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IMPORTANCE OF THE CALORIMETRIC-RESPIROMETRIC RATIO IN STUDYING INTERMEDIARY METABOLISM OF CULTURED MAMMALIAN CELLS*

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SUMMARY

Measurement of heat flux by cells in culture using direct calorimetry is undervalued partly because "onlookers" cannot understand its potential in signposting targets for elucidating the complexities of cellular physiology. 2C11-12 mouse macrophase hybridoma cells form a model system for studying immunological properties *in vitro* but little is known of their metabolic physiology. This study revealed that, as a statistical average, they increase in biomass with successive generations. Over the same time course, changes in the ratio of heat flux to oxygen flux (CR ratio) indicated a more intensive participation of glycolytic reactions to metabolic flux of "mature" cell cultures under aerobic conditions. Given this vital pointer, it was shown that increased lactate flux mostly accounted for CR ratios more negative than -470 $kJ.mol^{-1}O_2$. Succinate flux was also contributory to the CR ratio but the observed decrease in specific heat flux with increasing size could only be explained in terms of plasma membrane-limited metabolic flux with a high flux-control coefficient for glucose uptake. This cannot be the reason for more intensive anaerobic glycolysis, however, and there is some evidence that mitchondrial changes may be the cause.

INTRODUCTION

Scenario

It is a biochemical axiom that, in aerobic organisms, glycolysis is only the prelude to the citric acid cycle and electron-transport chain; pyruvate entering mitochondria and being completely oxidized to CO_2 and H_2O (ref. 1). Although this principle may not be true even for all tissues and organs of an animal (ref. 2), it has always been assumed that animal cells grown *in vitro* under fully aerobic conditions (see, for instance, refs. 3-4) undertake catabolism by complete oxidation principally of carbohydrates, but also of fatty and amino acids. Thus, in applying the energy balance method to animal cells in culture, there has been frequent neglect of the possible importance of aerobic glycolysis (see ref. 5), except in tumour cells (ref. 6). Quite simply, the accumulation or excretion of anaerobic endproducts under aerobic conditions have rarely been measured for cells (see ref. 5) because they are not *supposed* to happen in steady state. In this context, assays for glycolytic endproducts would be thought a wasteful exercise: yet there is a relatively simple approach through the application of calorimetry directly (heat measurements) and indirectly (commonly oxygen measurements) to studying cellular

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metabolism. In advocating the importance of the relationship between these two measurements, it is a necessary preliminary warily to tip-toe down a slippery "primrose path" and risk "everlasting damnation".

Simplicity

A direct calorimeter is thermodynamically a closed system which can only exchange thermal energy (heat) with its surroundings. In contrast, every form of indirect calorimetry is constituted as an open system in which there may be a free exchange of both matter and energy (including heat) with the environment. In a somewhat arbitrary fashion, this thermodynamic difference is frequently reconciled by regarding a closed system plus its environment as an isolated system with respect to energy (ref. 7). The total energy within such a system is, of course, the internal energy (U) and the difference in quantity of energy between two states of the system (ΔU) is separated into thermal energy (q) and work (w), the latter being non-thermal energy exchanged between two masses because of a force which is exerted between them:

$$\Delta U = U_2 - U_1 = (q - w) \qquad (1)$$

Most know that in an isobaric, isothermal closed system which performs no work on the environment other than that associated with a change in volume, ΔU is related to the change in enthalpy (ΔH),

$$\Delta H = \Delta U + P\Delta V \qquad (2)$$

According to eq.(2), ΔH and ΔU will have the same value for the biochemical reactions of cellular systems, most (all?) of which occur in dilute aqueous solution.

"Elementary, my dear Watson!" (Kemp!), do you say?

Development

Quite right but, in classical mechanics (ref. 8), work is defined as,

$$W = \int F.ds \qquad (3)$$

and force (F) as,

$$F = dp/dt \qquad (4)$$

where p is the momentum.

