Contribution by different fuels and metabolic pathways to the total ATP turnover of proliferating MCF-7 breast cancer cells

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For the past 70 years the dominant perception of cancer metabolism has been that it is fuelled mainly by glucose (via aerobic glycolysis) and glutamine. Consequently, investigations into the diagnosis, treatment and the basic metabolism of cancer cells have been directed by this perception. However, the data on cancer metabolism are equivocal, and in this study we have sought to clarify the issue. Using an innovative system we have measured the total ATP turnover of the MCF-7 breast cancer cell line, the contributions to this turnover by oxidative and glycolytic ATP production and the contributions to the oxidative component by glucose, lactate, glutamine, palmitate and oleate. The total ATP turnover over approx. 5 days was 26.8 μmol of ATP$\cdot 10^7$ cells$^{-1} \cdot$ h^{-1}. ATP production was 80% oxidative and 20% glycolytic. Contributions to the oxidative component were approx. 10% glucose, 14% glutamine, 7% palmitate, 4% oleate and 65% from unidentified sources. The contribution by glucose (glycolysis and oxidation) to total ATP turnover was 28.8%, glutamine contributed 10.7% and glucose and glutamine combined contributed 40%. Glucose and glutamine are significant fuels, but they account for less than half of the total ATP turnover. The contribution of aerobic glycolysis is not different from that in a variety of other non-transformed cell types.

Key words: glucose, glutamine, oleate, oxygen consumption, palmitate.

INTRODUCTION

The energy metabolism of cancer cells has been the focus of investigation since it was suggested 70 years ago that aerobic glycolysis (the production of lactate in the presence of oxygen) was a phenomenon peculiar to tumours [1]. Investigations into tumour intermediary metabolism since then have addressed many aspects, from aerobic glycolysis [2] to the interaction of p53 with the hexokinase promoter [3]. However, the one metabolic theme that dominates the literature, and which also influences the direction of basic research, treatment and diagnosis, is the overwhelming importance of glucose (via aerobic glycolysis), and to a lesser extent glutamine, as fuels for cancer cells. Consequently, a high proportion of studies on cancer cells are based on the perception that a feature of transformed cells is their high rate of aerobic glycolysis (for recent examples see [4–6]; the last two quote a 1956 paper by Warburg [6a] as evidence that there is increased glycolytic activity in cancer cells).

These perceived differences in metabolism between normal and cancerous tissue are now being exploited and used (i) to diagnose and monitor cancer, (ii) to treat cancer and (iii) as a basis for cancer research. The metabolic basis of diagnosis and monitoring is reflected in the increased recognition of the role of glucose-based positron emission tomography (PET) in oncology [7,8]. The principle of this technique [the uptake of a glucose analogue, fluorine-^{18}fluorodeoxyglucose (FDG)] is based upon the assumption that tumours take up more glucose than the surrounding non-cancerous tissue. There is no doubt that this is a valuable technique, but the efficacy varies with the study and the tissue (reviewed by Schiepers and Hoh [9]). For example, Rostom et al. [10] report a high success rate for breast primaries and metastases, but this is at odds with a study by Yutani et al. [11]. And PET was only 45% successful in detecting the primary tumours in patients with a variety of metastases [6]. Confounding the issue even further are recent and contradictory studies by Aloj et al. [12] and Chung et al. [13]. Aloj et al. [12] found that the uptake of FDG correlates inversely with the expression of two markers that are considered to reflect the potential of a cell to take up and use glucose, i.e. Glut-1 protein and hexokinase message (see below and Chang et al. [14]). Chung et al. [13], on the other hand, found that FDG uptake correlates with amounts of Glut-1 protein and message. These data suggest that the metabolic principles underlying the PET technique are not fully understood, and that they are likely to be more complex than previously thought.

There are recent therapeutic approaches to cancer that likewise are based on the perceived modification of metabolism in cancer cells. For example the anticancer drug 1-(2,4-dichlorobenzyl)-1*H*-indazol-3-carboxylic acid was developed on the premise that cancer cells are dependent upon a high rate of glycolysis [15]. Similar metabolically based rationales are the basis of antisense mRNA treatments that target glutaminase and glyceraldehyde-3-phosphate dehydrogenase [16,17], and the basis of a hexokinase-directed gene-targeting cancer therapy [18]. Basic research on the metabolism of the cancerous state is also concentrating on glycolysis and glutamine metabolism. For example, Mathupala et al. [3] investigated the role of p53 in the regulation of the putative obligate glycolysis that fuels cancer cells, and Mazurek et al. [19] reported changes in the glycolytic and glutaminolytic pathways that are linked to transformation.

