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Review

On the accuracy of results from microcalorimetric measurements on cellular systems [1]

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Abstract

During the last two decades, major developments have been made in microcalorimetry and in measurement techniques applied to living cellular systems. We have now reached a state where such measurements are often very easy to perform using precise commercial instruments. However, researchers should be reminded of certain risks in making systematic errors in such work. Some properties of the microcalorimeters used in work on cellular systems are briefly reviewed. Possible sources of errors and misinterpretations of results are discussed, and some test and calibration processes are described.

INTRODUCTION

When Stig Sunner introduced me to thermochemistry in the mid fifties, practically no calorimetric work was conducted on living cellular systems and except for combusion calorimeters used in routine work, calorimeters were home-built and usually operated without any electronics. Laboratories where calorimetry was practised were, with few exceptions, specializing in calorimetry and their students were well trained in the principles of heat measurements. We were constantly reminded that the results of calorimetric measurements can easily be impaired by systematic errors. Calibration and the application of correction procedures were essential parts of the experiments. It is fair to state that I found accurate calorimetry challenging but at times quite tedious. Since then, there have been major developments in calorimetric techniques leading to more sensitive instruments with much improved long-term stabilities, several new measurement functions and a high degree of automization. Many calorimetric designs have become commercially available, making it possible to conduct calorimetric measurements of high standards without spending years on the

[1] Updated version of the First Lavoisier Medal Lecture presented at the 7th Conference of the International Society for Biological Calorimetry, Royal Holloway and Bedford New College, University of London, Egham, Surrey, England, 9–11 April 1990.

necessary design and testing of these instruments. Furthermore, the analytical and the kinetic applications of calorimeters, in addition to their use as thermodynamic instruments, have been increasingly recognized during the last decades. This has contributed to the extension of the application of calorimetry to areas such as cell biology. In fact, we have now reached a state where calorimetric measurements on cellular systems can be conducted more or less automatically and with high precision using commercial instruments. However, the results of precise measurements can be inaccurate.

Commercially produced calorimeters are very easy to operate but their design, not least with respect to their calibration procedures, is typically far from 'transparent'. As may be expected, many users will therefore look upon their instruments as 'black boxes' operated by means of a few push buttons and a computer. Sometimes this approach can be perfectly acceptable but in other cases it may lead to significant errors or misinterpretations, even when the instruments are in good condition and used as recommended. Users of all kinds of calorimeters must be aware of the fact that practically all processes — physical, chemical or biological — give rise to heat effects. This property is most valuable as it makes calorimeters very powerful tools for the discovery and quantitative characterization of unknown or unexpected processes, for instance in biology. But this property can also lead to systematic errors and serious misinterpretations of calorimetric signals. In fact, calorimetry is more vulnerable to systematic errors and misinterpretations than most other measurement techniques. This is particularly important in microcalorimetry where heat effects due to, for instance, mechanical disturbances, adsorption, condensation–evaporation effects, and chemical or biological side-reactions can easily be of the same magnitude or larger than the process investigated. Users of calorimeters should therefore be acquainted with these problems as well as with the calibration procedures and test processes suitable in different experimental situations. By this I do not imply that very long training periods should be required before modern commercial instruments can be used successfully. But the risks of making systematic errors and misinterpretations of calorimetric signals should not be neglected in work on living cellular systems.

MICROCALORIMETERS

This report will focus on problems in connection with measurements in cell suspensions, in cells attached to solid supports, and in pieces of tissues. In such experiments, the heat production rate (the 'thermal power') is often, but not always, quite low. For vessel volumes in the order of a few cm^3, thermal powers are typically in the range or 10–100 μW. Therefore, microcalorimeters are normally used. The term 'microcalorimeter' is not

strictly defined, but in solution-reaction calorimetry it is common to use the 'micro' prefix when calorimeters have a sensitivity in the μW range. However, it is often suitable to use typical microcalorimeters at a much reduced sensitivity, for instance, in work with concentrated microbial suspensions, pieces of tissue and small animals, where the thermal power evolved may be larger by many orders of magnitude than the sensitivity of the instrument.