Power (P, unit W) has frequently been used as the expression for thermal energy (heat) but power is defined (ref. 8) as the time derivative of work

$$P = dW/dt \qquad (5)$$

and so is inappropriate in this context. It can also be misleading for the interpretation of results from direct calorimetry especially in terms of Gibbs energy changes. This has been clearly detailed by Gnaiger (ref. 9) who defines heat "evolution" (strictly "flux" - see soon!) as "the time derivative of the internal energy or enthalpy change in an irreversible process".

In some ways, it can be argued that it does not matter which term is applied to thermal energy measured by calorimetry because "insiders" know the sense, however loose the construction. Biological calorimetrists, nevertheless, make a general contribution to our knowledge of physiology by employing thermochemistry and should ensure that the term is precisely defined for "outsiders". Thus, the word "power" or any phrase incorporating it as an expression of thermal energy is confusing to them. "Rate of heat production" (refs. 10-11) could be satisfactory but does not directly convey its relationship to metabolism.

Flux

It has become customary to describe the chemical reactions of metabolism in terms of scalar fluxes and this has been embodied in the modern theory of metabolic control (ref. 12). The latter is central to our understanding of cellular physiology. In order to avail ourselves of the powerful tool of direct calorimetry in studying physiology, it is appropriate to express rate of heat dissipation (production), dQ/dt, in terms of unit mass or volume - scalar heat flux. The problem with this suggestion is that my now ancient Physics professor would have it that flux can only be vectoral, $J_Q = A^{-1}dQ/dt$ (6) where A is the area perpendicular to the plane (ref. 8). Nevertheless, Gnaiger (ref. 9) who does not know my mentor (thank goodness!), logically advocates scalar heat flux and this has the additional virtue of consistency with proponents of control theory (ref. 12). Acceptance of this proposal dictates that J is the dominant experimental symbol.

In an isothermal, isobaric, closed system, the algebraic sum of the enthalpies of all the metabolic reactions occurring within living material gives a net heat flux (Hess's Law of Constant Heat Summation). The flux of individual reactions is measured by specific analytical procedures but, in addition, pathways are subject to indirect calorimetric study, the most common of which being estimation of oxygen consumption in respiration.

Advocacy

In completely aerobic living material, total (scalar) heat flux, ${}_tJ_Q$ ($J.s^{-1}.m^{-3}$), is related to (scalar) catabolic oxygen flux, ${}_tQ_{O_2}$ ($mol\ O_2.s^{-1}.m^{-3}$), by appropriately derived theoretical oxycaloric equivalents, ${}_{\Delta_k}H_{O_2}$ ($J.mol^{-1}O_2$) (see ref. 13),

$$ {}_tJ_Q = {}_{\Delta_t}H_{O_2} \times {}_{\Delta_k}J_{O_2} \qquad (6) $$

where subscript k is the catabolic half-cycle. For a variety of substrates and conditions, the oxycaloric equivalents of aerobic respiration range from

-430 to -480 kJ.mol$^{-1}O_2$ (ref. 14).

Simultaneous measurement of heat and oxygen fluxes (ref. 14) gives the calorimetric-respirometric ratio (CR ratio),

$$\text{CR ratio} = {}_tJ_Q / {}_kJ_{O_2} \tag{7}$$

which was formerly called the (total) heat equivalent of oxygen $\Delta_t Q_{O_2}$ (ref. 15). It is the experimental counterpart to the oxycaloric equivalent, when no work is done (ref. 9). So, in solely aerobic cellular systems, the CR ratio should be within the known range of oxycaloric equivalents, -450 kJ.mol$^{-1}O_2$ ± 5% (ref. 14). In 1985, data for the heat and oxygen fluxes of LS-L929 mouse fibroblasts under conditions of complete aeration yielded a CR ratio of -836 kJ.mol$^{-1}O_2$ (ref. 16). Since this indicates that the cells did not have a balanced energy (enthalpy) budget, Kemp (ref. 5) analysed results from other laboratories for different cell types and found that greatly negative CR ratios were common: for instance, -649 kJ.mol$^{-1}O_2$ for rat hepatocytes (ref. 17) and -1132 kJ.mol$^{-1}O_2$ for resting human neutrophils (ref. 18). Unless there is poor oxygenation of the culture medium, CR ratios more negative than -500 kJ.mol^{-1} must be due to simultaneous aerobic and anaerobic catabolism (ref. 14), the latter measured by endproduct formation.

