Carbohydrate Formation from Various Precursors in Neonatal Rat Liver

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1. Measurements of the net synthesis of glucose plus glycogen from various precursors in slices of glycogen-depleted livers from rats at various stages of development indicated an increase in the gluconeogenic capacity after birth with L-lactate, oxaloacetate, a casein hydrolysate, L-serine, L-threonine, L-alanine and glycerol as substrates. 2. The highest rates of incorporation of ^{14}C -labelled precursors into glucose plus glycogen in slices of normal livers of rats of various ages were observed in such tissue preparations from neonatal animals for an amino acid mixture, L-alanine, L-serine and L-threonine. 3. The activities of rat hepatic L-serine dehydratase and L-threonine dehydratase increase rapidly after birth and show maxima about 20 days later. 4. The results provide further evidence of the increased capacity for hepatic gluconeogenesis in the neonatal period and suggest various sites of regulation of the process.

The newborn rat rapidly utilizes the store of hepatic glycogen that is built up before birth (Dawkins, 1966) and then proceeds to oxidize lipid as an energy source (Taylor, Bailey & Bartley, 1967). Rat milk (Dymsza, Czajka & Miller, 1964) is a high-fat diet and contains only a little carbohydrate, equivalent to no more than 10% of the calorie requirement (Hahn, Koldovský, Melichar & Novák, 1961b). The quantitative requirement of the neonatal animal for carbohydrate is, however, uncertain because many tissues can utilize free fatty acids and ketone bodies as alternative energy sources. An increased capacity for gluconeogenesis compared with the adult level has, nevertheless, been demonstrated in the neonatal rat (see reviews by Dawkins, 1966; Walker, 1968) by studies of isotopic incorporation into glycogen or glucose from pyruvate (Ballard & Oliver, 1963, 1965) or into glycogen from an amino acid mixture (Yeung & Oliver, 1967a). This evidence is supported by observations of increased activities of several hepatic enzymes involved in gluconeogenesis during the neonatal period. A rapid increase in the activity of cytoplasmic phosphopyruvate carboxylase (EC 4.1.1.32) (Ballard & Hanson, 1967; Yeung & Oliver, 1967b; Vernon & Walker, 1968) immediately after birth appears to be the most significant enzymic change that initiates the gluconeogenic capacity in rat liver (Yeung & Oliver, 1967b).

Pyruvate, used in most of the studies referred to above, is only a key intermediate in the process of gluconeogenesis from a few potential precursors (Krebs, 1964). The concentrations of pyruvate and

lactate in the rat liver fall sharply in the first 4 post-natal days (Burch, 1965). A high proportion of the amino acids derived from the proteins of milk (9.2g./100ml. according to Dymsza *et al.* 1964) is used in protein synthesis and probably only a little will be available for other purposes (Hahn, Koldovský, Křeček, Martínek & Vacek, 1961a). These amino acids must, however, represent, together with the glycerol released on hydrolysis of lipids, a major group of precursors for gluconeogenesis.

Krebs (1964) pointed out that sites of regulation of gluconeogenesis probably exist at the initial steps in the degradation of gluconeogenic amino acids. The development of the key enzymes involved and their regulatory role in neonatal gluconeogenesis are thus of interest. The work described in the present paper was designed to provide further evidence for the involvement of several metabolites, and amino acids in particular, as precursors for gluconeogenesis. In contrast with other studies on neonatal liver, the net formation of and the incorporation of label into total carbohydrate, i.e. glucose plus glycogen, was measured. Liver slices were used because size makes perfusion of neonatal rat liver impracticable. A preliminary account has been given (Vernon, Eaton & Walker, 1967).

MATERIALS AND METHODS

Animals. The rats were an albino Wistar strain; the normal dietary and weaning regimens and the method for assessing gestational age were as described by Vernon & Walker (1968). Adult animals were normal males about 3

months old. Animals to be starved were placed in wire-bottomed cages and allowed water *ad lib*. Phlorrhizin was dissolved in 0.1–1.0 ml. of aq. 50% (v/v) butane-2,3-diol, depending on the amount to be administered, and a dose of 1g./kg. body wt. was injected subcutaneously into the back as described by Krebs, Notten & Hems (1966b). The time sequences for these operations are given along with the results. Newborn rats were not treated with phlorrhizin because of the high mortality rate in preliminary experiments.

