Intracellular localization and properties of phosphate-dependent glutaminase in rat mesenteric lymph nodes

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1. Phosphate-dependent glutaminase was present at approximately similar activities in lymph nodes from mammals other than rat, and in thymus, spleen, Peyer's patches and bone marrow of the rat. This suggests that glutamine is important in all lymphoid tissues. 2. Phosphate-dependent glutaminase activity was shown to be present primarily in the mitochondria of rat mesenteric lymph nodes, and most of the activity could be released by detergents. 3. The properties of the enzyme in mitochondrial extracts were investigated. The pH optimum was 8.6 and the K_m for glutamine was 2.0 mM. The enzyme was activated by phosphate, other phosphorylated compounds including phosphoenolpyruvate, and also leucine: 50% activation occurred at 5, 0.2 and 0.6 mM for phosphate, phosphoenolpyruvate and leucine respectively. The enzyme was inhibited by glutamate, 2-oxoglutarate, citrate and ammonia, and by *N*-ethylmaleimide and diazo-5-oxo-L-norleucine; 50% inhibition was observed at 0.7 and 0.1 mM for glutamate and 2-oxoglutarate respectively. Some of these properties may be important in the control of the enzyme activity *in vivo*.

Although glutamine is not considered to be a dietary essential amino acid, it has several important roles in mammals (for reviews see Tate & Meister, 1973; Lund, 1980; Windmueller, 1982; Kovacevic & McGivan, 1983); in kidney, glutamine provides ammonia for acid/base balance (Pitts, 1973); in brain, it may function as a local store of glutamate, which is itself a neurotransmitter, and also for the production of γ -aminobutyrate (Quastel, 1979); and its utilization and oxidation is important, if not essential, for rapidly dividing cells (for review see Krebs, 1980). A key enzyme in the metabolism of this amino acid is phosphate-dependent glutaminase (EC 3.5.1.2) (referred to below simply as glutaminase). Lymphocytes, which can change rapidly from a resting state to rapid proliferation during an immunological challenge, possess a high activity of glutaminase, and this activity is further increased by immunological stimulation *in vivo* (Ardawi & Newsholme, 1982). To confirm that glutaminase is important in lymphoid tissue in general, the activity of this enzyme was measured in thymus, spleen, bone marrow and Peyer's patches of the rat as well as mesenteric lymph nodes of other mammals.

Glutaminase catalyses a non-equilibrium reaction in lymphocytes (see the Results and discussion section), so that its properties may be important in understanding how the enzyme activity is

controlled *in vivo* [see Newsholme & Crabtree (1979) for a review of approaches to metabolic control]. In all tissues so far investigated, glutaminase is localized within the mitochondria (see Katunuma *et al.*, 1967; Crompton *et al.*, 1973; Kalra & Brosnan, 1973; Pinkus & Windmueller, 1977), so that for interpretation of the properties of this enzyme in lymphocytes its intracellular localization must be known. The results of such an investigation, together with some properties of the enzyme, are reported below.

Materials and methods

Animals

Male Wistar albino rats (160–180 g) were obtained from Bantin and Kingman, Grimston, Hull, U.K. Rabbits and guinea pigs were obtained from University Park Farm, Northmoor, Oxford, U.K.; gerbils and hamsters were supplied by Intersimian, Abingdon, Oxon, U.K. Mice were bred in the Department of Biochemistry.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp., London W.5, U.K., except for the following: L-glutamine and hydrazine hydrate were obtained from BDH Chemicals, Poole, Dorset, U.K.; glycine and all inorganic reagents

were obtained from Fisons Scientific Apparatus, Loughborough, Leics., U.K.; bovine serum albumin, 6-diazo-5-oxonorleucine and thiol-blocking reagents were obtained from Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K.

Preparation of homogenates

Rats were killed by cervical fracture, and mesenteric lymph nodes, thymi and spleens were removed and weighed. Homogenates were prepared by extraction in micro ground-glass homogenizers (0.1 ml capacity) with 5 vol. of extraction medium at 0°C. The extraction medium consisted of 150 mM-potassium phosphate buffer (equimolar mixture of K_2HPO_4 and KH_2PO_4), 1 mM-EDTA and 50 mM-Tris/HCl, pH 8.6.

