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Maximum activities of key enzymes of glycolysis, glutaminolysis, pentose phosphate pathway and tricarboxylic acid cycle in normal, neoplastic and suppressed cells

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1. Maximal activities of some key enzymes of glycolysis, the pentose phosphate pathway, the tricarboxylic acid cycle and glutaminolysis were measured in homogenates from a variety of normal, neoplastic and suppressed cells. 2. The relative activities of hexokinase and 6-phosphofructokinase suggest that, particularly in neoplastic cells, in which the capacity for glucose transport is high, hexokinase could approach saturation in respect to intracellular glucose; consequently, hexokinase and phosphofructokinase could play an important role in the regulation of glycolytic flux in these cells. 3. The activity of pyruvate kinase is considerably higher in tumorigenic cells than in non-tumorigenic cells and higher in metastatic cells than in tumorigenic cells: for non-tumorigenic cells the activities range from 28.4 to 574, for tumorigenic cells from 899 to 1280, and for metastatic cells from 1590 to 1627 nmol/min per mg of protein. 4. The ratio of pyruvate kinase activity to 2 × phosphofructokinase activity is very high in neoplastic cells. The mean is 22.4 for neoplastic cells, whereas for muscle from 60 different animals it is only 3.8. 5. Both citrate synthase and isocitrate dehydrogenase activities are present in non-neoplastic and neoplastic cells, suggesting that the full complement of tricarboxylic-acid-cycle enzymes are present in these latter cells. 6. In neoplastic cells, the activity of glutaminase is similar to or greater than that of hexokinase, which suggests that glutamine may be as important as glucose for energy generation in these cells.

INTRODUCTION

It was first observed in the 1920s that tumour cells have a very high rate of lactate formation, even under aerobic conditions (see Warburg, 1956). Lack of a substantial Pasteur effect and a high rate of glycolysis even under aerobic conditions is a characteristic shared by other examples of dividing cells, albeit to a lesser extent than in tumour cells (see Krebs, 1972; Culvenor & Weidemann, 1976; Hume *et al.*, 1978). Not surprisingly there has been considerable discussion as to the underlying mechanism(s) for increased rates of glycolysis in tumour cells. For example, it has been observed that three enzymes of glycolysis, enolase, phosphoglyceromutase and lactate dehydrogenase, are phosphorylated on tyrosine residues in response to transformation of the cell with Rous sarcoma virus or upon exposure to growth factors, and the degree of phosphorylation appeared to correlate with the increase in rate of glycolysis (Cooper *et al.*, 1983). A correlation between the activity of pyruvate kinase and the malignancy of breast carcinomas has also been made (Ibsen *et al.*, 1982). However, none of these enzymes is considered to play a role in the regulation of glycolytic flux, at least in non-neoplastic cells (see Newsholme & Leech, 1983). Hence the significance of the phosphorylation of these glycolytic enzymes is unclear.

Lactate can be derived from glucose, via glycolysis, or from pyruvate produced during the partial oxidation of glutamine (glutaminolysis). Both glucose and glutamine can be used at high rates by tumour cells (see McKeehan, 1982; Kovacevic & McGivan, 1983), and it is considered that these processes are responsible for much of the ATP production in these cells. In general, it has been accepted that glycolysis is the most important process for ATP and lactate production in tumour cells (Lazo, 1981). This has received support from the evidence indicating that there is a strong correlation between rates of aerobic glycolysis and the degree of malignancy (see Weinhouse, 1972): thus Morris hepatomas that are slow-growing and well differentiated have lower glycolytic rates than the more advanced, high-growth-rate and poorly differentiated tumours. However, glutamine has also been considered to be a major fuel for tumours (see Kovacevic & McGivan, 1983) and also mammalian cells in culture (Zielke *et al.*, 1984). Thus, the precise quantitative importance of glycolysis and glutaminolysis for ATP formation, and indeed lactate formation, in such cells is by no means clear (see Reitzer *et al.*, 1979; Mares-Perlman & Shrago, 1988). It has also been observed that, in a wider range of tumour cells, rates of glutaminolysis may be proportional to degrees of malignancy (Kovacevic & Morris, 1972; Kovacevic & McGivan, 1983), and it has been suggested that glutaminolysis may be important for these cells for energy provision if glycolysis is inhibited, possibly owing to phosphorylation of pyruvate kinase (Singh *et al.*, 1974). Such phosphorylation could occur via a protein kinase that is the gene product of the *src* oncogene, pp60 *src* (Eigenbrodt & Glossmann, 1980) in cells transformed with Rous sarcoma virus or carrying the active oncogene. This suggestion has been made despite the lack of a mechanism by which changes in pyruvate kinase activity could modify the rate of glycolysis (Eigenbrodt & Glossmann, 1980). Thus all

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of these investigations suggest that there is lack of information about the relative importance of glutamine and glucose for energy provision in tumour cells. Such information will be essential in understanding the possible importance of changes in the activity of enzymes in the energy-providing pathways in these cells by processes such as phosphorylation.

