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Thermochemical analysis of intermediary metabolism in resting 2C11-12 mouse macrophage hybridoma cells

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Abstract

The calorimetric–respirometric (CR) ratio is a thermochemical device to establish whether given living organisms and cells possess a balanced aerobic energy (enthalpy) budget. For those with a CR ratio more exothermic than −500 kJ per mol O_2, the only tenable explanation must lie in simultaneous aerobic and anaerobic catabolism. The subject of the current investigation, resting 2C11-12 mouse macrophage hybridoma cells, went through nearly four generations when cultured for 48 h and in the final 12 h increased in average protein biomass from 0.45 to 0.68 ng per cell and in mean diameter from 12.6 to 15.4 μm. Singular to this period only, cellular mass density decreased by 15% and luminol-enhanced chemiluminescence, which had peaked at 24 h (7.2 cpm per cell), declined by over 50% to 2.8 cpm per cell.

Heat flux was 68 μW per g protein at 12 h but reduced from 60 to 38 μW per g protein between 36 and 48 h. Oxygen flux was above 7 nmol min^{-1} per g protein but declined to 5.8 at 36 h and 2.8 nmol min^{-1} per g protein at 48 h. These data resulted in CR ratios becoming more negative with time, being −514 kJ per mol O_2 at 24 h and −843 kJ per mol O_2 at 48 h. Lactate flux increased from 9.0 nmol min^{-1} per g protein at 12 h to 13.3 nmol min^{-1} per g protein at 48 h. Succinate flux was the most constant parameter at 0.6 nmol min^{-1} per g protein but did show a peak at 36 h of 1.2 nmol min^{-1} per g protein.

The molar amount of lactate and succinate produced per unit amount of oxygen consumed (Lac/O_2 and Succ/O_2 ratios) is used to calculate the catabolic heat changes per mol O_2 and gives results very close to the observed CR ratios. This indicates that catabolism in resting 2C11-12 cells is by integrated aerobic and anaerobic processes which become more intensively anaerobic as the cells increase in mass late in the growth curve.

INTRODUCTION

In 1984, de Baetselier's group [1] reported the formation of several cell lines by fusion of mouse peritoneal macrophages with lymphosarcoma cells. All lines retained enhanced chemiluminescence and several different types were selected each for one or two particular properties of

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macrophages. They are anchorage-dependent but have the advantage that, at sub-culture, they can be removed from the plastic surface by mechanical agitation, rather than by using an enzyme such as trypsin.

Of course the primary reason for generating these "immortalized" cells was to obtain a model system for studying the immunological properties of completely functional cells in vitro. It is by no means certain, however, that basic metabolism is equivalent in vivo and in vitro but that should be of little concern providing the respiratory burst measured by chemiluminescence is a relative constant between treated and control cultures. Nothing is known of the optimal conditions for growing these cells in vitro beyond the fact that those presently advised appear to be satisfactory. Viewed from the standpoint of cellular physiology, however, it would be a desirable adjunct to our knowledge of the immunological responses that more is understood of their basic metabolism and, in particular, catabolic pathways. Obviously, the respiratory burst [2] as well as the other more usual processes in growing cells [3] require energy, ultimately from exogenous substrates and preferably under aerobic conditions. Limitation of catabolic substrates and/or oxygen can seriously affect ATP production and result in the accumulation and/or excretion of toxic catabolic products, such as lactate [4].

Conventional assays of catabolic pathways throughout the growth of a culture is a daunting prospect but there is a relatively simple method of indicating that cells are only utilizing aerobic processes and, if not, clues to possible reasons. This is the simultaneous measurement of heat flux by direct calorimetry and oxygen flux by indirect calorimetry [5, 6], as first advocated by Gnaiger [7]. Heat flow dQ/dt ($J\,s^{-1}$), and (scalar) heat flux J_Q ($J\,s^{-1}\,m^{-3}$), are related to rate of oxygen consumption and (scalar) catabolic oxygen flux ${}_kJ_{O_2}$ by appropriately derived theoretical oxycaloric equivalents, $\Delta_kH_{O_2}$ [9]. For a variety of substrates, oxycaloric equivalents of aerobic respiration range from −430 to −480 kJ per mol O_2 [5]. The experimental equivalent has been termed the calorimetric–respirometric (CR) ratio [5]

