Journal of Thermal Analysis and Calorimetry, Vol. 60 (2000) 831–843

'GIE ME AE SPARK O' NATURE'S FIRE'* AN INSIGHT INTO CELL PHYSIOLOGY FROM CALORIMETRY

R. B. Kemp

The Institute of Biological Sciences, Edward Llwyd Building, The University of Wales, Penglais Aberystwyth, SY23 3DA, United Kingdom

Abstract

A formal explanation of Mayer's enthalpy balance method as applied to cell physiology is given with particular reference to striated muscle tissue. The approach is then extended to animal cells growing in a bioreactor, showing its use to optimise their growth and the production of therapeutic proteins, as well as defining the correct physiological conditions. Attention is then drawn to the value of the calorimetric-respirometric ratio in defining the anaerobic pathways operating simultaneously with respiration under fully aerobic conditions. An important field now is to resolve the metabolic costs of cell signalling cascades, an area that could demonstrate the coming importance of integrated circuit calorimetry.

Keywords: animal cells, calorimetry, cell signalling cascades, drug action, enthalpy balance, heat-flow rate, integrated circuits, therapeutic proteins

Introduction

The importance of calorimetry to the study of animal physiology was recognised over 200 years ago by the founder of modern chemistry and one of the earliest pioneers in calorimetry, Lavoisier [1]. With the mathematician Laplace, he calculated the total heat produced by a guinea pig in a given period of time by applying the formula for the latent heat of melting to the quantity of melted water collected in the ice calorimeter. He also showed that oxygen was vital to animals in the experiment in which a perched bird sharing a bell jar with a lighted candle expired (keeled over) at the same time as the candle went out. The conclusion was that 'respiration is a combustion, a slow one to be precise'.

The physician Mayer first became interested in heat some 60 years after these experiments when he noted that the venous blood taken from European patients newly arrived in Java was a brighter red colour than that from people in Germany [2]. He was aware of Lavoisier's theory and regarded the changes in colour of the blood

* Robert Burns

1418–2874/2000/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

as a 'sensible sign' that there was an oxidation in it (actually, we now know most of the oxidative processes are in tissues other than blood, such as muscle). Since the temperature of man is constant, he reasoned that the heat produced must bear a quantitative relation to the heat loss – a relation, that is, to the temperature of the surrounding medium that explains the difference in blood colour. In 1842, following experiments with a horse that drove a machine, he deduced a value for the mechanical equivalent of heat and presented his theory of the conservation of energy. Mayer [2] developed a physiological theory of combustion in which there is a general balance between the amount of matter consumed and the evolution of heat, 'so as to occasion growth and the renewal of worn-out parts.' In this way, he first introduced the concept of the enthalpy balance method in terms of 'receipts and expenditure' that has proved so useful in determining several mechanisms in physiology.

The first successful proof of the application of the first law of thermodynamics to living systems was by Rubner [3] another 60 years later. He showed that a dog living for 45 days in a respiration calorimeter produced 72 588 kJ (18.7 W) of heat energy at the same time as it utilised 72 827 kJ of (net) chemical energy from nutrients, as calculated from the dog's respiratory metabolism ('slow combustion') and faecal and nitrogenous excretion. From this pioneering research, the enthalpy balance approach has been extended to tissues, especially striated muscle, and to cells originating in the body but adapted to grow in vitro. In the latter case, it is now possible to envisage an account being made of the cell signalling mechanisms that are so important in the homeostasis of cells in tissues and organs. The aim of this paper is to illustrate how the extraordinary advances made by Lavoisier, Mayer and others over the past two centuries will serve us well in the study of cell physiology into the next millenium. In so doing, I hope to convince the reader that the aptly named, famous Scottish poet Burns, who was a contemporary of Lavoisier, unintentionally made a prophetic excursion into science.