There is also evidence, in tumour cells, that the flux through the left-hand side of the tricarboxylic acid cycle (that is, from oxoglutarate to oxaloacetate) is much greater than that through the right-hand side (that is, from acetyl-CoA to oxoglutarate), owing to the high rate of glutaminolysis in these cells (Coleman & Lavietes, 1981). It is considered that most of the citrate produced by citrate synthase is transferred out of the mitochondria for biosynthetic purposes (Kelleher *et al.*, 1987). Yet there is little information concerning the activities of citrate synthase and NAD^+-linked and $NADP^+$-linked isocitrate dehydrogenase activities in tumour cells.

Systematic studies in the 1970s on the maximum activities of key enzymes of carbohydrate and fat metabolism in muscle provided information on the types of fuels utilized by different muscles and their maximum contribution to ATP formation to support contractile activity (see Newsholme *et al.*, 1980). This enabled a systematic and comprehensive analysis to be made of the fuels used by different muscles from different animals across the animal kingdom (see Newsholme & Leech, 1983). More recently, a similar approach in the study of fuels used by lymphocytes and macrophages has been carried out and has provided, for the first time, evidence that these cells can use glutamine and/or long-chain fatty acids for energy formation and, indeed, that these fuels could be quantitatively more important than glucose (Ardawi & Newsholme, 1982, 1985; Newsholme *et al.*, 1986, 1987). Until that work, it had been considered that glucose was the major, if not the only, fuel to be used by lymphocytes (see Hume & Weidemann, 1980). In addition, such studies have provided, for the first time, quantitative information on the rates of ATP production from different fuels for macrophages in culture (Newsholme & Newsholme, 1989). Furthermore, the quantitative details of the fuel requirements for these cells lead to important implications concerning the whole-body supply of glutamine (Newsholme *et al.*, 1988).

In order to provide similar information for neoplastic cells, the maximum activities of some enzymes of glycolysis, the tricarboxylic acid cycle and glutamine utilization have been measured in cells from various cell lines, and these are presented in this paper. It was considered that such information would allow preliminary answers to be made to questions about the relative importance of various fuels and their potential for generation of ATP in such cells. Previous work in this area has focused attention mainly on ascites-tumour and HeLa cells. In the present work, a variety of cells were used, and not all these were neoplastic: normal and suppressed hybrid cells (Harris *et al.*, 1969) have been used for comparative purposes.

The cells used in the present work were as follows. H.Ep.2 cells were originally a human epithelial cell derived from a carcinoma of the larynx (Moore *et al.*, 1955); they have similar neoplastic properties to the HeLa cells. MRC5 cells are normal fibroblasts from a human male embryonic lung of 4 months gestation (Jacobs *et al.*, 1970). Two hybrid pairs were included in the study: the 2B1 Col 1 TG (malignant) and 2B1 Col 1 (suppressed) pair resulted from the fusion of a HeLa cell with a human fibroblast (Klinger, 1980); the second pair, ESH100 TR1-2 (malignant) and ESH100 P6 (suppressed) evolved from a combination of a HeLa cell with a human keratinocyte (Peehl & Stanbridge, 1981). Rat cardiac tissue in culture gave rise to a range of cells, the first, chronologically, being the rcc-1 line (Moares, 1989). Spontaneously, this cell line first became tumorigenic, producing rcc-1T, and then metastatic, rcc-1m. Further to this, four separate clones were discovered within rcc-1. Three were included in this study, rcc-1-2, rcc-1-3 and rcc-1-4, of which rcc-1-2 exhibits slight tumorigenic activity, although the other two appear to be normal. All cells were anchorage-dependent, except for Daudi cells, which are derived from lymphocytes of patients with Burkitt's lymphoma. These cells grow in suspension; they are small cells that do not produce tumours in nude mice (Klein *et al.*, 1967).