Despite the obvious acceptance of the concept of a 'cancer metabolism', there are three problems with the metabolic data on cancer cells. First, in most of the studies, total ATP turnover has not been measured because oxygen consumption was not determined. For example, Petch and Butler [20] supplied CC9C10 murine hybridoma cells with only glucose and glutamine. They

Abbreviations used: PET, positron emission tomography; FDG, fluorine-^{18}fluorodeoxyglucose; FBS, fetal bovine serum; pO_2, partial pressure of oxygen; $\dot{V}O_2$, rate of oxygen consumption.

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assumed that glucose and glutamine utilization (through a variety of pathways) accounted for 100% of ATP turnover, and calculated contributions to total ATP turnover by these two fuels based on this assumption. The rate of oxygen consumption ($\dot{V}O_2$) was not measured and would almost certainly have been greater than that accounted for by the oxidation of glucose and glutamine, due to the oxidation of unknown (exogenous from the fetal calf serum, and endogenous) fuels (see [4], and for a discussion of this point see [21]). It is clear that this approach significantly underestimates total ATP turnover and results in inflated estimates of the contributions of the fuels offered. Second, even when $\dot{V}O_2$ has been measured (e.g. [4]), none of these studies was developed beyond the stage of providing cells with only one or two fuels. The problems associated with this approach are evident from studies on human platelets, which in the presence of only 5 mM glucose and 1 mM acetate will fuel 80% of their ATP turnover with acetate oxidation [22]. In plasma, however, where acetate concentration is only about 50 μM, and where there are numerous other fuels available at physiological concentrations, acetate oxidation only accounts for 9% of ATP turnover [23]. Third, because there are technical difficulties with obtaining metabolic data on any proliferating (and therefore long-term) cultured cell system, the available data are derived from short-term experiments that do not comprise a complete or several complete cell cycles.

In summary, there are no unequivocal data on fuel preferences by proliferating cancer cells in a physiological medium. These data are essential if we are to understand the processes that form the basis of the current diagnostic and treatment strategies, and will in turn facilitate the improvement of these strategies, and perhaps suggest new ones. In order to characterize comprehensively the metabolism of a cancer cell under physiological conditions, we used a culture system (developed by Guppy et al. [21]) which enables the quantification of energy budgets of proliferating cells in long-term culture. This system was applied to the human MCF-7 breast cancer cell line, a transformed, epithelium-derived, oestrogen-receptor-positive line that proliferates with oestrogen and is representative of a significant proportion of breast cancers [24]. Furthermore it is a relevant cell line for this study as the PET technique is used to detect breast cancers and to monitor the effect of treatment [25].

EXPERIMENTAL

Materials

All enzymes were purchased from Roche Molecular Biochemicals (Castle Hill, NSW, Australia). D-[6-^{14}C]Glucose (56 mCi/mmol), [U-^{14}C]palmitic acid (795 mCi/mmol), [1-^{14}C]oleic acid (55–57 mCi/mmol), L-[U-^{14}C]glutamine (229 mCi/mmol), L-[U-^{14}C]lactic acid sodium salt (152 mCi/mmol) and NCS™ were from Amersham Australia Pty. Ltd. (Castle Hill, NSW, Australia). [U-^{14}C]Oleic acid (700 mCi/mmol) was from NEN (Boston, MA, U.S.A.). Myxothiazol, Phenol Red and glutamine were from Sigma (Castle Hill, NSW, Australia). Dulbecco's modified Eagle's medium, Penstrep and fetal bovine serum (FBS) were from Invitrogen (Rockville, MD, U.S.A.).

Maintenance of cell culture

All experiments were performed using MCF-7 cells (A.T.C.C. no. HTB-22) within 20–30 passages of purchasing. Cells were maintained in a 37 °C CO_2 incubator, in T75 culture flasks at less than 90% confluence, in Dulbecco's modified Eagle's medium with the following additions: 6 mM glucose, 1.5 mM glutamine, Phenol Red (15 mg/l), Penstrep (10 ml/l) and 5% FBS.

Experimental design using air-tight glass culture flasks

The methods used in this study were developed, validated and then published in 1997 [21]. But the approach is unusual and, to our knowledge, is not one that has been used subsequently in any other study. Therefore it is described again here, but in a more concise form, including any variations from the original method.

