Pyruvate Dehydrogenase Activity in Hamster Small Intestine during Development

By CAROL M. SCHILLER

*Environmental Toxicology Branch, National Institute of Environmental Health Sciences,
Research Triangle Park, NC 27709, U.S.A.*

(Received 9 December 1976)

Total pyruvate dehydrogenase activities in hamster intestine increase from 40 nmol/min (munits) per g of intestine in the foetal animals to 460 munits/g in the adult, whereas the fraction of the enzyme in the active form increases from 34 to 42 % of the total activity over the same period. However, a complete conversion of the enzyme into the active form is observed in the neonatal animal immediately after birth. Results from experiments *in vitro* suggested that the activation of pyruvate dehydrogenase is controlled, in part, by the $[NAD^+]/[NADH]$ ratio. This proposal was tested *in vivo* by examining the proportion of the enzyme in the active form during conditions when the $[NAD^+]/[NADH]$ ratio was markedly altered, and the data show a direct relationship between the mitochondrial redox state and activity of the active form.

Pyruvate is converted into acetyl-CoA in the mitochondrion by pyruvate dehydrogenase (EC 1.2.4.1), an enzyme that can be regulated by activation and inactivation reactions which are catalysed by other enzymes of the pyruvate dehydrogenase multi-enzyme complex (Linn *et al.*, 1969*a,b*). Thus phosphorylation and the concomitant inactivation of the enzyme is catalysed by a $MgATP^{2-}$ -requiring kinase, and dephosphorylation and concomitant re-activation is catalysed by a Mg^{2+} - and Ca^{2+} -requiring phosphatase (Linn *et al.*, 1969*a,b*; Denton *et al.*, 1972; Siess & Wieland, 1972; Pettit *et al.*, 1972). Results obtained with a purified mammalian pyruvate dehydrogenase system indicate that the kinase and phosphatase maintain a steady-state activity of the complex, and that this activity is modulated by the pyruvate, K^+ (possibly NH_4^+), uncomplexed Mg^{2+} and Ca^{2+} concentrations (Linn *et al.*, 1969*a*; Hucho *et al.*, 1972; Reed *et al.*, 1973; Roche & Reed, 1974) as well as by the $[ATP]/[ADP]$, $[acetyl-CoA]/[CoA]$ and $[NAD^+]/[NADH]$ ratios (Pettit *et al.*, 1975; Cooper *et al.*, 1975).

The role of pyruvate dehydrogenase in mammalian tissues may be expected to change during development. The proportions of nicotinamide-adenine dinucleotides are altered by a greater tissue oxygenation in the neonatal animal (Ballard, 1971*a,b*). The oxygenation may favour the conversion of pyruvate dehydrogenase into the active form in the intact organ as a consequence of a change in mitochondrial redox state. In the present study, changes in the total and active pyruvate dehydrogenase activities have been measured in developing intestine, and the role of the

mitochondrial redox state in the regulation of the enzyme has been investigated.

Materials and Methods

Biochemicals

Cofactors, substrates and enzyme standards for the enzymic measurement of intestinal metabolites were obtained from Boehringer-Mannheim, New York, NY, U.S.A. Sodium $[1-^{14}C]$ pyruvate was obtained from New England Nuclear, Boston, MA, U.S.A. Bovine serum albumin (fraction V), essentially fatty acid-free, was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals

The hamsters were obtained as pregnant Syrian golden-hamster females, LVG/LAK strain, from Charles River/Lakeview, Wilmington, MA, U.S.A. The animals were specific-pathogen-free, and were fed *ad libitum* on Wayne Sterilizable Lab-Blox. The animals were dated from time of observed breeding, assuming a 16-day gestational period. Animals were killed by decapitation, intestinal samples were excised quickly and dropped into liquid N_2 . The frozen tissue samples were weighed and homogenized as a 7.5 % (w/v) homogenate in ice-cold assay buffer. The tissue homogenates were refrozen in liquid N_2 and thawed twice in ice. In the perinatal experiments, foetal tissue was taken approx. 12 h before birth and neonatal tissue was taken approx. 12 h after birth. In the experiments where the neonatal hamsters were made anoxic, the 1-day-old (approx. 36 h after birth)

animals were kept at 37°C in a N₂ atmosphere for 15 min before the tissue was excised and frozen.

