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Calorimetric studies of heat flux in animal cells

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Abstract

This overview concerning calorimetric studies of animal cells in vitro firstly highlights some of the thermodynamic principles underlying measurement of heat flux. The resurgence of such measurements is based on refined instrumentation and a growing awareness of the undoubted contribution heat flux measurements can make to the accumulating knowledge of cellular physiology. Direct calorimetry has been employed to investigate medical, pharmacological and toxicological problems, and can be a valuable diagnostic tool. It is advocated, however, that it exercises a greater scientific contribution when it is harnessed to indirect calorimetry, especially measurement of oxygen flux. The calorimetric–respirometric (CR) ratio is explained in terms of its value in thermochemical analysis of cellular physiology based on rigorous thermodynamics.

SOME THERMODYNAMIC PRINCIPLES

In 1966, Lenard and Singer [1] challenged the conventional Davson–Danielli–Robertson model for membrane structure using mainly a thermodynamic basis. Singer [2] later substantiated his alternative proposals by analysing the unfavourable dynamics of transferring amino acid ion-pairs from water to the lipid bilayer. To force this transformation would require a Gibbs energy of $+210$ kJ mol^{-1}. From this thermodynamic reasoning came the essential elements of the now familiar hypothesis for membrane structure [3].

The reasoning behind the Singer model forms an elegant illustration of applying thermodynamics in terms of Gibbs energy G, solely related to work W, and these parameters must not be confused with heat energy Q measured by calorimetry. In order to distinguish between work and heat it is helpful to regard the former as an 'exergy' [4]. Gnaiger [5] stressed that the heat measured by a calorimeter is the sum of dissipated energy, $\mathrm{d}D$, and bound energy $\mathrm{d}B$

$$\mathrm{d}Q = \mathrm{d}D + \mathrm{d}B \tag{1}$$

the former being related to exergy (Gibbs energy) by

$$\mathrm{d}G = \mathrm{d}W + \mathrm{d}D \tag{2}$$

and the latter related to exergy and enthalpy (H) energy by

$$dH = dG + dB \tag{3}$$

Only if the chemical process is completely reversible ($dD = 0$) will change in bound energy in a closed isothermal reaction system equate to the change in entropy (S), $dB = T\ dS$; and be equal to heat exchange [5]. Clearly this is not the case for cells which transform chemical energy irriversibly from substrates into products exchanged with the environment, including heat which is dissipated from the system.

The thermal change measured in an isothermal calorimeter is heat flow, $J_Q = dQ/dt$, and not power $P = dW/dt$ (see refs. 5 and 6), which is the product of flux and conjugated force $W = F/dt$ and $F = dp/dt$, where p is momentum [7]. This is clearly in the realms of nonequilibrium thermodynamics, which is most apposite to the chemical reactions of cells resulting in thermal changes—ergodynamics as one would have it [8]—but not those changes per se. Gibbs energy changes of chemical reactions and transformation to work can be addressed through measurements of thermal energy (see ref. 5) but calorimetry does not directly measure them. For the ensuing thermal discussion, therefore, the results of some other researchers have been shamelessly converted to the S.I. unit joule J, from watt (W, the appropriate S.I. unit for power), or from the "Wild West" version known as cal (1 cal = 4.184 J).

RENASCENT HEAT

The resurgence in calorimetric measurements of heat flux from living cellular systems can be traced back to the Wadsö-designed heat conduction microcalorimeters (now called Wad I) of the 1960s [9]. Some of the early data for isolated animal cells was unsatisfactory [10] largely because of difficulties in matching cellular metabolic and environmental requirements to the physical constraints of the measuring vessel (for a review, see ref. 11). Notable success was achieved, however, by Monti's group measuring thermal changes of whole blood and its individual cellular components [12,13]. Even greater instrumental accuracy and higher time resolution were reached in Wadsö's second generation machine (Wad II; see ref. 14) and results from using the two instruments and other lesser known models were analysed in 1987 by Kemp [15].

