

Glutamine metabolism in lymphocytes of the rat

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1. The metabolism of glutamine in resting and concanavalin-A-stimulated lymphocytes was investigated. In incubated lymphocytes isolated from rat mesenteric lymph nodes, the rates of oxygen and glutamine utilization and that of aspartate production were approximately linear with respect to time for 60 min, and the concentrations of adenine nucleotides plus the ATP/ADP or ATP/AMP concentration ratios remained approximately constant for 90 min. 2. The major end products of glutamine metabolism were glutamate, aspartate and ammonia: the carbon from glutamine may contribute about 30% to respiration. 3. When both glucose and glutamine were presented to the cells, the rates of utilization of both substances increased. Evidence was obtained that the stimulation of glycolysis by glutamine could be due, in part, to an activation of 6-phosphofructokinase. 4. Starvation of the donor animal increased the rate of glutamine utilization. The phosphoenolpyruvate carboxykinase inhibitor mercaptopicolinate decreased the rate of glutamine utilization by 28%; the rates of accumulation of glutamate and ammonia were decreased, whereas those of lactate, aspartate and malate were increased. 6. The mitogen concanavalin A increased the rate of glutamine utilization (by about 51%). The rate of [³H]thymidine incorporation into DNA caused by concanavalin A in cultured lymphocytes was very low in the absence of glutamine; it was increased about 4-fold at 1 μ M-glutamine and was maximal at 0.3 mM-glutamine; neither other amino acids nor ammonia could replace glutamine.

The pathway of glucose utilization in lymphocytes has been investigated in detail (Roos & Loos, 1973; Hume *et al.*, 1978), and it has been suggested that a function of the enhanced glycolytic rate during proliferation of such cells is the maintenance of elevated concentrations of glycolytic intermediates for macromolecular synthesis (Hume & Weidemann, 1979). There is also considerable evidence that glutamine utilization and oxidation are important, if not essential, for rapidly dividing cells (for review see Krebs, 1981), which is not surprising, since it provides nitrogen for a number of important precursors for the synthesis of macromolecules, including purine and pyrimidine nucleotides, amino sugars and some amino acids (Tate & Meister, 1973). The importance of glutamine metabolism in lymphocytes was indicated from the observations that these cells possess high activities of glutaminase and that this activity was increased during immunological challenge *in vivo* (Ardawi & Newsholme, 1982).

The rate of glutamine metabolism and the nature

of the pathway of glutamine utilization by lymphocytes have been investigated by measurement of the intermediates that accumulate on incubation of lymphocytes with this amino acid. The effects of concanavalin A, which stimulates mitogenesis, mercaptopicolinate, which inhibits phosphoenolpyruvate carboxykinase, and starvation of the donor animals on the rate and nature of glutamine metabolism in isolated incubated lymphocytes have been investigated. The effect of glutamine on the initiation of DNA synthesis in lymphocytes in culture was investigated by following the uptake of [³H]thymidine. The results of these studies are reported and discussed below.

Materials and methods

Animals

Male Wistar albino rats (160–180 g) were obtained from Batin and Kingman, Grimston, Hull, Yorks. HU11 4QE, U.K.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp. (London), London W5 2TZ, U.K.,

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except for the following: L-glutamine and hydrazine hydrate were obtained from BDH Chemicals, Poole, Dorset BH12 4NN, U.K.; D-glucose, glycine and all inorganic reagents were obtained from Fisons Scientific Apparatus, Loughborough, Leics. LE11 0RG, U.K.; concanavalin A and constituents for the RPMI-1640 medium were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, U.K.; Repelcoté and silicone oil were obtained from Hopkin and Williams, Chadwell Heath, Essex, U.K.; foetal bovine serum, cell-culture plates, streptomycin and penicillin were obtained from Flow Laboratories, Irvine KA12 8NB, Scotland, U.K. The serum was dialysed for 36 h against six changes of 100 vol. of non-sterile phosphate-buffered saline (see Culvenor & Weidemann, 1976) to remove glucose. 3-Mercaptopicolinate was generously given by Mr. R. Hems of the Metabolic Research Laboratory, Radcliffe Infirmary, Oxford.

