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Regulation of Hepatic L-Serine Dehydratase and L-Serine–Pyruvate Aminotransferase in the Developing Neonatal Rat

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1. The activities of L-serine dehydratase and L-serine–pyruvate aminotransferase were determined in rat liver during foetal and neonatal development. 2. L-Serine–pyruvate aminotransferase activity begins to develop in late-foetal liver, increases rapidly at birth to a peak during suckling and then decreases at weaning to the adult value. 3. L-Serine dehydratase activity is very low prenatally, but increases rapidly after birth to a transient peak. After a second transient peak around the time weaning begins, activity gradually rises to the adult value. Both of these peaks have similar isoenzyme compositions. 4. In foetal liver both L-serine dehydratase and L-serine–pyruvate aminotransferase activities are increased after injection *in utero* of glucagon or dibutyryl cyclic AMP. Cycloheximide or actinomycin D inhibited the prenatal induction of both enzymes and actinomycin D blocked the natural increase of L-serine dehydratase immediately after birth. Glucose or insulin administration also blocked the perinatal increase of L-serine dehydratase. 5. After the first perinatal peak of L-serine dehydratase, activity is increased by cortisol and this is inhibited by actinomycin D. After the second postnatal peak, activity is increased by amino acids or cortisol and this is insensitive to actinomycin D inhibition. Glucose administration blocks the cortisol-stimulated increase in L-serine dehydratase and also partially lowers the second postnatal peak of activity. 6. The developmental patterns of the enzymes are discussed in relation to the pathways of gluconeogenesis from L-serine. The regulation of enzyme activity by hormonal and dietary factors is discussed with reference to the changes in stimuli that occur during neonatal development and to their possible mechanisms of action.

The metabolic route by which L-serine is converted into glucose is a matter of some controversy. The involvement of L-serine dehydratase (EC 4.2.1.13), catalysing the formation of pyruvate and NH_3, has long been assumed to account for gluconeogenesis from L-serine. More recently an alternative pathway of glucose formation from serine via hydroxypyruvate, involving initial transamination by L-serine–pyruvate aminotransferase (EC 2.6.1.51), has been proposed (Rowsell *et al.*, 1969, 1972*a*). Support for such a pathway has come from the work of Lardy *et al.* (1969) and Metz *et al.* (1972) in the perfused liver, and evidence implicating L-serine–pyruvate aminotransferase in gluconeogenesis has come from studies showing increased enzyme activity under conditions associated with enhanced gluconeogenesis (Rowsell *et al.*, 1969, 1972*a*,*b*, 1973; Sallach *et al.*, 1972). There is considerable evidence that gluconeogenesis is increased in the suckling rat during neonatal development (see reviews by Walker, 1971; Snell & Walker, 1973*b*; Walker & Snell, 1973), and the capacity to convert serine into glucose in liver slices and in the perfused liver *in vitro* appears to parallel this activity (Vernon *et al.*, 1968; Snell, 1974). The present paper describes the patterns of development of L-serine dehydratase and L-serine–pyruvate aminotransferase in neonatal rat liver and discusses them in relation to the pathways of glucose synthesis from L-serine.

Previous work in this laboratory has demonstrated the importance of hormonal factors in controlling enzyme development in foetal liver, and the combined role of hormonal and dietary factors in regulating postnatal enzyme development (Snell & Walker, 1972*a*). The involvement of such factors in controlling the neonatal development of L-serine dehydratase and L-serine–pyruvate aminotransferase has been investigated and the results are reported here. Part of this work has been presented as a preliminary report (Snell & Walker, 1972*b*).

Materials and Methods

Materials

Cyclic AMP and its 6-*N*,2′-*O*-dibutyryl derivative, NADH, spinach leaf glyoxylate reductase (EC

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1.1.1.26) and rabbit muscle lactate dehydrogenase (EC 1.1.1.27) were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.; L-serine, L-thyroxine (sodium salt), L-tryptophan, sodium pyruvate, amino acids for 'amino acid mixture' (see below) and casein (Hammarsten quality) were from British Drug Houses Ltd., Poole, Dorset, U.K.; L-homoserine, pyridoxal 5'-phosphate and cycloheximide were from Sigma (London) Ltd., London S.W.6, U.K.; glucagon was from Eli Lilly and Co. Ltd., Basingstoke, Hants., U.K.; cortisol acetate ('Hydrocortisyl') was from Rousell Laboratories Ltd., London, U.K.; and actinomycin D and dithiothreitol were from Calbiochem, London W.1, U.K. All other chemicals were of AnalaR quality.

Animals and treatments

The rats were a Wistar albino strain fed *ad libitum* and were maintained, foetal ages were assessed, and prenatal hormone injections were performed *in utero* as previously described (Snell & Walker, 1972*a*). Adult animals were males weighing 200–250g. Neonatal animals removed from a litter for any reason were always replaced by marked animals from another litter of comparable age, care being taken to avoid using the marked animals in the rest of the experiment, so that individuals of the original litter continued to receive a 'normal' supply of milk.

Hormonal agents were administered intraperitoneally. The doses of glucagon, dibutyryl cyclic AMP, cortisol acetate, thyroxine, cycloheximide and actinomycin D were 0.05, 0.5, 0.125, 0.003, 0.010 and 0.005mg/foetus respectively, administered in a volume of 0.05ml. Agents were administered to postnatal rats at doses of 0.5, 5.0, 2.5, 0.05 and 50.0mg/100g body wt. for glucagon, dibutyryl cyclic AMP, cortisol acetate, actinomycin D and tryptophan respectively, unless otherwise indicated. Glucagon was dissolved in 1.6% glycerine–0.2% phenol and the cortisol acetate was dispersed in 0.9% NaCl with sodium carboxymethyl-cellulose and 0.9% benzyl alcohol; other agents were dissolved or dispersed in 0.9% NaCl. L-Tryptophan and glucose (in the case of newborn animals) were dissolved in 0.9% NaCl and administered by intraperitoneal injection. Glucose given to postnatal animals, casein and the 'amino acid mixture' were intubated intragastrically in 0.9% NaCl (Snell & Walker, 1972*a*). The amino acid mixture contained tryptophan, threonine, phenylalanine, arginine, leucine, histidine and isoleucine (all in the L-form) in equimolar amounts (620μmol/g of mixture) (Peraino *et al.*, 1965) and was adjusted to pH7.0 with 1M-NaOH before administration. The intragastric doses of the 'amino acid mixture' and glucose were 75 and 625mg/100g body wt. respectively, and casein was intubated as 0.5ml of a 15% (w/v) slurry/rat.

