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Review

Developments in cellular microcalorimetry with particular emphasis on the valuable role of the energy (enthalpy) balance method [1]

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Abstract

This review of microcalorimetric studies of animal cells includes muscle and nerve as well as blood tissue, non-erythroid cells and transformed cells. It highlights the wide range of heat flow rate ($\phi = dQ/dt$) data obtained for cells but points to their limited inherent value unless expressed per unit volume or biomass — scalar heat flux. Measurements of heat flow have been used in detecting clinical, pharmacological, toxicological and immunological changes — calorimetry as an analytical tool to assay metabolic activity in the face of pathological and xenobiosis-induced alterations.

Microcalorimetry is a powerful and non-destructive technique of itself but its true strength is revealed when combined with other analytical procedures to allow access to the energy (enthalpy) balance method, which has its basis in the First Law of Thermodynamics. Its early use in dissecting all the chemical sources of heat in the thermogenesis of muscle is summarized, together with more recent studies of the metabolic burst in phagocytosis and catalytic pathways in cultured, anchorage-independent T-lymphoma and LS-L929 mouse fibroblasts. The theoretical basis of the calorimetric–respirometric (CR) ratio is explained and its value in detecting anaerobic pathways in respiring cells is emphasized with reference to several cell types. Two ways of calculating ATP turnover (μmol ∞ ATP h^{-1}) are described, namely from heat flow and catabolic coupling flow; and there is opuscular reference to thermodynamic efficiency.

INTRODUCTION

The Lavoisier Lectures celebrate reflectively the extraordinary life of the founder of modern chemistry. Along the way, the eponym invented

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calorimetry and, from his findings with Laplace [1], concluded that the respiration of animals is a very slow combustion. It is appropriate, therefore, that this paper focuses on the role of modern calorimetry, with its high accuracy and high time-resolution, in the study of respiration and energy balances in animal eukaryotic cells.

After the Lavoisier–Laplace experiments on animal aerobic processes, the next "metremark" in the calorimetry of living material was the massive experiment by Dubrunfaut [2] which involves sufficient yeast for a 4-day fermentation of 21.4 m^3 of molasses solution containing 2559 kg of cane sugar. The temperature rose from 23.7 to 33.7°C and, after corrections, he calculated a total heat output of −1.42 GJ. This was 38% lower than that calculated from the enthalpy of formation of ethanol from sucrose with a stoichiometry described by the Gay–Lussac equation. There was a deficit in the amount of alcohol obtained and this caused problems in the interpretation of many calorimetry experiments on yeast in the latter half of the 19th century. The heat of fermentation of glucose is now known to be −138.6 kJ mol^{-1} [3]. From the metabolic enthalpy balance method derived from the first Law of Thermodynamics, if all 14964 mole-equivalents of glucose were fermented to ethanol, the value for enthalpy should be −2.07 GJ rather than −1.42 GJ. The difference in terms of glucose equivalents can probably be ascribed to the yeast cells *growing* in the molasses, using some of the sucrose as their carbon source [3]. This is a first inkling of the probable worth of the enthalpy balance method in calorimetric studies but yeasts, while eukaryotic, are not animals and strictly not cells but organisms; so their 20th century story must be left to Battley [3].

MUSCLE

In calorimetric terms, the most intensively studied animal system is muscle. Only ten years after Rubner [4] first provided conclusive evidence that the Law of the Conservation of Energy applied to living systems, Hill in 1912 published his classical paper on the thermogenesis of muscle using a differential calorimeter [5]. He recognised that a knowledge of Gibbs energy changes is central to understanding biological energetics and thus the Second Law of Thermodynamics was introduced to the subject. Although a muscle fibre is a syncytium, not a cell, the understanding of muscle energetics consequent to the research of Hill and others is a vital prelude to understanding thermochemical analyses of animal cells.

Striated muscle consists of bundles of multinucleate myofibres (syncytia) containing myofibrils of thick (myosin) and thin (mainly actin) filaments which interact to give contraction in the presence of Ca^{2+} ions [6]. The hydrolysis of ATP by myosin [7] drives the sliding mechanism of the two filaments, the force being generated in the actin–myosin crossbridges.

Myosin is an actin-activated ATPase [8] because the thin filament is required to release ADP from it and thus allow a fresh ATP to bind to it. Skeletal muscle converts chemical energy to mechanical work with very high efficiency — less than 50% of the available energy is converted to heat [7]. There is relatively little change in the level of ATP on contraction because supply is available from a phosphogen reservoir. The tightly coupled conversion of the phosphogen, creatine phosphate, to ATP is maintained close to equilibrium by highly active creatine kinase.

When a muscle is at rest, cytosolic $[Ca^{2+}]$ is low ($<10^{-7}$ M). On excitation of the sarcolemma, depolarization spreads to the sarcoplasmic reticulum and Ca^{2+} bound to calsequestrin is released through membrane channels into the sarcoplasm [9]. Ca^{2+} ions ($>10^{-3}$ M) interact with the troponin C complex and the consequent conformational change to the troponin I then permits actin to cross-bridge with myosin ADPP_i. When excitation ceases, Ca^{2+} is removed by an ATPase pump to the sarcoplasmic reticulum and troponin I resumes its inhibitory interaction with actin [9].

A vital feature of studies of muscle physiology has been to account for heat and work in terms of chemical reactions. Using an energy (enthalpy) balance equation

$$h = \sum n_i \Delta H_i \quad (1)$$

where h is the total enthalpy (heat + work), n_i is the number of moles of reaction i, and H is the enthalpy change per mole of reaction i.

Woledge [10] showed that much of the heat is derived from the splitting of creatine phosphate and the reactions which resynthesize it. The energy (enthalpy) balance method then stimulated research into the sources of the unexplained energy. This was 21% in the case of that produced during isometric tetanic contractions at 0°C of *Rana temporaria* sartorius muscles [10]. One of the contributors to this energy is the activity of muscle proteins in the myofibrils [11]. Another root at the start of the contraction is the movement of Ca^{2+} ions from binding sites in the sarcoplasmic reticulum to binding sites on troponin C and paravalbumin [12]. The reversal of this process when contraction is over involves ATP splitting (exothermic) by the calcium pump. This is part of the energetic cost of contraction, but not concurrent with it. The accompanying heat flow is less than expected because hydrolysis is coupled to an endothermic process which is expected, though not observed, to be transport of two Ca^{2+} ions for each ATP split in the sarcoplasmic reticulum.