For the isolation of Peyer's patches, most of the small intestine was removed and rapidly washed by forcing ice-cold 0.9% NaCl through the lumen, and was then cut longitudinally. Peyer's patches were identified along the whole length of the small intestine, excised, weighed and homogenized as described above. Bone marrow was prepared as described by Ford (1979) and extracted in the same extraction medium. Other animals were killed by cervical dislocation, and mesenteric lymph nodes were removed, weighed and prepared for enzyme measurements.

Preparation of mitochondria

Mesenteric lymph nodes (5.0 g) were homogenized in 9 vol. of extraction medium, which consisted of 250 mM-sucrose, 1 mM-EGTA, 5 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and 1% (w/v) bovine serum albumin, pH 7.4, for 2×10 s at 0°C in a Polytron homogenizer (PCU-2, at position 3). Homogenates were centrifuged at 600g for 5 min, followed by centrifugation of the resultant supernatant at 8500g for 15 min. The pellet was resuspended in the washing medium (extraction medium without EGTA) and washed twice by centrifugation at 8500g for 15 min. For study of the properties of glutaminase, mitochondria were prepared by the above procedure except that the centrifugation at 8500g was for 3 min in an Eppendorf micro-centrifuge and the resultant pellet was washed twice with washing medium and resuspended in the same medium in a small volume (0.35 ml). There was no marked difference between the activities of glutaminase in either of the mitochondrial preparations. Mitochondrial respiration was measured with a Clark-type oxygen electrode. Isolated mitochondria (3 mg/ml) were incubated in 3.1 ml of a medium consisting of 70 mM-sucrose, 220 mM-mannitol, 10 mM-Tris/HCl, 1.2 mM-KCl, 1% bovine serum albumin and 2 mM-potassium phosphate, pH 7.2. Mitochondria were found to be

well coupled, with respiratory-control ratios between 2.5 and 3.5, with 4 mM-succinate or 5 mM-glutamate/1.5 mM-malate as substrate.

Sucrose gradients

All sucrose solutions were prepared in 1% bovine serum albumin and 1 mM-EDTA at pH 7.0. For intracellular distribution of glutaminase, a continuous sucrose gradient (30–50%, w/v) was used. Rat mesenteric lymph nodes were homogenized in 9 vol. of isolation medium (see above) in a ground-glass homogenizer. Homogenates were centrifuged at 650g for 10 min, and the resultant supernatant was layered on the sucrose gradient and centrifuged at 40000g for 3 h.

For studies on mitochondria, a continuous sucrose gradient (30–60%) was used. Mitochondria from rat mesenteric lymph nodes (see above) were suspended in small volume (1.5 ml) of 300 mM-sucrose containing 1% (w/v) bovine serum albumin and 5 mM-Hepes, pH 7.0, and layered on the sucrose gradient and centrifuged at 40000g for 3.5 h.

Release of enzyme from the mitochondria

A volume of mitochondria (0.5 ml) was diluted with equal volume of detergent solution, incubated at 4°C for 20 min and centrifuged for 45 min at 40000g. Mitochondria were sonicated for two periods of 5, 10 or 15 s with a microprobe of an MSE 100 W ultrasonic disintegrator operating at an amplitude of 6 μ m, and centrifuged for 25 min at 40000g. Supernatants and resuspended pellets were assayed for glutaminase and glutamate dehydrogenase activities.

