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Microcalorimetric techniques for the investigation of living plant materials ☆

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Abstract

Microcalorimetric investigations of living cellular systems have been conducted on small animals, cells and tissues from animals and plants and on microbial systems, but it is only recently that more systematic work has begun on plant materials. Isothermal experiments together with temperature scanning experiments have been performed. In most cases simple static ampoules have been used as calorimetric vessels and practically all calorimetric investigations reported on plant systems have been conducted under dark conditions. It is felt that some microcalorimetric techniques currently used in studies of microbial and animal systems, and in other areas, could be usefully applied more generally in the plant field: stirred injection (titration) vessels, gas and liquid perfusion vessels and different photocalorimetric methods.

Keywords: Living system; Microcalorimetry; Photocalorimetry; Plant

1. Introduction

During the last few decades, developments in calorimetry have, to a significant extent, been concerned with microcalorimetric techniques for investigations of living systems such as small animals, cells and tissues from animals and plants and on microbial systems [1–3]. Calorimetric investigations of plant material have been concerned with seed germination processes and on investigations of cell cultures,

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tissue pieces and whole plants. Studies of seed germination and of plant tissue pieces were initiated some 40 years ago by Prat using Tian–Calvet microcalorimeters equipped with simple ampoules as reaction vessels [4]. Surprisingly, after Prat's pioneering work, only a few scattered reports in plant calorimetry appeared until the last years of the 1980s. At that time systematic development was initiated by a working party from Brigham Young University, Provo, Utah, and the University of California at Davis, directed by Hansen, Criddle and Breidenbach [5]. At the present time several groups are active in this field of calorimetry.

Recent calorimetric investigations in the plant field have been conducted on tissue pieces and, to some extent, on cell suspensions. Very few studies have been reported from work on whole plants. A recent example from that field is the macrocalorimetric investigation by Lamprecht et al. [6] on Voodoo lilies with their remarkable thermogenesis. Very few studies on seed germination processes have been reported. Notably, practically all calorimetric work on living plant systems has been conducted under dark conditions.

To a large extent the work on plant tissues and cell suspensions appears to be of direct practical interest and also of fundamental importance: the effect of stress factors such as high or low temperature, high salt concentration, pollutants and the response to hormones and other metabolic effectors [5]. Further, a significant part of recent work has been concerned with correlations between metabolic heat rates and long term growth rates for trees, an area of obvious practical and economic importance [5,7]. The heat production rate ("thermal power") of growing plants or plant tissues is often as high as that of animals or animal tissues (see Table 1). Calorimetric experiments on living material from plants therefore often can be carried out using moderately sensitive calorimeters although, in most cases, typical microcalorimeters are used. (In this paper, the poorly defined term "microcalorimeter" is used to indicate that the instrument sensitivity is in the range of one μW or better.)

Table 1
Examples of heat production rates P [a] from living systems

System	P/mW g^{-1} (wet weight)	Reference
Man	1.2	[8]
Mouse	8.0	[8]
Snake (25°C)	0.6	[9]
Frog (21°C)	0.2	[10]
Human skeletal muscle (resting, 37°C)	1	[11]
White adipose tissue (mouse, 37°C)	1	[12]
Barley roots (24°C)	1.4	[13]
Leaf segment (apple, 25°C)	3	[5]
Pineapple fruit	0.2	[14]
Voodoo lily (whole plant, non-flowering, 25°C)	1	[6]
Voodoo lily (whole plant, flowering, 25°C)	7	[6]

[a] Approximate values.

2. Microcalorimetric techniques

Microcalorimeters used in measurements on living systems normally are twin instruments of the thermopile heat conduction type [15]. Different types of calorimetric vessel are in use. Closed ampoules operated under static conditions (no stirring or other form of agitation) are very common, but it is often advantageous to employ perfusion vessels where liquid medium or a gas is allowed to flow through the vessel in which the biological system is retained. For suspensions of microorganisms, and for some animal cells, flow vessels (where the liquid medium together with the cells form the flow) are often suitable. Plant cells have a tendency to aggregate which can make flow vessels less useful in this field.

When a vessel is charged with a liquid containing suspended cells or tissue pieces, it is of great advantage if a reagent can be injected during the experiment. In such cases, and for other reasons, it is desirable that liquid phases are stirred, or agitated by some other means. Calorimetric measurements on living cellular systems are normally conducted under essentially isothermal conditions. However, for plant systems in particular, it has been demonstrated that temperature scanning measurements can also be important [5].

The present paper will concentrate on microcalorimetric techniques for measurements of plant systems. Part of the treatment will deal with instruments and methods actually used in work on plant materials. However, to a significant extent, the discussion is concerned with techniques not yet applied in calorimetry on plants but which are believed to be of interest for this field.

2.1. Static vessels

Microcalorimetric experiments with tissue pieces from plants are usually conducted in simple static calorimetric vessels. Tissues are often placed on a piece of filter paper wetted with medium or put into agar gel. The gas phase in a closed vessel will then be saturated with vapour from the medium. If the gas phase consists of air it can sustain respiration of a tissue sample for several hours: 1 cm^3 of air at room temperature contains about 8 μmol of O_2. Assuming that this amount is used to oxidize a carbohydrate to CO_2 and H_2O in a steady-state process where the heat flow is 100 μW, it will take about 11 h before all O_2 is consumed. The oxygen concentration can be varied within wide limits without any effect on the dark metabolic rate of plant materials [5]. If the gas phase initially consists of pure oxygen, the experimental period for uninterrupted aerobic conditions theoretically will be extended about five times. However, the concentration of CO_2 would then have reached a very high level, even if respiration had not ceased due to lack of nutrients in the medium or stored in the tissue. In particular when glass vessels are used, it can be convenient to periodically interrupt the dark experiment and illuminate the sample in order to recharge it with photosynthate products and to lower the CO_2 concentration.

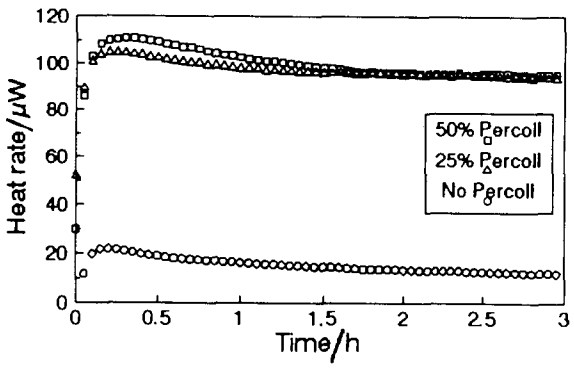

Fig. 1. The effect of medium density on the heat flow from a tomato cell culture in a static vessel. (Reproduced from Fontana et al. [17], with permission.)