Preparation of lymphocytes

Lymphocytes were prepared from rat mesenteric lymph nodes as described by Ardawi & Newsholme (1982). For cell-culture experiments, lymphocytes were prepared under sterile conditions.

Incubation procedures

Incubations were performed at 37°C in 10 ml Erlenmeyer flasks that had been silicone-treated. Freshly prepared lymphocytes (at a density of 5×10^7 cells) were incubated in a total volume of 1 ml of incubation medium, which consisted of phosphate-buffered saline (Culvenor & Weidemann, 1976) supplemented with 10% (v/v) dialysed foetal bovine serum, at pH 7.2. Flasks were gassed with 100% O₂ for 20 s and were shaken continuously (90 oscillations/min); incubations were initiated with either the substrate(s) alone or simultaneous additions of substrate and concanavalin A (at a concentration of $30 \mu\text{g}/10^7$ cells, which was found to produce the largest stimulation of lactate production when 5 mM-glucose was present). Incubations were terminated by addition of 200 μl of HClO₄ (25%, w/v) to the incubation flask and cooling the mixture to 0°C. Precipitated protein was removed by centrifugation at 8500 g for 3 min. The supernatant was neutralized with KOH, and KClO₄ removed by centrifugation at 8500 g for 3 min. In some experiments, cells were separated rapidly from the incubation medium by centrifugation through a layer of silicone oil into HClO₄ (see Halestrap & McGivan, 1979), and cells and medium were analysed separately.

Measurement of O₂ consumption

O₂ consumption was measured polarographically by means of a Clark-type oxygen electrode. Lymphocytes (5×10^7 cells/ml) were incubated in 3.1 ml of incubation medium at 37°C.

Assay of metabolites

Metabolites in neutralized extracts of cells, medium or cells plus medium were determined spectrophotometrically (with a Gilford recording spectrophotometer, model 240) by enzymic methods: glucose by the coupled hexokinase and glucose 6-phosphate dehydrogenase method as described by Bergmeyer *et al.* (1974a); glutamine and ammonia by the method of Windmueller & Spaeth (1974); glutamate by the method of Bernt & Bergmeyer (1974); lactate by the method of Gawehn & Bergmeyer (1974); aspartate and asparagine by the method of Bergmeyer *et al.* (1974b); alanine by the method of Williamson (1974); pyruvate by the method of Czok & Lamprecht (1974); 2-oxoglutarate by the method of Bergmeyer & Bernt (1974); malate by the method of Gutmann & Wahlefeld (1974); ADP and AMP by the method of Jaworek *et al.* (1974); ATP and glucose 6-phosphate by the method of Lamprecht & Trautschold (1974) (with 5 mM-D-glucose instead of the recommended 40 mM).

Cell culture

Lymphocytes were cultured in wells of round-bottomed micro-titre culture plates; 25 μl of cell suspension (5×10^5 cells) was placed in each well together with 175 μl of RPMI-1640 medium containing streptomycin (100 units/ml), penicillin (200 units/ml) and concanavalin A (1 $\mu\text{g}/\text{well}$). The cultures were incubated at 37°C in a 5% CO₂-incubator for 48 h. The proliferative response was determined by measuring the uptake of [³H]thymidine (0.5 $\mu\text{Ci}/\text{well}$) after an 18 h pulse. Cultures were harvested on to glass-fibre filters by using an automated cell harvester. The dried filters were added to scintillation vials containing 6 ml of scintillant [which contained 3.0 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of toluene], and radioactivity was measured in a liquid-scintillation counter (model L7200).

Expression of results

Changes in concentrations of substrates or metabolites during the incubation were determined from the net change between zero time and 60 min incubation. Rates of substrate utilization or metabolite production are expressed as $\mu\text{mol}/\text{h}$ per g dry wt. of cells, calculated from the finding that 10^{10} cells are equivalent to 0.815 ± 0.07 g dry wt. after drying at 50°C for 16 h.

