Liver enzymes of serine metabolism during neonatal development of the rat

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The developmental patterns of L-serine hydroxymethyltransferase, L-phosphoserine aminotransferase, L-serine aminotransferase and L-serine dehydratase were determined in rat liver. The results point to an increased capacity for serine biosynthesis *de novo* in the perinatal period. It is suggested that serine at this time, and also at weaning, may serve as a precursor, via the serine hydroxymethyltransferase reaction, for nucleotide biosynthesis to support the rapid phases of liver growth. The role of the alternative pathways of serine metabolism during neonatal development is discussed.

Serine, an important non-essential amino acid, may be formed endogenously from glucose in the liver and is then utilized in various pathways for biosynthetic purposes (Scheme 1) (Snell & Walker, 1973; Snell, 1980), as well as for direct incorporation into proteins. One of the most significant roles of serine in cellular metabolism during foetal and postnatal development may be in the provision of precursors and cofactors for nucleotide biosynthesis. The utilization of serine for this metabolic purpose is initiated by L-serine hydroxymethyltransferase (EC 2.1.2.1), which catalyses the transfer of a 'one-carbon unit' from serine to tetrahydrofolate with the formation of glycine and 5,10-methylenetetrahydrofolate. 5,10-Methylene-tetrahydrofolate is used as a carbon donor for thymidylate synthesis, as well as a precursor for other folate cofactors, which provide carbon for the synthesis *de novo* of purine nucleosides (for summary, see Krebs & Hems, 1976). In addition, the glycine formed can serve as a source of carbon and nitrogen for purine synthesis. No information on the neonatal development of serine hydroxymethyltransferase in rat liver has been published, although Sturman *et al.* (1975) have reported an increased incorporation of radioactivity from [3-¹⁴C]serine into DNA by slices from foetal human liver compared with adult human liver.

In the present study the activity of hepatic serine hydroxymethyltransferase was investigated during neonatal development, together with L-phosphoserine-2-oxoglutarate aminotransferase (EC 2.6.1.52) activity (involved in serine synthesis) and

L-serine-pyruvate aminotransferase (EC 2.6.1.51) and L-serine dehydratase (EC 4.2.1.13) activities (involved in alternative pathways of serine metabolism) (Scheme 1).

Experimental

Albino rats of an inbred isogenic Kx strain (New England Deaconess Hospital Breeding colony) were used. Foetal ages were determined from the weights of the embryos at the time of use within confidence limits of ± 0.24 day (Knox & Lister-Rosenoer, 1978). Adult rats were 60–90-day-old males, and immature rats were used without regard to sex. Animals were weaned at 23 days to Purina rat chow and water *ad lib*.

Adult rats were stunned and killed by decapitation with a guillotine; immature rats were killed by decapitation. Livers were rapidly excised, suspended in 9 vol. of ice-cold unbuffered 0.25 M-sucrose and homogenized with a glass Potter homogenizer equipped with a Teflon pestle. Tissue homogenates were diluted with 0.25 M-sucrose as appropriate and Triton X-100 was included to give a final concn. of 0.1%.

Serine hydroxymethyltransferase activity was assayed in 1% (w/v) tissue homogenates by a method based on that of Taylor & Weissbach (1965). L-Tetrahydrofolate was prepared as a 10 mM solution by dissolving in warm 60 mM-mercaptoethanol under an atmosphere of N₂. Solutions were dispensed into sealed 1 ml syringes, stored at -20°C and used within 3 weeks of preparation. Incubations, in a total volume of 0.5 ml, containing 2 mM-L-tetrahydrofolate, 12 mM-mercaptoethanol, 0.2 mM-pyridoxal 5'-phosphate, 60 mM-potassium phosphate buffer (pH 7.5) and tissue homogenate

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Scheme 1. Major pathways of hepatic serine metabolism