Sometimes it is suitable to submerge a tissue, for example a root, in a liquid medium during the calorimetric experiment. Respiration may then rather quickly make the medium anaerobic. If there is a gas phase above the liquid, oxygen will diffuse into the liquid and in the liquid phase. However, under static and isothermal conditions such diffusion processes are slow. Similarly to animal cells, plant tissue cells normally do not form stable suspensions in a static calorimetric vessel but will sediment. This can very quickly lead to anoxia and inadequate supply of nutrients even if there is a large gas phase. The amount of oxygen in 1 cm^3 of an aqueous medium in equilibrium with air at room temperature is about 0.27 μmol. If this amount is used to sustain a steady-state respiration process where the heat flow is 100 μW, the running time before the O_2 is consumed only will be about 20 min. Thus, if diffusion of oxygen from a gas phase to the (sedimented) plant material is slow, it is likely that the system will have turned anoxic before the calorimeter has equilibrated! In their early photocalorimetric experiments with algae, Magee et al. [16] showed that sedimentation could be slowed down by the addition of 0.1% agar to the medium without gel formation. More recently Fontana et al. [17] have devised an interesting method by which cells and tissues will float in liquid media and thus be in good contact with the gas phase. They supplied the standard medium with high concentrations (more than 25%) of Percoll (Pharmacia, Uppsala, Sweden), a medium used for cell separations. Fig. 1 shows results from three experiments where tomato cells were measured in a 1 cm^3 static vessel. In the case where no Percoll was added, a low, nearly constant, thermal power was observed. When the medium was supplied with 25% or 50% Percoll, values about five times higher were found. It seems likely that when the observation period started the sedimented cells were already under anoxic conditions.

2.1.1. Temperature scanning calorimetry

Plants grow at different temperatures and it is therefore often important to investigate the heat production of cells and tissues over a rather wide temperature

range. Such investigations, at high or low temperatures, can have the character of temperature stress experiments [5]. In the Introduction it was pointed out that most of the recent calorimetric investigations of plant materials have been conducted by the Provo–Davis group. In most cases they have used a temperature scanning calorimeter (Hart Scientific DSC 7707). This instrument is a heat conduction calorimeter equipped with four static vessels (volume 1 cm^3) of which one is employed as reference. When used with plant materials, the instrument is normally operated in the isothermal mode, but it has also been used in stepwise scanning, cycling and in continuous temperature scanning experiments. When used in the isothermal mode, the instrument has a rather large baseline noise compared with commercially available non-scanning microcalorimeters (± 3 μW) [18]. However, the ability to make very fast temperature changes is a significant advantage.

When living systems are investigated in continuous DSC experiments, the signals must be interpreted with care because contributions from heat capacity differences between sample and reference, and from thermally induced transitions, will be superimposed on the metabolic thermal power curve. Hansen and Criddle [19] have analysed these problems in some detail.

2.1.2. Simultaneous measurements of thermal power, CO_2 production and O_2 consumption

Calorimetry is often ideal as a general monitor for a complex physical, chemical or biological process. This technique will give a quantitative account, in terms of thermal power, of all significant part-processes. However, calorimetry does not identify the events. For this reason, it is often desirable to conduct specific analytical determinations of the reaction system in parallel with the calorimetric measurements. In experiments with plant material it is particularly important to determine rates of production of CO_2 and consumption of O_2 [5]. Measurements of concentration of O_2 and CO_2 in a closed vessel can be made, for example, by gas chromatographic analysis of samples extracted through a septum in the vessel. Electrode measurements can be conducted in closed vessels but will require a well stirred solution as reaction medium. Criddle et al. [20] have developed a method for the simultaneous determination of thermal power and changes of concentration of CO_2 and O_2 in a closed double calorimetric system (see also Ref. [21]). The method is based on the combination of results from two separate calorimetric measurements, conducted in parallel, and a measurement of the change of pressure in the closed system. A Hart DSC 7707 was used in these measurements. Two vessels are connected by PEEK tubing (which has a very low permeability for oxygen and carbon dioxide) (see Fig. 2). One of the vessels contains the plant material and the other, serving as CO_2 trap, is charged with 0.2 cm^3 of 0.4 M NaOH solution. A pressure sensor, SenSym SX01 (SenSym, Sunnyvale, California) is connected to the closed system. Carbon dioxide released from the plant tissue will diffuse to the vessel charged with NaOH solution, where it is absorbed, and the corresponding thermal power is recorded. When a steady-state condition has been established, the heat flow from the CO_2 trap will be proportional to the rate of CO_2 release from the biological sample. The proportionality factor is obtained from the known

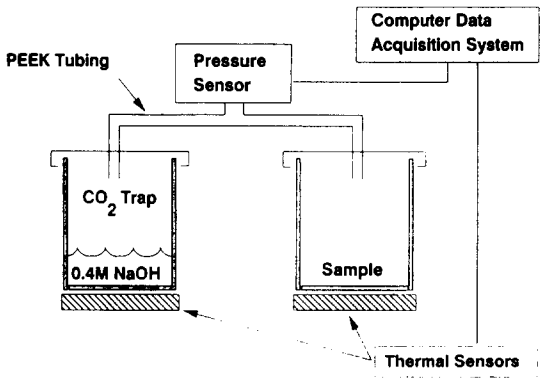

Fig. 2. Block diagram of DSC vessels (Hart 7707) modified to allow measurement of CO_2 and O_2 flux rates. (Reproduced from Criddle et al. [20], with permission.)

enthalpy data for the neutralization of CO_2 by NaOH or (better) by calibration experiments. If the gas volume of the double calorimetric system is known, results of the pressure measurements will lead to values for changes of the oxygen concentration.

In experiments with corn hypocotyl tissue, a nearly constant thermal power was recorded for the metabolic process 15 min after the start of the experiment. The second calorimeter, used as CO_2 trap and the pressure measurement, required a longer time to reach steady-state values (2.5 h). This was due to the rather slow diffusion of CO_2 between the two calorimeters. The accuracy by which the O_2 depletion rate can be measured was reported to be ± 1 pmol s^{-1} or $\pm 0.09\%$, whichever is larger. A similar technique was developed by the same group for use with a moderately sensitive large volume (78 cm^3), single vessel calorimeter (Hart Scientific 4242) (Fig. 3). The lid was modified to be pressure tight and the sample container was connected to the pressure sensor by use of PEEK tubing. With this instrument, calorimetric–respirometric experiments are conducted in two steps. Firstly the thermal power is measured for the metabolic process with no NaOH present in the vessel. When a steady-state value has been reached, a NaOH trap is added and the calorimeter will (after about 1.5 h of thermal equilibration) measure the sum of heat production rates for the metabolic process and the neutralization of CO_2. The difference between the two measured values for the thermal power will thus be proportional to the rate of CO_2 formation. The measured change of pressure will lead to a value for the rate of oxygen consumption. By this method O_2 depletion rates can be measured with an accuracy of ± 0.2 pmol s^{-1} or $\pm 0.4\%$, whichever is larger. As pointed out by Criddle et al. [21], it is important to select a growth medium which does not absorb CO_2. Also for this reason the volume of liquid medium should be kept small.

