Biochem. J. (1988) 250, 383–388 (Printed in Great Britain)

Metabolism of pyruvate by isolated rat mesenteric lymphocytes, lymphocyte mitochondria and isolated mouse macrophages

Rui CURI, Philip NEWSHOLME and Eric A. NEWSHOLME
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

1. The activities of pyruvate dehydrogenase in rat lymphocytes and mouse macrophages are much lower than those of the key enzymes of glycolysis and glutaminolysis. However, the rates of utilization of pyruvate (at 2 mM), from the incubation medium, are not markedly lower than the rate of utilization of glucose by incubated lymphocytes or that of glutamine by incubated macrophages. This suggests that the low rate of oxidation of pyruvate produced from either glucose or glutamine in these cells is due to the high capacity of lactate dehydrogenase, which competes with pyruvate dehydrogenase for pyruvate. 2. Incubation of either macrophages or lymphocytes with dichloroacetate had no effect on the activity of subsequently isolated pyruvate dehydrogenase; incubation of mitochondria isolated from lymphocytes with dichloroacetate had no effect on the rate of conversion of [1-^{14}C]pyruvate into $^{14}CO_2$, and the double-reciprocal plot of [1-^{14}C]pyruvate concentration against rate of $^{14}CO_2$ production was linear. In contrast, ADP or an uncoupling agent increased the rate of $^{14}CO_2$ production from [1-^{14}C]pyruvate by isolated lymphocyte mitochondria. These data suggest either that pyruvate dehydrogenase is primarily in the *a* form or that pyruvate dehydrogenase in these cells is not controlled by an interconversion cycle, but by end-product inhibition by NADH and/or acetyl-CoA. 3. The rate of conversion of [3-^{14}C]pyruvate into CO_2 was about 15% of that from [1-^{14}C]pyruvate in isolated lymphocytes, but was only 1% in isolated lymphocyte mitochondria. The inhibitor of mitochondrial pyruvate transport, α-cyano-4-hydroxycinnamate, inhibited both [1-^{14}C]- and [3-^{14}C]-pyruvate conversion into $^{14}CO_2$ to the same extent, and by more than 80%. 4. Incubations of rat lymphocytes with concanavalin A had no effect on the rate of conversion of [1-^{14}C]pyruvate into $^{14}CO_2$, but increased the rate of conversion of [3-^{14}C]pyruvate into $^{14}CO_2$ by about 50%. This suggests that this mitogen causes a stimulation of the activity of pyruvate carboxylase.

INTRODUCTION

It has been demonstrated, in both rat mesenteric lymphocytes and mouse peritoneal macrophages incubated *in vitro*, that rates of glucose and glutamine utilization are high, but the rates of complete oxidation of these substrates are low (Ardawi & Newsholme, 1983, 1985; Newsholme *et al.*, 1986, 1987). Since both substrates give rise to pyruvate as a common intermediate on the pathway to form acetyl-CoA for the tricarboxylic acid cycle, a simple explanation for this observation could be that the capacities for both glycolysis (from glucose) and glutaminolysis (i.e. partial oxidation of glutamine; see McKeehan, 1982) are very much greater than the capacity for pyruvate oxidation (i.e. pyruvate dehydrogenase activity) in both types of cell. The maximal capacity of glycolysis can be indicated from the maximal activity of 6-phosphofructokinase *in vitro*, that of glutaminolysis from the activity of glutaminase (see Ardawi & Newsholme, 1982, 1983; Newsholme *et al.*, 1986), and that of pyruvate oxidation from the activity of pyruvate dehydrogenase (Paul, 1979). The last of these has been measured in pig lymphocytes (Baumgarten *et al.*, 1983), but has not been reported for rat lymphocytes or murine macrophages. In addition, the rates of oxidation of pyruvate by these cells could be compared with the known rates of glucose and glutamine utilization.

