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Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages

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1. Maximum activities of some key enzymes of metabolism were studied in elicited (inflammatory) macrophages of the mouse and lymph-node lymphocytes of the rat. 2. The activity of hexokinase in the macrophage is very high, as high as that in any other major tissue of the body, and higher than that of phosphorylase or 6-phosphofructokinase, suggesting that glucose is a more important fuel than glycogen and that the pentose phosphate pathway is also important in these cells. The latter suggestion is supported by the high activities of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. However, the rate of glucose utilization by 'resting' macrophages incubated *in vitro* is less than the 10% of the activity of 6-phosphofructokinase: this suggests that the rate of glycolysis is increased dramatically during phagocytosis or increased secretory activity. 3. The macrophages possess higher activities of citrate synthase and oxoglutarate dehydrogenase than do lymphocytes, suggesting that the tricarboxylic acid cycle may be important in energy generation in these cells. 4. The activity of 3-oxoacid CoA-transferase is higher in the macrophage, but that of 3-hydroxybutyrate dehydrogenase is very much lower than those in the lymphocytes. The activity of carnitine palmitoyltransferase is higher in macrophages, suggesting that fatty acids as well as acetoacetate could provide acetyl-CoA as substrate for the tricarboxylic acid cycle. No detectable rate of acetoacetate or 3-hydroxybutyrate utilization was observed during incubation of resting macrophages, but that of oleate was 1.0 nmol/h per mg of protein or about 2.2% of the activity of palmitoyltransferase. 5. The activity of glutaminase is about 4-fold higher in macrophages than in lymphocytes, which suggests that the rate of glutamine utilization could be very high. The rate of utilization of glutamine by resting incubated macrophages was similar to that reported for rat lymphocytes, but was considerably lower than the activity of glutaminase.

INTRODUCTION

Lymphocytes and macrophages play an important role in the immune response, during which these cells can undergo increased rates of production and recruitment and alterations in function. Mature lymphocytes recirculate via blood and lymph through lymphoid tissues in a relatively quiescent state, until stimulated to proliferate by antigens. By contrast, macrophages are terminally differentiated end-cells in which the ability to proliferate is gradually lost (for review, see Gordon, 1986). Mature resident macrophages are found more widely distributed in haematopoietic, lymphoid and other tissues, where they exist as biosynthetically active cells. After an inflammatory or immunological stimulus, newly recruited macrophages with markedly different secretory and endocytic properties can accumulate in large numbers at specific sites. In spite of this functional heterogeneity, macrophages overall are characterized as motile, highly phagocytic, cells, which display marked plasma-membrane activity. Their versatile secretory activities, which include enzymes, hormone-like mediators of inflammation and toxic oxygen products, can be altered by stimuli such as phagocytic particles, microbial products and lymphokines acting on various macrophage plasma-membrane receptors.

Although many aspects of macrophage cell biology have been studied extensively, including metabolic aspects of the respiratory burst response (Babior, 1978; Karnovsky & Lazdins, 1978), very little is known about their intermediary metabolism. It was considered important to investigate systematically the types of fuels that could be used by macrophages, particularly since recent work has thrown new light on the fuels used by lymphocytes (see Ardawi & Newsholme, 1985). To this end, the maximum activities of the key enzymes involved in metabolism of glucose, glutamine, ketone bodies and fatty acids have been measured, together with the rates of utilization of these fuels by incubated mouse macrophages. Thioglycollate-elicited mouse peritoneal macrophages were chosen for this investigation, since they are readily available in reasonable yields as a homogeneous, stable and well characterized cell population (Cohn, 1978). In addition, the activities have also been measured in rat lymphocytes for direct comparison with those in the mouse macrophages.