In an accompanying paper [22] we report on a microcalorimetric (wet) gas perfusion vessel which is connected in series with a calorimetric CO_2 trap.

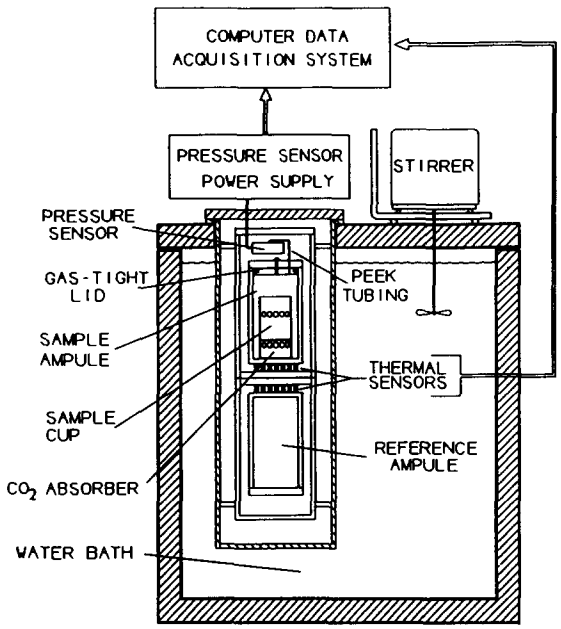

Fig. 3. Block diagram of the Hart Model 4242 calorimeter modified to allow measurement of CO_2 and O_2 flux rates. (Reproduced from Criddle et al. [20], with permission.)

2.2. *Stirred vessels, perfusion vessels*

Microcalorimetric techniques, where static closed ampoules are used as sample containers in biological experiments, can be simple and will be particularly suitable for serial analytical measurements. However, the use of stirred vessels or vessels where a gas or a liquid is allowed to perfuse through the chamber containing the biological material has important advantages. It will give the experimenter a large freedom in the design of the experiment; for example injections (titrations) can be made, electrodes and other devices can be used and sampling for analytical measurements is made simple. Further, such techniques make it possible to conduct experiments under more well defined and more constant conditions than are normally possible with a closed, static vessel. Microcalorimetric investigation on microbial and animal cells and tissue pieces are now often performed under non-static conditions, but such techniques have so far only been used in very few cases in work with plant materials.

2.2.1. *Liquid media*

Fig. 4 shows schematically the liquid perfusion vessel used by Lovrien and co-workers in their studies of the response of corn coleoptile tissue to indole acetic acid [23]. The tissue pieces are placed in the reaction chamber through which

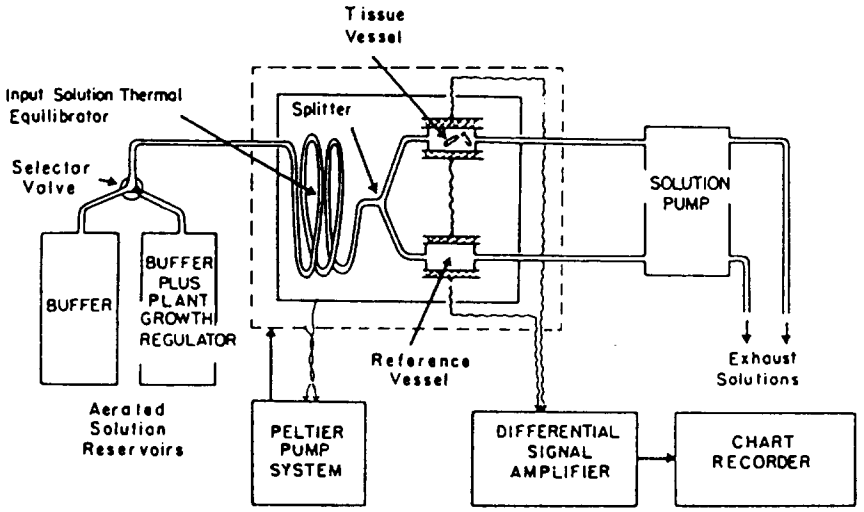

Fig. 4. A schematic diagram showing a liquid perfusion system. (Reproduced from Anderson et al. [23], with permission.)

thermally equilibrated medium, with or without the hormone present, flows continuously. Peristaltic pumps, placed after the calorimeter, control the flow rate. The oxygen level in the flowing liquid is maintained by bubbling air or oxygen through the reservoirs outside the calorimeter. (From experience in our laboratory, the medium may easily become oversaturated with gas which can lead to gas bubble formation in the vessel or in the flow line. This will seriously disturb the calorimetric signal when measurements are conducted at a high sensitivity.)

Gnaiger used a similar instrument (LKB 2107 flow sorption microcalorimeter) in series with a twin respirometer (Cyclobios, Austria) in work with small aquatic animals [24] (see Fig. 5). The O_2 electrodes in the twin respirometer continuously measured the oxygen concentration in the flow before and after passing the sample chamber.

In our laboratory we have developed a modular series of microcalorimetric vessels for use with a twin heat conduction microcalorimeter (LKB/Thermometric's TAM 2277) [25–29]. The vessels can consist of simple static ampoules made from steel or glass or they can be stirred vessels with possibilities for injection and perfusion of liquid media. The vessels are inserted into measurement position in the calorimeter by use of a stepwise equilibration procedure. Stirred vessels (Fig. 6) are made from acid proof steel where, in some cases, the inside is teflon coated. Sample containers of different functions and complexities are employed. Fig. 6(B) indicates a simple stirred injection vessel, volume 1 cm^3, designed for use as a titration vessel and for work with cell suspensions [26]. Injections of reagents are made by use of a thin injection needle a (i.d. 0.15 mm) introduced through a guide tube b. Baseline stability is about ± 0.3 μW (10 h) and the reproducibility of the baseline value, following recharging of the vessel, is about ± 0.5 μW. Normally experiments with