It is not an intention to dwell on the potential problems of cell culture (consult, for instance, refs. 16 and 17) but, of course, the unwary must take care to maintain optimum conditions in the calorimetric measuring vessel. As a recent illustration of the need for care, Bäckman [18] showed that heat flux of T-lymphoma cells was acutely dependent on pH, temperature and stirring rate. Relatively long-term unstirred cultures of cells can display decreased specific heat flux with time—the so-called "crowding effect"

[19,20]. The most probable reason is limited oxygen diffusion into cells which have sedimented to the bottom of the measuring vessel. The solubility in air-saturated pure water at the standard pressure of 101.325 kPa and 37°C is 2.102×10^{-7} mol cm^{-3} O_2 [21]. It is wise to remember, though, that the solubility of oxygen in physiological media is less than in pure water owing to the salting-out effect. Even so, this should present no difficulty to cells at relatively low density in a stirred medium [18] or for non-transformed cells in a contact-inhibited monolayer [22,23]. The same is also true for short-term (< 1800 s) registration of heat flux of cells using an ampoule-drop calorimeter [6]. But the local environment of cells which are either transformed (no contact inhibition) and, therefore, grow over one another [16], or have sedimented, may prevent access to dissolved oxygen. Cells then tune to more intensive anaerobic glycolysis [24], resulting in increased acid excretion ("ichor") into the limited interstices between cells in "heaps". Besides the energetic implications discussed later, accumulation of glycolytic acids proximal to the cell surface is toxic, and results in impaired viability and even death. Perhaps the best example of this effect can be derived, in a lateral way, from the "dreaded" infection of cell cultures by mycoplasma [15]. These are glycolytic "engines" (no mitochondria) which bind to the cell surface and are thought to decrease viability through local accumulation of ichor, causing a pH reduction. This is the cause of cell death.

Hansen's group [25] has made an ingenious suggestion for combating the Uriah (Heap) problem by floating cells obeying gravitational forces (sedimenting) to the surface of the culture medium using Percoll (Pharmacia). Perhaps the idea stemmed from heat measurements on brown adipocytes, which naturally float and exhibit totally aerobic catabolism [26], as judged from their calorimetric–respirometric (CR) ratio of -490 kJ mol^{-1} [24,27]. Fontana et al. [25] quote the experience of others [28] with white adipocytes ("bad", see ref. 15) but no CR ratios are available for these cells. At any rate, the technique was successful with baby hamster kidney (BHK) cells [25]. Heat measurements by flow calorimetry [29] do not suffer from the same problem, but one should be aware of possible gaseous diffusion from flow lines, especially if they are plastic [30].

HEAT MEASUREMENTS SECOND TO NONE

The two state functions in eqn. (3) originate from the fundamental relationship in the first law of thermodynamics

$$dH = dW + dQ \tag{4}$$

which implies a connection between enthalpy (H) and cellular metabolic reactions. Thus, heat flux measurements give an entry to understanding energy transformations in cells—bioenergetics. This avenue has been rarely

TABLE 1

Heat flux ${}_tJ_Q$ in animal cells with ranges or standard deviations for some cell types

Cell type	${}_tJ_Q$ (pJ s^{-1} per cell)	Authors	Ref. no.
kB	25	Cerretti et al.	69
HeLa-53G	31.2	McGuinness et al.	59
Human foreskin fibroblast	40 ± 10	Schaarschmidt and Reichert	22
Human keratinocytes SV-K14 (transformed)	83 ± 12	Reichert and Schaarschmidt	23
Keratinocytes	134 ± 35	Reichert and Schaarschmidt	23
Human T-lymphoma	8 ± 1	Schön and Wadsö	40
Human melanoma, H1477	80	Görman Nordmark et al.	39
LS-L929 fibroblasts	34 ± 3	Hoffner et al.	29
Human white adipocytes	49 ± 15	Monti et al.	46
Rat white adipocytes	40	Nilsson-Ehle and Nordin	32
Horse lymphocytes	8	Krakauer and Krakauer	33
Human lymphocytes	5	Bandmann et al.	31
Mouse lymphocyte hybridoma	30—50	Nässberger et al.	67
Hamster brown adipocytes	110	Nedergaard et al.	26
Human neutrophils	2.5 ± 0.3	Eftimiadi and Rialdi	48
Vero	27 ± 2	Schön and Wadsö	38
Bovine sperm	1.3 ± 0.1	Inskeep and Hammerstedt	36
Mouse macrophage hybridoma 2C11-12	32 ± 2	Kemp	6
Rat hepatocytes	329 ± 13	Nässberger et al.	20
3T3 mouse fibroblasts	17	Lönnbro and Schön	71