Krebs *et al.* (1966b) showed that for the measurement of the net synthesis of glucose plus glycogen it is essential to lower the initial carbohydrate content of the liver. The effectiveness of the present treatments (see the legend to Table 1 for further details) were such as to produce livers containing not more than 6 μ moles of glucose plus glycogen (expressed as glucose)/g. wet wt. of tissue for rats aged 3–4 days, 14–15 days, 28–29 days and adults. The newborn animals were aged 12–24 hr.; this is the stage immediately after that during which the liver glycogen content falls precipitously from the high concentration at birth (Shelley, 1961). The initial glucose plus glycogen concentrations in these newborn animals were nevertheless very variable, ranging between 4 and 15 μ moles of carbohydrate/g. wet wt. of tissue.

Materials. Sodium [2- 14 C]pyruvate, L-[U- 14 C]malic acid, L-[U- 14 C]alanine, L-[U- 14 C]serine, L-[U- 14 C]threonine, L-[U- 14 C]aspartic acid, L-[U- 14 C]proline and U- 14 C-labelled amino acid mixture were obtained from The Radiochemical Centre (Amersham, Bucks.). The components of the 14 C-labelled amino acid mixture (as given by the suppliers, with the percentages of the total radioactivity in parentheses) is: L-alanine (10.0), L-arginine (6.5), L-aspartic acid (9.0), L-glutamic acid (12.5), glycine (5.0), L-leucine (12.0), L-isoleucine (5.0), L-lysine (5.5), L-phenylalanine (7.0), L-proline (6.0), L-serine (5.0), L-threonine (6.0), L-tyrosine (3.5) and L-valine (7.0). This composition is not dissimilar to that of the 14 C-labelled protein hydrolysate from *Chlorella* protein used in the study on neonatal gluconeogenesis by Yeung & Oliver (1967a), except that the latter hydrolysate contains about 5% of radioactivity not associated with amino acids and whose possible involvement in gluconeogenesis is unknown.

Phlorrhizin, pure amino acids, pyridoxal phosphate and the Dowex 50W (X8; H⁺ form) and Dowex 4 (OH⁻ form) resins (both 20–50 U.S. mesh) were obtained from British Drug Houses Ltd. (Poole, Dorset). The commercial glucoamylase preparation was Agidex, a gift from Glaxo Laboratories Ltd. (Ulverston, Lancs.); this enzyme hydrolyses both α -(1 $\rightarrow$ 4)- and α -(1 $\rightarrow$ 6)-linkages and, in test experiments under the conditions employed below, hydrolysed 92–95% of glycogen. Muscle lactate dehydrogenase (EC 1.1.1.27) (360 units/mg.), peroxidase (EC 1.11.1.7) and NADH were from Boehringer Corp. (London) Ltd. (London, W.5), and glucose oxidase (EC 1.1.3.4) (type II) was from Sigma (London) Chemical Co. Ltd. (London, S.W.6). The casein hydrolysate was the 'Bacto vitamin-free casamino acids' prepared by Difco Laboratories (Detroit, Mich., U.S.A.) and obtained through Baird and Tatlock (London) Ltd. (Chadwell Heath, Essex).

Measurement of net gluconeogenesis. Liver slices from animals previously treated as described in the Results section were cut free-hand with a razor blade. The slices (approx. 100 mg. and weighed on a torsion balance) were

placed in 4 ml. of the saline medium of Krebs & Henseleit (1932) in a conventional Warburg flask and gassed with O₂ + CO₂ (95:5) at 37°. These and other conditions were as described by Krebs *et al.* (1966b). The substrate concentration was 10 mM and the incubation time was 1 hr. The procedures for stopping the reaction, homogenization of the whole contents of the flask, extraction of the glycogen, hydrolysis of the glycogen with glucoamylase and estimation of glycogen plus glucose (as glucose) with glucose oxidase were essentially as described by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963).

