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INTRACELLULAR DISTRIBUTION OF SOME ENZYMES OF THE GLUTAMINE UTILISATION PATHWAY IN RAT LYMPHOCYTES

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SUMMARY: In lymphocytes of the rat, pyruvate kinase, phosphoenolpyruvate carboxykinase and $NADP^+$-linked malate dehydrogenase (decarboxylating) are distributed almost exclusively in the cytosol whereas pyruvate carboxylase is distributed almost entirely in the mitochondria. For NAD^+-linked malate dehydrogenase and aspartate aminotransferase approximately 80% and 40%, respectively, are in the cytosolic compartment. Since glutaminase is present in the mitochondria, glutamine is converted to malate within the mitochondria but further metabolism of the malate is likely to occur in the cytosol. Hence pyruvate produced from this malate, via oxaloacetate and phosphoenolpyruvate carboxykinase, may be rapidly converted to lactate, so restricting the entry of pyruvate into the mitochondria and explaining why very little glutamine is completely oxidised in these cells despite a high capacity of the Krebs cycle.

INTRODUCTION: It has been demonstrated that in resting lymphocytes of the rat the rates of utilisation of both glucose and glutamine are high but that their oxidations are only partial; glucose is converted mainly into lactate, and glutamine into glutamate aspartate and lactate (1,2). This partial oxidation is similar to that of rapidly-dividing cells such as tumour cells (3). Since lymphocytes possess a respectable capacity of the Krebs cycle (4) and since fatty acids or ketone bodies can be oxidised, and provide a significant amount of energy (5), it is unclear why pyruvate derived from either glucose or glutamine should not be oxidised by the cycle. It is possible that the transport of pyruvate across the mitochondrial membrane is a slow process and/or the conditions in the cytosol favour a very low concentration of pyruvate (e.g. due to a higher $NADH/NAD^+$ concentration ratio than normal); then, if pyruvate production occurs in the cytosol, the rate of oxidation would be very low. Although this is likely to be the case for glycolysis in these cells, it is not

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known for glutamine. If the enzymes involved in the late stages of metabolism of glutamine are confined to the cytosolic compartment, then pyruvate derived from glutamine could be directed to form lactate rather than oxidation to CO_2; in addition oxaloacetate could be directed to form aspartate. Consequently it was considered important to study the subcellular distribution of enzymes involved in this metabolism, including phosphoenolpyruvate carboxykinase, pyruvate kinase, the NAD^+- and $NADP^+$-linked malate dehydrogenases and aspartate aminotransferase in lymphocytes.

Furthermore, until recently it has usually been assumed that the conversion of [1-^{14}C]-pyruvate to $^{14}CO_2$ is a direct quantitative indication of the activity of pyruvate dehydrogenase in tissues including lymphocytes (6). However, Myles et al. (7) have pointed out that, in tissues which possess a high activity of pyruvate carboxylase, conversion of 1-^{14}C-pyruvate to $^{14}CO_2$ could occur via pyruvate carboxylation rather than oxidative dehydrogenation. Since a high activity of pyruvate carboxylase is present in lymphocytes (4) it was also considered important to investigate the subcellular distribution of this enzyme.

MATERIALS AND METHODS: Rats, chemicals and biochemicals were obtained from sources given previously (1,4,8). Rats were killed by cervical fracture, and mesenteric lymph nodes isolated as described previously (1). Homogenates were prepared by extraction in micro-ground-glass homogenizers (1 ml capacity) with 5 vol of extraction medium at 0°C. For the preparation of mitochondria, mesenteric lymph nodes of male rats (1.0 g) were homogenized in 9 vol of extraction medium, which consisted of 250 mM-sucrose, 1 mM-EDTA, 5 mM-Hepes [4-(2-hydroxyethyl)-1-pipernazine-ethanesulphonic acid] and 1% (w/v) bovine serum albumin, pH 7.4, for 2 x 10s at 0°C in a Polytron homogenizer (PCU-2, at position 3). Homogenates were centrifuged at 600 g for 5 min, followed by centrifugation of the resultant medium (extraction medium without EGTA) and washed twice by centrifugation at 8500 g for 15 min. Enzymes were assayed as described previously (4). The activity of citrate synthase was used as a mitochondrial marker and that of lactate dehydrogenase as a cytosolic marker: with the most careful homogenisation of the cells 3.0% of citrate synthase activity was found in the cytosol and 4.5% of lactate dehydrogenase was found in the mitochondrial fraction. These values are assumed to represent cross-contamination.