The rate of pyruvate oxidation in intact cells or mitochondria is usually measured by following the conversion of [1-^{14}C]pyruvate into $^{14}CO_2$. However, it has been pointed out that, in tissues which possess pyruvate carboxylase activity, conversion of [1-^{14}C]-pyruvate into $^{14}CO_2$ could also occur via pyruvate carboxylation and subsequent reactions of the tricarboxylic acid cycle (Myles *et al.*, 1984). Since pyruvate carboxylase activity is present in both rat mesenteric lymphocytes and murine macrophages (Ardawi & Newsholme, 1983; Newsholme *et al.*, 1986), and since this enzyme is known to be present in the mitochondria of lymphocytes (Curi *et al.*, 1986) and macrophages (Newsholme *et al.*, 1987) it was considered important to attempt to estimate the contribution of pyruvate carboxylation to $^{14}CO_2$ production from [3-^{14}C]pyruvate in both incubated cells and isolated mitochondria. In addition, the effects of a number of compounds (e.g. ADP, malate, glutamine) on the rate of pyruvate oxidation and carboxylation have been investigated in the present work, to provide more information on these processes in mitochondria from lymphocytes.

The mitogen concanavalin A is known to increase the rate of pyruvate utilization by rat (Hume & Weidemann, 1980) and pig lymphocytes, and this has been assumed to be due to increased activity of pyruvate dehydrogenase (Baumgarten *et al.*, 1983). However, it seemed possible that this increased utilization of pyruvate could be due to a stimulation of pyruvate carboxylase rather than pyruvate dehydrogenase. To test this possibility, a study of the effect of concanavalin A on the rate of oxidation

Abbreviation used: CCCP, carbonyl cyanide trichloromethoxyphenylhydrazone.

of [1-^{14}C]- and [3-^{14}C]-pyruvate by rat mesenteric lymphocytes has been carried out, and the results are reported in this paper.

MATERIALS AND METHODS

Animals

Male Wistar albino rats, which were used to provide mesenteric lymph nodes, were obtained from Bantin and Kingman, Grimston, N. Humberside HU11 4QU, U.K. Elicited peritoneal macrophages were obtained from 12–16-week-old female mice of the C57 BL/C strain, bred in the Sir William Dunn School of Pathology, Oxford.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Mannheim, Lewes, Sussex, U.K., except for the following: alanine and nigrosine, from Sigma Chemical Co., Poole, Dorset, U.K.; the scintillant and all inorganic reagents, including A.R. water, from Fisons Scientific Apparatus, Loughborough, Leics., U.K.; [1-^{14}C]pyruvate from The Radiochemical Centre, Amersham, Bucks., U.K.; and [3-^{14}C]pyruvate from N.E.N. Research Products, Du Pont (U.K.) Ltd., Stevenage, Herts., U.K.

Preparation of lymphocytes and macrophages

Murine macrophages were obtained as described by Newsholme *et al.* (1986). Mesenteric lymph nodes were dissected from rats, and the lymphocytes were prepared as described by Ardawi & Newsholme (1982). Cells were suspended in phosphate-buffered saline (Culvenor & Weidemann, 1976) which had been oxygenated for 30 min under 100% O_2 (Ardawi & Newsholme, 1983).

Incubation procedure for lymphocytes and macrophages

The cells were incubated for the periods of time presented in the respective Tables, by the methods described for lymphocytes by Ardawi & Newsholme (1983) (except that 10% foetal-bovine serum was replaced by 1.5% defatted bovine serum albumin) and for macrophages, as described by Newsholme *et al.* (1987). There was little cell death during the incubations, since nigrosine was excluded from > 95% of the cells (Ardawi & Newsholme, 1983).