Lactate is generally the most common anaerobic product in mammalian cells. The dissipative catabolic enthalpy change for the net production of lactate from glucose, $\Delta_k H_{Lac}$, is -63 kJ.mol^{-1} when it is excreted into a bicarbonate buffer (ref. 15). The molar amount of lactate produced per unit amount of oxygen consumed (Lac/O_2 ratio) is an expression of the relative involvement of aerobic glycolysis (ref. 14). The theoretical catabolic heat change per mol O_2, $\Delta_k Q_{O_2}$ is then calculated as,

$$\Delta_k Q_{O_2} = \Delta_k H_{O_2} + \text{Lac}/O_2 \times \Delta_k H_{Lac} \tag{8}$$

It is important to note that the lactate component in eq. (8) is an addition. Analysis of published data using eq. (8) (see refs. 6, 14) revealed that CR ratios more negative than -500 kJ.mol^{-1} could usually be explained by the participation of aerobic glycolysis, with observed Lac/O_2 ratios higher than 1. Discrepancies between the Lac/O_2 and high CR ratios may be due to the formation of other glycolytic endproducts, such as pyruvate or succinate (ref. 15). A generalized equation can be written for the calculation of the heat change for all glycolytic endproducts per mol O_2,

$$\Delta_k Q_{O_2} = \Delta_k H_{O_2} + \sum_i i/O_2 \times \Delta_k H_i \tag{9}$$

where i is each individual endproduct.

Objective

In a thermochemical study of 2C11-12 mouse macrophage hybridoma cells (ref. 19) at 12 h after subculture, a CR ratio of -518 $kJ.mol^{-1}O_2$ was obtained, which could be explained entirely on the basis of the estimated Lac/O_2 ratio of 1.14 (ref. 20). More recent unpublished results for the CR ratio of cells further along the growth curve indicated more negative values. The present report confirms this result and examines possible reasons for the changes in terms of cell size, biomass, specific heat flux, endproduct formation and flux control theory.

METHODS

Cell Culture

2C11-12 mouse macrophage hybridoma cells (ref. 14) were cultured in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% (v/v) Antipodean foetal calf serum (Imperial Laboratories, Andover, Hants, U.K.),2mM glutamine, polymixin sulphate B (IO $U.cm^{-3}$) and gentamycin (59 $mg.dm^{-3}$). They were maintained by serial passage in plastic Petri dishes (78 cm^2). At sub-culture, cells were released from the substratum by mechanical agitation. They were counted by Coulter particle counter (Model D; Luton, U.K.) and by a calibrated low-radio-frequency dielectric method (ref. 21) using a two-terminal impedance analyser (Model 4192A; Hewlett-Packard, Milton Keynes, U.K.). Viability was determined by a fluorescent dye method (see ref. 22). Cells were then either sedimented by centrifugation (250 g for 300 s), washed in Dulbecco PBS, pH 7.2, containing glucose (5.5 mmol dm^{-3}) and resuspended in PBS for short-term experimentation or diluted to 10^5 cells cm^{-3} in fresh growth medium for culture in a humidified atmosphere containing 5% (v/v) CO_2 at 37°C. When supernatants were required for biochemical assays, they were taken prior to dissociation from the plastic, retrival of them being carried out in the appropriate medium.

Biomass estimations

Protein in cells washed free of serum was assayed by the Lowry colorimetric method (ref. 23) after precipitation by perchloric acid at a final concentration of 61 mmol dm^{-3} and subsequent solubilization in 40 mmol dm^{-3} NaOH (ref. 24). A calibration curve was constructed using similarly-treated bovine serum albumin and readings taken at 500 nm.