Cells were cultured in air-tight glass culture chambers which can be flushed and from which air and liquid samples can be taken. Cells were in culture for 5 days, during which time they underwent cell division about three times and cell numbers increased approx. 6-fold. The change in the partial pressure of oxygen (pO_2) and pH in the chambers over this time was maximally 5% and 0.23 units respectively. The cells were incubated with a mixture of fuels, one of which was spiked with the corresponding ^{14}C-labelled fuel. Initial and final measurements were made of lactate, $^{14}CO_2$, pO_2, cell numbers and time. From these measurements the total ATP turnover (oxidative from oxygen consumption and glycolytic from lactate production) was calculated. From the production of $^{14}CO_2$ the contribution of the spiked fuel to the oxidative ATP turnover in that particular experiment was calculated. The measurement of cell numbers over time allowed the construction of a growth curve and all measurements were expressed per the area under the growth curve (10^7 cells $\cdot$ h^{-1}) for the period of the experiment.

Each experiment required six glass culture chambers, labelled A–F, which had total volumes ranging from 405 to 530 ml. On day 0, experimental medium (50 ml; see the next section) was added to the cells (2×10^6). One ^{14}C-labelled fuel (approx. 20 μCi for glucose and 2 μCi for all other fuels) was added to flasks C–F and a liquid sample taken from flasks C–F for specific activity determination. All chambers were then flushed with 5% CO_2 in air and left for 24 h for the cells to attach. On day 1, flask A was used to determine an initial pH and cell count for the growth curve. The remaining flasks were flushed (5% CO_2 in air), and a liquid sample taken from flasks C–F for initial $^{14}CO_2$ d.p.m. determination in the liquid (all $^{14}CO_2$ in the gas having been flushed out). On day 2, the cell number in flask B was counted for the growth curve. Flasks C–F were left until day 5 when $^{14}CO_2$ in gas and liquid, pO_2 in gas, and pH and lactate in liquid were measured, and cell numbers were counted. The flasks were rocked for 20 min at room temperature before each measurement to ensure that the pO_2 in the liquid was the same as that in the gas. So each experiment comprising six flasks produced data ($n = 4$) for one fuel. The viability of the cells using Trypan Blue exclusion was never less than 97% at any time during any experiment.

Experimental medium

The experimental medium comprised Dulbecco's modified Eagle's medium containing 6 mM glucose, 1.5 mM glutamine, 0.09 mM palmitate, 0.05 mM oleate, Phenol Red (15 mg/l) and 5% FBS. These fuel concentrations are based on those used in a study of fuel utilization by human platelets in plasma [23], and are within the ranges found *in vivo*, with the exception of glutamine. In this case the concentration is approx. 3-fold higher than that found in human plasma in order to prevent a large percentage change in its concentration during the incubations (see Measurements and calculations section).

Stock FBS/fatty acids

All the experiments were carried out using the same bottle of FBS. Another bottle from the same batch (lot no. 1024481) was analysed for fatty acids by Dr J. Ballantyne (Zoology De-

partment, University of Guelph, Ontario, Canada). The concentrations of palmitate and oleate were between 45 and 49 μM in the undiluted FBS and were ignored. The stock FBS/fatty acid solution was prepared essentially as described by Guppy et al. [26] and diluted 20-fold to give 5% FBS and the fatty acid concentrations in the experimental medium described above.

