Pyruvate Kinase Isozymes from Rat Tissues

DEVELOPMENTAL STUDIES

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SUMMARY

Developmental and aging patterns of pyruvate kinase (PK) isozymes (PK 1, PK 2, PK 3, and PK 4) from rat liver, kidney, heart, and skeletal muscle were studied by electrophoretic, chromatographic and immunochemical techniques. During the first 2 weeks of postnatal life a decline of total PK activity occurred in liver, kidney, and heart followed by an increase to adult or higher than adult levels after weaning. Skeletal muscle PK increased steadily from very low levels before birth to steady state levels by the end of the 2nd month. It was demonstrated that changes in enzyme activity during development were the result of changes in the amount of enzyme protein.

Both PK isozymes from rat liver (PK 1 and PK 4) decreased during the first 2 weeks of postnatal life. Liver PK 1 sharply increased after weaning, whereas liver PK 4 remained at the level of 3 to 10% of the total enzymatic activity. PK 4, the only isozyme detected in fetal rat kidney, predominated throughout development, although PK 1, PK 2, and PK 3 appeared shortly after birth. A shift of PK 4 to PK 3 occurred in both cardiac and skeletal muscle with passage from the fetal to the neonatal stage of development. Adult skeletal muscle contains only PK 3, which is also the predominant form in cardiac muscle.

Antiserum prepared against purified PK 3 from skeletal muscle neutralized PK 2, PK 3, and PK 4 from kidney but did not cross-react with liver and kidney PK 1.

Developmental changes in total PK activity and in PK isozyme patterns in rat liver and kidney are discussed in terms of their possible significance in the regulation of gluconeogenesis.

The glycolytic enzyme pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40) exists in multiple molecular forms in various animal tissues (1-8). The molecular relationship of these forms has not yet been elucidated, thus leading to uncertainty regarding the nomenclature of the PK isozymes. The IUPAC-IUB Commission on Biochemical Nomenclature recently issued recommendations on the Nomenclature of Multiple Forms of Enzymes (1971) J. Biol. Chem. 246, 6127-6128. In this paper we follow these recommendations. The PK isozymes from rat tissues are thus designated as PK 1, PK 2, PK 3, and PK 4, in order of decreasing anodal mobilities. However, in references to specific work, the nomenclature as used by the authors will be cited to avoid misunderstanding.
suspending in 2.4 M ammonium sulfate. All other reagents were the best quality commercially available.

Animals—Sprague Dawley rats were housed in air conditioned rooms (24°C ± 1°C) on a schedule of alternating periods of light (7:00 a.m. to 7:00 p.m.) and dark (7:00 p.m. to 7:00 a.m.). The animals had Purina laboratory chow and water ad libitum. Fetal age was determined on the basis of fetal body weight (29). The number of animals in a litter was limited to a maximum of 10. Rats were weaned on the 22nd day of age. Sex was disregarded until the 12th postnatal day, after which only male rats were used. Rats were always killed between 8:00 a.m. and 9:00 a.m.

Tissue Preparation—Rats were decapitated and exsanguinated. Tissues were then removed, weighed, and kept on ice. Livers and kidneys (both cortex and medulla) were homogenized in a Potter-Elvehjem type homogenizer in 2 volumes, and hearts (ventricles) and skeletal muscle in 9 volumes of a cold solution containing 0.145 M KCl, 5 mM MgSO₄, and 1 mM EDTA, pH 7.4. In fetal and newborn rats all muscles from the hind legs were used for the skeletal muscle samples, whereas in older rats only the quadriceps femoris was used. The homogenates were centrifuged at 40,000 × g for 60 min and the clear, fat-free supernatants were used for the assay of enzyme activity.

Enzyme Assay—Pyruvate kinase activity was assayed by following the oxidation of NADH at 340 nm using a Gilford model 240 recording spectrophotometer (30). The assay mixture contained (final concentrations): 50 mM Tris-HCl buffer, pH 7.5, 75 mM KCl, 8 mM MgSO₄, 2 mM triethylamine P-enolpyruvate, 1.3 mM ADP, 0.15 mM NADH, and 1.0 unit of lactate dehydrogenase in a final volume of 2 ml. Temperature was strictly maintained at 25°C. Enzyme activities are expressed in units per gram of fresh tissue. One unit corresponds to the amount of enzyme that converts 1 pmole to P-enolpyruvate per min at 25°C.