L-Serine dehydratase assay

Homogenates (5%, w/v) of liver tissue were prepared in ice-cold 0.1M-potassium phosphate buffer, pH8.0, with a Potter-type homogenizer equipped with a Teflon pestle. Whole homogenates were assayed immediately for L-serine dehydratase activity. Reaction mixtures contained 200mM-L-serine, 0.17mM-pyridoxal 5'-phosphate, 50mM-potassium phosphate buffer, pH8.0, and tissue homogenate (0.2ml) in a total volume of 1.2ml and were incubated at 37°C under N_2. These assay conditions were found to be optimal (see also Greengard & Dewey, 1967). The reaction was initiated by the addition of L-serine,

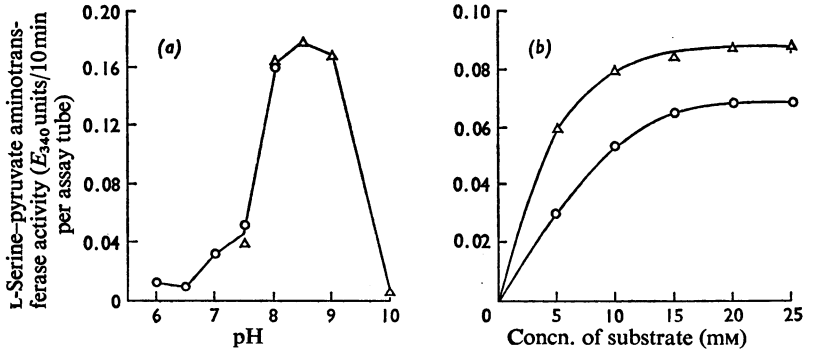

Fig. 1. *Properties of* L-*serine–pyruvate aminotransferase in crude liver homogenates*

L-Serine–pyruvate aminotransferase activity was assayed in crude liver homogenates from 10-day-old rats as described in the text. Activity was measured as a function of (*a*) pH of the assay, by using 0.1M-potassium phosphate buffer (○) or 0.1M-Tris buffer (△) at different pH values, and (*b*) substrate concentration, pyruvate (△) or serine (○), while keeping the co-substrate concentration constant at 20mM (each set of data was obtained in a separate experiment with a different liver homogenate). Activity is expressed in terms of extinction units (at 340nm)/10min per assay tube.

after preincubation of the other assay components together at 37°C for 3–5min. Portions (0.2ml) were removed at appropriate time-intervals (up to 15min) and were deproteinized with equal volumes of 10% (w/v) trichloroacetic acid. Pyruvate was measured in the protein-free un-neutralized supernatants by using lactate dehydrogenase as described by Rowsell *et al.* (1972*c*). Enzyme activities were linear with respect to incubation time and tissue concentration over the ranges used, and suitable control incubations showed negligible pyruvate formation in the absence of L-serine. Enzyme activities are expressed as μmol of pyruvate formed/min per g wet wt. of liver.

L-Serine–pyruvate aminotransferase assay

Homogenates (5%, w/v) of liver tissue were prepared (see above) in ice-cold 0.1M-Tris–HCl buffer, pH8.5. Incubations contained 20mM-L-serine, 20mM-pyruvate, 50mM-Tris–HCl buffer, pH8.5, and tissue homogenate (0.8ml) in a total volume of 1.6ml and were made at 37°C under N_2. The reaction was initiated by the addition of homogenate, after separate preincubation of homogenate and the rest of the assay system at 37°C for 2–3min. Portions (0.45ml) were removed at appropriate time-intervals (up to 15min) and were deproteinized with 0.5ml of 70% (w/v) $HClO_4$. After removal of the precipitated protein by centrifugation, the supernatant was neutralized with 4M-KOH and the precipitated $KClO_4$ was removed, again by centrifugation. Hydroxypyruvate in the neutralized supernatant was measured by the addition of 0.8ml of a batch reaction mixture [containing 4.5μmol of NADH and 0.05ml of glyoxylate reductase (2.75units) in 24ml of 0.1M-potassium phosphate buffer, pH7.4] to a total volume of 1.0ml. Extinctions were measured at 340nm after 10min.Optimal conditions for the assay of this enzyme have not previously been reported to our knowledge. Enzyme activity was linear with respect to incubation time up to at least 20min and activity was proportional to tissue concentration over the range 10–40mg of tissue/assay. Optimal activity was found at pH8.5 (Fig. 1*a*) and apparent saturating substrate concentrations were found at 20mM with both L-serine and pyruvate (Fig. 1*b*). No differences in these parameters were found between neonatal and adult liver homogenates. The addition of exogenous pyridoxal 5′-phosphate (0.17mM) to the aminotransferase incubations was without effect on enzyme activity at any age. Enzyme activities are expressed as μmol of hydroxypyruvate formed/min per g wet wt. of liver.

Cellulose acetate electrophoresis

Liver homogenates (35%, w/v) for ultimate electrophoresis were prepared in ice-cold medium containing 20mM-KCl, 5mM-Tris, 1mM-EDTA, 1mM-dithiothreitol and 1mM-pyridoxal 5′-phosphate, adjusted to pH8.6, and were centrifuged at 100000*g* for 60min. Portions (10μl) of the crude liver supernatant were applied to the centre of cellulose acetate strips (20cm×5.5cm; Oxoid Ltd., London SE1 9HF, U.K.) previously equilibrated with 50mM-barbitone buffer, pH8.6, containing 5mM-EDTA and 1mM-mercaptoethanol, under constant current (0.4mA/cm). Electrophoresis was continued for 4h at 2–4°C. The strips were stained for L-serine dehydratase activity by over-laying filter paper strips of similar dimensions previously soaked in staining medium containing 0.2M-L-serine, 0.4mM-pyridoxal 5′-phosphate, 0.57mM-NADH, lactate dehydrogenase (approx. 2units/ml), 0.15M-KCl and 0.1M-Tris, at pH8.4. Suitable blanks were run in which the assay medium lacked L-serine and lactate dehydrogenase. The stained strips were examined under a Hanovia u.v. lamp (maximum emission at 366nm), when regions of enzymic activity became visible as dark bands on a fluorescent background. Detection of L-homoserine dehydratase (EC 4.4.1.1) activity was carried out in a similar manner but with 0.2M-L-homoserine (which is converted into oxobutyrate, which also reacts with lactate dehydrogenase) in place of L-serine in the staining medium.

DEAE-cellulose chromatography

Liver homogenates (50%, w/v) were prepared as above and centrifuged at 100000*g* for 60min. Crude liver supernatant containing 30–40units of L-serine dehydratase activity was applied to a column (4cm×90cm) of DEAE-cellulose (Whatman DE-32) equilibrated with 10mM-potassium phosphate buffer, pH7.8, at 2–4°C. After washing with 200ml of 10mM-phosphate buffer, a linear gradient of phosphate buffer (10–150mM) containing 1mM-EDTA and 1mM-dithiothreitol was applied and 4.0ml fractions were collected. Enzyme activity in the fractions was assayed in a system, preincubated at 30°C for 2–3min, containing 0.2M-L-serine, 0.4mM-pyridoxal 5′-phosphate, 0.57mM-NADH, approx. 2 units of lactate dehydrogenase, 0.15M-KCl and 0.1M-Tris, pH8.4, in a total volume of 0.95ml. The assay was initiated with the addition of 0.05ml of column eluate and enzyme activity measured continuously at 340nm and 30°C. Activity is expressed as μmol of pyruvate formed/min.