Thermodynamics of the enthalpy balance method

Most scientists are familiar with the principles of classical thermodynamics, but living systems undertake an irreversible process of growth that can only be adequately described by non-equilibrium (irreversible) thermodynamics. Growth requires energy, a relatively small amount of which is stored in the accumulation and structuring of matter [4]. The rest of it is expended on maintaining the physiological state of the cell [5] and in the energy transformations concerned with the functions of the cytoskeleton [6]. For these processes, cells consume substrates in a series of redox reactions that possess high quality, useful energy known as Gibbs energy, dG. In the energy transformations required for maintenance and cytoskeletal activity, the quality is decreased and the remaining energy is dissipated as heat. The amount of internal work (dW) during the growth process is generally small and the energy conservation in biomass accumulation is modest. For these reasons, in the strict terms of irreversible thermodynamics [7], the growth of animal cells can be described as a dissipative rather than as a completely irreversible process, dD [8],

$$dG=dW+dD \quad (1)$$

In this case, the dissipated energy is related to the change in heat by,

$$dQ=dD+dB \quad (2)$$

where dB is the change in the bound energy, which is the change in entropy (dS) times the absolute temperature (dB=T dS). Equation (2) is recognised in the following form in relation to the change in enthalpy, dH [8],

$$dH=dG+dB \quad (3)$$

From the foregoing, it can be seen that the metabolism of a living system can be measured by the heat-flux, using calorimetry, when the absolute magnitude of the molar bound energy is negligible.

There are several types of calorimeter (consult for instance [9]), but those based on the heat conduction principle [10] have the greatest value in terms of assessing metabolism because they directly measure the heat-flow rate, Φ (Φ=dQ/dt, where Q is heat). Thus, they convey information about the kinetics, as well as the thermodynamics, of the biochemical processes taking place in living matter. Calorimetric determinations are made at constant temperature and, because the living material is normally in excess physiological solution with a gaseous phase, there is no change in pressure. Thus, according to the first law of thermodynamics, the sum of the enthalpies of the specific metabolic reactions in the living material, the enthalpic flux (J_H), should balance the heat produced by those reactions in size-specific (X) terms, $J_H/J_{\Phi/X}=1$; this ratio is known as the enthalpy recovery. Care has to be taken to account for all the side reactions that occur in complex systems bathed in a physiologically buffered aqueous phase with dissolved gases. It should be noted that, by definition, the reaction system does not exchange energy in the form of work within the boundary of the thermodynamic system (internal work).

The enthalpic flux is a general term that could be regarded as being terminologically equivalent to the biochemical phrase, metabolic flux [11], that is often loosely called 'metabolic activity' [12]. It is defined as,

$$J_H=\Delta_r H_B J_{B,r} \quad (4)$$

where $\Delta_r H_B$ is the molar reaction enthalpy and $J_{B,r}$ is the reaction (r) flux in which the subscript B indicates that any given reaction stoichiometry is divided by ν_B such that the stoichiometric form is obtained with $|\nu_B|=1$.

The first term on the right-hand side of Eq. (4) is the sum of the molar enthalpies of species i under the cell culture conditions,

$$\Delta_r H_B=\sum_i(\nu_i H_i) \quad (5)$$

where H_i is the partial molar enthalpy of species, i. However, this quantity is concentration-dependent and thus it is not possible to assign to it a value in a dynamic system, such as a living cell [11]. In practice, it is replaced by the concentration-indepen-

dent standard molar enthalpy, H_i^o, to give the aggregate standard molar enthalpy in Eq. (5).

The second term on the right-hand side of Eq. (4) is,

$$J_{B,r} = \frac{d_r \xi}{X dt} \tag{6}$$

where X is the biomass and ξ is the advancement or extent of the reaction,

$$d_r \xi = \frac{d_r n_i}{\nu_i} \tag{7}$$

where n_i is the amount of species, *i*. It is Eq. (6) that reveals the kinetic meaning to the term enthalpy flux.

It may seem to be an impossible task to summate the many thousands of reactions that take place in the cells of living material, but in practice it would appear that the enthalpy of anabolism is negligible [13]. Thus, only the catabolic reactions need to be considered and it would appear that there is only a very limited number of significant substrates that, while they participate in many reaction pathways, give only a few products. Thus, advantage can be taken of Hess's law of constant heat summation in making the calculations. Since there is a constant process of, in Mayer's terms, growth and renewal in living systems, the overall process can be described by a chemical equation, known as the growth reaction [14, 15]. The enthalpy balance method validates the description of such reactions.

When considering the reasons for the values of the enthalpy recovery that are other than unity, there are two possible causes in terms of enthalpy flux. If the yield is less than unity, then the thermochemical analysis of the energy transformations has failed fully to account for the calorimetric measured heat-flux. A value for the enthalpy recovery greater than unity indicates that there remained some undetected endothermic reactions. These are not the only reasons, however, for there can be changes in bound energy that would be detected as changes in enthalpy (Eq. (3)). As stated earlier, living matter constitutes an irreversible system in which there is no net work. Since the calorimetric measurements are at constant temperature and effectively at constant pressure, then the changes in enthalpy would be detected as heat, $\Delta H = Q_{T,P}$. The importance of this effect is illustrated by a brief account of the calorimetric studies of muscle.