MATERIALS AND METHODS

Animals

Male Wistar albino rats used in the preparation of homogenates from mesenteric lymph nodes, were obtained from Bantin and Kingman, Grimston, Hull, N Humberside HU11 4QE, U.K. Elicited peritoneal macrophages were obtained from 12–16-week-old female mice of the C57 BL/6 strain, bred in the Sir William Dunn School of Pathology, Oxford.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Mannheim, Lewes, Sussex, U.K., except for the following: 2-mercaptoethanol, 5,5′-dithiobis-(2-nitrobenzoic acid), L-alanine, acetoacetyl-CoA, iodoacetamide, DL-isocitrate and nigrosine were obtained from Sigma Chemical Co., Poole, Dorset, U.K.; the scintillant and all inorganic reagents were obtained from Fisons Scientific Apparatus, Loughborough, Leics. LE11 0RG, U.K.; Tes was obtained from Hopkin and Williams, Chadwell Heath, Essex, U.K.; and $NaH^{14}CO_3$ was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of lymphocytes and macrophages

Lymphocytes were prepared from rat mesenteric lymph nodes as described by Ardawi & Newsholme (1982). Macrophages were obtained from the peritoneal cavity of mice 4 days after injection of 1.5 ml of thioglycollate broth intraperitoneally, and purified by adherence to plastic Petri dishes (see Cohn, 1974). After the peritoneal cells had been resuspended in Dulbecco's modified Eagle's medium with 10% (w/v) foetal bovine serum and 20 μg of gentamicin/ml, supplemented with 2 mM-glutamine, cells were plated at a density of 2.5×10^7 cells/plate (Corning 100 mm-diam. tissue-culture dish) (equivalent to about 2×10^7 macrophages). After 4 h incubation at 37 °C, the tissue-culture dishes were washed three times with phosphate-buffered saline (Dulbecco & Vogt, 1954; pH 7.2–7.4) to remove non-adherent cells. Fresh tissue-culture medium was added, and the population was maintained in an incubator at 37 °C in air/CO_2 (19:1). After 24–48 h, cells (> 95% macrophages) were washed three times with phosphate-buffered saline and then gently scraped up into 2 ml of phosphate-buffered saline. Cells were centrifuged at 500 *g* for 5 min and resuspended in the relevant extraction buffer for the enzyme to be assayed. The protein content of the dishes at the time of assay was 2–4 mg/dish.

Preparation of homogenates

Homogenates of lymph nodes were prepared as described by Ardawi & Newsholme (1982). For the assay of most enzymes, macrophages were homogenized in a small ground-glass homogenizer (1 ml capacity) with 5–10 vol. of extraction medium at 0 °C. 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 800 *g* (in an Eppendorf micro-centrifuge) for 2 min and the resultant supernatant was used for assay. For the ketone-body-utilizing enzymes 3-oxoacid CoA-transferase and 3-hydroxybutyrate dehydrogenase (EC 2.8.3.5 and EC 1.1.1.30 respectively), homogenates were sonicated (for two separate periods of 2 s with a micro-probe of an MSE 100W ultrasonic disintegrator operating at an amplitude of 9 μm; the homogenates were maintained at 0–4 °C during sonication), after which they were centrifuged for 2 min at 8000 *g* and the resultant supernatants used. For the assay of oxoglutarate dehydrogenase (EC 1.2.4.2) and carnitine palmitoyltransferase (EC 2.3.1.2.1) it was important to use a mitochondrial preparation of both lymphocytes and macrophages; lymphocytes were homogenized for 2×10 s (at 0 °C) in a Polytron homogenizer (PCU-2, at position 3); macrophages were homogenized in a small ground-glass homogenizer; the homogenate was centrifuged at 300 *g* for 5 min, followed by centrifugation of the resultant supernatant at 8000 *g* for 2 min. The pellet was resuspended in extraction medium. The extraction media and assay methods were similar to or identical with those described by Ardawi & Newsholme (1982). Macrophages were incubated and rates of fuel utilization measured in general as described by Ardawi & Newsholme (1983) for lymphocytes. In all the enzyme assays (except for phosphorylase 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 (Crabtree *et al.*, 1979). The final volume of the assay mixtures in all cases was 1.0 ml. 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).

Assay of enzyme activities

Enzyme activities were measured as described previously (see Ardawi & Newsholme, 1982, for details or references), except that for the assays of aspartate aminotransferase and alanine aminotransferase (EC 2.6.1.1 and EC 2.6.1.2), as described by Sugden & Newsholme (1975), the concentrations of aspartate and alanine were lowered to 0.25, 0.5 and 1.0 mM in each assay (both enzymes from the macrophage were inhibited by high concentrations of the relevant amino acids), and for glucose-6-phosphate dehydrogenase (EC 1.1.1.49), as described by Bergmeyer *et al.* (1974), phosphogluconate dehydrogenase (EC 1.1.1.44) was added to the assay medium (1.2 units/assay). 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).

