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Development of Adenylate Kinase Isoenzymes in Rat Liver

By R. FILLER and W. E. CRISS*
Fels Research Institute, Temple University Medical School, Philadelphia, Pa., U.S.A.

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Total adenylate kinase activity was determined in developing rat liver. The activity was 18 units/g wet weight of tissue in foetal liver; this increased to 41 units/g immediately after birth and continued increasing until adult activities of 150 units/g were reached after two weeks. The adenylate kinase activity was separated into four isoenzymes. Only isoenzymes II and III were observed in foetal rat liver. Isoenzyme II activity was 2 units/g in the foetal liver and increased to 25 units/g in adult liver. Adenylate kinase III activity was 20 units/g in the foetal liver and increased to 118 units/g in adult liver. The possible role that adenylate kinase might have in regulating the energy flow in the developing liver cell is discussed.

Enzyme differentiation is the process whereby during the development of an organ in an animal, the organ acquires the quantitative and qualitative adult enzyme patterns (Greengard, 1969). Key enzymes in several metabolic processes have been found to undergo enzymic differentiation. These enzymes are involved in nitrogen metabolism (Wicks, Kenney & Lee, 1969), nucleic acid metabolism (Ove, Brown & Laszlo, 1969), glycolysis (Farina, Adelman, Lo, Morris & Weinhouse, 1968; Criss, 1969; Clark & Yochim, 1969), mitochondrial oxidation (deVos, Wilmink & Hommes, 1968), glycogen metabolism (Ballard & Oliver, 1963) and gluconeogenesis (Ballard & Hanson, 1967). Many of these enzyme shifts are simply quantitative changes (Wicks *et al.* 1969) that have been interpreted as the initiation or termination of a metabolic pathway in that tissue. However, some enzymes undergo shifts in their isoenzymic complements (Iwamura, Ono & Morris, 1968; Clark & Yochim, 1969; Jamdar & Greengard, 1970), thus making the overall metabolic interpretation more or less difficult, depending upon how much is known about the physiological role of each isoenzymic form.

The present report concerns the enzymic differentiation of four adenylate kinase isoenzymes during the development of the rat liver. The four isoenzymes have been identified in both rat liver and in a series of Morris hepatomas (Criss, Litwack, Morris & Weinhouse, 1970*a*; Criss, 1970).

* Present address: Department of Obstetrics and Gynaecology, University of Florida Medical School, Gainesville, Fla. 32601, U.S.A.

MATERIALS AND METHODS

Animals. Pregnant rats (CFN strain) were purchased from Carworth Farms, Rockland County, N.Y., U.S.A. They were maintained on commerical stock diet throughout the experiments. Time of birth was noted to within 5h and this was used in the calculation of the experimental results. Newborn and young rats were used without regard for sex.

Tissue preparation. The pregnant females were decapitated and the foetuses were removed within 2min. The foetal liver was removed without decapitation of the foetus. Newborn animals were either decapitated before removal of the liver, or the liver was removed without decapitation; the experimental results were the same. After excision, the liver tissue was cooled in a cold 25mM-sucrose solution containing 1mM-mercaptoethanol. The tissue was then blotted, weighed and homogenized in a glass coaxial homogenizer with a motor-driven Teflon pestle. It was homogenized in 5 vol. of 25mM-sucrose containing 1mM-mercaptoethanol. The homogenates were centrifuged for 1h at 2°C at 100000*g*. Samples of the supernatant were used for assay of adenylate kinase activity and protein, or for separation of the adenylate kinase isoenzymes by isoelectrofocusing. Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951).