The classical system of striated muscle

It is remarkable to consider that a century ago, the prevalent view among physiologists was that cells were tiny Carnot engines, converting the energy stored in a temperature gradient into a form of work. It was Hill in 1912 [16] who unequivocally established that the force, as it is now known in non-equilibrium thermodynamics, is due to the molar Gibbs energy. Over the next 50 years [17], he proceeded to elaborate on this theme using striated muscle as his experimental system.

A muscle consists of many large, syncytial cells called fibres. It was found that the heat-flow rate of striated muscle was 100 to 1000 times greater than when it was at rest. The initial heat lasts for a few seconds and is followed by a recovery period of up to an hour during which there is a continuous production of heat [18]. Unlike in 'non-muscle' cells, there is a considerable amount of external work done by this contractile system and an additional term, *h,* must be added to Eq. (4) to allow for the measured work. This component was held to account in the initial phase of heat production. By assays of the substrates and products, with calculation of the attendant aggregate enthalpy flux, it was shown that much of the initial heat was derived from the tightly coupled conversion of the phosphogen, creatine phosphate, to ATP to give the energy for the contraction. The same method indicated that the second, longer phase of lesser heat production was due to the restoration of the normal level of creatine phosphate, using ATP synthesised by the catabolic pathways.

Making an account of the chemical reactions by the enthalpy balance approach during the contraction-relaxation cycle gave an enthalpy recovery that failed to explain all the sources of the heat [18]. In the frog sartorius muscle, for instance, the unexplained heat was 21% of the total produced during the isometric tetanic contractions at 0°C [19] (Fig. 1). In order to consider the source(s) of this heat, it must be remembered that contraction is initiated by the depolarisation at the synapse spreading to the T-tubule system. This change causes the Ca^{2+} bound to the calsequestrin in the sarcoplasmic reticulum to be released into the sarcoplasm and bind to the troponin C [20]. It is the change in bound energy (Eq. (3)) caused by the movement of the ions that accounts for the majority of the unexplained heat [19]. In this respect, it has been shown that the majority of the heat produced by isolated neurons is also due to changes in bound energy caused by the ion movements that generate the action potential [21].

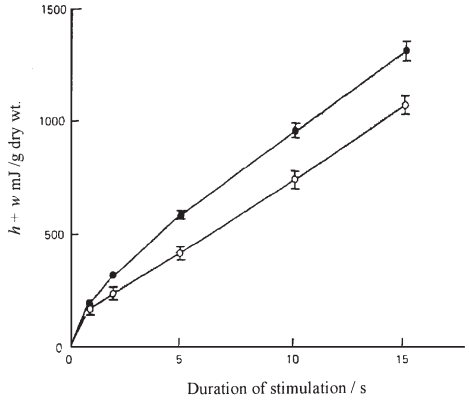

Fig. 1 This shows the heat+work produced (•) during a 15 s tetanus of a frog sartorius muscle at 0°C and the heat+work that is due to the measured PCr splitting (o). The bars show 1 SEM (Original data from [18]). (Reproduced from [19] with permission)

Growth of animal cells in culture

There is an increasing demand for the large-scale culture of animal cells in order to produce recombinant glycoproteins, monoclonal antibodies and vaccines. The problem has been that these cells have complex nutritional requirements and are sensitive to a deteriorating culture environment, a contributory cause of which is the catabolic products, particularly lactate and ammonia [6, 11, 22]. These factors have limited the cell growth and the production of the required macromolecules. In order to improve the yield, it is necessary to know more about the metabolic state of the cells while they are growing in batch culture. This has not been possible because there is a paucity of on-line biosensors [23].

Little attention has been paid to the possibility of heat-flux as a probe despite the fact that, as shown in Eqs (3) and (4), this variable measures the metabolic flux. Guan *et al*. [24] designed and assembled an on-line heat-flux probe. It consists of an ex situ flow microcalorimeter, through which there is pumped a continuous stream of cell suspension, and a radio frequency dielectric spectrometer to assay for the volume fraction of viable cells by measuring the capacitance of the cell culture.