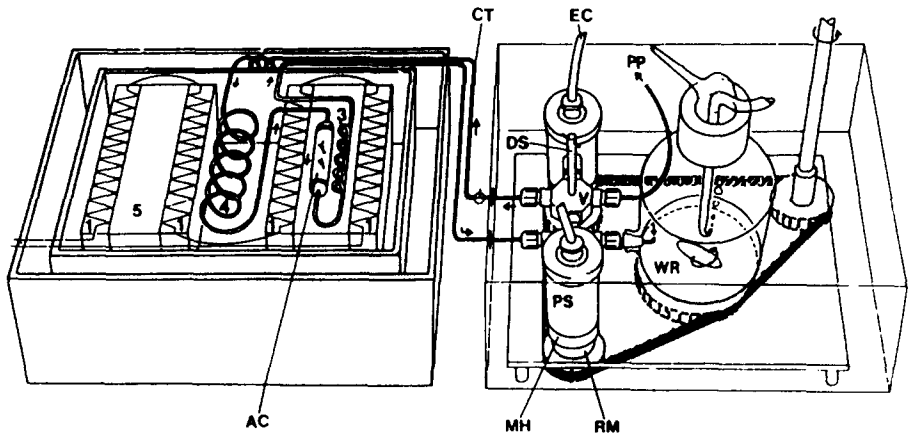

Fig. 5. A twin-flow respirometer (Cyclobios, Austria) connected to the LKB (flow) sorption calorimeter and used in experiments with small aquatic animals. AC, the sorption vessel, used as an animal chamber; CT, capillary tube; DS, drive shaft for switching the two four-way valves; EC, electrode cables of the polarographic oxygen sensor; MH, magnet housing; PP, outlet teflon tubing connected to the peristaltic microvalve; PS, polarographic oxygen sensor sleeve; RM, rotating magnet; V, four-way microvalve; WR, water reservoir for gas equilibration; 1, thermocouple plates; 3, 4, heat exchange coils; 5, static reference plate. (Reproduced from Gnaiger [24] with permission.)

this vessel are conducted with a gas phase above the liquid. Instead of the conventional stirrer indicated in Fig. 6(B), different types of rotating sample holders can be used [26,30]. Fig. 6(C) shows a vessel fitted with a "turbine" stirrer [26,28], which proved to be particularly useful for relatively tall vessels. The vessel shown in Fig. 6(C) can be employed as a liquid perfusion vessel. As indicated by the arrows, medium flows into the vessels through the hollow stirrer shaft and will leave the vessel through the space between the shaft and the surrounding steel tube (serving as a counter current heat exchanger; maximum flow rate is 20 cm^3 h^{-1}). Cells in suspension will be retained in the vessel, provided that their density is significantly higher than the medium [31]. The vessel also can be used as a stirrer injection vessel (with gas phase) in which case the lower part of the stirrer shaft is plugged. Fig. 6(D) shows the same vessel equipped with electrodes allowing simultaneous measurements of thermal power, pH and oxygen concentration [28]. For use with plant materials, a CO_2 electrode could be installed. In still another version of this vessel, optical cables are used to introduce light into the sample chamber [27] (see Fig. 11).

In another very similar modular system of insertion vessels, used in our laboratory for some time, the volume can be up to 20 cm^3 (diameter 28 mm), allowing more electrodes, or other sensors, to be installed. Vessels of this series recently were designed for use with plant materials as a gas perfusion vessel [22] and as a photocalorimetric vessel.

2.2.2. Gas perfusion

For measurements of living systems in a gaseous phase, it can be desirable to let air perfuse through the calorimetric vessel in order to avoid accumulation of CO_2

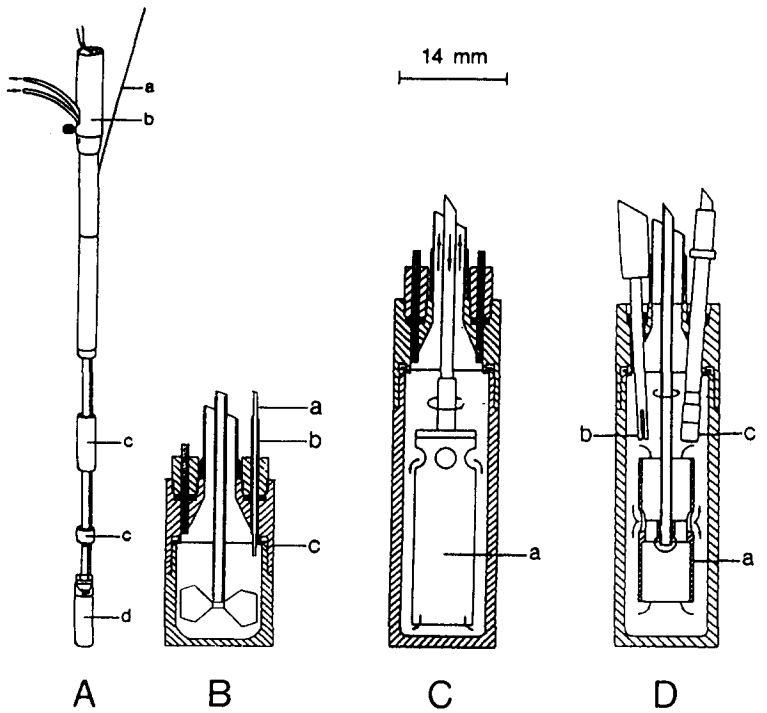

Fig. 6. A modular system of insertion vessels for use with Thermometric's 2277 microcalorimeter. (A) Picture of insertion vessel: a guide tube for injection needle; b, motor; c, brass bolts; d, sample compartment. (B) Section through a 1 cm^3 sample compartment with stirrer: a, injection needle, guide tube. (C) Section through a 3 cm^3 sample compartment for liquid perfusion experiments: a, turbine stirrer. (D) Section through a sample compartment equipped with electrodes for titration or perfusion experiments (titration needle not shown): a, tubine stirrer; b, pH electrode; c, O_2 electrode.

and depletion of O_2. Alternatively, it might be of interest to change the gas phase from air to air mixed with, for example, a pollutant gas. An important and sometimes difficult problem is then to avoid water vaporization or condensation in the calorimetric vessel.

Fig. 7 shows a simple and moderately sensitive semiadiabatic calorimeter (not a microcalorimeter) described by Heytler and Hardy [32]. It is one of the very few examples of gas perfusion calorimeters used with plant materials. The instrument was employed in measurements of N_2 fixation by soybean nodules. During the measurements the nodules were flushed with a controlled atmosphere, wetted by use of scrubbers outside the calorimeter, before finally being equilibrated (temperature, moisture content) in the thermostatic bath.