Enzyme assay. Adenylate kinase activity was measured spectrophotometrically in a coupled system as reported by Criss, Sapico & Litwack (1970*b*). The assay system was always preincubated for 1–2min before the reaction was initiated by adding AMP. A control cuvette containing enzyme but no AMP was used to determine the adenosine triphosphatase activity. In this assay the velocity was linear up to 5min and proportional to the amount of enzyme added. However, in extracts containing high concentrations of adenosine triphosphatase the assay became unreliable for the measurement of adenylate

kinase activity. Since most of the liver adenosine triphosphatase is associated with membrane fractions, a hypoosmotic extraction method was used to release adenylate kinase from subcellular compartments so that it could be measured as a 'soluble' enzyme in the absence of most of the liver adenosine triphosphatase (Criss, 1970). One unit of adenylate kinase was defined as the amount forming one μmol of ADP/min at 25°C.

Isoenzyme separation. The adenylate kinase isoenzymes were separated by using the method of isoelectrofocusing. A column of 110ml capacity containing Ampholine (LKB Instruments) to establish a pH gradient from 3 to 10 was used in all studies. Not more than 50mg of protein was placed in the column. The voltage was turned on to 100V (3–4mA current) for 24h and then increased to 300V (2–4mA current) for another 24–48h. A thiol such as β-mercaptoethanol or cysteine was used in the column to protect adenylate kinase isoenzyme II, which is readily oxidized. A more complete description of the method has been published (Criss *et al.* 1970*a*).

RESULTS

Adenylate kinase activity was about 15 units/g wet weight of tissue or 0.50 unit/mg of extracted protein in the foetal rat liver (Fig. 1). It began to increase just before birth. During the first day (within 2–8h) after birth, adenylate kinase activity increased to 41 units/g wet wt. or 0.90 unit/mg of protein and continued increasing linearly at a rate of about 10 units/day per g wet wt. or 0.15 unit/day per mg of protein. Adult concentrations of 150 units/g wet wt. or 2.2 units/mg of protein were reached at 10–14 days after birth.

Only adenylate kinase isoenzymes II and III (Criss, 1970) were found in the foetal liver (Fig. 2). Isoenzyme II activity was about 2 units/g in the foetal liver, increased to 4 units/g immediately after birth, and increased at a linear rate of 2 units/day per g until adult liver concentrations of 28 units/g were reached at 10–12 days of age. Isoenzyme III was 20 units/g in the foetal liver, increased to 35 units/g immediately after birth, and continued increasing at a linear rate of 10 units/day per g until adult liver concentrations of 115 units/g were reached at 10–12 days of age. Isoenzyme I could not be detected in the liver until 8–10 days of age and isoenzyme IV was not observed until the rat was 12–16 days old.

There was a shift in the adenylate kinase III/adenylate kinase II ratio during liver development (Fig. 2); these isoenzymes are respectively mitochondrial and cytoplasmic (Criss, 1970). The isoenzyme III/isoenzyme II ratio was about 10 in the foetal liver, decreased to 8 immediately after birth, and continued decreasing until the adult ratio of 4 was reached at 12 days of age. In adult liver, isoenzyme III was localized in the outer compartment of the mitochondria, whereas isoenzyme II was cytoplasmic in subcellular location

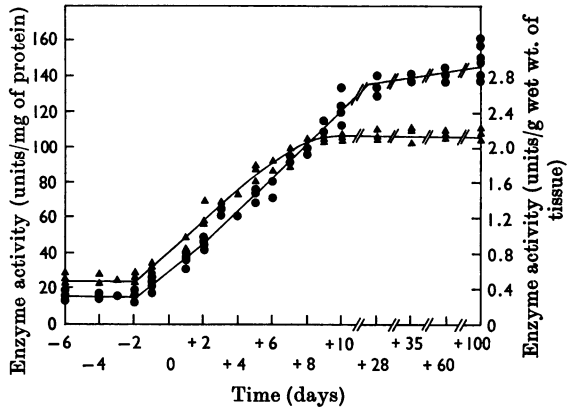

Fig. 1. Liver adenylate kinase activity during development. Activities are expressed in terms of tissue wet weight (●) and extracted protein (▲).

