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Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture

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1. The metabolism of mouse thioglycollate-elicited peritoneal macrophages was studied in culture for up to 96 h. 2. The rates of glycolysis, lactate formation and glutamine utilization were approximately linear with time for at least 80 h of culture. 3. The rates of glucose and glutamine utilization by cultured macrophages were approx. 500 and 90 nmol/h per mg of protein respectively. This rate of glucose utilization is at least 50% greater than that previously reported for macrophages during 60 min incubation in a shaking flask; and it is now increased by addition of glutamine to the culture medium. The rate of glutamine utilization in culture is similar to that previously reported for macrophages during 60 min incubation. The major end-product of glucose metabolism is lactate, and those of glutamine metabolism are CO_2, glutamate, ammonia and alanine. 4. Oleate was utilized by these cells: ^{14}C from [^{14}C]oleate was incorporated into CO_2 and cellular lipid. The highest rate of oleate utilization was observed when both glucose and glutamine were present in the culture medium. The presence of oleate in the culture medium did not affect the rates of utilization of either glucose or glutamine. Of the [^{14}C]oleate incorporated into lipid, approx. 80% was incorporated into triacylglycerol and only 18% into phospholipid. 5. The turnover rate for the total ATP content of the macrophage in culture is about 10 times per minute: the value for the perfused isolated maximally working rat heart is 22. This indicates a high metabolic rate for macrophages, and consequently emphasizes the importance of the provision of fuels for their function in an immune response.

INTRODUCTION

Very little work has been done on the metabolic profile of macrophages in culture. In general, studies have focused attention on metabolism that occurs during phagocytosis, particularly on the rates of glucose utilization, O_2 consumption and superoxide production (Hard, 1970; Karnovsky *et al.*, 1970; Stubbs *et al.*, 1973; Drath & Karnovsky, 1975; Michl *et al.*, 1976). A previous report from our laboratory (Newsholme *et al.* 1987) indicated that the macrophage utilizes glucose and glutamine at high rates, that the metabolism of these fuels is only partial, and that they have the ability to oxidize long-chain fatty acids, at least when the cells are taken out of a culture medium and incubated for periods of 30–90 min. The question arises whether these rates and types of metabolism represent that which occurs in culture, or whether they are a response to the 'shock' of removal from the culture medium and short-term incubation. Consequently, it was decided to investigate the rates of fuel utilization by macrophages in culture over several days, and the fate of such fuels. This is considered to be important, since the behaviour of the cells over several days is more likely to represent their behaviour in the peritoneal cavity.

Previous reports have described the rates of uptake and the fates in the macrophage of different classes of lipid. Low-density lipoprotein is taken up by the cells, and cholesterol is accumulated mainly as cholesterol ester (Fogelman *et al.* 1985). Long-chain fatty acids (oleate or palmitate) are taken up and incorporated into either phospholipid or triacylglycerol (Lokesh & Wrann, 1984; Von Hodenberg *et al.*, 1984). Since it had been established previously that fatty acids can be oxidized by macrophages (Newsholme *et al.*, 1987), it seemed important to attempt to measure the proportion of fatty acid incorporated into lipids and that which is oxidized, during culture of the macrophages.

MATERIALS AND METHODS

Animals

Thioglycollate-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. Asparaginase, L-alanine, L-glutamine, triethanolamine, sodium oleate and bovine serum albumin (fraction V) were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Hydrazine hydrate was obtained from BDH Chemicals, Poole, Dorset, U.K. All inorganic reagents and organic solvents were obtained from Fisons Scientific Apparatus, Loughborough, Leics., U.K. All radiochemicals and the tissue solubilizer were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., except for [3-^{14}C]pyruvate, which was obtained from New England Nuclear, Boston, MA, U.S.A. Liqui-

Abbreviation used: FCS, foetal-calf serum.

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scint (aqueous) and Betafluor (non-aqueous) scintillation cocktails were obtained from National Diagnostics, Somerville, NJ, U.S.A.

Cell-culture requirements

Tissue-culture dishes and plates and gentamycin were obtained from Flow Laboratories, Irvine, Scotland, U.K.

Eagle's minimum essential medium, and foetal-calf serum (FCS) were obtained from Gibco–Biocult, Paisley, Scotland, U.K. Phosphate-buffered saline A and B were obtained from Oxoid, Basingstoke, Hants., U.K. Thioglycollate broth was obtained from Difco Laboratories, Detroit, MI, U.S.A.