travelled by physiologists, the few of whom that are acquainted with calorimetry preferring the route of the instrument as a means of recording phenomenological changes. This worthy endeavour has identified, for instance, differences in blood cells [31] and white adipocytes [32] of humans in various clinical conditions; and the interaction of cells with primary messengers, such as noradrenaline [32] and antibodies [33,34]. And so much more, anon!

First, it is necessary to explain the differences in specific heat flux for cells of various origins (Table 1). The answer is complex because they are diversely remote from their stem [35]. Thus, some cell types are fully differentiated, for instance adipocytes [32], platelets [31] and sperm [36]; a few are freshly dissociated from tissues and organs of adults and embryos (e.g. hepatocytes; refs. 20 and 37); and the majority are established ("normal") or transformed cell lines. Established cell lines include Vero cells [38] and LS-L929 fibroblasts [29]; transformed cell lines include cells from such as melanoma [39] and lymphoma [40]. Many fully differentiated cells do not grow [35] whereas primary cultures and established cell lines go through several complete cycles from seeding to contact inhibition at

confluency (G_0; see refs. 17 and 41). Anchorage-dependent transformed cells at least partly escape contact inhibition of growth (G_0) and so are most susceptible to the Uriah effect, as are cells in tumours. It is notoriously difficult to synchronize the growth of animal cells in vitro, so heat flux and other measurements taken during the growth curve yield data averaged for cells at different phases of the cycle. This is true of much of the data in Table 1.

As an anchorage-dependent culture of an established cell line grows towards confluency (late exponential stage), the average size of the cells may increase because of the block to cell division (M phase; see ref. 17). The cells translate to the stationary phase G_0. So, apart from intrinsic differences in size between cell types [16], there is altered heterogeneity in this parameter through the growth curve of cultured cells.

Few calorimetrists have quoted heat flux ${}_tJ_Q$ in terms of (mean) cell size, yet it is obvious that one of the principal determinants of the quantity for this parameter must be exactly that—the "acorn and oak" principle [27]. Sizing (spherical) particles by Coulter counter [16], light scatter [6] or RF β-dispersion [42] holds few fears these days, and it is recommended that heat flux be quoted per unit volume as well as per cell. This will reveal another, more fundamental, determinant of scalar heat flux: what might loosely be termed the level of specific "metabolic activity"—dynamics. Leaving aside endogenous catabolic cybernetics, the level of exogenous supply of substrates is central to activity. Apart from gaseous diffusion, most primary catabolites gain access to the cell by facilitated diffusion or/and active transport, via cell surface receptors. The number of binding sites is expressed per unit area of plasma membrane, but little is known of possible changes during the cell cycle.

In studying the energetics of cellular systems, it is often stated that there is a directly linear relationship between size and biomass [43]: however, that assumes constant mass density $\rho = m/V$. There is evidence from data on 2C11-12 mouse macrophage hybridoma cells [6] that protein mass density decreases during the growth curve even though there are individual increases in both parameters. It is true to say, however, that such a decrease is not the "party line" for prokaryotes [43] and has not been systematically investigated for higher eukaryotes—animal cells [17].

What of metabolism, size and mass? Kleiber [44] showed that mass-specific oxygen flux decreases with increasing size in whole animals. Schön and Wadsö [38] gave data on the heat flux and protein mass of Vero cells at different densities. Analysing their results in Kleiber terms, on the assumption of constant mass density, revealed a marked influence of cell size on metabolic flux [27]. Results for 2C11-12 mouse macrophage hybridoma cells at two time points in their growth curve [6] confirmed this finding in terms of the specific relationship of both oxygen flux and heat flux to protein mass and cell size. A suggested explanation is that metabolic flux is

limited by cell surface area—the realms of flux-control theory! [45]. If the number of substrate-binding sites per unit area in the plasma membrane alters during the cell cycle, then the cell will have to resort to an alternative strategy of mobilizing endogenous reserves [6].