The enthalpy of vaporization of water at 25°C is as high as 2.4 mJ μg^{-1}. Thus, in a microcalorimetric experiment virtually no uncontrolled evaporation or condensation can be accepted. When measurements are conducted at a high sensitivity and a high gas flow rate, it can therefore be necessary to use a more exact liquid/vapour

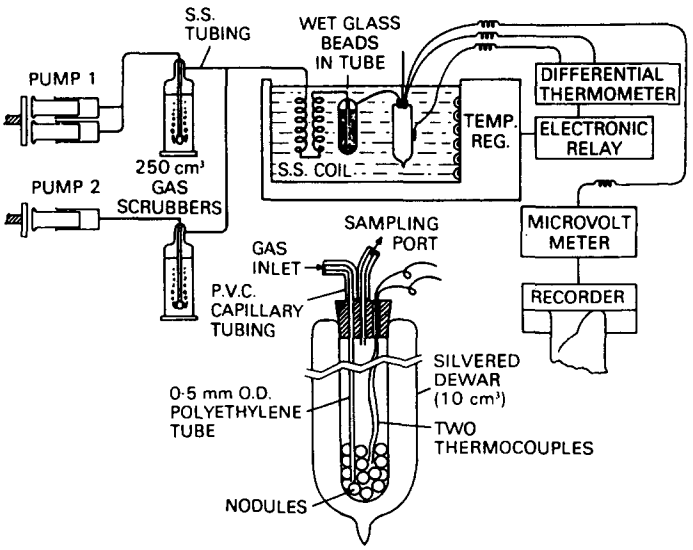

Fig. 7. Schematic picture of a simple gas perfusion calorimeter used for measurements of nitrogen fixation of soybean nodules. (Reproduced from Heytler and Hardy [32] with permission.)

equilibration procedure. Fig. 8 shows a perfusion vessel designed for use with a gas, liquid or segmented gas–liquid flow in a heat conduction microcalorimeter [33]. The fluid is equilibrated in a spiral-shaped steel tube (in thermal contact with the calorimeter heat sink) before reaching the sample container. When used with wet gas flow, a cotton thread, wetted with a suitable medium, is positioned inside the spiral tube. The gas will then gradually become temperature equilibrated and vapour saturated at a temperature very close to the one in the sample container. A related principle is employed with the gas perfusion vessel described in Ref. [22] and in Thermometric's microcalorimetric vessel (2254) primarily designed for vapour sorption measurements. With this latter vessel, the moisture content of the perfusion can easily be varied from 0% to 100% and it could be of interest to conduct metabolic experiments with plant materials under different moisture conditions. However, in such experiments it would be difficult to evaluate possible contributions to the calorimetric signal from evaporation or condensation processes in the vessel. In order to minimize the risk for uncontrolled processes of the kind, it seems preferable to work with gas flows in equilibrium with a medium contained in the vessel (for example liquid medium adsorbed on a piece of filter paper or an agar medium).

2.3. Photocalorimetry

Practically all calorimetric investigations on plant materials have been conducted in the dark. Criddle et al. [5] have pointed out that investigations carried out under

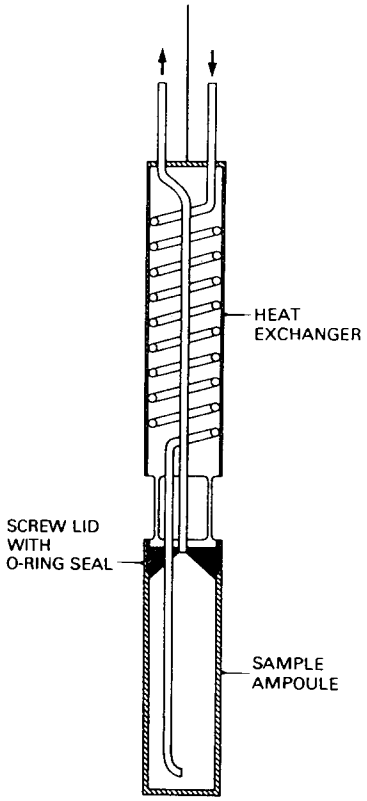

Fig. 8. A perfusion vessel for gas, liquid or segmented gas-liquid flow, designed for use with the LKB microcalorimetric system 2107 [33].

dark conditions in many instances may be preferable to experiments where photosynthesis will make the system more complex, for example in studies of growth rates and responses to some types of stress. Nevertheless, it is also felt important to conduct experiments with plants by use of photocalorimetry, both in studies of fundamental plant bioenergetics and, for example, in studies of different stress situations such as the effect of pollutant gases under light of different wavelength. Many photocalorimetric techniques have been described in the literature. In fact, by use of light guides, it is possible to transform almost any microcalorimeter used in chemistry or biology into a photocalorimeter. The introduction of light into a calorimetric vessel adds to the complexity of the instrument and the experimental procedure. However, with respect to photocalorimetric experiments on plant materials, the detailed analysis of the thermal measurements might be a more difficult problem. This appears to be an area where method work is required at the present time. Here some basic principles of an idealized calorimetric experiment combined with illumination will be outlined (see Ref. [34]).

2.3.1. Basic principles

In a photocalorimetric experiment, a certain amount of energy E_P is supplied to the calorimeter where some part will be transformed to heat Q. Other parts of the incident light energy might be reflected or (in the case of a transparent calorimetric vessel) transmitted to the surroundings (E_r and E_t, respectively). Likewise, if luminescence takes place and the system is transparent, radiant energy of this kind E_l will be lost to the surroundings. The enthalpy change for a photochemical process in the calorimeter $\Delta_P H$ is thus

$$\Delta_P H = E_P - Q - E_r - E_t - E_l \tag{1}$$

E_P is usually determined in a reference experiment where no photochemical process takes place. In analogy with Eq. (1), the energy balance in that experiment can be written as

$$0 = E_P - Q' - E'_r - E'_t - E'_l \tag{2}$$

where Q' is the heat quantity measured and E'_r, E'_t, and E'_l are energy quantities due to reflection, transmittance, and luminescence, respectively. Usually, the reflection terms in Eqs. (1) and (2) are small and, furthermore, nearly identical. When non-transparent calorimetric vessels are used, the two last terms in Eqs. (1) and (2) will be zero. Under such conditions, Eqs. (1) and (2) will lead to

$$\Delta_P H = Q' - Q \tag{3}$$

For the ideal case, where all light energy is transformed to heat in the reference experiment, Eq. (2) will lead to

$$E_P = Q' \tag{4}$$

The molar enthalpy change in the photochemical process, $\Delta_P H_m$, is

$$\Delta_P H_m = \Delta_P H / n \tag{5a}$$

or, under conditions where Eq. (3) will hold

$$\Delta_P H_m = (Q' - Q)/n \tag{5b}$$

where n is the amount of substance reacted in the photochemical process.