Preparation of thioglycollate-elicited macrophages

This was done as previously described (Newsholme *et al.*, 1986). After harvesting, the cells were resuspended in Eagle's minimum essential medium supplemented with 10% (v/v) FCS, 2 mM-glutamine and 20 μg of gentamycin/ml. These cells were plated at a density of $(2.0–2.5) \times 10^7$ cells per 100 mm-diam. tissue-culture Petri dish, or $(8–10) \times 10^5$ cells per well in a 24-well tissue-culture plate. After incubation for 4 h at 37 °C in air/CO_2 (19:1), the plates were washed vigorously with phosphate-buffered saline A (Culvenor & Weidemann, 1976) three times: all cells, except for the adherent macrophages, were lost in the washings. The plates were further incubated at 37 °C in air/CO_2 (19:1) for up to 96 h in the relevant culture medium (as described in Figure or Table legends). The volume of culture medium used was 25 ml for a 100 mm tissue-culture dish or 2 ml for a well in a 24-well plate. The biological viability of these cells over 96 h was determined to be > 95% by exclusion of Trypan Blue.

Analytical methods

Metabolite concentrations were determined enzymically by using a Gilford Stasar III spectrophotometer, as described previously (Newsholme *et al.*, 1987) except that alanine was measured by the method of Williamson (1974).

For the determination of radioactivity, 100 μl of sample (medium or cell lysate) was added to 900 μl of water, and to this mixture 10 ml of scintillation cocktail was added. The radioactivity was then measured in a Beckman liquid-scintillation counter (model LS 7500).

Lipid was extracted from the cells by standard procedures (see Newsholme, 1987). Separation was performed on t.l.c. plates with the solvent system hexane/diethyl ether/acetic acid (60:30:1, by vol.). The standards used for identification were triolein, cholesterol and oleic acid, and the positions of the standards on the t.l.c. plates were determined by a charring method. Phospholipids were identified by reaction with phosphomolybdic acid (see Hofman, 1962). The radioactivities associated with each class of lipid were determined by scraping the t.l.c. plate, and incubating the scrapings overnight in tissue solubilizer at room temperature. Then 16 ml of scintillation cocktail was added, and the radioactivity was measured in a Beckman liquid-scintillation counter (model LS 7500).

Spontaneous decomposition of glutamine

It is generally accepted that glutamine is unstable, but little work has been reported on this subject. Its stability was determined in culture medium incubated at 37 °C in air/CO_2 (19:1), in the absence of cells; at 2 mM-glutamine, it was found to decompose at a rate of 5%/day. This decomposition was taken into account in calculations of rates of glutamine utilization and production of ammonia and glutamate.

Expression of results

Rates of substrate utilization or product formation are expressed as nmol (or μmol) per mg of protein per unit time. Protein was assayed as described by Bradford (1976).

For experiments in which 100 mm tissue-culture dishes were used, four dishes were sampled for every experimental time point, and the experiment was repeated at least twice. For experiments in which 24-well plates were used, eight wells were sampled for every experimental time point, and the experiment was repeated at least twice. Identical and parallel incubations were performed so that the protein concentration could be measured at all time points.

Rates of metabolite utilization or production were identical whether the cells were in 100 mm culture dishes or in a 24-well plate.

All experiments involving radiolabelled substrates were carried out in 24-well plates.

Rates of utilization or production were obtained by removing samples of culture medium at a specific time point, measuring a concentration and subtracting from the zero-time concentration, or vice versa. Rates of incorporation into cellular material were obtained by disrupting cells with Nonidet P40 detergent and measuring the radioactivity associated with the cell lysate.

The apparent rates of oxidation of radiolabelled substrates were calculated as the difference between the radioactivity present at zero time and that in the medium plus that incorporated into cellular material at any particular time point. It is considered that the radioactivity not recovered is lost as $^{14}CO_2$, and hence it is termed apparent: it is expressed as nmol of substrate oxidized to CO_2 per mg of protein.

Culture media

Initial experiments were performed to identify the effect of FCS, if any, on rates of utilization of glucose and glutamine, and rates of production of some of the end-products of their metabolism. The final concentration of FCS was 5% (v/v) in all experiments (a concentration routinely used in cell-culture work). The FCS contains glucose, glutamine and fatty acid: but at an FCS concentration of 5%, the final concentrations of these substrates were less than 4% of those routinely used in utilization studies. In some experiments, FCS was replaced by 0.1% (w/v) bovine serum albumin (henceforth referred to as albumin), which had been defatted by the method of Chen (1967); preliminary experiments established that 0.1% albumin was the lowest concentration of protein that would sustain cell number over several days in culture in the presence of both glucose and glutamine. In the absence of both FCS and glucose, macrophages lysed very rapidly (1–12 h). It was not possible to maintain the viability of macrophages in culture for 24 h or longer without the presence of FCS plus glutamine or oleate.