Preparation of lymphocyte mitochondria

Rat mesenteric lymph nodes were dissected and washed several times with extraction buffer. The latter consisted of 210 mM-mannitol, 75 mM-sucrose, 10 mM-Tris/HCl and 0.5 mM-EDTA (K salt), pH 7.4. Lymph nodes were homogenized in this buffer in a manual homogenizer (Ardawi & Newsholme, 1982). The homogenate was centrifuged twice at 500 *g* for 5 min, and the supernatant was centrifuged at 8500 *g* for 10 min. The sediment was taken up in the incubation medium. On the basis of citrate synthase and lactate dehydrogenase activities in crude extracts, it was shown that 80% of the mitochondria was recovered and that the cytosolic contamination of the mitochondrial fraction was less than 1.5%. For lymphocyte mitochondria, in the present work, the stimulation of the rate of O_2 consumption (with succinate as substrate) by addition of ADP was 3.7-fold: the respiratory control ratio was 3.3.

Incubation procedure for lymphocyte mitochondria

Mitochondria from lymphocytes were incubated in the following medium: 75 mM-mannitol, 25 mM-sucrose, 100 mM-KCl, 0.5 mM-EDTA (K salt), 10 mM-Tris, 5 mM-potassium phosphate, 1% defatted bovine serum albumin, pH 7.4. The buffer was oxygenated with 100% O_2 before addition of mitochondria and for 30 s after the addition of substrate. Preliminary studies demonstrated that the optimum pH for pyruvate oxidation by rat lymphocytes is 7.4, and addition of $KHCO_3$ (0.1 mM) had no effect on the rate of conversion of [3-^{14}C]pyruvate into $^{14}CO_2$. The free Ca^{2+} concentration in the incubation medium was 0.7 μM [a $CaCl_2$/EGTA concentration ratio of 0.84 was used, as described by Portzehl *et al.* (1964) and Denton *et al.* (1978)]. The amount of mitochondria added to the incubation medium was sufficient to provide 0.5–1.0 mg of mitochondrial protein per incubation.

The $^{14}CO_2$ produced from [1-^{14}C]- or [3-^{14}C]-pyruvate was collected as described by Leighton *et al.* (1985). For metabolite measurements, $HClO_4$ was added to the incubation medium plus cells; the resultant precipitate was removed by centrifugation at 8500 *g* for 3 min in an Eppendorf micro-centrifuge. After removal of the precipitate, the supernatant was neutralized with 2 M-triethanolamine/KOH, cooled to 0 °C, and the $KClO_4$ was removed by centrifugation at 8500 *g* for 3 min (for details, see Ardawi & Newsholme, 1983). Pyruvate was measured as described by Czok & Lamprecht (1974), lactate as described by Eagle & Jones (1978), aspartate by the method of Bergmeyer *et al.* (1974) and alanine as described by Williamson & Mellanby (1974).

For measurement of pyruvate dehydrogenase activities in rat lymphocytes and murine macrophages, cells were extracted and the activity was measured as described by Coore *et al.* (1971) and modified by Denyer *et al.* (1986). Protein was assayed as described by Bradford (1976).

Expression of the results

The pyruvate dehydrogenase activities are presented as nmol of substrate utilized/min per mg of cell protein. Pyruvate consumption and decarboxylation rates are presented as nmol/mg of cell or mitochondrial protein.

RESULTS AND DISCUSSION

The activities of pyruvate dehydrogenase from rat lymphocytes and mouse macrophages are about 3 nmol/min per mg of protein (Table 1). A similar activity has been reported for lymphocytes from the pig (Baumgarten *et al.*, 1983). This activity is only about 5% of the maximum activities of PFK from these cells (Table 1), indicating that the capacity of glycolysis is markedly in excess of that for pyruvate oxidation. Similar conclusions concerning glutaminolysis are drawn from a comparison of pyruvate dehydrogenase activities with those of glutaminase (Table 1). To study this relationship further, the rates of oxidation of pyruvate by these cells have been measured with [1-^{14}C]pyruvate. These rates can be compared with the rates of utilization of glucose or glutamine: the rates of pyruvate oxidation (at a concentration of 2 mM in the medium) were 0.67 ± 0.03 (see below) and 0.75 ± 0.05 nmol/min per mg of protein for incubated lymphocytes and macrophages respectively; the rates of glucose utilization under similar conditions were 1.3 and 5.6 nmol/min per mg of protein, and for