Measurements and calculations

Measurements of pO_2 in gas, $^{14}CO_2$ in gas and liquid, lactate in liquid, calculations of oxygen consumption and the area under the growth curves were done as described in Guppy et al. [21]. Rates of fuel oxidation were calculated from the specific activities (d.p.m. $\cdot$ μmol^{-1}) of, and the rate of $^{14}CO_2$ production from, the spiked fuels. The rate of lactate production was calculated from the change in lactate concentration during the incubation. Total oxidative ATP turnover was calculated using the rate of oxygen consumption and an average P/O ratio (2.36) from Brand [27]. Glycolytic ATP turnover, and the contribution to total ATP turnover by lactate production were calculated by assuming a 1:1 stoichiometry between lactate and ATP production. The oxygen consumption attributed to the oxidation of each fuel was calculated assuming that the oxidation of 1 mol of glucose, glutamine, palmitate and oleate requires 6, 4.5, 23 and 26 mol of oxygen, respectively. This was converted into ATP turnover using the the average P/O ratio (see above). The percentage of each fuel used during the incubations was 0.898% glucose ($\pm$0.017 S.D.; $n = 4$), 6.48% glutamine ($\pm$0.17; $n = 4$), 11.2% palmitate ($\pm$0.29; $n = 4$), 9.73% [U-^{14}C]oleate ($\pm$0.53; $n = 7$) and 24.8% [1-^{14}C]oleate ($\pm$1.97; $n = 4$; see the Discussion for an explanation of the use of the two different oleate labels). If we had kept glutamine at physiological concentrations (approx. 0.4 mM) the percentage of glutamine used during the incubation would have been about 20%, which we decided was too high. The effect of myxothiazol on the rate of oxygen consumption was measured using short-term incubations, as described in Guppy et al. [23], and cells that had been in culture for 5 days. Myxothiazol (1 μM) inhibited the rate of oxygen consumption by 96.8$\pm$2.22% ($n = 4$; $\pm$S.D.), showing that essentially all of the oxygen consumption was mitochondrial.

A variation of the basic method

Glucose is oxidized through pyruvate, and pyruvate is in a complex equilibrium with intracellular and extracellular lactate. Therefore, if extracellular lactate is present at the start of the experiment, and is oxidized through pyruvate, a calculation of the rate of 'glucose' oxidation that uses the specific activity of extracellular glucose will underestimate the rate of carbohydrate (i.e. glucose+lactate) oxidation. To test whether unlabelled lactate (in the fetal calf serum and therefore present at the start of the incubations) was being oxidized, in one experiment both labelled glucose and lactate were added, to produce the same specific activity of the glucose and lactate pools, in terms of d.p.m./μmol of glucose.

Validation of the technique

The recovery of $^{14}CO_2$ (from added $^{14}HCO_3^-$) over a typical 5 day experiment was 108$\pm$2.22% ($n = 4$). This recovery takes into account the errors in all the processes involved in sampling, extracting and measuring the $^{14}CO_2$, and was taken into account in the calculations. The percentage of the radioactivity incorporated into the cell fraction during these experiments was 0.7$\pm$0.168% ($n = 4$).

Statistics

The Student's t test was used for any comparisons between means. Significant differences were defined as $P < 0.05$.

RESULTS

Cells were cultured in the air-tight flasks for an average of 121 h and over this period cell numbers, rates of oxygen consumption, rates of lactate production and the change in pH were measured. The mean values of these measurements from all experiments are shown in Table 1. Cell numbers increased by approx. 6-fold and the mean area under the growth curve is shown in Table 1. A growth curve incorporating all of the cell-number data is shown in Figure 1.

The rates of oxidation of four fuels were measured over the duration of the culture and are shown in Table 2. The rates of oxidation (μmol $\cdot$ 10^7 cells^{-1} $\cdot$ h^{-1}) vary from a low of 0.007 for oleate, to 0.012 for palmitate, 0.08 for glucose and a high of 0.15 for glutamine. There were no differences in the rates of oxygen consumption, 'glucose' oxidation or the total ATP turnover between labelled glucose and labelled glucose+lactate cultures

Table 1 General characteristics of the 5 day incubations

The data are mean values from all the experiments ($\pm$S.D.). Calculations are either described or cited in the Experimental section.

Characteristic	n	Mean$\pm$S.D.
Incubation time (h)	7	121$\pm$0.756
Area under growth curve (10^7 cells $\cdot$ h^{-1})	27	36.5$\pm$6.08
Rate of oxygen consumption (μmol $\cdot$ 10^7 cells^{-1} $\cdot$ h^{-1})	23	4.49$\pm$0.414
Rate of lactate production (μmol $\cdot$ 10^7 cells^{-1} $\cdot$ h^{-1})	27	5.39$\pm$0.946
Decrease in pH	5	0.2$\pm$0.04
Initial cell number (total $\times$ 10^7)	6	0.15$\pm$.033
Final cell number (total $\times$ 10^7)	27	0.87$\pm$0.17