At one time, it was thought that the active Na–K transport pump in the sarcolemma made a considerable contribution to muscle thermogenesis [13]. To test this hypothesis, it was shown that resting rat soleus muscle had a heat flux of -2.62 ± 0.06 mW (g wet wt.)$^{-1}$ [8]. Ouabain at 10 mmol dm^{-3} inhibited $^{22}Na^+$ efflux by 58% and $^{42}K^+$ influx by 30% and

decreased heat flux by -0.13 ± 0.01 mW (g wet wt.)$^{-1}$. This means that the pump made a small contribution of 4.7%. They went on to compute that the Gibbs energy transmitted to Na^+ and K^+ ions as they were actively transported across the plasma membrane (W) was 0.04 mW (g wet wt.)$^{-1}$ min^{-1}. According to Wilkie [14], the overall thermodynamic efficiency (ε) of the process, at steady-state conditions [15], is

$$\varepsilon = -\dot{W}/\dot{\xi}\,\Delta_r G \tag{2}$$

where $\Delta_r G$ is the total rate of expenditure of Gibbs energy involved in the process. $\dot{\xi} = d\xi/dt$ = velocity of reaction and $d\xi = dn_B/\upsilon_B$ = advancement where n is the amount of substance (mol) and υ is the whole stoichiometric number for entities B. Under conditions of aerobic metabolism, T times the entropy change for substrate oxidation is small compared with the enthalpy change [14], so heat flux, J_Q, is approximately equal to Gibbs energy flow rate. Thus, ΔQ should be approximately equal to $\Delta_r G$, and ε was therefore found to be 34%. On the premise that the maximum efficiency of the cellular energy conservation process is 65%, Chinet et al. [13] estimated that the minimum energetic efficiency of ATP utilization by the active Na–K transport process in mammalian striated muscle is 52%.

The small contribution to muscle thermogenesis by the active Na, K-ATPase was recently confirmed using guinea-pig heart ventricular trabeculae, the heat flow from which was inhibited by 4.1% by dihydro-ouabain [16].

By comparison with striated muscle, little calorimetric research has been performed on other forms of muscle. Lately, however, chemically skinned smooth muscle of guinea-pig *Taenia coli* has been incubated in a solution containing 3.2 mM MgATP and creatine phosphate, pH 6.9, and shown to produce at rest (p*Ca* 9) 0.40 ± 0.03 mW (g wet wt.)$^{-1}$. During maximal activation (p*Ca* 4.8), heat production increased to 1.12 ± 0.07 mW g^{-1}. It was further demonstrated that, with stepwise increments in $[Ca^{2+}]$ from p*Ca* 9 to 4.8, the energetic cost of force maintenance tended to increase at higher $[Ca^{2+}]$. After Ca^{2+} activation, forces still increased beyond the point at which the rate of heat production reached its maximum. Chemical skinning with 1% Triton X-100 eliminated oxidative and glycolytic catabolism and, of course, prevented any expression of the Ca^{2+} pump. So, the calorimetric measurements were reasonably consistent with the observed chemical breakdown of creatine phosphate. Because the chemical environment in skinned muscle is well defined, energy balance studies do not lead to "unexplained" heat values.

NERVE

Generation of the action potential in a nerve is accompanied by a biphasic change in heat flow, the first peak being exothermic and the second, endothermic. For a *Maia* (crab) non-myelinated nerve, the initial

exothermic heat after a single impulse was found to be $-11.7\,mJ\,g^{-1}$, and the subsequent endothermic heat was $+10\,mJ\,g^{-1}$ [18]. A similar experiment for rabbit non-myelinated fibres gave figures of 102.5 and 92.9 $\mu J\,g^{-1}$ respectively [19]. The mass-specific dissimilarity is probably owing to surface area differences. In both cases, the nerves possessed a residual heat, $1.7\,mJ\,g^{-1}$ for the crab nerve and $9.6\,\mu J\,g^{-1}$ for the rabbit. Deflections from the baseline continued for over 200 s compared with the biphasic peak of 500 ms. Ritchie [20] explained the thermal profile in terms of changes in entropy accompanying the charging and discharging of a condenser. The change in enthalpy on discharging a condenser of capacity C at a temperature T may differ considerably from the Gibbs energy change by an amount $T\Delta S$, which can be shown to equal $\Delta G(T/C)(\Delta C/\Delta T)$. If $\Delta C/\Delta T$ is positive, heat will be released on discharge; and if $\Delta C/\Delta T$ is negative, the entropy change will lead to an absorption of heat. For any substance $\varepsilon/\Delta T$ is zero near the absolute zero of temperature, ε being permittivity. As the temperature increases, ε also increases at first but starts to decrease after reaching a certain critical temperature, so that $\Delta\varepsilon/T$ is positive in the range from zero temperature until the critical temperature is reached, and thereafter is negative. Below the critical temperature, electrical polarization of the dielectric increases entropy; above this temperature, polarization decreases entropy. What happens in nerves thus depends on how the nerve membrane capacity varies with temperature. This is not known for crab and rabbit nerves but squid giant axon has a positive temperature coefficient for permittivity which, if also true of the nerves in question, means that entropy changes on discharge of the condenser would account for the bulk of the heat produced by the nerve. Ritchie [20] also speculated that the residual heat was due to activity of the sodium pump (Na^+, K^+-ATPase). ATP splitting is, of course, required for the coupled pumping of $Na^+$and K^+ ions to restore the resting potential of the nerve.

BLOOD CELLS

Little interest was shown in studying heat production of cells other than muscle and nerve until Wadsö's heat conduction flow microcalorimeter [21] was marketed and used to investigate thermal energy produced by blood cells [22]. Over the last 20 years, Monti's group (see reviews in refs. 23 and 24) has explored the metabolism of the various cell types in whole blood and has emphasised the reliable and valuable role of biothermograms in diagnosing many clinical conditions.

Human erythrocytes were found to produce $10\,fW\,cell^{-1}$ [22], a rate which was decreased by 50% when the cells were incubated with sodium fluoride, an inhibitor of glycolysis, and increased by 400% on exposure of erythrocytes to methylene blue, which stimulates the pentose phosphate pathway [23]. Erythrocytes from patients with various types of anaemia had a higher rate of heat production than those from healthy individuals [22].

This was also the case for cells from hyperthyroid patients (13 fW cell^{-1}) but medical treatment reduced heat production to normal values [25].