Two variants of Eagle's minimum essential medium were used for these experiments: minimum essential medium with Earle's salts (without L-glutamine) was

Table 1. Rates of substrate utilization and metabolite production by macrophages during 23 h culture

Macrophages were cultured and rates of utilization or production of metabolites were measured as described in the Materials and methods section: −indicates utilization. Glucose, when present, was used at an initial concentration of 5 mM, glutamine at 2 mM, FCS at 5% (v/v) and albumin at 0.1%. Results are presented as means ± S.E.M. for at least four separate culture dishes. The rates of production of lactate and ammonia in the absence of added substrate were subtracted before presentation of the data, except for the row marked *.

Additions to culture medium	Rates of utilization or production (nmol/h per mg of protein)					
	Glucose	Lactate	Glutamine	Ammonia	Glutamate	Aspartate
Glucose, glutamine and albumin	−570±22.5	888±23.6	−61±6.0	59±2.3	7.2±0.38	6.6±0.57
Glucose, glutamine and FCS	−561±22.8	920±21.0	−55±8.0	56±3.7	11±2.1	1.5±0.18
Glucose and albumin	−487±11.4	872±35	−	< 0.1	< 0.1	< 0.1
Glucose and FCS	−462±10.0	874±11.3	−	< 0.1	3.2±0.70	< 0.1
Glutamine and FCS	−	< 0.1	−89±5.8	109±3.4	18±1.9	3.1±0.36
FCS*	−	67±5.4	−	35±1.9	< 0.1	< 0.1

obtained from Gibco–Biocult, and an identical medium but without D-glucose was supplied by staff of the Tissue Culture Laboratory, Sir William Dunn School of Pathology, Oxford.

Oleate, when present, was added as a complex with albumin, as described by Newsholme (1987).

All media plus supplements, either separately or when mixed, were filtered through Millipore filters (pore size 0.22 μm) before use.

RESULTS AND DISCUSSION

The rates of glucose and glutamine utilization and of lactate, ammonia, glutamate and aspartate production by macrophages over a 23 h culture period are given in Table 1. Some important differences in the metabolism of glucose and glutamine are observed compared with 60 min-incubated macrophages, described by Newsholme *et al.* (1987). First, the rate of glucose utilization by cultured macrophages is very high (at least 50% greater than that reported for 60 min-incubated cells), and it is increased rather than decreased by the presence of glutamine. However, as in the incubated cells, almost all of the glucose utilized by macrophages is converted into lactate (Table 1). The rate of glucose utilization is about 10 times that of glutamine at 23 h of culture when both substrates are present together in the culture medium; it is approx. 5-fold greater when the two substrates are present separately at 23 h of culture. It should be noted that the rate of glucose utilization is high (about 50% of that of the maximally working perfused rat heart), and this rate is maintained over 3–4 days of culture.

The rate of glutamine utilization by macrophages at 23 h of culture is very similar to that observed in 60 min incubations, i.e. approx. 100 nmol/h per mg of protein. This rate is decreased both in culture and in the incubation to approx. 60 nmol/h per mg of protein when glucose is present in the medium. One important difference in the metabolic behaviour of macrophages in culture conditions compared with incubation is that in culture the rates of production of glutamate and aspartate, the major end-products of glutamine metabolism during incubation, are much lower (Table 1). It is possible, however, that the difference is more apparent than real: thus most of the glutamate produced by the incubated macrophage remains within the macrophage, and is not lost to the incubation medium, and hence the intracellular concentration of glutamate can be very high (Newsholme *et al.*, 1987). This gives the impression that in these latter cells glutamate is an end-product of glutamine metabolism, but this may not be the case. From a comparison of the results with the incubation and culture experiments, it is suggested that the macrophage has a high capacity to take up glutamine and 'trap' it, at least for a period of time, as glutamate; and in this way the glutamate acts as an intracellular store for both energy formation and provision of precursors for biosynthesis. If glutamine/glutamate metabolism is important in these cells for, e.g., ATP production and/or provision of nitrogen for biosynthesis, a decrease in the extracellular concentration of glutamine could seriously impair the functioning of these cells at a critical time during the immune response. An intracellular 'store' of glutamine/glutamate could be of considerable importance in the maintenance of the normal functioning of the macrophage at such times.

There were no statistically significant differences between rates of glucose or glutamine utilization, and of lactate or ammonia production, when macrophages were cultured in the presence of 5% FCS compared with 0.1% albumin (Table 1). Therefore all subsequent experiments were performed in the presence of 5% FCS.

The rates of glucose utilization and lactate production by cultured macrophages are linear with time over a period as long as 80 h (Fig. 1). Addition of oleate did not alter glucose metabolism over this period (Fig. 1).