Results and discussion

O₂ consumption

The rate of respiration by lymphocytes isolated from rat mesenteric lymph nodes in the absence of added substrate was almost 1.0 $\mu\text{mol}/\text{min}$ per g dry

wt. (Table 1), which is similar to that reported for rat thymocytes (Hume *et al.*, 1978), one-sixth the rate for rat spleen slices (Suter & Weidemann, 1975) and about one-tenth the rate observed for isolated enterocytes (Watford *et al.*, 1979a; Porteous, 1980). Addition of 5.0 mM-glucose or 2 mM-glutamine led to a 40% or 44% increase in the O₂ consumption respectively, which was linear over the period of incubation (60 min). Similar increases were observed after addition of acetoacetate or 3-hydroxybutyrate (5 mM). Addition of 2 mM-pyruvate, -2-oxoglutarate or -succinate increased the rate of respiration by approx. 60% over the endogenous rate (Table 1). O₂ consumption was not stimulated by asparagine, alanine, lactate or ethanol (results not shown).

Adenine nucleotide concentrations

The concentrations of adenine nucleotides were measured in lymphocytes as soon as possible after isolation from mesenteric lymph nodes and then at various times after incubation in the normal incubation medium containing 5 mM-glucose. The concentration of ATP, the total nucleotide concentration and the ATP/AMP concentration ratio were

maintained throughout 90 min of incubation (Table 2). Indeed, there may be a slight increase in the total nucleotide concentration. This is in contrast with the situation found in rat enterocytes, in which the total adenine nucleotide concentration and the [ATP]/[AMP] ratio decreased rapidly during incubation (Watford *et al.*, 1979a). These results, together with the fact that rates of O₂ consumption, glucose utilization and lactate production were linear over 60 min of incubation (results not shown), indicate that isolated rat mesenteric lymphocytes provide a satisfactory system for short-term metabolic studies.

Metabolites formed from glucose and/or glutamine

Lymphocytes utilized glucose at a rate of about 0.6 μmol/min per g dry wt. (Table 3), which is about 9% of the maximum hexokinase activity (Ardawi & Newsholme, 1982). This rate is similar to that reported for thymocytes (Culvenor & Weidemann, 1976; Yassmeen *et al.*, 1977; Hume *et al.*, 1978). About 70% of the glucose utilized could be accounted for as lactate and pyruvate. The amounts of glutamate, aspartate and alanine produced, above the endogenous amounts, were small (Table 3). Assuming that glucose carbon contributed only to the production of lactate plus pyruvate, it was calculated that glucose contributed about 48% to respiration (assuming also that the endogenous rate of lactate production was not affected by metabolism of glucose). If, however, glutamate, glutamine, alanine and aspartate and all the lactate were produced from the glucose utilized, no glucose carbon would be available for oxidation.

The rate of glutamine utilization by incubated lymphocytes was about 2.7 μmol/min per g dry wt., which is about 8% of the maximum glutaminase activity in lymphocytes (Ardawi & Newsholme, 1982). This rate is similar to that reported for rat kidney slices (Vinay *et al.*, 1980), but lower than that for colonocytes (Roediger, 1982) and enterocytes of the rat (Watford *et al.*, 1979a). The major end products were glutamate and aspartate (Table 3). This is in contrast with intestinal preparations, which produce alanine rather than aspartate from glutamine (see, e.g., Hanson & Parsons, 1977;

Table 1. Oxygen consumption by isolated mesenteric lymphocytes of the rat

Values of O₂ consumption are presented as means ± s.e.m., with the numbers of separate experiments given in parentheses.