Despite their diminutive size, human platelets were shown to produce considerably more heat (61 fW cell^{-1}) than their big brothers [23]. Cells from hypothyroid patients produced significantly less heat (51 fW cell^{-1}) but medical treatment restored normal values. The far greater sized lymphocytes recorded heat evolution of 2.2 pW cell^{-1} [26] while its leucocyte relative, the polymorphonuclear neutrophil or granulocyte "weighed in" with 3.5 pW cell^{-1}, both suspended in platelet-poor plasma [26]. Monti has used calorimetry as a diagnostic tool for investigating pathological conditions in white cells [23, 24]. Lymphocytes from patients with non-Hodgkin lymphoma (NHL) [27] gave significantly higher power values (3.7 pW cell^{-1}) than those from healthy humans or patients who were responding to clinical treatment (2.5 pW cell^{-1}). In addition, it was shown that elevated lymphocyte metabolism as indicated by greater heat production in NHL patients is associated with a higher degree of malignancy [28]. This indicated that calorimetry can be used to assess the prognosis of the disease.

Although patients with chronic lymphocytic leukaemia show symptoms of fever and sweating, their lymphocytes produce less heat than those from healthy patients [29]. It was concluded that the chemical effects were due to the vastly increased white cell population rather than cellular hyper-metabolism. Lymphocytes from patients with hyperthyroidism [30] do, however, produce more heat (3.4 pW cell^{-1}) than those from "normal" humans, an effect similar to that for erythrocytes. Clearly, the thyroid hormones influence the metabolic rate of blood cells in general. This observation is strengthened by the finding that lymphocytes from patients with acromegaly have a higher rate of heat production than controls [31]. Acromegaly is a disease with increased production of growth hormone, often caused by tumors of the pituitary gland [24].

It is somewhat surprising that the clinical potential of calorimetry has not been exploited in hospitals outside Monti's home base in Lund, Transylvania!

NON-ERYTHROID CELLS

Early studies on non-erythroid cells in vitro encompassed primary cultures of embryonic chick fibroblasts [32], established cell lines [33] and transformed cells [34], which gave heat flows of 3, 25 and 58 pW cell^{-1}, respectively. There were early difficulties in matching calorimetric vessels to the precise requirements of delicate cells from solid tissues or anchorage-dependent culture [35] but a catalogue has now been written [36–39] showing heat flows for cells from bovine sperm (1.3 pW cell^{-1}) [39], through 3T3 mouse fibroblasts (17 pW cell^{-1}) [40], rat white adipocytes

(40 pW cell^{-1}) [41] and human keratinocytes (83 pW cell^{-1}) [42], to hamster brown adipocytes (110 pW cell^{-1}) [43] and rat hepatocytes (329 pW cell^{-1}) [44]. It is obvious that one of the reasons for these great differences is size, so it would be helpful if heat measurements could be stated in terms of scalar heat flux, ${}_tJ_Q$ (W m^{-3}). Data would then relate more closely to metabolic (mostly catabolic) flux and reveal interesting diversity between cells. For instance, a human foreskin fibroblast (40 pW cell^{-1}) [45] is a quarter the volume of a rat hepatocyte but only produces an eighth of the heat. Care must be taken in such comparisons to eliminate the possibility of differences in the mass:volume ratio, $\rho = m/V$, because protein mass may be the important factor [46]. It has been shown [37] for both Vero cells [47] and 2C11-12 mouse macrophage hybridoma cells [46], that there is a very marked influence of cell size on heat flux expressed per unit protein content. This is reminiscent of Kleiber's finding for whole animals [48] that mass-specific oxygen flux decreases with increasing size. It should also be remembered that volume alters during the cell cycle and this would affect metabolic flux if plasma membrane substrate receptors with high flux-control coefficients are limited during cell growth [46].

In terms of heat production, there is some indication that naturally transformed (cancer) and experimentally transformed cells give higher values. Attention has already been drawn to leukaemic cells (see earlier and ref. 34) and it has been reported that virally transformed (SV-K14) keratinocytes [42] have a higher heat flow (83 pW cell^{-1}) than their normal counterparts (40 pW cell^{-1}). Unfortunately, there have been no measurements of size in such studies, so it is not possible to comment on flux differences. Elevated heat production has been said to indicate higher metabolic activity [34] but that is intentionally a loose phrase. First of all, it should be realized that the anabolic contribution to heat production of micro-organisms [49, 50] and animal cells [51] is negligible, so heat flux essentially is related to catabolic processes. In fully coupled systems, such processes are geared solely to ATP production with stoichiometric coupling coefficients for glucose catabolism aerobically (ν_{ATP/O_2}) of 6 and anaerobically to lactate ($\nu_{ATP/Lac}$) of 1 [52]. So, higher metabolic (catabolic) activity results from and equates to increased demand for ATP which can be met by elevated aerobic and/or anaerobic processes. The heat flux per mole ATP differs little between pathways for a given substrate but the carbon flux is markedly dependent on availability of oxygen and ability to utilize it. Naturally and experimentally transformed cells have greater demands for ATP synthesis than normal cells and, in the case of tumor cells at least [53], appear to meet it mostly by more intensive glycolysis rather than greater respiration. To illustrate the point, human leukaemic cells, Molt 4 which, at an initial inoculum of 1×10^5 cells cm^{-3}, evolved heat at 21 pW cell^{-1} for 48 h, produced 1.8 mole lactate per mole of glucose, a glycolytic conversion which is nearly stoichiometric [54]. Finally, a system

which is uncoupled, for instance brown adipocytes [55], or decoupled, for example futile substrate cycling in rat hepatocytes [56], from ATP demand in the strict sense [57], will of course produce more heat concomitant with increased ATP synthesis.

pH and oxygen

Among all the possible factors in cellular heat measurements, two — bulk phase pH and dissolved oxygen — have been highlighted by recent studies. When T-lymphoma cells, CCRF-CEM, were suspended in a balanced salts solution with 20 mmol dm^{-3} HEPES in the pH range 6.7–7.6, there was a linear relationship between rate of heat flow and pH of 21% per pH unit [58]. The suspension medium was inadequate to support growth but cells incubated short-term (20 min) with growth medium showed a more acute pH dependence of 61% [59]. In long-term growth culture (28 h), the optimum pH of the bulk phase was 7.2, lower and higher values causing a decrease in growth rate [60]. It was also found that pH decreased with increasing total heat produced per cm^3 of cell suspension. This is probably a reflection of the high rate of aerobic glycolysis in the cells, with lactate being excreted into the bulk phase (medium). Changes in heat flow with pH may be due to pH sensitivity of the glycolytic rate [60] but the exact cause of the influence of bulk phase pH on intracellular pH is not known.