Protein Determination—Protein was determined either by the biuret method (31) or by the method of Lowry et al. (32) as modified by Munro and Fleck (33) with crystalline bovine serum albumin as standard.

Electrophoresis—Electrophoresis was performed on cellulose polyacetate strips (Sephaphore III, 2.5 × 15 cm, gelman Instrument Co., Ann Arbor, Mich.) as described by Susor and Rutter (34). Tissues were homogenized (liver and kidney 1:2, w/v; heart and skeletal muscle 1:9, w/v) in the medium described by Susor and Rutter (34) minus dithiothreitol. Except where stated otherwise, electrophoresis was carried out at 0–4°C for 2 hours at 17 volts per cm in a medium containing 20 mM Tris-chloride buffer, pH 7.5, 0.5 M sucrose, 0.1 mM FDP, 100 mM 2-mercaptoethanol.

DEAE-cellulose Chromatography—PK isozymes from rat liver during development were separated and quantitated by a chromatographic procedure similar to that described by Lo et al. (35). Sucrose, 0.25 m in 0.02 m triethanolamine buffer, pH 7.5, was used instead of mannitol. Sucrose was found to stabilize the specific liver isozyme (PK 1) during purification (4). Column chromatography gave quantitative recovery of PK isozymes from adult rat liver, but the recovery was only about 90% in rats less than 1 month of age. A satisfactory recovery of PK isozymes during that developmental period was obtained employing the following procedures. Twenty milliliters of DEAE-cellulose slurry previously equilibrated in the above buffer was placed into a small Buchner funnel (diameter 45 mm) and packed using a slight negative pressure. Livers were homogenized in the buffer (1:2, w/v), centrifuged as above, and 0.8 ml of the supernatant was applied to the resin. The resin was then washed several times with the buffer. A slight negative pressure was used for filtration. In this way PK 4, the isozyme that was not adsorbed by the resin, was separated. The adsorbed enzyme activity (PK 1) was eluted by 0.5 m KCl in buffer. Separated isozymes were always checked by electrophoresis. The whole procedure was carried out in the cold room (0–4°C).

Immunological Procedures—PK from rat skeletal muscle was purified as described by Tanaka et al. (3). Attempts to purify PK from rat liver by the procedure of Tanaka et al. (3) were unsuccessful. The final skeletal muscle preparation had a specific activity of 382 units per mg of protein at 25°C, and showed a single symmetrical peak during sedimentation in the ultracentrifuge. One major protein band and a faint minor band were seen on disc gel electrophoresis. Antibody against this isozyme was prepared by injecting New Zealand white rabbits with the purified enzyme. Two milligrams of the enzyme preparation were emulsified with an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.) and injected into the toe pads and the back. The procedure was repeated 2 weeks later. Four weeks after the first injection the same amount of the enzyme without adjuvant was injected subcutaneously in the back. Six to 8 weeks after the first injection rabbits were bled by cardiac puncture. The antisera were precipitated with ammonium sulfate (0 to 50% saturation), the precipitate was dissolved in 0.15 m NaCl and dialyzed against the same solution for 24 hours. The volume of antisera was finally adjusted to the initial volume with 0.15 m NaCl and stored at −20°C until used.

Immunoprecipitation was performed as follows: tissues were homogenized in 4 volumes of a solution containing 0.14 m KCl, 5 mM MgSO₄, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.5. The samples were centrifuged at 105,000 × g for 1 hour and the supernatants were used for the reaction with antisemur. In order to determine the percentage of total liver or kidney PK that could be neutralized with anti-PK 3 serum, the respective tissue supernatants of known enzyme activity were mixed with a surplus of antibody, incubated for 30 min at room temperature, and then for 18 hours at 4°C. The samples were then centrifuged for 15 min at 27,000 × g, and the supernatants were assayed for enzyme activity. The enzyme activity not neutralized, corresponds to the major liver isozyme (PK 1) which does not cross-react with anti-PK 3 serum (3). For each sample two control tubes were run in parallel, one containing control serum and the other homogenizing medium instead of antisemur.