The aim of the study was therefore to provide quantitative information on the maximum catalytic activities of some key enzymes involved in glucose and glutamine metabolism for each cell line, and to determine if differences in activities exist in different types of neoplastic cells. Maximum enzyme activities from previous work on rat lymphocytes and mouse macrophages are included for comparative purposes.

MATERIALS AND METHODS

Materials

All chemicals, biochemicals, enzymes and cell-culture requirements were obtained from sources given previously (Ardawi & Newsholme, 1982, 1983; Newsholme *et al.*, 1986; Newsholme & Newsholme, 1989).

Preparation of cells

Cells were grown in Dulbecco's modified Eagle's medium + 10% (v/v) foetal-calf serum in tissue-culture flasks. They were rinsed three times with phosphate-buffered saline (PBS), pH 7.2 (Culvenor & Weidemann, 1976), and then removed from the flask with PBS/1 mM-EDTA and washed three times with PBS (3×10 min centrifugation at 500 *g*). Cells grown in suspension (Daudi) were harvested by centrifugation and prepared as above. Cells were resuspended in 3–5 vol. of the appropriate extraction buffer, giving a final concentration of about 5–10 mg of protein/ml. This suspension was homogenized in a 1 ml glass homogenizer at 0 °C, with 20–30 passes of the pestle. Enzyme assays were performed on this homogenate.

Extraction and enzyme assay

Activities were measured by monitoring the change in A_{340} for all enzymes except citrate synthase and pyruvate carboxylase, for which the change in A_{412} was monitored. Preliminary experiments were carried out with these cells to provide optimal conditions for extraction and measurement of maximal activities as described by Crabtree *et al.* (1979). On the basis of these experiments, some of the conditions given in published methods have been modified slightly, so that the complete details of assay buffers for these enzymes are given.

Hexokinase, lactate dehydrogenase, pyruvate kinase, isocitrate dehydrogenase, aspartate and alanine aminotransferases were extracted in the following medium:

50 mM-triethanolamine/HCl, 1 mM-EDTA, 2 mM-$MgCl_2$, 26 mM-mercaptoethanol, pH 7.5.

For assay of hexokinase, the solution contained 75 mM-Tris, 7.5 mM-$MgCl_2$, 0.8 mM-EDTA, 1.5 mM-KCl, 4 mM-mercaptoethanol, 0.02 mM-$NADP^+$, 0.125 mM-ATP, 10 mM-glucose, glucose-6-phosphate dehydrogenase (0.01 mg/ml), creatine kinase (0.05 mg/ml), 7.8 mM-phosphocreatine and 0.05% Triton at pH 7.5, in a final volume of 1 ml. Homogenate was added to start the reaction.

For assay of lactate dehydrogenase, the medium contained 60 mM-triethanolamine/HCl, 0.17 mM-NADH, 0.4 mM-pyruvate and 0.05% Triton, at pH 7.5. The final volume was 1 ml. Homogenate was added to start the reaction.

For assay of pyruvate kinase, the medium contained 160 mM-triethanolamine/HCl, 10 mM-$MgCl_2$, 80 mM-KCl, 0.17 mM-NADH, 5 mM-ADP, 0.05% Triton, 5 mM-phosphoenolpyruvate and lactate dehydrogenase (25 μg/ml), at pH 7.35. Homogenate was added to start the reaction.

For assay of isocitrate dehydrogenase, the medium contained 80 mM-Pipes, 8 mM-$MgCl_2$, 2 mM-NAD^+, 1 mM-$MnCl_2$, 2 mM-ADP, 0.05% Triton, 1.12 mM-citrate and 0.15 mM-isocitrate, at pH 7.1. The final volume was 1 ml. Isocitrate was added to start the reaction. The $NADP^+$-linked isocitrate dehydrogenase activity was assayed similarly, with the substitution of 0.5 mM-$NADP^+$ for NAD^+.

Aspartate aminotransferase and alanine aminotransferase were assayed by the method of Sugden & Newsholme (1975). Glutamate dehydrogenase was assayed as described by Ardawi & Newsholme (1982). Glucose-6-phosphate dehydrogenase and phosphogluconate dehydrogenase were assayed as described by Newsholme *et al.* (1986).