Enzyme assays

Glutaminase was assayed either by a sampling method (Curthoys & Lowry, 1973) or by a coupling assay that monitors the rate of ammonia formation by coupling to the indicator enzyme glutamate dehydrogenase (Kvamme *et al.*, 1970). For the sampling method, the assay medium contained 150 mM-phosphate buffer (equimolar mixture of K_2HPO_4 and KH_2PO_4), 0.2 mM-EDTA, 50 mM-Tris/HCl, 20 mM-glutamine and 10–20 μ l of mitochondria preparation [treated immediately before assay with 1% (v/v) Triton X-100] at pH 8.6 in final volume of 1.0 ml. The assay was initiated by addition of freshly prepared glutamine, and the medium was incubated at 37°C for 10 min (preliminary experiments established that the time course was linear for at least 10 min). The reaction was stopped by addition of 0.2 ml of 25% (w/v) $HClO_4$, and protein was removed by centrifugation; the supernatant was neutralized with $KHCO_3$, and the concentration of glutamate determined as described by Bernt & Bergmeyer (1974). For the coupling assay, the assay medium contained

100mM-potassium phosphate (equimolar mixture of K_2HPO_4 and KH_2PO_4), 8mM-glutamine, 20mM-2-oxoglutarate, 0.12mM-NADH, rotenone (10 μ g/ml), 10 units (μ mol/min) of glutamate dehydrogenase (in glycerol) and 10–20 μ l of mitochondrial preparation [treated immediately before assay with 1% (v/v) Triton X-100] at pH 8.0 in final volume of 1.0ml. The rate of NADH oxidation was followed at 340nm and was linear for 35min. Glutamate dehydrogenase (EC 1.4.1.2) was assayed as described by Williamson *et al.* (1967), citrate synthase (EC 4.1.3.7) as described by Alp *et al.* (1976), lactate dehydrogenase (EC 1.1.1.27) as described by Zammit & Newsholme (1976), succinate dehydrogenase (EC 1.3.99.1) as described by Cooney *et al.* (1981), and 5'-nucleotidase (EC 3.1.3.5) as described by Ray (1970), except that P_i formation was measured (Fiske & SubbaRow, 1925). All enzyme assays were performed in a Gilford recording spectrophotometer (model 240). Protein was determined by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and discussion

Glutaminase activity was present in mesenteric lymph nodes of all six mammals investigated. The activities ranged from 4.6 to 9.2 μ mol/min per g fresh wt. (Table 1). All the lymphoid tissue investigated possessed glutaminase activity (Table 1). The highest was found in Peyer's patches, which are considered to be important sites for local antigenic activity in the intestine (Taylor, 1980). The activities in lymphoid tissue are similar to that reported for cells of the small intestine (Budohoski *et al.*, 1982), a tissue in which glutamine utilization is known to be quantitatively important (Wind-

mueller, 1982). It is suggested that glutamine metabolism is important for lymphoid tissue in general, in confirmation of previous findings with lymphocytes (Ardawi & Newsholme, 1982, 1983).

Intracellular distribution of glutaminase

Glutaminase is localized within the mitochondria, probably attached to the inner side of the mitochondrial inner membrane, in a number of tissues, including kidney (Curthoys & Weiss, 1974), liver (Kalra & Brosnan, 1973), small intestine (Pinkus & Windmueller, 1977) and Ehrlich ascites-tumour cells (Kovacevic, 1974). The present work suggests a similar localization of the enzyme in lymphocytes (Table 2). Marker enzymes were used to indicate cytosol (lactate dehydrogenase), plasma membrane (5'-nucleotidase) and mitochondria (glutamate dehydrogen-

Table 1. Activities of glutaminase in lymphoid tissue of different animals

Activities are presented as means $\pm$ S.E.M.; the numbers of separate animals used are given in parentheses. For details see the text.

Animal	Tissue	Glutaminase activity (μ mol/min per g fresh wt. at 37°C)
Gerbil	Mesenteric lymph node	5.6 $\pm$ 1.20 (3)
Guinea pig	Mesenteric lymph node	6.0 $\pm$ 0.45 (5)
Hamster	Mesenteric lymph node	6.6 $\pm$ 0.70 (4)
Mouse	Mesenteric lymph node	9.2 $\pm$ 0.46 (6)
Rabbit	Mesenteric lymph node	4.6 $\pm$ 0.78 (3)
Rat	Mesenteric lymph node	8.7 $\pm$ 0.43 (15)
	Thymus	6.0 $\pm$ 0.96 (6)
	Spleen	6.1 $\pm$ 0.91 (5)
	Peyer's patches	10.6 $\pm$ 1.00 (5)
	Bone marrow	3.7 $\pm$ 0.15 (6)