Citrate synthase (EC 4.1.3.7) and pyruvate carboxylase (EC 6.4.1.1) were assayed by measuring the rate of change in A_{412}, ketone-body-utilizing enzymes by measuring the rate of change in A_{303}, and the remainder of the enzymes by measuring 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 (EC 3.5.1.2) and phosphoenolpyruvate carboxykinase (EC 4.1.1.49), which were determined at 37 °C.

Expression of results

All enzyme activities and rates of fuel utilization are expressed as nmol of substrate utilized/min per mg of protein. Protein was assayed as described by Bradford (1976). This may account for some differences in the enzyme activities in lymphocytes reported in this work from those reported previously (Ardawi & Newsholme, 1982), since in that previous work protein was measured by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Glycolysis and the pentose phosphate pathway

The activities of some of the glycolytic enzymes in lymphocytes and macrophages are given in Table 1. The activity of hexokinase (EC 2.7.1.1) in mouse macrophages is considerably greater than that in rat lymphocytes

Table 1. Activities of some enzymes of carbohydrate, ketone-body, fatty acid and amino acid metabolism in macrophages of the mouse and lymphocytes of the rat

Lymphocytes and macrophages were prepared and extracted and enzymes were assayed as described in the Materials and methods section. Activities were measured at 25 °C, except for glutaminase (37 °C). The results are presented as means ± S.E.M. for at least 12 separate measurements for macrophages (from two separate batches of macrophages from six separate animals for each batch) or six separate animals for lymphocytes.

Enzyme	Enzyme activity (nmol/min per mg of protein)	
	Lymphocytes	Macrophages
Hexokinase	17.3 ± 0.31	76 ± 1.4
Phosphorylase	3.8 ± 0.04	3.0 ± 0.11
6-Phosphofructokinase	25.7 ± 0.69	22.8 ± 1.0
Pyruvate kinase	403 ± 9.9	447 ± 39.3
Lactate dehydrogenase	823 ± 64	764 ± 13.4
Glucose-6-phosphate dehydrogenase	17.5 ± 0.48	34.5 ± 2.6
6-Phosphogluconate dehydrogenase	21.1 ± 0.29	23.8 ± 0.74
Citrate synthase	63.7 ± 1.6	108 ± 3.3
Oxoglutarate dehydrogenase	5.1 ± 0.08	10.1 ± 0.69
3-Oxoacid CoA-transferase	19.9*	36.6 ± 0.63
Acetoacetyl-CoA thiolase	27.4*	15.5 ± 0.68
3-Hydroxybutyrate dehydrogenase	4.8*	0.17 ± 0.02
Carnitine palmitoyltransferase	0.47 ± 0.05	0.75 ± 0.12
Phosphate-dependent glutaminase	39.4 ± 1.3	152 ± 5.5
Glutamate dehydrogenase	34.0 ± 1.6	98.6 ± 3.0
$NADP^+$-linked malate dehydrogenase	3.5 ± 0.26	< 0.001
Phosphoenolpyruvate carboxykinase	4.9 ± 0.35	7.6 ± 0.51
Pyruvate carboxylase	2.5 ± 0.19	4.7 ± 0.34
Aspartate aminotransferase	67.4 ± 4.8	118 ± 9.7
Alanine aminotransferase	10.3 ± 2.5	4.4 ± 0.16
NAD^+-linked malate dehydrogenase	683 ± 16.6	452 ± 10.6

* Data from Ardawi & Newsholme (1982).