**Results**

Developmental Pattern of Pyruvate Kinase Activity—Changes in total activity of PK in rat tissues during development and aging are shown in Fig. 1. Late fetal liver PK activity was 30 to 40% lower than the enzyme level of young adults (50 to 60 days). During the first 2 weeks of postnatal life it decreased, reaching a minimum by the 15th day. After that activity rose about 4-fold and attained a maximum between the age of 40 and 50 days. Thereafter, enzyme activity decreased slightly with increasing age. High PK activities in kidney occurred before birth, at birth, and between 30 and 40 days after birth. Similar to the liver pattern, a minimum was observed between 15 and 20 postnatal days. A steady decrease of kidney enzyme activity occurred with increasing age. However, a slight rise was noted in the two oldest groups. Cardiac muscle PK activity was highest 3 days before birth. It then dropped to a minimum about 12 to 15 days after birth, reached the adult
FIG. 1. Developmental and aging changes in pyruvate kinase activity of rat tissues. The results are means ± 1 S.E. of 4 to 8 animals. Where standard error is not indicated the results are means of two or three pooled tissue samples each consisting of 6 to 12 animals.

Level by Day 30, and maintained that level thereafter. PK activity of skeletal muscle rose steadily from very low levels at birth to approximately 20-fold higher levels in adult animals. When the enzyme activities of the different tissues were expressed per mg of supernatant protein, similar changes were observed during development. Since the relative liver weight (g/100 g, body weight) changes during development and during dietary and hormonal alterations, liver enzyme activities are frequently reported as total activities per 100 g of body weight. When liver PK activity was expressed on this basis, essentially the same pattern as shown in Fig. 1 was obtained, except that a more pronounced decrease in activity occurred with increasing age. In adult rats (60 days) there were no significant sex differences in the activity of PK in any of the tissues studied.

To examine the possibility of the presence of activators or inhibitors of enzyme activity in tissue extracts at different ages (particularly around the 15th and 50th postnatal days), mixing experiments were done. Liver, kidney, and heart supernatants of the two age groups described were first assayed separately. Then, equal volumes of the respective tissue supernatants were mixed and assayed. The results of these assays were those predicted by addition of the mixture and thus ruled out the presence of any activators or inhibitors. Similar experiments were conducted with skeletal muscle supernatants at ages 5 and 50 postnatal days, and the results were the same as for the other tissues. These experiments suggest that the measured activities reflect the amount of enzyme protein and that the influence of allosteric or other effectors under the conditions of the assay is negligible.

Electrophoretic Patterns of Pyruvate Kinase Isozymes of Rat Tissues during Development—Sisor and Rutter (34) noted that FDP added to the electrophoresis buffer enabled the separation of PK isozyme A (PK 3) from isozyme C (PK 4). In their experiments FDP changed the mobility of PK 4 towards the cathode without affecting the mobility of PK 3. To stabilize the enzyme during electrophoresis they employed a high concentration of 2-mercaptoethanol (0.1 M). We tested the effect of FDP and 2-mercaptoethanol on the mobility and electrophoretic separation of PK isozymes from adult rat liver, kidney, skeletal muscle, and heart (Fig. 2). Compared with the complete electrophoresis buffer (A), the omission of 2-mercaptoethanol (B) affected neither mobility nor separation of PK isozymes. When FDP was omitted (C), PK 3 from skeletal and cardiac muscle moved from the origin towards the cathode, thus displaying the electrophoretic mobility of PK 4, whereas the mobilities of the liver and kidney isozymes were unaffected. The omission of both FDP and 2-mercaptoethanol (D) had essentially the same effect as the omission of FDP alone but, in addition, the kidney PK isozymes were not clearly separated. Therefore, in our experience FDP changed the electrophoretic mobility of skeletal and cardiac muscle PK from a cathodal (PK 4) to a neutral position (PK 3) in contrast to the results of Susor and Rutter (34). FDP is known to activate PK 1, but not PK 3 (3). Thus, we have the puzzling situation that when FDP binds PK 3 it alters the charge on the protein without affecting the activity, but when FDP binds PK 1 it alters activity without affecting charge.