Phosphofructokinase was extracted in the following buffer: 50 mM-Tris, 1 mM-EDTA and 5 mM-$MgCl_2$, pH 8.2. The assay medium contained the following: 50 mM-Tris, 6 mM-$MgCl_2$, 200 mM-KCl, 1 mM-mercaptoethanol, 2 mM-AMP, 1 mM-ATP, 0.17 mM-NADH, 0.15 mM-fructose 6-phosphate, 0.05% Triton, aldolase (0.05 mg/ml), triosephosphate isomerase (0.0125 mg/ml) and glycerol-phosphate dehydrogenase (0.0125 mg/ml) at pH 7.6. Homogenate was added to start the reaction.

Citrate synthase was extracted in 50 mM-Tris/1 mM-EDTA, pH 7.4. The assay medium contained the following: 50 mM-Tris, 0.2 mM-5,5′-dithiobis-(2-nitrobenzoate), 25 μM-oxaloacetate, 5 μM-acetyl-CoA and 0.05% Triton, at pH 7.5. The total volume was 1 ml.

Glutaminase was extracted in the following buffer: 150 mM-potassium phosphate, 50 mM-Tris and 1 mM-EDTA, pH 8.6. The assay medium contained the following: 20 mM-potassium phosphate, 20 mM-glutamine, 28 mM-Tris, 5 mM-EDTA and 0.05% Triton, at pH 8.2. The total volume was 1 ml, and the reaction was started by addition of homogenate. This medium was incubated for 10 min at 37 °C, and the reaction was then stopped with 200 μl of ice-cold 25% (w/v) $HClO_4$. The supernatant was assayed for glutamate. Preliminary experiments established that the rate of glutamate formation was linear with time for at least 10 min.

Pyruvate carboxylase was extracted in the following buffer: 300 mM-sucrose, 50 mM-triethanolamine/HCl and 1 mM-EDTA, pH 7.4. Pyruvate carboxylase was assayed in the following medium: 100 mM-triethanolamine/HCl, 5 mM-$MgCl_2$, 0.2 mM-5,5′-dithiobis-(2-nitrobenzoate), 2.5 mM-ATP, 0.75 mM-acetyl-CoA, 50 mM-$NaHCO_3$, 5 mM-phosphocreatine, 0.05% Triton, 1 mM-pyruvate, creatine kinase (20 μg/ml) and citrate synthase (12.8 units/ml), at pH 7.4. The final volume was 1 ml and the reaction was started by addition of homogenate.

Expression of results

Enzyme activities are expressed as nmol/min per mg of protein. Activities were measured on different batches of cells, that is, separate cultures, which were grown at different times and extracted and assayed at different times, and results are presented as means ± S.E.M. Protein was determined by the method of Bradford (1976).

RESULTS AND DISCUSSION

As might be expected with such different cells, there is considerable variability in the activities of hexokinase and 6-phosphofructokinase (7.8–109 nmol/min per mg of protein for hexokinase and 0.96–64.3 nmol/min per mg of protein for 6-phosphofructokinase) (Table 1). Although it is usually considered that these cells, especially neoplastic cells, exhibit high rates of glycolysis, the maximum activities of hexokinase are not greater in most of these cells than those in normal cells, such as white and brown adipocytes or heart cells [see Cooney *et al.* (1981) for activities in normal cells]. This is also the case for phosphofructokinase. Thus any differences in the rate of glycolysis in neoplastic cells compared with normal cells may be reflected more in the control of glycolysis than in its capacity. Although the highest activities of hexokinase increase in the sequence non-tumorigenic, tumorigenic and metastatic cells, there is only a small difference in the range of activities, which are 7.8–51 (if rcc-1-2 is considered normal), 11.5–84.7 and 12.8–109 nmol/min per mg of protein respectively. Unfortunately, only two of the cell lines used in this work were metastatic, H.Ep.2 and rcc-1m, and the difference in hexokinase activity is large, 12.8 compared with 109 nmol/min per mg of protein. If indeed glycolysis is important in these cells, this finding suggests that the absolute rate is not important for metastasis.