1, Glycolysis; 2, phosphoglycerate dehydrogenase (EC 1.1.1.95); 3, phosphoserine aminotransferase (EC 2.6.1.52); 4, phosphoserine phosphatase (EC 3.1.3.3); 5, serine aminotransferase (EC 2.6.1.51); 6, D-glycerate dehydrogenase (EC 1.1.1.29); 7, glycerate kinase (EC 2.7.1.31); 8, gluconeogenesis; 9, serine dehydratase (EC 4.2.1.13); 10, glycogenesis; 11, pyruvate dehydrogenase complex; 12, citric acid cycle; 13, lipogenesis; 14, serine hydroxymethyltransferase (EC 2.1.2.1); 15, purine biosynthesis; 16, thymidylate synthase (EC 2.1.1.45). *: enzymes assayed in the present study.

(final concn. 2 mg of tissue/ml), were kept at 37°C for 5 min and the reaction was initiated by the addition of 20 mM-L-[3-¹⁴C]serine (0.01 mCi/mmol). After incubation for 20 min, the reaction was terminated by the addition of 0.3 ml of 1 M-sodium acetate (pH 4.5). To the tubes were added 0.2 ml of 0.1 M-formaldehyde and 0.3 ml of 0.4 M-dimmedone (5,5-dimethylcyclohexane-1,3-dione) dissolved in 50% (v/v) ethanol, and after being covered they were heated in a boiling-water bath for 5 min. After cooling for 5 min in ice and warming to room

temperature, the radioactive formaldehyde-dimmedone adduct was extracted with toluene by shaking for 10 min. After brief centrifugation in a bench centrifuge at room temperature to separate the aqueous toluene layer, 3 ml of the toluene layer was removed and added to 10 ml of Aquasol for radioactivity counting in a Packard scintillation counter at 75% efficiency. Homogenate-free blanks were subtracted from experimental assay values. A radioactive standard of 0.01 μ Ci (1 μ mol) of L-[3-¹⁴C]serine in 3 ml of toluene was also counted in

10 ml of Aquasol. Enzyme activity is expressed as units (μmol of formaldehyde formed/min) per g of tissue.

L-Phosphoserine aminotransferase activity was assayed in 10% (w/v) tissue homogenates by a method based on that of Walsh & Sallach (1966). Phosphohydroxypyruvic acid was prepared from the tricyclohexylammonium salt of the dimethylketal by the method of Ballou (1960) and neutralized by the addition of solid NaHCO_3 . Incubations, in a total volume of 1.5 ml, containing 2.5 mM-phosphohydroxypyruvate, 10 mM-L-glutamate, 0.01 mM-pyridoxal 5'-phosphate, 45 mM-sodium pyrophosphate buffer (pH 8.2), 36 mM-EDTA and 0.05 mM-mercaptoethanol were kept at 37°C for 2 min and the reaction was initiated by the addition of tissue homogenate (final concn. 40 mg of tissue/ml). Samples were removed from the incubation at time intervals up to 10 min and deproteinized with 20% (v/v) HClO_4 . After neutralization with 0.2 M-KOH, 2-oxoglutarate in the samples was determined enzymically by using glutamate dehydrogenase (Bergmeyer & Bernt, 1963). Blanks (incubations from which L-glutamate was omitted) were subtracted from experimental assay values. Enzyme activity is expressed as units (μmol of 2-oxoglutarate formed/min) per g of tissue.

L-Serine-pyruvate aminotransferase and L-serine dehydratase were assayed at 37°C as described by Snell & Walker (1974), and activities are expressed as units per g of tissue.

L-[3- ^{14}C]Serine (56 mCi/mmol; batch 38) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.) and was free of [^{14}C]glycine as judged by paper chromatography with butan-1-ol/acetone/diethylamine/water (20:20:3:10, by vol.) as the development solvent.

Aquasol was from New England Nuclear Corp. (Boston, MA, U.S.A.). L-Tetrahydrofolate, pyridoxal 5'-phosphate, L-serine, tricyclohexylammonium phosphohydroxypyruvic acid dimethylketal, 3-mercaptoethanol, dimedone and L-glutamate were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); NADH, glutamate dehydrogenase, glyoxylate reductase and sodium pyruvate were from Boehringer-Mannheim Co. (Indianapolis, IN, U.S.A.). Other chemicals were of reagent grade.