The solubility of oxygen in water is 2.102×10^{-7} mol cm^{-3} at 101.325 kPa and 37°C [61] and even less in culture media because of the salting-out effect. As an instance of this phenomenon, normal human plasma has a Bunsen oxygen solubility of 0.0214 at 37°C, which is 89.6% that of water [62]. If cellular oxygen flux exceeds availability, then obviously the cells will be stressed in these hypoxic conditions and in terms of catabolism resort to greater participation of glycolysis, leading to more production, accumulation and excretion of lactate. This, in turn, would lower bulk phase pH which, as stated earlier, can act as a negative feedback to the glycolytic pathway. This can occur with cells at high concentrations in suspension, for instance blood cells [63], but is more generally evident when cells are allowed to sediment under batch conditions. For instance, murine macrophages at 30×10^6 and 60×10^6 cells cm^{-3} manifested a sharp decrease in heat flow with time [64]. This was ascribed to the "crowding effect", a phrase originally coined to describe density-dependent inhibition of oxygen consumption [65]. Although oxygen dissolved in the bulk phase culture medium may be adequate, the micro-environment surrounding "crowded" cells rapidly becomes depleted, leading to hypoxia. This reason could be ascribed to the finding that the heat flow of mouse lymphocyte hybridoma cells decreased with increasing cell concentration [66]. It should be emphasised, however, that this is not an absolute rule. Molt 4 cells did not exhibit any appreciable alteration of heat flow during the major part of

the log growth phase [54]. An interesting general pointer is that the critical level of oxygen tension for lymphoma cells is a p_{O_2} of 2 kPa ($\approx 2 \times 10^{-8}$ mol cm^{-3}) [60].

Oxycaloric equivalents

It has often been convenient in studies of intermediary metabolism by cells in vitro, only to take indirect calorimetric measurements (oxygen) and neglect direct ones (heat), relying on an assumption of singularly aerobic glycolysis. In this case, oxygen flux ($_rJ_{O_2}$) is related to heat flux ($_rJ_Q$) by the appropriate oxycaloric equivalent, $\Delta_rH_{O_2}$ [57]

$$_rJ_Q = \Delta_rH_{O_2}{}_rJ_{O_2} \tag{3}$$

Oxycaloric equivalents are the enthalpy changes of the catabolic half-cycle, e.g. glucose to HCO_3^- and H^+, and do not include any coupled processes such as ATP production; that is, no work is done so the net efficiency is zero. They are calculated from standard enthalpies of formation, $\Delta_fH^{\ominus\prime}$ [57], and, for given substrates, are the same as values obtained by bomb calorimetry, $\Delta_cH^{\ominus\prime}$, but adjusted to a particular aqueous solution. In aerobic glucose catabolism, the equivalent for glucose at pH 7 is -469 kJ mol^{-1} O_2.

The theoretical oxycaloric equivalent is the *expected* ratio of calorimetric measured heat flux and respirometric oxygen flux, CR ratio

$$\text{CR ratio} = {}_rJ_Q/{}_rJ_{O_2} \tag{4}$$

Hamster brown adipocytes suspended in glucose-containing saline gave a ratio of -490 kJ mol^{-1} O_2 [55] (see also reviews in refs. 37 and 38), indicating fully aerobic metabolism; but many cell types in completely aerated culture media have ratios more exothermic than this, and as high as -1100 kJ mol^{-1} O_2 [30] (see reviews in refs. 37 and 38). In some cases, for instance rat heptatocytes cultured in saline containing 25 mmol dm^{-3} fructose [67], highly negative CR ratios have been ascribed to increased futile cycling which would consume ATP and result in decreased ATP production for other more "useful" requirements. A similar explanation has been invoked for other examples of highly negative CR ratios, for instance resting human neutrophils [68] in which the catabolic half-cycle is uncoupled from ATP production, reducing the efficiency of the latter. Because oxycaloric equivalents are calculated on the assumption of zero net efficiency, irrespective of intermittent efficiencies of ATP production in the phosphorylation reaction [69], increased futile cycling and uncoupling cannot be the causes of highly exothermic CR ratios.

The correct reason is the activation of anaerobic catabolism [57]. In simultaneous aerobic and anaerobic glycolysis, the catabolic heat change per mol O_2, $\Delta_r H_{(ox+anox)}$, is calculated by adding to the appropriate oxycaloric equivalent the molar amounts of glycolytic end-products (p) formed per unit amount of oxygen consumed (p/O_2 ratio) with the requisite dissipative catabolic enthalpy changes of end-product ($\Delta_r H_p$)

$$\Delta_r H_{(ox+anox)} = \Delta_r H_{O_2} + p/O_2 \times \Delta_r H_p \quad (5)$$

The most common end-product in mammalian cells is lactate, the $\Delta_r H_{Lac}$ [57] for which is $-63\ kJ\ mol^{-1}$ if it is excreted into a bicarbonate buffer [52], $-77\ kJ\ mol^{-1}$ into 20 mM HEPES buffer [51] and $-59\ kJ\ mol^{-1}$ into a phosphate buffer [70]. Calculations from experimental data have shown aerobic glycolysis to lactate alone as the reason for the highly exothermic CR ratios in some cell types [37, 38]. But for others, for instance 2C11-12 mouse macrophage hybridoma cells [46], the calculation for lactate failed to achieve a complete reconciliation. When this is the case, $\sum_p p/O_2 \times \Delta_r H_p$, where p is each individual end product [38], can be substituted in eqn. (4). In the case of 2C11-12 cells, succinate was found to be excreted into the medium [46]. Adding the Succ/O_2 ratio multiplied by the enthalpy change of succinate excreted into bicarbonate buffer ($\Delta_r H_{Succ} = -152\ kJ\ mol^{-1}$ at $\Delta_b H'_{H^+} = -25$ [52]) gave a $\Delta_r H_{(ox+Lac+Succ)}$ value very similar to the CR ratio.

It is unwise, of course, to read across from in vitro results in the in vivo situation without eliminating the possibility that the conditions under which the former were obtained produced artifacts. For instance, calorespirometric measurements of unstirred cultures may indicate intensive glycolysis only because sedimentation can induce hypoxic conditions [63, 64, 66]. Taking a clue from the fact that hamster brown adipocytes float on culture media and were shown to have a fully aerobic CR ratio [55], Hansen's group [71] added the PVP-coated silica sol Percoll (Pharmacia) to growth medium to give different densities. BHK fibroblasts were found to float at 20% (w/v) Percoll and gave the optimal heat flow of $15\ pW\ cell^{-1}$. It is already recognised that tumour cells [52] and lines derived from them, for instance T-lymphoma [58–60] undergo intensive aerobic glycolysis. It is now increasingly clear that many normal cells freshly obtained from animals or established in vitro as a line, conduct simultaneous aerobic and anaerobic catabolism even in fully aerobic conditions [72].