The rates of glutamine utilization and ammonia production by cultured macrophages, over at least 90 h, are presented in Fig. 2. The presence of glucose decreased the rate of utilization of glutamine for the whole prolonged period of culture. When glucose was present in the culture medium, the rate of ammonia production was identical with the rate of glutamine utilization. However, when glucose was omitted from the culture medium, not only was the rate of glutamine utilization markedly increased, but the rate of ammonia production was increased over and above that predicted from the increment in the rate of glutamine utilization (Fig. 2). Thus the fate of the glutamine nitrogen appears to be

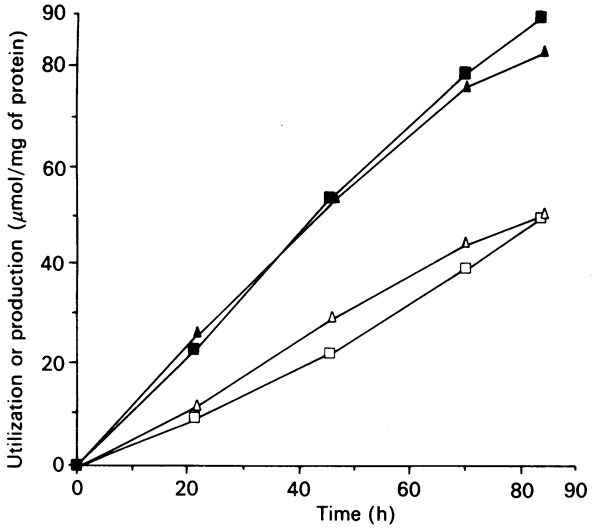

Fig. 1. Glucose utilization (△, □) and lactate production (▲, ■) with time by macrophages in culture

Macrophages were cultured on 24-well plates as described in the Materials and methods section: culture medium contained 5% FCS. Each point represents the mean of results from four separate culture plates; S.E.M. values are not given, but are less than 10% of means: ■, □, 5 mM-glucose plus 2 mM-glutamine present in culture medium; ▲, △, 5 mM-glucose, 2 mM-glutamine plus 0.3 mM-oleate present in the culture medium.

ammonia (Fig. 2) and alanine (Fig. 3). The ammonia probably results from the action of glutaminase, and alanine is probably formed by transamination of pyruvate, which is derived from glucose (the rate of alanine production decreases markedly when glucose is omitted from the culture medium; Fig. 3).

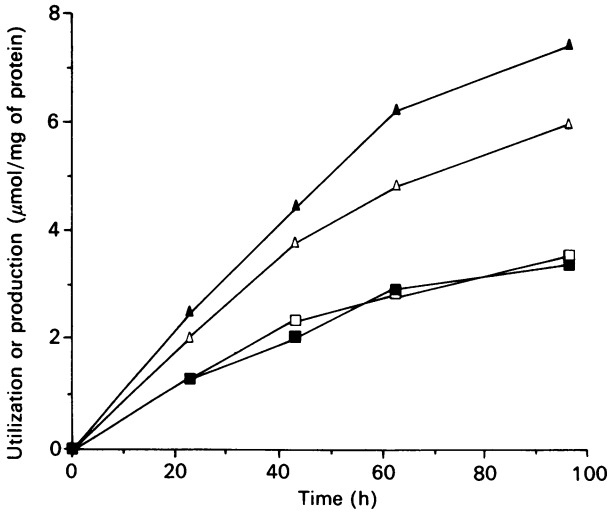

Fig. 2. Glutamine utilization (△, □) and ammonia production (▲, ■) with time by macrophages in culture

Macrophages were cultured in 100 mm culture dishes as described in the Materials and methods section: culture medium contained FCS (5%). Each point represents the mean of results from four separate culture dishes: S.E.M. values are not given, but are less than 10% of means: ▲, △, 2 mM-glutamine present; ■, □, 2 mM-glutamine and 5 mM-glucose present.

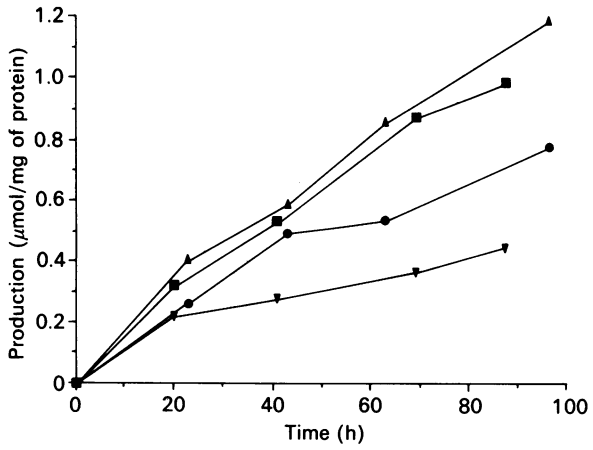

Fig. 3. Formation of glutamate (▲, ●) and alanine (■, ▼) with time by macrophages in culture

Macrophages were cultured in 100 mm tissue-culture dishes as described in the Materials and methods section. All culture media contained 5% FCS. The points represent the means of at least four separate culture dishes: S.E.M. values are less than 10% of means. Glutamate and alanine productions when cells incubated with glutamine alone are indicated by (▲) and (▼) respectively, and those from glutamine plus glucose are indicated by (●) and (■).