$$\text{CR ratio} = J_Q/{}_kJ_{O_2} \tag{1}$$

In solely aerobic cellular systems, the CR ratio should be within the range of oxycaloric equivalents, −450 kJ per mol O_2 (±5%). This energy (enthalpy) balance method also detects anaerobic catabolism under aerobic conditions in that CR ratios more negative than about −500 kJ per mol O_2 can only be due to simultaneous processes, aerobic and anaerobic [5]. It has previously been demonstrated for several cell types that lactate, the most common anaerobic product, can explain much of the discrepancy [9], even in conditions shown to be fully aerobic. There is preliminary evidence that this phenomenon occurs in mouse macrophage hybridoma cells and this paper reports CR ratios at discrete points in the

growth of serially passaged cells as well as assays for lactate and succinate.

It is important to relate these estimations to biomass. Different methods of assessment were chosen; protein content and cell size. As well as being intrinsically valuable and enabling data to be quoted as scalar flux, the estimations could indicate any changes in mass density ($\rho = m/v$) during succeeding generations of one serial passage. It follows that the relationship to thermochemical data is in terms of scalar rather than vectoral flux.

The type of mouse macrophage hybridoma cell chosen for this study was 2C11-12. It has been originally cloned to retain Fc receptor expression, Fc phagocytosis and 1L-1 secretion while retaining enhanced chemiluminescence on trigger and modulation (activation) of resting cells [10]. It was this latter property that was used to monitor the efficacy of cultures but, apart from this test, the present study was confined to resting cells.

MATERIALS AND METHODS

Cell culture

2C11-12 mouse macrophage hybridoma cells [1] were cultured in plastic Petri dishes (78 cm^2) using RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) including 10% (v/v) foetal calf serum (Imperial Laboratories, Andover, Hants, UK), glutamine (2 mmol l^{-1}), polymixin sulphate B (10 kU l^{-1}) and gentamycin (59 mg l^{-1}; Gibco, Uxbridge, UK). Dishes were placed in a CO_2/air incubator at 37°C. At sub-culture (48 h), cells were removed from the plastic by mechanical agitation and counted by Coulter particle counter (Model D; Luton, UK). Viability was determined by a fluorescent dye method [11].

For short-term experimentation, cells were centrifuged (250 *g* for 300 s), washed in Dulbecco PBS, pH 7.2, containing glucose (5.5 mmol l^{-1}) and glutamine (2 mmol l^{-1}), and resuspended in that solution. When used for serial passage, the cells were diluted to 1.25×10^5 cells cm^{-3} in fresh growth medium and cultured at 37°C in a humidified atmosphere including 5% (v/v) CO_2.

Biomass estimations

Cell size as a measure of biomass was estimated using a laser-light Skatron Argus flow cytometer (Skatron Ltd., Newmarket, Suffolk, UK) with a low angle (<15°) light scattering detector [12]. When particle sizes are significantly greater (>25×) than the wavelength of the light, then scattering obeys Lorenz–Mie principles [13]. The extent of light scattered in a forward direction is strongly dependent on cell size [14] but a

calibration curve is required; this was constructed using monodisperse latex particles (Dyno Particles, Lillestrøm, Norway) in an appropriate range. In order to reduce small-diameter debris, PBS was filtered at 0.22 μm and an additional filter (Millipore) was placed in the sheath-fluid line.

Protein in cells washed free of serum was assayed by the Lowry colorimetric method (Sigma test kit; ref. 15) after precipitation with 61 mmol per litre of perchloric acid and subsequent solubilization in 40 mmol per litre of NaOH.

Direct calorimetry

An LKB Model 10700 heat conduction twin ampoule microcalorimeter was used to measure heat flow by the cells. The ampoules (5 cm^3) were made of stainless steel. One contained 2 cm^3 cell suspension (2×10^6 cells cm^{-3}) in PBS with 5.5 mmol per litre of glucose and 2 mmol per litre of glutamine while the other acted as a blank with 2 cm^3 medium only. Equilibration to 37°C in the instrument usually took 1200 s and registration of plateaux, 900 s. Amplification was set at 10 μV on a Keithley 150 B microvolt ammeter. In addition to external electrical calibration, internal calibration was performed with a chemical reaction [16].