Cell size as a measure of biomass was estimated by the dielectric method (see above) and by flow cytometry using a laser-light Skatron Argus instrument (Skatron Ltd., Newmarket, Suffolk) with a low-angle (<15°) light scattering detector (ref. 25). In principle, Lorenz-Mie scattering occurs

when particle sizes are significantly greater (>20 x) than the wavelength of the light (ref. 26). The extent of light scattered in a foreward direction is strongly dependent on cell size but the relationship to "channel" number is non-linear (ref. 27); so a calibration curve was constructed using monodisperse latex particles (Dyno Particles, Lillestrøm, Norway) in an appropriate range. Channel numbers were converted to equivalent cell diameters using a spreadsheet method on a Viglen II HDE microcomputer, which also controlled the cytometer. In order to reduce small-diameter debris, PBS was filtered at 0.22 μm and an additional filter (Millipore) was placed in the sheath-fluid line.

Calorimetry

Having relegated my replica Lavoisier-Laplace (1783) calorimeter (see ref. 28 for results) to the museum, heat flux measurements were conducted using the unique Johansson (J)-modified LKB 2107 heat conduction instrument (ref. 29) of the stainless-steel ampoule (5 cm^3) kind (see ref. 30). Exotic machines of the BAM, TAM (Thermometrics, Järfälla, Sweden) or whatever-acronym type (ref. 31) will not be allowed until mature (old!) - say, 2001!

The characteristics of the 2107 system are historically well-documented (see refs. 29-31) but, to be specific for J-2107, 2 cm^3 cell suspension at 2 x 10^6 cm^3 PBS was loaded into one ampoule and 2 cm^3 PBS placed in the reference vessel, thus leaving a void volume of 3 cm^3. Equilibration to 37°C took about 1800 s depending on ambient conditions, and registration of plateaux, 1200 s. Amplification was set at 10 μV on the trusty Keithley 150B microvolt ammeter. No one seems to agree on a totally suitable method for chemical calibration of an ampoule calorimeter but the J-2017 is regularly calibrated using cholinesterase (ref. 16).

Oxygen Measurements

Oxygen flux of cells in suspension was determined simultaneously to that of heat by direct calorimetry using a Clarke electrode in a YS1 oxygen monitor (ref. 16). Cells (2 x 10^6 cm^{-3}) in PBS (4 cm^3) were equilibrated to 37°C for 900 s and oxygen consumption measured for a similar time. In relating oxygen (and, of course, heat) flux to the solubility of oxygen in PBS, a benchmask figure to recall is that the solubility of oxygen in air-saturated pure water at the standard pressure of 101.325 kPa and 37°C is 2.102 x 10^{-7} $mol.cm^{-3}$ (ref. 32). Due to the salting-out effect, the concentration of oxygen in physiological saline is less than in pure water but, for the purpose of checking the maintenance of aerobic conditions in short-term experiments, it is permissible to omit the required correction factors.

Sugar uptake

This was assessed by adding [U-^{14}C] glucose at 60 µmol.dm^{-3} to 5 cm^3 cell suspension in PBS (2 x 10 cells.cm^{-3}) containing 5.5 mmol.dm^{-3} "cold" glucose and a range of 6-deoxyglucose concentrations from 1 to 10 mmol.dm^{-3}. After incubation for 15 min at 37°C, 500 mm^3 samples were taken, filtered using Whatman GF/C glass fibre filters and washed using 2 x 5 cm^3 PBS. [^{14}C]Glu was counted as described previously (ref. 33) and, simultaneously, 3-phosphoglycerate measured enzymically by a standard procedure (ref. 34).

Miscellaneous essays

Lactate and succinate respectively were measured in cells and supernatants by a modified Barker technique (ref. 33) and an enzymic procedure (ref. 34).