A hypothesis to explain the high rates of both glycolysis and glutamine utilization in rapidly dividing cells, including tumour cells, fibroblasts and enterocytes, and in those cells with the potential for rapid cell division (such as lymphocytes), has been put forward (Newsholme *et al.*, 1985*a*,*b*). The hypothesis proposes that the high rates of these pathways provide ideal conditions for precise regulation of the rates of processes that utilize intermediates of these pathways for biosynthesis; these intermediates include glucose 6-phosphate (for ribose phosphate formation) and glycerol 3-phosphate (for phospholipid synthesis) in glycolysis, and glutamine utilization will provide glutamine and aspartate for purine and pyrimidine synthesis. In a previous paper concerning macrophage metabolism (Newsholme *et al.*, 1987), it was suggested that the observed high rates of glucose and glutamine utilization by macrophages incubated for 60 min could also be attributed to the need for precision in the regulation of biosynthetic pathways, even though peritoneal-elicited macrophages are terminally differentiated. Macrophages are characterized by high rates of protein secretion and membrane recycling (for review, see Werb, 1984), for which high rates of protein and lipid biosynthesis may be required. The high rate of protein synthesis will require high rates of mRNA synthesis, for which purines, pyrimidines and ribose phosphate may be required, and new lipid synthesis will require glycerol 3-phosphate for the phospholipid backbone. The results in the present paper give further weight to the above hypothesis, by presenting evidence that high rates of glutamine and glucose utilization by macrophages occur over several days in conditions that more closely mimic those occurring *in vivo*.

The presence of oleate (0.3 mM) in the culture medium had no effect on the rate of glutamine utilization for at least 96 h of culture (results not shown).

The time courses of glutamate, aspartate and alanine production in the cultures containing glutamine were

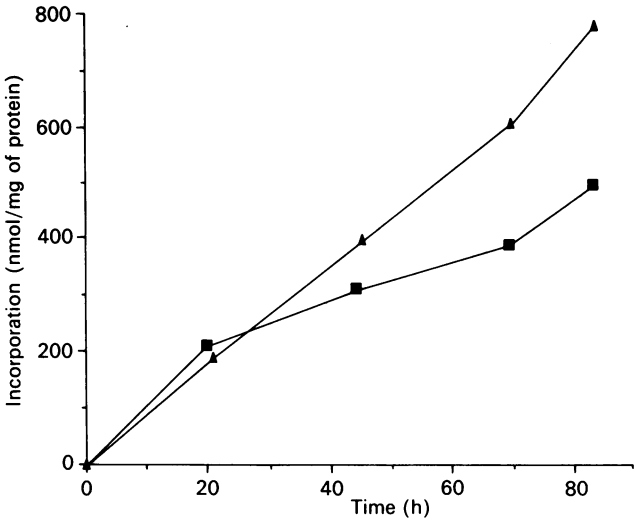

Fig. 4. Incorporation of ^{14}C from [2-^{14}C]glucose (▲) or [U-^{14}C]-glutamine (■) into cell material of macrophages in culture with time

Macrophages were cultured in 24-well plates as described in the Materials and methods section. Glucose was present at 5 mM (0.5 μCi/ml), and glutamine at 2 mM (0.36 μCi/ml). The points represent the means of at least four separate culture plates; S.E.M. values are not given, but are less than 10% of means.

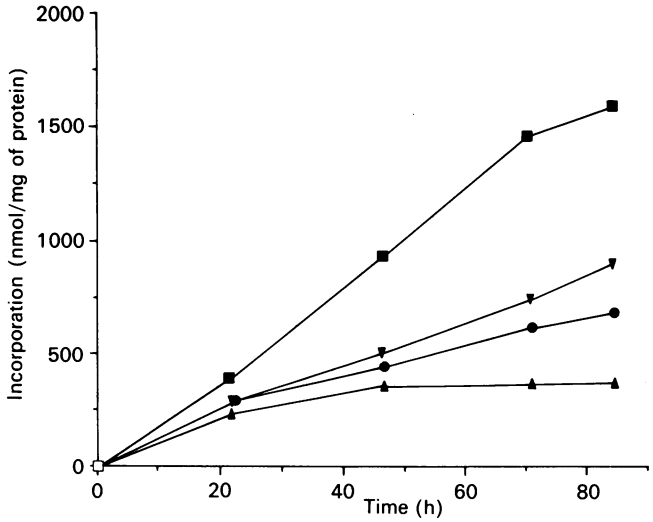

Fig. 5. Incorporation of ^{14}C from [1-^{14}C]oleate into cell material of macrophages in culture with time