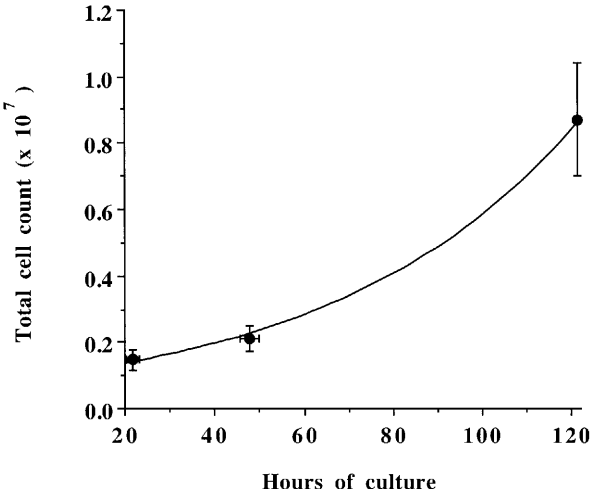

Figure 1 Combined growth-curve data from all the experiments from which the data are derived

The data for the first two time points were taken from flasks A and B for each experiment. The 120 h time point is the mean of the counts in flasks C–F from all of the experiments. The error bars are S.D., and the S.D. for the time axis of the 120 h point is contained within the point. The equation of the line is $y = 0.094 \times 10^{0.0079x}$, $r = 0.998$.

Table 2 Rates of fuel oxidation

The calculations are described in the Experimental section. Means ± S.D are shown; $n \geqslant 4$.

Fuel	Rate of oxidation (μmol · 10^7 cells^{-1} · h^{-1})
Glucose oxidation (without labelled lactate)	0.075 ± 0.007
Glucose oxidation (with labelled lactate)	0.085 ± 0.005
Glutamine oxidation	0.148 ± 0.01
Palmitate oxidation	0.012 ± 0.001
[U-^{14}C]Oleate oxidation	0.007 ± 0.0004
[1-^{14}C]Oleate oxidation	0.013 ± 0.001

Table 4 Energy budget of the MCF-7 cell

All calculations are described in the Experimental section. Means ± S.D. are shown.

Calculation	*n*	Mean ± S.D.
Total ATP turnover (μmol · 10^7 cells^{-1} · h^{-1})	23	26.8 ± 2.62
Oxidative (%)	23	79.2 ± 1.94
Glycolytic (%)	23	20.8 ± 1.94
Contribution to oxidative ATP turnover (%) by ...		
Glucose oxidation (without labelled lactate)	4	10.1 ± 0.414
Glucose oxidation (with labelled lactate)	4	11.2 ± 0.604
Glutamine oxidation	4	13.5 ± 0.607
Palmitate oxidation	4	6.75 ± 0.541
[U-^{14}C]Oleate oxidation	7	4.0 ± 0.378
Oxidative ATP turnover unaccounted for by fuel oxidation (%)*		64.6

* Glucose with labelled lactate was used for this calculation.

(see Table 3). However there were small but significant differences between these two cultures for all of the other measurements. Table 3 also shows the data from the cultures using [U-^{14}C]oleate and [1-^{14}C]oleate. There were no differences between the percentages of oxidative and glycolytic ATP turnover, but there were differences between all the other measurements and between the measured rates of fuel oxidation (Table 2). The differences manifesting between the two oleate labels are addressed in the Discussion.

Table 4 shows the total ATP turnover of the MCF-7 cell. ATP production is 80 % oxidative and 20 % glycolytic. Contributions to the oxidative component by oxidation of the four fuels are approx. 10 % by glucose, 14 % by glutamine, 7 % by palmitate, 4 % by oleate and 65 % from unidentified sources. The contribution by glucose (glycolysis and oxidation) to total ATP turnover is 28.8 %, glutamine contributes 10.7 % and glucose and glutamine contribute 40 %. The change in glucose concentration was measured in three flasks from three different experiments. The mean ratio of Δlactate production/Δglucose consumption was 1.56 ± 0.152 ($n = 3$).

DISCUSSION

The culture system we have used to generate these metabolic data is representative of the physiological state in a number of ways. Unlike most culture systems it contains a variety of fuels, including carbohydrate, amino acids and fatty acids. These fuels are present at concentrations either within the physiological range or, in the case of glutamine, at a concentration at most only 3-fold higher than the physiological range. The concentration of each of the fuels changes maximally by 12 % during the culture period, the cells are attached to a substrate for the whole of the experiment, they proliferate and pass through the cell cycle, they show almost 100 % viability throughout the culture period and are in the presence of Phenol Red, which acts as a weak oestrogen [28]. Technologically the method is complex and demanding, but considering the amount of processing that is involved, the data are precise. These data are, therefore, thought to be an accurate representation of the metabolism of a breast cancer cell with respect to the four fuels offered. It is timely to note here that if oxygen consumption had not been measured in these experiments, and if it was assumed that glucose and glutamine utilization accounted for 100 % of ATP turnover, total ATP turnover would have been calculated as 10.6 rather than 26.8 μmol · 10^7 cells^{-1} · h^{-1} (see Table 4), and 100 % of this turnover would have been accounted for by glucose (73 %) and glutamine (27 %) utilization.

