ON THE ONTOGENY AND INTERACTIONS OF PHOSPHOFRUCTOKINASE IN MOUSE TISSUES

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Abstract—1. The distribution and interactions of phosphofructokinase isozymes with cellular structure have been studied in the major tissues of the mouse during development.

2. The ontogenic patterns of isozymes which were obtained were consistent with those observed for other species and are interpreted in terms of the presence of three genes and three homotetrameric forms of the enzyme (A, B, and C) in the tissues of the mouse.

3. In addition, the data provides a clear indication that interactions between the enzyme and cellular structure are appreciable in all major tissues and at all stages of development, with all isozyme types exhibiting such interactions.

4. The significance of the study of subcellular interactions of these isozymes in contributing to a comprehensive physiological rationale for this mammalian enzyme and its multiple forms is discussed.

INTRODUCTION

Phosphofructokinase (PFK; EC 2.7.1.11), a tetrameric enzyme, is the largest enzyme of the glycolytic sequence in mammals, with subunit molecular weights of around 85,000, and is generally recognized as exerting a major regulatory influence in carbohydrate metabolism (Hoffman, 1978). While a considerable amount of research has been directed towards investigations of the kinetics of this enzyme and susceptibility towards a great variety of effectors, there remains some dispute for most mammalian species, as to the exact number of electrophoretically distinct forms of PFK in the various tissues, due in large part to the difficulty in resolving PFK isozymes by standard electrophoretic techniques (Davidson et al., 1983). Nevertheless most literature on this subject is consistent with the interpretation that PFK exists in mammalian tissues in three major homotetrameric forms, PFK-A, B, and C, with PFK A predominating in muscle, PFK B predominating in liver and PFK C predominating in the brain (Davidson et al., 1983; Vora, 1982; Defaria et al., 1978; Gonzalez et al., 1975; Foe and Kemp, 1974; Khoja and Kellett, 1983; Kurata et al., 1972; Tsai and Kemp, 1973).

Also, in recent years there has been an increasing amount of attention directed towards the association of glycolytic enzymes with subcellular structure (Masters, 1984; Masters and Holmes, 1975). In the case of PFK the extent of the available evidence for such interactions varies considerably in individual tissues. With liver and kidney, for example, no major reports indicating PFK interactions to subcellular material are available in the literature, whereas a number of reports do exist which support PFK interactions in muscle and brain (Masters, 1981; Baquer et al., 1975; Knulf, 1978). In skeletal muscle PFK has been shown, along with other glycolytic enzymes to be localized in the relaxed muscle fibre, predominantly within the isotropic zones (Sigel and Petic, 1969; Dolken et al., 1975). Also a number of reports have shown PFK to bind to F actin and to reconstituted thin filaments (Clarke and Masters, 1974, 1976) and the extent of binding of PFK to the particulate fraction of homogenates has been shown to increase along with an increase in glycolytic activity following the electrical stimulation of ovine hind limb (Masters, 1981) and bovine psoas (Clarke et al., 1980) muscle. In addition, a report exists indicating that rabbit muscle PFK is activated by actin (Liou and Anderson, 1980). In short, evidence is available indicating the likely interaction of PFK to actin filaments in vivo and is consistent with such interactions increasing during periods of increased muscle activity.

For the brain, Baquer et al. (1975) has demonstrated a considerable degree of pelletable PFK activity in rat brain homogenates from birth to the adult animal, with the activity in the adult brain being as much as 70-75% bound. In addition two reports in the literature indicate likely subcellular sites for brain PFK interactions. Knulf (1978) indicates that the particulate PFK of brain is associated with synapticosomal membranes (it should be noted also that high concentrations of actin are present in these regions of the brain (Reid and Masters, 1985) and Craven et al. (1974) has presented some evidence consistent with a mitochondrial location for brain PFK.