Macrophages were cultured in 24-well plates as described in the Materials and methods section. Culture media contained 5% FCS. Oleate was present at 0.3 mM (0.25 μCi/ml): ●, no addition; ▼, 5 mM-glucose; ▲, 2 mM-glutamine; ■, 5 mM-glucose plus 2 mM-glutamine. Each point represents the mean of four separate culture plates; S.E.M. values are not given, but are less than 10% of means.

measured. These compounds are known to be end-products of glutamine metabolism in cells that utilize glutamine (e.g. cells of the small intestine, lymphocytes, macrophages, colonocytes: see Ardawi & Newsholme, 1985; Hanson & Parsons, 1980). The time course of aspartate production is not given, since the amount of aspartate produced was always less than 5% of the amount of glutamine utilized (in cultures longer than 24 h): this is in contrast with the results with 60 min-incubated macrophages (Newsholme *et al.*, 1987). The rate of alanine production is highest when both glucose and glutamine are present in the culture medium (Fig. 3). The decrease in the rate of alanine production when glucose is omitted is almost identical with the increased rate of ammonia production (see Figs. 2 and 3). The percentage of glutamine metabolized to glutamate is not changed by the presence or absence of glucose in the culture medium.

The time courses of incorporation of ^{14}C from [2-^{14}C]-glucose or [U-^{14}C]glutamine into the cell contents of cultured macrophages over 84 h culture are given in Fig. 4. The rates of incorporation of both glucose and glutamine were approximately linear over this period. The incorporation of both substrates was measured in optimal culture conditions (i.e. glucose, glutamine and FCS were all present). The rate of incorporation of glucose into cell contents was slightly higher than that of glutamine.

The time courses of incorporation of [1-^{14}C]oleate into cell contents of cultured macrophages over a time course of 84 h in several conditions of culture are given in Fig. 5. It has previously been shown (Lokesh & Wrann, 1984) that the rate of oleate incorporation into macrophages approaches saturation at approx. 0.2 mM; in the present studies a concentration of 0.3 mM was used; in previous studies this latter concentration provided a maximal rate of oleate oxidation (Newsholme *et al.*, 1987). When both glutamine and glucose were present in the culture medium, the rate of incorporation of oleate into cell contents was considerably higher than if either or both of these components were omitted from the culture medium. The highest rate of incorporation of ^{14}C from [^{14}C]oleate is at least 2-fold greater than the rate from either [^{14}C]glucose or [^{14}C]glutamine; and yet the rates of utilization of these latter substrates are 25- and 2-fold higher than that for oleate respectively (Newsholme, 1987).

The results of a series of experiments to determine the rate of incorporation into cell contents and apparent oxidation rates of [2-^{14}C]glucose and [U-^{14}C]glutamine in different culture conditions during 82 h of culture are given in Table 2. For both glucose and glutamine, it is shown that the apparent rate of oxidation is higher than the rate of incorporation into cellular contents for these substrates. This apparent oxidation rate for glucose is approx. 5% of the rate of utilization of glucose by these cells (cf. data in Table 1 with those in Table 2) and that incorporated into cellular material is only 2%. The remainder is lost to the medium largely as lactate. In contrast, the apparent rate of oxidation of glutamine is a high proportion of the rate of utilization (74%). This is in contrast with the behaviour of macrophages during incubation (see Curi *et al.*, 1988) (however, see below). Of the remainder of the glutamine, approx. 8% is incorporated into cell material and about 20% is lost to the medium (as glutamate, alanine and aspartate).

The time courses of apparent oleate oxidation under different conditions are shown in Fig. 6. As might be expected, if oleate is oxidized to satisfy some of the energy requirement of the cell, the highest rate was

Table 2. Incorporation of ^{14}C from [^{14}C]glucose or [^{14}C]glutamine into cellular material or $^{14}CO_2$ and effects of unlabelled glucose, glutamine or oleate

Incorporation of radiolabelled substrate into cellular material or CO_2 was measured as described in the Materials and methods section. Glucose was present at an initial concentration of 5 mM, glutamine at 2 mM and oleate at 0.3 mM. Specific radioactivities are given in the legend to Fig. 4.

