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Maximum activities of some enzymes of glycolysis, the tricarboxylic acid cycle and ketone-body and glutamine utilization pathways in lymphocytes of the rat

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1. The maximum activity of hexokinase in lymphocytes is similar to that of 6-phosphofructokinase, but considerably greater than that of phosphorylase, suggesting that glucose rather than glycogen is the major carbohydrate fuel for these cells. Starvation increased slightly the activities of some of the glycolytic enzymes. A local immunological challenge *in vivo* (a graft-versus-host reaction) increased the activities of hexokinase, 6-phosphofructokinase, pyruvate kinase and lactate dehydrogenase, confirming the importance of the glycolytic pathway in cell division. 2. The activities of the ketone-body-utilizing enzymes were lower than those of hexokinase or 6-phosphofructokinase, unlike in muscle and brain, and were not affected by starvation. It is suggested that the ketone bodies will not provide a quantitatively important alternative fuel to glucose in lymphocytes. 3. Of the enzymes of the tricarboxylic acid cycle whose activities were measured, that of oxoglutarate dehydrogenase was the lowest, yet its activity (about 4.0μmol/min per g dry wt. at 37°C) was considerably greater than the flux through the cycle (0.5μmol/min per g calculated from oxygen consumption by incubated lymphocytes). The activity was decreased by starvation, but that of citrate synthase was increased by the local immunological challenge *in vivo*. It is suggested that the rate of the cycle would increase towards the capacity indicated by oxoglutarate dehydrogenase in proliferating lymphocytes. 4. Enzymes possibly involved in the pathway of glutamine oxidation were measured in lymphocytes, which suggests that an aminotransferase reaction(s) (probably aspartate aminotransferase) is important in the conversion of glutamate into oxoglutarate rather than glutamate dehydrogenase, and that the maximum activity of glutaminase is markedly in excess of the rate of glutamine utilization by incubated lymphocytes. The activity of glutaminase is increased by both starvation and the local immunological challenge *in vivo*. This last finding suggests that metabolism of glutamine via glutaminase is important in proliferating lymphocytes.

It has been known for many years that rapidly dividing cells utilize glucose at a high rate (Warburg, 1956; Roos & Loos, 1973); more recently evidence has been obtained that glutamine oxidation may also be important for such tissues (for review see Krebs, 1981). Glucose metabolism in lymphocytes, which can undergo rapid cell division, has been investigated in some detail (Cooper *et al.*, 1963; Hume *et al.*, 1978), but the pathway of metabolism of glutamine and other fuels (such as ketone bodies) and their quantitative relationship to that of glucose have not been investigated.

It has been established that, for muscle, a quantitative indication of the maximum capacity of some metabolic pathways can be obtained from the maximum catalytic activity *in vitro* of key enzymes in those pathways (Newsholme *et al.*, 1980; Cooney *et al.*, 1981). The maximum activities of the following enzymes have been measured in lymphocytes to provide information about the capacities of some metabolic pathways: hexokinase (EC 2.7.1.1) for the pathway of glycolysis from glucose; glycogen phosphorylase (EC 2.4.1.1) for the pathway of glycolysis from glycogen; oxoglutarate dehydrogenase (EC 1.2.4.2) for the tricarboxylic acid cycle. In addition, the activities of 3-oxo acid CoA-transferase (EC 2.8.3.5) and 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), provide qualitative information on the ability to oxidize ketone bodies (Beis *et al.*, 1980). The activity of phosphate-de-

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pendent glutaminase (EC 3.5.1.2.) (hereafter termed 'glutaminase'), has been measured to provide information on the glutamine-utilization pathway, although there is no evidence that this activity can be used to indicate the maximum flux through this pathway (see the Results and discussion section). The activities of other related enzymes have also been measured to provide more information about metabolism of lymphocytes. Since starvation causes changes in carbohydrate, protein and fat metabolism in the whole animal (see Ruderman, 1975), the effect of this condition on these enzyme activities has been measured to indicate possible changes of fuel utilization and to compare the results with those of a similar study on the rapidly dividing cells of the small intestine (Budohoski *et al.*, 1982). Finally, the effect of a graft-versus-host reaction, used as a model of a local immunological challenge *in vivo*, has been investigated on the activities of some of these enzymes in lymphocytes from the popliteal lymph nodes. The results are reported and discussed below.