HEAT FLUX AND DIVERSE AGENTS

Apart from the early exploratory work (see ref. 11), much of the data collected in Table 1 was part of an investigation of the value of heat flow numbers in blood disorders [13] or other clinical conditions, for example the infamously "bad" white adipocytes (refs. 32, 46 and a review, ref. 14) and truly bad tumour cells, for instance from patients with non-Hodgkin lymphoma [47]. Another strand of research has been to follow the classical route for specialists in metabolic biochemistry and expose cells to inhibitors. At appropriate concentrations, heat flux of LS-L929 cells was inhibited by potassium cyanide and 2,4-dinitrophenol [29]. Human neutrophils activated by phorbol-12-myristate-13-acetate (PMA) showed reduced heat flux when incubated in the presence of *N*-ethylmaleimide, sodium fluoride [48] and sodium azide [49]. NaF and cyanide were employed by Loike et al. [19] in their investigation of glucose oxidation using heat flux changes [14]. These powerful tools have been supplemented by the use of cytochalasin B on both these cells and horse lymphocytes [33]. This form of the agent inhibits glucose transport through the plasma membrane but also disrupts the cytoskeleton. Its metabolic effect should be compared with that of the D form, which causes solely cytoskeletal disorder [19] through its action on microfilaments.

The search for investigative methods in pharmacology and toxicology has led to researchers advocating calorimetric measurements on the basis that heat is related to enthalpy [see eqn. (4)]. The path was signposted by numerous studies of bacteria (see review, ref. 50) and the effects on whole aquatic animals of the antibiotics streptomycin and neomycin [51]. The aminoglycoside antibiotic gentamycin caused an increased heat flux from human blood cells only at concentrations above therapeutic levels [52]. T-lymphoma cells exposed to this antibiotic in the recommended dose range exhibited an unchanged heat flux [18]. Cycloheximide, an antibiotic which inhibits protein synthesis, reduced the heat flux of human lymphocytes at a concentration of 1 mmol dm^{-3} [33]. The same antibiotic at a lower concentration (0.07 mmol dm^{-3}) had no effect on murine macrophages [19], but obviously concentration and time of exposure are both important factors, the second being particularly important for anabolic inhibitors which only act indirectly on the catabolic half-cycle, as defined by Gnaiger [8].

There have been studies of the effect on animal cells of mitogenic lectins. The heat flux of horse lymphocytes was increased by exposure to a

low concentration of concanavalin (Con) A (1 μg cm^{-3}) but decreased by this lectin at an order of magnitude higher concentration [33]. In the same phenomenon as for PMA on neutrophils [48], low concentrations of Con A activated the lymphocytes. Borrebaeck and Schön [53] studied the anti-proliferative action of phytohaemagglutinin (PHA) isolectins on human lymphocytic leukaemia cell lines. Among other findings, they showed that the isolectin PHA-L_4 (0.1 μg cm^{-3}) strongly and quickly (< 30 min) inhibited heat flux, but that a 200-fold increase in concentration of the E_4 isolectin was required to obtain the same effect. PHA was not cytotoxic but it did alter metabolic pathways at the same time as inhibiting cellular DNA synthesis and growth.

An important class of drugs is the β-adrenoceptor blockers. Monti's group has investigated the effect of several of them on thermogenesis by human muscle tissue biopsies [54]. Using a perfusion microcalorimeter, it was found that, for instance, propranolol reduced heat production by 25%. In contrast to this example of a non-selective β-blocker a β_1-selective drug atenolol had no effect on heat flux at its therapeutic dose. This was also the case for the partial β_2-agonist and β-adrenoceptor blocker pindolol. Thus, these workers concluded that blockade of sympathetic β_2-receptors was the cause of reduced thermogenesis. Because body temperature falls when one is asleep [55], the "take-home" message is do not take β_2-blockers and sleep outdoors in Lapland!