Oxygen measurements

Oxygen uptake by cells was determined at the same time as that of heat with a Clark-type electrode in a YS1 oxygen monitor [17]. A 4 cm^3 aliquot of cell suspension was equilibrated to 37°C for 1200 s and oxygen consumption measured for 900 s.

Miscellaneous assays

Lactate and succinate respectively were measured in cells and supernatants after oxygen measurements by a modified Barker technique (Sigma, ref. 18) and an enzymic procedure [19]. Results for 24, 36 and 48 h were obtained by subtraction of the previous total.

Chemiluminescence

This was measured at the same time as heat and oxygen using a Packard 6500 48-channel Picolite luminometer. Cells (2×10^4 cm^{-3}) were suspended in veronal-buffered solution containing 200 μmol per litre of Ca^{2+}, 500 μmol per litre of Mg^{2+}, 0.2 mg per cm^3 of BSA, 20 U per cm^3 of recombinant interferon (INF-γ-R) and 5.5 mmol per litre of glucose. Chemiluminescent emission [10] in 1 cm^3 aliquots was recorded for 30 min

after addition of 100 μmol per litre of luminol (5'-amino-2,3-dihydro-1,4-phthalazinedione; Boehringer, Mannheim, Germany) and the triggering agent, phorbol-12-myristate-13-acetate (PMA; 1.6 mmol l^{-1}).

Chemicals

Except where otherwise stated, these were obtained at the highest grade from Sigma.

RESULTS

The growth curve for resting 2C11-12 mouse macrophage hybridoma cells is shown in Fig. 1. Cell density decreased during the first 6 h in culture and only attained the original inoculum level at 12 h. Thereafter,

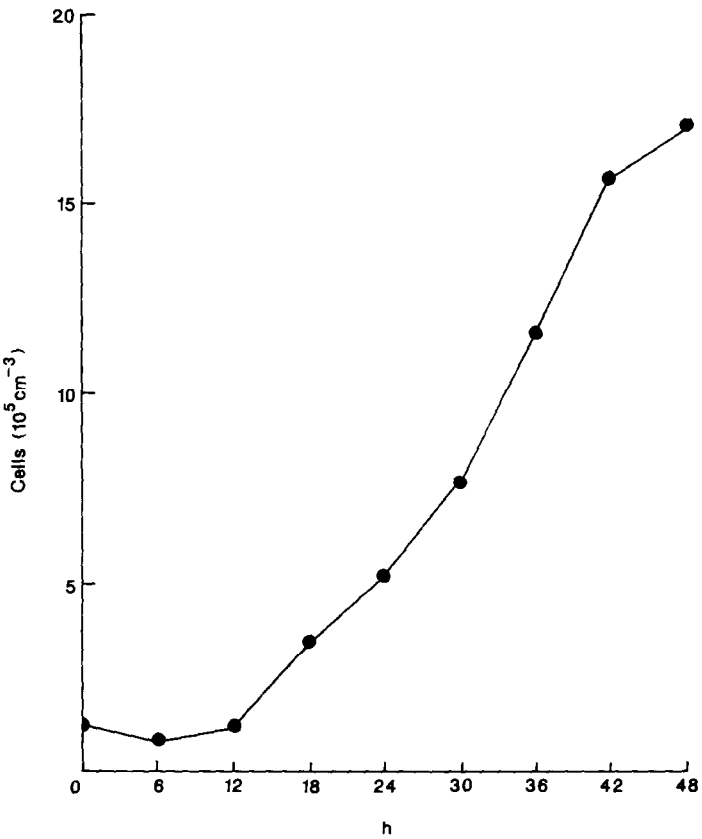

Fig. 1. Typical growth curve of resting 2C11-12 mouse macrophage hybridoma cells. They were grown for 48 h at 37°C in RPMI 1640 medium with 10% (v/v) calf serum. The initial inoculum was 1.25×10^5 cells cm^{-3}. Counts by Coulter particle counter were made every 6 h. Note the initial decline in viable cells and the decrease in growth from the exponential phase after 42 h.