Table 2. Relative activities of glutaminase and marker enzymes in fractions of rat mesenteric lymph nodes prepared on a sucrose gradient

The fractions were prepared from a crude homogenate and enzyme activities were measured as described in the Materials and methods section. Enzyme activities in the crude homogenate were as follows (nmol/min per mg of protein, at 25°C): glutaminase, 27 $\pm$ 1.0; lactate dehydrogenase, 646 $\pm$ 13; glutamate dehydrogenase, 110 $\pm$ 6; citrate synthase, 165 $\pm$ 10; succinate dehydrogenase, 11 $\pm$ 1.5; 5'-nucleotidase, 2.1 $\pm$ 0.1. Results are presented as means $\pm$ S.E.M. of percentage of activity in the crude homogenate of three separate experiments, each performed with two separate sucrose gradients.

Sucrose gradient (%, w/v)	Enzyme activities (% recovered of that in crude homogenate)					
	Glutaminase	Lactate dehydrogenase	Glutamate dehydrogenase	Citrate synthase	Succinate dehydrogenase	5'-Nucleotidase
10	0	82.7 $\pm$ 1.8	0	0	0	3.7 $\pm$ 0.3
10–30	0	11.7 $\pm$ 1.2	7.7 $\pm$ 1.5	13.3 $\pm$ 1.8	9.0 $\pm$ 0.6	19.0 $\pm$ 0.6
30–35	3.7 $\pm$ 0.9	5.7 $\pm$ 0.9	0	7.0 $\pm$ 0.6	0	56.0 $\pm$ 2.1
35–40	11.3 $\pm$ 0.9	0	0	0	0	10.0 $\pm$ 1.2
40–45	53.0 $\pm$ 1.2	0	65.7 $\pm$ 1.8	56.7 $\pm$ 1.8	62.3 $\pm$ 1.5	8.3 $\pm$ 2.1
45–50	32.0 $\pm$ 1.2	0	27.3 $\pm$ 0.3	23.9 $\pm$ 0.6	28.7 $\pm$ 1.3	3.0 $\pm$ 1.2

Fig. 1. Activities of glutaminase and glutamate dehydrogenase in fractions of rat mesenteric-lymph-node mitochondria prepared on a sucrose gradient

Mitochondria and fractions were prepared, and enzyme activities were measured, as described in the Materials and methods section. Enzyme activities in the mitochondrial homogenate were as follows (nmol/min per mg of protein at 25°C): ●, glutaminase, 25; ○, glutamate dehydrogenase, 88.

ase, citrate synthase, succinate dehydrogenase), as in the work of Jacques (1973) and Wattiaux (1973).

The distribution of glutaminase in fractions from mitochondria of lymphocytes prepared by sucrose-gradient centrifugation closely followed that of the mitochondrial marker enzyme glutamate dehydrogenase (Fig. 1).

Glutaminase was not released from the mitochondria by hypo-osmotic solution (2 mM-MgCl₂/10 mM-KH₂PO₄, pH 7.6) or freeze-thawing. Sonication of mitochondria resulted in the release of 80% of the glutamate dehydrogenase, but only 50% of glutaminase. Treatment of mitochondria with 0.05% Triton or Lubrol resulted in the release of 12 and 3% respectively of the enzyme. At 0.5% of these detergents, more than 50% of the glutamin-

ase was released, whereas about 80% of the glutamate dehydrogenase was released (Table 3). Increased concentrations of detergent result in a greater proportion of glutaminase being released. It seems likely that this enzyme is bound to the mitochondrial inner membrane, but can be released by strong detergents. This is similar to the enzyme from other tissues (Kalra & Brosnan, 1974; Curthoys *et al.*, 1976).