In the present investigation, the extent of interaction of PFK with structural elements has been detailed in all the major tissues of the mouse at sequential stages of development so as to allow further definition of the biological significance of these associations. Analyses of the isozyme status of the enzyme and the latency during development have also been carried out, and changes in these parameters have been correlated with the changing metabolic emphasis of these tissues during ontogeny.

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MATERIALS AND METHODS

Chemicals
All biochemicals were obtained from Sigma Chemical Company, St. Louis, MO. Other chemicals used in these investigations were all of reagent grade purity.

Animals
Mice of the Quackenbush strain were used for the developmental studies in this paper. The animals were bred in the laboratory of the authors from sexually mature mice supplied by the Central Animal Breeding House, University of Queensland, Brisbane, Australia. These mice have a gestation period of nineteen days and were bred in such a manner as to enable an accurate specification of the developmental age of the animals (Rugh, 1968). Throughout the matings described, the mice were maintained on a standard laboratory diet with drinking water ad libitum.

Preparation of tissues
Animals used in these experiments were sacrificed by cervical dislocation or decapitation.

For those developmental stages near and after birth, livers, kidneys, brain and skeletal muscle were removed, rinsed in water, dried on filter paper and kept on ice until homogenized. For early foetal animals (seven days before birth), only the liver and brain tissues could be extracted in amounts sufficient for the type of enzyme analyses employed in this work.

After dissection, tissues were finely minced with scissors, weighed and homogenized in 10 volumes of ice cold homogenizing buffer. The homogenizing buffer contained 0.32 M sucrose, 1 mM EDTA, 10 mM mercaptoethanol, 0.01% trypsin inhibitor, 1 mM phenylmethylsulphonyl fluoride and 10 mM tris-HCl, pH 7.4. Homogenates were prepared via the use of a glass tube and motor driven teflon pestle. The homogenates were then spun at 100,000 g for 1 h at 4°C, using a 50 Ti rotor in a Beckman ultracentrifuge. The supernatant so obtained was diluted with an equal volume of extraction buffer, which was identical to the homogenizing buffer except that it contained 0.2 mM ATP and 0.2 mM F6P in addition. Enzyme activities determined for this fraction were defined as "soluble" activities.

The 100,000 g pellets from the above spin were extracted by adding extraction buffer, equal in volume to that in which the tissue was homogenized, to the pellet, and re-suspending by brief use of the Ultra Turrax homogenizer. This resuspended pellet was then centrifuged at a low speed (600 g for 15 min at 4°C), the supernatant from this spin kept on ice, and the pellet extracted a second time by a final spin was discarded and the two low speed supernatants were obtained using the procedures outlined above. In this way, the activities and isozyme patterns for these fractions defined as "bound" activities and isozymes. Electrophoresis was performed on tissue samples using cellulose acetate plates (Helena Laboratories, Beaumont, TX), with a buffer system based on that used by Defaria et al. (1978).

The plates were presoaked in, and electrophoresis carried out with, a buffer containing 50 mM glycylglycine pH 8.1, 1 mM ATP, 2 mM MgCl2, 50 mM KCl, 1.0 mM EDTA and 1.0 mM KCN was incorporated in the assay mix so as to inhibit non specific dehydrogenase activities, particularly for the "bound" tissue fractions. Control assays were carried out with F6P in the assay mixture for both the "soluble" and "bound" fractions of the mature female animal tissue and these assays indicated that the changes in absorbance at 340 nm under the assay conditions without substrate were negligible.

Total protein in the various samples were determined by the method of Peterson (1977) with bovine serum albumin as standard. PKF activities were finally expressed as the soluble, bound or total activities per total mgms of protein. The assay data for each tissue and each developmental stage has been expressed as the mean ± SFM for three or more experiments.

Determination of Aldolase Isozyme Patterns
Electrophoresis was performed on tissue samples using cellulose acetate plates (Helena Laboratories, Beaumont, TX), with a buffer system based on that used by Defaria et al. (1978).