It is of interest that in some cells the activity of hexokinase is greater than that of 6-phosphofructokinase (similar to macrophages); if maximum activities *in vitro* are indicative of the catalytic capacities for these two enzymes *in vivo*, it suggests that these cells might contain higher concentrations of glucose 6-phosphate and fructose 6-phosphate than in normal cells, and the concentration of glucose 6-phosphate might be sufficient to inhibit markedly the activity of hexokinase. If the capacity for glucose transport is high in these cells, which may well be the case for tumour cells (White *et al.*, 1981; Weber *et al.*, 1984), the combination of high rates of glucose transport and inhibition of hexokinase could lead to considerable accumulation of intracellular glucose, so that the concentration of the latter might approach that in the extracellular compartment. In this case, the intracellular glucose concentration may exceed considerably the K_m of hexokinase for glucose (usually about 0.1 mM), so that this enzyme would approach saturation with pathway substrate: that is, hexokinase would become flux-generating for glucose utilization and for glycolysis (see Newsholme & Leech, 1983). Since

Table 1. Activities of some glycolytic enzymes in various cell lines

Results are presented as means ± S.E.M. for at least eight separate batches of cells: [a] indicates tumorigenic cells, [b] metastatic cells.

Cells	Enzyme activities (nmol/min per mg of protein)			
	Hexokinase	Phosphofructokinase	Pyruvate kinase	Lactate dehydrogenase
H.Ep.2[b]	12.8 ± 1.9	42.6 ± 9.5	1627 ± 120	3775 ± 33.0
MRC5	11.0 ± 1.1	44.9 ± 4.6	547 ± 50	221 ± 19.0
ESH TR1-2[a]	11.5 ± 3.79	21.6 ± 4.0	899 ± 150	2590 ± 559
ESH P6	20.0 ± 1.36	6.71 ± 0.7	28.4 ± 9.1	415.6 ± 49.0
2B1 TG[a]	17.1 ± 1.7	8.09 ± 1.2	1194 ± 255	1024 ± 194
2B1	14.7 ± 1.3	10.9 ± 1.2	313.6 ± 13.6	1078 ± 37.0
rcc-1	62.5 ± 14.0	0.96 ± 0.13	174 ± 5.0	28.5 ± 2.5
rcc-1-2	51.0 ± 4.4	29.4 ± 7.1	373 ± 39.0	1815 ± 664
rcc-1-3	35.2 ± 2.3	7.9 ± 1.96	398 ± 19.0	943 ± 104
rcc-1-4	39.9 ± 7.8	7.5 ± 2.16	217 ± 38.0	718 ± 70.6
rcc-1t[a]	84.7 ± 13.5	40.4 ± 2.0	1280 ± 40.0	7978 ± 441
rcc-1m[b]	109 ± 14.7	64.3 ± 8.0	1590 ± 12.0	1579 ± 90.0
Daudi	7.8 ± 3.0	45.3 ± 4.0	504 ± 154	1528 ± 305
Mesenteric lymphocytes	17.3	25.7	403	823
Macrophages	76.0	22.8	447	764

phosphofructokinase can communicate with hexokinase via the near-equilibrium of phosphoglucoisomerase and the glucose 6-phosphate inhibition of hexokinase, phosphofructokinase would become a key regulatory enzyme for glycolysis in these cells, in contrast with the suggestion by Bosca & Corredor (1984). Further work on the properties of hexokinase and 6-phosphofructokinase, and particularly on the concentrations of glycolytic intermediates in these cells, is required to test this hypothesis; such work would be of importance to permit a better understanding of the regulation of the flux through this important pathway in neoplastic cells.

It is likely that, in some of these cells, some glucose 6-phosphate will be metabolized by the pentose phosphate pathway. The activities of the first two key enzymes of this pathway, glucose-6-phosphate dehydrogenase and phosphogluconate dehydrogenase, are presented in Table 2: the maximum activities are very variable. Indeed, in some neoplastic cells, particularly rcc-1m and H.Ep.2, the maximum activities of the pentose-phosphate-pathway enzymes are very high. However, the low activities in other cells, and the variability in activity, suggest that the high rates of glycolysis are not related to the need to supply precursor for the pentose phosphate pathway.

Table 2. Activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in various cell lines

Activities are presented as means ± S.E.M. for at least six separate batches of cells: [a] indicates tumorigenic cells, [b] metastatic cells.