Materials and methods

Animals

Male Wistar albino rats (160–180g) and other male rats [DA × PVG (65–75g); PVG (160–180g)] were obtained from Batin and Kingman Ltd., Grimston, Hull, Yorks. HU11 4QE, U.K. The former animals provided mesenteric lymph nodes and the latter popliteal lymph nodes.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Mannheim G.m.b.H., Lewes, Sussex, U.K., except for the following: 2-mercaptoethanol, 5,5′-dithiobis-(2-nitrobenzoic acid), L-alanine, acetoacetyl-CoA, iodoacetamide, DL-isocitrate and Nigrosine were obtained from Sigma Chemicals, Poole, Dorset, U.K.; the scintillant and all inorganic reagents were obtained from Fisons Scientific Apparatus, Loughborough, Leics. LE11 0RG, U.K.; Tes (2-{[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]-amino}ethanesulphonic acid) was obtained from Hopkin & Williams, Chadwell Heath, Essex, U.K.; and $NaH^{14}CO_3$ was obtained from The Radiochemical Centre, Amersham, Bucks. HP7 9LL, U.K.

Preparation of lymphocytes

Lymphocytes were prepared from rat mesenteric lymph nodes by teasing the lymph nodes over a fine stainless-steel mesh into phosphate-buffered saline at pH 7.2 and 0°C (see Culvenor & Weidemann, 1976). The cells were rapidly filtered through a small absorbent cotton-wool plug, which was rinsed once with cold phosphate-buffered saline. The cells were centrifuged at 400*g* for 10min (twice) and were then resuspended in phosphate-buffered saline. Erythrocytes were removed by a brief (5–10min) exposure of cell pellet to 1ml of Tris-buffered NH_4Cl (Boyle, 1968). The number of viable cells present was determined by the Trypan Blue exclusion test; dilutions were made in 0.1% Trypan Blue and the proportion of viable cells present after 5min was determined with a haemocytometer; viability was always 85–90%. All glassware was silicone-treated before use.

Graft-versus-host reaction

Ford *et al.* (1970) have established that the popliteal-lymph-node weight assay is more sensitive for indicating the extent of the graft-versus-host reaction in rats than is the spleen weight assay. Cell inocula, obtained from highly inbred donors (DA × PVG rats), consisted of lymphocytes isolated from mesenteric lymph nodes. These (10^7 cells) were injected (under light anaesthesia) into the foot pad of one leg of the young F_1-hybrid recipients (DA × PVG) to act as a control. Syngeneic cells, isolated from parental (PVG) mesenteric lymph nodes, were injected into the foot pad of the other leg of the F_1 hybrid to produce the graft-versus-host reaction. Hence each F_1 recipient rat carried a graft-versus-host lymph node in one leg while the other leg carried the control lymph node. Then 7 days later rats were killed and popliteal lymph nodes were removed and weighed. Index of enlargement was determined as the ratio weight of graft-versus-host node/weight of control node.

Preparation of homogenates

Animals were killed by cervical dislocation and mesenteric or popliteal lymph nodes were carefully removed and weighed. For the assay of most enzymes, lymph nodes or lymphocytes were homogenized in a very small ground-glass homogenizer (0.1ml capacity) with 5–10vol. of extraction medium at 0°C. Preliminary experiments established that no significant differences existed between enzyme activities measured in homogenates of lymphocytes or homogenates of lymph nodes (on a dry-weight or protein basis). Hence, most activities were measured on homogenates of lymph nodes. The whole homogenate was used for enzyme assays without further treatment except for the following: for the assay of phosphorylase (EC 2.4.1.1), homogenates were centrifuged at 8000*g* (in an Eppendorf micro-centrifuge) for 2min, and the resultant supernatant was used for assay; for ketone-body-utilizing enzymes and NAD^+- and $NADP^+$-linked isocitrate dehydrogenase (EC 1.1.1.41, EC 1.1.1.42, respectively), homogenates were sonicated (for two separate periods of 2s with a microprobe of an MSE 100W ultrasonic disintegrator operating at

an amplitude of 6μm; the homogenates were maintained between 0 and 4°C during sonication), after which they were centrifuged for 2min at 8000g and the resultant supernatants used. For the assay of oxoglutarate dehydrogenase, it was important to use a mitochondrial preparation; this was obtained by homogenization of lymphocytes for two periods of 10s (at 0°C) in a Polytron homogenizer (PCU-2, at position 3) and centrifugation of the homogenate at 300g for 5min, followed by centrifugation of the resultant supernatant at 8000g for 2min. The pellet was resuspended in extraction medium.