RESULTS AND DISCUSSION: The activities of citrate synthase, lactate dehydrogenase, pyruvate kinase, pyruvate carboxylase, phosphoenolpyruvate-carboxykinase, NAD^+-linked malate dehydrogenase, $NADP^+$-linked malate de-

TABLE 1 - Distribution of some enzymes involved in pyruvate metabolism and marker enzymes between mitochondria and cytosol in lymphocytes of rat

Enzyme	Enzyme activities		
	Whole homogenate (μmol/min per g fresh wt. lymph node)	% recovered in cytosol	% recovered in mitochondria
Lactate dehydrogenase	82.7 ± 4.13(13)	100	0
Citrate synthase	6.5 ± 0.12(8)	0	100
Pyruvate kinase	50.3 ± 1.05(5)	102 ± 3.0	1.0 ± 0.9
Pyruvate carboxylase	0.62± 0.63(14)	3.1 ± 1.3	94.4 ± 5.8
Phosphoenolpyruvate carboxykinase	0.58± 0.04(8)	96.9 ± 1.8	5.1 ± 1.8
$NADP^+$-linked malate dehydrogenase (decarboxylating)	0.28± 0.02(9)	99.5 ± 2.9	1.4 ± 1.4
NAD^+-linked malate dehydrogenase	100 ± 7.1 (9)	82.1 ± 5.7	22.9 ± 6.6
Aspartate aminotransferase	12.4 ± 0.85(12)	41.2 ± 1.4	60.8 ± 2.6

Results are presented as means ± S.E.M. with number of separate animals used given in parenthesis.

hydrogenase (decarboxylating) and aspartate aminotransferase were measured in the whole homogenate and in the cytosolic and mitochondrial compartments of lymphocytes (Table I). For calculation of the distribution of the enzyme in the two compartments, it has been assumed that lactate dehydrogenase and citrate synthase are markers for the cytosolic and mitochondrial compartments respectively. It was found that almost all the pyruvate kinase, phosphoenolpyruvatecarboxykinase and $NADP^+$-linked malate dehydrogenase activities were in cytosol whereas almost all of the pyruvate carboxylase activity was mitochondrial (Table 1). About 80% of the NAD^+-linked malate dehydrogenase was cytosolic whereas aspartate aminotransferase activity was approximately evenly distributed between the two compartments. Since previous work with lymphocytes has demonstrated that glutaminase is a mitochondrial enzyme (8) it is likely that the glutamine pathway in the mitochondria is as follows: glutamine → glutamate → oxoglutarate → succinyl CoA succinate → fumarate → malate. Some of the malate may be converted to oxaloacetate which could be transaminated with glutamate to produce oxoglutarate and aspartate. The remainder of the malate could be transported into the cytosol where it could suffer the following fate:

conversion to oxaloacetate, which would be either transaminated with glutamate catalysed by the cytosolic aspartate aminotransferase or converted to phosphoenolpyruvate via the carboxykinase for formation of pyruvate and hence lactate via pyruvate kinase and lactate dehydrogenase. This distribution of the enzymes should minimise the formation of pyruvate from glutamine in the mitochondrion and hence explain why glutamine is only partially oxidised in these cells. Of interest in the fact that in tumour cells, in which glutamine is also only partially oxidised, $NADP^+$-linked malate dehydrogenase is found mainly in the mitochondria (9) in contradistinction to the present findings with lymphocytes. In order to explain the failure to oxidise glutamine completely in tumour cells, it is proposed that the intramitochondrial $NADP^+$-linked malate dehydrogenase is inhibited by ATP (10). It is unclear why the enzyme distribution should be different in the two cells but that of the lymphocyte may represent a more stable arrangement to prevent complete oxidation of glutamine.

Ardawi and Newsholme (8) demonstrated that phosphoenolpyruvate was a potent activator of lymphocyte glutaminase and suggested that if the carboxykinase was present in the mitochondrion it could play an important role in the regulation of glutaminase. The present finding suggests that this is unlikely; cytosolic phosphoenolpyruvate might, however, enter the mitochondria via the adenine nucleotide translocator (11).

As in all other tissues (e.g. kidney, liver) pyruvate carboxylase is present exclusively in the mitochondria. This enzyme may play an important role in provision of oxaloacetate for the Krebs cycle, the substrate for which could be obtained from the β-oxidation of fatty acids. Another possible role of the high activity of pyruvate carboxylase in the mitochondria of these cells may be to produce oxaloacetate for transamination to aspartate. The importance of aspartate in such cells is to provide precursors for the formation of pyrimidines during cell division. The distribution of this enzyme calls into question any fluxes through the

pyruvate dehydrogenase reaction that are measured in isolated mitochondria on the basis of the conversion of 1-^{14}C-pyruvate into $^{14}CO_2$ (6).

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