Where possible, n is determined by analysis, but it may be calculated if the overall quantum yield at the actual wavelength, ϕ_λ, is known. ϕ_λ is defined by

$$\phi_\lambda = n/N \tag{6}$$

where N is the total amount of photons supplied to the reaction system

$$N = E_P/L(hc/\lambda) \tag{7a}$$

or, when Eq. (4) is an acceptable approximation

$$N = Q'/L(hc/\lambda) \tag{7b}$$

Here L is Avogadro's number, h is Planck's constant, c is the vacuum speed of light and λ is the (average) wavelength of the light. Combination of Eqs. (5b), (6), and (7b) will lead to

$$\Delta_P H_m = (1 - Q/Q')(Lhc/\lambda)/\phi_\lambda \tag{8}$$

and

$$\phi_\lambda = nLhc/\lambda Q' \tag{9}$$

When Eq. (8) is used to calculate $\Delta_P H_m$, it is important that the photocalorimetric experiment and the experiment where ϕ_λ is determined are run under closely similar conditions.

2.3.2. Calorimetric techniques

Two main methods are used to introduce light into a calorimetric vessel: through a window made from glass or quartz or by use of light guides consisting of fibres, fibre bundles or solid rods, made from a polymer, quartz or glass. Liquid-filled flexible tubes are also used. Flexible light guides can be of great advantage from the point of view of instrument design and use. However, the transmittance of light through a flexible guide may vary significantly if its shape is changed. Therefore, it is essential that the light guide has a well-defined and fixed position in the instrument.

Several types of experimental assemblies are used in solution photocalorimetry as shown schematically in Fig. 9. Both macro- and micro-photocalorimeters have often been arranged according to the simple model I. Reference and reaction experiments are conducted separately, which requires that the light source and other parts of the calorimetric system are very stable. In model II, a second calorimeter (or some other instrument for measurement of light energy) is connected in series with a transparent photocalorimetric vessel and used for measurement of fluorescence or directly transmitted light. In model III, the incident light is split into two beams directed to each of the vessels in a twin calorimeter. In the vessel of the reference calorimeter C_R, all light energy is transformed to heat. If the differential system forms a perfect twin (thermally and with respect to the split light beam), the quantity $Q' - Q$ (Eq. (3)) will be obtained directly. However, the required high degree of twinning can be difficult to achieve and model IV might therefore be easier to realize. In this type, one light beam is directed intermittently to each of the vessels in the twin calorimeter. In model V, one part of the split beam is used to illuminate the sample in the photo reaction calorimeter (C_P). The other part of the light beam is measured in a separate reference calorimeter (C_R) where the light energy is quantitatively transformed to heat. In practice, and as indicated in the figure, both calorimeters C_P and C_R are usually part of twin instruments with no light supplied to their internal reference vessels, C_r. Alternatively, some non-calorimetric instrument, for example a photodiode, may be used instead of the reference calorimeter C_R. The photocalorimetric assembly V is first calibrated by experiments where both C_P and C_R are charged with a photoinert medium leading to quantitative transformation of radiant energy to heat (Q_0 and Q_0' respectively). The ratio

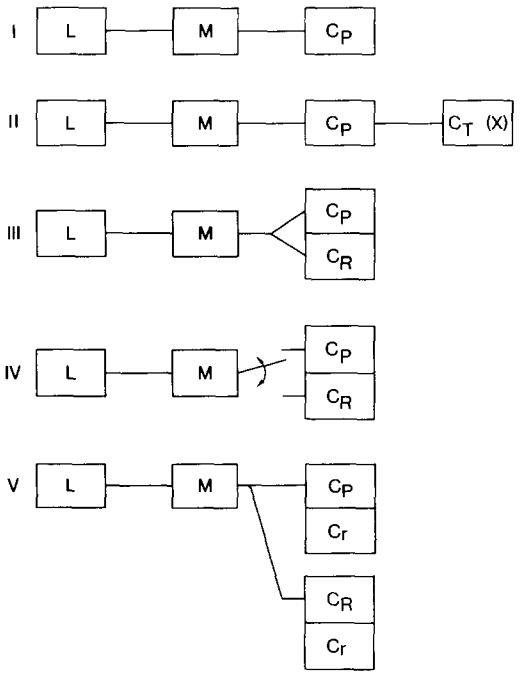

Fig. 9. Different types of solution photocalorimetric assemblies (see text). L, light source; M, monochromator, lenses, filters; C_P, photocalorimeter, C_T, photoinert calorimeter measuring transmitted light; C_R, photo-inert reference calorimeter; X, non-calorimetric device for measurement of radiant energy; C_r, twin reference vessel (no light supplied). Adapted from Teixeira and Wadsö [34].

between the measured heat quantities is an instrument constant (which may vary with the wavelength) a_λ.

$$\alpha_\lambda = Q_0/Q'_0 \tag{10}$$

The photocalorimetric assembly V thus can be used to continuously measure the radiant power supplied to reaction calorimeter C_P even if the radiant power from the light source fluctuates (cf. Eq. (4))

$$E_P = \alpha_\lambda Q' \tag{11}$$

2.3.3. A few practical designs

As long ago as 1939 Daniels and co-workers reported an interesting heat conduction photocalorimeter used for investigation of the quantum efficiency of photosynthesis in algae [16] (model II, Fig. 9). A cylindrical calorimetric vessel made from quartz was used. Light was introduced through one of the planar end walls and was partly absorbed by the cells. Heat evolved in the vessels was measured by use of a thermopile. Part of the light was transmitted through the rear end wall of the vessel and was measured by a separate thermopile.

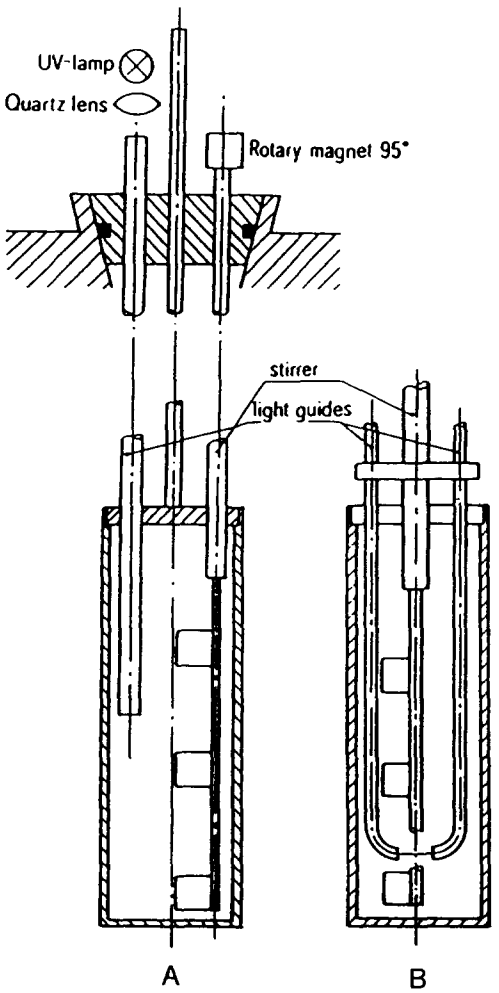

Fig. 10. Large vessels, volume 100 cm^3, of a Calvet microcalorimeter transformed (a) to photocalorimetric vessels and (b) to a vessel for simultaneous measurement of heat flow and optical density (cell concentration). (Reproduced from Schaarschmidt and Lamprecht [35] with permission.)