The physiological and, of course, pharmacological actions of all drugs are of major interest, but none more so than for antineoplastic formulations. The drug should be selectively toxic to tumour cells rather than normal cells, instead of vice versa. For obvious reasons, measuring heat flux on its own could be a rapid and sensitive means of indicating cytotoxicity; and even more so if associated with determination of oxygen consumption (see refs. 6, 8, 14, 24 and 27). Schön and Wadsö [56] paved the way by demonstrating that the dose–response effect of methotrexate on the heat flux of T-lymphoma cells was correlated with more conventional cytotoxicity data, in this case trypan blue dye exclusion. This was followed by a calorimetric study on a range of anti-tumour drugs, which further underscored the potential value of heat flux measurements in predicting cytotoxicity [57]. The value of this calorimetric type of assay is enormous, not least because society is questioning the morals of exploiting animals; in addition, many of the pharmacological models that use whole animals are simply not sound in scientific terms [58].

Kemp [15] reported unpublished data of Thorén and Holma originating from a novel calorimetric cytotoxicity test for metal and non-metal particles. Manganese dioxide particles decreased the heat flux of rabbit alveolar macrophages, whereas titanium dioxide particles had no effect. These results were similar to those using the conventional fluorescein diacetate viability assay. However, quartz dioxide particles had no effect on cell

viability but caused increased heat flux (Thorén, unpublished results). This may be an early sign of disease—pulmonary fibrosis.

More recently, Barisas and coworkers have developed an acute toxicity test based on the heat flux of the S3G strain of HeLa cells and human lymphocytes [59]. They reported data from dose–response curves for 10 toxicants, including the heavy metals Hg, Cd, Cu, Pb, Cr, V and As, and calculated the concentrations that were effective in inhibiting heat flux by 50% (EC_{50}). They also compared the findings with results from conventional toxicity assays and heat flux from the chemiluminescent bacterium *Photobacterium phosphoreum*. The comparison was favourable to the increased sensitivity of calorimetry, this obviously being reflected in the ability to detect deleterious change in cellular metabolism at low concentrations of toxicant. In the case of Hg, it was found by using fluoresence photobleach recovery methods that the heavy metal induces formation of large plasma membrane protein aggregates which affect lateral mobility in the fluid mosaic lipids of the membrane [3]. This approach should commend itself to all dedicated "heatophilics" who realise that we cannot live by calorimetry alone!

CALORIMETRIC–RESPIROMETRIC RATIOS AND THEIR IMPLICATIONS

It is hoped that the reader by now has been persuaded to acknowledge the value of essentially non-specific heat measurements in studying specific processes in cellular physiology. This revelation needs to be reinforced by explaining that the non-specific can be coupled to the specific by simultaneous assays of more traditional input/output indicators of metabolism; for instance, oxygen consumption, carbon dioxide production, turnover of glycolytic endproducts, and phosphorylation potential, $\log[ATP]/[ADP] \times [P_i]$. A post-dawn thunderbolt for some: but, since at least calorimetric and polarographic determinations are not destructive to cells and tissues, Pliny's harmless thunderbolt!

The most conventional assay of metabolic flux in aerobic living systems is oxygen flux, J_{O_2} [60]. Its equivalence to heat flux is central to thermochemical analysis, and is obtained by applying appropriately derived theoretical oxycaloric equivalents $\Delta_k H_{O_2}$ (see ref. 30)

$$J_Q = \Delta_k H_{O_2} \times_k J_{O_2} \tag{5}$$

where subscript k is the catabolic half-cycle. In a totally aerobic system with a simple and single energy source (say, glucose), the theoretical oxycaloric equivalent can be calculated as an enthalpy change from the stoichiometry of the half-cycle using the tabulated enthalpies of formation (see refs. 24 and 61). With glucose as the only substrate for cells, and assuming that O_2 and CO_2 are exchanged with the gas phase, the oxycaloric equivalent is -469 kJ mol^{-1} O_2 [24]. But animal cells in vitro are rarely