As shown in Fig. 3 most developmental changes of PK isozymes in rat tissues, as detected by electrophoresis, occurred during the last several prenatal days and early in postnatal life. In fetal liver, PK 4 was most prominent 5 days before birth; but, in addition, there were two anodal bands with a mobility similar to that of PK 1. These two anodal bands became more prominent 2 days before birth while displaying a
Fig. 2. The effect of FDP and 2-mercaptoethanol on electrophoretic mobility and separation of PK isozymes from rat tissues. Liver (L), kidney (K), skeletal muscle (M), and heart (H) homogenates were prepared, and electrophoresis was performed as described in "Experimental Procedure." A, complete electrophoresis buffer was used; B, 2-mercaptoethanol was omitted; C, FDP was omitted; D, both FDP and 2-mercaptoethanol were omitted.

slight decrease in anodal mobility. At birth the slower moving anodal band decreased in intensity and completely disappeared 2 days after birth. Later PK 1 became increasingly prominent, while PK 4 decreased. During late fetal life the liver contains a considerable proportion of hematopoietic cells, the number of which decreases markedly towards the end of gestation (36, 37). The slower moving anodal band, that disappeared after birth, may represent the PK isozyme from erythropoietic cells. Evidence in support of this came from an experiment in which adult rat liver and washed rat erythrocytes were mixed and homogenized. The supernatant was subjected to electrophoresis, and the pattern of isozymes thus obtained was almost identical with that of rat liver 2 days before birth.

In kidney, PK 4 was the predominant form throughout life; it was the only form present before birth. Two days after birth PK 1 appeared. From the 4th postnatal day on, PK 2 and PK 3 were present as very faint bands. They became much more prominent later in life. To our knowledge these four distinct electrophoretic forms of adult rat kidney PK have not been previously described. All our experiments were performed with homogenates of whole kidney. However, kidney cortex yielded an electrophoretic pattern identical to that of whole kidney.

We have recently observed five molecular forms of PK in adult rat intestinal mucosa.

Fetal rat heart exhibited only PK 4. During the first 4 days of postnatal life, the enzyme displayed a mobility between PK 3 and PK 4. From 14 days of age, in addition to the major isozyme (PK 3), PK 4 was also present as a faint band.

During fetal and neonatal development of skeletal muscle a transition of PK 4 to PK 3 occurred with the appearance of intermediate forms. Adult skeletal muscle revealed only PK 3.

Quantitation of Rat Liver and Kidney Pyruvate Kinase Isozymes by DEAE-cellulose Chromatography—Lo et al. (35) used DEAE-cellulose column or batch chromatography to separate PK isozymes from rat liver and various transplantable hepatomas. We employed similar chromatographic procedures for quantitation of rat liver PK isozymes during development. Identity of the separated isozymes was verified by electrophoresis. Table I shows the total recovery and the percentage of recovered PK activity as PK 4 and PK 1, respectively. During the last 4 fetal days and first 10 postnatal days PK 4 accounted for 33 to 47% of the total liver PK activity. Then, mainly as the result

\[ J. \text{Osterman and P. J. Fritz, manuscript in preparation.} \]
TABLE I
Developmental pattern of liver pyruvate kinase isozymes as separated by DEAE-cellulose column chromatography

Details of the separation are described in “Experimental Procedure.” PK 4 refers to the activity that was not adsorbed onto the resin. PK 1 was eluted with 0.5 M KCl in the buffer. For each time point the range of values obtained is reported from two or three separate experiments. Each liver sample was a pool of 2 to 18 livers. Single values represent the data obtained by column chromatography.