Chemicals

Except for gentamycin (Gibco, Uxbridge, U.K.), these were obtained at the highest grade from Sigma. [U-^{14}C]glucose was purchased from Amersham. Water was singly distilled in an all-glass apparatus after deionization in an Elgastat.

RESULTS AND DISCUSSION

For the purposes of clarity and advocacy, the results have been stripped down to two crucial breakpoints in the growth curve of a 2C11-12 cell culture; those are the onset of the exponential phase (12 h) and close to its demise (48 h). The data-hungry can eat previous results (ref. 20) or salivate for more to be published elsewhere.

From Table 1, it is seen that the number of cells at 12 h of culture had barely increased from the initial inoculum of 10^5.cm^{-3}. This was probably

TABLE 1

Increase in cell numbers and changes in biomass with time for resting 2C11-12 mouse macrophage hybridoma cells.

Time (h)	cells.cm^3	Protein mass (ng.cell^{-1} ± SD)	Lorenz-Mie diameter (µm ± SD)
12	1.2 x 10^5	0.47 ± 0.07	12.9 ± 0.5
48	1.7 x 10^6	0.68 ± 0.09	15.4 ± 1.1

owing to the trauma of subculture (ref. 3), especially in these cells which retain the parent immunological property of high sensitivity to foreign

agents (ref. 19). The exponential phase over the next 36 h progressed through almost 4 generations showing they had not undergone differentiation and were still in the resting state.

Of great interest from the data in Table 1 was the fact that the mean biomass of cells at 48 h was significantly greater than at 12 h. It had increased by 45% (t-test : $P<0.01$) in terms of protein content and by 20% (t-test : $P<0.001$) with respect to the mean cellular diameter. The change in the latter indicates that the volume fraction of cells at 48 h was a remarkable 74% greater than in the early exponential phase; an observation confirmed qualitatively by the dielectric increment of the RF β-dispersion (refs. 20-21). Cutting out the jargon, these results infer that cellular protein does changes proportional to size during the growth of 2C11-12 cells.

Heat and oxygen fluxes were determined simultaneously (Table 2) on samples of the same cell cultures used to ascertain growth characteristics and showed

TABLE 2

Change in heat flux ${}_{t}J_{O_2}$, and oxygen flux, ${}_{k}J_{O_2}$, during the growth of resting 2C11-12 mouse macrophage hybridoma cells; with resultant CR ratios.

Time (h)	Heat flux ($pJ.s^{-1}.cell^{-1}$ ± SD)	Oxygen flux (10^{-15} $mol.min^{-1}.cell^{-1}$ ± SD)	CR ratio ($kJ.mol^{-1},O_2$)
12	32 ± 2	3.7 ± 0.3	-514
48	26 ± 2	1.9 ± 0.4	-843

that both parameters decreased between 12 h and 48 h in culture. Oxygen flux, however, showed a greater fall than did heat flux which resulted in a more negative CR ratio at 48 h when compared to that at 12 h (Table 2). Even the value at the earlier time indicates a cellular metabolic state involving participation of anaerobic processes (ref. 14) but the ratio at 48 h clearly demonstrates an even more intensive share for glycolysis in catabolism. Conventional explanations for this change involve poor oxygenation of the medium and the so-called "crowding effect" (ref. 14). The former cannot be true for cultures grown in unsealed Petri dishes and subjected to short-term assay (see Methods). "Crowding" has been invoked to explain anaerobic glycolysis in cells at high concentrations (see refs. 36-37 and reviews, refs. 5-6) when sedimentation or absence of contact inhibition can cause oxygen concentration to be a limiting factor. Neither is true of the present experimental system, as can easily be shown by a simple calculation (see

Methods).