Assay of pyruvate dehydrogenase

The activities were determined by the stoichiometric release of ¹⁴CO₂ from [1-¹⁴C]pyruvate (Taylor *et al.*, 1973); all buffers and the assay mixture were prepared as described for this technique. After 2 min of preincubation of 0.3 ml of assay mixture at 37°C, 0.1 ml of tissue homogenate was injected into the assay vial and incubated for an additional 2 min at 37°C. The reaction was terminated by the injection of 0.8 ml of citrate/phosphate buffer, pH 3. Each assay was performed in triplicate. Results were corrected for blank values obtained from vials in which 0.1 ml of homogenizing buffer replaced the tissue homogenate. This assay method was linear with time and protein concentration within the

ranges used. The optimum activation conditions for intestinal pyruvate dehydrogenase were determined to be exposure to 15 mM-MgCl₂ at 37°C for 2.5 min. Samples of intestinal tissue were assayed before and after Mg²⁺ activation to determine the active and total pyruvate dehydrogenase activities. A unit of activity is 1 μ mol/min.

Measurements of metabolites and calculations of redox state

Samples (30–90 mg/30 ml of liquid N₂) of frozen intestine were homogenized in ice-cold 5% (v/v) HClO₄. After the protein was removed by centrifugation at 1000 g for 5 min, the supernatant fluids were neutralized to pH 7.0 with KOH. The clear extracts were then used as samples for the specific enzymic determinations of various intermediates: lactate, pyruvate, β -hydroxybutyrate and aceto-

Fig. 1. Changes in total pyruvate dehydrogenase activity in intestine from developing hamster. Activities are expressed as either munits/mg of intestinal protein (○) or munits/g of intestine (●). Values are means $\pm$ S.E.M. for eight determinations. B, Birth; A, adult.

Fig. 2. Changes with development in hamster intestine in the percentage of pyruvate dehydrogenase as the active form or the amount of active enzyme

Active pyruvate dehydrogenase activity is expressed as a percentage of the total activity (○) or as munits/g of intestine (●). Values are means $\pm$ S.E.M. for eight determinations. B, Birth; A, adult.

acetate (Denton & Halperin, 1968; Williamson & Corkey, 1969). The cytosolic and mitochondrial $[NAD^+]/[NADH]$ ratios were calculated from the $[lactate]/[pyruvate]$ and $[\beta\text{-hydroxybutyrate}]/[acetoacetate]$ values respectively by the methods previously described (Williamson, 1969; Ballard, 1971a,b), by using the values of 1.11×10^{-4} for $K_{lactate \text{ dehydrogenase}}$ and 4.93×10^{-2} for $K_{\beta\text{-hydroxybutyrate dehydrogenase}}$.

Measurements of mitochondrial marker enzymes

β -Hydroxybutyrate dehydrogenase and citrate synthase activities were measured spectrophotometrically in the whole homogenates from foetal and neonatal hamster intestine by the methods of Williamson (1969) and Schiller *et al.* (1974) respectively.

Protein analysis

Total protein concentrations in the intestinal homogenates were determined by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and Discussion

Changes in activity during development

Total activity in intestine increases markedly after birth, reaches a plateau before weaning, and then decreases somewhat to the adult value of 460 munits/g of intestine (Fig. 1). This pattern is also observed if the data are plotted as munits/mg of intestinal protein. It is probable that the increase in pyruvate dehydrogenase activity with age parallels a general increase in the mitochondrial activity in intestine, since malate dehydrogenase, aspartate aminotransferase, citrate synthase and flavoprotein-L-glycerol 3-phosphate dehydrogenase measured in isolated mitochondria all show a similar increase in activity in the newborn-hamster intestine (C. M. Schiller & R. Walden, unpublished work). However, the real functional importance of intestinal pyruvate dehydrogenase may change independently of alterations in the activities of other mitochondrial enzymes as a result of the interconversion between the active and inactive forms of pyruvate dehydrogenase. The intestinal active enzyme increases sharply after birth, reaches a plateau and decreases to the adult value (206 munits/g of intestine) (Fig. 2). This pattern is in sharp contrast with that observed for liver, where the active pyruvate dehydrogenase remains relatively constant, i.e. 100–200 munits/g of liver, throughout development (Knowles & Ballard, 1974). Also, Fig. 2 shows that the proportion of enzyme in the active form reaches a peak of 100% during the perinatal period and then declines to the adult value of about 40–60%. This perinatal increase in the percentage of enzyme in the active form is unlike the

gradual postnatal decline seen in liver (Knowles & Ballard, 1974). It is therefore likely that changes in the amount of active pyruvate dehydrogenase contribute to the changeover from glycolysis in the foetus to oxidative decarboxylation after birth.