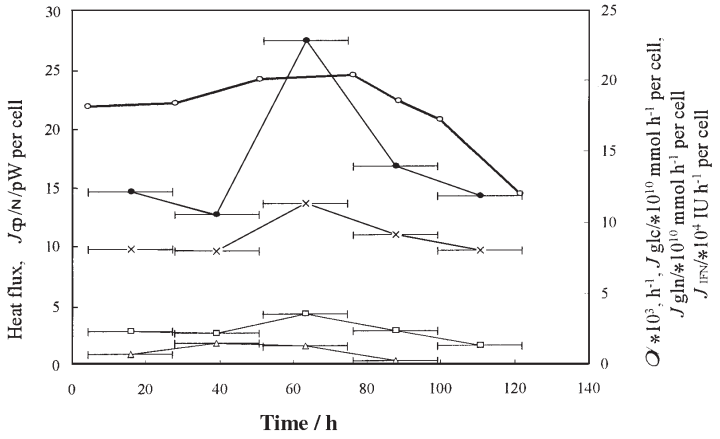

Fig. 2 Comparison of the heat-flux ($J_{\Phi/N}$) with the fluxes of glucose (J_{glc}), glutamine (J_{gln}) and IFN-γ (J_{IFN}), as well as the specific growth rate (μ) during the batch cultivation of CHO 320 cells in suspension. Heat-flux (o), glucose flux (□), glutamine flux (Δ), IFN-γ flux (×) and specific growth rate (•). The bars indicate the period over which the discrete off-line measurements were made to give the individual average values for fluxes. (Reproduced from [24] with permission)

The new probe was used to monitor the growth of genetically engineered CHO 320 cells, producing recombinant interferon-γ (IFN-γ) in batch culture, and the results were compared *vs.* changes in the concentrations of the major substrates and products [25] (Fig. 2). As described earlier, the chemical events depicted in Fig. 2 can be described as a generalised growth reaction (r) that relates the material fluxes of substrates and products to the enthalpy flux:

$$\nu_{S_1}\text{glucose}+\nu_{S_2}\text{glutamine}+\nu_O O_2 \rightarrow \nu_X\text{biomass}+\nu_p\text{IFN-}\gamma+\nu_L\text{lactate}+\nu_C CO_2+$$
$$+\nu_N NH_3+\nu_H H_2O+\nu_X\left(\int_{t_1}^{t_2} J_{H,r}\,dt\right) \quad (8)$$

in which there is the explicit use of the stoichiometric coefficients, ν_i for the ith species. It will be noticed in Fig. 2 that the fluxes change with time, but the stoichiometric coefficients constitute a set of constant values only for a given metabolic state and, thus, must change to reflect different such states [25]. This means that a given stoichiometric reaction only applies as long as there are no alterations in the yield [24], including that of heat ($Y'_{Q/X}$). It formalises the biothermochemical events of the batch culture shown in Fig. 2.

As established earlier, any given growth reaction can be validated by the enthalpy balance approach. Bearing in mind the fact that the enthalpy of anabolism is negligible [13, 14], the growth reaction depicted in Eq. (8) neglects the contribution of the amino acids other than glutamine. This is oxidised principally to lactate (glutaminolysis [26]), as well as being incorporated into the biomass, mainly in the nucleic acids. Stoichiometric catabolic half-reactions can be written from the data depicted in Fig. 2 for each time period [27]. For instance, at 52–76 h the half-reaction for the redox reaction of glucose and glutamine to form lactate, ammonia and carbon dioxide was:

$$C_6H_{12}O_6+0.085C_5H_{10}N_2O_3+0.64O_2=1.91C_3H_6O_3+0.68CO_2+0.71NH_3+0.43H_2O \quad (9)$$

The aggregated enthalpy flux for these half-reactions was determined from the difference between the standard enthalpies of formation ($\Delta fH°$) of the products and the reactants, together with the reaction flux, $J_{B,r}$ (Eq. (6)). From the data for the time period used to construct Eq. (9), the heat-flux was 23.1 pW per cell and the calculated enthalpy flux was 23.6 pW per cell, an enthalpy recovery of 1.02. On this firm basis, it was possible to construct the growth reaction as follows:

$$C_6H_{12}O_6+0.377C_5H_{10}N_2O_3+0.44O_2=$$
$$3.52CH_{2.0}O_{0.82}N_{0.18}+1.3C_3H_6O_3+0.46CO_2+0.12NH_3+0.29H_2O \quad (10)$$