| Additions to the incubation | Rate of O ₂ consumption (μmol/h per g dry wt.) |
|---|--|
| None | 55.8 ± 1.91 (7) |
| 5 mM-Glucose | 93.6 ± 5.09 (6) |
| 2 mM-Glutamine | 99.6 ± 7.96 (7) |
| 5 mM-Glucose plus 2 mM-glutamine | 108.1 ± 1.46 (7) |
| 5 mM-Glucose, 2 mM-glutamine plus 2 mM-glutamate | 99.7 ± 5.12 (5) |
| 2 mM-Pyruvate | 130.5 ± 10.1 (6) |
| 2 mM-2-Oxoglutarate | 135.3 ± 6.21 (6) |
| 2 mM-Succinate | 144.5 ± 3.19 (4) |
| 5 mM-3-Hydroxybutyrate | 98.0 ± 13.0 (6) |
| 5 mM-Acetoacetate | 89.0 ± 3.92 (5) |

Table 2. Concentration of adenine nucleotides in incubated mesenteric lymphocytes of the rat

Lymphocytes were incubated for the times indicated; the incubations were terminated as described in Materials and methods. Values are presented as means ± s.e.m., with the numbers of separate experiments given in parentheses.

| Incubation time (min) | Concentration (μmol/g dry wt.) | | | Total adenine nucleotides | Ratio [ATP]/[AMP] |
|--------------------------|--------------------------------|----------------|-----------------|------------------------------|----------------------|
| | ATP | ADP | AMP | | |
| 0 | 7.8 ± 0.14 (3) | 1.4 ± 0.04 (3) | 0.66 ± 0.01 (3) | 9.9 | 11.8 |
| 30 | 8.9 ± 0.08 (3) | 1.8 ± 0.11 (3) | 0.64 ± 0.03 (3) | 11.3 | 13.9 |
| 60 | 8.9 ± 0.06 (3) | 1.6 ± 0.12 (3) | 0.82 ± 0.03 (3) | 11.3 | 10.8 |
| 90 | 9.6 ± 0.26 (3) | 1.3 ± 0.05 (3) | 1.0 ± 0.03 (3) | 11.9 | 9.6 |

Table 3. Rates of utilization of glucose and glutamine and production of glutamate, aspartate, alanine, asparagine, ammonia, pyruvate and lactate by isolated incubated rat mesenteric lymphocytes

Rates are given as means \pm S.E.M., with the numbers of separate experiments given in parentheses. A negative sign indicates utilization. Differences in rates between incubations with glucose and glucose plus glutamine for glucose utilization and pyruvate and lactate production between incubations with glutamine and glucose plus glutamine for glutamine utilization and for remainder of the rates of production of other metabolites that are statistically significant (Student's *t* test) are indicated by * ($P < 0.01$) or ** ($P < 0.001$).

| Additions to incubation | Rates of utilization or production ($\mu\text{mol/h}$ per g dry wt.) | | | | | | | | | |
|--------------------------------------|---|-----------------------|---------------------|----------------------|----------------------|---------------------|--------------------|----------------------|---------------------|--|
| | Glucose | Glutamine | Glutamate | Alanine | Aspartate | Asparagine | Ammonia | Pyruvate | Lactate | |
| None | — | -6.24 ± 1.74 (6) | 5.51 ± 1.52 (6) | — | 14.2 ± 2.5 (6) | — | 12.5 ± 2.7 (6) | 0.80 ± 0.18 (6) | 13.2 ± 3.6 (6) | |
| Glucose (5 mM) | -37.1 ± 1.1 (11) | 8.81 ± 2.69 (4) | 13.2 ± 1.3 (7) | 2.58 ± 0.64 (5) | 20.1 ± 5.6 (6) | — | 27.0 ± 5.0 (4) | 3.73 ± 1.20 (8) | 54.2 ± 2.2 (10) | |
| Glutamine (2 mM) | — | -160 ± 7.7 (12) | 99.0 ± 3.2 (8) | 1.9 ± 0.1 (6) | 43.2 ± 5.7 (8) | 4.03 ± 1.9 (8) | 99.7 ± 9.1 (8) | 8.09 ± 0.71 (7) | 6.39 ± 1.10 (8) | |
| Glucose (5 mM) plus glutamine (2 mM) | -60.8 ± 1.3 (9)** | -222 ± 10.6 (9)** | 142 ± 8.9 (7)** | 3.52 ± 0.70 (6)* | 69.0 ± 3.7 (8)** | 6.89 ± 1.62 (8) | 119 ± 1.8 (7) | 8.98 ± 0.81 (9)* | 116 ± 5.5 (9)** | |