ENERGY (ENTHALPY) BALANCES

Attention has already been drawn to the value of the enthalpy balance method in studying microbial growth [3] and muscle energetics [10]. In

recent years, this approach has been extended to other cell types and its valuable application (see eqn. (1)) is now illustrated by reference to human neutrophils [68] and T-lymphoma cells [60].

Mammalian phagocytic cells of their various types have in common the ability to kill foreign microbes after engulfing them, using metabolites of oxygen and hydrogen peroxide. In order rapidly to produce these metabolites on activation, oxygen is reduced primarily using NADPH as a hydrogen donor, but also probably NADH [73]. NADPH is generated by the pentose phosphate pathway (PPP) from glucose-6-phosphate (G6P). There are two competing demands of this pathway, NADPH and ribose 5-phosphate. For the phagocyte metabolic burst, the former is generated by two sets of reactions with the following stoichiometrics: firstly, when G6P is completely oxidized to CO_2

$$G6P + 12NADP^+ + 7H_2O \rightarrow 6CO_2 + 12NADPH + 12H^+ + P_i$$

and, secondly, when G6P is oxidized to pyruvate (Pyr)

$$3G6P + 6NADP^+ + 5NAD^+ + 5P_i + 8ADP \rightarrow$$
$$5Pyr + 3CO_2 + 6NADPH + 5NADH + 8ATP + 2H_2O + 8H^+$$

In the presence of mitochondria, pyruvate can enter the tricarboxylic acid cycle according to the stoichiometry

$$Pyr + 4NAD^+ + FAD + GDP + P_i + 2H_2O \rightarrow$$
$$3CO_2 + 4NADH + FADH_2 + GTP + 2H^+$$

Otherwise pyruvate can be converted to lactate

$$Pyr + NADH + H^+ \rightarrow Lac + NAD^+$$

NADPH oxidase is an enzyme which traverses the plasma membrane and catalyses the divalent reduction of oxygen, with NADPH as the hydrogen donor

$$NADPH + O_2 \xrightarrow[\text{oxidase}]{\text{NADPH}} O_2^- + NADP^+ + H^+$$

(binding site in cytoplasm) (extracellular) (plasma membrane) (extracellular) (cytoplasm)

Eftimiadi and Rialdi [68] resuspended fresh human neutrophils in Gey's saline containing 11 mM glucose and thermochemically characterized them in both the resting state and activated by phorbol-12-myristate-13-acetate (Table 1). The heat flow from resting neutrophils was steady at

TABLE 1

Thermochemical characterization of fresh human neutrophils interpreted and summarized from Eftimiadi and Rialdi [68]

	Resting	PMA-activated ($10\ \mu g\ cm^{-3}$)	NaF ($20\ mmol\ dm^{-3}$)	PMA + NaF
Heat flow ($pW\ cell^{-1}$)	2.5	9.8	3.4	3.9
Oxygen consumption ($fmol\ h^{-1}\ cell^{-1}$)	7.95	60	25.0	28.7
Lactate production ($fmol\ h^{-1}\ cell^{-1}$)	111	103	6.2	6.3
Carbon dioxide evolution ($fmol\ h^{-1}\ cell^{-1}$)	–	48	23.4	27
CR ratio ($kJ\ mol^{-1}\ O_2$)	1132	588	489	490
Respiratory quotient	–	0.8	0.93	0.94
Lac/O_2 ratio	14	1.7	0.25	0.22
Calculated $\Delta_r H_{(ox+Lac)}$	1348	577	484	482
ATP consumption in burst = $1.64\ fmol\ h^{-1}\ cell^{-1}$				

The calculated "resting" heat ($pW\ cell^{-1}$) is 1.8 (glycolysis) + 1.0 (PPP) = 2.8; the actual value was 2.5. The calculated total "burst" heat ($nW\ cell^{-1}$) is 4.03 (glycolysis) + 19.52 (PPP) = 23.55; the actual value was 23.5.

$2.5\ pW\ cell^{-1}$. For the enthalpy balance, an oxycaloric equivalent of $-488\ kJ\ mol^{-1}\ O_2$ (cf. ref. 57) was chosen for the complete oxidation of glucose and an enthalpy change of $58.5\ kJ\ mol^{-1}$ Lac was assigned to the formation of lactate from glucose. From radiocarbon studies for $^{14}CO_2$ evolution from [1-^{14}C] and [6-^{14}C] glucose, it was shown that respiratory CO_2 was exceedingly low. Applying eqn. (1) to the data showed that PPP and glycolysis accounted for ("explained") all the heat produced by resting neutrophils. The contribution of the TCA cycle was very small because neutrophils have few mitochrondria. The importance of glycolysis in catabolism of these cells was emphasised by calculating the CR and Lac/O_2 ratios (Table 1). Applying the latter to eqn. (4) gives a calculated $\Delta_r H_{(ox+Lac)}$ of $-1348\ kJ\ mol^{-1}\ O_2$ (cf. experimental ratio in Table 1) which may explain the slight discrepancy in estimating the energy balance.

Neurophils activated with $10\ \mu g\ cm^{-3}$ PMA produced a heat burst of $23.5\ nW\ cell^{-1}$ in 45 min (Table 1). Calculations of enthalpy balance from glucose carbon 1/carbon 6 ratios and lactate estimations, together with known enthalpy changes of the pathways, showed that PPP was responsible for the majority of the burst ($19.52\ nW\ cell^{-1}$), with a minor contribution from glycolysis ($4.03\ pW\ cell^{-1}$). The calculated total was virtually the same as the actual measurement, indicating that only the two pathways were involved in the burst. The predominance of PPP was also illustrated by the less negative CR ratio in activated cells (Table 1) compared with that in the

resting state, the enthalpy balance method showing that the experimental Lac/O_2 ratio fully accounted for the observed ratio.