Effects of changing redox state on pyruvate dehydrogenase activity

Although the factors responsible for the regulation of pyruvate dehydrogenase *in vivo* have not been elucidated, it is clear from experiments with the purified pyruvate dehydrogenase complex that a decrease in the $[NAD^+]/[NADH]$ ratio increases the proportion of the phosphorylated form of the enzyme (Linn *et al.*, 1969a). The activity of pyruvate dehydrogenase kinase is inhibited by NAD^+ ; conversely, $NADH$ inhibits pyruvate dehydrogenase phosphatase, and this inhibition is reversed by NAD^+ (Pettit *et al.*, 1975; Cooper *et al.*, 1975). The calculated free $[NAD^+]/[NADH]$ ratio in the intestinal cytosol increases from 37 in the foetus to 131 in the newborn, whereas the increase in the calculated mitochondrial free $[NAD^+]/[NADH]$ ratio was from 18 to 28 at the same developmental stages (Table 1). At these two stages, the total pyruvate dehydrogenase activity decreases from 151 to 121 munits/g of intestine, the activity of the active form increases from 35 to 85 munits/g of intestine and the percentage of pyruvate dehydrogenase in the active form increases from 22 to 75% (Table 1). These changes agree with the developmental patterns described here and also with the changes in calculated $[NAD^+]/[NADH]$ ratios. The activities of other mitochondrial enzymes have been measured in tissue from the same animals in order to observe any general trend during the postnatal period. β -Hydroxybutyrate dehydrogenase activity remains relatively constant, whereas citrate synthase increases slightly (Table 1). The protein content also remains constant (Table 1). It is concluded that intestinal pyruvate dehydrogenase conversion does respond during the perinatal period to oxygenation in a manner that would be expected from observations *in vitro* (Linn *et al.*, 1969a).

A reversal of the oxygenation process associated with birth was simulated by exposing neonatal animals to a N_2 atmosphere for 15 min before excising the tissue. The effects of hypoxia are demonstrated in Table 2; the calculated cytosolic free $[NAD^+]/[NADH]$ ratio fell from 167 to 157 and the mitochondrial ratio fell from 42 to 16 in the treated newborns. Under these conditions, the total pyruvate dehydrogenase decreased from 113 to 82 munits/g of intestine, the active enzyme activity decreased from 113 to 56 munits/g of intestine, and the percentage of pyruvate dehydrogenase in the active form decreased from 108 to 67%. The effects of hypoxia on the other mitochondrial enzymes were that the β -hydroxybutyrate dehydrogenase increased slightly and the

Table 1. *Changes in hamster intestine at the perinatal period*

Enzyme activities are expressed as munits/g of intestine for pyruvate dehydrogenase and as units/g of intestine for β -hydroxybutyrate dehydrogenase and citrate synthase. Metabolite contents are expressed as $\mu\text{mol/g}$ of intestine. Values are means $\pm$ S.E.M. for between 8 and 12 determinations. * $P < 0.01$, † $P < 0.05$ for difference between foetal and neonatal values. For other differences $P > 0.05$. These P values were determined by the Mann-Whitney U test. Details of experimental design, measurements and calculations are given in the Materials and Methods section.

	Foetus (12h before birth)	Newborn (12h after birth)
Total pyruvate dehydrogenase activity	151 $\pm$ 17	121 $\pm$ 20
Active pyruvate dehydrogenase	35.0 $\pm$ 7.1	85.3 $\pm$ 9.5*
% of pyruvate dehydrogenase as the active form	21.9 $\pm$ 2.7	74.9 $\pm$ 9.2*
β -Hydroxybutyrate dehydrogenase	0.935 $\pm$ 0.136	0.863 $\pm$ 0.087
Citrate synthase	9.27 $\pm$ 0.74	11.5 $\pm$ 0.7
Protein (mg/g of tissue)	57.1 $\pm$ 3.9	52.4 $\pm$ 2.6
Lactate	32.5 $\pm$ 1.3	12.1 $\pm$ 1.1*
Pyruvate	0.131 $\pm$ 0.033	0.169 $\pm$ 0.016
Calculated $[\text{NAD}^+]/[\text{NADH}]$ ratio (cytosol)	37.0 $\pm$ 8.8	131 $\pm$ 16*
β -Hydroxybutyrate	0.075 $\pm$ 0.005	0.078 $\pm$ 0.019
Acetoacetate	0.063 $\pm$ 0.003	0.095 $\pm$ 0.009*
Calculated $[\text{NAD}^+]/[\text{NADH}]$ ratio (mitochondria)	17.6 $\pm$ 1.9	28.3 $\pm$ 3.3†

Table 2. *Changes in hamster intestine after exposure of newborns to a N_2 atmosphere*

Details of the experimental design, measurements and calculations of the data for 1-day-old (approx. 36 h after birth) hamsters, are given in the Materials and Methods section. Enzyme activities and concentrations are as in Table 1. Values are the means $\pm$ S.E.M. for between 8 and 12 determinations. * $P < 0.01$, † $P < 0.05$ for difference between normal and treated values. For other differences $P > 0.05$.