The simple photocalorimetric vessel reported by Schaarschmidt and Lamprecht [35] (Fig. 10(A)) is a typical example where an existing microcalorimeter (a Calvet microcalorimeter with 100 cm^3 vessels) has been transformed into a photocalorimeter. The light guide was a solid quartz rod, diameter 10 mm. The stirred vessel was used for investigations of the sensitivity of yeast cells to UV radiation. Fig. 10(B) shows a vessel equipped with two thinner quartz rods that were bent to a distance of 5 mm. Primarily, this vessel was not designed for use in photocalorimetric measurements but rather for the simultaneous determination of heat production and optical density of cell suspensions. One of the quartz rods was illuminated, the

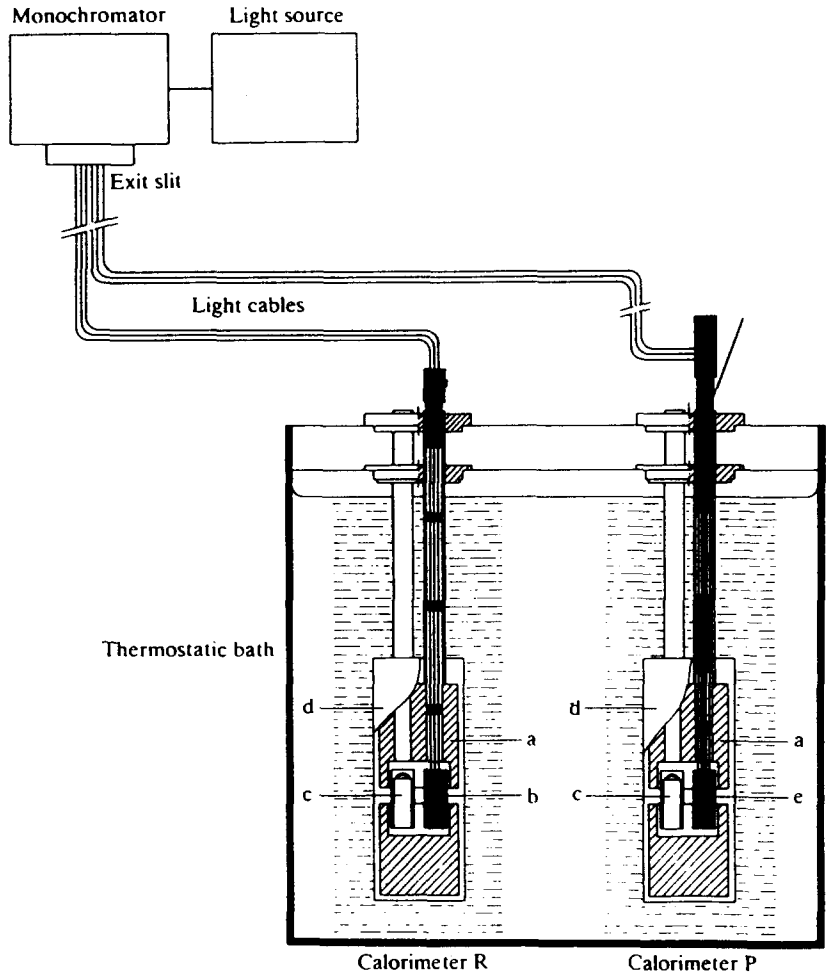

Fig. 11. Schematic picture of a photocalorimetric system according to model V (Fig. 9). Twin microcalorimeter P is used for the photochemical measurements and twin microcalorimeter R serves as a photo-inert reference. For each calorimeter: a, heat sink; b, photo-inert reference vessel; c, (internal) reference; d, steel can; e, photochemical reaction vessel. (Adapted from Teixeira and Wadsö [27].)

light passed through the 5 mm gap and through the other rod, bringing it to a photomultiplier. Different types of photocalorimeters have been used to study the chemistry of vision, including a typical solution–reaction microcalorimeter (LKB rotating batch microcalorimeter) fitted with quartz fibre light guides by Cooper and Converse [36]. That instrument is represented by model IV, Fig. 9.

Fig. 11 shows a photocalorimetric system according to model V. Six optical cables of the plastic, single-fibre type, diameter 1 mm, were used to guide light to the photocalorimetric vessels of two twin heat conduction calorimeters (Thermometric's TAM). The reaction vessel in calorimeter P is a stirred titration–perfusion

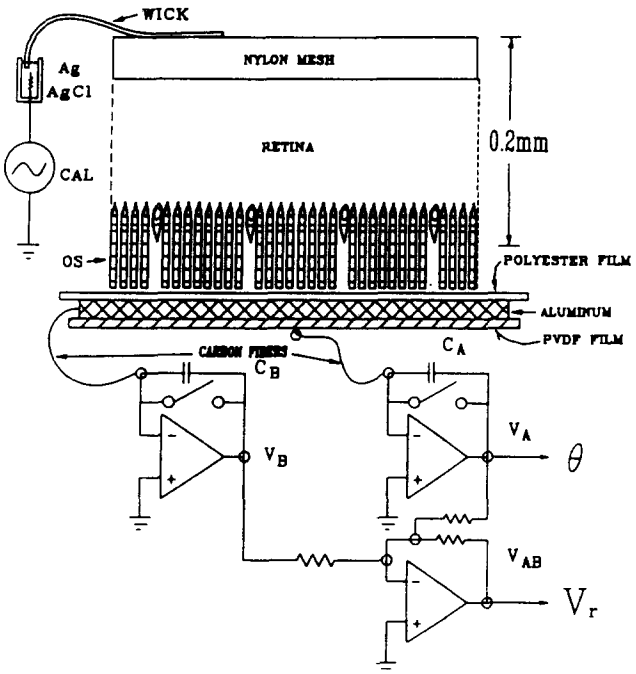

Fig. 12. Schematic diagram of a "pyroelectric" photocalorimeter used for investigations of retina. Operational amplifiers clamped the lower PVDF electrode and the Al foil and upper PVDF electrode at ground potential by current through 220 pF capacitors (C_A and C_B). Pyroelectric current from the PVDF flowed through C_A and C_B in opposite directions. A weighted sum of voltages V_A and V_B, V_{AB} was adjusted to be independent of temperature but responsive to transretinal potentials through the polyester film capacitance and the grounded wick on the retina. A voltage ramp in series with the wick calibrated V_{AB}. V_A gave the pyroelectric current integrated in C_A. V_A and V_{AB} were differentiated and recorded digitally as $\dot{\theta}$ and $\dot{V}_r$. After subtracting preamplified input currents, $\dot{\theta}$ and $\dot{V}_r$ were integrated numerically to yield changes in temperature θ, and in transretinal potential V_r. This figure was reproduced from W.A. Hagins et al. [39] with permission.

vessel (cf. Fig. 6(C)). The other calorimeter R, serving as the photoinert reference, used a simple light absorption vessel.

