The Effects of Starvation and Experimental Diabetes on Phosphoenolpyruvate Carboxykinase in the Guinea Pig

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1. Approx. 85% of liver phosphoenolpyruvate carboxykinase is associated with the mitochondrial fraction in the fed guinea pig. Enzyme activity is unchanged in diabetes, but doubles during starvation. In contrast with earlier reports, both cytoplasmic and mitochondrial activities were found to be increased. 2. In kidney cortex, total enzyme activity is increased in both starved and diabetic animals. These changes are associated with increases in the cytoplasmic activity alone. 3. In diabetic animals the mean blood-glucose concentration was 23.1 mm. Other blood metabolites were lower than those in the rat, and the animals did not show significant ketosis. 4. Changes in the rates of gluconeogenesis from lactate and propionate paralleled those in phosphoenolpyruvate carboxykinase activity.

The enzyme phosphoenolpyruvate carboxykinase [GTP-oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32] typically exhibits a biphasic intracellular distribution in mammals, including man (Hanson & Garber, 1972). Although the rat has been the major experimental animal in investigations of glucose synthesis, the virtual absence of a mitochondrial phosphoenolpyruvate carboxykinase activity means that this species may not be wholly typical. This has prompted a number of perfusion studies (Arinze *et al.*, 1973; Jomain-Baum *et al.*, 1973; Söling *et al.*, 1970, 1973) with livers from guinea pigs, which, although herbivorous, are both experimentally convenient and resemble man with respect to the activities of phosphoenolpyruvate carboxykinase and other enzymes (Söling *et al.*, 1970).

Previous studies of the intracellular distribution of phosphoenolpyruvate carboxykinase have shown that a substantial proportion of the total activity is mitochondrial, but have differed quantitatively (Nordlie & Lardy, 1963; Holten & Nordlie, 1965; Söling *et al.*, 1970). Much of the discrepancy may be attributed to the failure of some workers to take account of cross-contamination between cell compartments and of 'losses' of enzyme activity in the low-speed pellet during fractionation. As a consequence, the increase in phosphoenolpyruvate carboxykinase activity in the starved animal has been ascribed solely to changes in the cytoplasmic 'iso-enzyme', although there is strong evidence to indicate that both enzymes play a role in supporting glucose synthesis, at least in the perfused liver.

This report demonstrates that the increase in phosphoenolpyruvate carboxykinase activity in the guinea pig deprived of food is attributable to changes in both the cytoplasmic and mitochondrial enzymes. Neither this enzyme nor net gluconeogenesis from major substrates is increased in the non-ketotic streptozotocin-diabetic animal.

Materials and Methods

Animals

Male guinea pigs (Dunkin–Hartley strain, from a local supplier), 350–600 g, were allowed food (standard guinea-pig diet, Grain Harvesters Ltd., Wingham, Kent, U.K.) and water *ad libitum* unless otherwise specified. Diabetes was induced as follows. Animals deprived of food for 48 h were injected subcutaneously with insulin (6 i.u./kg body wt. in 0.9% NaCl). After 4–5 h the animals were injected intracardially with streptozotocin (200 mg/kg body wt. in 50 mM-sodium citrate, pH 5.1). They were then allowed free access to food and water, and were used 3 days later.

Materials

Radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. Enzymes and biochemicals were from the Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. PCS was obtained through Hopkin and Williams, Chadwell Heath, Essex, U.K. Triton X-100, heparin and 3-(*N*-morpholino)propanesulphonic acid were from Sigma (London) Chemical Co., Norbiton, Surrey, U.K. Insulin and streptozotocin were gifts from Boots Pure Drug Co., Nottingham, U.K., and Dr. D. C. N.

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Earl, ICI Ltd., Alderley Park, Cheshire, U.K., respectively. All other reagents were of the purest grade available from standard suppliers.

Enzyme-distribution experiments

Liver cell suspensions were treated with digitonin as described by Zuurendonk & Tager (1974), except that 0.75 ml of cells was added to 2.5 ml of 0.1% digitonin/buffer solution, and the incubation time was 5 min at 0°C. The pellet after centrifugation was dispersed in 2 ml of 0.25 M-sucrose/20 mM-3-(*N*-morpholino)propanesulphonate/3 mM-EDTA, pH 7.0, containing 0.2% Triton X-100. A further sample of the original cell suspension was also diluted 1:1 with the same solution. All samples were frozen and thawed 3 times (liquid N₂, and water at 37°C), and centrifuged at 12000g for 2 min (Eppendorf 3200 centrifuge); the supernatants were stored in ice and assayed the same day.