Results

L-Serine dehydratase and L-serine-pyruvate aminotransferase activities during development

The pattern of neonatal development of L-serine dehydratase in rat liver was essentially the same as that reported by Snell & Walker (1974) in a Wistar albino rat strain, except that adult activities were about 5-fold higher in the present study. Thus activity was less than 1% of the adult value in foetal (20 days of gestation) liver and, apart from a transient increase in activity on the first day after birth to the adult value, activity until day 15 *post partum* was always less than 30% of that in the adult (Fig. 1a). Activity increased on day 15 to reach about twice the adult value on day 20, and then declined to that of the adult. In contrast, serine aminotransferase activity was present at about 30% of the adult value in late-foetal liver and increased immediately after birth to a value that was about twice that of the adult throughout the suckling period, before declining to the adult value (Fig. 1a). This pattern was essentially the same as that observed previously in a Wistar albino rat strain (Snell & Walker, 1974).

Fig. 1. Developmental formation of enzymes of serine metabolism in rat liver

The activities of (a) L-serine dehydratase (O) and L-serine-pyruvate aminotransferase (●) and (b) L-serine hydroxymethyltransferase (O) and L-phosphoserine-2-oxoglutarate aminotransferase (●) were assayed as described in the Experimental section and are all expressed as units (μmol of product formed/min) per g of fresh tissue. The points indicate the means of five to eight determinations at each age and bars show $\pm$ S.E.M. Abbreviations: B, birth; A, adult, 60–90-day-old male rats.

L-Serine hydroxymethyltransferase and L-phosphoserine-2-oxoglutarate aminotransferase activities during development

Serine hydroxymethyltransferase activity showed a peak in the late-foetal liver, to a value about 50% of that in the adult. Activity declined after birth to a trough in the suckling period, before the rise to the adult value commenced around weaning. Phosphoserine aminotransferase activity showed a peak in the perinatal period (at about twice the adult value), but then declined progressively during postnatal development.

Discussion

The developmental patterns for serine aminotransferase and serine dehydratase (Fig. 1a) are in agreement with previous studies (Rowell *et al.*, 1973; Snell & Walker, 1973, 1974). They are consistent with a negligible role for serine dehydratase in serine metabolism during development until weaning, when the amino acid may be directed towards lipogenesis and glycogenesis or used for oxidative energy metabolism (Scheme 1). On the other hand, the pattern of serine aminotransferase during development suggests that it plays a significant role in directing serine towards hepatic glucose formation during the postnatal suckling period, when gluconeogenesis from serine by this gluconeogenic pathway is increased compared with the adult (Snell, 1974, 1975, 1976). D-Glycerate dehydrogenase, another enzyme involved in the pathway of gluconeogenesis via hydroxypyruvate (Scheme 1), shows a very similar developmental pattern to that observed for serine aminotransferase (Johnson *et al.*, 1964).

The developmental patterns of phosphoserine aminotransferase and serine hydroxymethyltransferase suggest a limited role in serine metabolism during the suckling period, but a more significant role in the foetal-perinatal period (Fig. 1b). Phosphoserine aminotransferase is involved in the pathway of biosynthesis of serine *de novo* from 3-phosphoglycerate produced in glycolysis (Ichihara & Greenberg, 1957; Walsh & Sallach, 1966). Previous developmental studies have shown that both phosphoglycerate dehydrogenase (Johnson *et al.*, 1964) and phosphoserine phosphatase (Jamdar & Greengard, 1969) activities are high in the late-foetal-perinatal period, but decrease rapidly postnatally to the adult value. The pattern of development of phosphoserine aminotransferase in the present study is similar to that of these other enzymes and suggests that the capacity for serine biosynthesis *de novo* is particularly high in late-foetal rat liver compared with the adult (see Scheme 1). In part, this enhanced biosynthetic capacity can be viewed in terms of an increased provision of

serine for incorporation into proteins during the rapid phase of foetal liver growth. However, the fact that such growth is essentially hyperplastic in nature suggests that serine may also be synthesized at this time to provide nucleotide precursors for RNA and DNA formation. The finding of high phosphoserine aminotransferase (Snell & Knox, 1979) and phosphoserine phosphatase (Knox *et al.*, 1969) activities in neoplastic tissues is consistent with this suggestion.

