Subcellular Distribution of Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase in Dog Liver and Kidney (36815)

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Pyruvate carboxylase (PC) (EC 6.9.1.1) and phosphoenolpyruvate (PEPck) carboxykinase (EC 4.1.1.32) are two key enzymes present in the gluconeogenic pathway. They enable the energetically unfavorable steps in reverse glycolysis to be bypassed and glucose to be synthesized from lactate, pyruvate and certain amino acids (1). The subcellular distribution of these two enzymes has implications for control of gluconeogenesis. Their localization varies with species as shown by Nordlie and Lardy (2), and it has been shown that at least in rat liver, the soluble and mitochondrial forms are immunologically different (3); only the soluble form is inducible by hormones or starvation (2). The distribution of PEPck ranges from total activity in the supernatant of mouse and hamster liver, to total mitochondrial activity in rabbit liver (2, 5, 6) while PC always is predominately mitochondrial (4). The role of mitochondrial PEPck is still unknown. In conjunction with studies in our laboratory on regulation of gluconeogenesis, and in view of the extensive use of dogs in pharmaceutical testing and medical research, we deemed it to be of importance to determine the distribution of PC and PEPck in dog liver and kidney.

Methods. The animals used in this experiment were full-grown male mongrel dogs routinely used by our Physiology Department. The tissue was removed while the dog was under Nembutal anesthesia and quickly placed in cold medium. The assay of PC was essentially that of Doedhar and Mistry (7). Tissue homogenates were prepared in cold medium containing 0.25 *M* sucrose, 20 m*M* tris(hydroxymethyl)aminomethane, 1 m*M* reduced glutathione, and 1 m*M* ethylenediaminetetraacetate. The tissue was homogenated for 3 min in a glass homogenizer using a loosely fitting Teflon pestle supported in an ice bath. The homogenate was then divided into two parts and from one part nuclei and mitochondria were isolated according to the method of Johnson and Lardy (8). The 10% homogenate was centrifuged at 600*g* for 10 min, the supernatant was decanted and used for mitochondrial isolation and the nuclear pellet was washed twice with 10 ml of medium. The nuclear pellet was then resuspended in 2.5 ml medium/10 ml original homogenate, to give fraction N. The nuclear supernatant was centrifuged at 15,000*g* for 5 min to obtain the mitochondrial pellet, washed 2× with 20 ml medium and resuspended in 2 ml medium/10 ml homogenate to give fraction M. Fractions N and M were then sonicated at 20 W for 90 sec on a ultrasonics sonifier Model W185D and centrifuged at 30,000*g* for 30 min on a Sorvall refrigerated centrifuge Model RC2-B with SS-34 rotor. The supernatants were diluted by 1:1 and used for the assay. The soluble fraction was obtained by centrifugation of the second half of the 10% homogenate at 105,000*g* for 1 hr in a Beckmann Model L2-65B ultracentrifuge with a Type 40 fixed angle rotor. The supernatant was diluted 1:1 and used for the assays. Protein was determined by the biuret method (9). An aliquot of the sample was added to a test tube followed by 0.5 ml of deoxycholate (20 mg/ml) which removed interference from lipid. Water was added to make 2.5 ml and 2.5 ml biuret reagent was added. The absorbance was read after 30 min at 540 nm on a Gilford Model 240 spectrophotometer. A few grains of KCN were then added to each blue sample and

TABLE I. Subcellular Distribution of PEPck and PC in Dog Liver and Kidney.

Cell fraction	PEPck		PC	
	μmoles/min/g tissue	μmoles/min/mg protein	μmoles/min/g tissue	μmoles/min/mg protein
Soluble				
Liver	0.56	0.007	None	None
Kidney	0.44	0.011	None	None
Nuclear				
Liver	0.20	0.009	0.395	0.018
Kidney	0.11	0.013	None	None
Mitochondrial				
Liver	1.05	0.060	0.508	0.029
Kidney	1.09	0.074	0.587	0.040
Total				
Liver	1.81		0.903	
Kidney	1.64		0.587	

after the color disappeared, the samples were corrected for this blank reading.