The plates were presoaked in, and electrophoresis carried out with, a buffer containing 50 mM glycylglycine pH 8.1, 1 mM ATP, 2 mM MgCl2, 50 mM KCl, 1.0 mM EDTA, 1.0 mM KCN, 0.15 mM NADH, 60 mM KCl, 1.0 mM F6P (fructose-6-phosphate), 1.0 mM ATP, 2.0 mM MgCl2, 1.0 mM aldolase, 7.5 U/ml of fructose-1, 6 diphosphate dehydrogenase and 22.5 U/ml of triose phosphate isomerase plus 7.2 mg/ml of agar. Plates were stained in this manner in a moist chamber at 37°C for an appropriate period. Subsequently this overlay was peeled off and the position of the isozymes were visualized as clear bands on a dark background by the use of a second overlay.

Latent activities
The majority of the assay and electrophoretic data in this paper were obtained using the procedures outlined above. In addition, however, the possibility of extracting further latent activity was studied when 0.1% triton X-100 was included in the extracting buffer.

RESULTS

Skeletal Muscle
Figure 1 illustrates the changes in the total PKF activities and the soluble and bound PKF activities for mouse skeletal muscle with development. From Fig. 1 it would appear that mouse skeletal muscle PKF activity increases quite dramatically over the neonatal period, continues to increase up to one week postnatal, levels out to 2 weeks postnatal and then decreases quite sharply to the 3 weeks postnatal stage.

In regard to the percentage of PKF activity bound with development, skeletal muscle displays a rise in the percentage of PKF activity which is bound over the neonatal period, a slight decrease to 1 week
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![Graph](image1)

**Fig. 1.** Partitioning of PFK activity in mouse skeletal muscle during development. Soluble and bound activities were prepared from tissue samples and assayed as described in the Materials and Methods section. (●) Soluble activity, (▲) bound activity, (□) total activity.

postnatal and then a substantial increase to almost 50% bound in the 3 week postnatal animal. By the adult stage, however, binding drops off to around 30% PFK bound.

The isozyme data for skeletal muscle (Fig. 2) indicates that PFK A₂ predominates in both the soluble and bound fractions of this tissue from the newborn to the adult stages. For muscle and for the other tissues studied with development, brain, kidney and liver, represented in Fig. 2, the PFK isozyme patterns for the 1, 2 and 3 week postnatal animals were also examined, but were found to be equivalent to the patterns seen in the adult tissues and so this data is not shown. The muscle isozyme data also indicates some PFK A–C hybrid activity in the neonatal and adult muscles and shows that the PFK found in the bound fractions tends to be slightly less anodal (i.e. there is apparently more of the A type) than the PFK seen in the muscle soluble fractions.

**Brain**

Figure 3 shows a plot of the total PFK activity of the brain with development. The activity is seen to be relatively low during the early foetal period (~7 days gestation) and to stay low until 1 day before birth. Over birth the activity rises slightly and then remains level again until 1 week postnatal. From 1 week postnatal until 3 weeks postnatal (weaning) the PFK activity rises quite dramatically in the brain. From weaning, the brain shows a slight increase in PFK activity to reach adult values. Adult male and female activities are similar.

With regard to the binding of PFK in this tissue, the data for brain indicates that at ~7 days gestation 30% of the PFK activity present is bound (Fig. 3). By the neonatal period, this rises to 35%, then declines quite sharply to around 20% by 1 week postnatal.

![Graph](image2)

**Fig. 2.** Diagrammatic representation of the soluble (S) and bound (B) isozymes observed during development of mouse skeletal muscle, brain, kidney and liver. The soluble and bound fractions were prepared, subjected to electrophoresis and stained for PFK activity as described in the Materials and Methods section. In addition the adult mouse soluble PFK isozyme pattern for heart is shown so as to illustrate the possible location of the C₄ isozyme under the electrophoretic conditions used.
The "bound" activity then rises to 30–35% again at 2 weeks postnatal, before declining to adult values of around 25%. When latent activities are taken into consideration though (Table I), the developmental relativities assume a different profile. For example, at -7 days gestation, determination of PFK latent activity in the brain indicated that latent activities are very low at this early stage of development. For the new born and mature female stages, however, the latent activities of brain PFK are quite high, and indicate that in the new born around 40% of the brain PFK is bound and by the adult stage this rises to about 45%. It is not unreasonable to assume latent activities of the brain represents bound activity for reasons presented elsewhere (Reid and Masters, 1985).