Cells	Enzyme activities (nmol/min per mg of protein)	
	Glucose-6-phosphate dehydrogenase	6-Phosphogluconate dehydrogenase
H.Ep.2[b]	18.4 ± 2.0	31.1 ± 3.3
MRC5	18.8 ± 1.5	16.3 ± 1.6
ESH-TR1-2[a]	1.9 ± 0.13	3.8 ± 0.08
2B1 TG[a]	2.2 ± 0.22	1.2 ± 0.04
2B1	1.8 ± 0.14	0.4 ± 0.11
rcc-1-2	7.6 ± 1.8	8.0 ± 0.54
rcc-1-3	0.48 ± 0.12	2.6 ± 0.37
rcc-1-4	0.15 ± 0.02	0.60 ± 0.02
rcc-1t[a]	8.4 ± 1.3	54.8 ± 3.6
rcc-1m[b]	41.9 ± 0.2	46.0 ± 2.4
Daudi	0.23 ± 0.02	27.9 ± 1.5
Lymphocytes	17.5	21.1
Macrophages	34.5	23.8

The activity of lactate dehydrogenase is extremely variable, ranging from 28.5 to 1528 nmol/min per mg of protein for the normal and suppressed cells (if Daudi cells are included in this category), and 1024 to 7978 nmol/min per mg for the neoplastic cells. These activities are considerably higher than those of hexokinase in the same cell: hence the maximum activity of the dehydrogenase will be considerably higher than the glycolytic flux. These very high activities in the neoplastic cell lines suggest that this enzyme will compete very favourably for pyruvate, particularly in respect to the mitochondrial-membrane pyruvate transporter. Consequently, under normal conditions, very little, if any, pyruvate would be expected to enter the mitochondria from the cytosol. This could be one explanation for the very low rate of glucose oxidation in neoplastic cells.

One particular common feature from this study is that the activity of pyruvate kinase is high in almost all cells (except ESH P6 cells); the range of activities is 174–1627 nmol/min per mg of protein, excluding ESH P6 cells (Table 1). However, in contrast with all other activities measured in this work, the activities of pyruvate kinase appear to be capable of separation into three classes: for non-tumorigenic cells the activity ranges from 28.4 to 547, for tumorigenic cells the range is from 899 to 1280, and for metastatic cells the range is from 1590 to 1627. It is thus of interest that, on the basis of the activities of this enzyme, it is possible to distinguish between tumorigenic and metastatic cells. The significance of this is not known,

Table 3. Activities of citrate synthase, NAD$^+$- and NADP$^+$-linked isocitrate dehydrogenase and pyruvate carboxylase in various cell lines

Activities are presented as means ± S.E.M. for at least six separate batches of cells: [a] indicates tumorigenic cells, [b] metastatic cells.

Cell line	Enzyme activities (nmol/min per mg of protein)			
	Citrate synthase	Isocitrate dehydrogenase		Pyruvate carboxylase
		NADP$^+$-linked	NAD$^+$-linked	
H.Ep.2[b]	179.0±13.8	24.3±3.3	12.0±3.0	3.6±1.1
MRC5	86.4±6.4	43.2±4.9	9.2±1.0	25.3±2.1
ESH TR1-2[a]	71.2±9.7	8.2±1.2	10.2±2.4	21.2±6.1
ESH P6	39.7±2.3	1.6±0.11	–	4.9±0.16
2B1 TG[a]	62.1±2.12	9.0±0.46	6.8±0.46	8.7±0.49
2B1	38.6±5.8	12.1±0.28	8.5±3.1	13.4±2.07
rcc-1-2	70.5±2.6	17.5±0.78	4.2±0.1	–
rcc-1-3	37.8±3.7	4.0±0.48	0.75±0.09	–
rcc-1-4	44.5±1.6	5.6±0.06	0.52±0.07	–
rcc-1t[a]	74.1±0.3	50.5±7.0	9.1±3.5	27.9±0.04
rcc-1m[b]	50.7±4.0	36.6±2.0	4.5±2.8	18.9±4.9
Lymphocytes	63.7	26.9	6.3	2.5
Macrophages	108	27.4	5.8	4.7

but it reinforces the view that this enzyme may play an important role in neoplastic cells. Since glycolysis divides into two at the triose phosphate level, the flux through pyruvate kinase should be twice that through 6-phosphofructokinase, so that, to compare the two activities, the activity of the latter enzyme should be multiplied by 2. For neoplastic cells, the mean of the activity ratio, pyruvate kinase/2 × phosphofructokinase, is 22.4. It could be argued that such a high ratio is simply characteristic of cells that have a high glycolytic flux capacity. However, the ratio reported for a variety of muscles, in some of which the glycolytic flux can also be very high (see Newsholme & Leech, 1983), is much lower: for muscle from 60 different animals, the mean ratio was 3.8 (Zammit *et al.*, 1978). This finding suggests that, if much of this catalytic activity of pyruvate kinase is active in neoplastic tissues, the concentration of phosphoenolpyruvate will be very low, and it is therefore suggested that this glycolytic intermediate could have an inhibitory role in the proliferation process. It has, however, been shown that the concentration of phosphoenolpyruvate is not different between normal and transformed cells (Singh *et al.*, 1974). Nonetheless it may be possible that, for most of the cell cycle, the activity of the enzyme is inhibited, but at a critical stage it is activated, which then will result in a marked decrease in the level of phosphoenolpyruvate, perhaps to permit key processes in proliferation to occur.