Isotope incorporation experiments. The conditions of incubation of tissue slices prepared from normal untreated animals were similar to those used above except that the incubation medium contained approx. 100 mg. of slices, 20 mM-substrate and 1 μ C of radioactive precursor in 2 ml. of the saline incubation medium. The reaction was stopped after 1 hr. by the addition of 1 ml. of 0.81 M-HClO₄ and a procedure similar to that described above was followed. Suitable portions (0.1–0.5 ml.) of the total homogenate were diluted to 2 ml. with 0.27 M-HClO₄ and were used for the estimation and hydrolysis of glycogen. At the end of the glucoamylase treatment samples (1 ml.) of the protein-free filtrate were adjusted to pH 7.0–7.5 with NaOH and HCl (with narrow-range pH paper) and the volumes made up to 1.2 ml. Portions (0.5 ml.) of this neutralized extract were run on columns (11 cm. $\times$ 0.5 cm.) containing a 1:1 (w/w) mixture of Dowex 50W (X8; H⁺ form) and Dowex 4 (OH⁻ form) resins and washed into the columns with 0.5 ml. of water. After standing for 1 hr. the columns were washed with 5 ml. of water. Glucose appeared in this eluate, which was collected directly into scintillation vials; the eluate was evaporated to dryness *in vacuo* in a desiccator containing conc. H₂SO₄ and pellets of NaOH. The residue was dissolved in 0.2 ml. of water, and 5 ml. of Ditol (Herberg, 1960) was added. Radioactivity was counted at about 70% efficiency in a Nuclear-Chicago model no. 725 automatic scintillation spectrometer, and counts were corrected by the channels-ratio procedure to 100% efficiency.

Control experiments performed in the presence of salt concentrations identical with those present in the normal incubation procedure and with [14 C]glucose showed that the recovery of glucose by this procedure was 98–101% over the range 200–2000 counts/min.; the addition of appropriate amounts of non-radioactive gluconeogenic precursors had no effect on these recoveries. A second set of control experiments with appropriate concentrations and radioactivities of labelled precursors showed that the following percentages of the initial counts/min. applied to the mixed ion-exchange columns were not retained by the columns: pyruvate, 0.76; L-malate, 0.29; L-alanine, 0.57; L-amino acid mixture, 0.33; L-aspartate, 0.58; L-serine, 0.63; L-threonine, 0.56; L-proline, 0.46. Preliminary experiments with all the radioactive precursors showed that the rate of incorporation was directly proportional to time during the 60 min. incubation period. The nature of the product eluted from the mixed ion-exchange resins was examined by descending chromatography on Whatman no. 1 paper for 40 hr. in propan-1-ol-ethyl acetate-water (7:1:2, by vol.). The chromatograms were scanned with a Packard Radiochromatogram Scanner and indicated a single radioactive spot having the same *R_F* as [14 C]glucose controls.

Enzyme assays. All the enzymes were assayed in liver supernatant preparations obtained by centrifugation at

100000g for 30 min. of 33½% (w/v) homogenates in 0.25M-sucrose. Both L-serine dehydratase (EC 4.2.1.13) and L-threonine dehydratase (EC 4.2.1.16) activities were assayed by a continuous method involving reduction by NADH in the presence of lactate dehydrogenase of the reaction products pyruvate and α -oxobutyrate respectively. Because both of the enzymes have very low affinities for their substrates, the substrate concentration used was fixed at the non-saturating concentration of 100mM. The general conditions of assay were those of Holzer, Boll & Cennamo (1963). The incubation mixtures contained (final concentrations) in a total volume of 1ml.: triethanolamine buffer, pH 7.9 (50mM), mercaptoethanol (10mM), L-serine or L-threonine (100mM), pyridoxal phosphate (0.1mM), lactate dehydrogenase (18 units), NADH (0.5mM) and 0.1ml. of enzyme preparation. The reaction mixtures were preincubated in the absence of substrate for 5 min. at 30°, and then the substrate was added and the rate of NADH oxidation measured by a Gilford model 2000 Multiple Sample Absorbance Recorder attached to a Unicam SP.500 Spectrophotometer. Alanine transaminase (EC 2.6.1.2) was assayed in the same liver preparations by the method of Bergmeyer & Bernt (1963) as used by Yeung & Oliver (1967a).