Discussion. As previously shown the localization of PC and PEPck vary with the species studied (2, 10–12). The dog seems to be no exception. Pyruvate carboxylase activity in liver mitochondria represented about 60% of the total activity with the remaining activity found in the nuclear fraction. In the kidney all the PC activity was found in the mitochondria (Table I). The pattern of distribution of liver and kidney PEPck appears to be similar to rabbit and guinea pig liver (2). We found that 60–70% of the activity is mitochondrial, 15–16% is nuclear, and 25–30% is soluble.

The location of PC and PEPck have implications for the control of gluconeogenesis. Since PC is located predominately in the mitochondria (4), glucose synthesized from pyruvate and lactate must arise from mitochondrially formed oxaloacetate. Depending on the location of PEPck, phosphoenolpyruvate (PEP), an obligatory precurser in gluconeogenesis from TCA cycle intermediates, can be formed in the mitochondria, the cytosol, or both. PEP is capable of rapid translocation across the mitochondrial membrane (13). The question is, which compartment is important for PEP synthesis under conditions of stimulated gluconeogenesis?

In the conventional picture proposed for rat liver where PEPck is strictly cytosolic, the carbon skelton of pyruvate is shuttled to the cytosol in the form of TCA intermediates such as malate and fumarate, and in the presence of glutamate, asparate. These compounds also carry reducing equivalents needed for glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (14, 15). Fatty acid oxidation produces high levels of mitochondrial reducing equivalents and these are probably one way in which fatty acid oxidation stimulates gluconeogenesis. A mitochondrial PEPck which requires oxaloacetate availability could not participate in the fatty acid oxidation stimulated gluconeogenic reponse since the mitochondrial redox favors malate synthesis from OAA. Therefore we must postulate another role for mitochondrial PEPck or show that the redox of the mitochondria is not as conventionally depicted; the latter possibility has been suggested (16). Earlier work from this laboratory (17) indicated that fatty acid oxidation as well as several other experimental conditions known to elevate the mitochondrial NADH/NAD^+ ratio inhibited PEP synthesis is isolated intact guinea pig liver mitochondria which contain a large fraction of the total PEPck activity of guinea pig liver. Fatty acid oxidation has also been shown to inhibit gluconeogenesis in perfused liver (18, 19). This study suggests that dog may be similar to the guinea