Gnaiger (ref. 15) first pointed to the value of measuring glycolytic endproducts in the case of CR ratios being more negative than -470 kJ.mol^{-1}. As is seen in Table 3, lactate accumulation and excretion doubled in 2C11-12

TABLE 3

Lactate, $_kJ_{Lac}$, and succinate, $_kJ_{Succ}$, for growing 2C11-12 mouse macrophage hybridoma cells, with corresponding i/O_2 ratios, see eqs. (8) and (9), and calculated heat change per mol O_2 $\Delta_k Q_{O_2}$, which should be compared with the experimental CR ratios in Table 2.

Time (h)	Lactate flux (10^{-15} mol.min^{-1}.cell^{-1} ± SD)	Lac/O_2 ratio	Lac$\Delta_k Q_{O_2}$
12	4.2 ± 0.6	1.14	-540
48	9.0 ± 1.2	4.74	-769
	Succinate flux (10^{-15} mol.min^{-1}.cell^{-1} ± SD)	Succ/O_2 ratio	Lac+Succ $\Delta_k Q_{O_2}$
12	0.3 ± 0.704	0.08	-552
48	0.4 ± 0.04	0.22	-803

cells during the growth period. When combined with reduced oxygen flux (Table 2), this resulted in an increased Lac/O_2 ratio which indicates a greater relative extent of anaerobic glycolysis in late exponential phase. Calculating from the ratios the heat change per mol O_2 ($\Delta_k Q_{O_2}$) on the assumption that lactate was the only glycolytic endproduct, gave an adequate explanation for the CR ratio at 12 h (see refs. 6, 14) but failed fully to account for the more negative CR ratio at 48 h. The difference may have been due to the formation of other glycolytic endproducts (ref. 15) and the first additional assay to be attempted, that for succinate, did go someway to accounting for the discrepancy (Table 3).

CR ratios more negative than -500 kJ.mol^{-1} certainly can be explained by simultaneous aerobic and anaerobic catabolism and not by low efficiency of net ATP production (see Gnaiger, this volume). There is now the need to discover why the involvement of glycolytic reactions under aerobic conditions became greater with time in culture.

The first hint to a possible solution came from analysis of the calorimetric data of Schön and Wadsö (ref. 38) for cultured Vero cells. This showed that heat flux of these cells decreased with increasing protein mass per cell (ref. 6). A similar change in specific heat flux was also observed in 2C11-12 cells (see Tables 1 and 2) in terms both of protein mass and, more dramatically, cell size. Besides echoing Kleiber's "rule" (ref. 39) that in whole animals specific oxygen flux decreases with increasing size, the results suggest that metabolic flux is limited by the surface area of the cell (see ref. 12). All catabolic substrates are transported through the plasma membrane by facilitated diffusion and/or active symporters (see ref. 1) using carriers. If there is a more-or-less constant number of carriers per plasma membrane area during this cell volume increase, then substrate (glucose) availability will become rate-limiting necessitating resort to the alternative strategy of utilizing endogenous reserves such as glycogen and even amino and fatty acids through degradation to acetyl CoA.

One of the essential prerequisites for this hypothesis is that the glucose uptake system has a high flux-control coefficient. This is defined as the fractional change in pathway flux divided by the fractional change in the activity of the relevant enzyme; and it is a quantitative representation of enzyme (carrier) control over flux. [^{14}C]glucose uptake and 3-phosphoglycerate (3-PG) production by 2C11-12 cells was measured in the presence of the isomeric inhibitor, deoxyglucose (1-10 mM). Compared with the maximum uptake rate of 6.5 pmol [^{14}C]glu.min^{-1} per mg protein, 2 mM deoxyglucose caused a 45% reduction in [^{14}C]glu uptake and 27% inhibition of 3-PG production. Overall, the flux-control coefficient was indeed high at 0.73 (dimensionless). So, surface-area-limited metabolic flux could possibly explain the adherence by these cells to Kleiber, but does not give a reason for them becoming bigger with successive generations - the "Topsy" effect so despised of white adipocytes (see ref. 5).