Portions of whole liver and of kidney cortex were homogenized in 0.25 M-sucrose/20 mM-3-(*N*-morpholino)propanesulphonate/3 mM-EDTA, pH 7.0, and 0.25 M-sucrose/3.4 mM-Tris/1 mM-EGTA, pH 7.4, respectively (10 ml/g wet wt.).

Mitochondrial fractions were prepared as described by Chappell & Hansford (1969). The pellets and samples of original homogenates were treated as above.

Enzyme activities were measured in homogenates, postmitochondrial supernatants and mitochondrial pellets in each case.

Phosphoenolpyruvate carboxykinase was assayed at 37°C by the method of Seubert & Huth (1965), as modified by Pogson & Smith (1975). Lactate dehydrogenase and glutamate dehydrogenase were assayed at 30°C (Longshaw *et al.*, 1972). Distributions of phosphoenolpyruvate carboxykinase activities between cytoplasmic and mitochondrial compartments were calculated after corrections for recovery of lactate dehydrogenase and glutamate dehydrogenase activities and for cross-contamination of fractions, assuming that these enzymes are exclusively located in the cytoplasm and mitochondria respectively. The unit of enzyme activity is defined as that amount which converts 1 μ mol of substrate into product per min at the stated temperature.

Incubations

Liver cells were prepared and incubated as described previously (Elliott *et al.*, 1976), except that lactate, albumin and hyaluronidase were omitted from the perfusion medium. Rates of gluconeogenesis were linear from 30 to 90 min of incubation; results are expressed as nmol of glucose formed/mg dry wt. over this period. All determinations were in triplicate; glycogen was measured, in parallel incubations 'stopped' with 30% (w/v) KOH, by the method of Randle *et al.* (1964).

[¹⁴C]Glucose was isolated from neutralized extracts as follows: 0.5 ml portions were added to 3 cm $\times$ 0.5 cm columns of Amberlite CG-400 (acetate form; 200 mesh) and were washed through with 2 ml of water. Appropriate samples were counted in PCS in a Packard 3375 liquid-scintillation counter. Preliminary experiments, involving conversion of [¹⁴C]glucose into [¹⁴C]gluconate with glucose oxidase, followed by ion-exchange chromatography, indicated that more than 90% of the counts in the original eluate were attributable to [¹⁴C]glucose.

Blood samples

Animals were decapitated, and 2 ml samples were immediately deproteinized with 1 ml of 6% (w/v) HClO₄. Samples were subsequently treated and assayed as described by Schein *et al.* (1971). Results have been corrected for recoveries after deproteinization. A further sample of blood was treated with solid heparin (approx. 1 mg) and centrifuged immediately for plasma-glucose determination (Krebs *et al.* 1963).

Results and Discussion

The digitonin-treatment technique for the separation of mitochondrial and cytoplasmic compartments of isolated liver cells (Zuurendonk & Tager, 1974) permits the ready and quantitative recovery of intracellular enzyme activities while minimizing cross-contamination. The activities of lactate dehydrogenase (the marker for the cytoplasmic compartment) in cells from fed, 48 h-starved and diabetic guinea pigs were respectively 124 $\pm$ 16 (mean $\pm$ S.E.M. for four determinations), 125 $\pm$ 18 (7) and 158 $\pm$ 21 (7) units/g wet wt. The corresponding activities for the mitochondrial marker, glutamate dehydrogenase, were 310 $\pm$ 23 (4), 382 $\pm$ 39 (7) and 347 $\pm$ 44 (7) units/g wet wt. respectively. These values are similar to those measured in the intact liver. The recoveries of both activities after fractionation were quantitative. The lactate dehydrogenase activity in the mitochondrial fraction (i.e. the contamination with cytoplasm) amounted to 8.7 $\pm$ 0.6% (18 observations; range 3.8–12.7%); that of glutamate dehydrogenase in the supernatant (i.e. the contamination of cytoplasm with the mitochondrial fraction) was 2.0 $\pm$ 0.4% (17 observations; range 0.2–6.1%).