Results

Developmental patterns

L-Serine–pyruvate aminotransferase activity, measured in whole liver homogenates, shows a developmental profile (Fig. 2) very similar to that observed by Rowsell *et al.* (1973), using slightly different assay conditions and a different strain of rats. L-Serine dehydratase activity, measured in liver homogenates, shows a much more complex

pattern of development, which is further complicated by a seasonal variation in enzyme activity (Fig. 3). This seasonal variation involved not only differences in enzyme activity at any age but also small temporal shifts in the pattern of development. For example, adult activity in February–March is about twofold higher than in August–September, and the second postnatal peak of activity occurs somewhat earlier (at 15 days *post partum*) in August–September than in February–March (at 18 days *post partum*) (Fig. 3). The time of year at which various experiments were carried out is given in the legends of the appropriate Tables and Figures. There is some disagreement in the literature as to the pattern of development of L-serine dehydratase; some workers have reported a peak of activity in developing rat

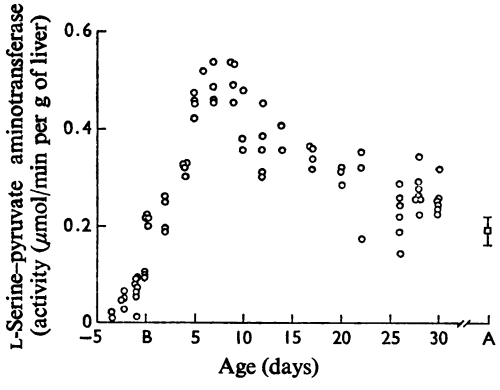

Fig. 2. *Activity of* L-*serine–pyruvate aminotransferase in whole liver homogenates as a function of age of the rat*

Each point represents a single determination on the liver pooled from two animals (less than 5 days *post partum*) or from a single liver (from animals older than 5 days *post partum*). The adult value (□) is the mean of nine determinations on liver from rats 8–10 weeks old and vertical bars show ±S.E.M. Activity was determined as described in the text and is expressed as μmol of hydroxypyruvate formed/min per g of liver. B, birth; A, adult.

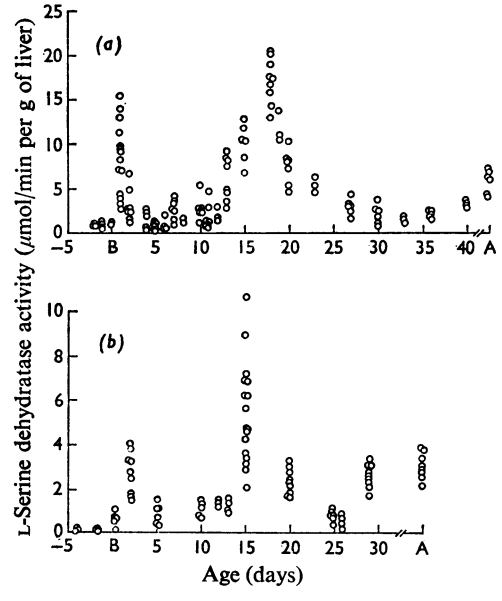

Fig. 3. *Activity of* L-*serine dehydratase in whole homogenates assayed in* (*a*) *February–March and* (*b*) *August–September as a function of age*

Each point represents a single determination on the liver pooled from two animals (less than 5 days *post partum*) or from a single liver (from animals older than 5 days *post partum*). Activity was determined as described in the text and is expressed as μmol of pyruvate formed/min per g of liver. B, birth; A, adult (8–10 weeks old).

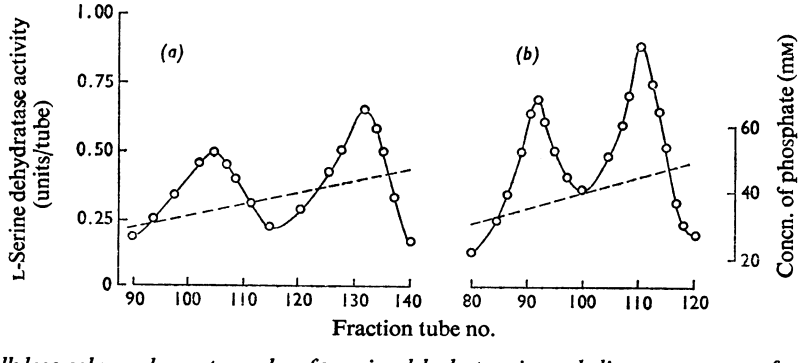

Fig. 4. *DEAE-cellulose column chromatography of* L-*serine dehydratase in crude liver supernatants from* (*a*) *15-day-old and* (*b*) *2-day-old rats*

The chromatographic procedure and the collection and assay of fractions (4.0ml) are described in the text. ——, Enzyme activity (units/tube); – – – –, concn. of phosphate in eluate.

Table 1. *Hormonal effects on rat liver* L-*serine–pyruvate aminotransferase activity in foetal and* 34*-day-old rats*

Foetal rats were injected intraperitoneally *in utero* 2 days before term with various hormonal agents as described in the text. Weaned 34-day-old rats were injected intraperitoneally with glucagon, dibutyryl cyclic AMP, cortisol, thyroxine or actinomycin D at dosages of 0.5, 5.0, 10.0, 0.5 and 0.1 mg/100g body wt. respectively. In all cases, L-serine–pyruvate aminotransferase was assayed 24h after injection of the hormonal agent. Activity is expressed as the mean ± S.E.M., with the number of observations in parentheses. Significance of results: $*P<0.001$.

Hormonal agent	L-Serine–pyruvate aminotransferase activity (μmol/min per g of liver)	
Age (days)	... −2	34
Control (0.9% NaCl)	0.096 ± 0.006 (16)	0.200 ± 0.010 (19)
Glucagon	0.255 ± 0.011 (6)*	0.835 ± 0.081 (6)*
Dibutyryl cyclic AMP	0.308 ± 0.005 (4)*	0.932 ± 0.076 (6)*
Cortisol	0.095 ± 0.016 (5)	0.181 ± 0.018 (6)
Thyroxine	0.100 ± 0.018 (5)	0.230 ± 0.026 (6)
Dibutyryl cyclic AMP + actinomycin D	0.105 ± 0.015 (4)	0.215 ± 0.024 (6)

liver at 2 days *post partum* (Yeung & Oliver, 1971) and others only at 20–26 days *post partum* (Vernon *et al.*, 1968; Evered & Roffe, 1971; Goswami *et al.*, 1972).

Adult rat liver L-serine dehydratase occurs as two isoenzymes which are subject to differential regulation by hormonal and dietary factors (Inoue & Pitot, 1970; Inoue *et al.*, 1971). The complex developmental pattern of L-serine dehydratase observed in the present work might be attributed to the differential development of these isoenzyme forms. Chromatography of a crude liver supernatant from 15-day-old rats (August–September) on DEAE-cellulose revealed two peaks of activity on elution with a KCl gradient (Fig. 4*a*). Peak I (eluted at 35mM-phosphate buffer) represents about 44% of the total recovered activity and peak II (eluted at 44mM-phosphate buffer) about 56%. The total recovery was 80–85%. The isoenzyme pattern is similar to that found by Inoue & Pitot (1970) after DEAE-cellulose chromatography of liver supernatant from adult rats fed on a high-protein diet or injected with tryptophan. Chromatography of a crude liver supernatant from 2-day-old rats (August–September) (i.e. when the first postnatal peak of total L-serine dehydratase activity occurs) gave a similar isoenzyme pattern to that of the 15-day-old rats, with peak I accounting for 40% of the total recovered activity and peak II about 60% (Fig. 4*b*). The total recovery was 80–85%.