The extraction medium for hexokinase consisted of 50mM-triethanolamine/HCl, 1mM-EDTA, 2mM-$MgCl_2$ and 30mM-mercaptoethanol at pH7.0. The same extraction medium was used for pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27), except that the pH was 7.6 and 7.5 respectively. For 6-phosphofructokinase the extraction medium was as described by Opie & Newsholme (1967). The extraction medium for phosphorylase consisted of 35mM-glycerol 2-phosphate, 20mM-NaF, 1mM-EDTA and 30mM-mercaptoethanol at pH6.2 (Cornblath *et al.*, 1963). For the assay of citrate synthase (EC 4.1.3.7), the extraction medium consisted of 25mM-Tris/HCl and 1mM-EDTA at pH7.4 (Sugden & Newsholme, 1975). For assay of oxoglutarate dehydrogenase, the extraction medium consisted of 250mM-mannitol, 5mM-Tes (potassium salt) and 1mM-EGTA at pH7.4 (Cooney *et al.*, 1981). For $NADP^+$-linked isocitrate dehydrogenase, glutamate dehydrogenase (EC 1.4.1.2), aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2), the extraction medium consisted of 50mM-triethanolamine/HCl, 1mM-EDTA, 5mM-$MgCl_2$ and 30mM-mercaptoethanol adjusted to pH7.5 with KOH. For assay of NAD^+-linked isocitrate dehydrogenase, the extraction medium was the same as that used for $NADP^+$-linked enzyme, except 2.5mM-ADP was included (Sugden & Newsholme, 1975). For assay of $NADP^+$-linked malate dehydrogenase (EC 1.1.1.40), the extraction medium consisted of 20mM-Tris/HCl, 10mM-dithiothreitol, 1mM-$MgCl_2$, 100mM-KCl and 250mM-sucrose at pH7.6 (Newsholme & Williams, 1978). For both pyruvate carboxylase (EC 6.4.1.1) and phosphoenolpyruvate carboxykinase (EC 4.1.1.32), the extraction medium consisted of 300mM-sucrose, 50mM-triethanolamine/HCl and 1mM-EDTA at pH7.4 and 7.1 respectively (Crabtree *et al.*, 1972). For the assay of 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase (EC 2.3.1.9), the extraction medium consisted of 10mM-Tris/HCl, 3mM-$MgCl_2$, 1mM-EDTA, 1mM-mercaptoethanol and 300mM-sucrose at pH7.4 (Williamson *et al.*, 1971; Beis *et al.*, 1980). For D-3-hydroxybutyrate dehydrogenase, the extraction medium consisted of 50mM-triethanolamine/HCl, 1mM-EDTA, 2mM-$MgCl_2$ and 30mM-mercaptoethanol at pH7.5 (Beis *et al.*, 1980). For assay of glutaminase, the extraction medium consisted of 150mM-potassium phosphate buffer (equimolar mixture of K_2HPO_4 and KH_2PO_4), 1mM-EDTA and 50mM-Tris/HCl at pH8.6.

Assay of enzyme activities

Enzyme activities were measured as described previously: hexokinase as described by Crabtree & Newsholme (1972), 6-phosphofructokinase as described by Opie & Newsholme (1967), pyruvate kinase as described by Zammit *et al.* (1978), lactate dehydrogenase as described by Zammit & Newsholme (1976), phosphorylase as described by Bergmeyer *et al.* (1974), citrate synthase, NAD^+- and $NADP^+$-linked isocitrate dehydrogenase as described by Alp *et al.* (1976), oxoglutarate dehydrogenase as described by Cooney *et al.* (1981), 3-oxo acid CoA-transferase, acetoacetyl-CoA thiolase and 3-hydroxybutyrate dehydrogenase as described by Williamson *et al.* (1971), glutaminase as described by Curthoys & Lowry (1973), glutamate dehydrogenase as described by Williamson *et al.* (1967), $NADP^+$-linked malate dehydrogenase as described by Newsholme & Williams (1978), phosphoenolpyruvate carboxykinase as described by Ballard & Hanson (1967), pyruvate carboxylase as described by Crabtree *et al.* (1972), aspartate aminotransferase and alanine aminotransferase as described by Sugden & Newsholme (1975).