RESULTS

The incorporation of [2-¹⁴C]pyruvate into glucose plus glycogen (Fig. 1) in neonatal rat liver is similar to the pattern of incorporation into glycogen alone described by Ballard & Oliver (1963). It parallels closely the phosphopyruvate carboxylase activities reported previously (Vernon & Walker, 1968); this correlation has also been noted by Yeung & Oliver (1967b). Under the same conditions the incorpora-

tion of L-malate is very low. Because isotope incorporation in this type of system is no proof of net synthesis (see, e.g., Krebs, Hems, Weidemann &

Fig. 1. Incorporation of [2-¹⁴C]pyruvate (○) and L-[U-¹⁴C]-malate (□) into glucose plus glycogen in rat liver slices as a function of age. The substrate concentration in each case was 20mM and the incubation medium (2ml.) contained approx. 1μC of radioactivity. Further details are given in the text. The rates of incorporation are expressed as percentages of the initial counts/min. in the incubation medium incorporated/hr./100mg. of liver. Each point represents the mean of five determinations and the vertical bar shows $\pm$ S.E.M., when large enough to record. Δ , Mean glucose + glycogen content of rat liver slices. T, Term; A, adult.

Table 1. *Gluconeogenesis from various precursors in liver slices from rats of various ages*

The adult males, 28–29-day-old and 14–15-day-old rats were starved overnight and treated with phlorrhizin 6 hr. before being killed. The 3–4-day-old rats were not starved, but were treated with phlorrhizin similarly. The newborn rats were 12–24 hr. old and were not treated before being killed. Slices were incubated for 1 hr. in the presence of 10mM-substrate as described in the text. The endogenous gluconeogenesis in the absence of added substrate is given in the first line; all other results are corrected for this endogenous formation of carbohydrate. The results are given as means $\pm$ S.E.M. with the numbers of observations in parentheses.

Substrate added	Gluconeogenesis [μ moles of glucose + glycogen (expressed as glucose) formed/min./g. wet wt. of tissue]				
	Newborn	3–4 days old	14–15 days old	28–29 days old	Adult males
None	0.12 $\pm$ 0.02 (22)	0.12 $\pm$ 0.03 (22)	0.10 $\pm$ 0.01 (36)	0.10 $\pm$ 0.01 (21)	0.12 $\pm$ 0.03 (12)
D-Fructose	0.63 $\pm$ 0.06 (19)	0.79 $\pm$ 0.12 (5)	1.09 $\pm$ 0.16 (8)	0.71 $\pm$ 0.11 (12)	1.00 $\pm$ 0.14 (3)
Sorbitol	0.07 $\pm$ 0.03 (9)	0.31 $\pm$ 0.08 (5)	0.70 $\pm$ 0.09 (6)	0.67 $\pm$ 0.11 (11)	
Glycerol	0.09 $\pm$ 0.02 (12)	0.08 $\pm$ 0.02 (8)	0.30 $\pm$ 0.02 (6)	0.35 $\pm$ 0.03 (13)	0.53 $\pm$ 0.02 (3)
L-Lactate	0.19 $\pm$ 0.04 (11)	0.33 $\pm$ 0.02 (6)	0.48 $\pm$ 0.11 (6)	0.25 $\pm$ 0.04 (10)	0.29 $\pm$ 0.08 (6)
Oxaloacetate	0.16 $\pm$ 0.03 (6)	0.15 $\pm$ 0.04 (8)	0.32 $\pm$ 0.04 (11)	0.19 $\pm$ 0.02 (4)	0.19 $\pm$ 0.03 (6)
Casein hydrolysate	–0.08 $\pm$ 0.01 (5)	0.07 $\pm$ 0.02 (8)	0.07 $\pm$ 0.01 (6)	0.14 $\pm$ 0.02 (6)	0.13 $\pm$ 0.01 (8)
L-Alanine	0.06 $\pm$ 0.03 (11)	0.02 $\pm$ 0.05 (5)	0.08 $\pm$ 0.07 (5)	0.17 $\pm$ 0.05 (5)	0.27 $\pm$ 0.03 (6)
L-Serine	0.12 $\pm$ 0.05 (3)	0.16 $\pm$ 0.03 (11)	0.22 $\pm$ 0.07 (5)	0.15 $\pm$ 0.03 (5)	0.17 $\pm$ 0.01 (6)
L-Threonine	0.01 $\pm$ 0.01 (3)	0.04 $\pm$ 0.03 (7)	0.05 $\pm$ 0.02 (6)	0.05 $\pm$ 0.02 (5)	0.07 $\pm$ 0.02 (6)
L-Aspartate	–0.08 $\pm$ 0.02 (15)	–0.07 $\pm$ 0.03 (9)	0.03 $\pm$ 0.01 (6)	0.05 $\pm$ 0.02 (6)	0.03 $\pm$ 0.03 (4)
L-Glutamate	0.00 $\pm$ 0.02 (6)	–0.06 $\pm$ 0.02 (5)	0.04 $\pm$ 0.02 (20)	0.01 $\pm$ 0.02 (9)	
L-Proline	–0.02 $\pm$ 0.02 (5)	0.02 $\pm$ 0.01 (8)	0.03 $\pm$ 0.01 (6)	0.06 $\pm$ 0.02 (6)	0.04 $\pm$ 0.01 (11)