Nor does it explain the increasingly intensive participation of glycolytic reactions under aerobic conditions with successive generations. The marked rise in lactate flux (Table 3) can only be due to either a change in the lactate dehydrogenase isozyme system towards "hyperactivity" or an interference with entry to the mitochondrial citric acid cycle after formation of pyruvate and possibly even at the initial step of the oxidative decarboxylation of pyruvate to acetyl CoA by the pyruvate dehydrogenase complex within the mitochrondrial matrix. In fact, there is some preliminary electron microscope evidence for mitochondrial damage in 2C11-12 mouse macrophase hybridoma cells but that is another, as yet unvalidated, story.

Enough of speculation! The crucial message is that, "an die freude", the combined measurements of heat and oxygen fluxes for these cells has revealed an intriguing physiological problem.

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REFERENCES

1 L. Stryer, Biochemistry, 2nd edn., Freeman, San Francisco, 1981.
2 J.M. Shick, J. Widdows and E. Gnaiger, Calorimetric studies of behavior, metabolism and energetics of sessile intertidal animals, Am. Zool., 28 (1988), 161-181.
3 R.I. Freshney, Culture of animal cells and manual of basic techniques, 2nd edn., A.R. Liss, New York, 1987.
4 R. Baserga, Cell Growth and Division, IRL Press, Oxford, 1989.
5 R.B. Kemp, Heat dissipation and metabolism in isolated animal cells and whole tissues/organs, in: A.M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, Wright, Bristol, 1987, pp. 147-166.
6 R.B. Kemp and E. Gnaiger, Aerobic and Anaerobic Energy Flux in Cultured Animals Cells, in: W. Wieser and E. Gnaiger (Eds.), Energy Transformations in Cells and Animals, Georg Thieme Verlag, Stuttgart, 1989, pp. 91-97.
7 E.H. Battley, Energetics of microbial growth, Wiley, New York, 1987.
8 I. Mills, P. Cvitas, K. Homann, N. Kallay and K. Kuchitsu, Quantities, units and symbols in physical chemistry, Blackwell, Oxford, 1988.
9 E. Gnaiger, Physiological calorimetry: heat flux, metabolic flux, entropy.
10 P. Bäckman, Effects of experimental factors on the metabolic rate of T-lymphoma cells as measured by microcalorimetry, Thermochim. Acta, 172 (1990) 123-130.
11 P. Lönnbro and A. Schön, The effect of temperature on metabolism in 373 cells and SV40-transformed 3T3 cells as measured by microcalorimetry, Thermochim. Acta, 172 (1990) 75-80.
12 H.V. Westerhoff, A.K. Groen and R.J.A. Wanders, Modern theories of metabolic control and their applications, Biosci. Reports 4)1984), 1-22.
13 E. Gnaiger, Calculation of energetic and biochemical equivalents of respiratory oxygen consumption, in: E. Gnaiger and H. Forstner (Eds.), Polarographic Oxygen Sensors, Aquatic and Physiological Applications, Springer-Verlag, Berlin, Heidelberg, New York, 1983, pp. 337-345.
14 E. Gnaiger and R.B. Kemp, Anaerobic metabolism in aerobic mammalian cells : information drawn from the ratio of calorimetric heat flux and respirometric oxygen flux, Biochim. biophys. Acta 1016 (1990) 328-332.