The activity of citrate synthase in these various cells is fairly constant, although in H.Ep.2 cells it is at least 2-fold higher than in other cells. The activities of both NAD$^+$- and NADP$^+$-linked isocitrate dehydrogenase are present in all cells studied (Table 3). The activity of NAD$^+$-linked enzyme is particularly variable (0.52–12.0). There is less variability in the total NAD$^+$ + NADP$^+$-linked isocitrate dehydrogenase activities (4.7–59.6). In this present work, the activities of oxoglutarate dehydrogenase were not measured, but, since glutamine utilization probably occurs in these cells (see below), the enzyme is expected to be present. These findings therefore suggest that a full complement of tricarboxylic-acid-cycle enzymes are present in all of these cells. This is of interest, since there is considerable evidence that in tumour cells very little oxidation of acetyl units occurs via the classical tricarboxylic acid cycle. Thus, for many tumour cells (and also for lymphocytes and macrophages: see Newsholme *et al.*, 1987; Newsholme & Newsholme, 1989), it is well established that they carry out oxidation of glutamine only via the left-hand side of the tricarboxylic acid cycle (that is oxoglutarate to oxaloacetate) so that the oxidation of glutamine is only partial (glutaminolysis) (Kovacevic & Morris, 1972; McKeehan, 1982; Kovacevic & McGivan, 1983). Since the full complement of enzymes of the tricarboxylic acid cycle are present in these cells, the question arises as to the mechanism for the apparent inhibition of the right-hand side of the cycle (i.e. acetyl-CoA to oxoglutarate) in these cells. Previous work has put forward reasons for this failure (e.g. use of acetyl-CoA for cholesterologenesis), but no mechanism for regulation of the initial stages of the tricarboxylic acid cycle is known.

Although the activities of citrate synthase and total isocitrate dehydrogenase in aerobic muscles (e.g. rat heart and pigeon pectoral muscle) (Newsholme & Leech, 1983) are approximately an order of magnitude higher than those in tumour cells, the ratios of activities of citrate synthase/phosphofructokinase, or isocitrate dehydrogenase/phosphofructokinase, are not very different (comparing data provided in Tables 1 and 3 with those in Newsholme & Leech, 1983). These results support the view that it is not enzymic capacity that is responsible for a low rate of oxidation by the tricarboxylic acid cycle. This implies that metabolic control of the activities of these enzymes may be responsible. Knowledge of this mechanism may be of some considerable importance in understanding energy formation in these cells.

In the neoplastic cells investigated in this work, glutaminase activity is present with, in most cases, a maximum activity greater than that of hexokinase (cf. data in Table 1 with those in Table 4). In general, the activities of glutaminase are approximately similar to those reported previously for lymphocytes. This suggests that

Table 4. Activities of glutaminase, glutamate dehydrogenase, alanine aminotransferase and aspartate aminotransferase in various cell lines.

Activities are presented as means ± S.E.M. for at least six separate batches of cells: [a] indicates tumorigenic cells, [b] metastatic cells.

Cell line	Enzyme activities (nmol/min per mg of protein)			
	Glutaminase	Glutamate dehydrogenase	Alanine aminotransferase	Aspartate aminotransferase
H.Ep.2[b]	68.6±1.2	54.3±5.3	37.0±6.1	64.6±8.0
MRC5	45.5±7.6	140.8±8.9	34.8±3.45	35.5±0.19
ESH TR1-2[a]	26.1±2.9	25.7±5.7	38.0±12.2	22.7±3.3
ESH P6	19.8±1.7	133.3±24.5	232.5±55.7	19.1±3.3
2B1 TG[a]	73.2±13.4	16.4±3.6	10.0±2.8	42.6±9.2
2B1	58.7±3.0	32.5±6.1	4.5±0.77	17.6±2.5
rcc-1	–	15.8±3.2	–	–
rcc-1-2	11.7±2.8	4.4±1.5	18.6±4.6	39.1±4.1
rcc-1-3	38.4±2.8	19.0±4.2	32.5±10.6	33.0±2.0
rcc-1-4	5.4±0.94	18.9±6.1	19.0±0.91	13.1±2.0
rcc-1t[a]	41.2±4.1	–	–	448±9.5
rcc-1m[b]	61.8±4.7	–	–	59.5±1.2
Daudi	84.4±1.7	16.4±0.6	1.9±0.39	30.3±4.1
Lymphocytes	39.4	34.0	10.3	67.4
Macrophages	152	98.6	4.4	118