Fig. 2. Incorporation of various $\text{U-}^{14}\text{C}$ -labelled amino acids into glucose plus glycogen in rat liver slices as a function of age. In each case the final concentration of amino acid was 20mM; an average molecular weight of 130 was assumed for the amino acid mixture and for the casein hydrolysate. Each incubation medium (2ml.) contained approx. $1\mu\text{C}$ of radioactivity. Further details are given in the text. (a) Incorporation of a $\text{U-}^{14}\text{C}$ -labelled L-amino acid mixture diluted to a final concentration of 20mM with an amino acid mixture of the same composition (for details see the Materials and Methods section) into glucose plus glycogen. (b), (c), (d), (e) and (f), Incorporation of L-[$\text{U-}^{14}\text{C}$]alanine, L-[$\text{U-}^{14}\text{C}$]serine, L-[$\text{U-}^{14}\text{C}$]threonine, L-[$\text{U-}^{14}\text{C}$]aspartate and L-[$\text{U-}^{14}\text{C}$]proline respectively into glucose plus glycogen in the presence of 20mM-amino acid (○) or 20mM-casein hydrolysate (□). The rates of incorporation are expressed as percentages of the initial counts/min. in the incubation medium incorporated/hr./100mg. of liver. Each point represents the mean of three to five determinations and the vertical bar shows $\pm$ S.E.M., when large enough to record. T, Term; A, adult.

Speake, 1966a) and perfusion of neonatal rat liver is not possible, liver slices were used in an attempt to demonstrate increased net synthesis of glucose plus glycogen in neonatal rat liver.

Table 1 shows the compiled results of a series of experiments in which liver slices from animals of several different selected ages were incubated in the presence of 12 different substrates. The values for adult male animals are similar to, but a little lower than, those given by Ross, Hems & Krebs (1967). This difference is almost certainly accounted for by the fact that the rats used by Ross *et al.* (1967) had been starved for a longer period (48 hr.); the sex difference noted for mice by Krebs *et al.* (1966b) may also be contributory. The different conditions of the animals at various ages, as indicated in the Materials and Methods section and in the legend to Table 1, rule out statistical analyses of the differences with age in many instances, but the results permit several conclusions to be drawn. The net synthesis of glucose plus glycogen from the amino acids was, in general, very low. The net synthesis from L-serine was highest at 14–15 days, whereas that from the casein hydrolysate or from L-threonine was particularly low in the newborn animals. The pronounced increase with age in the synthesis from L-alanine is also noteworthy. Very low rates of net synthesis, similar to those shown for L-glutamate, were also recorded for succinate, L-glutamine and L-valine.