N-Ethylmaleimide (0.1 mmol dm^{-3}) completely inhibited the metabolic burst [68] but sodium fluoride, which inhibits glycolysis, caused a burst and is known to stimulate PPP. The peak of the expected thermal profile did not occur for cells treated with NaF, so heat production and oxygen consumption was less than in PMA-activated cells and Eftimiadi and Rialdi [68] speculated that this was "because of inhibition of some NaF-sensitive peroxide-producing pathway". In other words, they knew not why! In any case, similar suppression by NaF also occurred to the PMA-activated burst (Table 1), further proving that PPP was solely responsible for it. The effectiveness of NaF in inhibiting glycolysis was demonstrated by the further reduced negative (exothermic) CR and Lac/O_2 ratios. Incorporating the latter in eqn. (4) demonstrated that PPP plus a very small amount of glycolysis totally accounted for the neutrophil PMA-activated metabolic burst.

Another study relying heavily on the enthalpy balance method was that by Wadsö's group on the thermochemical characterization of the anchorage-independent human cancer cell line T-lymphoma, CCRF-CEM [51, 60]. These cells were grown in suspension in RPM1 1640 medium supplemented with 10% (v/v) calf serum and containing 300 mg dm^{-3} glutamine and 5.5 mmol dm^{-3} glucose. Most crucially [58–60], the medium was buffered to pH 7.17 ± 0.03 with 20 mmol dm^{-3} HEPES. The heat flow was 12.2 pW $cell^{-1}$ at pH 7.17 (Table 2), considerably more than normal lymphocytes (2.2 pW $cell^{-1}$) [26] but markedly less than cell lines from solid tumours, for instance human adenocarcinoma gastric cells, SGc 7901

TABLE 2

Thermochemical characterization of human T-lymphoma cells interpreted and summarized from Bäckmann [51]

Heat flow	12.2 pW $cell^{-1}$
Oxygen consumption	0.98 fmol min^{-1} $cell^{-1}$
CR ratio	747 kJ mol^{-1} O_2
Glucose consumption	1.67 fmol min^{-1} $cell^{-1}$
Lactate production	3.8 fmol min^{-1} $cell^{-1}$
Lac/O_2 ratio	3.9
Pyruvate production	0.07 fmol min^{-1} $cell^{-1}$
Pyr/O_2 ratio	0.07
$\Delta_r H_{(ox+Lac+Pyr)}$	469 + 246 + 10 = 725
Glutamine consumption	0.28 fmol min^{-1} $cell^{-1}$
Glutamine production	0.05 fmol min^{-1} $cell^{-1}$
Aerobic catabolic carbon flux	3%
ATP content	3.2 ± 0.4 amol $cell^{-1}$
ATP turnover	160 amol ∞ ATP s^{-1} $cell^{-1}$

(40 pW cell^{-1}) [74] and human melanoma cells (80 pW cell^{-1}) [75]. From heat and oxygen measurements, it was possible to calculate the CR ratio which was so exothermic as to show an intensive participation of glycolytic reactions under aerobic conditions. Bäckman [51] estimated lactate and pyruvate production (Table 2) and, from their ratios to oxygen consumption, it is calculated from eqn. (4) that the combined aerobic and anaerobic enthalpy change ($\Delta_r H_{ox+Lac+Pyr}$) is 734 kJ mol^{-1} O_2, very close to the CR ratio. There is thus a full account of the terminal glycolytic processes.

The molar enthalpy changes, $\Delta_r H_{O_2}$, of oxidative catabolism are relatively similar [57] and, if several are proceeding simultaneously, they contribute proportionally to the CR ratio. Bäckman [51] calculated that with HEPES as buffer, the oxycaloric equivalents for complete oxidation of glucose is −503 kJ mol^{-1} O_2, and for glutamine to lactate, −463 kJ mol^{-1}, and to pyruvate, −445 kJ mol^{-1}. From radiocarbon studies, he showed that most of the glucose underwent glycolysis to lactate ($\Delta_r H_{Lac}$ = −76.5 kJ mol^{-1} in HEPES buffer). L-glutamine, conversely, was more importantly a respiratory substrate, with 23% oxidized to $^{14}CO_2$ and the rest accumulating as pyruvate and lactate (Table 2). The first step in glutaminolysis is deamination to glutamate ($\Delta_r H_{Glu}$ = −22 kJ mol^{-1} in HEPES buffer) and radioisotope studies showed that 23% was metabolised to this amino acid which, in glutaminolysis, is further deaminated before entering the TCA cycle. The molar enthalpy changes and the biochemical data were inserted into the enthalpy balance equation (eqn. (1)) to give a calculated heat flow of 7.8 pW cell^{-1} compared with the actual heat flow of 12.2 pW cell^{-1} (Table 2). Of the "unexplained" heat flow, 4.2 pW cell^{-1} could be reckoned from the oxidation of unknown substrates by oxygen consumption surplus to the catabolism of glucose and glutamine, using an average oxycaloric equivalent of −450 kJ mol^{-1} O_2 [57]. It will be recalled that T-lymphoma cells were grown in medium containing calf serum. Mammalian cells are said to "prefer" amino acids and fatty acids to carbohydrates for respiratory catabolism [76] and they are abundant in serum. As an interesting aside, this study provides a case study for those with intracellular partitions and regulation on their minds [77].

Bäckman's study [51] illustrates two further points. Firstly, because the enthalpy changes per mole of carbon are 20 times higher for oxidative processes than for glycolysis, the former account for a large part of the heat flux but only a minor fraction of the carbon flux. Oxidative processes were responsible for 60% of the heat flow but only 3% of the carbon flux in T-lymphoma cells [51]. Secondly, because in catabolic processes molar enthalpic energy changes are similar to molar Gibbs energy changes [78], heat flow from catabolic reactions reflect the amount of potential energy stored as ATP. Each pathway has a mechanistic molar stoichiometry for ATP, and it is thus possible to calculate the molar ATP yield for the

pathways. For those operating in T-lymphoma cells, the range was 12.6–13.5 μmol ATP J^{-1}. D-glucose and L-glutamine catabolism contributed 47% and 17% respectively to the energy requirements of these cells. Using an average or 13 μmol J^{-1} for catabolism of these two substrates, it was calculated that the total cellular ATP turnover was 1.6×10^{-16} mol s^{-1} (Table 2). According to Berry et al. [77], it is now generally accepted that mammalian thermogenesis occurs almost entirely in association with scalar chemical reactions involving the synthesis or breakdown of ATP [79].