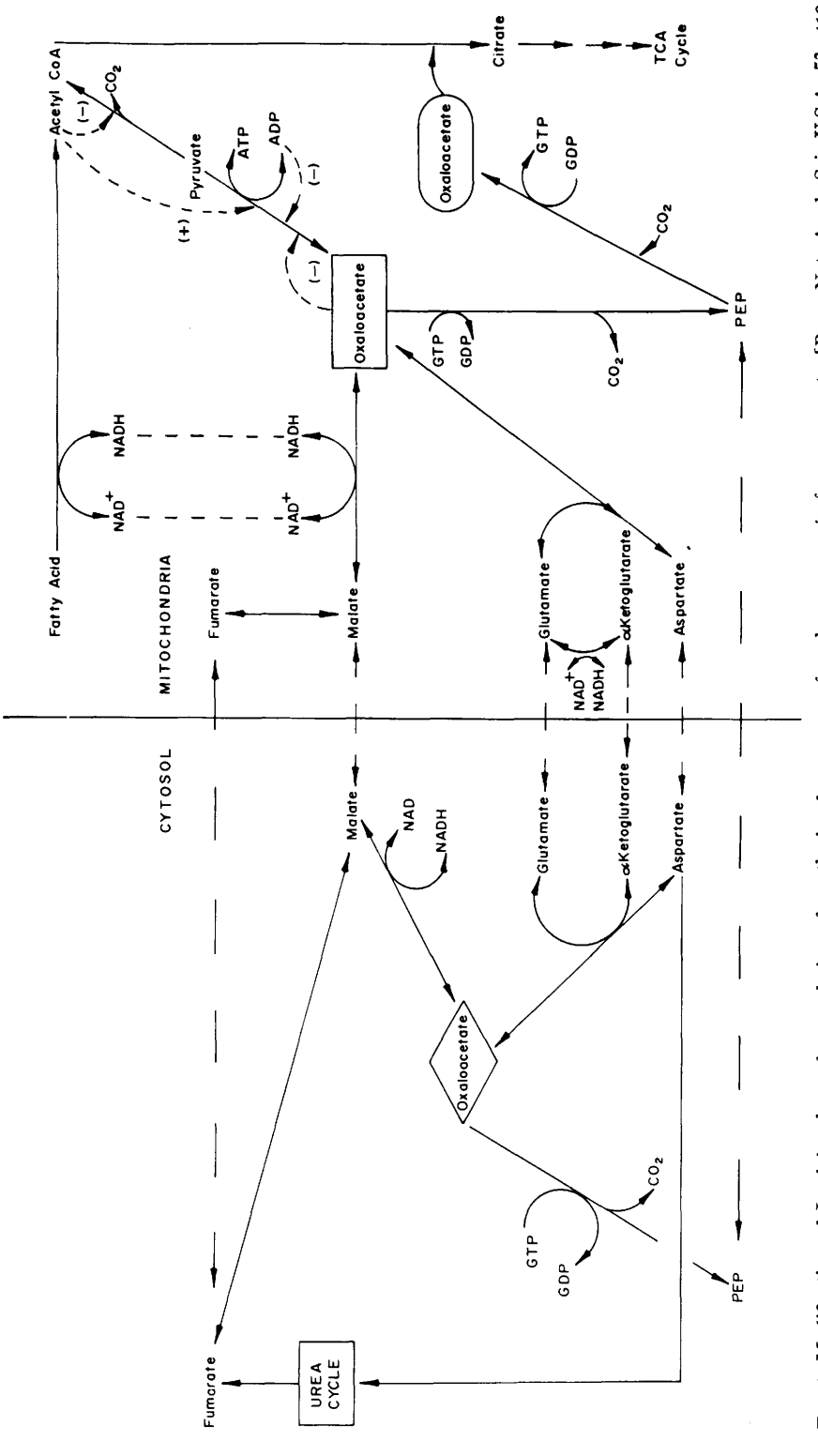

FIG. 1. Modification of Lardy's scheme for regulation of synthesis of precursors for gluconeogenesis from pyruvate [Proc. Nat. Acad. Sci. U.S.A. 53, 410 (1965)]. We are hypothesizing that PEP synthesis in dog is similar to that from guinea pig. The primary determiner of mitochondrial PEP synthesis is the pool size or availability of oxaloacetate which is regulated by the NADH/NAD^+ ratio. Increasing the NADH/NAD^+ ratio by fatty acid oxidation for example favors malate formation from oxaloacetate and inhibits PEP formation. A reversible mitochondrial PEPck would enable PEP to serve as a storage form of oxaloacetate which could supply a second pool of oxaloacetate to replenish the TCA cycle. This would allow for citrate synthesis when the NADH levels were high and thus the primary pool of oxaloacetate low.

pig, rabbit, and human (20) in the mechanism by which mitochondria regulate synthesis of gluconeogenic precursors. Figure 1 shows a schematic representation of a proposed mechanism for this regulation.

Summary. The distribution of PC and PEPck in dog liver and kidney cortex has been determined. Kidney PC is totally mitochondrial and liver PC is 60% mitochondrial. Kidney and liver PEPck are also predominately of mitochondrial origin (60–70%), while 15–16% is nuclear, and 25–30% is in the soluble fraction. It is concluded that since this distribution is similar to that found in guinea pig, rabbit, and human, the mitochondrial regulation of gluconeogenic precursor synthesis may also be similar.

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