Properties of glutaminase

For the study of the properties of the enzyme, mitochondria were prepared from rat mesenteric lymph nodes and the activity was released by addition of 1% (v/v) Triton to the assay medium. Mitochondria from rat mesenteric lymph nodes retained about 80% of glutaminase activity when maintained at 4°C in 100 mM-potassium phosphate buffer, pH 8.6, for 60 min, and less than 50% after 24 h. The effects of pH, substrate concentration and some activators or inhibitors were investigated.

The pH optimum was 8.6, with activity maintained over a broad pH range of 7–10 (results not shown).

The K_m for glutamine, obtained from a double-reciprocal plot, was about 2.0 mM, which is similar to that reported for the enzyme from brain (Svenneby *et al.*, 1970), kidney (Curthoys *et al.*, 1976), small intestine (Pinkus & Windmueller, 1977) and ascites-tumour cells (Kovacevic, 1974), but much lower than that reported for the liver (Huang & Knox, 1976).

Activators and inhibitors

The concentration of glutamine, glutamate and ammonia were measured in freeze-clamped mesenteric rat lymph nodes (Ardawi, 1983); assuming equal distribution of these metabolites in cytosolic and mitochondrial compartments, the ΔG for the glutaminase reaction was calculated as -16.08 kJ/mol. This indicates that the enzyme catalyses a non-equilibrium reaction and therefore the properties of the enzyme may form the basis for a theory of control *in vivo*.

It is well established that phosphate is an activator of glutaminase (Sayre & Roberts, 1958; Svenneby, 1972; Curthoys *et al.*, 1976; Pinkus & Windmueller, 1977). The effects of phosphate and other compounds on glutaminase activity from lymphocytes are shown in Table 4. Phosphate and other phosphate-containing compounds activated the enzyme: at equimolar concentrations, ATP, CTP, GTP, ITP and phosphoenolpyruvate were as effective as, or more effective than, phosphate. Sulphate and leucine also activated the enzyme (the sampling assay was used to investigate the effect of leucine). The effects of phosphate,

Table 3. Effect of sonication and different detergents on the release of glutaminase and glutamate dehydrogenase from rat mesenteric lymph node mitochondria

Preparation of mitochondria and enzyme assays are described in the Materials and methods section. The percentages of glutaminase and glutamate dehydrogenase activities released were based on the amounts contained in the initial mitochondrial sample (i.e. 2.5 $\mu\text{mol/min}$ and 3.1 $\mu\text{mol/min}$ respectively).

Treatment	Glutaminase			Glutamate dehydrogenase		
	$(\mu\text{mol/min})$		(% released)	$(\mu\text{mol/min})$		(% released)
	Supernatant	Pellet		Supernatant	Pellet	
Sonication						
2 $\times$ 5 s	1.10	1.05	44	1.45	1.38	46
2 $\times$ 10 s	1.28	0.97	51	2.10	1.10	66
2 $\times$ 15 s	1.13	0.72	45	2.55	0.42	81
Triton						
0.05%	0.30	2.13	12	1.19	1.85	38
0.10%	0.73	1.52	29	1.92	1.11	61
0.50%	1.53	0.81	61	2.76	0.30	88
1.00%	1.90	0.32	76	2.83	0.20	90
Lubrol						
0.05%	0.075	2.03	3	1.54	1.30	49
0.10%	0.35	1.82	14	2.05	0.93	65
0.50%	1.38	0.87	55	2.30	0.63	73
1.00%	1.55	0.72	62	2.36	0.54	75

Table 4. Activation of lymphocyte glutaminase by various compounds

Mitochondria from rat mesenteric lymph nodes were used as the source of glutaminase, which was assayed by the continuous assay, except for the effect of leucine, when the sampling assay was used (see the Materials and methods section). The assay buffer consisted of 50mM-Tris/HCl and 250mM-sucrose, pH 8.0. Under these conditions glutaminase activity was very low (6.71 nmol/min per mg of protein at 25°C). All compounds were added at a concentration of 2.0mM unless otherwise indicated.