Calorimetric principles

The principles and practical design of the microcalorimeters used in biological work have recently been reviewed [1, 2]. There follows here a very brief summary and discussion of the basic principles and properties of such instruments.

Calorimeters useful for studies of heat quantities q and heat production rates, $P = \mathrm{d}q/\mathrm{d}t$, normally fall into one of the groups: (1) adiabatic or quasi-adiabatic calorimeters or (2) heat conduction calorimeters. At present, (thermopile) heat conduction calorimeters are by far the most important type employed in measurements on cellular systems.

In an ideal adiabatic calorimeter there is no heat exchange between the calorimetric vessel and the surroundings. With these calorimeters, it is the change in temperature of the reaction system ΔT that is measured

$$q = \varepsilon_a \Delta T \tag{1}$$

$$P = \varepsilon_a(\mathrm{d}T/\mathrm{d}t) \tag{2}$$

The proportionality constant ε_a ('the calibration constant') is determined experimentally and is, in the ideal case, equal to the heat capacity of the reaction vessel and its contents. Thus, the calibration constant of an adiabatic calorimeter will change if the heat capacity of the vessel system changes. Therefore, if a reaction vessel is charged with a uniform cell suspension, the measured values for ΔT and $\mathrm{d}T/\mathrm{d}t$ will be essentially the same regardless of the amount of cells (it is assumed that the heat capacity of the vessel is much smaller than that of its contents). A process studied with an adiabatic calorimeter is conducted non-isothermally, but for a sensitive instrument the temperature change can be small enough to be neglected or corrected for.

In the ideal heat conduction calorimeter, the heat released is quantitatively transferred from the reaction vessel to a surrounding heat sink (usually a metal block). With such calorimeters it is a property proportional to the total heat flow rate that is measured. Normally, the heat flow rate is recorded by use of a 'thermopile wall' positioned between the calorimeter vessel and the heat sink. The temperature difference over the thermopile will give rise to a potential signal U proportional to the heat flow rate, $\mathrm{d}q/\mathrm{d}t$. At steady state, the heat flow rate is identical to the thermal power

produced in the vessel

$$P = \mathrm{d}q/\mathrm{d}t = \varepsilon_c U \tag{3}$$

where ε_c is the calibration constant. When ΔT and U change with time, the heat content of the vessel will also change, leading to

$$P = \varepsilon_c(U + \tau\, \mathrm{d}U/\mathrm{d}t) \tag{4}$$

where τ is the time constant of the instrument. In the ideal case, the time constant is defined by

$$\tau = C/K \tag{5}$$

where C is the heat capacity of the vessel and its contents, and K the heat conductivity through the thermopile. Equation (4) is derived under the assumption that there are no significant temperature gradients in the vessel. When such gradients are significant, equations with two or more time constants should be used [2, 3]. The integrated form of such equations as well as eqns. (3) and (4) give the simple expression

$$q = \varepsilon_c \int U\, \mathrm{d}t \tag{6}$$

provided that the thermopile potential at the initial and the final integration time are the same (normally the baseline value, $U = 0$).

It should be observed that the expression for P at steady state, eqn. (3), and the integrated expression (6) are independent of the heat capacity of the reaction system and the vessel, unlike corresponding equations for adiabatic instruments, eqns. (1) and (2). But the expression for the thermal power under non-steady-state conditions depends on the time constant (5) and thus on the heat capacity and the heat conductivity of the vessel system. For modern instruments, the measured potential–time curves are commonly transformed on line to power–time curves (a 'black box' procedure!). If the time constant of the instrument is not exceptionally large, the term $\tau\, \mathrm{d}U/\mathrm{d}t$ of eqn. (4) can often be neglected in biological experiments and the expression for the thermal power is accurately described by the simple eqn. (3). Under such conditions, the directly recorded potential–time curve will thus accurately describe the kinetics of the process.