Table 1. Activities of pyruvate dehydrogenase and other enzymes in rat lymphocytes and murine macrophages

Pyruvate dehydrogenase activities were determined in mitochondria prepared from macrophages or lymphocytes after incubation at 20 min for 37 °C; cells were also incubated with CCCP or dichloroacetate for a similar period. Other activities were obtained from Newsholme *et al.* (1986) and have been re-calculated for 30 °C; 6-phosphofructokinase activity is reported as C_3 units for ease of comparison with that of PDH.

Cells	Addition to incubation medium	Enzyme activities (nmol/min per mg of protein at 30 °C)				
		Pyruvate dehydrogenase	6-Phospho-fructokinase	Glutaminase	Pyruvate carboxylase	Oxoglutarate dehydrogenase
Lymphocytes	None	3.3±0.11	78	59	3.7	7.6
	Dichloroacetate (100 μM)	3.5±0.12	–	–	–	–
	CCCP (10 μM)	4.9±0.16	–	–	–	–
Macrophages	None	3.2±0.26	68	228	7.0	15.1
	Dichloroacetate (100 μM)	3.2±0.04	–	–	–	–
	CCCP (10 μM)	2.9±0.09	–	–	–	–

glutamine utilization were 5.5 and 1.7 nmol/min per mg of protein, for lymphocytes and macrophages respectively. Hence rates of glucose utilization by lymphocytes and glutamine utilization by macrophages are not markedly in excess of rates of pyruvate oxidation from added pyruvate. This suggests that the low rates of oxidation of pyruvate, derived from either glucose or glutamine, by these cells under these conditions, are due to a low concentration of pyruvate, probably caused by competition for pyruvate by lactate dehydrogenase: the activity of the latter is more than 200-fold higher than that of pyruvate dehydrogenase in macrophages or lymphocytes (see Newsholme *et al.*, 1986).

The maximum activity of pyruvate dehydrogenase in both lymphocytes and macrophages is lower than that of oxoglutarate dehydrogenase (Table 1). Since the latter enzyme may indicate the maximum flux through the tricarboxylic acid cycle, this observation supports the view that substrates other than pyruvate may enter the cycle at the level of acetyl-CoA in both sets of cells; one possibility is fatty acids (Ardawi & Newsholme, 1984; Newsholme *et al.*, 1987).

Pyruvate dehydrogenase from mammalian tissues can be activated when the cells or tissues are incubated with dichloroacetate (see Whitehouse *et al.*, 1974). In contrast, incubation of macrophages, lymphocytes or mitochondria isolated from lymphocytes with dichloroacetate had no effect on the subsequent activity of pyruvate dehydrogenase (Table 1), and there was no effect of 2 mM-dichloroacetate on the rate of conversion of [1-^{14}C]pyruvate into $^{14}CO_2$ in incubated lymphocyte mitochondria (Table 2) (under similar conditions, dichloroacetate doubled the rate of $^{14}CO_2$ production in isolated hepatic mitochondria, results not shown). This finding is in contrast with that of Baumgarten *et al.* (1983), who demonstrated a 30% increase in activity of pyruvate dehydrogenase by dichloroacetate in pig lymphocytes. Dichloroacetate is considered to activate pyruvate dehydrogenase by inhibiting the interconversion-cycle enzyme, pyruvate dehydrogenase kinase, by

Table 2. Effects of various compounds on the rates of conversion of [1-^{14}C]pyruvate or [3-^{14}C]pyruvate to $^{14}CO_2$ by mitochondria isolated from mesenteric rat lymphocytes

Mitochondria were prepared as described in the Materials and methods section. The concentration of pyruvate was 0.5 mM. The results are presented as means±S.E.M. for four separate incubations.

