The Activities and Intracellular Distribution of Nicotinamide–Adenine Dinucleotide Phosphate–Malate Dehydrogenase, Phosphoenolpyruvate Carboxykinase and Pyruvate Carboxylase in Rat, Guinea-Pig and Rabbit Tissues

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1. Measurements are presented of the activity and intracellular distribution of phosphoenolpyruvate carboxykinase, pyruvate carboxylase and NADP–malate dehydrogenase in rat, guinea-pig and rabbit liver and kidney cortex, together with previously obtained measurements of these enzymes in adipose tissue. 2. In all three tissues pyruvate carboxylase activity was greatest in the rat and lowest in the rabbit. 3. Guinea pig and rabbit were very similar to each other with respect to the extramitochondrial–mitochondrial distribution of phosphoenolpyruvate carboxykinase in all three tissues. 4. NADP–malate dehydrogenase was present in all three tissues in the rat, present in kidney cortex and adipose tissue in the guinea pig and absent from all tissues examined in the rabbit.

Three enzymes which catalyse the interconversion of C_3 and C_4 compounds are believed to play a role in the metabolism of a number of mammalian tissues. In the rat, which is the most extensively examined species, pyruvate carboxylase (EC 6.4.1.1) and phosphoenolpyruvate carboxykinase (EC 4.1.1.32) have been assigned a role in gluconeogenesis, and pyruvate carboxylase and NADP–malate dehydrogenase (EC 1.1.1.40) a role in lipogenesis. Knowledge of the occurrence, activity and intracellular distribution of these enzymes is desirable in any discussion of metabolic control concerning these processes. It is generally accepted that pyruvate carboxylase has a mitochondrial location. NADP–malate dehydrogenase has an almost exclusively extramitochondrial location in a number of tissues and species, though the occurrence of this enzyme appears to be somewhat uneven (Gul & Dils, 1969; Stanley, 1972). Phosphoenolpyruvate carboxykinase appears to have a dual location. Initially it was reported that most of the activity was extramitochondrial in rat liver, 50% was mitochondrial in guinea-pig liver and 100% was mitochondrial in rabbit liver (Nordlie & Lardy, 1963; Lardy, 1965). Since then, there have been reports from various laboratories of an extramitochondrial, adaptive phosphoenolpyruvate carboxykinase in rabbit liver (Johnson *et al.*, 1970, 1973; Garber & Hanson, 1971*b*) and of a higher percentage of the total activity of phosphoenolpyruvate carboxykinase that is mitochondrial in normally fed guinea pigs [76% (Söling *et al.*, 1970) or 73% (Garber & Hanson, 1971*a*)].

In the present study, the intracellular distribution of phosphoenolpyruvate carboxykinase in rabbit and guinea-pig liver has been reinvestigated to establish whether these species differ or are similar in the distribution of this enzyme under normal conditions. To minimize possible adaptive changes, both rabbits and guinea pigs were maintained under identical conditions and were fed on the same diet. Measurements of phosphoenolpyruvate carboxykinase activity in rat tissues are included for comparison. In view of the growing interest in renal gluconeogenesis (see Kurokawa & Rasmussen, 1973) similar measurements were also made in kidney cortex from these three species. Measurements of the activities of pyruvate carboxylase and NADP–malate dehydrogenase are also included.

Materials and Methods

Chemicals

These were all obtained and treated as described by Saggerson (1974*a*).

Animals

Female Wistar rats, female guinea pigs and male rabbits were bred in the animals colony at University College. The rats were fed on diet 41B (Bruce & Parkes, 1949) and the guinea pigs and rabbits on diet SG1 (Oxoid Ltd., London S.E.1, U.K.) supplemented with cabbage. All animals had water *ad libitum*. At death, rats, guinea pigs and rabbits

weighed 150–200 g, 600–800 g and 1.7–2.1 kg respectively.

Preparation of tissue extracts

Animals were killed by cervical dislocation and tissues cut into small pieces in ice-cold extraction media. For determinations of enzyme activity other than NADP-malate dehydrogenase the extraction medium was 0.14 M-sucrose containing 50 mM-potassium phosphate buffer, pH 7.2, in which tissues were homogenized in an ice-cold motor-driven Potter-Elvehjem homogenizer fitted with a Teflon pestle (clearance 0.6 mm). This medium was especially chosen because of its reported stabilization of glutamate dehydrogenase in tissue extracts (Walter & Anabitar, 1971). For the determination of NADP-malate dehydrogenase activity tissues were homogenized in ice-cold 0.25 M-sucrose containing 20 mM-Tris-HCl buffer, pH 7.4, in an Ultra-Turrax homogenizer (Jankeund Kunkel, Staufen i. Breisgau, West Germany). After homogenization, all tissue extracts were centrifuged for 1 min at 700g_{av}. and the supernatant was centrifuged at 38000g_{av}. for 30 min at 4°C. The resulting 38000g_{av}. supernatants were designated 'S' fractions. The pellets ('M' fractions) from the 38000g_{av}. centrifugation of sucrose-phosphate buffer extractions were suspended in more of the same medium and subjected to ultrasonication at 0°C for four 30 s periods over 4 min. Pellets from the 38000g_{av}. centrifugation of sucrose-Tris buffer extractions were discarded.