SOME SOURCES OF SYSTEMATIC ERRORS

Mechanical effects

In calorimetric work on living material, the value of the thermal power is usually measured. It is then important not only that the calorimeter baseline is stable during an experiment, but also that its value is the same

during the control experiment (where it is established) and in the experiments with cells. However, for thermopile heat conduction calorimeters it is quite common that small but significant and unpredictable baseline shifts occur when a vessel is inserted into a calorimeter or when a permanently installed batch vessel is manipulated during cleaning and charging procedures. The origin of such effects is often obscure but they appear, at least sometimes, to be due to relaxation effects in the thermopiles or in other parts of the instrument, e.g. in O-ring seals [4]. Frequent tests of the reproducibility of the baseline value should be made in order to assess realistic uncertainties for the measured thermal power. When the P value is small, $\leqslant 10\ \mu W$, it is common that the reproducibility of the baseline value limits the precision of the P value, see refs. 4 and 5. This is not often discussed in reports or mentioned in advertisements for commercial instruments. In microcalorimetry, it is normally not advisable to start a mixing process by breaking a glass ampoule or opening a mechanical valve inside the vessel. The heat effects connected with such operations are generally poorly reproducible.

When a calorimetric liquid is stirred or agitated by rotation or rocking of the vessel assembly, there will usually be significant contributions from liquid friction effects to the observed thermal power. The same will occur in flow vessels. Such thermal effects will not cause any problems, provided the effects are constant and that relevant control experiments can be carried out where the heat-producing cellular material is not present. But in some cases problems are difficult to overcome. For instance, suspensions of red blood cells at their normal concentration, about 40 vol.%, are much more viscous than the medium (plasma or a buffer solution). In such cases, where the biological material causes the effect, it is difficult to find a suitable reference solution or suspension which will accurately mimic the flow properties of the cell suspension. Another problem that is difficult to overcome completely can arise if a cell suspension changes viscosity during the experiment, e.g. in a microbial growth experiment. As a general rule, flow rates and stirring rates should be kept as low as possible. This is also advantageous from the point of view of possible damages to the cell material caused by mechanical action on them.

Evaporation and condensation

Water is always present in biological experiments and the enthalpy of vaporization is very large, $\Delta_{vap}H(25°C) = 44.0\ kJ\ mol^{-1}$, or about 2.4 mJ per μg of water. Clearly, virtually no uncontrolled evaporation or condensation can be tolerated in experiments carried out at the μW level. Possible distillation effects or changes in the gas phase composition must be considered. Gas phase problems are avoided if there is no gas present, which sometimes gives flow or perfusion calorimeters an extra advantage.

Leakage effects due to poor packing rings or pinholes can be detrimental for a calorimetric experiment, but such effects are usually irregular and will hardly cause any systematic errors.

Sedimentation and adhesion

Partial or complete sedimentation of cells during a calorimetric experiment may lead to poorly defined power values. 'Crowding effects' have been reported for some types of cells. In other cases, where 'crowding effects' are not in operation, the metabolic rate can be affected by local depletion of oxygen and the differences in pH which develop as a result of the very high cell concentration in the sediment. It is therefore felt that, in general, static experiments with cell suspensions should be avoided. The use of 'turbine' stirrers [1, 6] and vertically oscillating discs [7] appear to be particularly useful for keeping cell suspensions (including cells attached to microcarriers [8]) at a uniform concentration. In flow calorimetry, cells may sediment in the flow lines or in the flow vessel, leading to erroneous values of the thermal power [9]. Sometimes sedimentation effects cannot be distinguished from adhesion effects (see below). Problems with sedimentation of cells in flow calorimetry, particularly with yeast cells, have led to the construction of special flow vessels [1].

Many cells adhere easily to the walls of experimental vessels. Sometimes this is intentional, e.g. in work with monolayers of tissue cells. In other cases, adhesion effects can cause serious problems. The adhesion process itself is accompanied by an increased metabolic activity and a significant heat burst. Furthermore, the metabolic activity of attached cells may be different from that of the cells in suspension. In flow experiments, cells can adhere to the flow line before they reach the calorimetric vessel and cause the measured power to be lower than that corresponding to the initial cell concentration. If the cells adhere preferentially to the flow vessel, power values that are too high will be recorded.