Addition(s) to incubation medium	Rate of $^{14}CO_2$ production (nmol/20 min per mg of mitochondrial protein) from:		Ratio of [3-^{14}C] oxidation to [1-^{14}C] oxidation (%)
	[1-^{14}C]Pyruvate	[3-^{14}C]Pyruvate	
None	62.6±1.96	0.60±0.15	1.0
Dichloroacetate (2 mM)	61.6±0.14	0.48±0.05	0.8
CCCP (10 μM)	113±1.2	1.29±0.16	1.1
CCCP (10 μM) plus dichloroacetate (1 mM)	96.2±8.0	1.5±0.20	1.5
ADP (0.5 mM)	85.0±1.41	4.6±0.56	6.7
Malate (0.5 mM)	63.1±0.78	0.11±0.03	0.17
Glutamine (0.5 mM)	43.5±0.70	0.18±0.07	0.4
Glutamate (0.5 mM)	40.6±1.0	0.31±0.05	0.7
Glutamine (0.5 mM) plus ADP (0.5 mM)	84.2±0.04	0.39±0.08	0.5
Glutamine (0.5 mM) plus CCCP (10 μM)	108±2.1	0.72±0.06	0.6
α-Cyano-4-hydroxycinnamate (100 mM)	11.6±0.81	0.11±0.02	0.9
α-Cyano-4-hydroxycinnamate (100 μM) plus CCCP (10 μM)	9.3±0.35	0.11±0.04	1.2

Table 3. Effect of concanavalin A on conversion of [1-^{14}C]- and [3-^{14}C]-pyruvate into $^{14}CO_2$ at various times of incubation of mesenteric lymphocytes of the rat

Cells were incubated and $^{14}CO_2$ collected as described in the Materials and methods section. Results are presented as means ± S.E.M. for four separate experiments. Statistical significance of difference between control and concanavalin-stimulated rates (Student's t test) is indicated by *($P < 0.05$).

Time of incubation (min)	[^{14}C]Pyruvate conversion into $^{14}CO_2$ (nmol/mg of protein)			
	[1-^{14}C]Pyruvate		[3-^{14}C]Pyruvate	
	Control	Concanavalin A	Control	Concanavalin A
30	21.7±2.5	22.2±0.3	2.1±0.05	3.1±0.07*
60	42.1±2.5	41.5±0.4	8.2±1.10	12.0±0.50*
90	52.9±1.4	53.8±0.9	16.9±1.6	23.9±4.4

binding to the allosteric pyruvate-binding site; pyruvate is a pronounced inhibitor of this enzyme, and hence is a (substrate) activator of pyruvate dehydrogenase. A double-reciprocal plot of [1-^{14}C]pyruvate conversion into $^{14}CO_2$ against pyruvate concentration in mitochondria is linear over a wide range of pyruvate concentrations (0.025–2.0 mM) (results not shown). If pyruvate caused activation of pyruvate dehydrogenase, this plot would not be linear at the higher concentrations of pyruvate (Dixon & Webb, 1979). The K_m for pyruvate oxidation from this plot was 80 μM, which is similar to the reported K_m of rat heart pyruvate dehydrogenase (37 μM) (see Denton *et al.*, 1975). In contrast with the lack of effect of dichloroacetate, the uncoupling agent carbonyl cyanide trichloromethoxyphenylhydrazone (CCCP) resulted in a small increase in the activity of the extracted enzyme (Table 1). Consistent with this effect, CCCP increased the rate of [1-^{14}C]pyruvate conversion into $^{14}CO_2$ in isolated lymphocyte mitochondria by almost 2-fold (Table 2). These findings can be interpreted in at least two ways. First, the activity of pyruvate dehydrogenase in lymphocytes may not be controlled by an interconversion cycle, but only by end-product inhibition by NADH (competitive with NAD^+) and/or acetyl-CoA (competitive with CoASH) as a result of reductive acetylation of lipoate with concomitant accumulation of the pyruvate dehydrogenase–hydroxyethylthiamin pyrophosphate carbanion complex (see Cooper *et al.*, 1974; Randle *et al.*, 1978; Randle, 1981). Secondly, the enzyme in the lymphocytes or mitochondria used in this work may be primarily in the active form, so that dichloroacetate or pyruvate would not be expected to have any effect.