There are three aspects of the data that are surprising considering the perception of cancer metabolism that was mentioned in the Introduction. First, the ATP in an MCF-7 cell is generated mostly (80 %) by oxidative metabolism. (Note that the P/O ratio used in this study was a lower one than that used classically. The contribution by oxidative metabolism will therefore, if anything, be underestimated compared with most previous studies. The P/O ratio used will not however affect the contributions of the fuels to total oxidative ATP turnover, as varying this ratio will affect total ATP turnover and contributions to total ATP turnover equally.) Aerobic glycolytic ATP production contributes only 20 % to the total ATP production. This value may be as high as 24 % if there is glucose passing through glycolysis and not being either oxidized or detected as lactate (this could be the case as the mean ratio of Δlactate production/Δglucose consumption was 1.56; see the Results section).

Table 3 Comparison between experiments using labelled glucose only and labelled glucose and lactate, and between experiments using [U-^{14}C]oleate and [1-^{14}C]oleate

Means ± S.D are shown; $n \geqslant 4$.

Measurement	Labelled glucose	Labelled glucose + lactate	[U-^{14}C]Oleate	[1-^{14}C]Oleate
Rate of oxygen consumption (μmol · 10^7 cells^{-1} · h^{-1})	4.47 ± 0.46	4.57 ± 0.327	4.39 ± 0.415†	3.72 ± 0.253
Total ATP turnover (μmol · 10^7 cells^{-1} · h^{-1})	26.2 ± 2.65	28.1 ± 1.72	25.6 ± 2.19†	21.8 ± 1.25
Oxidative ATP turnover (%)	80.4 ± 0.881*	76.7 ± 0.918	80.8 ± 0.953	80.5 ± 1.15
Glycolytic ATP turnover (%)	19.6 ± 0.814*	23.4 ± 0.929	19.2 ± 0.968	19.5 ± 1.15
Fuel oxidation (% contribution to oxidative ATP turnover)	10.1 ± 0.41*	11.2 ± 0.604	4.0 ± 0.378†	8.83 ± 1.08
Fuel used (%)	0.9 ± 0.017*	0.762 ± 0.039	9.73 ± 0.53†	24.2 ± 1.97

* Significant difference ($P < 0.05$) between labelled glucose and labelled glucose + lactate measurements.
† Significant difference ($P < 0.05$) between [U-^{14}C]oleate and [1-^{14}C]oleate measurements.

Table 5 Volumes, total ATP turnover, ATP turnover due to glucose of various cell types and the volume-adjusted metabolic rate in units of μmol of ATP · 10^7 cells^{-1} · h^{-1} · (cell volume)$^{-1}$

Volumes are from the cited reference, or from blood-bank data (platelets), or are calculated from the diameters (estimated using a haemocytometer), assuming that the cells are spherical. Units of ATP turnover are μmol of ATP · 10^7 cells^{-1} · h^{-1}.

Cell	Reference	Volume (fl)	Total ATP turnover	ATP turnover due to glucose (%)*	Volume-adjusted total ATP turnover	Volume-adjusted ATP turnover due to glucose*
MCF-7	This study	4000	26.8	7.7 ± 30.2	0.0067	0.0019
Platelet	[23]	9	0.025	0.0067 ± 26.8	0.0028	0.00074
Jurkat	M. Guppy, unpublished work	1437	5.34	2.08 ± 39.0	0.0037	0.0014
Resting thymocyte	[38]	300	0.997	0.216 ± 21.6	0.0033	0.0007
Proliferating thymocyte	[38]	840	2.24	1.506 ± 67.0	0.0027	0.0018
J2E	[39]	2500	6.70	1.41 ± 21.0	0.0027	0.00056
H9C2	[40]	2500	12.8	ND	0.0051	ND

* These values comprise glucose oxidation and glycolysis.