Addition to assay mixture	Increase in glutaminase activity (fold)
None	0
Phosphate (100mM)	7.35
Phosphate	1.76
ATP	2.79
CTP	2.06
GTP	2.03
ITP	2.13
Glucose 6-phosphate	1.25
Fructose 6-phosphate	1.32
Phosphoenolpyruvate	2.43
Leucine	1.98
Sulphate	2.72

phosphoenolpyruvate and leucine were studied further: a plot of percentage activation against phosphate concentration is sigmoidal, with a 50% effect at about 5mM-phosphate (Fig. 2). The plot of glutaminase activity against phosphate concentra-

Fig. 2. Effect of phosphate on rat mesenteric-lymph-node glutaminase activity

Activity was measured as described in the Materials and methods section.

tion is more sigmoidal for the enzyme from lymph nodes than that from other tissues (for example Goldstein, 1967; Chiu & Boeker, 1979). This suggests that changes in the concentration of phosphate may be important in control of the activity *in vivo*. The 50% effect of phosphoenolpyruvate occurred at 0.2mM; the 50% effect of leucine

Table 5. *Inhibition of lymphocyte glutaminase by various compounds*

Mitochondria from rat mesenteric lymph nodes were used as the source of glutaminase assayed by the continuous assay, except for the experiments involving 2-oxoglutarate and NH_4Cl , in which the sampling assay was used (see the Materials and methods section). The assay buffer consisted of 50 mM-Tris/HCl, 100 mM-potassium phosphate and 250 mM-sucrose, pH 8.0. Under these conditions glutaminase activity was 51.6 nmol/min per mg of protein. All compounds were added at a concentration of 2.0 mM unless otherwise indicated.

Addition to assay mixture	Inhibition (%)
2-Oxoglutarate	97
Glutamate	72
Citrate	35
NH_4Cl	32
Succinate	24
Mersalyl (1.0 mM)	92
N-Ethylmaleimide (1.0 mM)	89

occurred at about 0.6 mM in the presence of phosphate. The effects of these compounds occur at physiological concentrations, suggesting that they might be important in regulation of this enzyme *in vivo*. Although the effect of phosphoenolpyruvate on the enzyme is probably due to the phosphate group, it is possible that changes in the concentrations of both phosphate and phosphoenolpyruvate in the mitochondria control the activity of glutaminase; indeed the mitochondria concentration of phosphoenolpyruvate may be higher than that of phosphate. Changes in the mitochondrial concentration of leucine may also be important: indeed, leucine stimulates glutamine metabolism in isolated liver cells (Baverel & Lund, 1979).

Glutamate is known to be a potent inhibitor of glutaminase from various sources (Krebs, 1935; Sayre & Roberts, 1958; Goldstein, 1966; Svenneby *et al.*, 1970; Crompton *et al.*, 1973; Shapiro *et al.*, 1982). Glutaminase from lymphocytes is also inhibited by glutamate (Table 5), and the inhibition is competitive with respect to glutamine; 50% inhibition occurred at 0.7 mM-glutamate. Marked inhibition was produced by 2-oxoglutarate, 50% inhibition occurring at approx. 0.1 mM (sampling assay was used). Citrate and NH_4Cl also caused inhibition (Table 5). The metabolism of glucose by lymphocytes and their stimulation by concanavalin A both increase the rate of glutamine metabolism, and hence the activity of glutaminase (Ardawi & Newsholme, 1983). Candidates for regulation of this enzyme include increased concentration of leucine or phosphoenolpyruvate and/or decreased concentrations of glutamate or 2-oxoglutarate. Since there is evidence that stimula-

Table 6. *Inhibition of lymphocyte glutaminase by 6-diazo-5-oxo-L-norleucine*

Mitochondria from rat mesenteric lymph nodes were used as the source of glutaminase; these were preincubated for 20 min at 22°C in solutions containing 50 mM-Tris/HCl, 30 mM-potassium phosphate and 250 mM-sucrose, pH 7.6, and various concentrations of 6-diazo-5-oxo-L-norleucine, as indicated below (but absent from control), and then glutaminase was assayed by the continuous assay (see the Materials and methods section). In some experiments, 20 mM-glutamine was included in the preincubation period. The 100% activity of glutaminase (control) under standard assay conditions was 46.52 nmol/min per mg of protein at 25°C.