Of the non-amino acid substrates tested, the rate of glucose plus glycogen formation from L-lactate (Table 1) showed a peak value at 14–15 days, but this is not significantly higher ($0.1 < P < 0.2$) than that at 28–29 days because of the wide scatter of values recorded. A similar peak value was observed for oxaloacetate and this was significantly higher than that for adults ($P < 0.05$). The rate of fructose conversion was surprisingly high in newborn animals, but sorbitol was a poor substrate in these animals. Glycerol was a poor substrate in the newborn and 3–4-day-old animals compared with later ages.

The incorporation of label from certain individual amino acids into glucose plus glycogen (Fig. 2) was studied under two sets of conditions. In one, the slices (approx. 100 mg. wet wt.) were incubated with 20 mM-amino acid and 1.0 μ C of radioactivity in 2 ml. of medium. In the other the same tracer amount of radioactive amino acid was present, but the incubation medium contained 20 mM-casein hydrolysate, this concentration referring to the sum of the concentrations of the amino acids and assuming an overall molecular weight of 130. In these latter conditions, the specific radioactivity of the substrates will be much higher (15–20-fold) and this fact clearly results in a higher incorporation of label into glucose plus glycogen in liver slices at all ages

Fig. 3. Activities of L-serine dehydratase (Δ) and L-threonine dehydratase ($\circ$) in the liver supernatant as a function of age. Each point represents the mean of four to eight determinations and the vertical bar shows $\pm$ s.e.m., when large enough to record. Details of the methods are given in the text. T, Term; A, adult.

from alanine, aspartate and proline (Figs. 2b, 2e and 2f respectively). Serine and threonine (Figs. 2c and 2d) are different in this respect, probably owing to the very low affinities of the L-serine dehydratase and L-threonine dehydratase for their substrates (e.g. Nakagawa, Kimura & Miura, 1967). The incorporation of label by foetal liver slices was very low from all the substrates tested. The developmental pattern for incorporation from the amino acid mixture (Fig. 2a) is copied, with certain quantitative variations, by the profiles for the five individual amino acids examined (Figs. 2b–2f). In all cases the ability to incorporate label increased rapidly in the early postnatal livers. The profiles for the incorporation of alanine, serine and threonine showed pronounced peaks some days after birth.

Enzyme activities. Measurement of the activities of L-serine dehydratase and L-threonine dehydratase in liver samples from animals of various ages revealed that these enzymes develop immediately after birth and have maximum activities about 20 days later (Fig. 3). Hepatic cytoplasmic alanine transaminase activity was found to be very low during the early neonatal period and to increase during weaning in a manner analogous to that described by Yeung & Oliver (1967a).

DISCUSSION

The existence of permeability barriers in liver slices to a number of precursors of gluconeogenesis was noted by Krebs *et al.* (1966b). Ross *et al.* (1967)

examined this problem further and obtained substantially higher rates of gluconeogenesis from many precursors, including many amino acids, with the perfused liver; there must be a barrier at the outer liver cell membrane (Hems, Stubbs & Krebs, 1968) to these metabolites that is no longer effective in preparations involving homogenization. This permeability problem, along with the difficulties in preparing the animals at the different ages in exactly the same way before they are killed, makes the net synthesis experiments reported in this paper of limited value. Nevertheless they indicate lower rates of gluconeogenesis in the newborn animals from several precursors and rates higher than in the adult for lactate and oxaloacetate at 14–15 days, just before weaning. The newborn animals were at the age range during which the phosphopyruvate carboxylase activity is rising rapidly; considerable variations in results at this time are thus to be expected.