The isozyme data (Fig. 2) for the brain "soluble" and "bound" fractions throughout development appeared as an unresolved A-C plus B-C hybrid set in all cases. Thus no major differences between brain isozymes at different developmental stages or between the "soluble" and "bound" fractions were detected.

Kidney

Figure 4 shows a plot of the total PFK activity with development for the kidney. The data indicates quite a steep peak of PFK activity at birth, with a substantial decrease in activity during the first postnatal week. From this first postnatal week the PFK activity tends to level out to adult values. The adult male and female activities appear to be similar.

Figure 4 also shows the PFK supernatant and pellet activities with development. These plots illustrate that the supernatant (soluble) and extracted pellet (bound) fractions show much the same developmental pattern as does the plot for the total activity. For the kidney just before (-1 day) and at birth, PFK binding is very high 55–56% but this binding drops off rapidly over the first week postnatal and slowly levels out to adult values of 20–30% bound.

The electrophoretic data (Fig. 2) indicate that in the adult mouse kidney PFK B–C hybrid isozymes predominate. The neonatal kidney isozyme pattern appears to be the same as the "soluble" activity isozyme forms for both the new born and mature kidney, and the PFK patterns of the "bound" fractions appear to be the same as the "soluble" activity isozyme forms for both the new born and mature kidney. While the resolution of the kidney PFK isozymes at all developmental stages was insufficient to provide definitive information it appeared that in early development PFK C type activity predominates over B type activity, while later the reverse is true.

Liver

Figure 5 shows a plot of the total PFK activity in liver with development. PFK activity is quite high in the early foetus, but then there is quite a dramatic drop in activity until just before birth. Over the first week postnatal, there is a further dramatic drop in PFK activity from 1 week postnatal to 2 weeks postnatal there is a levelling out of activity followed by a gradual decrease to adult values. The adult male activities appear to be slightly higher than those for the adult female.
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Fig. 5. Partitioning of PFK activity in liver during development. Soluble and bound activities were prepared from tissue samples and assayed as described in the Materials and Methods section. (●) Soluble activity, (▲) bound activity, (□) total activity.

With regard to the extent of binding of this enzyme in liver, the most striking observation from Fig. 5 is the high percentage of "bound" PFK throughout development (50–60% bound at all stages). There appears to be a drop from 1 week prenatal to the neonatal period, a rise in the percentage bound to a 1 week postnatal and then a gradual decline to adult values. At 1 week postnatal, 65–70% of the PFK activity is bound.

The PFK isozyme data (Fig. 2) indicated that in the early foetal liver (−7 days) a PFK pattern less anodal than the B, isozyme was detected in both the "soluble" and "bound" liver fractions. From the neonatal period on however, it appeared that PFK B, predominated (along with some B–C hybrid activity) in the "soluble" and "bound" fractions. It should be noted at this point that while assay data clearly indicated extensive binding of PFK in the kidney and liver tissues, it proved difficult to obtain clear isozyme patterns for the bound liver and kidney fractions. For this reason the isozyme patterns for the bound fractions of the liver and kidney are shown as less intense than are the soluble patterns.

**DISCUSSION**

The status of the multiple forms of phosphofructokinase activity is less well defined than with most other glycolytic enzymes, and for this reason initial comment on the present data is directed towards the designation of these isozymes in this investigation. As indicated in Fig. 2, the resolution of the individual forms of activity was often not complete, but it should be noted that this result represents a general feature of the electrophoretic resolution of this enzyme rather than a particular technical deficiency. PFK patterns are often difficult to interpret because of overlapping positions of the hybrid sets and the difficulties of resolution. But the present observations are consistent with reports in other mammalian species and the relative designations have been made on the basis of the present results and the majority of these comparative data (Davidson et al., 1983; Vora, 1982; DeFaria et al., 1978; Gonzalez et al., 1975; Khajo and Kellett, 1983; Kurata et al., 1972; Isei and Kemp, 1973).