The concentrations of certain substances, such as hormones and drugs, used in work with cells can be very small, while the inside surface area of a microcalorimetric vessel can be quite large. This may lead to significant vessel adsorption effects.

Oxygen starvation

In many types of calorimetric experiments with aerobic systems, it is difficult to arrange for a sufficient supply of oxygen [9]. Water in equilibrium with air dissolves about 0.2 μmol of oxygen per cm^3 at 37°C. Thus, 1 cm^3 of aqueous medium will contain enough oxygen for the complete oxidation of about 33 nmol of glucose, corresponding to a heat

production rate of about 10 μW during 2 h. If the medium is equilibrated with pure oxygen instead of air (which from a physiological point of view may not be acceptable), the amount of oxygen will increase by a factor of approximately 5. Thus, with dilute cell suspensions and with sensitive calorimeters, it is possible to make rather long experiments with aerobic cells without any transfer of oxygen to the medium during the experiment. It is often very desirable to be able to monitor the oxygen concentration in the medium. In flow calorimetry this can be done by use of an electrode placed in the flow line [9–11]. It is important that the electrode is positioned as close as possible to the flow cell. Oxygen electrodes have also been used inside batch macrocalorimetric vessels, see, for example, ref. 12. Recently, a miniaturized oxygen electrode placed inside a stirred microcalorimetric batch vessel was used for measurements on cultured tissue cells [13].

High rates of oxygen consumption lead to high heat production rates and one may then use comparatively vigorous agitation of an oxygen–liquid system; however, this might damage some types of cells. A different experimental approach is to use vessels with a large contact area between the liquid phase and the gas phase, in combination with moderate agitation [14]. In cases where the cellular material is floating (adipose tissue, fat cells), significant amounts of oxygen can be transferred from a gas phase even if the medium is not agitated. In flow calorimetry it can be suitable to use a mixed (segmented) flow of gas and liquid, for which several flow vessels have been designed [1, 9].

Two other calorimetric measurement problems with gas–liquid systems should be mentioned. As stated above, the enthalpy of vaporization of water is very high. A stream of air perfusing through a calorimetric vessel can thus cause substantial cooling effects, even if it is close to equilibrium with the calorimetric liquid. In experiments performed at a high heat-production rate, the problem can be avoided by a suitable humidifier in series with the flow vessel. In perfusion experiments at the μW level, where gas bubbling in addition to oxygenation might be useful as a means of mild agitation, special precautions must be taken. A segmented flow of gas–liquid, in combination with an adequate heat exchanger, can provide for an efficient equilibration of the vapour phase [9].

Another calorimetric problem deals with the fact that the enthalpy of solution of gas into the medium cannot be neglected ($\Delta_{sol}H(25°C) = -12.0\,kJ\,mol^{-1}$). It is therefore often important to know if oxygen consumed by cells in suspension is replaced by transfer of oxygen from a gas phase in the calorimeter vessel. With efficient agitation of the system, this can probably be assumed to be the case. However, the situation in a static reaction vessel will be questionable, in particular if the oxygen consumption rate is high. For this reason, it is sometimes preferable to avoid the gas phase completely, by the use of a flow or perfusion vessel. Note that

a corresponding problem can also occur for systems where carbon dioxide is formed.

Special problems can arise with oxygen consumption and carbon dioxide formation in long timescale measurements on microbial systems on soil. A calorimetric technique using a permeable silicone rubber membrane has been developed [9].

Effect of pH

The metabolic rate and thus the rate of heat production from a cellular system can be very sensitive to changes in pH. As an extreme case, it has been shown that the thermal power from suspensions of erythrocytes at around pH 7.4 will change by about 135% per pH unit [15]. Most cells produce metabolites which effect the pH value, and a significant pH change is common during the course of an experiment even if the medium is well buffered. Initial and final pH values should therefore be measured in all calorimetric experiments with living cellular systems. Even better is to monitor continuously the pH in the calorimetric vessel throughout the experiment by use of an electrode positioned in the vessel or, in flow calorimetry, in the flow line. Recently, it was shown that a miniature pH electrode (together with an oxygen electrode) can be placed in a stirred microcalorimetric vessel without causing significant disturbance of the heat measurements [13].