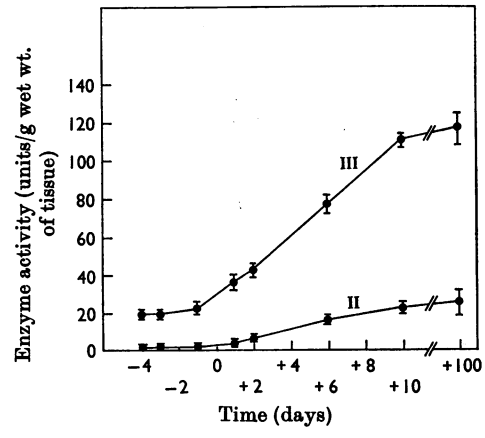

Fig. 2. Adenylate kinase isoenzyme activity during liver development. Only isoenzymes II and III are shown.

(Criss, 1970). Because of the fragility of foetal liver mitochondria, the location of isoenzyme III could not be conclusively determined in the foetal liver.

DISCUSSION

The birth process suddenly alters the normal energy supply of the foetal animal. Before birth, the foetus received a substantial concentration of glucose across the placental membrane. After birth, the young animal is suddenly faced with the task of maintaining a high enough concentration of glucose in the blood to maintain brain metabolism.

This appears to be accomplished by the initiation or increase in certain key liver enzymes, particularly the enzymes involved in gluconeogenesis (Jamdar & Greengard, 1970) and amino acid catabolism (Wicks *et al.* 1969), near term. The net result is a maintenance of a minimum concentration of blood glucose during the switch in energy supply.

Atkinson and his colleagues (Atkinson, 1968; Klugsoyr, Hageman, Fall & Atkinson, 1968; Shen, Fall, Walton & Atkinson, 1968) have postulated that the adenylate charge acts as the energy mediating system of the cell and is capable of switching metabolic pathways on or off. Adenylate kinase is obviously extremely important in maintaining the adenylate charge, because the observed concentrations of the adenine nucleotides change (Bucher, Kryci, Russmann, Schnitger & Wesemann, 1964; Ballard, 1970; Start & Newsholme, 1968) in such a way as to implicate the equilibrium constant of the adenylate kinase reaction (Markland & Wadkins, 1966). Thus the change in the activity of adenylate kinase during liver development would be in good agreement with this postulate. When a tissue is under a fairly constant energy supply, such as glucose to foetal liver tissue or erythrocytes, very little adenylate kinase activity (or ability to maintain energy homeostasis) is observed, but when the energy supply suddenly changes, as at birth, the liver adenylate kinase activity (or ability to maintain energy homeostasis) is increased.

Ballard (1970) measured the concentrations of adenine nucleotides and calculated the changes in the adenylate charge during the development of rat liver tissue. In the intact liver cell, he observed a fourfold increase in the ATP/ADP ratio, a sixfold increase in the ATP/AMP ratio, and an increase in the adenylate charge from 0.71 to 0.91 when comparing pre- and post-delivery. During this time-interval we observed a doubling in the total liver adenylate kinase activity and the beginning of a shift in the adenylate kinase isoenzyme III/isoenzyme II ratio. Since the apparent equilibrium constant for purified adenylate kinase is near 0.8 (Markland & Wadkins, 1966) and the apparent equilibrium constant calculated from determinations *in vivo* of the postnatal liver is always greater than 1 (Ballard, 1970), it would appear that the adenylate kinase activity does not increase rapidly enough to maintain a constant overall adenylate charge immediately after birth. Also, since there was a large change in the [ATP]/[AMP] ratio during this interval of liver development (the latter change would not occur if adenylate kinase was the only contributor in regulating the adenylate charge), it is likely that at birth other components in addition to adenylate kinase are involved in shifting the adenylate charge. This increase in adenylate charge may be necessary for the process of gluconeogenesis to occur. However, the concurrent decrease in the mitochondrial (III)/cytoplasmic (II) adenylate kinase isoenzyme ratio (Criss, 1970) may cause a subcellular shift in the adenylate charge which cannot be observed by examining total concentrations of adenine nucleotides in the intact tissue.

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