The total recovered activity of phosphoenolpyruvate carboxykinase in cell-fractionation experiments was similar to that found in the whole tissue (Table 1). This, with the dehydrogenase data, demonstrates further that cells prepared by this method are intact and do not 'leak' during perfusion and centrifugation procedures. The biphasic nature of phosphoenolpyruvate carboxykinase distribution in guinea-pig liver has been confirmed. Approx. 85% of total activity is associated with the mitochondrial

Table 1. *Distribution of phosphoenolpyruvate carboxykinase activities in liver and kidney cortex of fed, 48 h-starved and diabetic guinea pigs*

Procedures and assay methods were as described in the text. Results are means $\pm$ S.E.M.; the numbers of observations are shown in parentheses. *P* (versus fed controls): * <0.025 ; ** <0.01 ; *** <0.005 ; † <0.001 ; other differences are not significant.

	Enzyme activity			Cytoplasmic enzyme activity (% of total)
	(units/g wet wt.)	(units of cytoplasmic enzyme/g wet wt.)	(units of mitochondrial enzyme/g wet wt.)	
Liver cells				
Fed (3)	20.7 $\pm$ 2.4	2.7 $\pm$ 0.3	17.5 $\pm$ 2.1	13.7 $\pm$ 1.7
Starved (4)	44.5 $\pm$ 4.9**	9.4 $\pm$ 1.1***	33.1 $\pm$ 3.7*	23.4 $\pm$ 0.8***
Diabetic (5)	23.0 $\pm$ 1.7	3.5 $\pm$ 0.5	19.3 $\pm$ 1.7	15.5 $\pm$ 1.6
Whole liver				
Fed (3)	22.9 $\pm$ 0.5	3.5 $\pm$ 0.1	17.4 $\pm$ 1.0	16.9 $\pm$ 1.1
Kidney cortex				
Fed (6)	10.5 $\pm$ 0.5	2.1 $\pm$ 0.1	8.4 $\pm$ 0.4	20.2 $\pm$ 0.6
Starved (4)	20.3 $\pm$ 0.7†	10.7 $\pm$ 0.9†	9.6 $\pm$ 1.0	52.3 $\pm$ 4.1†
Diabetic (4)	14.8 $\pm$ 1.6**	6.6 $\pm$ 1.1†	8.0 $\pm$ 1.4	44.6 $\pm$ 4.9†

Table 2. *Blood metabolites in fed, 48 h-starved and diabetic guinea pigs*

Samples were obtained and assayed as described in the text. Results are means $\pm$ S.E.M.; the numbers of observations are given in parentheses. *P* (versus fed controls); * <0.01 ; ** <0.001 ; other differences are not significant.

	Concentration (μ mol/ml) or ratio		
	Fed	Starved	Diabetic
Plasma			
Glucose	9.2 $\pm$ 0.3 (9)	9.2 $\pm$ 0.3 (11)	23.1 $\pm$ 2.1 (8)**
Whole blood			
L-Lactate	0.71 $\pm$ 0.10 (6)	0.62 $\pm$ 0.03 (4)	1.95 $\pm$ 0.16 (4)**
Pyruvate	0.071 $\pm$ 0.010 (6)	0.078 $\pm$ 0.004 (4)	0.139 $\pm$ 0.020 (4)*
Lactate/pyruvate	10.0 $\pm$ 0.4 (6)	8.0 $\pm$ 0.5 (4)*	14.6 $\pm$ 1.4 (4)*
β -Hydroxybutyrate	0.020 $\pm$ 0.004 (6)	0.172 $\pm$ 0.021 (4)**	0.187 $\pm$ 0.037 (4)**
Acetoacetate	0.090 $\pm$ 0.005 (6)	0.169 $\pm$ 0.016 (4)**	0.075 $\pm$ 0.016 (4)
β -Hydroxybutyrate acetoacetate	0.22 $\pm$ 0.05 (6)	1.0 $\pm$ 0.1 (4)*	2.7 $\pm$ 0.5 (4)**

fraction. Total activity is doubled in liver cells from 48 h-starved animals. The present measurements agree with earlier reports (Nordlie & Lardy, 1963; Söling *et al.*, 1970) that the cytoplasmic activity is increased during starvation.

It has been claimed that the mitochondrial enzyme content of both guinea pigs and rabbits does not vary with nutritional conditions (Hanson & Garber, 1972; Söling & Kleineke, 1976), although a close examination of some of the data does not clearly support this view (Söling *et al.*, 1970; Johnson *et al.*, 1973). Our results show that the guinea pig resembles the sheep (Taylor *et al.*, 1971) and the pig (Swiatek *et al.*, 1970) in the responsiveness of the mitochondrial enzyme to starvation.