The rate of utilization of pyruvate (at 2 mM-pyruvate) by isolated lymphocytes was 2 nmol/min per mg of protein (at 37 °C); the rates of formation of lactate, aspartate and alanine were 0.9, 0.3 and 0.1 nmol/min per mg and the remainder was converted into CO_2. The rate of conversion of [1-^{14}C]pyruvate (at 2 mM-pyruvate in the incubation medium) into $^{14}CO_2$ was 0.7 and that of [3-^{14}C]pyruvate into $^{14}CO_2$ was 0.11 nmol/min per mg of protein. Hence the rate of pyruvate dehydrogenation is calculated to be approx 0.6 nmol/min per mg of protein, or about 10% of the maximum activity of pyruvate dehydrogenase (when calculated for 30 °C). The rate of [3-^{14}C]pyruvate oxidation by lymphocytes was about 10–30% of that for [1-^{14}C]pyruvate (Table 3). A similar percentage has been reported for incubated macrophages (Newsholme *et al.*, 1987). In contrast, in the isolated mitochondria from lymphocytes, the rate of [3-^{14}C]-pyruvate conversion into $^{14}CO_2$ was only about 1% of that from [1-^{14}C]pyruvate (Table 2). Furthermore, of the total pyruvate utilized by mitochondria, almost all could be accounted for as $^{14}CO_2$ from [1-^{14}C]pyruvate (Table 4). This suggests that the activity of pyruvate carboxylase is much higher in intact cells than in isolated mitochondria, and that some factor is responsible for activation of the enzyme in the intact cell (e.g. acetyl-CoA).

Since mitochondria can be readily prepared from lymphocytes in sufficient quantity to carry out incubation studies, the effects of a variety of factors on the rate of oxidation of [1-^{14}C]pyruvate by isolated mitochondria have been investigated (Table 2). The factors include ADP, malate and glutamine: ADP was used, since it is known, in general, to stimulate the rate of oxidation of a number of mitochondrial substrates and is known to stimulate that of glutamine in lymphocyte mitochondria (R. Curi & E. A. Newsholme, unpublished work); malate usually increases the rate of pyruvate oxidation in mitochondria from many different tissues; and glutamine is known to be an important fuel for these cells (see the Introduction). Addition of ADP increased the rate of oxidation of [1-^{14}C]pyruvate, which could be explained by a decrease in the NADH/NAD^+ concentration ratio in the mitochondria, owing to stimulation of respiration. The effect of ADP on the rate of oxidation of [3-^{14}C]-pyruvate was more marked: it increased the rate 6-fold. This may be partly explained by a stimulation of the

Table 4. Pyruvate utilization and conversion of [1-^{14}C]pyruvate into CO_2 into incubated lymphocyte mitochondria

The values are given as means ± S.E.M.

Period of incubation (min)	Rates (μmol/mg of mitochondrial protein)	
	Pyruvate utilization	[1-^{14}C]Pyruvate conversion into $^{14}CO_2$
5	30.2±3.3	26.3±3.8
10	52.3±1.9	46.0±4.2
20	62.3±2.2	62.6±2.0

tricarboxylic acid cycle by ADP and a decrease in the concentration of oxaloacetate and malate, so that the specific radioactivity in these cycle precursors could be increased.