Watford *et al.*, 1979a; Porteous, 1980). The rates of glutamine utilization and the production of aspartate were almost linear for 60 min (Fig. 1). The concentration of glutamine that produced 50% of maximal rate of utilization in isolated incubated lymphocytes is about 2.5 mM (Fig. 2). Glutamate, aspartate, asparagine, alanine, lactate, pyruvate and ammonia were all produced from glutamine. Of the glutamine utilized, ammonia production accounted for about 30% of the glutamine nitrogen, which, since the glutaminase reaction produces ammonia, suggests that the glutamate is metabolized via a transaminase reaction rather than by glutamate dehydrogenase. This is expected from the low activity of the latter enzyme in lymphocytes (Ardawi & Newsholme, 1982). For the glutamine metabolized beyond the stage of glutamate, about 70% was accounted for as aspartate and only about 3% as alanine. This finding is expected from the maximum activities of aspartate aminotransferase and alanine aminotransferase in rat mesenteric lymph nodes (Ardawi & Newsholme, 1982). Calculations based on the formation of ^{14}C from [^{14}C]glutamine (results not shown) indicate that it contributes about 30% to respiration. Furthermore,

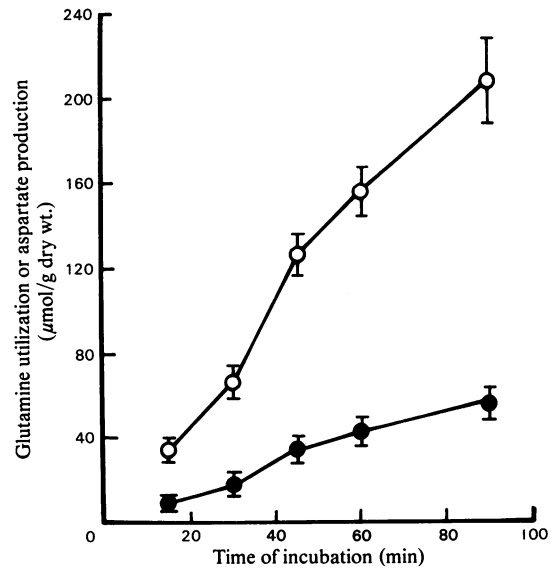


Fig. 1. Time course of glutamine utilization (○) and aspartate production (●) by incubated mesenteric lymphocytes

Incubations of lymphocytes were terminated at the times indicated, and glutamine utilization or aspartate production was measured as described in the Materials and methods section. Initial concentration of glutamine was 2 mM. Results are presented as means of four separate experiments; bars represent \pm S.E.M.

glutamine increased O₂ consumption by lymphocytes to a similar extent to that with glucose (Table 1).

The formation of aspartate, asparagine and ammonia from glutamine is probably required for biosynthetic processes (see Tate & Meister, 1973); the present results enable the rate of glutamine utilization by the total lymphocyte population *in vivo* to be calculated; this is about 3 μmol/h (assuming that a 280g rat possesses 5 × 10⁸ lymphocytes). Apart from glutamine produced in the lumen of the intestine, most of the glutamine required by the rapidly dividing cells is obtained from muscle, which is known to be capable of releasing about 42 μmol/h (see Schröck *et al.*, 1980). Hence the magnitude of glutamine release by muscle exceeds enormously that required by the lymphocytes.