Enzyme assays

Glutamate dehydrogenase (EC 1.4.1.2), which was used as a mitochondrial marker, lactate dehydrogenase (EC 1.1.1.27), which was used as an extramitochondrial marker, and NADP-malate dehydrogenase were assayed spectrophotometrically at 25°C and phosphoenolpyruvate carboxykinase and pyruvate carboxylase were assayed radiochemically at 30°C. All the experimental methods were as described by Saggerson (1974a).

Results and Discussion

General considerations

It was the intention of this study to bring together information about the activities and intracellular distributions of these three enzymes in kidney cortex, liver and, in addition, adipose tissue. Some of the results are novel, some are not. Metabolic parameters of this kind can, however, be more easily correlated and interrelated when obtained simultaneously in the same study. At present comparative information on rates of glucose

Table 1. Activities of enzymes in rat, guinea-pig and rabbit liver, kidney cortex and adipose tissue

The results are means $\pm$ s.e.m. For liver and kidney, activities are expressed as μ mol/min per g wet wt., and for adipose tissue as nmol/min per 100 μ g of DNA. Glutamate dehydrogenase and lactate dehydrogenase were used as mitochondrial and extramitochondrial marker enzymes respectively. The extramitochondrial and mitochondrial activities of phosphoenolpyruvate carboxykinase and pyruvate carboxylase were derived from the activities measured in 'S' and 'M' fractions corrected for cross-contamination of these fractions. The values in parentheses represent percentage distributions of activity between compartments. Data for adipose tissue are from Saggerson (1974a,b). —, Activity not detectable or becomes zero after correction for cross-contamination of fractions. n.d., Not determined.

Tissue	Animal	Glutamate dehydrogenase		Lactate dehydrogenase		NADP-malate dehydrogenase		Phosphoenolpyruvate carboxykinase		Pyruvate carboxylase		No. of animals
		% activity in 'S' fraction	Activity	% activity in 'M' fraction	Activity	Extramitochondrial activity	Mitochondrial activity	Extramitochondrial activity	Mitochondrial activity	Extramitochondrial activity	Mitochondrial activity	
Liver	Rat	14.3	39.8 $\pm$ 3.3	3.9	222.1 $\pm$ 7.2	0.46 $\pm$ 0.11	n.d.	0.29 $\pm$ 0.06 (74)	0.10 $\pm$ 0.02 (26)	—	2.80 $\pm$ 0.50	6
	Guinea pig	14.9	46.8 $\pm$ 1.3	3.5	61.3 $\pm$ 4.4	—	n.d.	0.44 $\pm$ 0.07 (21)	1.60 $\pm$ 0.09 (79)	—	1.40 $\pm$ 0.15	6
Kidney cortex	Rabbit	22.9	20.9 $\pm$ 3.3	4.0	136.5 $\pm$ 8.3	—	n.d.	0.15 $\pm$ 0.01 (18)	0.68 $\pm$ 0.09 (82)	—	0.85 $\pm$ 0.10	5
	Rat	21.5	31.6 $\pm$ 2.6	3.0	96.9 $\pm$ 6.0	0.48 $\pm$ 0.03	n.d.	1.96 $\pm$ 0.25 (100)	—	—	3.10 $\pm$ 0.80	6
Adipose tissue	Guinea pig	23.1	10.2 $\pm$ 0.9	2.8	78.5 $\pm$ 2.4	0.08 $\pm$ 0.01	n.d.	0.28 $\pm$ 0.06 (20)	1.15 $\pm$ 0.07 (80)	—	1.50 $\pm$ 0.12	6
	Rabbit	26.6	4.3 $\pm$ 0.4	2.4	49.1 $\pm$ 2.1	—	n.d.	0.57 $\pm$ 0.03 (29)	1.38 $\pm$ 0.08 (71)	—	0.85 $\pm$ 0.10	5
	Rat	6.4	147.1 $\pm$ 12.4	4.2	2816 $\pm$ 177	599.9 $\pm$ 38.9	—	8.70 $\pm$ 1.03 (83)	1.77 $\pm$ 0.16 (17)	—	146.5 $\pm$ 15.0	6
	Guinea pig	5.5	220.7 $\pm$ 13.5	2.3	657.7 $\pm$ 48.9	69.7 $\pm$ 4.1	—	0.30 $\pm$ 0.09 (4)	7.39 $\pm$ 0.57 (96)	—	19.25 $\pm$ 1.15	8
	Rabbit	7.1	27.5 $\pm$ 3.1	4.9	2552.3 $\pm$ 327.3	—	—	2.63 $\pm$ 1.19 (6)	42.60 $\pm$ 1.89 (94)	—	2.75 $\pm$ 0.70	5

production or rates of lipogenesis by both liver and kidney-cortex preparations in reliable preparations *in vitro* (e.g. perfusion systems or intact isolated cells) do not appear to be available for all three species, except for measurements of glucose production by isolated perfused livers (Arinze *et al.*, 1973). In general therefore it is not possible as yet to attempt to relate the measured enzyme activities to measured rates of operation of the gluconeogenic and lipogenic pathways.