With respect to the foetal form of PFK in the mouse, the brain and liver patterns (−7 days) (Fig. 2), provide indications that early in development there is a common PFK isozyme pattern which is quite complex, consisting of a mixture of A, B and C activities, which is not dissimilar to that observed in the adult brain. No previous reports in the literature clearly identify a unique foetal PFK isozyme pattern for any species, but it is of interest to note that work with tissues from human foetuses (12–40 weeks gestation) indicates that early in the development of the muscle, heart and brain tissues of this species, quite complex patterns are also observed involving all 3 forms of PFK, A, B and C (Davidson et al., 1983). In this respect, then, the data for the mouse and for human tissues are similar, and support an interpretation that the foetal PFK isozyme pattern exists in a much more complex pattern than that seen for other glycolytic enzymes (Reid and Masters, 1975, a–d).

With regard to the developmental properties of PFK in muscle, there is evidence in the literature for a change in the isozyme status of the enzyme during the neonatal to early postnatal stages of development (Davidson et al., 1983; Defaria et al., 1978; Thrasher et al., 1981). With human, guinea pig and rabbit tissues, for example, the switch is from AC to A, type activity (Davidson et al., 1983; DeFaria et al., 1978) while the rat exhibits an apparent B, to A, shift (Thrasher et al., 1981).

There is some support in the literature, too, for the occurrence of a substantial rise in the PFK activity of skeletal muscle during the postnatal stages of development (Davidson et al., 1983; Defaria et al., 1981; Dunaway, 1983), and such an increase would be consistent with the substantial postnatal increases seen for other glycolytic enzymes in this tissue (Reid and Masters, 1985, a–c). With the present studies, though, the data fails to support the expected postnatal increase and the reasons for this appear to relate to the unique properties of PFK in this tissue. Muscle PFK showed a variability in analysis which was not evident in any other tissue (Figs 3–5), or with any other glycolytic enzyme studied (Reid and Masters, 1983, a–d), and gives every indication that the activity present in vivo is in some way being degraded, inhibited or masked, especially in the late postnatal states of development. Despite appreciable attention being directed towards this problem during these studies, the causation was not fully elucidated; but it is worthy of note, that there is considerable evidence in the literature of similar developmental variation of the muscle enzyme (DeFaria et al., 1978; Gonzalez et al., 1975), and a number of authors have commented that muscle PFK is a particularly difficult enzyme to study because of the extraordinary lability of the enzyme (Bronstein and Knoll, 1981; Clarke and Masters, 1973; Westrin and Backman, 1983).
For the reasons referred to above, there are difficulties in interpreting the general trends in PFK-muscle binding data. Nevertheless, the combination of data from this investigation and from the literature, provide indications that there is a PFK A-C to A, isozyme switch as muscle develops, and it seems clear that PFK A, is best suited of the PFK isozymes for binding to F-actin filaments. Taken together, then, the potential for interactions between PFK and actin appear to increase with muscle development. As with the other glycolytic enzymes that have been studied to date (Reid and Masters, 1985; a-d), the indications are that during development in muscle, the appropriate glycolytic isozymes are established, the amount of F-actin filaments increases and appropriate systems are produced to regulate metabolite levels and continue activation states. Thus the system develops the capacity to switch to an extensive binding of glycolytic enzymes to F-actin during periods of high rates of anaerobic contraction in adult muscles.