Electrophoresis on cellulose acetate strips resolved crude liver supernatant from 2- and 15-day-old rats into three bands of enzymic activity migrating 0.3, 1.2 and 2.3cm from the origin towards the anode. The slowest band stained more intensely than the fastest, and the middle band the least. Essentially identical electrophoretic patterns were obtained for both of these ages, which correspond to the two postnatal peaks of L-serine dehydratase activity (Fig. 3*b*). The multiple forms of L-serine dehydratase may be explained, in part, by separation of L-homoserine dehydratase from L-serine dehydratase, since the former enzyme protein possesses some activity towards L-serine (Hoshino *et al.*, 1971). Staining of cellulose acetate strips for L-homoserine dehydratase gave a single band of activity coincident in mobility with the fastest band of L-serine dehydratase activity. The neonatal development of L-homoserine dehydratase shows a rise in activity beginning in the foetal period, reaching the adult value in the first day after birth and persisting at this value throughout life (Snell, 1973*a*). This explains why the fastest band is always present in electrophoretograms of neonatal liver supernatant, and further, this pattern suggests that L-homoserine dehydratase plays no part in determining the complex developmental pattern of L-serine dehydratase activity. The two slower-migrating bands were devoid of L-homoserine dehydratase activity, and activity with respect to L-homoserine in the two peaks from DEAE-cellulose chromatography was 6% or less of that with L-serine as substrate. Thus, both column chromatography and electrophoresis suggest the presence of two isoenzyme forms of L-serine dehydratase but give no support to the possibility that their differential development is responsible for the complex nature of the developmental pattern of total activity observed.

Hormonal regulation of L-*serine–pyruvate aminotransferase in foetal and adult rat liver*

A number of hormones were tested for their effect on L-serine–pyruvate aminotransferase activity in foetal rats after administration *in utero* and in young adult rats. Cortisol and thyroxine were without effect on enzyme activity, but glucagon or dibutyryl cyclic AMP markedly increased activity measured 24h later both in foetal and also in adult animals (Table 1). The time-course of adaptation after a single intraperitoneal injection of glucagon into adult rats is shown in Fig. 5. An increase

in L-serine–pyruvate aminotransferase activity in normal adult rats 24h after glucagon administration has been observed previously (Rowsell *et al.*, 1969, 1973). The effect of glucagon appears to be mediated via cyclic AMP and, at both ages, increase in activity owing to the latter is blocked by the simultaneous administration of actinomycin D (Table 1).

Hormonal regulation of L-serine dehydratase in foetal and perinatal rat liver

Cortisol and thyroxine injected *in utero* into foetal rats (2 days before term) has no effect on L-serine dehydratase activity assayed 5h later [0.13±0.02(6) and 0.15±0.01(6) respectively; cf. 0.14±0.02(9) μmol/min per g of liver for 0.9% NaCl-injected controls]. Glucagon markedly increased activity to 0.98±0.03(9) ($P<0.001$), as shown previously (Greengard & Dewey, 1967; Yeung & Oliver, 1971). This effect of glucagon is mediated via cyclic AMP, and actinomycin D and cycloheximide block the stimulation of enzyme activity by dibutyryl cyclic AMP (Table 2).

The increase in L-serine dehydratase activity that takes place immediately after birth is evident within 12h after surgical delivery in both suckled and unsuckled neonatal rats (Table 3) and so is not dependent on postnatal maternal factors or feeding. However, the increase in unsuckled animals was somewhat greater than in the fed rats ($P<0.05$). In both groups of rats the rise in enzyme activity is blocked completely by actinomycin D (Table 3). The rise in L-serine dehydratase activity after birth is

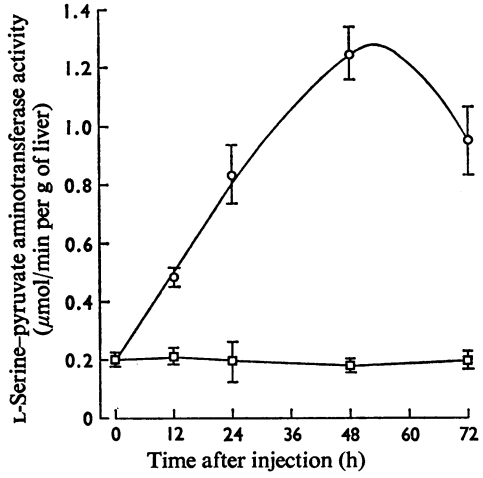

Fig. 5. *Stimulation of L-serine–pyruvate aminotransferase activity in adult rats by glucagon in vivo as a function of time*

Adult male rats (100–120g body wt.) were injected intraperitoneally at zero time with 0.5mg of glucagon/100g body wt. (○) or 0.9% NaCl (□) and killed at the time-intervals indicated. Each point and vertical bar is the mean ±S.E.M. of four determinations.

Table 2. *Effect of cycloheximide and actinomycin D on L-serine dehydratase induction in foetal rat liver*

Foetuses were injected intraperitoneally *in utero* with glucagon or dibutyryl cyclic AMP as described in the text. Some littermates also received actinomycin D or cycloheximide in addition to the hormonal agent and further saline-injected littermates served as controls. Animals were injected on the twenty-first day of gestation and killed 12h later. The pooled liver from two foetuses was used for assay as described in the text. Enzyme activity (February–March) is recorded as the mean ±S.E.M., with the number of observations in parentheses. Significance of results: *$P<0.001$.

Treatment	L-Serine dehydratase activity (μmol of pyruvate formed/min per g of liver)
Control (0.9% NaCl)	0.70±0.12 (12)
Glucagon	3.82±0.54 (4)*
Glucagon+actinomycin D	0.65±0.20 (4)
Dibutyryl cyclic AMP	4.05±0.91 (4)*
Dibutyryl cyclic AMP +actinomycin D	0.50±0.17 (6)
Dibutyryl cyclic AMP +cycloheximide	0.68±0.18 (4)

Table 3. *Effect of actinomycin D on L-serine dehydratase development in newborn rat liver*

Term foetuses were delivered by caesarian section and injected with 0.9% NaCl (controls) or actinomycin D as described in the text. Some litters were kept in an incubator at a temperature of 35±1°C and others were placed with a lactating foster-mother and allowed to suckle. Animals were killed at 0h and 12h after injection and individual livers used for assay as described in the text. Enzyme activity (February–March) is recorded as the mean±S.E.M., with the number of observations in parentheses. Significance of results compared with controls at 0h: *$P<0.001$.