| Age (days) | Recovery | Percent of recovered enzyme activity as PK 4 | % | PK 1 | % |
|-----------|----------|---------------------------------------------|---|------|---|
| -4        | 92–94    | 32.4–35.0                                   | 65.0–67.6 |
| -1        | 94–96    | 36.2–40.0                                   | 60.0–63.8 |
| 1         | 98–99    | 40.2–47.5                                   | 52.5–53.8 |
| 5         | 93–100   | 44.7–49.5                                   | 50.5–55.3 |
| 10        | 94–95    | 33.3–37.0                                   | 63.0–66.7 |
| 15        | 90–91    | 29.8–31.8                                   | 69.2–70.2 |
| 19        | 86–83    | 15.0–15.7                                   | 84.3–85.0 |
| 20        | 98–100   | 5.4–7.0                                     | 93.0–95.0 |
| 29        | 96–101   | 5.1–8.2                                     | 91.8–94.9 |
| 43        | 105.6    | 2.0                                         | 98.0 |
| 75        | 98.5     | 2.5                                         | 97.5 |
| 205       | 105.6    | 3.4                                         | 96.6 |
| 355       | 102.5    | 3.0                                         | 97.0 |
| 70        | 94–99    | 10.2–12.0                                   | 88.0–89.8 |

Fig. 5. DEAE-cellulose chromatography of rat kidney pyruvate kinase. Kidneys from five adult rats were homogenized in 2 volumes of 0.01 M Tris-HCl, 0.5 M sucrose, 0.1 mM FDP, 1 mM dithiothreitol, pH 7.5. Ten milliliters of 40,000 X g supernatant (148.0 units) were applied to the column (25 X 2.5 cm) that had been equilibrated with the same buffer. Pooled PK activity of the first peak not retained in the resin contained 71% of applied activity. The column was then developed with the linear concentration gradient of 0 to 0.5 M KCl in buffer. The second pooled peak of activity had 9% of applied enzyme activity. The whole supermatant and separated two peaks of activity were subjected to electrophoresis as described in “Experimental Procedure.” The whole procedure was carried out at 0–4°C.

Fig. 4. Pyruvate kinase isozyme activities of rat liver during development. The isozyme activities (means) of PK 1 and PK 4 are derived from the data in Fig. 1 and Table I.

of a dramatic accumulation of PK 1 after weaning, PK 3 decreased to a level of about 3% and remained at this level thereafter. In fetal liver and during the first day or two of postnatal life both anodal bands of PK activity (Fig. 3) contributed to the activity designated as PK 1. Developmental changes of liver PK activity are thus characterized by both changes in the total enzymatic activity (Fig. 1) and in the distribution of PK

isozymes (Table I). Fig. 4, derived from the data in Fig. 1 and Table I, shows the pattern of two liver PK isozymes during development.

In adult rats, fasting for 72 hours resulted in a 60% decrease in total liver PK activity. Under these conditions the percentage of PK 4 increased to about 11% mainly as the result of a substantial loss of PK 1. Separation of adult rat kidney PK isozymes by the same column chromatographic procedure used for the liver isozymes resulted in recoveries between 15 and 30%, PK 4 accounting for about 80% and PK 1 for 20% of the recovered activity. PK 2 and PK 3 were lost during chromatography. In order to stabilize the kidney enzymes during chromatography, several different solutions were tried. The best recovery was obtained with a solution of the following composition: 0.01 M Tris-HCl, 0.5 M sucrose, 0.1 mM FDP, and 1 mM dithiothreitol, pH 7.5. A similar medium has been used recently for the separation of kidney PK isozymes by Jiménez de Asúa et al. (38). Using this solution we recovered 80% of the applied activity, 90% of the recovered activity as PK 4 and 10% as PK 1, 2, and 3 (Fig. 5). Electrophoresis showed that the unadsorbed fraction was PK 4, whereas the peak recovered with salt elution contained PK 1, PK 2, and PK 3. Small amounts of kidney tissue at younger age groups and relatively poor recoveries during chromatography precluded the use of this technique for the quantitation of kidney PK isozymes during development. More information on that question was obtained by the immunological method.
In order to determine whether this in-

isozyme profile of kidney PK.