If fresh cell-free medium is continuously perfused through a stirred calorimetric vessel, the pH can be kept constant. This technique can also be applied in experiments with pieces of tissue submerged in a medium and with cells adhered to some solids (the wall of the reaction vessel, a plastic film, or microcarriers) and with rather heat cells such as yeast cells. But light cells may be lost with the medium leaving the vessel. In all perfusion experiments there will be a dilution of the extracellular metabolites produced by the cells. This can decrease the precision of the analytical determinations.

Calibrations

In addition to the different types of problems discussed above, the possibility of significant calibration errors should not be neglected. It is my impression that many commercial manufacturers of calorimeters, and their users, do not give enough weight to this problem. Calorimeters are usually calibrated by the release of electrical energy in a resistor positioned in a strategic place in the instrument. The actual measurement of electrical energy or power is today a trivial procedure which easily can be made with an accuracy far exceeding the requirements of biological experiments. The problem is rather to make sure that the electrical energy will produce a heat

flow rate in the calorimetric system that is closely comparable with that of the process studied. For practical reasons, microcalorimeters used in biological work are not always well suited for strict comparisons; for instance, it is sometimes difficult to produce or to use a calibration heater of an ideal design, or to place it in the best position. It is then important to have suitable test processes available using which the electrical calibration values can be checked. In modern reaction and solution microcalorimetry, it is not uncommon that electrical calibrations are avoided and replaced by chemical ones which may better mimic the process studied.

Examples of suitable chemical test and calibration processes, and some problems in the use of electrical calibrations with microcalorimeters of the heat conduction type, were recently discussed in some detail [1, 16]. With the focus on work in cell biology, these matters will be reviewed in the next two paragraphs.

SOME TEST AND CALIBRATION PROCESSES

In most calorimetric experiments with living cells, it is their rate of heat production or the thermal power which is measured. In a stirred suspension of cells the thermal power is released in all parts of the liquid system. Frequently, electrical calibration heaters have a compact form and even if they are placed in the best position available, their heat flow rate patterns may not be very closely comparable to that from the cell suspension. The same often applies to flow vessels used with cell suspensions. In order to test the accuracy of electrical calibration procedures for such instruments, we have designed a series of heat-producing liquids in which the thermal power changes slowly and predictably with time [16, 17]. In these reaction systems, triacetin is hydrolysed in aqueous solutions containing different amounts of imidazole and acetic acid. So far, five such solutions have been characterized, see Tables 1 and 2. The hydrolysis reactions are complex processes and their thermodynamic and kinetic properties have not been

TABLE 1

Composition of test solutions for the hydrolysis of triacetin [a]

Solution	Buffer composition [a], mass of HOAc in g	Mass of triacetin per 100 g of buffer in g
A	10.00	10.000
B	16.00	5.000
C	18.00	3.600
D	20.00	3.600
E	24.00	3.600

[a] All buffer solutions were prepared by adding acetic acid to 100 g of water and 27.23 g of imidazole [17].

TABLE 2

Triacetin reaction mixtures: values for the constants in eqn. (7)

Test solution	T/K	$a/(\mu W\,g^{-1})$	$b/(10^{-4}\,\mu W\,g^{-1}\,s^{-1})$	$c/(10^{-10}\,\mu W\,g^{-1}\,s^{-2})$
A [a]	310.15	90.66	3.63	8.1
A [b]	298.15	34.32	0.62	1.2
B [a]	310.15	35.35	1.16	2.3
B [a]	298.15	13.35	0.26	1.0
C [a]	310.15	21.80	0.79	3.5
D [a]	310.15	16.00	0.45	1.1
D [b]	298.15	5.19	0.08	0.4
E [a]	310.15	7.25	0.16	0.5

[a] Ref. 17.
[b] Ref. 18.