Additions to incubation medium	Rate of incorporation into (μmol/82 h per mg of protein)	
	Cell material	CO_2
[2-^{14}C]Glucose plus glutamine	0.78±0.05	2.1±0.13
[2-^{14}C]Glucose plus glutamine plus oleate	1.01±0.08	2.4±0.19
[2-^{14}C]Glucose plus oleate	0.37±0.05	3.2±0.43
[U-^{14}C]Glutamine	0.43±0.03	4.5±0.31
[U-^{14}C]Glutamine plus glucose	0.51±0.01	3.2±0.03
[U-^{14}C]Glutamine plus glucose plus oleate	0.35±0.01	3.2±0.06
[U-^{14}C]Glutamine plus oleate	0.35±0.04	5.3±0.54

observed when glucose and glutamine were omitted from the culture medium, whereas the lowest rate was observed when both glucose and glutamine were present. The highest rate of oleate oxidation reported here is approx. 10 times that reported for 60 min-incubated macrophages (Newsholme *et al.*, 1987).

Calculations have been made on the rate of ATP formation provided by each of these fuels in the macrophage (Table 3). It can be calculated that oleate accounted for all of the energy requirements of the cells when it was the sole substrate, and as low as 22% in the additional presence of glucose and glutamine.

It was calculated that, when cultured in the presence of both glucose and glutamine, approx. 25% of the oleate taken up by the cells was oxidized and 75% incorporated into cellular material. It seemed likely that oleate would be incorporated into various lipid classes, and these were separated and the radioactivity measured. In addition, it was decided to investigate the incorportion of ^{14}C from radiolabelled glucose, pyruvate and glutamine into the lipid classes for comparison with oleate. The results are presented in Table 4. Less than 2% of ^{14}C from glutamine that was incorporated into cell constituents was found in the lipid classes analysed (results not shown). In contrast, of the ^{14}C from glucose, pyruvate, or oleate that was incorporated into cell constituents, it was found that 27.7, 71.5 and 99.5% respectively were incorporated into lipid (results not shown). Of the radioactivity associated with lipid, approx. 80% of oleate is incorporated into triacylglycerol, about 18% is incorporated into phospholipid, and 2% is recovered as non-esterified fatty acid. It was surprising that the major proportion is incorporated into triacylglycerol, since phospholipid turnover is considered to be high in macrophages. It is possible that triacylglycerol represents a major fuel store for these cells, and whenever fatty acids are available they are taken up and incorporated into lipid to act as an important fuel reserve. It remains an interesting question whether excessive uptake of fatty acid and excessive triacylglycerol formation are possible in these cells and, if so, whether they are deleterious to the function of the cells.

Pyruvate is incorporated to an approximately equal extent into all four classes of lipid (cholesterol, fatty acid, phospholipid and triacylglycerol), whereas glucose is incorporated mainly into phospholipid and triacylglycerol. This difference suggests that the glucose carbon is incorporated into the glycerol moiety rather than the fatty acid moiety of both the triacylglycerol and the phospholipid molecules. From the rates of incorporation of radiolabel into triacylglycerol and phospholipid, under the conditions of cell culture used in this work (Figs. 4 and 5), it is clear that the rate of synthesis of lipid from precursors such as pyruvate or glucose is low, whereas the rate of incorporation of extracellular fatty acid into cellular lipid is high (and the incorporation of label from glucose was approx. 7 times that from pyruvate; results not shown).

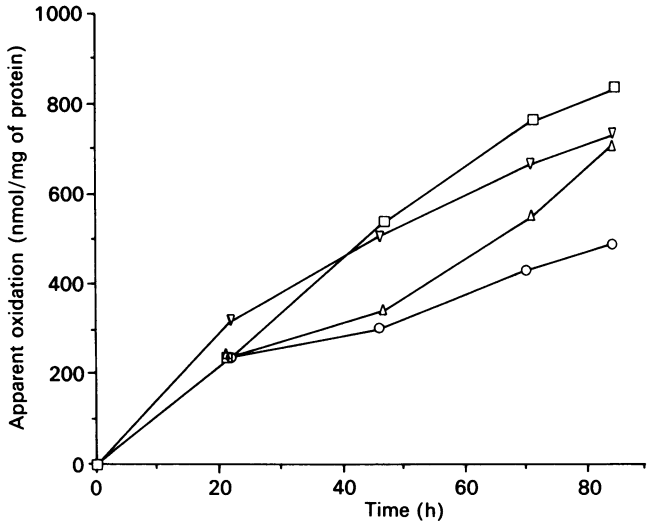

Fig. 6. Apparent oxidation of [1-^{14}C]oleate by macrophages in culture against time

Macrophages were cultured in 24-well plates, and $^{14}CO_2$ was measured as described in the Materials and methods section. Culture media contained 5% FCS. Oleate was present at 0.3 mM (0.25 μCi/ml): □, no addition; ▽, 5 mM-glucose; △, 2 mM-glutamine; ○, 5 mM-glucose plus 2 mM-glutamine. Each point represents the mean of four separate culture plates; S.E.M. values are not given, but are less than 10% of means.