Treatment	L-Serine dehydratase activity (μmol of pyruvate formed/min per g of liver)	
	Unsuckled	Suckled
Controls (0.9% NaCl), at 0h	0.82±0.11 (6)	1.11±0.09 (7)
Controls (0.9% NaCl), at 12h	4.93±0.46 (6)*	4.41±0.48 (7)*
Actinomycin D, at 12h	0.99±0.08 (6)	1.30±0.12 (7)

partially blocked by glucose injection, but glucose has no effect ($P<0.05$) on the glucagon-induced increase in enzyme activity after birth (Table 4; see also Greengard & Dewey, 1967; Yeung & Oliver, 1971). Insulin given at birth completely suppresses the natural increase in activity (Table 4).

Hormonal and dietary regulation of L-*serine dehydratase in postnatal rat liver*

The second phase of development of L-serine dehydratase activity in rat liver (Fig. 3) takes place at or soon after the commencement of weaning. A number of agents were tested for their effect on L-serine dehydratase activity at 11 days *post partum*, before the postnatal peak of activity occurs, and at 21 days *post partum*, after the postnatal peak of activity (Table 5). Glucagon or dibutyryl cyclic AMP were without effect at 11 or 21 days. Cortisol or tryptophan both increased enzyme activity at either age. Cortisol was somewhat more effective than tryptophan at 11 days, but both agents were about equally effective at 21 days. The administration of cortisol and tryptophan together (both agents were given at their maximally effective dose) gave no greater an increase in enzyme activity than cortisol given alone at either age. A single intubation of an amino acid mixture produced a similar increase in activity to administration of tryptophan at either age. The effect of cortisol and amino acid mixture administered together at 11 days *post partum* was not additive. The intubation of casein for 3 days increased L-serine dehydratase activity in 21-day-old animals, and when this treatment was combined with cortisol administration the effect on enzyme activity was again not additive. The lack of effect of glucagon or dibutyryl cyclic AMP on L-serine dehydratase activity at 11 or 21 days *post partum* (Table 5) was also apparent at 10, 26 and 40 days *post partum* (Table 6), despite the marked effect of these agents in foetal and newborn animals and at 4 days *post partum* (Tables 2, 4 and 6). Cortisol, which was ineffective in foetal animals, was effective at all postnatal ages, but especially so at 10 days *post partum*, i.e. shortly before the second developmental peak of

Table 4. *Effects of glucagon and glucose on* L-*serine dehydratase development in newborn rat liver*

Term foetuses were delivered by caesarian section and injected immediately with 0.9% NaCl (controls), insulin (0.04i.u./rat), glucagon (50μg/rat), glucose (25mg/rat) or glucagon+glucose. Animals injected with glucose or insulin received a further injection 3h after the initial treatment and all animals were killed at 6h. L-Serine dehydratase activity was assayed (August–September) as described in the text and recorded as the mean±S.E.M., with the number of observations in parentheses. Significance of results compared with controls at 6h: $*P<0.001$.

	L-Serine dehydratase activity (μmol of pyruvate/min per g of liver)
Controls (0.9% NaCl), at zero time	0.25±0.03 (8)
Controls (0.9% NaCl), at 6h	1.56±0.14 (8)
Glucose, at 6h	0.67±0.09 (6)*
Glucagon, at 6h	4.28±0.36 (6)*
Glucagon+glucose, at 6h	3.84±0.42 (6)*
Insulin, at 6h	0.29±0.02 (6)*

Table 5. *Stimulation of* L-*serine dehydratase activity in postnatal rat liver*

Animals were injected intraperitoneally with the indicated agents, except for casein and the amino acid mixture, which were intubated intragastrically. Casein intubation was carried out for 3 days (i.e. 19–21 days *post partum* inclusive), after which some animals received a single intraperitoneal injection of cortisol, and all the animals were killed 12h later. The amino acid mixture was administered in a single intubation; some animals also received a single intraperitoneal injection of cortisol at the same time, and all the animals were killed 12h later. Animals injected intraperitoneally with hormonal agents were killed 12h later. In all cases saline-injected littermates served as controls. L-Serine dehydratase was assayed (February–March) as described in the text and recorded as the mean±S.E.M., with the number of observations in parentheses. –, not determined. Significance of results: $*P<0.001$; $**P<0.01$.

Treatment	L-Serine dehydratase activity (μmol of pyruvate/min per g of liver)	
Postnatal age (days)	... 11	21
Controls (0.9% NaCl)	2.60±0.36 (12)	5.18±0.32 (12)
Glucagon	2.45±0.18 (6)	—
Dibutyryl cyclic AMP	2.80±0.15 (6)	4.82±0.24 (4)
Cortisol	10.84±0.72 (5)*	11.49±1.06 (5)*
Tryptophan	5.42±0.66 (5)*	9.22±0.60 (4)*
Cortisol+tryptophan	10.46±0.64 (4)*	10.73±0.83 (4)*
Amino acid mixture	5.90±0.38 (4)*	8.82±0.92 (4)**
Cortisol+amino acid mixture	11.23±0.46 (4)*	—
Casein	—	11.03±0.52 (4)*
Cortisol+casein	—	10.12±0.74 (4)*

Table 6. *Effect of developmental age on the response of* L-*serine dehydratase to hormonal agents in postnatal rat liver*

Animals were killed 12h after a single intraperitoneal injection of the indicated agent, and saline-injected littermates served as controls. The ages selected for comparison were : 4 days *post partum*, shortly after the first developmental peak of activity; 26 days *post partum*, in the period after the second developmental peak but before weaning is complete; and 40 days *post partum*, when animals are fully weaned individuals. L-Serine dehydratase was assayed (February–March) as described in the text and recorded as the mean ± S.E.M., with the number of observations in parentheses. Significance of results: $*P<0.001$.

	L-Serine dehydratase activity (μmol of pyruvate/min per g of liver)			
Postnatal age (days) ... Treatment	4	10	26	40
Control (0.9% NaCl)	2.37±0.10 (9)	5.52±1.05 (6)	1.56±0.19 (6)	1.91±0.27 (5)
Glucagon	4.33±0.11 (6)*	5.37±0.22 (5)	1.96±0.19 (5)	2.41±0.32 (5)
Cortisol	5.63±0.22 (6)*	19.53±1.10 (5)*	4.91±0.43 (6)*	2.11±0.19 (5)

Table 7. *Effect of developmental age on the response of* L-*serine dehydratase to cortisol after actinomycin D treatment in postnatal rats*

Animals were injected intraperitoneally with cortisol with or without a prior injection of actinomycin D (30min before cortisol) and killed 12h later for assay as described in the text. Saline-injected littermates served as controls. Enzyme activity (February–March) is recorded as the mean ± S.E.M., with the number of observations in parentheses. Significance of results: $*P<0.001$.