15 E. Gnaiger, Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power, J. Exp. Zool., 228 (1983) 471-490.
16 S.E.S. Hoffner, R.W.J. Meredith and R.B. Kemp, Estimation of heat production by cultured cells in suspension using semi-automated flow microcalorimetry, Cytobios, 42 (1985) 71-80.
17 I.G. Jarrett, D.G. Clark, O.H. Filsell, J.W. Harvey and M.G. Clark, The application of microcalorimetry to the assessment of metabolic efficiency in isolated rat hepatocytes, Biochem. J., 180 (1979) 631-638.
18 C. Eftimiadi and G. Rialdi, Increased heat production proportional to oxygen consumption in human neutrophils activated with phorbol-12-myristate-13-acetate, Cell Biology, 4)1982) 231-244.
19 P. De Baetselier, P. Brys, E. Vercauteren, K. Mussche, R. Hamers and E. Schram, Generation and analytical applications of luminescent macrophate cell lines, in: L.J. Kricka, P.E. Stanley, G.H.G. Thorpe and T.P. Whitehead (Eds.), Analytical Applications of Bioluminescence and Chemiluminescence, New York Academic Press, 1984, p. 287.
20 R.M. Kemp and R.W.J. Meredith, Thermal studies of isolated animal cells, Cytobios, 1990 (in press).
21 C.L. Davey, D.B. Kell, R.B. Kemp and R.W.J. Meredith, On the audio- and radio-frequency dielectric behaviour of anchorage-independent, mouse L929-derived LS fibroblasts, Bioelectrochem. Bioenerg., 20 (1988) 83-90.
22 R.W. Kemp, R.W.J. Meredith, S. Gamble and M. Frost, A rapid cell culture technique for assessing the toxicity of detergent-based products *in vitro* as a possible screen for eye irritancy *in vivo*, Cytobios, 36 (1983) 153-159.
23 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with the Folin phenol reagent, J. biol. Chem., 193 (1951) 265-275.
24 M.J. Dunn, R.B. Kemp and A.H. Maddy, The similarity of two high-molecular weight polypetides of erythrocyte spectrin, Biochem. J., 173 (1978) 197-205.
25 R.J.G. Carr, R.G.W. Brown, J.G. Rarity and D.J. Clarke, Laser light scattering and related techniques, in: Biosensors: Fundamentals and Applications, A.P.F. Turner, I. Karube and G.S. Wilson (Eds.), Oxford University Press, Oxford, 1987, pp. 679-701.
26 A.L. Koch, Estimation of size of bacteria by low-angle light-scattering measurements; theory, J. Microbiol. Meth., 5 (1984) 221-235.
27 G.C. Salzman, Light scattering analysis of single cells, in: Cell Analysis, vol. 1, N. Catsimpoolas (Ed.), Plenum Press, New York, 1982, pp. 111-143.
28 R.B. Kemp, Microcalorimetric studies of tissue cells *in vitro*, in: A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, pp. 113-130.
29 P. Monk and I. Wadsö, Design and testing of a flow calorimeter, Acta Chem. Scand., 22 (1968) 1848-1852.
30 I. Wadsö, Some problems in calorimetric measurements on cellular systems, in: A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, New York, 1980, pp. 247-274.
31 I. Wadsö, Calorimetry techniques, in: A.M. James (Ed.), Thermal and energetic studies of cellular biological systems, John Wright, Bristol, 1987, pp. 34-67.
32 H. Forstner and E. Gnaiger, Calculation of equilibrium oxygen concentration (appendix A), in: E. Gnaiger and H. Forstner (Eds.), Polarographic Oxygen Sensors, Aquatic and Physiological Applications, Springer-Verlag, Berlin, 1983, pp. 321-333.
33 M.J. Dunn and R.B. Kemp, Studies on carbohydrate metabolism in the aggregation *in vitro* of embryonic chick cells, Cytobios, 7 (1973) 127-145.

34. H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Academic Press, New York, 1974.
35 J.D. Loike, S.C. Siverstein and J.M. Sturtevant, Application of differential scanning microcalorimetry to the study of cellular processes: heat production and glucose oxidation of murine macrophages, Proc. Natn. Acad. Sci. U.S.A., 78 (1981) 5958-5962.
36 L. Nässberger, L. Truetsson and M. Monti, Microcalorimetric studies of hybridoma cells, Biol. Cell, 62 (1988) 33-37.
37 A. Schön and I. Wadsö, Microcalorimetric measurements on tissue cells attached to microcarriers in stirred suspension, J. Biochem. Biophys. Meth. 13 (1986) 135-143.
38 M. Kleiber,The Fire of Life; an introduction to animal energetics, Wiley, New York, 1961, pp. 454.