In a previous review of the data on PFK activity changes in rat brain, Dunaway (1983) concluded that all such reports consistently indicate little change before birth, but a substantial increase in activity at weaning to reach adult levels. The data from this present investigation (Fig. 3), for PFK activity changes in mouse brain, then, compare closely with these reports for the rat, and in terms of activity, at least, rat and mouse brain may be said to show similar behaviour with respect to PFK ontogeny. A further point that is relevant in regard to this topic is the description of brain PFK isozymes. While Dunaway (1983) has indicated that the majority of PFK in rat brain is of the A, type, it should be noted that data has been presented by other workers which is at variance with this conclusion. Kurata et al. (1972), have shown that the predominant form of PFK present in adult rat brain is anodal to the PFK A, band, for example, and while these workers were not able to identify whether these activities were A-B hybrids or A-C hybrids, it is of interest that the form they observed was not of the A, type and that the data that they reported is consistent with the observations made for mouse brain in this work (Fig. 2). With regard to species other than the rat, it may also be noted that there is other evidence that PFK C type activity appears to predominate in the adult rabbit, guinea-pig and mouse brains (DeFaria et al., 1978; Gonzalez et al., 1975; Foe and Kemp, 1984; Tsai and Kemp, 1973). On the basis of these observations then, there would seem to be appreciable reason to question whether PFK A, does predominate in rat brain, but the answer to this question may have to await an improvement in the procedures which are presently available for the resolution of these isozymes. In regard to the rationale for the large postnatal increase of PFK activity in brain, it is known that at the time PFK is increasing in activity in the brain (along with other glycolytic enzymes), the rat and mouse brain undergo a growth spurt, and there is a proliferation of glial cells, nerve endings and dendrites (Reid and Masters, 1985; a, b). Thus the glycolytic potential of the brain needs to increase to meet the energy needs of these developmental processes and also to allow them to function properly once formed. In addition, it should be remembered that mice are normally weaned at 21 days after birth and go on to a high carbohydrate diet. Thus the brain reduces its consumption of ketone bodies and must have developed sufficient glycolytic capacity, by this time, to utilize glucose as its predominant energy source.

It is not so easy, however, to explain other aspects of mouse brain PFK ontogeny. The reason why the brain utilizes the particular PFK isozymes it does, is not clear. For example, it appears that the brain chooses a combination of A and C isozymes with A type activity predominating in some species and C type activity predominating in others. In the human brain, for example, PFK A type activity certainly predominates (Davidson et al., 1983; Vora, 1982). Whereas in the mouse (Fig. 2), the rabbit (Foe and Kemp, 1984) and possibly the rat (Kurata et al., 1972), PFK C type activity is present in greater quantity than A type activity. Whether PFK C and A types have separate locations and functions in neurones (and perhaps glial cells) is not known, as yet, but would be useful additional information in this situation. The subcellular site of PFK interactions also merits further definition. Whether PFK in brain is interacting with mitochondria or actin, an increase of such binding with development can in part be explained by an increase in the number of binding sites with development, because both the number of mitochondria and the quantity of pelletable actin increase in the brain with development (Pysh, 1970; Schmitt et al., 1977). Many of the mitochondria in the mature brain are located in synaptosomes (Wilson, 1972) and much of the actin remains polymerized in the mature brain probably because it is concentrated in the nerve endings and spines (Reid and Masters, 1985). PFK, then, may be localized in the brain synapse regions so as to provide rapid energy production for action-associated movements in these regions. It may also be localized to the mitochondria so as to utilize mitochondrial ATP along with HK (Reid and Masters, 1985 d), so as to help provide pyruvate to feed the mitochondria. The supply of pyruvate from glucose for the mitochondria is of course most important in the brain as glucose is by far the predominant energy source for this tissue. In a sense, then, three separate functions can be visualized for PFK in brain. The enzyme could be involved in the efficient supply of pyruvate to mitochondria, in the efficient production of fructose-1,6-biphosphate for use elsewhere in the cell, or in the rapid production of energy at brain synapse regions. Possibly PFK isozymes predominantly of the C type (C,, C,A, C,A, and B-C hybrids) interact in brain with the mitochondria or to actin near the mitochondria so as to help either to feed the mitochondria pyruvate or to produce F1, 6DP for elsewhere in the cell. Brain PFK predominantly of the A type would then bind actin and be located at the synaptosomal membrane or in spines, and would take part in the rapid production of energy by a similar mechanism to that found in white muscles. Certainly, as may be seen from the above ideas, the quantification and localization of the different PFK isozymes in brain neurones of a number of species is a worthwhile project for further, detailed investigation.
With kidney, it is not possible to compare the present data for PFK ontogeny with respect to activity or isozyme changes, to other such reports for mammalian kidney samples because no such reports are readily available. It is however, of interest and value to discuss the possible rationale for the kidney PFK data observed in the mouse (Figs 2 and 4). It would seem that high PFK activities occur at the neonatal stages and during the first few days postnatal because glycolysis is important for the kidney's energy production at this time with mitochondrial numbers being low during this period (Clark, 1957).

