more male than female children had post-mortem examinations during the study period. However, sex was not statistically related to HCA. The mode of feeding was also unevenly distributed among the study population, because those who died during the first few days of life fed poorly (and were often premature) and those who died at an older age were generally well fed. Therefore, although feeding was statistically related to HCA, this relationship was due to the highly significant correlation between feeding and gestational and postnatal age ($r = .76$, $P < 0.01$). Because of the correlations just described, the effect of feeding alone on the development of enzyme activity could not be determined. Although the time interval after death at which the autopsy was performed varied between 5–48 h, the results showed no effect of this variable (multiple regression analysis).

The most influential factors affecting the development of HCA were weight at death ($r = .73$, $P < 0.01$), postnatal age ($r = .66$, $P < 0.01$) and gestational age ($r = .61$, $P < 0.01$). This analysis was complicated, however, by the significant interrelationships between postnatal and gestational age and weight at death (older infants tended to be more gestationally mature and to weigh more); and between gestational age and postnatal age (infants who died at an older age were usually full-term). In order to disentangle the effects of the three variables on HCA, multiple regression analysis was completed. This showed that the combination of gestational age and postnatal age were the two most important factors influencing HCA (multiple $r = .75$, $P < 0.01$). Therefore the data were further analyzed to document the effects of postnatal and gestational age on HCA.

HEPATIC CYSTATHIONASE ACTIVITY IN PRE-TERM INFANTS

Sixteen of the 20 premature infants sampled were less than 32 wk gestation at birth and all were less than 2 wk of age at the time of death (Table 2). The mean HCA was 40.1 nmoles cysteine/mg protein/h, which was 23% of the control value (control mean HCA=171.5). Although postnatal age affected the development of HCA ($r = .46$, $P < 0.05$), only 2 premature infants were older than 7 days of age when they died, therefore, comment must be withheld on the later postnatal development of HCA in the premature infant. Neither gestational age (24–36 wk) nor birthweight affected HCA. Six infants died in the first day of life. Their mean HCA was 25.6 nmoles cysteine/mg protein/h (range 0–59.9) which was 15% of the control value.

Table 2. *Hepatic cystathionase activity in premature and full-term infants during the first 14 days of life*

	Gestational age (wk)	Postnatal age (d)	Birth weight (g)	Liver weight (g)	Hepatic cystathionase (nmoles cysteine/mg protein/h)	% of control activity[3] (%)
Premature (20)	30 ± 1[1]	3 ± 1	1420 ± 145	49	40.1 ± 5.3[2]	23.4 ± 3.1[2]
Full-term (18)	39 ± 0	4 ± 1	3296 ± 206	118	78.1 ± 10.2	45.7 ± 6.0

[1] Mean ± S.E.

[2] Full-term > premature, $P < 0.01$.

[3] Control hepatic-cystathionase activity = 171.5 ± 14.5 nmoles cysteine/mg protein/h.

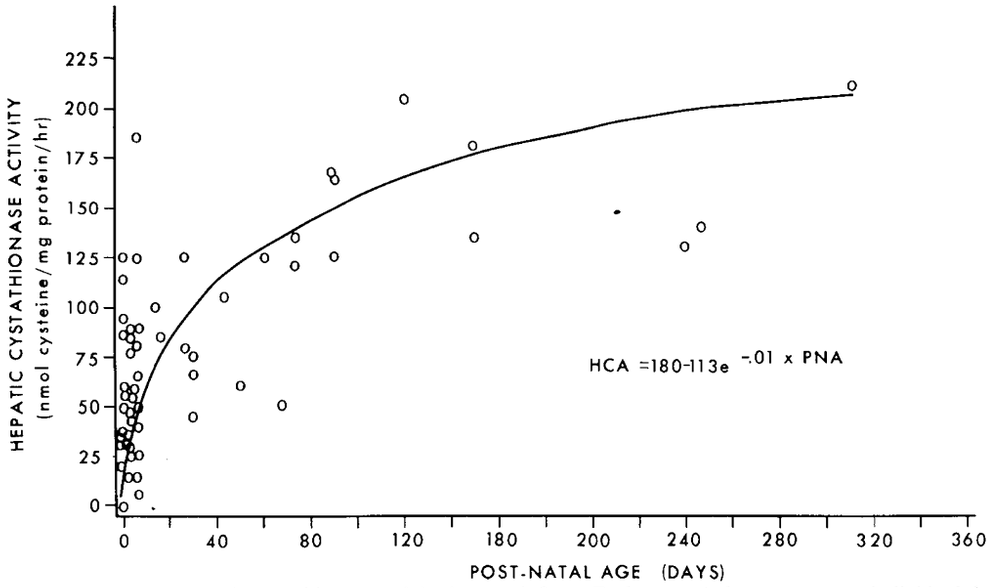

Fig. 2. The effect of postnatal age on hepatic cystathionase activity in term infants. Each open circle represents an individual data measurement.