The most obvious fact that emerges from Eq. (10) is that the cellular requirement for glucose and glutamine at this particular stage in their growth (52–76 h) was 2.65:1, whereas the medium contained these substrates in the ratio of 5.5:1. This obviously explains why glutamine was exhausted before glucose and gave the first basis to the design of an improved medium that extended to many of the other components in it, particularly the amino acids [25]. The use of this new medium resulted in higher specific growth and an improved yield of IFN-γ while the cells consumed markedly less glucose (Table 1). There was also a 50% reduction in the lactate flux and this probably contributed to the improved cell growth and productivity. The decreased amount of lactate produced was probably due to a reduced need for biosynthetic precursors from glucose because more of these substrates were supplied in the medium. Thus,

the physiology of the cells was maintained at a higher state for a longer period because the environmental conditions were better suited to the cells.

Table 1 Representative average material fluxes of the major metabolites and the specific growth rate (μ) for CHO 320 cells growing in the original and the improved media in the period of 36 to 60 h during batch culture in a bioreactor (modified from data in [25])

Media	Fluxes				
	μ/h^{-1}	Glucose/ mol s^{-1} per cell	Glutamine/ mol s^{-1} per cell	Lactate/ mol s^{-1} per cell	IFN-γ/ IU h^{-1} per cell
Improved	0.05	$4.1\cdot10^{-17}$	$1.7\cdot10^{-17}$	$5.3\cdot10^{-17}$	$6.1\cdot10^{-4}$
Original	0.03	$5.8\cdot10^{-17}$	$1.8\cdot10^{-17}$	$1.1\cdot10^{-16}$	$4.8\cdot10^{-4}$

The use of the growth reaction offered an opportunity to verify the reasonable assumption in Eq. (8) that in one sense heat-flux can be considered as being equivalent to a stoichiometric coefficient [24]. It will be seen from Eq. (8) that growth can be represented by the set of stoichiometric coefficients with that for IFN-γ being included with biomass because, as products of translation, they cannot be separated [25]:

$$\vec{\nu}=(\nu_{S_1}\nu_{S_2}\nu_O 1(\text{cell})\nu_L\nu_C\nu_N\nu_H Q) \tag{11}$$

where Q is the integral of the heat-flow rate. Since Eq. (3) emphasises that there is a one-to-one corresponding relationship between the metabolic flux and the stoichiometry of the growth reaction, it follows that:

$$\frac{1}{X}\frac{\mathrm{d}\xi}{\mathrm{d}t}\leftrightarrow\vec{\nu} \tag{12}$$

Since it has already been shown in Eq. (4) that there is a first law equivalence between metabolic flux and heat-flux, J_{th}, Eq. (12) can be converted as:

$$\nu_i=f(J_{th}) \tag{13}$$

The strength of this relationship can be seen from data for the oxidation of the substrates in relation to heat-flux for the following stoichiometric ratios in the period 0–120 h depicted in Fig. 2.

$$\nu_{Glc}/\nu_O=0.0581J_\Phi^2-2.0949J_\Phi+19.663 \tag{14}$$

$$\nu_{Gln}/\nu_O=0.0279J_\Phi^2-1.045J_\Phi+9.8561 \tag{15}$$

From Eqs (14) and (15), Fig. 3 is drawn to illustrate that, for the specified range, heat-flux is a monotonically increasing function of these ratios and therefore a valid probe of metabolic flux.

Advantage could be taken of the heat-flux probe to maintain a batch culture for a long period of time by a small decrease in the trace from the on-line biosensor triggering the software-controlled addition of more nutrients. Early intervention in such a fed batch culture would ensure the continued production of target protein. The success of this approach is shown in Fig. 4, depicting the addition of additional medium. On-line

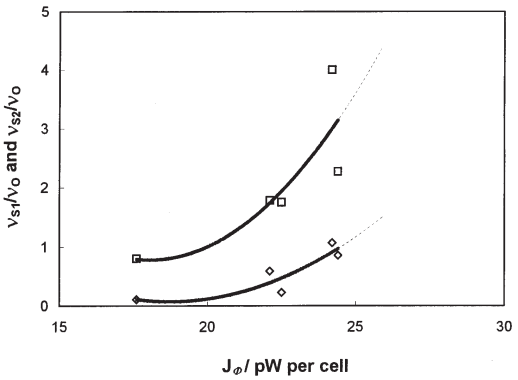

Fig. 3 The heat-flux over a specified set of values is compared *vs.* the stoichiometric ratios for the consumption of glucose (◇) and glutamine (□) to oxygen