Indeed, the greater absolute change occurs in the mitochondrial activity, although the relative cytoplasmic enzyme concentration rises to 23.4%. The increases in mitochondrial activity cannot be ac-

counted for simply on the basis of changes in liver weight; the DNA contents of liver cells from fed, starved and diabetic guinea pigs were 10.3 $\pm$ 0.6(3), 9.5 $\pm$ 2.2(4) and 10.0 $\pm$ 1.9 (five observations) μ g/mg dry wt. respectively. Cells from diabetic guinea pigs contain similar phosphoenolpyruvate carboxykinase concentrations and distribution to 'fed' cells.

In guinea-pig kidney cortex (Table 1), the mitochondrial phosphoenolpyruvate carboxykinase activity again predominates, being 80% of the total in the fed animal. Total activity, however, is increased in both 48 h-starved and streptozotocin-diabetic guinea pigs. In both cases, the increases are entirely attributable to changes in the cytoplasmic activity. It is noteworthy that the proportions of cytoplasmic and mitochondrial enzyme activities differ markedly in liver and kidney, and that this is especially evident in starved and diabetic animals.

We have attempted to correlate changes in enzyme

Table 3. *Net rates of gluconeogenesis in fed, 48 h-starved and diabetic guinea pigs*

Incubations, assays and calculations were as described in the text. Results are corrected for changes in cellular glycogen during incubation. The numbers of animals are given in parentheses; results are means $\pm$ S.E.M. *P* (versus fed controls); * <0.05 ; ** <0.025 ; *** <0.001 ; all other differences are not significant.

Substrate	Glucose formed (nmol/h per mg dry wt.)		
	Fed	Starved	Diabetic
L-Lactate (10mM)	101 $\pm$ 19 (4)	173 $\pm$ 20 (4)**	134 $\pm$ 10 (5)
Propionate (10mM)	111 $\pm$ 17 (3)	124 $\pm$ 33 (4)	128 $\pm$ 16 (5)
L-[U- ¹⁴ C]Lactate (10mM)	48 $\pm$ 5 (5)	117 $\pm$ 11 (4)***	93 $\pm$ 8 (4)***
[1- ¹⁴ C]Propionate (10mM)	31 $\pm$ 7 (5)	36 $\pm$ 8 (4)	48 $\pm$ 4 (4)*

activity with simultaneous changes in blood metabolites (Table 2) and, more particularly, in hepatic gluconeogenesis (Table 3).

Plasma-glucose concentrations were similar in both fed and starved animals, and increased approx. 2½-fold in diabetic guinea pigs. Starvation alone increased blood ketone-body concentrations and caused a 5-fold increase in the β -hydroxybutyrate/acetoacetate ratio. In contrast, diabetic animals exhibited increases in lactate, pyruvate and β -hydroxybutyrate, but not acetoacetate, concentrations; the ratios of lactate to pyruvate and especially of β -hydroxybutyrate to acetoacetate were significantly elevated (Table 2). In general, however, the concentrations of the metabolites studied were, except for glucose, considerably lower than those in rats under similar conditions (Ballard & Hanson, 1969). The discrepancy between our results and those of Söling *et al.* (1970) for the fed and starved guinea pig may be, in part at least, attributable to the use of pentobarbital anaesthesia by the latter workers (C. I. Pogson, unpublished work).

Liver phosphoenolpyruvate carboxykinase and net gluconeogenesis are both increased in starved and diabetic rats (Exton *et al.*, 1972; Pogson & Smith, 1975; Söling & Kleineke, 1976). Similarly, the enzyme and gluconeogenesis from lactate and propionate are increased in cells from starved guinea pigs (Table 3), although the magnitude of this change is less than in the rat. In contrast, cells from diabetic guinea pigs show no increase in either phosphoenolpyruvate carboxykinase content or gluconeogenic rate from these substrates.

Although our observations confirm the report by Lundquist *et al.* (1975) in showing that there is considerable weight loss in these animals (approx. 20%, mostly muscle, over 3 days), the streptozotocin-induced diabetes here described is clearly non-ketotic, suggesting that this animal model may have some application in the study of human hyperosmolar non-ketotic diabetes. Nevertheless, the present results indicate clear differences in the metabolic responses of the rat and guinea pig to both starvation and diabetes. Similar studies elsewhere have shown

that the rabbit is again distinct from both these species in this respect (Garber & Hanson, 1971; Huibregtse & Ray, 1975; Huibregtse *et al.*, 1976). In the absence of reliable specifically human data it is not easy to decide which of these species, if any, represents the best model for specific aspects of carbohydrate metabolism in man. It follows that due caution should be exercised in extrapolating from one species to another.

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