When both glutamine plus glucose were presented to the lymphocytes, the rates of utilization of

both substrates were increased and the rate of lactate production was doubled (Table 3). All of the increased glutamine utilized can be accounted for as glutamate and aspartate. All of the increased-glucose utilized can be accounted for as lactate. The increase in the rate of glycolysis caused by glutamine is accompanied by a decrease in the concentration of glucose 6-phosphate (Table 4). These findings indicate that the activity of 6-phosphofructokinase was increased by glutamine; this could have been caused by the increased concentration of AMP (Table 4), a known activator of the enzyme, or an increased concentration of aspartate, which is also an activator of lymphoid tissue 6-phosphofructokinase (Hickman & Weidemann, 1975).

Of the intermediates produced from glutamine or glucose by the lymphocytes, the largest proportion was found in the medium; 80% of the glutamate, 90% of the lactate and 88% of the ammonia were present in the media of the incubation system.

Starvation of the donor rats for 48h caused an increased rate of glutamine utilization by isolated lymphocytes (Table 5). In addition, the rates of formation of ammonia, glutamine, aspartate and lactate were also increased (Table 5). These effects are predicted by the effect of starvation on the maximum activity of glutaminase in lymphocytes (Ardawi & Newsholme, 1982), but are in contrast with the effect of starvation on glutamine metabolism by intestinal preparations (Hanson & Parsons, 1980).

Effects of 3-mercaptopycolinate on glutamine metabolism

On the basis of maximal enzyme activities, it has been suggested that the conversion of oxaloacetate into phosphoenolpyruvate, catalysed by phosphoenolpyruvate carboxykinase, is one reaction involved in the pathway for glutamine oxidation in lymphocytes (Ardawi & Newsholme, 1982). 3-Mercaptopycolinate has been shown to be a reasonably specific inhibitor of phosphoenolpyruvate carboxykinase (Kostos *et al.*, 1975; Robinson & Oei, 1975; Jomain-Baum *et al.*, 1976). The effect of this inhibitor on the rate of glutamine utilization

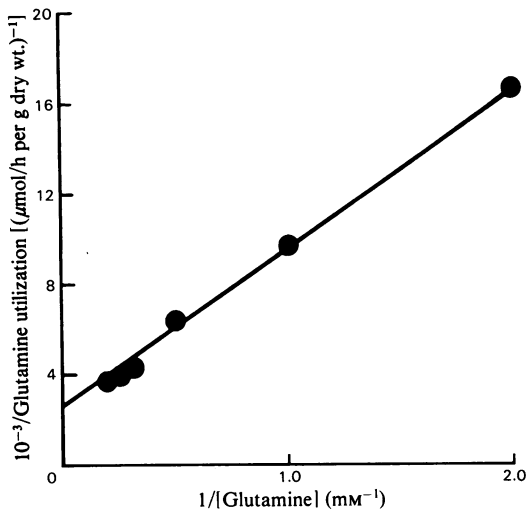


Fig. 2. Double-reciprocal plot of rates of glutamine utilization by incubated mesenteric lymphocytes. Incubations were carried out as described in the Materials and methods section. Results are the means of two separate experiments.

Table 4. Effect of glutamine on the concentrations of adenine nucleotides and glucose 6-phosphate in incubated mesenteric lymphocytes of rat

Isolated lymphocytes were incubated (5 × 10⁷ cells) for 60 min in total volume of 1 ml of incubation medium. Concentrations are presented as means ± S.E.M. for ten separate cell preparations. A statistically significant difference (Student's *t* test) from control values is indicated by * (*P* < 0.01) or ** (*P* < 0.005).

| Addition to incubation | Concentration (μmol/g dry wt.) | | | |
|----------------------------------|--------------------------------|------------|------------|---------------|
| | Glucose 6-phosphate | ATP | ADP | AMP |
| 5 mM-Glucose | 0.14 ± 0.018 | 8.9 ± 0.22 | 1.9 ± 0.10 | 0.79 ± 0.050 |
| 5 mM-Glucose plus 2 mM-glutamine | 0.077 ± 0.016** | 8.4 ± 0.21 | 1.9 ± 0.11 | 0.96 ± 0.037* |