(Table 1); indeed, the activity of hexokinase in macrophages is as high as that in any other major tissue of the body (compare these activities with those presented in Table 1 of Cooney & Newsholme, 1982). The activity reported here for the macrophage is considerably higher than that reported by Kiyotaki *et al.* (1984): in the present work an ATP-regenerating system is included in the assay to lower the concentration of ADP, which inhibits hexokinase. Similarly to lymphocytes, but unlike most other tissues, the hexokinase activity in the macrophage is considerably higher than that of phosphorylase, suggesting that glycogen is not an important fuel for these cells: this suggestion is in contrast with that by Gudewicz & Filkins (1976). In macrophages, the maximum activity of hexokinase is considerably greater than that of 6-phosphofructokinase (EC 2.7.1.11), which is unusual (see Cooney & Newsholme, 1982). This probably reflects the quantitative importance of the pentose phosphate pathway, a suggestion that is supported by the high activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Table 1). The activities of the last two enzymes are approximately similar to those reported by Kiyotaki *et al.* (1984). It should be noted that the activity of glucose-6-phosphate dehydrogenase in the mouse macrophage is almost 8-fold greater than that in mouse liver (compare data in Table 1 with those in Newsholme *et al.*, 1979). Axline *et al.* (1980) have shown that the activities of 6-phosphofructokinase and pyruvate kinase (EC 2.7.1.40) are increased severalfold when macrophages are cultured under anaerobic conditions, which suggests a greater dependence on glycolysis under these conditions. The rate of glucose utilization in incubated 'resting' macrophages is considerably lower than the maximum activities of either hexokinase or 6-phosphofructokinase (cf. results in Table 1 with those in Table 2). This suggests either that the rate may be dramatically increased, for example during phagocytosis or production of toxic oxygen products, or that the transport of glucose into the cell provides a major limitation in the rate of glycolysis. Nonetheless the rate of glucose utilization reported here for elicited macrophages is high in comparison with other tissues; the rate in isolated soleus muscle of the rat is approx. 100 nmol/h per mg of protein (Challiss *et al.*, 1986) and that for rat brain *in situ* is approx. 300 nmol/h per mg of protein (Hawkins *et al.*, 1971). This high rate of glycolysis may play a role in the provision of the optimal conditions for sensitivity of the biosynthetic processes that utilize glycolytic intermediates, as discussed by Newsholme *et al.* (1985*a*,*b*). The activities of these enzymes reported in Table 1 are approximately similar to those reported by Axline *et al.* (1980) for cells from aerobic culture.

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 O_2 consumption) and hence aerobic metabolism (Read *et al.*, 1977; Cooney *et al.*,

1981; Newsholme & Paul, 1983). In the present work, the maximum activities of oxoglutarate dehydrogenase and citrate synthase in macrophages are high in comparison, for example, with those in lymphocytes (Table 1). Furthermore, the activity of oxoglutarate dehydrogenase in a very aerobic tissue, rat heart, is only about 2–4-fold higher than that in mouse macrophage (cf. data in Table 1 with those reported by Cooney *et al.*, 1981). This indicates that the cycle is important for energy generation in macrophages. Simon *et al.* (1977), on the basis of results with antimycin A, have concluded that 75% of total oxygen consumption of the macrophage is due to the mitochondrial electron transport which will be provided with electrons from the tricarboxylic acid cycle. These findings raise the question of the substrates that could provide acetyl-CoA in these cells. It appears to be tacitly assumed that glucose is the major, if not the only, fuel for macrophages and other cells capable of phagocytosis; and lack of a marked stimulation of [^{14}C]glucose conversion into $^{14}CO_2$ by phagocytosing cells had led to the conclusion that energy generated by the cycle, in contrast with that generated by glycolysis, is unimportant for phagocytosis (Roos & van der Stijl-Neijenhuis, 1980). For this reason the activities of key enzymes involved in the metabolism of some other possible substrates for the cycle were investigated.

Ketone-body and fatty acid utilization

In the present work it has been found that the activity of 3-oxoacid CoA-transferase in macrophages is twice as high as that in lymphocytes, but the activity of 3-hydroxybutyrate dehydrogenase is very low (Table 1). However, at a concentration of 3 mM, there was no detectable rate of utilization of either ketone body by incubated macrophages (Table 2).

In muscle tissue, the activity of carnitine palmitoyltransferase provides a quantitative indication of the maximum capacity to oxidize fatty acids (Crabtree & Newsholme, 1972). In macrophages, it was found that the rate of utilization of oleate (Table 2) is considerably lower than the activity of the palmitoyltransferase (Table 1), which suggests that the rate could be substantially increased under conditions when the energy demand is higher (e.g. during phagocytosis). There is considerable evidence of a high turnover of phospholipids in macrophages, which is probably related to membrane recycling and eicosanoid formation (Aderem *et al.*, 1985; Bonney *et al.*, 1979). However, a high turnover of phospholipids will require not palmitoylcarnitine but palmitoyl-CoA, so that it is unlikely that the carnitine palmitoyltransferase activity is an index of phospholipid turnover. The acetyl units from fatty acid oxidation could be used for synthesis of cholesterol, but the activity of the key enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-CoA synthase, is less than 10% of that of palmitoyltransferase when expressed as C_4 units (see Knight *et al.*, 1983).