Secondly, although glucose (both oxidative and glycolytic metabolism) is the highest contributor of the four fuels to ATP production, it contributes only 30 % of the total ATP production. Similarly the contribution to total ATP production by glutamine is the second highest of the four fuels, but is 11 %. The combined metabolism of glucose and glutamine accounts for less than half of the total ATP turnover. The only other transformed cell to be studied using this method is the J2E cell, a transformed erythroid precursor [21]. That study showed a similar oxidative/glycolytic ratio, and a negligable contribution to ATP turnover by glucose oxidation. A low P/O ratio was also used for the calculations in that study, but it was done using only glucose and glutamine at 25 and 4 mM respectively. A recent study of AS-30D hepatoma cells again showed these cells to be mainly (98 %) oxidative [4], although the study was done either without fuel or with only one fuel at a time, and the oxidation of individual fuels was not measured. So contrary to the accepted perception, recent data suggest that cancer cells are mainly oxidative and that glucose and glutamine are significant, but not the only fuels.

The third surprising aspect of the data is that the oxidation of unidentified fuels accounts for 65 % of the oxidative ATP turnover, and 51 % of the total ATP turnover. All oxygen consumption was a result of fuel oxidation in the electron-transport system, as shown by the complete inhibition of oxygen consumption by myxothiazol. So, half of the ATP production cannot be accounted for by the utilization of the four fuels supplied. This is quite different from another similar study in which only 26 % of the total ATP turnover was unaccounted for in platelets [23]. The reasons which may partially explain the higher unidentified percentage in the MCF-7 cells are (i) acetate accounts for 9 % of total ATP turnover in platelets and was not present in our incubations and (ii) palmitate oxidation accounts for 21 % of total ATP turnover in platelets compared with only 5.4 % in the MCF-7 cell. The contribution by oleate oxidation to total ATP turnover in the MCF-7 cell (3.2 %; using the data from the U-^{14}C-labelled cultures) is actually very similar to that in the platelet (7.2 % [23]), as the use of [1-^{14}C]oleate (as in the platelet study) overestimates oleate oxidation if the fatty acid is not oxidized completely. This is shown in Table 3 where the use of [1-^{14}C]oleate resulted in an overestimation of the rate of oleate oxidation by 2.2-fold. The overestimation in the platelet was 1.5-fold so the percentage contribution of oleate oxidation to total ATP turnover in the platelet is actually 4.8.

A possible contender for the unidentified fuel is lactate, which is in the FBS and which was present in the initial cultures at 1.1–1.7 mM. Lactate is oxidized by numerous cells and tissues [29–31]. Our experiment in which both glucose and lactate were labelled (Table 3) did show an increased 'glucose' oxidation compared with the culture in which only glucose was labelled. But this increase, although significant, is trivial in terms of the unidentified oxidizable fuel source. However there are approx. 120 mg of amino acids in the FBS in the incubation (the protein in FBS varies from batch to batch). This is distributed approximately equally between protein and amino acids (but there is no free glutamine other than what we added). If we assume that all this is in the form of glutamine, only 2.6 mg of 'amino acid/protein' would need to be oxidized to account for the remainder of the oxygen consumption. There is also triacylglycerol in FBS (75 mg/100 ml). Only 60 % of the triacylglycerol in the incubation would need to be oxidized to account for the remaining oxygen consumption. The only other possibilities for alternative fuels in these experiments comprise endogenous ones. These have been mentioned regularly in the literature for the past 30 years [32–34], and some candidates (glucose, the ketones acetoacetate and β-hydroxybutyrate, and glycogen) were identified in a recent study on AS-30D hepatoma cells [4]. Endogenous fuels could also include any or all of protein, triacylglycerol, free fatty acids and amino acids. A more comprehensive study, similar to the one done on platelets [23], is required to resolve this issue and to account for all the oxygen consumption.

Comparisons between this study and other metabolic studies on MCF-7 and other breast cancer cells are difficult as the data from the previous studies are equivocal and suffer from the same shortcomings as mentioned earlier, i.e. total ATP turnover was not measured and the cells were not offered a physiologically relevant medium in terms of fuels. Hugo et al. [35] report a similar Δlactate production/Δglucose consumption ratio (of 1.72) to the one calculated in this study (1.56), but did not measure glucose oxidation. NMR studies show that MCF-7 cells are more sensitive to glucose deprivation than to electron-transport inhibitors [36], but this is probably because the Pasteur effect (an increase in the rate of glycolysis at low or zero oxygen concentrations) can compensate for the inhibition of oxidative phosphorylation if glucose is available. Ting et al. [37] report that rates of glucose consumption and aerobic glycolysis are not different between normal and cancerous (primary) breast cells. Comparisons with data from other transformed and non-transformed cells is again difficult as comprehensive energy budgets, especially with a range of fuels, are virtually non-existent. But on the basis of what is available, the accumulating data suggest that the MCF-7 cell is no more aerobically glycolytic than a wide range of other non-transformed cell types, including lens epit-