glutamine is an important substrate in all these cells. It is established that some tumour cells can utilize glutamine at a high rate; the activities presented here suggest that this will also be the case for all the cells investigated in the present work.

The activity of glutamate dehydrogenase is either similar to or lower than that of glutaminase, which suggests that some of the glutamate produced via the glutaminase reaction will be metabolized via transamination rather than by dehydrogenation. Whether alanine or aspartate is produced will depend on the relative activities of the two aminotransferase enzymes and the concentrations of the two keto acids (pyruvate and oxaloacetate). In general, the activities of aspartate aminotransferase are similar to or greater than those of alanine aminotransferase (Table 4). It would be expected that the neoplastic cells would have a higher activity of aspartate aminotransferase, to favour the formation of aspartate, a necessary precursor for the formation of pyrimidine nucleotides. In this context, it is interesting that high activities of pyruvate carboxylase (Table 3) are present in these cells, in some cases higher than those reported for lymphocytes or macrophages. This suggests that, provided that some pyruvate is available, oxaloacetate can always be produced. The role of pyruvate carboxylase may therefore be to maintain a high concentration of mitochondrial oxaloacetate in comparison with that of pyruvate to favour the aspartate aminotransferase reaction and hence the formation of aspartate rather than alanine. This would be facilitated if a large proportion of aspartate aminotransferase was present in the mitochondrial compartment. This is indeed the case for lymphocytes (Curi *et al.*, 1986).

The presence of considerable activities of glutaminase in the neoplastic cells (Table 4) suggests that the pathway of glutaminolysis will be utilized for energy provision in these cells, provided that glutamine is available. If glutaminase activity provides an index of the capacity for glutaminolysis, the present work suggests that the capacity is similar to or higher than that in lymphocytes (Table 4). Since the flux through this pathway in lymphocytes is more than two orders of magnitude greater than the demand for nitrogen for biosynthetic purposes (see Szondy & Newsholme, 1989), this finding suggests that the same may be the case for neoplastic cells, and this supports the role of glutaminolysis for energy production. However, it is also consistent with the proposal that high rates of glutaminolysis, and indeed glycolysis, provide optimal conditions for precise regulation of the biosynthetic processes that use intermediates of glutaminolysis or glycolysis. This explanation was first put forward to explain the high rates of glutaminolysis and glycolysis found in lymphocytes and macrophages (see Newsholme *et al.*, 1985*a*,*b*, 1987; Newsholme & Newsholme, 1989); for example, glutamine and aspartate are part of the glutaminolytic pathway, and are used for purine and pyrimidine nucleotide formation; glucose 6-phosphate is an intermediate in glycolysis, and is used for ribose 5-phosphate formation (see Newsholme *et al.*, 1985*a*,*b*). This precision in regulation is known as 'branched-point sensitivity' in metabolic control (Crabtree & Newsholme, 1985): if, for example, an increase in the rate of the biosynthetic pathway of 25.5-fold was required, then an increase in one specific regulator of this pathway by 25.5-fold would cause this precise increase in flux if branched-point sensitivity was applicable, and provided that the response between the regulatory enzyme and the regulator was linear. Such precision in regulation may be necessary at critical times during the cell cycle.

When the range of activities of glutaminase are compared for non-tumorigenic, tumorigenic and metastatic cells, there is overlap between the three groups: ranges are 5.4–84.1, 26.1–73.2 and 61.8–68.6 nmol/min per mg of protein respectively. As for glycolysis, this finding suggests that, if the rate of glutaminolysis increases from non-tumorigenic to tumorigenic and finally to metastatic cells, this is more likely to be due to control of key enzymes of the pathway than to differences in the

capacity. These findings suggest that a detailed study of rates of glucose and glutamine utilization in these various cells would be of considerable importance to understand more of the role and control of glycolysis and glutaminolysis in these different cell lines.

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