TABLE 1

Cell numbers, biomass and enhanced chemiluminescence of resting 2C11-12 mouse macrophage hybridoma cells grown in culture for 48 h[a]

Time (h)	Cells ($\times 10^5$ cm^{-3})	Protein (ng cell^{-1} ($\pm$ SD))	Diameter (μm ($\pm$ SD))	Mass density (ng μm^{-3})	Chemiluminescence (cpm per cell ($\pm$SD))
12	1.2	0.47 ± 0.07	12.9 ± 0.5	418	6.2 ± 0.4
24	5.1	0.51 ± 0.06	13.4 ± 0.4	405	7.2 ± 0.5
36	11.6	0.45 ± 0.05	12.6 ± 0.7	430	6.4 ± 0.4
48	17.0	0.68 ± 0.09	15.4 ± 1.6	356	2.8 ± 0.3

[a] Data are from a minimum of five experiments, with standard deviations (SD); cells at 48 h were subcultured to act as zero time for the subsequent culture.

the cells were in exponential phase, passing through approximately 4 generations until about 40 h, at which point there was little further increase in numbers.

Four time intervals were chosen for intensive investigation of biomass and the results are depicted in Table 1. The average cell diameter as determined by Lorenz–Mie scattering was statistically similar for at least the first 36 h in culture but then significantly increased (t-test: $P < 0.001$) by more than 20% in the next 12 h. The volume fraction of course increased to a much greater extent ($\approx$80%).

The second measure of biomass, cellular protein, also remained similar from 12 to 36 h and then increased to a highly significant degree (t-test: $P < 0.001$) at 48 h (50%: see Table 1). The different percentage changes are reflected in an altered protein mass/volume ratio ρ, and amounted to an overall reduction of 15% in 48 h, mostly in the final time period.

The biological activity (respiratory burst) of the cells at the different times was measured from the luminescent degeneration of reactive oxygen metabolites enhanced by luminol. As is seen in Table 1, resting 2C11-12 cells which had been triggered by PMA and modulated by INF-γ-R at all four stages of the growth curve were active but not in a consistent manner. Peak light emission was highest at 24 h and lowest at 48 h. Data at each time were significantly different (t-test: $P < 0.01$).

Heat and oxygen fluxes were measured using aliquots of the same cell cultures employed to study growth characteristics. Both metabolic parameters decreased (Table 2) but the downward trend in oxygen flux began after 24 h and accelerated, whereas reduced heat flux was only evident at 48 h. The resulting CR ratios became more negative as the culture grew more confluent, indicating an increased intensity of anaerobic metabolism.

Analysis of lactate combined from cells and supernatants showed (Table 2) that, even at an early stage in culture growth, lactate production occurred in appreciable amounts. With time, the flux increased and was 50% greater in the final 12 h period than in the first one. The other

TABLE 2

Heat flow, fluxes of heat, oxygen, lactate and succinate, and CR ratio of resting 2C11-12 mouse macrophage hybridoma cells cultured for 48 h [a]

Time (h)	Heat flow (pJ s^{-1} per cell (±SD))	Oxygen flux (nmol min^{-1} per g protein (±SD))	Heat flux (μW per g protein)	CR ratio (kJ per mol O_2)	Lactate flux (nmol min^{-1} per g protein (±SD))	Succinate flux (nmol min^{-1} per g protein (±SD))
12	32 ± 2	7.9 ± 0.3	68	−514	9.0 ± 0.6	0.6 ± 0.04
24	33 ± 2	7.3 ± 0.4	64	−534	8.1 ± 0.5	0.6 ± 0.04
36	32 ± 2	5.8 ± 0.3	60	−640	11.1 ± 0.6	1.2 ± 0.03
48	26 ± 2	2.8 ± 0.4	38	−843	13.3 ± 1.2	0.6 ± 0.04

[a] Data are the average from at least five experiments, with standard deviations (SD); Cells were subcultured at 48 h and acted as zero time for the next culture; CR ratios were calculated from figures with two decimal places.

anaerobic endproduct measured, succinate, was found in cells and supernatants throughout the culture period (Table 2), its flux peaking in the period between 24 and 36 h.

It is worth noting that there was some evidence of a decrease in specific heat flux with increasing protein mass (see Table 2).