As development proceeds, the kidney's dependence on glycolysis decrease while its gluconeogenic potential increases—behaviour which is consistent with the low PFK activities shown for the more mature kidney. Certainly the B form of PFK which increases with kidney development (Fig. 2) is more suited to a cell that wishes to carry out gluconeogenesis, than are the other forms of PFK, (Vora, 1982; Tsai et al., 1975).

Perhaps the most important observation with respect to PFK binding in mouse kidney though is the degree of binding during the neonatal period of development. The neonatal period is also the stage of kidney development when a peak of total PFK activity is seen. Fig. 4. The kidney depends largely on glycolysis for energy at this time and the kidney cells are involved in many dynamic activities (Clarke, 1957) which in turn almost certainly involve actin (Reid and Masters, 1985a). The association of PFK with actin filaments, then, would allow this enzyme to be localized at the appropriate intracellular sites of energy need in the kidney cells. The PFK isozymes present in the kidney "soluble" and "bound" fractions at this time are of the B-C hybrid type and this data is consistent with interactions between the PFK B-C hybrids and actin or other subcellular structure in this tissue. As development continues, the percentages of activity bound decreases and since no major isozyme changes occur in this postnatal period, one must assume that the binding decreases due to a reduction in binding sites and/or a decrease in the need for glycolysis. Both of these events probably apply. As the kidney develops beyond the neonatal stage, the numbers of mitochondria in the kidney cells increase and so the cell is not so dependent on glycolysis for energy production. Also as the kidney cells fully differentiate and cease to divide regularly, their need for movement functions involving actin filaments no doubt decrease. Thus the drop in PFK binding after would seem to be due to the cell environment decreasing the amount of stable actin filaments available for binding and because of a drop in the cells need for a mobile localized anaerobic energy producing unit.

With regard to the developmental behaviour of PFK in liver, it may be noted that Dunaway (1983) has reviewed reports on PFK activity changes in rat liver with development and a number of interesting points are raised by this work. An initial point is that Dunaway's work indicates that there is a rapid decrease in liver PFK activity during the first 24 hr after birth, which would certainly fit in with the early postnatal gluconeogenic role of this tissue. It would seem then, that the dramatic fall in mouse liver PFK activity seen in the present investigation (Fig. 5), over the first week postnatal, actually represents an even more dramatic fall over the first 24 hr postnatal followed by a more gradual decrease in activity in the 2–7 days postnatal period. The other interesting point raised by Dunaway's report is that they observed PFK A, activity in the neonatal and foetal rat liver. For the mouse liver, at no developmental stage was any PFK A, detected, but A-B hybrids did appear to be present in liver samples in addition to PFK B, plus B-C hybrid activities (Fig. 2). PFK ontogeny of the hepatocytes, then, can be explained as follows. In the early foetal liver PFK activities are high and most probably of the PFK B-C hybrid type. By the neonatal period PFK activities have fallen somewhat and PFK B, activity predominates. Following birth there is a dramatic drop in activity in the first postnatal week after which the activity levels out to adult values. No dramatic alterations in hepatocyte PFK isozyme content appears to take place in the postnatal period.