exposed to glucose alone; except for solely metabolic studies, they are bathed in a complex medium which includes serum or its artificial chemically defined substitute [16,17,62]. If demand for an exogenous source exceeds supply (medium depletion), then cells will resort to endogenous reserves. Examples are glycogen through glycolysis to the citric acid cycle, and fatty acids and amino acids through acetyl CoA to the same oxidative cycle. In the same demand/supply (cybernetic) terms, animal cells in vitro seem to utilize any of the exogenous carbohydrates, fatty acids and amino acids in complex media and it is by no means certain that they have a predilection for carbohydrates—aren't they wise? In fact, there is a body of evidence that mammalian cells in vitro "prefer" fatty acids to carbohydrates in oxidative catabolism [17]. These subtleties are of physiological importance, but they are encompassed within a small range of oxycaloric equivalents. Calculations show that these theoretical equivalents vary by only $\pm 5\%$, from -430 to -480 kJ mol^{-1}, for a variety of catabolic substrates under different "normal" physiological conditions [8,24,63].

The simultaneous measurement of heat and oxygen fluxes gives the calorimetric–respirometric ratio [24,27]

$$\text{CR ratio} = J_Q / {}_k J_{O_2} \tag{6}$$

which was formerly called the (total) heat equivalent of oxygen $\Delta_t Q_{O_2}$ under conditions when no work is done [8]. It is important to realise, therefore, that the "R" refers solely to oxygen flux (input) and not respiratory output (e.g. carbon dioxide). The respiratory quotient (molar gas exchange ratio CO_2/O_2) is only unity for carbohydrates such as glucose, and not for fatty and amino acids [24].

The foregoing analysis is essentially the energy (enthalpy) balance method [8] based on the principle of the First Law of Thermodynamics. If the CR ratio of a cell type is -450 kJ mol^{-1} $O_2 \pm 10\%$, then it is reasonable to state that it has a balanced aerobic energy (enthalpy) budget. Berry et al. [64] relied on this assumption when developing the idea that aerobic catabolism in isolated rat primary hepatocytes is not tightly coupled to ATP generation. Some years before, it had been shown that hepatocytes from rats on a normal diet had a CR ratio of -542 kJ mol^{-1} O_2 with glucose as substrate (ref. 65; also see review, ref. 15) and -497 kJ mol^{-1} O_2 with no exogenous substrate [66]. These figures were obtained by analysis of their data [15]. A CR ratio 20% more negative than the value for a balanced aerobic energy (enthalpy) budget needs to be treated with caution. Different conditions were observed for the latest study [64] but no CR ratio is available, apparently because calorimetry is too "cumbersome"!

The assumption that the rate of oxygen flux gives an acceptable approximation to heat flux [64,67] must be tested for each cell type. It is clear from Table 2 that hamster brown adipocytes have a balanced aerobic energy (enthalpy) budget [26], but this is not the case for some animal cells in

TABLE 2

Calorimetric–respirometric (CR) ratio for some cell types

Cell type		CR ratio (kJ mol^{-1} O_2)	Authors	Ref. no.
LS-L929 fibroblasts		−836	Hoffner et al.	29
Bovine sperm		−542	Inskeep and Hammerstedt	36
Rat hepatocytes	basal	−542	Jarrett et al.	65
	25 mmol dm^{-3} fructose	−649		
Hamster brown adipocytes		−490	Nedergaard et al.	26
Human neutrophils	basal	−1132	Eftimiadi and Rialdi	48
	PMA activated	−588		
Lymphocyte	3×10^5 cells cm^{-3}	−770	Nässberger et al.	67
T-lymphoma		−617	Borrebaeck and Schön	53

culture. If the original data are interpreted by the energy balance method in terms of CR ratios [18,15,24,27], then manifestly some cells are operating under different rules (Table 2) or no rules at all. Values less than −500 kJ mol^{-1} O_2 and as low as −1132 kJ mol^{-1} O_2 must be explained by thermochemical rationale.