DISCUSSION

The growth curve for 2C11-12 cells (Fig. 1) illustrates three salient points. First, there was no net increase in cell numbers at 12 h. Indeed, there was a decline at 6 h. It is likely that this was at least partly due to the mechanical trauma of subculture [4]. The supposition could be particularly applicable to these cells which retain the parent property of high sensitivity to foreign agents [1, 10]. Clearly, there was a major change because the average protein biomass and diameter of the cells had decreased from those at 48 h, the time of subculture (Table 1). It seems likely that a fraction of the total cell population at zero time, which is equivalent to 48 h in culture, was of large cells and this has skewed the average biomass and cell diameter. It is supposed that these cells died and lysed in the first few hours, a suggestion which is now under investigation.

After the 12 h stage, the cell cultures clearly demonstrated an exponential phase of growth which had probably started before that time and perhaps lasted 30 h. The second salient point is that the period was short compared with that of many cell lines [3, 4] because the cell cycle was relatively rapid. This was probably due to the origins of the hybrid in a lymphosarcoma cell [1].

The onset of contact inhibition of growth normally occurs in G_1 of the cell cycle, the cells then being designated as G_0 type [3, 20]. This clearly occurred sometime between 42 and 48 h. Arrest at this phase clearly implies that cells are relatively small, having just undergone mitosis. The third feature of the growth curve, assumption of G_0, is associated with the atypical finding that at least a proportion of the cells were very large, had a reduced capacity for "respiratory burst" (Table 1) and, as has been seen in Table 2, were intensively anaerobic.

Biomass has a simple definition, of course, but deciding which parameter to use with respect to the definition can pose problems. A non-destructive technique is to be preferred and cell size by particle counter, dielectric methods [21] or flow cytometry [12] are well founded but rely on the assumption that the mass/volume ratio ρ is constant. While true of most animal cell types and bacteria investigated, it was certainly not the case for yeast [22] and these 2C11-12 cells. ρ was only the same with experimental limits for the first 36 h in culture but then decreased by 17% up to 48 h (Table 1). This was only discovered by measuring a macromolecular parameter, protein, and it may be an

important cautionary tale in these days of biotechnological exploitation of animal cells [23].

Heat flux of the cells was similar to that of other established cell lines (see reviews 9, 21). In the past, figures have usually been given on a "per cell" basis but there is a clear need to normalize to biomass. This is most expressed as simply volume, given constant mass density. Only in this way can energy flux differences between cell types be truly reflected by data [24]. For instance, at present it is not known whether the heat flow for 3T3 mouse fibroblasts (19 pJ s^{-1} per cell; see ref. 25) is lower than that of 2C11-12 cells (32 pJ s^{-1} per cell) because of less biomass or smaller overall metabolic flux.

As can be seen in Table 2, heat flux was constant within experimental error for 36 h in culture and declined by 20% in the final 12 h. The decrease in oxygen flux, however, began some time after 24 h and, by 48 h, was only half that of readings during the early stages of growth. This was reflected in more negative CR ratios at 36 and 48 h, and was traced to increased lactate production (Table 2). The more intensive share for anaerobic catabolism (glycolysis) is often explicable in terms of either poor oxygenation of the medium or the so called "crowding effect" [9, 26]. The latter is generally attributed to one of two causes. Firstly, cells originally in suspension, sediment on top of one another so that available dissolved oxygen is acutely limited by restricted interstices between cells. This was reported for calorimetric studies of murine macrophages [27], lymphocyte hybridoma cells [28] and Vero cells [29]. Secondly, in the case of transformed cells which have lost contact inhibition, the cells grow over one another, with the same effect of limited available oxygen in restricted intercellular spaces. Care should be taken, however, not necessarily to label this as anomalous because it is well documented that tumour cells have a high glycolytic capacity with a mechanism able to integrate respiration and glycolysis [30].

Turning to 2C11-12 cells, it is established that, in general, cell cultures in Petri dishes do not suffer oxygen deprivation in 96 h [4]. The dead space of air above the medium is more than sufficient for recruitment of oxygen to the dissolved state. Bearing in mind the fact that the solubility of oxygen in pure water at the standard pressure of 101.325 kPa and 37°C is 1.102×10^{-7} mol cm^{-3} [31], then a simple calculation using an air : medium ratio of 5 : 1, gives a thousand-fold oxygen surplus for 17×10^5 cells cm^{-3} in 48 h. No allowance has been made for the salting-out effect, which is shorthand for the fact that the concentration of oxygen in physiological saline is less than in pure water; but this effect is markedly less than one order of magnitude.