Clearly, a rationale for the high PFK activities of the foetal liver may be in relation to the energy needs of these foetal cells which are largely met by glycolysis at this time. It would also seem appropriate for the liver to produce a relatively glycolytic isozyme for PFK at this early stage rather than a gluconeogenic isozyme. The presence of PFK B-C hybrid activity at this time then seems appropriate. As the neonatal period approaches, the liver hepatocytes prepare for their critical early postnatal gluconeogenic function, and so we see in these cells a drop in activities and a switch to the highly regulated B, (gluconeogenic) isozyme.

Liver PFK displays a high percentage of interactions throughout development. The binding appears to be highest for the early foetal liver (—7 days gestation) and for the 1 week postnatal liver. The binding sites for PFK in liver are not known, but it would be most consistent with ideas developed in this manuscript, if the liver PFK were to interact with both actin and with the liver mitochondria. There are several results in the literature of actin filaments adjacent to mitochondria (Bradley and Satir, 1979; Pardo et al., 1983), and by being localized near the mitochondria the PFK B-C hybrids would be able to utilize mitochondrial ATP to produce F1,6DP which can then be utilized by the aldolase to pyruvate kinase sequence in the cell to meet various localized energy needs. Glycolytic energy production is, of course, very important to early liver foetal cells. In the early postnatal liver cells, gluconeogenesis becomes an important process and PFK B, is the predominant PFK isozyme in the postnatal hepatocytes. The mitochondrial localization of PFK B, during the postnatal period would be most useful for a number of reasons. If the hepatocyte wishes to produce glycolytic energy, PFK would be in an appropriate position to utilize mitochondrial ATP. However, if the cell wishes to carry out gluconeogenesis (a process that most likely is localized near mitochondria, as it is an energy requiring process and the enzymes involved in the reverse of the PK glycolytic step involves a major input by the mitochondria), then the PFK B, would be appropriately localized so as to be inhibited and so work in with the localized control mechanisms
which turn off glycolysis and turn on gluconeogenesis. The peak of PFK binding at 1 week postnatal could then reflect the importance of gluconeogenesis at this time in the liver.

Overall, then, the major aim of this work has been to clarify the developmental progressions of the multiple forms of PFK in mouse tissues and to investigate the interactions of these isozymes with cellular structure. Evidence has been provided that considerable interactions between all the isozymes of PFK to subcellular material take place and that these interactions mainly involve actin filaments of the different tissues. Some evidence is present also, that PFK A activity in muscle displays preferential binding characteristics but in toto the data argues for appreciable interactions of all the isozymes to subcellular material. Such interactions are viewed as allowing PFK to be localized at points in the cell requiring anaerobic energy production or to allow PFK to be localized near mitochondria so as to either utilize ATP generated by the mitochondria, or to respond to control mechanisms in the cell that bring about a switch from glycolysis to gluconeogenesis. Despite considerable recent research into the phosphorylation of PFK, much remains to be learnt about the control of this enzyme (Foe and Kemp, 1984; Hue, 1982; Foe and Kemp, 1982; Foe et al., 1983). It may be noted that some previous studies have already led to the conclusion that PFK phosphorylation may be more significant in its effects on the localization of the enzyme within the cell than on the kinetics of PFK (Bazaes et al., 1982; Soling and Brand, 1981), and have pointed to the dynamic equilibrium between subunits and the role of this phenomenon and subcellular concentrations to the regulation of PFK activity (Luther et al., 1985; Bosca et al., 1985). It is argued from the present studies that the different isozymes of PFK may take up different locations in the cell, each isozyme form suiting a particular location and in turn a particular function, and such considerations, it is suggested, if studied in combination with the influence of effectors, and of phosphorylation and oxidation events on these isozymes, should lead to a fuller understanding of the rationale of this multiplicity.

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