HEPATIC CYSTATHIONASE ACTIVITY IN FULL-TERM INFANTS

In the first 2 wk of life, mean HCA was 78.1 nmoles cysteine/mg protein/h (18 infants), while for those who died in the first day of life (5 infants), the mean HCA was 85.7. During the first year of life, there was a significant positive correlation between increasing postnatal age and HCA ($r = .64$, $P < 0.01$). By plotting the graph of postnatal age versus HCA, an exponential curve results (Fig. 2). The control HCA value of 171 nmoles cysteine/mg protein/h was achieved by 240 days, and 50% of control activity was achieved by 28 days of age. A significant but weaker correlation between increasing gestational age (37–42 wk) and HCA was also observed ($r = .49$, $P < 0.01$).

OTHER FACTORS AFFECTING HEPATIC CYSTATHIONASE ACTIVITY

The mothers of five prematurely born infants in the study received corticosteroids at or just prior to the delivery of their infants. In addition, seven other infants received corticosteroids as a therapeutic medication at some point prior to their death. Since it has been shown *in vitro* that corticosteroids induce cystathionase activity (7), the results from these twelve infants were dependently analyzed by group t test (Table 3). Maternal medication had no effect on the development of cystathionase activity; however, infants who received corticosteroids tended to have greater enzyme activity compared to matched controls, although the results are not statistically significant. The greatest HCA in a premature infant (90 nmoles cysteine/mg protein/h), which was 40% higher than the next closest value, was observed in the only infant who received total parenteral nutrition with added cysteine hydrochloride. This value was excluded from the premature group when statistical analyses were performed.

CYSTATHIONASE ACTIVITY IN KIDNEY, ADRENAL AND PANCREAS

Ten kidneys, twelve adrenals and nine pancreases were analyzed for cystathionase activity (Table 4). Adrenal cystathionase showed the greatest activity, while renal cystathionase also showed high levels of activity. Pancreatic activity was the lowest of the organs studied. Enzyme activity in the three organs did not correlate with either gestational or postnatal age.

DISCUSSION

Cystathionase activity in liver is dependent on both gestational age and postnatal age. In addition, this study shows that kidney and adrenals have considerable activity which does not change with gestational or postnatal age.

Table 3. *In vivo effect of corticosteroids on hepatic cystathionase activity*

	Gestational age	Postnatal age	Hepatic cystathionase activity
	(wk)	(days)	(nmoles cysteine/mg protein/h)
Infants receiving corticosteroids ($n = 7$)	38.1 ± 0.4[1]	3.0 ± 1.0	83.2 ± 20.1
Matched controls[2] ($n = 14$)	38.6 ± 0.5	3.4 ± 1.0	64.6 ± 11.1
Infants of mothers receiving corticosteroids ($n = 5$)	28.8 ± 1.0	3.8 ± 1.0	37.0 ± 9.3
Matched controls ($n = 9$)	28.1 ± 0.9	3.6 ± 0.5	38.1 ± 6.3

[1] Mean ± S.E.
[2] Controls matched for gestational and postnatal age.

Table 4. *Cystathionase activity in kidney, adrenal and pancreas*

Organ	Gestational age	Cystathionase activity
	(wk)	(nmoles cysteine/mg protein/h)
Kidney ($n = 10$)	34.7 ± 1.7	47.3 ± 6.5[1]
Adrenal ($n = 12$)	35.3 ± 1.5	80.3 ± 14.8
Pancreas ($n = 9$)	34.0 ± 1.8	17.6 ± 4.5

[1] Mean ± S.E.

The higher HCA at birth in full-term versus preterm infants is consistent with the observed development of other enzyme systems which become more active with increasing gestational age (*e.g.*, lipid and protein digestive enzymes) (6). Unlike the gradual increase in HCA over the first few months of life that was observed in the full-term infants, there is a more marked increase during the first 2 wk of life in the premature infant.