In all the enzyme assays (except for phosphorylase, NAD^+- and $NADP^+$-linked isocitrate dehydrogenase and the ketone-body-utilizing enzymes), 0.05% (v/v) Triton X-100 was added to the assay system to complete the extraction of the enzymes. The final volume of the assay mixtures in all cases was 1.0ml.

Citrate synthase and pyruvate carboxylase were assayed by following the rate of change in A_{412}, ketone-body-utilizing enzymes by following the rate of change in A_{303}, and the remainder of the enzymes were assayed by following the rate of change in A_{340}. All spectrophotometric measurements were performed in a Gilford recording spectrophotometer (model 240) at 25°C, except for glutaminase, which was determined at 37°C. For all enzymes studied, preliminary experiments established that extraction and assay procedures were such as to produce maximum enzyme activities (see Crabtree *et al.*, 1979).

Expression of results

All activities are expressed as μmol of substrate utilized/min per g dry wt. of tissue and as nmol of substrate utilized/min per mg of protein. For

determination of dry weight, lymph nodes were dried in an oven at 50°C for 18h; the mean ratio wet wt./dry wt. was 4.0.

Results and discussion

Glycolysis

The activities of some of the glycolytic enzymes in lymphocytes are given in Table 1. The activity of hexokinase in lymphocytes is similar to that in many vertebrate muscles (Surholt & Newsholme, 1981) and similar to those reported in thymus and spleen by Hickman (1973). This finding indicates the quantitative importance of the pathway of glycolysis from glucose in lymphocytes. However, unlike most muscles, the activity of phosphorylase is lower than that of hexokinase, indicating that endogenous glycogen is not an important fuel for the lymphocyte. The activity of 6-phosphofructokinase is considerably higher than that of phosphorylase, but is only twice that of hexokinase, supporting the view that glucose is the major carbohydrate that is used by this tissue. As in muscle, the activities of pyruvate kinase and lactate dehydrogenase are much higher than those of hexokinase or phosphofructokinase. Starvation increases slightly the activities of some of the glycolytic enzymes (Table 1), which is in contrast with its effects on the glycolytic enzymes in the absorptive cells of the intestine (Budohoski *et al.*, 1982). The latter cells also possess glucose 6-phosphatase and fructose bisphosphatase activities, which may play a role in the regulation of the glycolytic flux. These activities were, however, undetectable in lymphocytes.

The effect of a local immunological challenge on these activities is shown in Table 2; the activities of all the glycolytic enzymes that were measured increased, those of 6-phosphofructokinase and pyruvate kinase by more than 100%. This finding emphasizes the importance of this pathway in the response of the lymphocyte to an immunological challenge.

Tricarboxylic acid cycle

In muscle, the maximum activity of oxoglutarate dehydrogenase provides a reasonable quantitative index of the maximum capacity of the tricarboxylic acid cycle (as measured by maximum oxygen

Table 1. *Effect of starvation on the activities of some key enzymes of carbohydrate, ketone-body and amino acid metabolism in mesenteric lymph nodes of the rat*

Lymph nodes were extracted and enzymes assayed as described in the Materials and methods section. Activities were measured at 25°C, except for glutaminase and phosphoenolpyruvate carboxykinase, which were measured at 37°C. They are presented as means ± S.E.M., with the numbers of separate animals used given in parentheses. The statistical significance (Student's *t* test) of the difference in activity between fed and starved animals is indicated by $*P < 0.05$, $**P < 0.02$, $***P < 0.005$.