Table 2. Rates of utilization of glucose, glutamine, ketone bodies and oleate by isolated incubated mouse macrophages

Macrophages were prepared as described in the Materials and methods section and were incubated for 60 min as described for lymphocytes by Ardawi & Newsholme (1983), except that 10% foetal bovine serum was replaced by 1.5% defatted bovine serum albumin. Results are presented as means ± S.E.M., with numbers of separate experiments given in parentheses. Each experiment consisted of at least three separate incubations. The value given for oleate is calculated from $^{14}CO_2$ produced from [^{14}C]oleate at a final concentration of 0.5 μCi/ml of incubated medium. The $^{14}CO_2$ was collected as described by Leighton *et al.* (1985).

Substrate	Rate of fuel utilization (nmol/h per mg of protein)
Glutamine (2 mM)	102 ± 5.03 (6)
Glucose (5 mM)	339 ± 15.8 (6)
Oleate (0.5 mM)	1.0 ± 0.05 (3)
Acetoacetate (3 mM)	< 0.2 (3)
Hydroxybutyrate (3 mM)	< 0.2 (3)

Glutamine metabolism

Glutamine is known to be an important fuel for a number of rapidly dividing cells (e.g. enterocytes, tumour cells, fibroblasts, reticulocytes; see Lund, 1980; Krebs, 1981) and also resting lymphocytes and thymocytes, which have the potential for rapid cell division (Ardawi & Newsholme, 1983; Brand *et al.*, 1984). These cells are characterized by a high activity of glutaminase. The pathway of glutamine metabolism in the lymphocyte has been proposed to include the following enzymes: glutaminase, glutamate dehydrogenase, aspartate aminotransferase, phosphoenolpyruvate carboxykinase, NAD^+-malate dehydrogenase and oxoglutarate dehydrogenase (Ardawi & Newsholme, 1982, 1985). These and related enzyme activities were measured in the mouse macrophage in the present work and are reported in Table 1. The activity of glutaminase in the macrophage is about 4-fold greater than that in the lymphocyte. The activity of glutamate dehydrogenase in the macrophage 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 an aminotransferase(s), rather than glutamate dehydrogenase, is important in the conversion of glutamate into oxoglutarate in macrophages. Similarly to the lymphocyte, 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 macrophages. In contrast with the lymphocyte, the activity of $NADP^+$-linked malate dehydrogenase is very low (< 0.01 nmol/min per mg of protein), suggesting that phosphoenolpyruvate carboxykinase catalyses the major reaction by which intermediates of the tricarboxylic acid cycle are converted into pyruvate for conversion into lactate or for complete oxidation (see Goldstein & Newsholme, 1976). Hence it is possible that glutamine provides acetyl-CoA for complete oxidation by the tricarboxylic acid cycle in macrophages.

The rate of glutamine utilization by incubated mouse macrophages is about 100 nmol/h per mg of protein, which is considerably lower than the maximum activity of glutaminase (almost 152 nmol/min per mg of protein); this finding suggests either that under other conditions (e.g. increased protein-secretory activity) the rate of glutamine utilization will be dramatically

increased by incubated macrophages or that the rate of this pathway might be limited by transport of glutamine across the cell or the mitochondrial membrane. Nonetheless the rate of glutamine utilization even in this resting state is high; it is similar to that of rat lymphocytes and only about one-third that of enterocytes or kidney tubules (see Ardawi & Newsholme, 1985).

It has been proposed that a major role for the high rate of glutamine utilization in lymphocytes and enterocytes, which have the potential for rapid cell division, is to provide the kinetic basis for a satisfactory control mechanism for provision of metabolism intermediates for biosynthetic pathways, especially the provision of intermediates for purine and pyrimidine synthesis (Newsholme *et al.*, 1985*a*,*b*). Thioglycollate-elicited mouse peritoneal macrophages do not divide under the conditions of cell cultivation used here and would respond only sluggishly to specific growth factors such as CSF-1 (Gordon, 1986). However, macrophages are known to have a very high rate of protein turnover (Hammer & Rannels, 1981), and hence a high turnover of mRNA may occur in these cells, for which purine and pyrimidine synthesis will be required.

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