The present investigation shows cystathionase activity in the kidney and especially the adrenals, with low or zero activity in the majority of pancreases analyzed. It has been shown previously that the human fetal kidney has achieved two-thirds of adult levels by the second trimester (5). However activity in pancreases or adrenals has not been previously determined in man (11). Although the number of samples analyzed was few, the kidney and adrenal samples did not show the same developmental re-

sponse to gestational and postnatal age as was demonstrated for the hepatic samples.

The practical importance of documenting the development of the transsulfuration enzyme pathway relates to decisions regarding the optimal nutrient composition of infant diets, especially formulations for parenteral feeding. Presently, intravenous formulations are based primarily on adult needs and most amino acid solutions are cysteine–free because of its low solubility (14). The present data show that cystathionase activity is considerably greater than previously appreciated, and if adequate methionine is present, the absence of cysteine may not be of concern. Furthermore this high endogenous cystathionase activity would explain the observation that infants fed intravenous formulations free of cysteine but adequate in methionine demonstrate nitrogen retention and weight change comparable to cysteine supplemented infants and similar to rates observed *in utero* (13, 14, 15).

REFERENCES AND NOTES

1. Albanese, A. A., Holt, L. E., Jr., Brumback, J. E., Jr., Frankston, J. E., and Irby, V.: Observations on a diet deficient in both methionine and cysteine in man. Bull. Johns Hopkins Hospt., *74:* 308 (1944).
2. Gaitonde, M. K.: A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem. J., *104:* 627 (1967).
3. Gaull, G. E., Rassin, D. K., and Sturman, J. A.: Enzymatic and metabolic studies of homocystinemia: effects of pyridoxine. Neuropadiatre, *1:* 199 (1969).
4. Gaull, G. E., Von Berg, W., and Raiha, N. C. R.: Development of mammalian sulfur metabolism. Absence of cystathionase in human fetal tissues. Pediatr. Res., *6:* 538 (1972).
5. Gaull, G. E., Von Berg, W., Raiha, N. C. R., and Sturman, J. A.: Development of methyltransferase activity of human fetal tissues. Pediatr. Res., *7:* 527 (1973).
6. Greengard, O.: Enzymatic differentiation in mammalian liver. Science, *63:* 891 (1969).
7. Heinonen, K., and Raiha, W. R.: Induction of cystathionase in human foetal liver. Biochem. J., *144:* 607 (1974).
8. Lowry, O. H., Rosebrough, N. J., Fan, A. L., and Randell, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem., *193:* 265 (1951).
9. Pascal, T. A., Gillam, B. M., and Gaull, G. E.: Cystathionase: immunochemical evidence for absence from human fetal liver. Pediatr. Res., *6:* 773 (1972).
10. Statistical Analysis Systems Users Guide. General Linear Models/reference. (SAS Institute, Raleigh, 1979).
11. Sturman, J. A., Rasin, D. K., and Gaull, G. E.: Distribution of transsulfuration enzymes in various organs and species. Int. J. Biochem., *1:* 251 (1970).
12. Sturman, J. A., Gaull, G., and Raiha, N. C. R.: Absence of cystathionase in human fetal liver; is cysteine essential? Science, *169:* 74 (1970).
13. Zlotkin, S. H., Bryan, M. H., and Anderson, G. H.: Cysteine supplementation to cysteine-free intravenous feeding regimens in newborn infants. Am. J. Clin. Nutr., *34:* 914 (1981).
14. Zlotkin, S. H., and Anderson, G. H.: Amino acid requirements and sources during total parenteral nutrition. In: Nutrition in Clinical Surgery, Eds. M. Dietel and M. V. Kaminski, pp. 75–82 (Wilkins and Wilkins Co., Baltimore, MD, 1980).
15. Zlotkin, S. H., Bryan, M. H., and Anderson, G. H.: Intravenous nitrogen and energy intakes required to duplicate in utero nitrogen accretion in prematurely born infants. J. Pediatr., *99:* 115 (1981).

16. Address reprint requests to: Dr. G. Harvey Anderson, Department of Nutrition and Food Science, Fitzgerald Building, University of Toronto, Toronto, Ontario M5S IA8.
17. The work presented was conducted by Dr. Zlotkin in partial fulfillment of the requirements of the Ph.D. degree. Current Address: Division of Clinical Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, M5G IX8.
18. This research was supported by grants from the Medical Research Council of Canada and Abbott Laboratories, Montreal, Quebec.
19. Received for publication March 16, 1981.
20. Accepted for publication June 11, 1981.

0031-3998/82/1601-0065$2.00/0

Printed in U. S. A.