If the observations by Christensen & Clifford (1963) of an intensification of the hepatic accumulation of amino acids during the first day after birth in the guinea pig are applicable to the newborn rat, such a change could have a bearing on the isotope incorporation experiments. The effect of the size of the intracellular pool of amino acids on the results of these experiments is not likely to be serious, however. The intracellular pool size will, from the data of Wise & Oliver (1967), be less than 10% of the total amino acid pool. In the experiments in which the individual amino acid concentration in the incubation medium was 20mM, this concentration will exceed that in the tissue slice even though it is less than the total amino acid concentration in the slice (this being about 38mM, assuming a mean amino acid molecular weight of 130 and distribution of amino acid throughout the whole tissue). In the experiments with 20mM-casein hydrolysate, the total tissue amino acid concentration is higher than that in the incubation medium, but the concentrations of the individual amino acids will be more nearly comparable.

These rates of incorporation are likely to be a reasonable estimate of the actual rate of gluconeogenesis in liver slices, because there is fair agreement between the rates of synthesis of glucose plus glycogen when calculated as $\mu\text{moles formed/min./g.}$ of liver from the incorporation data and the rates for net synthesis quoted for similar adult rat liver slices by Ross *et al.* (1967). The value of $22.8 \mu\text{moles of } [2\text{-}^{14}\text{C}]\text{pyruvate incorporated into glucose plus glycogen/hr./g. of liver}$ for adult rats for slices in a high- Na^+ medium also bears comparison with the values of 12.1 and $13.4 \mu\text{moles/hr./g. of liver}$ for the rates of incorporation of $[2\text{-}^{14}\text{C}]\text{pyruvate}$ into glycogen and into glucose respectively for similar slices in a high- K^+ medium (Ballard & Oliver, 1963, 1965).

It is therefore reasonable to conclude that there is a rapid increase in gluconeogenesis from amino acids into glucose plus glycogen after birth that roughly parallels the rapid rise in phosphopyruvate carboxylase activity. This increase is quicker than the increase in gluconeogenesis reported by Yeung & Oliver (1967a), who used a very low amino acid concentration in the incubation medium and obtained a much lower rate of incorporation; formation of glucose rather than glycogen may occur in the first few days after birth, when the glucose 6-phosphatase activity is very high (Vernon & Walker, 1968).

Comparison of the absolute rates of isotope incorporation under the two sets of incubation conditions is difficult. The specific radioactivity for the individual amino acid varied on average about 20-fold between the two types of experiments, yet the rates did not differ by more than about twofold (Fig. 2). This may be partly explained as follows. When the casein hydrolysate is used, there might be some competition for entry between certain amino acids; there will almost certainly be dilution of intermediates before the phosphopyruvate carboxylase step if the latter is rate-controlling, and the more balanced supply of amino acids may favour protein synthesis to the debit of gluconeogenesis.

The known metabolic pathways of gluconeogenesis (Krebs, 1964) suggest that all amino acids to be converted into carbohydrate proceed via oxaloacetate and the phosphopyruvate carboxylase step. The development of this enzyme could therefore be a sufficient site of control in the initiation of the overall process from amino acids in the newborn rat. Protein synthesis in the liver must occur at all stages of development, however, so that additional sites of control in the initial steps of amino acid degradation could be expected. This might be so for, e.g., alanine and serine, where the rates of incorporation (Fig. 2b and 2c respectively) at all ages are lower than that of pyruvate, the immediate product of their catabolism. Yeung & Oliver (1967a) listed facts on the development of enzymes involved in the degradation of several amino acids, and the development of the enzymes L-serine dehydratase and L-threonine dehydratase reported in the present paper (and also briefly noted by Greengard & Dewey, 1967) extends this list. There is a good correlation between these two activities and the isotope incorporation data for the respective amino acids (Figs. 2c and 2d respectively). The confirmation of the developmental activity profile for cytoplasmic alanine transaminase raises an as yet unexplained discrepancy between this enzyme activity and the net synthesis results for alanine (Table 1) on the one hand and the rate of incorporation of $[^{14}\text{C}]\text{alanine}$ into carbohydrate (Fig. 2b) on the other.

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