analysed in any detail. However, it was shown that, under isothermal conditions, their thermal power–time curves can be described accurately by

$$P = a - bt + ct^2 \tag{7}$$

where t is the time after mixing triacetin with the aqueous solution, and a, b, and c are constants. Values for the constants at 25 and 37°C are given in Table 2. The thermal power production isothermally by the reaction mixtures changes very slowly and, for low values ($P \leq 40\,\mu W\,g^{-1}$), almost linearly with time, see Fig. 1. The accuracy of the P values predicted by

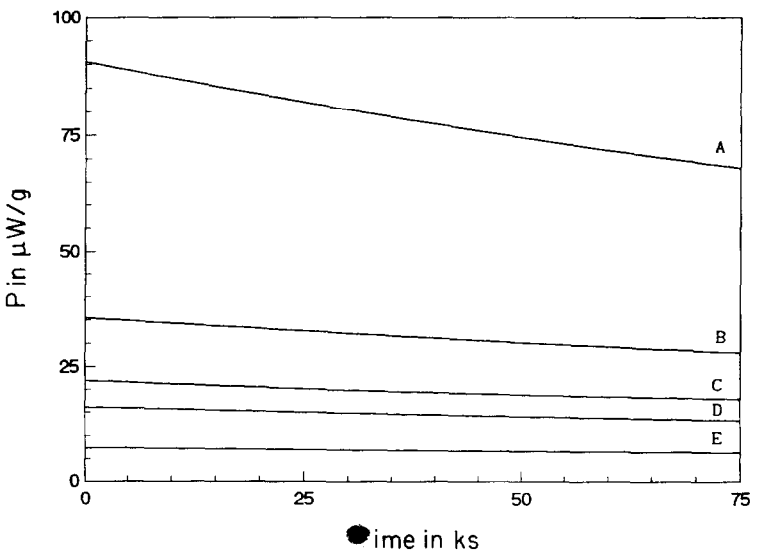

Fig. 1. Thermal power P versus time for the hydrolysis of triacetin mixtures (Table 1) at 37°C: ks = kiloseconds; 1 ks = 16.67 min (from ref. 16).

eqn. (7) is estimated to be better than ±1% for at least 20 h. Because the decline in thermal power is very slow, it is not necessary in practice to measure the time very accruately. But the temperature coefficients are high: for the conditions investigated so far, dP/dT is 5–10% per degree. The reaction mixtures can be stored for a long time in a freezer (about −27°C) with only a small decrease in their P values at room temperature [16]. For instance, for solution A (Tables 1 and 2) for which P decreases comparatively fast (from 90.7 to 89.3 $\mu W g^{-1}$ during the first hour), the power value measured at 25°C after storage for 1 month at −27°C will be about 2% lower than that predicted by eqn. (7).

We have found radioactive probes (Am, Monsanto) to be useful for tests and calibrations of some types of vessels used for measurements of thermal power. However, the trade in such probes (and their transfer across national borders) is restricted and they cannot be expected to come into common use.

Photoreduction of KFe(III) oxalate is useful as a test reaction in photo-microcalorimetry. A new enthalpy value for this process was recently determined [18].

A few processes useful in general biocalorimetry should also be mentioned. Dilution of aqueous solutions of sucrose and of propanol, and various neutralization processes, have proved to be accurate and convenient in tests and calibrations of microcalorimeters, for instance in biochemical reaction calorimetry [16]. The binding of Ba^{2+} to 18-crown-6 is recommended as a test reaction in connection with titration experiments where both the equilibrium constant and the enthalpy change can be evaluated [16].

SOME PROBLEMS WITH CERTAIN TYPES OF VESSELS

Many types of microcalorimetric vessels have proved to be useful in studies on living systems. Simplified diagrams of the most important types used with thermopile heat conduction calorimeters are shown in Fig. 2. Some problems concerning their calibration will be pointed out.