The utilization of serine for nucleotide biosynthesis is initiated by serine hydroxymethyltransferase (Scheme 1). It is noteworthy therefore that the activity of this enzyme shows a peak in late-foetal liver (Fig. 1b). Sturman *et al.* (1975) have reported increased incorporation of [3-¹⁴C]serine into DNA in human foetal liver slices compared with the adult, which is consistent with a foetal elevation of serine hydroxymethyltransferase activity to provide 5,10-methylenetetrahydrofolate for thymidylate synthesis. Elevations in DNA concentration, DNA polymerase activity and [³H]thymidine incorporation into DNA by liver slices are found in perinatal rat liver (Yeoh & Oliver, 1971, 1972). In addition, developmental peaks of activity of enzymes involved in the biosynthesis of pyrimidines and purines *de novo* are also found in the perinatal period (for references, see Snell, 1980). It seems that the perinatal peak of serine hydroxymethyltransferase activity may be associated with the utilization of serine, synthesized *de novo*, for the provision of carbon and nitrogen for increased pyrimidine and purine nucleotide synthesis.

During the last days of gestation there is a decrease in total liver cell number that is due, in large part, to a loss of haematopoietic cells, which constitute a high proportion of the cellular population of foetal-rat liver (Greengard *et al.*, 1972). The decrease in the number of total cells per g in homogenates of liver from this strain of rats (Machovich & Greengard, 1972) was not closely paralleled by the perinatal decline of phosphoserine aminotransferase or serine hydroxymethyltransferase activities seen in the present study. Therefore it seems unlikely that these enzyme activities are involved predominantly in the metabolism of non-parenchymal liver tissue at this age.

The increase in serine hydroxymethyltransferase activity that occurs around weaning (Fig. 1b) may be associated with the generalized increase in the capacity for amino acid oxidation in rat liver at this time (see Snell, 1980, for review). On the other hand, it is known that liver growth in the first 2 weeks after birth is relatively slow, but that at weaning there is a phase of rapid growth involving a 3.5-fold increase in organ weight in 2 weeks (Brosnan *et al.*, 1978). This growth spurt is, in part, due to a phase of hyperplasia and is reflected in a 2.5-fold increase in

total liver DNA and a 50% increase in liver RNA concentration (Brosnan *et al.*, 1978). The developmental increase in serine hydroxymethyltransferase activity over this period could again be associated with its role in providing nucleotide precursors. The provision of serine would be from exogenous dietary sources, since protein intake also increases at weaning (Snell & Walker, 1972), and so there would be no requirement for increased serine biosynthesis *de novo* at this time.

The present study shows shifts in the direction of serine metabolism during neonatal development, as judged by the changing developmental patterns of enzymes involved in alternative metabolic pathways. Serine biosynthesis *de novo* is increased in the late-fetal liver, when presumably trans-placental supply is inadequate for the anabolic requirements for serine in nucleotide biosynthesis and protein synthesis to support rapid growth. During suckling, hepatic serine metabolism is diminished, perhaps to conserve the amino acid for general body protein synthesis and growth. The increased capacity for gluconeogenesis from serine at this time has been discussed fully elsewhere (Snell, 1974, 1976, 1980). At weaning, a rapid phase of liver growth may again involve serine as a precursor for nucleotides as well as for protein synthesis, with the diet serving as the amino acid source. After weaning, serine may be utilized for oxidative energy, lipogenesis, glucose and glycogen formation and for nucleotide biosynthesis as physiological situations demand.

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