Table 5. *Effects of concanavalin A, 3-mercaptopycolinate and starvation of the donor animal on the metabolism of glutamine by incubated mesenteric lymphocytes of the rat*

Lymphocytes were incubated (5×10^7 cells) for 60 min in 1 ml of incubation medium containing 2 mM-glutamine. Rates of utilization (indicated by minus sign) or production are presented as means \pm S.E.M., with the numbers of experiments with separate cell preparations given in parentheses. Statistical significance of difference between control and experimental values (Student's *t* test, based on difference between means) is indicated by * ($P < 0.01$), ** ($P < 0.01$), or *** ($P < 0.001$).

| Condition of animal or addition to medium | Rates of utilization or production ($\mu\text{mol/h}$ per g dry wt.) | | | | | | | |
|---|---|-----------------------|---------------------------|---------------------------|---------------------------|------------------------|----------------------------|--------------------|
| | Glutamine | Glutamate | 2-Oxoglutarate | Aspartate | Ammonia | Pyruvate | Lactate | Malate |
| None | -157 ± 7.5 (16) | 111 ± 5.9 (16) | 2.32 ± 0.13 (5) | 42.0 ± 5.2 (9) | 93.8 ± 8.26 (13) | 7.96 ± 0.61 (9) | 6.47 ± 1.60 (8) | — |
| Concanavalin A (150 $\mu\text{g/ml}$) | -237 ± 14.6 *** (11) | 109 ± 4.5 (6) | 1.72 ± 0.09 ** (6) | 59.4 ± 2.6 *** (6) | 174 ± 12.1 *** (6) | 6.92 ± 1.10 (8) | 42.7 ± 5.80 *** (8) | — |
| None (6) | -156 ± 5.6 | 120 ± 3.4 | — | 35.0 ± 2.82 | 102 ± 5.6 | — | 5.65 ± 2.54 | 0.45 ± 0.16 |
| 3-Mercaptopycolinate (0.5 mM) (6) | -112 ± 4.7 *** | 56.7 ± 10.7 *** | — | 51.3 ± 6.14 * | 79.5 ± 3.00 *** | — | 19.5 ± 3.40 *** | 1.17 ± 0.20 ** |
| Fed (3) | -164.8 ± 7.6 | 95.5 ± 4.36 | — | 37.4 ± 2.04 | 93.8 ± 7.61 | — | 8.6 ± 0.5 | — |
| Starved (4) | -203.7 ± 10.6 * | 127.0 ± 9.91 * | — | 48.8 ± 2.50 *** | 133.90 ± 14.72 *** | — | 14.0 ± 2.61 ** | — |

and the concentration of metabolites of the glutamine pathway in lymphocytes were therefore investigated. The inhibitor decreased the rate of glutamine utilization by about 28%, and the rates of formation of both glutamate and ammonia were also decreased (Table 5). In contrast, the rates of formation of lactate, aspartate and malate were increased by the inhibitor (Table 5). 3-Mercaptopycolinate had similar effects on the rate of glutamine metabolism and accumulation of intermediates in isolated kidney tubules of the rat (Watford *et al.*, 1979b, 1980). The findings suggest that phosphoenolpyruvate carboxykinase plays a role in the pathway of glutamine metabolism in lymphocytes. The accumulation of aspartate and malate is expected from the inhibition of the carboxylase, and the decreased rate of utilization of glutamine could be explained by inhibition of mitochondrial glutamine transport, possibly by 2-oxoglutarate (Goldstein, 1976) or inhibition of glutaminase by glutamate (Curthoys & Shapiro, 1982). The increased accumulation of lactate suggests that other enzymes, such as 'malic' enzyme or oxaloacetate decarboxylase, are also involved in glutamine metabolism.