Table 3. Calculations of the contributions of glucose, glutamine and oleate to the energy requirement of cultured peritoneal macrophages

It was assumed that the following numbers of mol of ATP can be generated from the metabolism of 1 mol of substrate as shown below:

Glucose	38 mol of ATP	(CO_2 as end-product)
Glucose	2 mol of ATP	(lactate as end-product)
Glutamine	24 mol of ATP	(CO_2 as end-product)
Oleate	146 mol of ATP	(CO_2 as end-product)

It is assumed that ATP generation is maximal in the presence of glucose, glutamine and oleate. ATP generation is calculated from the rate of conversion of glucose into CO_2 and lactate (proportions indicated in the text), glutamine into CO_2 and oleate into CO_2, after 82 h in culture. Experimental details are given in the Materials and methods section. Data are calculated from the results of at least three separate experiments; values for S.E.M. were less than 10% of the mean.

Substrates present	ATP generated (μmol/82 h per mg of protein)	Contribution of substrate to total ATP generation (%)	Percentage of maximum ATP generation
5 mM-glucose	173	54	100
2 mM-glutamine	76	24	
0.3 mM-oleate	71	22	
5 mM-glucose	166	68	76
2 mM-glutamine	78	32	
5 mM-glucose	199	65	96
0.3 mM-oleate	105	35	
2 mM-glutamine	127	55	72
0.3 mM-oleate	104	45	
2 mM-glutamine	107	100	33
0.3 mM-oleate	122	100	38

Table 4. Percentage distribution of radioactivity into various lipid components of macrophages after 68 h culture with radiolabelled glucose, glutamine, pyruvate or oleate

Cells from 36 wells were combined and the lipids extracted as described in the Materials and methods section. Oleate, glutamine and glucose (of which only one was labelled) were all present in the culture medium used. Pyruvate, however, was added to medium deficient in any other substrate. Glucose was present at an initial concentration of 5 mM, pyruvate at 1 mM and oleate at 0.3 mM. Specific radioactivities are given in the legend to Fig. 4, except for pyruvate, which was present at 1.0 μCi/ml. Results are presented as means from at least three separate experiments; S.E.M. values were less than 10% of means.

Radiolabelled substrate	Radioactivity (% of total)				
	Fatty acid	Triacylglycerol	Phospholipid	Cholesterol	Unidentified lipids
[2-^{14}C]Glucose	< 0.1	33.3	62.5	4.2	< 0.1
[3-^{14}C]Pyruvate	10	19.7	29.0	38.3	2.9
[1-^{14}C]Oleate	1.8	78.8	17.5	2.9	0.6

It is noteworthy that, on the basis of the highest O_2-consumption rates by incubated macrophages measured by Newsholme *et al.* (1987) (almost 160 nmol/h per mg of protein), the rate of ATP formation (assuming a P/O ratio of 3) is approx. 100 μmol/82 h per mg of protein. This is similar to the rate of ATP generation calculated for oleate or glutamine utilization when presented singly to the cells, but perhaps only 50% of that when more than one fuel is present in the culture medium. Perhaps in culture O_2-consumption rates can be twice those measured in an incubation system. Alternatively, and interestingly, perhaps metabolites other than those measured in the present work are produced from oleate and glutamine, and they decompose to provide $^{14}CO_2$ and so interfere in the present interpretations.

From the concentration of ATP present in the peritoneal macrophage (6.5 nmol/mg of protein: Newsholme *et al.*, 1987) and the calculated rate of ATP formation (65 nmol/min per mg of protein; Table 3) it can be calculated that the ATP content of this cell is turned over 10 times per minute: for the maximally working perfused rat heart, under aerobic conditions, the value is 22 (calculated from data in Newsholme & Leech, 1983). Even if based on O_2-consumption data from Newsholme *et al.* (1987), the value for the macrophage is about 5 times per minute. Thus the elicited peritoneal macrophage must be considered to be an extremely metabolically active cell that satisfies its energy requirements by a mixture of anaerobic glycolysis-from-glucose and aerobic glutamine and fatty acid oxidation. The high

demands for such fuels by macrophages and possibly other cells of the immune system may explain, in part, the increased rates of protein degradation in skeletal muscle and increased rates of adipose-tissue lipolysis during injury, trauma, sepsis and burns (see Newsholme *et al.*, 1988). This point emphasizes the importance of knowledge of the biochemical nutrition of the cells of the immune system, and raises questions about the significance of such nutrition during pathological conditions, including such diverse conditions as atherosclerosis and sepsis.

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