Vessel A pictures a closed ampoule with liquid and gaseous phases but with no stirring and no possibility to add reagents during a measurement (a 'static vessel'). As was pointed out earlier, sample vessels of this kind should be avoided whenever cell sedimentation is a problem. Their function is also much limited because no reagent can be added during a measurement, but they are convenient to use and problems connected with effects from mechanical disturbances and evaporation–condensation effects are minimized. They can usually be calibrated electrically with an acceptable accuracy, in particular when the heater is positioned inside the vessel (which is usually not the case) and the heat flow is well 'equalized' by the wall of the vessel (or a vessel holder) before reaching the thermopile.

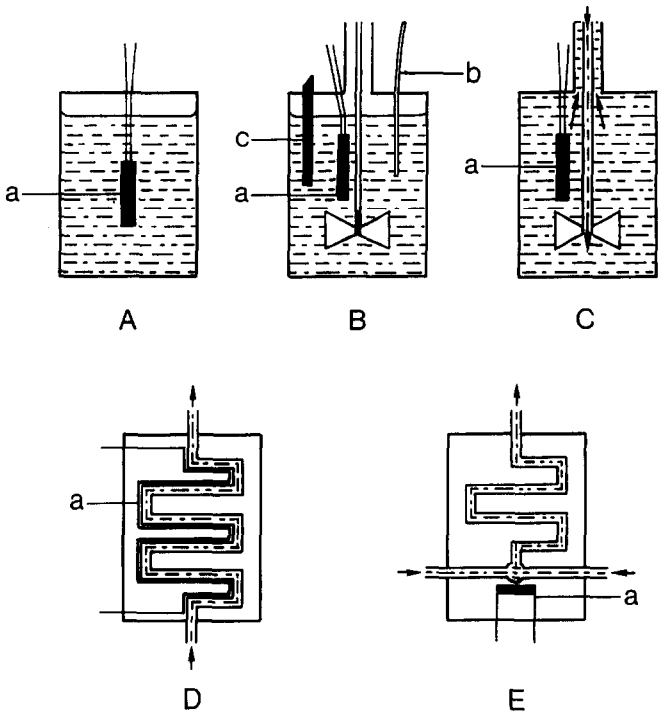

Fig. 2. Schematic picture of some important types of vessels used with thermopile heat conduction microcalorimeters. A, closed static ampoule; B, reaction (titration) vessel with stirrer; C, perfusion vessel; D, flow-through vessel; E, flow-mixing vessel. a, electrical heater; b, injection tube; c, other devices, e.g. electrodes, optical fibres.

The triacetin reaction mixtures are well suited for test and calibration of such vessels.

Vessel B represents the case where the liquid content is stirred and where reagents can be added during a measurement. Other devices, such as electrodes, may also be present. Such vessels can be far superior than static vessles in physiological work. But they are less ideal from a calorimetric point of view because the fraction of the heat flow passing through the thermopile will be smaller than in vessel A. It is our experience that vessels of type B can still be calibrated accurately by use of insertion heaters, provided that the agitation of the liquid is efficient. Suitable test (calibration) reactions are the triacetin reaction (thermal power measurements) and the dilution of propanol or sucrose solutions (heat quantities evolved at injections). We have found propanol solutions particularly easy to handle. The dilution of sucrose may serve as an excellent test for the efficiency of a mixing process, because aqueous sucrose solution (approx. 15–25%) can be rather difficult to mix with water.

Vessel C represents a stirred vessel where a liquid flow (pure medium or a cell suspension) enters the vessel through the stirrer shaft (a 'perfusion

vessel') [5]. The incoming and outgoing flow serves as a counter-current heat exchanger which is in thermal contact with a surrounding thermostated bath. Such flow(-through) vessels can also be equipped with injection tubes and can then serve as mixing vessels in continuous or stopped flow mode. In work with tissue pieces, it can be suitable to support the samples by special holders attached to the stirrers or forming the stirrers [5, 19]. Calibration problems are similar to those of vessel B. The calibration constant can be highly dependent on the flow rate and it is very important that the heater has a suitable design and position. Sometimes heaters are in poor thermal contact with the liquid medium but in good contact with the thermopile. In such cases, substantial errors in the calibration constant can result, in particular at high flow rates.