	Enzyme activities			
	Fed		Starved	
	(μmol/min per g dry wt.)	(nmol/min per mg of protein)	(μmol/min per g dry wt.)	(nmol/min per mg of protein)
Hexokinase	7.3 ± 0.37 (8)	16.2 ± 2.01	9.0 ± 0.29***	17.4 ± 0.56
Phosphorylase	1.8 ± 0.09 (6)	7.9 ± 0.40	3.0 ± 0.16***	10.7 ± 0.94***
6-Phosphofructokinase	14.4 ± 0.64 (8)	41.7 ± 1.93	14.8 ± 0.13	36.7 ± 1.97
Pyruvate kinase	90.6 ± 4.0 (6)	98.2 ± 6.3	120.4 ± 14.8*	131.7 ± 6.9***
Lactate dehydrogenase	397 ± 13.2 (6)	652 ± 50.5	443 ± 11.1	700 ± 32.6
Citrate synthase	41.0 ± 0.87 (8)	167 ± 24.7	32.0 ± 3.42	113 ± 13.2*
NAD^+-linked isocitrate dehydrogenase	3.9 ± 0.36 (7)	9.2 ± 0.75	—	—
$NADP^+$-linked isocitrate dehydrogenase	2.4 ± 0.28 (7)	3.5 ± 0.35	—	—
Oxoglutarate dehydrogenase	2.1 ± 0.14 (15)	—	1.71 ± 0.41	—
3-Oxo acid CoA-transferase	0.97 ± 0.13 (8)	19.9 ± 4.15	1.15 ± 0.10	20.1 ± 4.62
Acetoacetyl-CoA thiolase	1.4 ± 0.10 (8)	27.4 ± 3.1	1.7 ± 0.20	25.9 ± 3.6
3-Hydroxybutyrate dehydrogenase	0.36 ± 0.04 (8)	4.8 ± 0.7	0.40 ± 0.05	4.9 ± 0.89
Phosphate-dependent glutaminase	34.8 ± 1.75 (15)	—	49.3 ± 1.47***	—
Glutamate dehydrogenase	13.2 ± 0.93 (12)	—	18.3 ± 0.58*	—
$NADP^+$-linked malate dehydrogenase	0.56 ± 0.05 (9)	—	—	—
Phosphoenolpyruvate carboxykinase	2.7 ± 0.57 (7)	10.1 ± 0.14	3.4 ± 0.63*	13.7 ± 0.65
Pyruvate carboxylase	7.8 ± 0.47 (6)	32.8 ± 2.55	8.0 ± 0.40	27.7 ± 3.57
Aspartate aminotransferase	10.5 ± 0.95 (9)	129 ± 8.4	—	—
Alanine aminotransferase	0.66 ± 0.07 (9)	10.3 ± 2.49	—	—

Table 2. *Maximal activities of enzymes in control and graft-versus-host-reaction popliteal lymph nodes*
Enzyme activities are presented as means ± S.E.M., with the numbers of separate animals used in parentheses. Since only one enzyme activity could be measured in a single lymph node, the mean index of enlargement (see the Materials and methods section) is given for each enzyme. The statistical significance (Student's *t* test) of the difference in activity between control and graft-versus-host lymph nodes is indicated by $*P < 0.001$. All enzymes were assayed at 25°C, except for phosphate-dependent glutaminase (37°C).

	Enzyme activities (μmol/min per g fresh wt.)		
Enzyme	Control	Graft-versus-host	Index of enlargement
Hexokinase	2.49 ± 0.09 (10)	3.83 ± 0.23*	11.20 ± 1.22
6-Phosphofructokinase	2.18 ± 0.02 (12)	5.52 ± 0.33*	11.45 ± 1.07
Pyruvate kinase	33.31 ± 1.51 (8)	68.30 ± 6.83*	12.53 ± 1.12
Lactate dehydrogenase	71.99 ± 6.04 (7)	124.5 ± 6.26*	13.57 ± 1.01
Citrate synthase	7.98 ± 0.14 (7)	12.37 ± 0.57*	12.61 ± 0.67
Phosphate-dependent glutaminase	8.17 ± 0.77 (7)	14.03 ± 0.62*	13.68 ± 0.95

consumption) and hence aerobic metabolism (Read *et al.*, 1977; Cooney *et al.*, 1981; Newsholme & Paul, 1982). In the present work, the maximum capacity of the tricarboxylic acid cycle in lymphocytes (oxygen consumption, when glucose was the sole substrate, was 1.6 ± 0.09 μmol/min per g dry wt. at 37°C, suggesting a capacity of the cycle of about 0.5 μmol/min per g) is considerably less than the maximum activity of oxoglutarate dehydrogenase (about 4.0 μmol/min per g at 37°C; Table 1). Nonetheless, the activity of this enzyme is the lowest of the enzymes of the tricarboxylic acid cycle studied in the present work (i.e. citrate synthase and isocitrate dehydrogenase). It is possible that the rate of the cycle increases towards the maximum activity of oxoglutarate dehydrogenase when the lymphocytes are in the proliferative stage after an immunological challenge. Indeed, the effect of a local immunological challenge (the graft-versus-host reaction) is to increase the activity of citrate synthase (Table 2), and this provides the first, albeit indirect, evidence of the importance of the tricarboxylic acid cycle in cell division.