	L-Serine dehydratase activity (μmol of pyruvate/min per g of liver)			
Postnatal age (days) ... Treatment	4	13	19	23
Control (0.9% NaCl)	2.37±0.10 (9)	3.58±0.63 (4)	8.28±0.18 (4)	5.40±0.48 (5)
Cortisol	5.63±0.22 (6)*	11.15±0.58 (6)*	13.95±1.00 (6)*	14.47±0.84 (5)*
Cortisol+actinomycin D	2.18±0.31 (6)	4.92±0.31 (6)	12.59±0.57 (6)*	12.70±0.54 (5)*

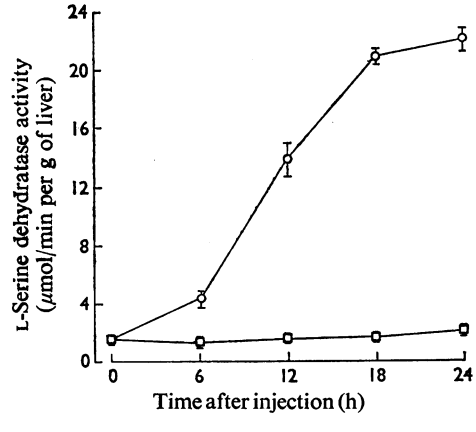

Fig. 6. *Time-course of the stimulation by cortisol of* L-*serine dehydratase activity in* 10-*day-old neonatal rats*

The 10-day-old rats were given a single intraperitoneal injection of cortisol (○) or 0.9% NaCl (controls) (□) at zero time and were killed at various time-intervals after injection. L-Serine dehydratase activity was assayed (February–March) as described in the text and is expressed as μmol/min per g of liver. Each point and vertical bar represents the mean ± S.E.M. of four to six observations.

L-serine dehydratase activity. The acquisition of responsiveness to cortisol postnatally may be related to the developmental increase of dexamethasone-binding capacity of liver cytosol after birth (Cake *et al.*, 1973). Neither cortisol nor glucagon had any significant effect ($P>0.05$) on L-serine dehydratase activity in 40-day-old animals within 12h of injection. The time-course of the stimulation of L-serine dehydratase activity by cortisol in 10-day-old rats is shown in Fig. 6. Similar time-courses were found after amino acid intubation or tryptophan injection (results not shown). The response to cortisol at 4 days *post partum* was completely blocked by actinomycin D and at 13 days was inhibited by 80% (Table 7). However, after the natural developmental stimulation of L-serine dehydratase activity (i.e. at 19 and 23 days *post partum*), the further increase in activity elicited by cortisol was insensitive to actinomycin D inhibition. A similar situation appears to hold for the tryptophan-mediated increase in L-serine dehydratase activity (Table 8). At 8 days *post partum*, before the natural developmental rise in activity occurs, the response to tryptophan is blocked by actinomycin D, but at 22 days *post partum*, after the second post-

Table 8. *Effect of actinomycin D on tryptophan stimulation of* L-*serine dehydratase activity in postnatal rat liver*

Animals were injected intraperitoneally with tryptophan with or without a prior injection of actinomycin D (30min before tryptophan) and killed 12h later for assay as described in the text. Saline-injected littermates served as controls. Enzyme activity (February–March) is recorded as the mean±S.E.M., with the number of observations in parentheses. Significance of results: $*P<0.001$.

	L-Serine dehydratase activity (μmol of pyruvate/min per g of liver)	
Postnatal age (days) ...	8	22
Treatment		
Controls (0.9% NaCl)	1.30±0.11 (5)	4.60±0.26 (5)
Tryptophan	4.40±0.55 (5)*	7.22±0.63 (4)*
Tryptophan+actinomycin D	1.30±0.03 (5)	8.91±0.77 (5)*

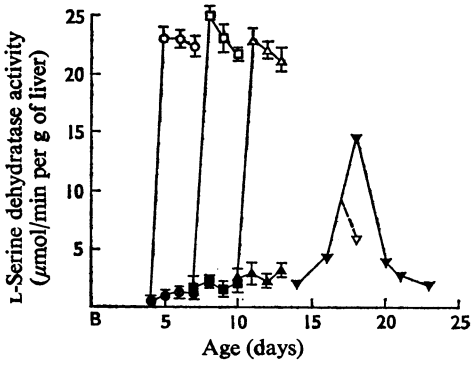

Fig. 7. *Time-course of the stimulation by cortisol of* L-*serine dehydratase activity in neonatal rats at different ages after birth*

Rats at 4 (●, ○), 7 (■, □) or 10 (▲, △) days *post partum* were given a single intraperitoneal injection of cortisol (○, □, △) or 0.9% NaCl (●, ■, ▲) (controls) as described in the text. Animals were killed at 24h intervals after injection and L-serine dehydratase activity (expressed as μmol/min per g of liver) was determined (February–March) as described in the text. Each point and vertical bar (●, ■, ▲, ○, □, △) represents the mean±S.E.M. of determinations on three to five rats. Also shown is the natural time-course of L-serine dehydratase activity in untreated animals from a single litter (▼). Two littermates were intubated intragastrically with glucose at 6-h intervals at 17 days and killed at 18 days (▽). Each point (▼, ▽) is the mean of two determinations.

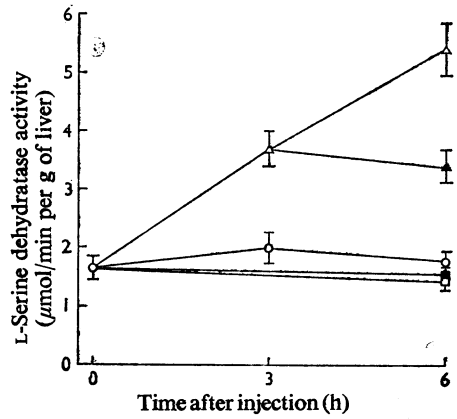

Fig. 8. *Effect of glucose intubation on the stimulation by cortisol of* L-*serine dehydratase activity in 9-day-old neonatal rats*

The 9-day-old rats were given a single intraperitoneal injection of cortisol (△) or 0.9% NaCl (○) (controls) at zero time. Some rats were also given a single intragastric intubation of glucose at zero time together with an injection of cortisol (■) or 0.9% NaCl (□), and others injected with cortisol at zero time were given a single intragastric intubation of glucose at 3h (▲). Animals were killed as indicated and L-serine dehydratase activity was determined (February–March) as described in the text. Each point and vertical bar represents the mean±S.E.M. of four to six determinations.

natal peak of activity the effect of tryptophan is unaffected by actinomycin D treatment.

A unique feature of the developmental pattern of L-serine dehydratase is the atypical transient nature of the second postnatal increase in activity (cf. Snell & Walker, 1972*a*, 1973*b*; Snell, 1973*b*). An examination of the time-course of the increase in L-serine dehydratase activity over the succeeding 3 days after a single injection of cortisol to 4-, 7- and 10-day-old rats is shown in Fig. 7. In each case, L-serine dehydratase activity was increased markedly 24h after cortisol injection, but activity was not appreciably decreased from this value up to 2 days later. In contrast, the natural development of L-serine dehydratase activity shows a return almost to basal values within 2 days of the peak of activity found at 18 days *post partum* (Fig. 7). Factors other than cortisol alone might control the developmental activity, and glucose is known to suppress adaptive increases of L-serine dehydratase in the adult rat (Pitot & Peraino, 1963; Söling *et al.*, 1969). The

natural increase in L-serine dehydratase activity at 18 days *post partum* was suppressed in animals intubated intragastrically with glucose at 6-h intervals over the preceding 24h (Fig. 7). The effect of glucose on the cortisol-induced increase of L-serine dehydratase was also examined (Fig. 8). A single intubation of glucose into 9-day-old rats had no effect on enzyme activity measured 6h later, but completely prevented an increase by cortisol. When glucose was administered 3h after cortisol injection, i.e. when L-serine dehydratase activity was already increasing, a further rise in enzymic activity was arrested (Fig. 8).