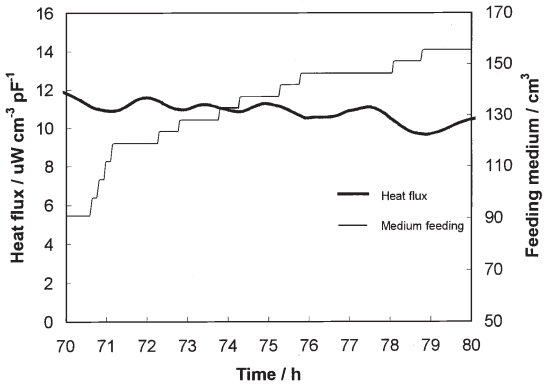

Fig. 4 This shows a small section of the heat profile for a fed-batch culture (from 70 to 80 h) to illustrate that the medium feeding was triggered by the declining heat-flux values over the 1-h assessment period. The heat-flux was restored, to a varied extent, by this feeding strategy

data from continuously stirred tank reactors is noisy in electronic terms. Although this means that smoothing of the combined calorimetric and dielectric signals by the moving average technique must be employed, it cannot be allowed that the trigger is so sensitive to change in signal that the nutrient is added in a manner similar to that for continuous cultures. The result is that the culture slowly deteriorates with time, as can be seen by the declining heat flux measurement over a 10-h period (Fig. 4).

Signalling pathways

In just the same way as signal transduction in nerves was a primary concern in animal physiology for 50 years, so the myriad of signal cascades in cells is an important topic

to cell physiologists at the dawn of the new millenium. The cell surface has receptors for a range of different primary messengers, from hormones and growth factors to steroids and cytokines. It is not possible to detect the enthalpy of binding of the messenger to the receptor on the plasma membrane of living cells. The heat produced by the triggered cascade should be capable of measurement, however, because the process usually involves multiple phosphorylation steps that require the intracellular synthesis of ATP from ADP by catabolic pathways.

Eftimiadi and Rialdi [28] mimicked the phagocytic respiratory burst in human neutrophils with a phorbol ester and showed, by the enthalpy balance method, that the increased heat-flux was due to a greater intensity in the hexose monophosphate shunt for producing the necessary NADPH for the formation of the lethal oxygen species that kill foreign organisms (and cause inflammation). The same explanation was found for the phorbol-triggered respiratory burst in 2C11-12 mouse macrophage hybridoma cells [29]. There were very considerable (tenfold) changes in heat-flux due to the involvement of the complete cell.

The alteration to the heat-flow rate that occurs with the cellular interaction of most primary messengers is likely to be much smaller, however, and not so amenable to detection. Nevertheless, Schön and Walum [30] have shown that the human growth hormone (hGH), at a physiologically valid concentration, increased the initial heat-flow rate of the Ba/F3:6 lymphoid cells transfected with the hGH receptor, in order to increase the number of them at the cell surface. The change in heat-flow rate was 'immediate, albeit rather slow,' and amounted to 10% within 1 h. It was not due to increased cell numbers, because animal cells have doubling times of many hours, rather than minutes. There was a later, second-phase rise in heat-flow rate, however, that correlated with the hormone-induced increase in cell proliferation. It is necessary now to make account of this change in terms of metabolism and verify it by the enthalpy balance method. This will be a valuable contribution to cell physiology.

The way forward

As part of an increasing awareness that heat-flux measures the metabolic activity of cells, it is to be hoped that the pharmaceutical industry will adopt the probe that uses this property to monitor large-scale, animal cell cultures. Even better, it should be recognised that the ideal solution to the problem would be to associate this probe with one that estimates the oxygen uptake rate (OUR) of the cells, a powerful combined tool first recognised 200 years ago by Lavoisier! The calorimetric-respirometric (CR) ratio is the experimental equivalent of the calculated oxycaloric equivalent. It has a value calculated from the heats of formation for carbohydrates, amino acids and fatty acids of 450±5% kJ mol^{-1} O_2 [31]. This similarity is due to Thornton's established regularity [32] for the combustion of carbon compounds that gives an average of 115 kJ per equivalent of the available electrons [33]. A CR ratio close to the oxycaloric equivalent for a particular substrate or set of substrates indicates that only aerobic processes are taking place but a significantly more negative (more exothermic) ratio indicates that anaerobic processes are taking place in the cells. It has been shown that this

is surprisingly frequent in animal cells in vitro [22] with the most common product being lactate (see earlier) from the Embden–Meyerhof–Parnes (glycolytic) pathway. The intensity of this pathway appears to be due to the need by cells for biosynthetic precursors not found, or at least present in inadequate amounts, in the medium [24]. Assays of the anaerobic substrates and products and calculations of the contributions of the respective pathway will give the basis of an enthalpy balance [31].