affected neither total activity nor the

remaining 8 to 10% of enzyme activity

appeared shortly after birth (Fig. 3). From the 15th postnatal
day 90 to 92% of total kidney PK activity could be neutralized
in the supernatants of liver and kidney and that PK 4 from liver
shows that after the immunoprecipitation test PK 1 was present
in skeletal muscle PK. Since electrophoresis of kidney PK
isozymes disclosed four bands of activity (PK 1 to 4) it was of
interest to determine their immunochemical behavior. Fig. 6
shows that after the immunoprecipitation test PK 1 was present
in the supernatants of liver and kidney and that PK 4 from liver
and PK 2, PK 3, and PK 4 from kidney were neutralized. We
used this method for the quantitation of PK isozymes from rat
liver and kidney during development. As shown in Table II
developmental pattern of liver PK isozymes obtained by immu-
nochemical method agreed well with our chromatographic
results (Table I). However, in adult rats immunoprecipitable
PK 4 accounted for 10% of the total liver PK activity, whereas
chromatographically separated PK accounted for only 3%.

Rat kidney at birth contained only PK 4, whereas PK 1 appeared
shortly after birth (Fig. 3). From the 15th postnatal
day 90 to 92% of total kidney PK activity could be neutralized
with PK 3 antibody, the remaining 8 to 10% of enzyme activity
being PK 1. Fasting affected neither total activity nor the
isozyme profile of kidney PK.

Comparison of Young and Adult Skeletal Muscle Pyruvate
Kinase by Immunoprecipitation—Skeletal muscle PK undergoes
almost a 20-fold increase in activity during the first 2 months of
postnatal life (Fig. 1). In order to determine whether this in-
crease was due to change in catalytic activity of the enzyme or
to differences in the amount of enzyme protein at different ages,
the experiment shown in Fig. 7 was performed. A slight dif-
fERENCE between young and adult skeletal muscle PK was found
in that 17% more antibody was required to precipitate the same
amount of enzyme activity from young as compared with adult
rats. However, since the same amount of protein was precipi-
tated at equivalence point was 0.117 mg ( Anonymous) and 0.121 mg (o),
respectively.

### Table II

**Immunoprecipitation of liver and kidney PK isozymes during development**

| Age      | Enzyme activity in liver as: | Enzyme activity in kidney as: |
|----------|-----------------------------|-----------------------------|
|          | PK 4 | PK 1 | PK 4 | PK 1 |
| days     |      |      |      |      |
| -2       | 54   | 46   | 100  | 0    |
| Newborn  | 35   | 65   | 90   | 0    |
| 15       | 25   | 75   | 90   | 10   |
| 35       | 17   | 83   | 90   | 10   |
| 75       | 10   | 90   | 90   | 10   |
| 70 (after 72 hours of fasting) | 24 | 76 | 90 | 10 |

* At these ages the activity precipitated with anti-PK 3 serum includes also PK 2 and PK 3.

Quantitation by Immunoprecipitation of Rat Liver and Kidney
Pyruvate Kinase Isozymes during Development—Tanaka et al. (3)
employed an immunoprecipitation technique to study PK
isozymes from rat tissues. They showed that the major liver
PK isozyme, type L (PK 1) does not cross-react with anti-type
M antibody (anti-PK 3). Since electrophoresis of kidney PK
isozymes disclosed four bands of activity (PK 1 to 4) it was of
interest to determine their immunochemical behavior. Fig. 6
shows that after the immunoprecipitation test PK 1 was present
in the supernatants of liver and kidney and that PK 4 from liver
and PK 2, PK 3, and PK 4 from kidney were neutralized. We
used this method for the quantitation of PK isozymes from rat
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**DISCUSSION**