The observed Uriah effect (see refs. 19 and 67) gives the vital clue—simultaneous and intensive participation of anaerobic catabolism (glycolysis) with aerobic processes [24]. The most common anaerobic end product is lactate, and its production from glucose and excretion into a bicarbonate buffer is accompanied by a catabolic enthalpy change $\Delta_k H_{Lac}$ of −63 kJ mol^{-1} [8,24]. In simultaneous processes, this change must be added to the oxycaloric equivalent as a ratio between the molar amount of lactate produced per unit amount of oxygen consumed (Lac/O_2; see ref. 24). The theoretical catabolic heat change per mol of O_2 $\Delta_k Q_{O_2}$ is then calculated as

$$\Delta_k Q_{O_2} = \Delta_k H_{O_2} + Lac/O_2 \times \Delta_k H_{Lac} \tag{7}$$

The ratio in eqn. (7) is comparable with the respiratory quotient. In the same way, it indicates the relative extent of aerobic glycolysis. In passing, it is worth remembering that the CO_2/O_2 ratio is 1 only for certain carbohydrates catabolized under solely aerobic conditions [24]. Simultaneous measurement of CO_2 and heat fluxes [48,68], even when associated with the appropriate oxycaloric equivalent(s), needs careful interpretation.

As can be seen in Table 3, the observed Lac/O_2 ratios largely explain CR ratios more negative than −500 kJ mol^{-1} O_2. The thermochemical reasoning has been more fully explained elsewhere [24] but there are general lessons. To illustrate, it was shown that LS-L929 mouse fibroblasts in a Vibromixer culture vessel had a CR ratio of −836 kJ mol^{-1} O_2 (Table

TABLE 3

The calculated catabolic heat change per mol of O_2 ($\Delta_k Q_{O_2}$), on the assumption that lactate is the only glycolytic end-product produced by cells cultured in an aerobic environment, compared with experimental CR ratios

Cell type		CR ratio (kJ mol^{-1} O_2)	$\Delta_k Q_{O_2}$	Lac/O_2
Human neutrophils:	resting	−1132	−1352	14.0
	PMA-activated	−588	−577	1.72
T-lymphoma		−617	−581	1.75
LS-L929 fibroblasts		−633	−601	2.1
Mouse macrophage hybridoma 2C11-12		−518	−540	1.14

2; ref. 29). This indicated intensive anaerobic glycolysis under supposedly aerobic conditions. A change in vessel design to allow direct oxygen purge reduced the CR ratio to −633 kJ mol^{-1} O_2 (Table 3; ref. 27). There was still simultaneous aerobic and anaerobic catabolism, which was due to lactate production. A low density of lymphocyte hybridoma cells (6×10^4 cm^{-3}) incubated in a calorimeter for 1 h registered a CR ratio of −570 kJ mol^{-1} O_2 [67] on interpreting the data. Although the cells were not stirred, it would be surprising if Uriah had descended on so few cells so quickly. Certainly, there was sufficient bulk phase dissolved oxygen. It is perhaps not unreasonable that cells of the same type at higher density (3×10^5 cm^{-3}) succumbed to Uriah (Table 2), but there is no lactate datum.

Possible insight may be derived from a recent study showing that the CR ratio of 2C11-12 mouse macrophage hybridoma cells under fully aerobic conditions became more negative late in the exponential phase of growth [6]. This was proved to be caused mainly by increased lactate production. Possibly homeostatic control by hormones and other regulatory factors is lost in cells in vitro. As mentioned earlier, it is well established that anaerobic glycolysis is frequently more prominent in cultured cells [16,17,62].

One of the reasons for possible discrepancies between the Lac/O_2 ratio and a highly negative CR ratio could be the formation of other glycolytic end products. A general equation has been written for calculation of the heat change for all end products per mol of O_2 [6]

$$\Delta_k Q_{O_2} = \Delta_k H_{O_2} + \sum_i i/O_2 \times \Delta_k H_i \qquad (8)$$

where i is each individual end product. There is some evidence of succinate production in 2C11-12 cells [6].

Researchers have invoked uncoupling [29] and decoupling by futile cycling (refs. 65 and 70; see Table 2) to explain values more exothermic than −600 kJ mol^{-1} O_2, and then ascribe the phenomenon to low

efficiency of net ATP production [67,70]. Gnaiger and Kemp [24] reasoned that an increase in CR ratio above the theoretical enthalpy equivalent of oxygen consumption cannot be explained on this basis. Futile cycling is no different to any other dissipative maintenance process with a net efficiency of zero. Compensation for a low ATP/O_2 ratio on uncoupling is by substrate-level phosphorylation, generating ATP.

But for so much more on this, I must "bounce" the reader into a review by Gnaiger [63] on concepts of efficiency.

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