As a diagnostic tool in the pharmaceutical industry, calorimetry suffers from low throughput at a time when there is the robotic monitoring of the effects of compounds on animal cells growing in 384–well, and even 1536–well, microtitre plates. In this regard, GlaxoWellcome is already experimenting with infrared thermography [34] for C3H10T1/2 adipocytes that produce considerable heat. It was found possible to detect changes in the heat caused by the β_3-adrenergic agonists, CL316243 (anti-obesity agent) and troglitazone (antidiabetic drug). However, the detection limit is two to three orders of magnitude less than for microcalorimetry (not to mention nanocalorimetry facilitated by improved amplifiers).

A small thermoelectric device [35] has been demonstrated to have a detection limit 100-fold lower than that for thermography. It is a hybrid biosensor with the cells attached to a highly sensitive, thin-film, thermopile transducer consisting of antimony and bismuth metals in regular patterns to form pairs of thermocouple junctions on a thin (38 μm) Mylar© support. In its initial form, it was envisaged as an immunoassay system and consisted of living mast cells with the IgE antigen recognition system, bound by natural extracellular matrix proteins to the active sensing junctions. However, a multiplexed version [35] has been promoted that would meet the requirements for the high throughput of physiological information.

It seems likely that recent developments in integrated circuit (IC) calorimetry [36] will allow, in comparison to conventional microcalorimeters, (i) small sample mass; (ii) a lower detection limit; (iii) high resolution for a succession of thermal events; (iv) faster equilibration time; and (v) the probability of multiple-channel equipment to match microtitre plates. As can be seen in Fig. 5, the integrated circuit consists of a thin Si-chip with a rim for stabilisation, assembled in a massive chip carrier. The Si-chip is a planar structure with a self-supporting membrane. The sensitive

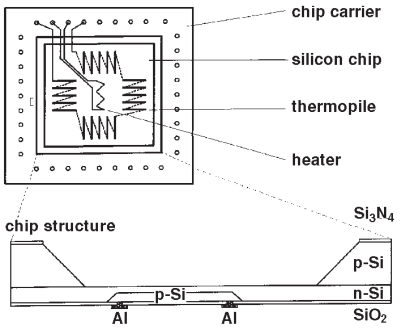

Fig. 5 Scheme of a silicon chip with an integrated thermopile and calibration heater (reproduced with permission from [37])

sphere for heat detection is in the middle of the membrane that contains a Joule calibration resistor. The thermopile is integrated between the sensitive sphere and the rim. This measures the temperature difference given as a consequence of the heat-flow through the membrane with the thermocouples. A number of different types of IC calorimeter have been designed from a simple liquid-batch model to more complex flow calorimeters and even a temperature scanning version [37]. Although these have only been used to measure heat changes in chemical reactions until now, there seems no reason why adaptations cannot be made for the IC calorimeter to be employed for cellular physiological and pharmacological investigations. In this way, it should be possible to extend and expand the application of calorimetry into the next millenium. Then we shall truly reflect the contentment of Burns in the couplet of his Epistle to Lapraik,

Gie me ae spark o' Nature fire,
That's a' the learning I desire.

* * *

The author wishes to record his appreciation to Dr. Y. Guan for many fruitful discussions that clarified the ideas given in this paper. He is also grateful to the Biotechnology and Biological Sciences Research Council for financial support in grants numbered 2/3680, 2/TO3789 and 2/E10985.