Concn. of inhibitor (mM)	Activity (% of control)	
	In the presence of glutamine	In the absence of glutamine
0.7	100	100
0.5	86	43
1.0	60	16
2.0	42	3

tion by concanavalin A increases the activity of 2-oxoglutarate dehydrogenase (Ardawi & Newsholme, 1983), which would cause a decrease in the concentrations of 2-oxoglutarate and glutamate, this could be responsible for an increase in the activity of glutaminase in a similar manner to that proposed for the kidney (Goldstein, 1976; Goldstein & Boylan, 1978; Strzelecki & Schoolwerth, 1981; Curthoys & Shapiro, 1982).

The thiol-blocking reagents *N*-ethylmaleimide and mersalyl (1.0 mM) inactivated (>90%) glutaminase when they were preincubated with the mitochondrial preparation at 37°C for 20 min in 100 mM-potassium phosphate buffer (pH 8.0). The inhibitory effect of thiol-blocking reagents on glutaminase has been observed previously (Sayre & Roberts, 1958; Kvamme & Olsen, 1979; Joseph & Meijer, 1981). 6-Diazo-5-oxo-L-norleucine has been shown to inhibit various glutaminases (Hartman, 1968; Holcenberg *et al.*, 1978; Shapiro *et al.*, 1979) by interacting at a site specific for the binding of glutamine; it also inhibits lymphocyte glutaminase (Table 6).

The following compounds were found to have little or no effect on glutaminase: ADP, AMP, aspartate, acetoacetate and bicarbonate, glucose, lactate, fumarate, adenosine, IMP, glycine, asparagine, serine, valine, isoleucine and 4-methyl-2-oxopentanoate.

Effect of age and altered physiological states

The activity of lymphocyte glutaminase from newly weaned rats (20 days of age) was found to be about 14.6 $\mu\text{mol/min}$ per g fresh wt. measured at

Table 7. *Effect of acidosis on the activity of glutaminase in various tissues of the rat*

Rats were made acidotic by the addition of 280 mM-NH₄Cl to their drinking water for 7 days. Homogenates of lymphoid tissues were prepared as described in the Materials and methods section. Homogenates of other tissues were prepared in 6–10 vol. of extraction medium with a Polytron (at position 4), for two separate periods of 10–20 s at 0°C. Glutaminase was assayed by measuring the rate of glutamate formation (Curthoys & Lowry, 1973). Activities were measured at 37°C, and are presented as means ± S.E.M., with the numbers of separate animals used given in parentheses.

Tissue	Glutaminase activity ($\mu\text{mol/min per g fresh wt.}$)	
	Control	Acidosis
Mesenteric lymph nodes	8.57 ± 0.43 (7)	7.92 ± 0.33 (10)
Thymus	5.72 ± 0.56 (4)	5.07 ± 0.63 (4)
Spleen	6.21 ± 0.78 (4)	6.10 ± 0.85 (4)
Liver	5.11 ± 0.57 (5)	6.89 ± 0.52 (5)
Heart	4.11 ± 0.66 (4)	5.12 ± 0.81 (4)
Kidney	24.27 ± 0.84 (8)	82.58 ± 3.9 (8)

37°C, which was about 65% higher than that in adult rats. The adult activity was present by the age of 28 days. The activity was increased by starvation of the donor rat for 48 h (see Ardawi & Newsholme, 1982), but was not affected by chronic acidosis in contrast with the enzyme from the kidney but similar to that from heart and liver (Table 7). Hence the increased demand for glutamine by kidney is unlikely to be met by decreased rates of glutamine utilization in lymphoid or other tissues, but must be achieved by increased rates of glutamine formation in muscle (see Schröck *et al.*, 1980).

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