Discussion

Developmental patterns and serine metabolism

The apparent disagreement among various groups on the pattern of development of L-serine dehydratase deserves some comment. The sharp peak of activity observed by Yeung & Oliver (1971) at 2 days *post partum* was also found in the present work when assays were carried out in August–September (Fig. 3*b*). However, assays made in February–March showed low L-serine dehydratase activity at 2 days *post partum*, with the peak of activity occurring about 24h after birth (Fig. 3*a*). Depending on the time of year at which assays were made, this may account for the failure of other workers (Vernon *et al.*, 1968; Goswami *et al.*, 1972) to observe the early postnatal peak of L-serine dehydratase. The development curve described by Yeung & Oliver (1971) did not continue long enough into postnatal life to detect the late postnatal peak of L-serine dehydratase, but this was observed in the present work at 15 days (August–September) or 18 days (February–March) *post partum*, and by others at 20 (Vernon *et al.*, 1968), 21 (Goswami *et al.*, 1972) or 26 (Evered & Roffe, 1971) days *post partum*. The variation in the timing of this second postnatal peak may again be connected with the time of the year at which assays were made and/or the age at which animals were weaned. A seasonal variation in L-serine dehydratase activity has also been reported briefly in adult rat liver (Lefauconnier *et al.*, 1973). Seasonal variations in activity have been described for tyrosine aminotransferase (Kenney, 1963) and phosphoenolpyruvate carboxykinase (Suda *et al.*, 1973), and it is noteworthy that for both these enzymes activity was also higher (by about twofold) in February–March than in August–September.

The pattern of development of L-serine dehydratase activity is similar to that found for a number of enzymes involved in amino acid metabolism (see Snell, 1973*b*; Snell & Walker, 1973*b*), with a peak of activity in the early neonatal period followed by further changes around the time of weaning. Thus for the majority of the suckling period the enzymic potential for the catabolism of serine via L-serine dehydratase is depressed below adult values (Fig. 3). L-Serine–pyruvate aminotransferase, on the other hand, shows a pattern of development which is very similar to that exhibited by enzymes known to be involved in gluconeogenesis (Walker, 1971; Snell & Walker, 1973*b*), namely a sustained elevated activity throughout the suckling period and decreasing to the adult value at weaning (Fig. 2). This developmental pattern correlates well with the capacity for gluconeogenesis *in vitro* from serine measured in liver slices during neonatal development (Vernon *et al.*, 1968) or in the perfused neonatal liver (Snell, 1974) and suggests that gluconeogenesis from serine proceeds via the aminotransferase pathway in the neonatal rat. The next enzyme in this pathway, D-glycerate dehydrogenase, catalysing the reduction of hydroxypyruvate to glycerate (see Snell, 1974), shows a similar developmental pattern to L-serine–pyruvate aminotransferase (Johnson *et al.*, 1964). Although the activity of L-serine–pyruvate aminotransferase is considerably elevated above the adult value in neonatal rat liver, it is lower in comparison with that of L-serine dehydratase at all ages. However, the very high K_m for serine of L-serine dehydratase (50–100mM; see Snell & Walker, 1973*b*) relative to plasma concentrations in the neonatal animal (0.2–0.4mM; Snell & Walker, 1973*b*) or adult liver concentrations (about 2μmol/g; Bojanowska & Williamson, 1968; Christophe *et al.*, 1971) makes it probable that the activity obtaining *in vivo* is only a small fraction of that assayed *in vitro* under optimal conditions.

The precise relationship between the pathway initiated by L-serine dehydratase and that involving L-serine–pyruvate aminotransferase in directing serine towards glucose synthesis is still far from clear. Apparently it is the aminotransferase pathway which responds to the metabolic situation of the neonatal rat, but this is not so for all glucogenic circumstances (e.g. starvation and severe alloxan-induced diabetes; Sallach *et al.*, 1972).

Regulation of perinatal enzyme development

Both L-serine dehydratase and L-serine–pyruvate aminotransferase are prematurely induced by glucagon (acting via cyclic AMP) *in utero* (Tables 1 and 2). Since cycloheximide or actinomycin blocked the prenatal induction of both enzymes (Tables 1 and 2) and actinomycin blocked the natural increase of L-serine dehydratase immediately after birth (Table 3), the increase in enzyme activities in both situations is probably due to the synthesis of enzyme protein *de novo*.

The pronounced hypoglycaemia in the first hours of postnatal life (Snell & Walker, 1973*a*; Girard *et al.*, 1973*b*) may be the natural stimulus for the release of glucagon in the newborn rat, since the

decrease in plasma glucose is accompanied by a rise in plasma concentrations of glucagon (Girard *et al.*, 1972). However, the reported insensitivity of the pancreatic α-cells of the newborn rat to acute variations in glucose concentration (Girard *et al.*, 1973*a*, 1974) suggests the operation of other factors in initiating glucagon release. If this is so the effect of glucose to block partially the natural development of L-serine dehydratase at birth (Table 4) is unlikely to be due to suppression of glucagon release. Indeed the effect is more likely to be mediated by glucose-induced insulin secretion, for insulin administered at birth completely blocked the postnatal development of L-serine dehydratase (Table 4). The failure of glucose to block the glucagon-potentiated induction of L-serine dehydratase after birth (Table 4) further suggests that glucose is acting indirectly in this situation. The inhibition by glucose of a postnatal rise in hepatic cyclic AMP concentration (Butcher & Potter, 1972) is also consistent with the glucose effect being mediated via insulin secretion, which would oppose any glucagon-induced increase in cyclic AMP (Exton *et al.*, 1972; Kuster *et al.*, 1973). The rat has very high plasma insulin concentrations at birth which decrease dramatically during the first hour of life (Girard *et al.*, 1973*b*), so the inhibitory effect of insulin on the development of L-serine dehydratase and some other enzymes in the newborn rat (Dawkins, 1963; Yeung & Oliver, 1968; Wicks, 1969; Girard *et al.*, 1973*c*) may have a physiological relevance. It appears that the rapid increase in L-serine dehydratase and other enzymes immediately after birth is due to an increase in hepatic cyclic AMP concentration brought about by a fall of plasma insulin and rise of plasma glucagon concentrations. However, this does not exclude the possibility that insulin may also have other more direct actions on enzyme synthesis or may even be involved in the virtual absence of enzyme degradation noted immediately after birth for phosphoenolpyruvate carboxykinase (Philippidis *et al.*, 1972). It is also possible that a block in enzyme degradation may play a role in the rise of L-serine dehydratase activity after birth, since enzyme synthesis (measured immunologically) shows a large increase between parturition and 6h *post partum* but a much smaller increase between 6h and 48h, when the greatest increase in enzyme activity occurs (Miura & Nakagawa, 1970). Reintroduction of enzyme degradation could then account for the decline of L-serine dehydratase activity after the peak at 2 days *post partum* that occurs in spite of hepatic cyclic AMP concentrations remaining elevated until at least 10 days *post partum* (Novak *et al.*, 1972; Christoffersen *et al.*, 1973).