References

1 A. L. Lavoisier and P. S. de Lalpace, Mémoire sur la Chaleur, 1780, (Trans. H. Guelac), Neale Watson, New York 1982.
2 J. R. Mayer, in E. L. Youmans, The Correlation and Conservation of Forces, Appleton, New York 1865, p. 316.
3 M. Rubner, Die Gesetze des Energieverbrauchs bei der Ernährung, Franz Deutiche, Leipzig and Vienna 1902.
4 L. Garby and P. S. Larsen, Bioenergetics: Its Thermodynamic Foundations, Cambridge University Press, 1995, p. 16.
5 J. A. Roels, Energetics and Kinetics in Biotechnology, Elsevier, Amsterdam 1983, p. 91.
6 R. B. Kemp and Y. Guan, Thermochim. Acta, 309 (1998) 63.
7 I. Prigogine, Introduction to Thermodynamics of Irreversible Processes, 3rd Edit., Wiley-Interscience, New York 1967, p. 27.
8 E. Gnaiger, Thermochim. Acta, 151 (1989) 23.
9 M. E. Brown (Ed.), in Handbook of Thermal Analysis and Calorimetry, Vol. 1, Principles and Practice, Elsevier, Amsterdam 1998.
10 R. B. Kemp, in M. E. Brown (Ed.), Handbook of Thermal Analysis and Calorimetry, Vol. 1, Principles and Practice, Elsevier, Amsterdam 1998, Ch. 14, p. 557.
11 R. B. Kemp, P. M. Evans and Y. Guan, J. Thermal Anal., 49 (1997) 755.
12 R. B. Kemp, in E. E. Bittar and N. Bittar (Eds), Principles of Medical Biology, Vol. 4, Cell Chemistry and Physiology, Part III, JAI Press, Greenwich, Connecticut, USA 1996, p. 303.
13 E. Battley, Can. J. Microbiol., 41 (1995) 388.
14 E. Battley, Energetics of Microbial Growth, Wiley, New York 1987, p. 255.
15 E. Battley, Physiol. Plant., 13 (1960) 192.

16 A. V. Hill, J. Physiol., 44 (1912) 466.
17 A. V. Hill, Trails and Trials in Physiology, Arnold, London 1965.
18 R. C. Woledge, N. A. Curtin and E. Homsher, Energetic Aspects of Muscle Contraction, Academic Press, London 1985.
19 R. C. Woledge, in W. Weiser and E. Gnaiger (Eds), Energy Transformations in Cells and Organisms, Georg Thieme, Stuttgart 1989, p. 36.
20 R. B. Kemp, Thermochim. Acta, 219 (1993) 17.
21 J. M. Ritchie, Prog. Biophys. Mol. Biol., 26 (1973) 147.
22 R. B. Kemp and Y. Guan, Thermochim. Acta, 300 (1997) 199.
23 W. Zhou and A. Mulchandani, ACS Symp. Ser., 613 (1995) 88.
24 Y. Guan, P. M. Evans and R. B. Kemp, Biotechnol. Bioeng., 58 (1998) 464.
25 Y. H. Guan and R. B. Kemp, Cytotechnol., 30 (1999) 107.
26 E. A. Newsholme, B. Crabtree amd M. S. M. Adawi, Quart. J. Exp. Physiol., 70 (1985) 473.
27 Y. H. Guan and R. B. Kemp, J. Biotechnol., 69 (1999) 95.
28 C. Eftimiadi and G. Rialdi, Cell Biophys., 4 (1982) 231.
29 R. B. Kemp, A. Belicic-Kolsek, S. Hoare, C. Schmalfeldt, C. Townsend and P. M. Evans, Thermochim. Acta, 250 (1995) 259.
30 A. Schön and E. Walum, in Abstracts, 37th Meeting of the American Society for Cell Biology, Washington DC, 13–17 December, 1997, A3479.
31 E. Gnaiger and R. B. Kemp, Biochim. Biophys. Acta, 1016 (1990) 328.
32 W. M. Thornton, Phil. Mag., Sixth Series, 33 (1917) 196.
33 L. E. Erickson, in A. M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, Wright, Bristol 1987, p. 12.
34 M. A. Paulik, R. G. Buckholtz, M. E. Lancaster, W. S. Dallas, E. A. Hull-Ryde, J. E. Weiel and J. M. Lenhard, Pharm. Res., 15 (1998) 944.
35 V. B. Pizziconi and D. L. Page, Biosensors Bioelectronics, 12 (1997) 287.
36 J. Lerchner, G. Wolf, A. Torralba and V. Torra, Thermochim. Acta, 302 (1997) 201.
37 J. Lerchner, A. Wolf and G. Wolf, J. Therm. Anal. Cal., 57 (1999) 241.