Effect of concanavalin A on glutamine metabolism

Mitogen-induced lymphocyte transformation has provided a useful model for the study of the metabolic events associated with non-specific antigenic stimulation of lymphocytes. A mitogen (e.g. concanavalin A) triggers a series of biochemical events which ultimately result in lymphoblastic transformation and cell division (for review, see Wedner & Parker, 1976). The presence of concanavalin A in the incubation medium increased the rate of glutamine utilization and the accumulation of ammonia by about 51% and 81% respectively (Table 5). However, there was no change in the rate of formation of glutamate, whereas that of aspartate was increased; there was a particularly marked increase in the concentration of lactate (Table 5). In these experiments, the concentration of 2-oxoglutarate was also measured, since this may be a regulator of glutamine metabolism (Goldstein & Boylan, 1978; Strzelecki & Schoolwerth, 1981; Curthoys & Shapiro, 1982). From these studies there is no doubt that concanavalin A stimulates glutamine metabolism, which may explain, in part, the marked increase in the concentration of lactate (Zielke *et al.*, 1980), although it could have arisen from increased endogenous glucose metabolism (Hume & Weidemann, 1979). It is unclear how the pathway of glutamine metabolism in the lymphocyte is controlled, but the decrease in the total concentration of oxoglutarate (Table 5) suggests that the activity of oxoglutarate dehydrogenase is

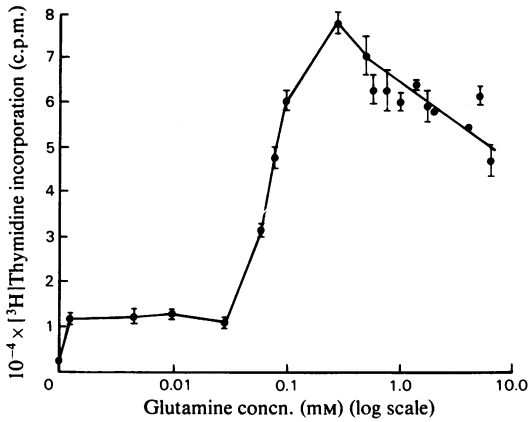


Fig. 3. Effect of glutamine concentration on $[^3\text{H}]\text{thymidine}$ incorporation in concanavalin-A-stimulated rat mesenteric lymphocytes in cultures

Cells (5×10^5) were cultured in RPMI-1640 medium (free of glutamine) as described in the Materials and methods section. Various concentrations of glutamine were added at the start of the cell culture. The values shown are means of four separate experiments and the bars represent \pm S.E.M.

increased by concanavalin A, and this could increase glutamine utilization via the decrease in concentration of 2-oxoglutarate, and lead to an increased rate of glutamine transport into the mitochondria or the activity of glutaminase, as suggested for the pathway in the kidney (Goldstein, 1976; Curthoys & Shapiro, 1982).

Effect of glutamine concentration on $[^3\text{H}]\text{thymidine}$ incorporation into concanavalin A-stimulated lymphocyte cultures

The incorporation of $[^3\text{H}]\text{thymidine}$ is used as an index of DNA synthesis, and hence of proliferation of lymphocytes. In the absence of glutamine, $[^3\text{H}]\text{thymidine}$ incorporation into lymphocytes cultured for 48 h was very low and was similar to that observed in the absence of concanavalin A. The effect of increasing the glutamine concentration in the culture medium is shown in Fig. 3. Addition of $1 \mu\text{M}$ -glutamine caused a 4-fold increase in $[^3\text{H}]\text{thymidine}$ incorporation. Maximum rates of incorporation were observed at 0.3 mM-glutamine. Above this concentration the rate decreased slightly. The effect of glucose was not investigated in the present work, but a similar response to that of glutamine has been observed with glucose in rat thymocytes (Hume *et al.*, 1978).

Glutamine could not be replaced by other amino acids; at a concentration of 2 mM in the culture medium, arginine, asparagine, aspartate, glutamate, glycine, histidine, proline, serine, or NH_4Cl , stimulated $[^3\text{H}]\text{thymidine}$ incorporation to only 10–20% of that produced by 2 mM-glutamine.

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