TOWARDS EFFICIENCY OF CELLULAR ENERGY TRANSFORMATION

L929-derived LS mouse fibroblasts are adapted to grow in suspension using a low calcium medium. Under standard conditions in a Vibromixer flask, the doubling time was found to be 22.5 h but the cells had a highly exothermic CR ratio of -836 kJ mol^{-1} O_2 [80]. It was possible that the intensive participation of glycolysis was at least in part owing to poor aeration because the cells were stirred in large volumes normally for batch harvest and simply pumped to the microcalorimeter. Improvements to the design of the culture vessel and continuous aeration reduced the exothermic CR ratios to -633 kJ mol^{-1} O_2 [81] and increased heat flow per cell from 34 [81] to 43 pW (Table 3), resulting in an improved generation time of 18.3 h. The major end-product of glycolysis was, not surprisingly, lactate but there was a small contribution from pyruvate (Table 3), possibly as a result of glutaminolysis, a pathway more recently recognised as important in cells [83]. The calculated total enthalpy change, $\Delta_r H_{(ox+Lac+Pyr)}$, was very similar to the observed CR ratio and showed that these cells undergo simultaneous aerobic and anaerobic glycolysis. There is also evidence that LS cells can metabolise the lactate aerobically [81] and that replacement of glucose in the medium by lactate reduced the CR ratio to less than -500 kJ mol^{-1} O_2. A similar result has been found for rat hepatocytes which had a CR ratio of -461 and -477 kJ mol^{-1} O_2 for endogenous metabolism in respectively the absence and presence of purified bovine serum albumin [84], but a ratio of -563 kJ mol^{-1} O_2 when incubated in Krebs–Henseleit buffer containing 40 mmol dm^{-2} glucose [85]. This was reduced to -471 kJ mol^{-1} O_2 by addition of lactate and pyruvate at appropriate steady state levels.

The ATP content of LS cells was estimated at 5.1 fmol $cell^{-1}$ (Table 3), somewhat greater than human neutrophils (1.2 fmol $cell^{-1}$) [68] and the tiny bovine sperm (0.12 fmol $cell^{-1}$) [86], and surprisingly much more than T-lymphoma cells (0.003 fmol $cell^{-1}$) [51]. Taking the average ATP yield per J calculated for the latter of 13 μmol J^{-1}, this means a turnover time of 0.02 s compared with 9.1 s for LS cells (Table 3).

TABLE 3

Thermochemical data and fluxes of adenine nucleotides for anchorage-independent L929-derived LS mouse fibroblasts suspended in Dulbecco phosphate and bicarbonate buffered saline, pH 7.4 ± 0.01 and 37°C, containing 5.5 mmol dm^{-3} D-glucose and 2 mmol dm^{-3} L-glutamine; summarized from Kemp [81, 82]

Adenine nucleotide and inorganic phosphate content (μmol g^{-1} protein)	
ATP	7.1 ± 0.4
ADP	1.5 ± 0.1
AMP	1.25 ± 0.1
P_i content	4.6 ± 0.3
ATP/ADP ratio	4.5 ± 0.3
Adenylate energy charge	0.8 ± 0.02
Phosphorylation potential (M^{-1})	1405 ± 220
Actual Gibbs energy change of ADP phosphorylation, $_eG_{ATP}$ (1 mmol dm^{-3} Mg^{2+}) (kJ mol^{-1})	49.2
Protein (pg $cell^{-1}$)	490
Heat flow (pW $cell^{-1}$)	43
Heat flux (mW g^{-1} protein)	60
Oxygen flux (nmol s^{-1} g^{-1} protein)	95
CR ratio (kJ mol^{-1} O_2)	632
Lactate flux (nmol s^{-1} g^{-1} protein)	200
Lac/O_2 ratio	2.1
Pyruvate flux (nmol s^{-1} g^{-1} protein)	16
Pyr/O_2 ratio	0.17
$\Delta_r H_{(ox+Lac+Pyr)}$	469 + 132 + 24 = 625
ATP turnover [a] (μmol ∞ ATP s^{-1} g^{-1} protein)	0.78
ATP turnover [b] (μmol ∞ ATP s^{-1} g^{-1} protein)	0.80

[a] Calculated from heat flux (see text).
[b] Calculated from the catabolic coupling flow (see text).

Calculating the phosphorylation potential by the classical equation [87] gives a value of 1405 M^{-1} (Table 3) but the same data can be used to express the conjugate ergobolic force of ADP phosphorylation to ATP — the actual Gibbs energy change, $\Delta_e H_{ATP}$ [88]

$$\Delta_e G_{ATP} = \Delta_e G^{\ominus\prime} + RT \ln\left(\frac{ATP}{ADPP_i}\right) \quad (6)$$

where $\Delta_e G^{\ominus\prime}$ is the standard Gibbs energy of ADP phosphorylation (30.54 kJ mol^{-1}) and subscript e denotes an energy-conserving (ergobolic) reaction [52, 88].

Knowledge of the ergobolic force is necessary to considerations of efficiency and to Gnaiger's concept of power and economy in cells [88]. Central to this idea is the statement that total cellular energy flow is functionally related to the coupling of input to output flows and of input forces to compensatory forces. In the overall process, the high driving force

of the catabolic half-reaction is coupled to the ergobolic reaction, which is in turn coupled to the anabolic half-reaction [52]. Each has a force and a flow and the product of them is power. The partner to ergobolic force is ergobolic flow expressed as the rate of ATP production, ${}_{e}\dot{N}$ (μmol ATP h^{-1}).

Catabolic flow, ${}_{k}\dot{N}_{l}$, is measured as catabolic substrate consumption ${}_{k}\dot{N}_{s}$, as the rate of oxygen consumption ${}_{k}\dot{N}_{O_2}$, or as the rate of accumulation and excretion of end-products ${}_{k}\dot{N}_{p}$ [88]. The catabolic input force is expressed as the Gibbs energy change per mole of substrate, $\Delta_{k}G_{s}$. Simplifying the case of LS cells to glucose as the substrate, the Gibbs energy change is -2903 kJ mol^{-1} [89]. Because 6 moles of O_2 are consumed per mole of glucose in aerobic catabolism, the catabolic force of oxygen, $\Delta_{k}G_{O_2}$, is $-2903/6 = -484$ kJ mol^{-1}. This value is very similar to the oxycaloric equivalent of glucose, -469 kJ mol^{-1}, for reasons of small entropy change as discussed by Prigogine [78]. In passing, this means that, for aerobic catabolism *only*, measurement of heat dissipation directly yields the catabolic power [90].