The similarity of the activities of NAD^+-linked and $NADP^+$-linked isocitrate dehydrogenase activities is also observed for mammalian brain and skeletal muscle, but not for heart (Sugden & Newsholme, 1975; Alp *et al.*, 1976). Starvation decreases the activities of both citrate synthase and oxoglutarate dehydrogenase, suggesting a decreased capacity of the cycle under these conditions. This is in contrast with the effect of starvation on cells of the small intestine, in which these activities were increased (Budohoski *et al.*, 1982).

Ketone-body utilization

The enzymes involved in ketone-body metabolism are considered to catalyse near-equilibrium reactions, so that they cannot be used to provide a quantitative indication of the rate of ketone-body utilization. However, it is clear from the activities presented in Table 1 that they are well below those of hexokinase or phosphofructokinase, which is in complete contrast with the findings in brain and muscle (Sugden & Newsholme, 1973; Beis *et al.*, 1980), two tissues in which ketone-body utilization is known to be quantitatively important. Hence ketone bodies may not provide a quantitatively important alternative fuel to glucose in lymphocytes (see also Hume *et al.*, 1978).

Glutamine and amino acid metabolism

Glutamine is known to be an important fuel for a number of rapidly dividing cells (e.g. enterocytes, tumour cells, fibroblasts, reticulocytes; see Krebs, 1981). The pathway for glutamine metabolism in the enterocyte has been proposed to include the following enzymes: glutaminase, glutamate dehydrogenase, phosphoenolpyruvate carboxykinase, $NADP^+$–malate dehydrogenase, oxoglutarate dehydrogenase, aspartate aminotransferase and alanine aminotransferase (Watford *et al.*, 1979; Hanson & Parsons, 1980). These activities were measured in the lymphocyte in the present work and are reported in Table 1. The activity of glutaminase in rat lymphocytes is similar to the activity reported in the absorptive cells of the small intestine (Budohoski *et al.*, 1982). The activity of glutamate dehydrogenase in the lymphocyte is similar to that of glutaminase (calculated at 25°C), which is in contrast with the kidney, in which the dehydrogenase activity is 10 times that of glutaminase (see Newsholme *et al.*, 1982). This suggests that aminotransferase reactions rather than glutamate dehydrogenase are important in the conversion of glutamate into oxoglutarate in lymphocytes. The activity of aspartate aminotransferase is high, whereas that of alanine aminotransferase is low, suggesting that formation of aspartate is more important than the formation of alanine in lymphocytes. The activities of both $NADP^+$-linked malate dehydrogenase and phosphoenolpyruvate carboxykinase, which are required to convert intermediates of the tricarboxylic acid cycle into pyruvate for complete oxidation (see

Goldstein & Newsholme, 1976; Snell & Duff, 1977), are low and much lower than that of glutaminase. This finding suggests that the mitochondrial NAD^+-linked malate dehydrogenase might be important in converting malate into pyruvate in lymphocytes in a similar manner to that in the enterocyte (see Watford *et al.*, 1979; Hanson & Parsons, 1980). The rate of glutamine utilization by incubated lymphocytes (isolated from the mesenteric lymph nodes) is 2.62 μmol/min per g dry wt. (or about 0.8 μmol/min per g fresh wt.), which is considerably lower than the maximum activity of glutaminase of almost 35 μmol/min per g dry wt.; this suggests that the activity of this enzyme is not a good quantitative index of the maximum capacity for glutamine utilization by the lymphocyte and that the rate of this pathway might be limited by transport of glutamine across the cell or the mitochondrial membrane.

Despite this apparent excess of glutaminase activity, it is increased in the mesenteric lymph node by starvation (Table 1) and in the popliteal lymph node by an immunological challenge (Table 2). The importance of glycolysis in lymphocytes has been realized for some years, so that it is not surprising that the capacity of this pathway appears to be increased in response to an immunological challenge (see above). However, the increase in the maximum activities of citrate synthase and glutaminase suggests that both the tricarboxylic acid cycle and the glutamine utilization pathways are important in provision of energy and biosynthetic precursors for proliferation of the lymphocytes. Direct studies on the rates of these pathways in lymphocytes isolated from graft-versus-host-reaction lymph nodes will be necessary to indicate the precise quantitative importance of these pathways during proliferation.

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