Since one of the parent cells of the hybrid was a lymphosarcoma cell, it was possible that the cells grew in overlay. Visual examination gave no hint of this phenomenon.

This leaves the possibility of oxygen deprivation during the short-term measurements of heat and oxygen fluxes. Recorder traces from the Clarke electrode showed no tendency to tail-off, which is the usual sign of limited oxygen. It is possible that sedimentation by gravity might have influenced the measurement of heat but it had little effect on the CR ratio at 12 h, which was only 45 kJ per mol O_2 less negative (Table 2) than the theoretical oxycaloric equivalent for glucose ($-469\ kJ\ mol^{-1}$). It was a constant factor for all determinations, so the decrease in heat flux at 48 h was real. A lactate estimation for the contents of a calorimeter ampoule at 12 h gave a reading of 4.7×10^{-15} mol min^{-1} per cell, within the experimental error for lactate in cell cultures (Table 2).

The range of theoretical oxycaloric equivalents (-450 kJ per mol O_2 ($\pm 5\%$)) is the enthalpy change only of catabolism and excludes coupled processes such as ATP production, anabolism or mechanical work [5]. It is totally dissipative and has zero net efficiency [32]. Brown adipocytes, which float on culture medium, gave a CR ratio of -490 kJ per mol O_2 [33]. Within experimental limits, this indicates a balanced aerobic energy (enthalpy) budget. Resting 2C11-12 cells are characterized by CR ratios more exothermic than $-500\ kJ\ mol^{-1}$ and this cannot be interpreted in terms of decreased efficiency of ATP production (see ref. 30) but rather the involvement of glycolytic reactions under aerobic conditions, with accumulation or excretion of anaerobic endproducts. This has frequently been reported for tumour cells (see ref. 30) but there is increasing evidence for simultaneous aerobic and anaerobic catabolism generally in mammalian cells in vitro (see ref. 5). In particular, analysis of heat and oxygen measurements for LS-L929 fibroblasts [17], hepatocytes [34], sperm [35] and both resting and activated neutrophils [36] showed them to have highly negative CR ratios [9, 24].

The most common anaerobic endproduct in mammalian cells is lactate. Net production of it from glucose is accompanied by a dissipative catabolic enthalpy change $\Delta_k H_{Lac}$ of -63 kJ per mol lactate if it is excreted into a bicarbonate buffer [8]. The molar amount of lactate produced per unit amount of oxygen consumed (Lac/O_2 ratio) indicates the relative extent of aerobic glycolysis. The catabolic heat change per mol O_2 $\Delta_r H_{(ox+anox)}$ for simultaneous aerobic (ox) and anaerobic (anox) catabolism is then calculated as modified from ref. 5

$$\Delta_r H_{(ox+anox)} = \Delta_k H_{O_2} + Lac/O_2 \times \Delta_k H_{Lac} \quad (2)$$

where r is the metabolic reaction for any stoichiometry defined by the Lac/O_2 ratio (note the + sign).

Taking the oxygen and lactate measurements in Table 2 and, through the Lac/O_2 ratios, applying eqn. (2) gives calculated catabolic heat changes per mol O_2 (Table 3). These are slightly more negative than the CR ratio at 12 h, the same at 24 h and an inadequate total explanation for

TABLE 3

Observed CR, lactate/O_2 and succinate/O_2 ratios for cultured resting 2C11-12 mouse macrophage hybridoma cells at 4 growth time points; the catabolic heat change per mol O_2 ($\Delta_r H_{(ox+anox)}$) for simultaneous aerobic and anaerobic catbolism is calculated for lactate (Lac) and succinate (Succ)

Time (h)	CR ratio (kJ per mol O_2)	Lac/O_2 ratio	$\Delta_r H_{(ox+Lac)}$ (kJ per mol O_2)	Succ/O_2 ratio	$\Delta_r H_{(ox+Lac+Succ)}$ (kJ per mol O_2)
12	−514	1.14	−540	0.08	−552
24	−534	1.11	−538	0.08	−550
36	−640	1.92	−590	0.20	−620
48	−843	4.74	−769	0.22	−803

CR ratios at 36 h and 48 h. It is likely that other glycolytic endproducts may have been formed, such as pyruvate, acetate or succinate [8].