The pyruvate-transport inhibitor cyano-4-hydroxycinnamate (Halestrap, 1975) decreased the rate of [1-^{14}C]-pyruvate oxidation by 80%, suggesting that pyruvate requires a specific transporter in lymphocyte mitochondria, as in other cells. However, in contrast with the response of mitochondria from many other cells, the addition of malate (0.5 mM) to the incubation medium had no effect on the rate of [1-^{14}C]pyruvate oxidation. This suggests that these mitochondria can maintain a sufficient concentration of oxaloacetate to satisfy the cofactor requirement of the tricarboxylic acid cycle; this might be brought about by the high activity of pyruvate carboxylase (Table 1). The presence of glutamine or glutamate decreased the rate of [1-^{14}C]pyruvate oxidation by approx. 30% (Table 2), which may be explained by a decrease in the specific radioactivity of the pyruvate in the mitochondria, since glutamine (and therefore glutamate) can be converted into pyruvate (see Ardawi & Newsholme, 1985; Curi *et al.*, 1986). Glutamine inhibited the rate of [3-^{14}C]pyruvate oxidation by 77%, and in the presence of ADP the inhibition by glutamine was more than 91% (Table 2). The mechanism is not known. Addition of glutamate had a smaller effect, but this may be due to a low rate of transport into the mitochondria.

In previous reports, evidence has been presented that concanavalin A can acutely stimulate pyruvate oxidation (Hume *et al.*, 1978; Hume & Weidemann, 1980); and Baumgarten *et al.* (1983) considered that this was due to a stimulation of pyruvate dehydrogenase by an increase in the mitochondria concentration of Ca^{2}. In the present work, we have shown that incubation with concanavalin A, at a concentration previously shown to cause mitogenesis in cultured lymphocytes (Ardawi & Newsholme, 1983) did not change the rate of [1-^{14}C]pyruvate conversion into $^{14}CO_2$: it did, however, increase the rate of [3-^{14}C]pyruvate conversion into $^{14}CO_2$ (Table 3). These results indicate that, in the present work, concanavalin A increases pyruvate utilization via a stimulation of pyruvate carboxylation, rather than via a stimulation of pyruvate dehydrogenation. It is possible that the lack of effect of concanavalin A on pyruvate dehydrogenation in the present work, in contrast with the findings of Baumgarten *et al.* (1983), is due to the different concentration of concanavalin A: in the present work the concentration was 10 μg/ml. Alternatively, it is possible that, in the present work, most of the pyruvate dehydrogenase was in the active form, so an effect of concanavalin A would not be expected. It should be noted that concanavalin A has been reported to activate pyruvate dehydrogenase from adipocytes (Mukherjee *et al.*, 1980). Nonetheless we consider that the stimulation of pyruvate carboxylation is of physiological importance in these cells, possibly to provide oxaloacetate for aspartate formation: the latter will be required for pyrimidine synthesis.

Until recently it had been been assumed that pyruvate was the most important substrate for oxidation by the tricarboxylic acid cycle in the mitochondria of lymphocytes (see Hume *et al.*, 1978; Hume & Weidemann, 1980; Baumgarten *et al.*, 1983). However, it is now known that other compounds can enter the tricarboxylic acid cycle via acetyl-CoA in these cells (e.g. fatty acids, ketone bodies), and that glutamine is oxidized by part of the tricarboxylic acid cycle (Ardawi & Newsholme, 1983, 1984, 1985). These findings suggest that pyruvate oxidation may play only a minor role in the provision of ATP for these cells, and it is tempting to speculate that pyruvate dehydrogenase is controlled by end-product inhibition (by NADH or acteyl-CoA; see above) rather than via a complex interconversion cycle involving a pyruvate dehydrogenase kinase and a phosphatase.

We are grateful to the late Professor R. R. Porter, F.R.S., for his interest and encouragement. R. C. was the recipient of a Fellowship from CNP_q (Conselho Nacional de Desenvolvimento Cientifico & Tecnologico, Brasil), and P. N. was the recipient of a SERC Training Award. We are grateful to Dr. David Hayes and Dr. Alan Kerbey for help and advice with mitochondrial preparations and assay of pyruvate dehydrogenase respectively.