helia, smooth muscles, embryos and lymphocytes (see Table 5 and [21]). The contribution by glutamine is similar to that in rat thymocytes, but higher than that in human platelets, and the contribution by glucose oxidation is higher (2–3-fold) than it is in human platelets or rat thymocytes, but also higher than the contribution by glucose oxidation in the transformed J2E cell [21] and Jurkat cell (M. Guppy, unpublished work). The combination of glucose and glutamine contributes 40 % of the total ATP turnover in the MCF-7 cell, 31 % in the platelet, 38 % in the resting thymocyte and a massive 86 % in the proliferating thymocyte [23,41] (in the thymocyte study only glucose and glutamine were available to the cells).

So the paucity of data makes it difficult generally to link cell types with metabolic patterns, and the exercise is confounded by the effect of proliferation on quantitative and qualitative aspects of metabolism, and by the fact that transformed cells are often rapidly proliferating. On the basis of the data herein, and the various supporting studies mentioned above, we suggest that the concept of a 'cancer cell metabolism' fuelled mainly by glucose and glutamine is an oversimplification of a complex process. Glucose and glutamine are certainly significant fuels, but they account for less than half of the total ATP turnover, and the contribution of the major glucose utilization pathway, aerobic glycolysis, is not different from that in a variety of other non-transformed cell types. Differences between MCF-7 cells and non-transformed cells may become more obvious in the presence of oestrogen rather than Phenol Red. In addition, there is evidence to suggest that the environment of solid cancers *in vivo* may be relatively hypoxic for at least some of the time [42]. So, rates of glucose utilization in transformed cells may be higher *in vivo* due to the Pasteur effect, and on top of this, the effect may be disproportionately large in transformed cells.

There are important implications of these data for the glucose-based PET technique, but the issues are complex. Observed rates of glucose utilization by a tissue (as in a PET scan) will not only depend upon the percentage of metabolic rate that is accounted for by glucose utilization. It will also reflect the volume-adjusted total ATP turnover rate of the cell. This point is specifically mentioned by Lowe and Naunheim [8]. The volume-adjusted total ATP turnover is relatively high in the MCF-7 cell, as is the volume-adjusted glucose utilization compared with that of a variety of other cell types, including proliferating thymocytes (Table 5). But (i) these values in the MCF-7 cell are also higher than the respective values in the Jurkat cell, which is itself transformed, (ii) some cell types have an inherently low metabolic rate regardless of whether they are transformed or not and (iii) most of the values in Table 5 derive from short-term measurements of cell preparations, and we have found that the total ATP turnover in the MCF-7 cells was considerably lower (results not shown) when the cells were in suspension in a short-term (40 min) incubation.

The most feasible and testable hypothesis that can be gleaned from the available data is that cancer cells do take up glucose at a higher rate than normal cells, but that this is not associated with an increased glycolytic rate. Certainly, recent data show that FDG uptake is faster in colon cancer cells than in normal colon cells [13], and there are data showing that in mice, Glut-1 overexpression is associated with increased uptake of 2-deoxyglucose in muscle, and with increased whole-body glucose disposal [43]. But there is no evidence that increased glucose uptake in a particular tissue is associated with increased flux of that glucose through glycolysis. The evidence only shows that Glut-1 overexpression is associated with increased levels of glucose 6-phosphate and increased rates of glycogen synthesis [43]. What is required to clarify this confusion are measurements of glucose uptake and glycolytic flux (using the ^3H_2O method), on a wild-type and Glut-1-overexpressing tissue.

This study has generated data that will stimulate a reappraisal of our current perception of 'cancer cell metabolism', and perhaps a reappraisal of our current culture media. In addition it has raised questions about the identification of unknown fuels that contribute to cellular metabolism, and the mechanism involved in the efficacy of glucose-based PET in detecting cancers. These issues will be clarified when comprehensive energy budgets of cancer cells are available, at various oxygen tensions. Once available, these data may suggest new strategies for diagnosis and treatment.

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