A generalized form of eqn. (2) can be written for the calculation of the heat change for all glycolytic endproducts per mol O_2

$$\Delta_r H_{(ox+anox)} = \Delta_k H_{O_2} + \sum_i i/O_2 \times \Delta_k H_i \quad (3)$$

where i is each individual endproduct [6]. The succinate estimations shown in Table 2 were inserted into eqn. (3) and a dissipative catabolic enthalpy change for succinate $\Delta_k H_{Succ}$ of $-152\,kJ\,mol^{-1}$ for succinate excreted into a bicarbonate buffer [8] was assumed. As will be seen in Table 3, the calculated catabolic heat changes for the cells at 36 h and 48 h are now very close to the observed CR ratios but there still could be a failure to identify all the glycolytic endproducts at 48 h. Other endproducts, such as pyruvate, require measurement.

Choice of the appropriate figures for catabolic enthalpy changes is a source of error in calculations based on eqns (2) and (3). For instance, $\Delta_k H_{Lac}$ is −80 rather than −63 kJ per mol Lactate, when the acid is buffered within the cell. Interpretation of measurements of glycolytic endproducts formed by cells grown in complex media containing serum is also difficult. Nevertheless, combined heat and oxygen measurements give insight to the catabolic processes of 2C11-12 cells in which there is clearly an involvement of glycolytic reactions under aerobic conditions. It is also intriguing that the glycolytic reactions became more intense with time in culture.

It is not known why 2C11-12 mouse macrophage hybridoma cells or, for that matter, other cell lines [9, 24] adopt the strategy of simultaneous aerobic and anaerobic catabolism. It is worth especially noting another mouse hybridoma cell, that of the lymphocyte (6×10^4 cells cm^{-3}), had a CR ratio of −570 kJ per mol O_2. The phenomenon is found also in normal, transformed and tumour-derived cell lines [9], as well as freshly

dissociated cells, such as rat hepatocytes [34]. It can only be surmised that the effect is due to loss of "whole-animal" homeostatic control.

The changes in the final 12 h were foreshadowed in the previous 12 h by the decrease in oxygen flux and a similar rise in percentage lactate production. Although glucose uptake in these cells had a high flux control coefficient at 0.73 [6] and, therefore, cell surface area could be a limiting factor, there was no increase in cell diameter until after 36 h. The inhibition in oxygen flux and, therefore, respiration with accumulation of glycolytic endproducts, could be due to an increase in activity of the lactate dehydrogenase isozyme system, but this has yet to be demonstrated for these cells. Alternatively, an interference with entry to the mitochondrial citric acid cycle is a possibility. This could be even at the initial step of the oxidative decarboxylation of pyruvate to acetyl CoA by the pyruvate dehydrogenase complex within the mitochondrial matrix. Under normal circumstances, acetyl CoA is transferred to the cytosol by a series of reactions, the concomitant of which is the conversion of $NADP^+$ to NADPH. This is the primary electron donor for the reduction of oxygen during the respiratory burst [2]. A further source is the pentose phosphate pathway. NADPH oxidase catalyzes formation of the microbicidal superoxide anion which can be dismutated to hydrogen peroxide by superoxide dismutase. The degeneration of these labile oxygen components results in chemiluminescence which can be amplified by luminol. The decreased light emission by 2C11-12 cells at 48 h (Table 1) may be due, therefore, to limited NADPH resulting from reduced activity of pyruvate dehydrogenase possibly because of mitochondrial damage.

Less chemiluminescence was associated with decreased mass-specific heat flux. Similarly, specific oxygen flux decreases with increasing size in whole animals [37] and it has been shown [9] that the same is true of the calorimetric data for cultured Vero cells [29]. It is not suggested that decreased mass-specific heat flux in resting cells is responsible necessarily for a reduced respiratory burst on activation but that there should be an investigation of the coincidence.

In conclusion, the results from this investigation into the thermochemical characteristics of resting 2C11-12 mouse macrophage hybridoma cells growing under aerobic conditions have shown CR ratios more negative than possible from applying theoretical oxycaloric equivalents. This is principally due to intensive production of lactate as a glycolytic endproduct simultaneously with aerobic catabolism. The full rationale for this occurrence requires further experimentation but the current study illustrates that calorespirometry is a senstive tool for elucidating the thermochemical mechanisms of metabolic flux.

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