	Normal	Treated
Total pyruvate dehydrogenase activity	113 $\pm$ 11	81.5 $\pm$ 7.4
Active pyruvate dehydrogenase	113 $\pm$ 8	55.8 $\pm$ 12.1*
% of pyruvate dehydrogenase as the active form	108 $\pm$ 15	66.9 $\pm$ 10.4†
β -Hydroxybutyrate dehydrogenase	6.53 $\pm$ 0.86	7.78 $\pm$ 0.21
Citrate synthase	14.7 $\pm$ 1.3	10.3 $\pm$ 0.4
Protein (mg/g of tissue)	74.1 $\pm$ 7.5	74.3 $\pm$ 1.8
Lactate	20.4 $\pm$ 1.6	25.8 $\pm$ 3.6
Pyruvate	0.372 $\pm$ 0.050	0.394 $\pm$ 0.047
Calculated $[\text{NAD}^+]/[\text{NADH}]$ ratio (cytosol)	167 $\pm$ 19	157 $\pm$ 23
β -Hydroxybutyrate	0.068 $\pm$ 0.013	0.146 $\pm$ 0.015*
Acetoacetate	0.105 $\pm$ 0.012	0.100 $\pm$ 0.006
Calculated $[\text{NAD}^+]/[\text{NADH}]$ ratio (mitochondria)	41.6 $\pm$ 12.0	16.3 $\pm$ 2.4*

citrate synthase decreased. A similar increase in the specific activity of β -hydroxybutyrate dehydrogenase during the neonatal period has been described in liver (Ballard, 1971a). The protein concentration remained constant.

Studies *in vitro* have shown that pyruvate dehydrogenase regulation is sensitive to changes in the concentrations of a number of physiological metabolites (Cooper *et al.*, 1975). Likewise, intestinal tissue taken from animals during the perinatal period contains pyruvate dehydrogenase activity that is sensitive to changes in redox state *in vivo*. The conversion of pyruvate dehydrogenase into its active form immediately after birth and after exposure to hypoxia is in the direction predicted by the concomitant change in

the calculated mitochondrial free $[\text{NAD}^+]/[\text{NADH}]$ ratio. Undoubtedly, as experimental measurements *in vivo* are refined and expanded, other metabolic control parameters may also be implicated and their effects on regulation of pyruvate dehydrogenase *in vivo* verified.

I thank Ms. S. Gipson and Mr. R. Walden for their excellent technical assistance in performing some of the assays.

References

- Ballard, F. J. (1971a) *Biochem. J.* **121**, 169–178
- Ballard, F. J. (1971b) *Biochem. J.* **124**, 265–274

- Cooper, R. H., Randle, P. J. & Denton, R. M. (1975) *Nature (London)* **257**, 808–809
- Denton, R. M. & Halperin, M. L. (1968) *Biochem. J.* **110**, 27–38
- Denton, R. M., Randle, P. J. & Martin, B. R. (1972) *Biochem. J.* **128**, 161–163
- Hucho, F., Randall, D. D., Roche, T. E., Burgett, M. W., Pelley, J. W. & Reed, L. J. (1972) *Arch. Biochem. Biophys.* **151**, 328–340
- Knowles, S. E. & Ballard, F. J. (1974) *Biol. Neonate* **24**, 41–48
- Linn, T. C., Pettit, F. H. & Reed, L. J. (1969a) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 234–241
- Linn, T. C., Pettit, F. H., Hucho, F. & Reed, L. T. (1969b) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 227–234
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Pettit, F. H., Roche, T. E. & Reed, L. J. (1972) *Biochem. Biophys. Res. Commun.* **49**, 563–571
- Pettit, F. H., Pelley, J. W. & Reed, L. J. (1975) *Biochem. Biophys. Res. Commun.* **65**, 575–582
- Reed, L. J., Pettit, F. H., Roche, T. E. & Butterworth, P. J. (1973) in *Protein Phosphorylation in Control Mechanisms* (Huijing, F. & Lee, E. Y. C., eds.), pp. 83–95, Academic Press, New York
- Roche, T. E. & Reed, L. J. (1974) *Biochem. Biophys. Res. Commun.* **59**, 1341–1348
- Schiller, C. M., Taylor, W. M. & Halperin, M. L. (1974) *Can. J. Biochem.* **52**, 813–821
- Siess, E. A. & Wieland, O. H. (1972) *Eur. J. Biochem.* **26**, 96–105
- Taylor, S. I., Mukherjee, C. & Jungas, R. L. (1973) *J. Biol. Chem.* **248**, 73–81
- Williamson, J. R. (1969) in *The Energy Level and Metabolic Control in Mitochondria* (Papa, S., Tager, J. M., Quagliariello, E. & Slater, E. C., eds.), pp. 385–400, Adriatica Editrice, Bari
- Williamson, J. W. & Corkey, B. E. (1969) *Methods Enzymol.* **13**, 434–513