Figure 2D indicates a flow-through vessel made from a metal tube or consisting of a groove cut in a metal plate. The tube, in a zig-zag pattern or formed as a spiral, is in good thermal contact with the surrounding thermopile [1, 9]. Such vessels, used in many studies with suspensions of living cells, are difficult to calibrate electrically. As indicated in the figure, the calibration heater should preferably be spread out along the reaction tube (in most cases it is not) but even with this arrangement problems will remain. As for vessel C, the electrical calibration constant is typically very dependent on the liquid flow rate. Furthermore, when a heat-producing flow leaves the heat exchange unit, its temperature is ideally the same as that of the calorimetric heat sink, but there may be a significant increase in the temperature of the liquid during its passage through the connecting tube to the calorimetric vessel (this can also apply to vessels of type C). If the thermal power per unit of volume is known, the value for the 'efficient volume' of flow-through vessels at different flow rates can be calculated [4]. The use of a heat-producing liquid, such as the triacetin reaction mixtures, seems to be the best method for tests and calibration experiments with this type of flow vessel.

Figure 2E represents a flow-mixing vessel made from metal tubes. It consists of two inlet tubes, a mixing point (mixing chamber) and a zig-zag or spiral-shaped reaction-outlet tube. The mixing point and the reaction-outlet tube must be in good thermal contact with the thermopile. Such vessls are used in two modes: in continuous-flow mixing and in stopped-flow experiments. When the continuous-flow mixing technique is used, it is important that the mixing process is completed in the mixing chamber or immediately thereafter. In experiments not involving cells or when cells are activated with some reagents, the reaction time must be much shorter than the residence time of the reaction mixture in the vessel. If these conditions are not realized, it is very difficult to assign representative values for the calibration constant and for the amount of material reacted. The flow rates of the two liquids are in most cases kept constant which leads to constant thermal power values. Electrical calibrations using heaters in close

proximity to the mixing area will normally mimic very closely the heat evolution from mixing and dilution processes and from fast chemical reactions. Sucrose dilution is a particularly suitable test process because the mixing efficiency is critical for such vessels and will often be a limiting factor in their performance. If a cell suspension is mixed with another liquid, one must also consider the problems typical for the flow-through vessel in Fig. 2D.

A very useful feature of all flow vessels is that they normally work without a gas phase. Therefore, evaporation–condensation problems will be avoided. Flow-through or perfusion vessels are sometimes operated with a mixed liquid–gas flow (segmented flow in the tube vessels) which can be useful to prevent oxygen starvation. But in such experiments the thermopile signal will be disturbed, presumably due to evaporation–condensation effects caused by minor pressure variations in the flow lines. It should be observed that the heat produced by a uniform cell suspension in a flow vessel will decrease in proportion to the gas phase present.

CONCLUSIONS

Work on living cellular systems represents a vast field for calorimetric investigations. In some cases, such efforts concentrate on thermodynamic aspects; in other cases the calorimeters are mainly used as analytical monitors. In all types of experiments, it is of course important that the physiological conditions in the calorimetric vessel are adequate and well defined. This can often pose problems but great progress has been made during recent years in adjusting calorimetric instruments and experimental procedures to the requirements of biological systems. In this connection, it is considered important that methodological studies are carried out on different cell systems where the influence on their heat production from variations in experimental parameters, such as temperature, cell concentration, pH and other medium effects, storage conditions, calorimetric techniques, etc, is recorded. In addition to its basic importance, such information is valuable when results from different investigations are compared.

Thermodynamic experiments on the molecular level require accurate calorimetric measurements combined with analyses of the chemical changes accompanying the biological processes. It is felt that we should make these analytical determinations more detailed and more accurate than is typically the case today.

Finally, a few words about the exciting possibilities of using microcalorimetry as an analytical tool in the fields of clinical and pharmacological sciences, in studies on biocompatibility connected with medical techniques, and in many types of applications in agriculture and forestry. In the further development of microcalorimetric techniques in such areas, I

believe it is important to conduct the studies as much as possible on a molecular thermodynamic level. This will lead to a better understanding of the chemical origins of the recorded calorimetric signals: long-term progress can hardly be made by empirical correlations alone.

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