The results are presented in Table 1. This also includes, for reference purposes, data on enzyme activities in adipose tissue from the three species taken from Saggerson (1974a,b). Care was taken to ensure gentle treatment during tissue homogenization of liver and kidney for preparation of the fractions. Even so it was found, particularly with kidney, that there was a fairly high proportion of the mitochondrial marker in the cytoplasmic fraction. The extramitochondrial and mitochondrial activities of phosphoenolpyruvate carboxykinase and pyruvate carboxylase presented in Table 1 are values corrected for cross-contamination of fractions. In all cases the percentage of the total pyruvate carboxylase activity found in the 'S' fraction was less than the percentage of the total glutamate dehydrogenase activity in that fraction. In liver the percentage of pyruvate carboxylase activity in the 'S' fraction was 14.2% for rat, 0.9% for guinea pig and 5.0% for rabbit. The corresponding values for kidney were 20.7% for rat, 17.1% for guinea pig and 14.3% for rabbit. These results appear to suggest that pyruvate carboxylase is not stable in the 'S' fractions of guinea-pig and rabbit liver under the conditions used.

Phosphoenolpyruvate carboxykinase

In rabbit and guinea pig maintained under identical well-fed conditions, the percentage distribution of phosphoenolpyruvate carboxykinase between mitochondrial and extramitochondrial compartments is the same for these two species in liver, kidney cortex and adipose tissue. The actual percentage of phosphoenolpyruvate carboxykinase that is extramitochondrial in rabbit and guinea-pig adipose tissue was considerably lower than in liver and kidney cortex. Presumably this reflects the fact that adipose tissue is not a gluconeogenic tissue. The mainly extramitochondrial distribution of phosphoenolpyruvate carboxykinase found in rat liver was essentially the same as that found previously (Nordlie & Lardy, 1963; Lardy, 1965). The apparent absence of a mitochondrial activity of phosphoenolpyruvate carboxykinase in rat kidney cortex was surprising. This finding is relevant to gluconeogenic studies undertaken in this tissue. The activity of rat

renal phosphoenolpyruvate carboxykinase has been measured by Scrutton & Utter (1968), though no intracellular distribution study appears to have been performed and it is unclear whether the reported activities relate to renal cortex or to whole kidney. Longshaw *et al.* (1972) have presented results concerning the intracellular distribution of guinea-pig renal-cortex phosphoenolpyruvate carboxykinase, also using glutamate dehydrogenase as a mitochondrial marker. They found only 41% of the enzyme activity to be mitochondrial. The results of the present study are at variance with those of Longshaw *et al.* (1972), possibly reflecting differences in feeding and maintenance of animals, or differences in stability of marker enzymes in tissue extracts. It is important in distribution studies of this kind that tissue extracts be made in buffers in which the stability of marker enzymes has been established (Walter & Anabitarte, 1971). There do not appear to have been any previous measurements of the renal activity of phosphoenolpyruvate carboxykinase in the rabbit. The total activities of liver phosphoenolpyruvate carboxykinase in the present study were lower than those generally presented (Lardy, 1965; Scrutton & Utter, 1968; Böttger *et al.*, 1969; Söling *et al.*, 1970; Garber & Hanson, 1971a,b). However, the assay used in the present study (in the direction of phosphoenolpyruvate carboxylation) gives rates that are approximately one-eighth of those in the opposite direction at the pH value used (Chang *et al.*, 1966).

Pyruvate carboxylase

It is noteworthy that in liver, kidney cortex and adipose tissue the measured activities of pyruvate carboxylase varied between the three species in the order: rat > guinea pig > rabbit. The lower activity of this enzyme in the guinea pig and especially in the rabbit compared with the rat was, however, far more apparent in adipose tissue than in liver and kidney cortex. This presumably reflects the proposed dual role of this enzyme in both gluconeogenesis and lipogenesis. It seems reasonable to speculate that pyruvate carboxylase may play little or no part in lipogenesis in the rabbit.

NADP-malate dehydrogenase

The uneven distribution of NADP-malate dehydrogenase between tissues in the three species is noteworthy. This enzyme was present in liver, kidney cortex and adipose tissue of the rat (Table 1) and is also active in lactating mammary gland of this species (Gul & Dils, 1969). In guinea pig NADP-malate dehydrogenase activity is not measurable in liver and mammary gland (Stanley, 1972), but can be

detected in kidney cortex and adipose tissue (Table 1). In the rabbit this enzyme would not appear to be measurable in any of these four tissues (Table 1; see also Gul & Dils, 1969; Stanley, 1972).

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