The link between ergobolic and catabolic flows and forces is provided by the mechanistic molar stoichiometry, $\upsilon_{ATP/i}$, where i is the catabolic reactant. Gnaiger [88] defines the catabolic force efficiency, ${}_{k}f$, and flow efficiency, ${}_{k}j$, as the ergobolic/catabolic flow and force ratio, respectively, normalized for the ATP stoichiometry

$${}_{k}f = \frac{-\Delta_{e}G}{(\Delta_{k}G_{i}/\upsilon_{ATP/i})} \tag{7a}$$

$${}_{k}j = \frac{{}_{e}\dot{N}}{({}_{k}\dot{N}_{i}\upsilon_{ATP/i})} \tag{7b}$$

In eqn (7a and b), the two expressions in brackets are the catabolic coupling force and flow, respectively, normalized for the mechanistic ATP-coupling stoichiometry [52]

$$\Delta_{k}G = \Delta_{k}G_{i}/\upsilon_{ATP/i} \tag{8a}$$

$${}_{k}\dot{N} = {}_{k}\dot{N}_{i}\upsilon_{ATP/i} \tag{8b}$$

From eqns. (7b) and (8b), it is seen that for the fully coupled process (${}_{k}j = 1$), the ergobolic rate of ATP production, ${}_{e}\dot{N}_{i}$, is numerically identical to ${}_{k}\dot{N}$, which is then the rate of ATP turnover (μmol ∞ ATP h^{-1}). From the data available for LS cells (Table 3) and using the ATP stoichiometric coefficient for oxygen of $37/6 = 6.17$ ATP/O_2 and, for glycolysis, the mechanistic stoichiometries of 1.0 ATP/Lac and 1.0 ATP/Pyr, ATP turnover is $(95 \times 6.17) + (200) + (16) = 802$ nmol ATP s^{-1} g^{-1} protein, with

a turnover time of 8.85 s which compares favourably with that calculated from heat flux measurements (9.1 s).

If LS cells only catabolised glucose aerobically, the aerobic catabolic force efficiency is obtained from eqn. (7a)

$$_{k}f = \frac{49.2}{484/6.17} = \frac{49.2}{78.4} = 0.63$$

According to Gnaiger [88], the product of flow efficiency and force efficiency is the input–output power efficiency or ergodynamic efficiency η, which equals the force efficiency at $_{k}j = 1$

$$_{k}\eta = {}_{k}j_{k}f = \frac{-{}_{e}P}{{}_{k}P} \tag{9}$$

where $_{e}P$ is ergobolic power output and $_{k}P$ is catabolic power output. From this point, it is possible to discuss power and economy strategies [88] but the LS cell system must first receive further thermochemical characterization.

TOXICOLOGICAL ASPECTS

Calorimetry has increasing application to the study of pharmaceutical drugs and other bioactive agents on animal cells [36, 38]. In most of the work in this field, heat measurement has been regarded simply as a non-destructive and unspecific indicator of metabolic activity, the active compound causing changes in total enthalpy, $\Delta_{t}H_{Met}$. In this fashion, it was found that the anti-psoriasis drug, anthralin, reduced the heat flow of human keratinocytes from 83 pW cell^{-1} to 7 pW cell^{-1} at a concentration of 0.5 μmol dm^{-3}, but unfortunately also caused 13% cell death [91]. When testing a series of anthralin analogues and derivatives, it was discovered that DT 86 at the same concentration reduced heat to <1 but had only a 2% effect on viability.

An important class of drugs is the β-adrenoceptor blockers and the effect of several of them at their therapeutic doses has been tested on the thermogenesis of human muscle tissue biopsies [92]. A non-selective β-blocker, propranolol, reduced heat production by 25%, whereas the β_{1}-selective atenolol and the β_{2}-agonist pindolol had no effect. Mitogenic lectins have also been assayed by cellular heat production. Concanavalin A increased heat flow at 1 μg cm^{-3} but caused a decrease at 10 μg cm^{-3}. Low concentrations of this lectin caused metabolic activation. The anti-proliferative activity of phytohaemagglinin (PHA) isolectins on T-lymphoma cells [93] was foreshadowed by the finding that 0.1 μg cm^{-3} PHA-L_4 strongly and quickly (<30 min) inhibited heat flow but that a 200-fold increase in concentration of the E_4 isolectin was required to produce the same effect. Schön and Wadsö [94] went on to study the heat

effect of the antineoplastic drug, methotrexate, and showed that the dose-dependent response on T-lymphoma cells was correlated with trypan blue exclusion. This type of investigation was then extended to other cancer drugs, Ara-C, cisplatin, vinblastine, chlorambucil and prednimustine, and correlations were found between microcalorimetric response and cell death [95], suggesting that the technique is a reliable assay system for this type of drug.

As has been established for human neutrophils [68] and 2C11-12 mouse macrophage hybridoma cells [96], a burst of heat production is an excellent measure of activated phagocytic cells and it is reasonable to suppose that the heat profile will differ both qualitatively and quantitatively depending on the nature of the triggering agent. This supposition was borne out in the phagocytosis of pseudo-immune complexes formed by staphylococcal protein A in the presence of serum [97]. Different protein A/IgG ratios produced dissimilar "power–time" curves. In a more recent study [98], it was shown that the increase in heat production of human neutrophils was directly proportional to the number of *Saccharomyces cerevisiae* cells *and* to the concentration of opsonizing serum. In a different approach, the heat production of rabbit alveolar macrophages [99] was used as a short-term test for the cytotoxicity of metal and non-metal particles [100]. Manganese dioxide particles were found strongly to decrease heat flow whereas those of titanium dioxide had no effect. Quartz particles increased metabolic activity. Further in this catalogue, heat flow of the S3G strain of HeLa cells has been utilized to study the dose–response curves of the heavy metals Hg, Cd, Cu, Pb, Cr, V and As, obtaining the concentrations effective in inhibiting heat production by 50% [101]. The results had the same rank order as more conventional assays but were more sensitive. A similar conclusion was drawn for the investigation into using the CR ratio of LS cells to predict the human toxicity of acetaminophen (paracetamol), aspirin, diazepam, digoxin and ethanol [81, 102]. All in all, microcalorimetry seems to be a promising and sensitive technique to employ in the developing field of cytotoxicology.

CONCLUSION

This critical review of the more important contributions of calorimetry and, especially, enthalpy balances to our knowledge of cellular physiology underlines the potential of heat measurements. It is the Gibbs energy changes that are central to an understanding of bioenergetics [103]; physiological calorimetry only supplies a limited input to this understanding. Nevertheless, measurements of enthalpy changes do complement valuably our knowledge of the thermodynamics of cellular metabolism.

For the scientist in a hurry an extended summary of this review is published elsewhere [104].

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