In the four rat tissues studied quantitative changes in the
distribution of PK isozymes occur during prenatal and postnatal
development. In the case of liver and kidney, these changes
may be related to the control of glycolysis and gluconeogenesis.
Developmental patterns of carbohydrate-metabolizing enzymes
of rat liver and kidney show many similarities (39, 40). The
gluconeogenic pathway becomes active in both organs shortly
after birth apparently because of a combination of factors in-
cluding increase in tissue oxygen, increase in P-enolpyruvate-
carboxykinase, and decrease in PK (22, 41, 42). The activity
of the rate-limiting gluconeogenic enzymes remains above adult
levels during the first 2 weeks of postnatal life (22, 42, 43).
Maximal glucose production from pyruvate in rat kidney cortex
slices was observed on the 16th postnatal day (42). Our results
indicate that both liver and kidney PK activities decline during
this time and attain minimal levels around the 15th postnatal
day. At this stage of rat development, the mean value for liver
PK 1 activity is 6.0 units per g of tissue, and for PK 4 is 2.5 units,
whereas for kidney the mean values are 1.4 units for PK 1 and
10.3 units for PK 4. It is possible that due to its allosteric
properties, liver PK 1 is largely inactive at the tissue concentra-
tions of FDP, P-enolpyruvate, ATP and amino acids present during conditions of active gluconeogenesis (18). In contrast, liver PK 4, which is less sensitive to inhibition by ATP and is not activated by FDP, probably represents the enzymatically active form of PK at this developmental stage. Unless its in vivo activity was much smaller than that observed under conditions of our assay, considerable P-enolpyruvate recycling would occur.

The situation with respect to the potential role of PK isozymes in the regulation of gluconeogenesis in the kidney is less clear. The major kidney isozyme (PK 4) is not affected by ATP and FDP, but displays a cooperative effect with respect to P-enolpyruvate, like the major liver isozyme (38). It is also inhibited by several amino acids (38). The minor rat kidney cortex PK isozyme, which has been chromatographically separated by Jiménez de Asúa et al. (38), displayed kinetic features of the major hepatic isozyme (PK 1). Our chromatographic separation under similar conditions revealed that the enzymatic activity recovered during salt elution contained three electrophoretically distinct forms (PK 1, PK 2, and PK 3). Furthermore, our study indicates that PK 2, PK 3, and PK 4 are antigenically related since anti-PK 3 serum neutralized them all.

The only previous attempt to quantitate changes in PK isozymes during development was reported recently by Middleton and Walker (28). These authors utilized differences in kinetic properties of the two rat liver PK isozymes to estimate isozyme relationships. Our results are in general agreement with theirs, although they found that PK 4 (type M) decreased sharply during late fetal life and reached its low steady state level immediately after birth, whereas our data indicated that there was little change in the level of this isozyme during late fetal life. Susor and Rutter (34) recently observed five electrophoretically distinct forms of PK in newborn rat liver. We have observed repeatedly only three PK isozymes from fetal and newborn rat liver, and our results suggest that the isozyme with intermediate electrophoretic mobility probably originates from erythroplastic cells within the liver. Two other laboratories have investigated quantitative differences in the PK isozyme levels of adult rat liver. Tanaka et al. (3) used an immunochemical technique and reported that the type M isozyme (PK 4) accounted for 35% of the total liver PK activity and the type L isozyme (PK 1) accounted for 65%. Lo et al. (35), employing DEAE-cellulose batch chromatography, recovered 62% of the total PK activity, 8% as PK 4 (Isozyme 1 in their designation) and 54% as PK 1 (Isozyme 2). Our data on developmental changes in the total rat liver PK activity are for the most part consistent with previous reports (20, 22).

The physiological significance of the sharp decline in cardiac muscle PK during late fetal life and in the second postnatal week is unclear. The observed shifts in pattern of PK 3 to PK 4 during perinatal period are in agreement with the data of Susor (27).

Development of mammalian skeletal muscle is accompanied by dramatic increases in many glycolytic and other muscle specific enzymes (21, 25, 44, 45). The contractile activity of the muscle seems to be an important factor in determining the time during development at which the activities of the enzymes of special significance for muscle (adenylate kinase, AMP-deaminase and creatine phosphokinase) rise sharply to adult levels (45). In agreement with this observation we observed that in atrophic leg muscles of a 23.5-year-old rat a drastic reduction of total PK activity was accompanied by the appearance of PK 4.4 This isozyme had previously only been observed during the fetal and neonatal period of rat skeletal muscle. Our data on developmental changes of total rat skeletal muscle PK are consistent with a previous report (25), and our observation of a shift of PK 4 to PK 3 in this tissue during fetal to neonatal period is in agreement with the recent findings of Susor (27).

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