REFERENCES

Ardawi, M. S. M. & Newsholme, E. A. (1982) Biochem. J. **208**, 743–748

Ardawi, M. S. M. & Newsholme, E. A. (1983) Biochem. J. **212**, 835–842

Ardawi, M. S. M. & Newsholme, E. A. (1984) Biochem J. **217**, 289–296

Ardawi, M. S. M. & Newsholme, E. A. (1985) Assays Biochem. **21**, 1–44

Baumgarten, E., Brand, M. D. & Pozzan, T. (1983) Biochem. J. **216**, 359–367

Bergmeyer, H.-U., Gawehn, K. & Grassl, M. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 2127–2131, Academic Press, London and New York

Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254

Cooper, R. H., Randle, P. J. & Denton, R. M. (1974) Biochem. J. **143**, 625–641

Coore, H. G., Denton, R. M., Martin, B. R. & Randle, P. J. (1971) Biochem. J. **125**, 115–127

Culvenor, S. G. & Weidemann, M. J. (1976) Biochim Biophys. Acta **437**, 354–363

Curi, R., Newsholme, P. & Newsholme, E. A. (1986) Biochem. Biophys. Res. Commun. **138**, 318–322

Czok, R. & Lamprecht, W. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1446–1451, Academic Press, London and New York

Denton, R. M., Randle, P. J., Bridges, B. J., Copper, R. H., Kerbey, A. L., Pask, H. T., Severson, D. L., Stansbie, D. & Whitehouse, S. (1975) Mol. Cell. Biochem. **1**, 27–53

Denton, R. M., Richards, P. A. & Chin, J. G. (1978) Biochem. J. **176**, 899–906

Denyer, G. S., Kerbey, A. L. & Randle, P. J. (1986) Biochem. J. **239**, 347–354

Dixon, M. & Webb, E. C. (1979) Enzymes, 3rd edn., pp. 137–138, Longman, London

Eagle, P. C. & Jones, J. B. (1978) Anal. Biochem. **88**, 475–484

Halestrap, A. P. (1975) Biochem. J. **148**, 85–96

Hume, D. A. & Weidemann, M. J. (1980) Mitogenic Lymphocyte Transformation, Elsevier/North-Holland, Amsterdam

Hume, D. A., Vijayakumar, E. K., Schweinberger, F., Russell, L. M. & Weidemann, M. J. (1978) Biochem. J. **174**, 711–716

Leighton, B., Budohoski, L., Lozeman, F. J., Challiss, R. A. J. & Newsholme, E. A. (1985) Biochem. J. **227**, 337–340

McKeehan, W. L. (1982) Cell Biol. Int. Rep. **6**, 635–650
Mukherjee, S. P., Mukherjee, C. & Lynn, W. J. (1980) Biochem. Biophys. Res. Commun. **93**, 36–41
Myles, D. D., Strong, P. & Sugden, M. C. (1984) Biochem. J. **218**, 997–998
Newsholme, P., Curi, R., Gordon, S. & Newsholme, E. A. (1986) Biochem. J. **239**, 121–125
Newsholme, P., Gordon, S. & Newsholme, E. A. (1987) Biochem. J. **242**, 631–636
Paul, J. M. (1979) D.Phil. Thesis, University of Oxford
Portzehl, H., Caldwell, P. C. & Ruegg, J. C. (1964) Biochem. Biophys. Acta **79**, 581–591
Randle, P. J., Sugden, P. H., Kerbey, A. L., Radcliffe, P. M. & Hutson, W. J. (1978) Biochem. Soc. Symp. **43**, 47–67
Randle, P. J. (1981) Curr. Top. Cell. Regul. **18**, 107–128
Whitehouse, S., Cooper, R. H. & Randle, P. J. (1974) Biochem. J. **141**, 761–774
Williamson, D. H. & Mellanby, J. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1836–1839, Academic Press, London and New York

Received 7 May 1987/15 October 1987; accepted 28 October 1987