Regulation of postnatal enzyme development

Dietary components have been shown to play a role in the postnatal development of certain enzymes around the time of weaning (Walker & Eaton, 1967; Vernon & Walker, 1968; Lockwood *et al.*, 1970; Greengard & Jamdar, 1971; Snell & Walker, 1972*a*), as also have hormones (Greengard, 1970, 1971). A combined or related action of both hormonal and dietary factors have been shown sometimes to be important in regulating enzyme development at this time (Jamdar & Greengard, 1970; Greengard & Dewey, 1971; Greengard & Jamdar, 1971; Snell & Walker, 1972*a*). In some instances corticosteroids have been found to effect a necessary actinomycin D-sensitive developmental step before the stimulation of the appearance of enzyme activity by the dietary component in a step insensitive to actinomycin D (Jamdar & Greengard, 1970; Greengard & Dewey, 1971). An explanation of this is that stimulation of the transcription of messenger for the enzyme precedes in time the translation of the messenger into enzymically active protein, and that these two processes are controlled by hormonal and dietary factors respectively.

The present results with L-serine dehydratase are in some ways consistent with the hypothesis but, generally speaking, present a rather more complex pattern of developmental enzyme regulation. Thus, cortisol was shown to stimulate enzyme activity before the natural increase at about 18 days *post partum* by a mechanism involving transcriptional regulation (i.e. actinomycin D-sensitive), whereas tryptophan stimulated enzyme activity after 18 days *post partum* by a mechanism involving post-transcriptional regulation (i.e. actinomycin D-insensitive) (Tables 7 and 8). However, before 19 days *post partum* other agents (amino acid mixture and tryptophan) were also able to stimulate L-serine dehydratase activity, albeit not as effectively as cortisol, and by an actinomycin D-sensitive mechanism (Tables 5 and 8). Similarly, after 19 days *post partum* not only dietary agents (amino acid mixture, casein and tryptophan), but also cortisol, were able to stimulate enzyme activity by an actinomycin D-insensitive mechanism (Tables 5 and 7). These data obtained in whole-animal experiments do not show unequivocally that the action of cortisol precedes that of dietary amino acids. This would be a reasonable interpretation to make, however, since the functioning of the pituitary–adrenal axis (and consequent potential for stimulating corticosteroid secretion) appears to precede the increased dietary intake of protein (and increased availability of amino acids) associated with weaning (see Snell & Walker, 1972*a*). On the other hand, the present data do show that before weaning regulation of enzyme activity is dependent on transcription, whereas after weaning this is controlled at the post-transcriptional level. The action of amino acids, particularly tryptophan, at the post-transcriptional level may be related to their

effects on polyribosome aggregation (Munro, 1970; Sidransky, 1972). However, it has also been demonstrated that tryptophan can stimulate tryptophan pyrrolase (Schimke *et al.*, 1965), tyrosine aminotransferase (Cihak *et al.*, 1973) and phosphoenolpyruvate carboxykinase (Treadow & Khairallah, 1972; Ballard & Hopgood, 1973) activities by a mechanism involving stabilization of the enzyme and inhibition of enzyme degradation. Such a mechanism would also be insensitive to inhibition by actinomycin D and could account for the lack of effect of the inhibitor observed in the 22-day-old neonatal rats (Table 8).

The premature stimulation of L-serine dehydratase by cortisol leads to high induced enzyme activity in 24h, which decrease gradually over the succeeding 2 days (Fig. 7). The natural increase in L-serine dehydratase, on the other hand, shows a marked decline from maximal activity at 18 days *post partum* to near pre-induced values by 20 days *post partum* (Fig. 7). At this later age, at which the natural induction of the enzyme occurs, other factors apparently operate to make the time-course of the elevation much more short-lived. The rat is in the process of weaning to a solid diet that results in a marked increase in the ingestion of carbohydrate, particularly glucose. Glucose markedly depresses L-serine dehydratase induction in the adult, and induction of a number of other enzymes effected by various stimuli (Peraino & Pitot, 1964; Peraino *et al.*, 1966; Söling *et al.*, 1969; Pestana, 1969; Treadow & Khairallah, 1972; Greengard & Dewey, 1973). Now glucose given at 17 days *post partum* considerably depressed L-serine dehydratase activity 24h later (Fig. 7). Moreover, the premature induction of L-serine dehydratase by cortisol in 9-day-old rats was completely inhibited by the simultaneous administration of glucose (Fig. 8). Previous work with the protein-depleted adult rat has shown that the repression by glucose involved the complete cessation of L-serine dehydratase synthesis and a stimulation of enzyme degradation (Jost *et al.*, 1968) and a similar mechanism could account for the rapid repression seen in the neonatal rat. When the administration of glucose was delayed until 3h after cortisol injection in the neonatal rat, the continued rise in enzyme activity was completely prevented (Fig. 7). At this time the further rise of L-serine dehydratase activity is no longer sensitive to inhibition by actinomycin D (results not shown), suggesting that the action of glucose may well be at the translational level of enzyme synthesis, as suggested for the adult (Jost *et al.*, 1968). The effect of glucose is unlikely to be mediated via cyclic AMP, since enzyme activity at this time is insensitive to changes in glucagon or cyclic AMP concentrations (Tables 5 and 6).

After the abrupt postnatal peak of L-serine dehydratase shortly after weaning commences, enzyme activity gradually rises to attain the final adult value and this is probably associated with the increased dietary intake of protein (from about 10 to 20%) that occurs during weaning (Snell & Walker, 1972*a*). Above a dietary content of about 10% protein, L-serine dehydratase activity in young adults increases rapidly with the protein content of the diet (Hurvitz & Freedland, 1968; Mauron *et al.*, 1973). Adaptation of L-serine dehydratase to such a dietary change in protein requires about 5 days, for a shift from a 12%- to a 60%-protein diet (Reynolds *et al.*, 1971). However, the accompanying increase in glucose intake during weaning would tend to repress the increase in enzyme activity. Glucose completely prevents the amino acid induction of L-serine dehydratase in the adult, but prior treatment with cortisone (as we suggest will have occurred naturally during the neonatal development of enzyme activity) allows induction by amino acids to continue in the presence of glucose, although at a much lower rate (Peraino *et al.*, 1966).

A more precise interpretation of the physiological stimuli and molecular mechanisms involved in determining the pattern of development of enzymes in the postnatal rat will require studies *in vitro* using isolated liver systems. The present results on L-serine dehydratase reinforce the principle that during postnatal development hormonal agents are important in initiating enzyme synthesis but that dietary influences are superimposed on these hormonal effects to determine the final pattern of enzyme development. The hormonal effects may be acting at the level of gene activation and transcription, whereas dietary factors may modify these effects, perhaps by acting at the post-transcriptional stage of enzyme synthesis or on the turnover of enzyme protein.

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