Figs. 10 and 11 represent typical solution photocalorimeters believed to be useful in work with different plant materials.

A very different type of photocalorimeter of potential interest for the plant field was first developed by Tasaki and co-workers [37,38]. Their instrument makes use of a pyroelectric film (PVDF) as sensor for fast temperature changes caused by a short (a few microseconds) light pulse on, for example, a piece of biological tissue (retina, nerve). The calorimetric signal, which is an electric potential proportional to the rate of change of the temperature of the PVDF film, has a time resolution approaching 10^{-3} s. Therefore, it seems as if such calorimeters could be important for studies of rather fast kinetics in connection with photoinduced processes in plant materials. Fig. 12 shows schematically an instrument of this kind reported by Hagins et al. [39] and used in studies of retinal rods from frog.

3. Conclusions

During the time span of only a few years, microcalorimetric techniques applied to plant cells and tissues have made a major step forward [5,6]. One may expect that these successful developments will continue, no least due to the fact that several types of investigations in this field appear to be of immediate practical importance. It is felt that recent advances in isothermal microcalorimetry which are currently applied to microorganisms and animal (including human) cellular systems, and to non-living systems, offer interesting possibilities which should be more explored in the plant field: stirred reaction vessels like those used in titration microcalorimetry, gas and liquid perfusion microcalorimetry and different kinds of photomicrocalorimetry. Problems with possible evaporation/condensation processes in gas perfusion experiments with plant tissues need more attention. Method work related to the interpretation of results from photochemical experiments with plant tissues is needed. An important area for isothermal microcalorimetry is applications where the instruments are used as "process monitors" for living as well as for non-living systems. It is felt that such techniques will develop in a direction where increasingly they will be more integrated with other continuous analytical techniques. In some cases, sensors of different types can be placed in the calorimetric vessel or in a flow line immediately after a perfusion vessel. However, such multifunctional instrument systems are bound to be complex and are vulnerable to technical problems. A simple static ampoule used as a calorimetric vessel will always be the most dependable process monitor for plant tissues and will be well suited for use with possible future multichannel analytical machines. Therefore, it is important to investigate the shortcomings of such simple vessels in comparative measurements using vessels or instrument systems where there is a better control of the physiological processes.

References

[1] A.E. Beezer, Biological Microcalorimetry, Academic Press, London, 1980.
[2] A.M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, Wright, Bristol, 1987.
[3] I. Lamprecht, W. Hemminger and G.W.H. Höhne, Thermochim. Acta, 193 (1991) (special issue).
[4] H. Prat, in H.D. Brown (Ed.), Biochemical Calorimetry, Academic Press, New York, 1969, p. 181.
[5] R.S. Criddle, R.W. Breidenbach and L.D. Hansen, Thermochim. Acta, 193 (1991) 67.
[6] I. Lamprecht, K. Drong, B. Schaarschmidt and G. Welge, Thermochim. Acta, 187 (1991) 33.
[7] L.D. Hansen, Thermochim. Acta, 250 (1995) 215.
[8] M. Kleiber, The Fire of Life, Wiley, New York, 1961, p. 200.
[9] I. Lamprecht and F.-R. Matushka, Thermochim. Acta, 94 (1985) 161.
[10] C. Schulz, M. Thuy and G. Wegener, Thermochim. Acta, 187 (1991) 71.
[11] B. Fagher, M. Monti and I. Wadsö, Clin. Sci., 70 (1986) 63.
[12] E.S. Hansen and J. Knudsen, Biochim. Biophys. Acta, 721 (1982) 418.
[13] R.S. Criddle, L.D. Hansen, R.W. Breidenbach, M.R. Ward and R.C. Huffaker, Plant Physiol., 90 (1989) 53.
[14] E. Iversen, E. Wilhelmsen and R.S. Criddle, J. Food Sci., 54 (1989) 1246.

[15] I. Wadsö, in A.M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, Wright, Bristol, 1987, p. 34.
[16] J.L. Magee, T.W. DeWitt, E.C. Smith and F. Daniels, J. Am. Chem. Soc., 61 (1939) 3529.
[17] A.J. Fontana, L.D. Hansen, R.W. Breidenbach and R.S. Criddle, Thermochim. Acta, 172 (1990) 105.
[18] L.D. Hansen and R.S. Criddle, Thermochim. Acta, 154 (1989) 81.
[19] L.D. Hansen and R.S. Criddle, Thermochim. Acta, 160 (1990) 173.
[20] R.S. Criddle, A.J. Fontana, D.R. Rank, D. Peige, L.D. Hansen and R.W. Breidenbach, Anal. Biochem., 194 (1991) 413.
[21] R.S. Criddle, R.W. Breidenbach, D.R. Rank, M.S. Hopkin and L.D. Hansen, Thermochim. Acta, 172 (1990) 213.
[22] P. Bäckman, R.W. Breidenbach, P. Johansson and I. Wadsö, 9th ISBC Conf., Berlin-Schmerwitz, Germany, 1994, S16.
[23] P.C. Anderson, R.E. Lovrien and M.L. Brenner, Planta, 151 (1981) 499.
[24] E. Gnaiger, J. Exp. Zool., 228 (1983) 471.
[25] J Suurkuusk and I. Wadsö, Chem. Scr., 20 (1982) 155.
[26] N.M. Görman, J. Laynez, A. Schön, J. Suurkuusk and I. Wadsö, J. Biochem. Biophys. Methods, 10 (1984) 187.
[27] C. Teixeira and I. Wadsö, J. Chem. Thermodyn., 22 (1990) 703.
[28] P. Bäckman and I. Wadsö, J. Cell Physiol., 150 (1992) 99.
[29] P. Bäckman, M. Bastos, L.-E. Briggner, D. Hallén, P. Lönnbro, S.-O. Nilsson, G. Olofsson, A. Schön, J. Suurkuusk, C. Teixeira and I. Wadsö, Pure Appl. Chem., 66 (1994) 375.
[30] P. Lönnbro and P. Hellstrand, J. Physiol., 440 (1991) 385.
[31] A. Schön and I. Wadsö, J. Biochem. Biophys. Methods, 13 (1986) 135.
[32] P.G. Heytler and R.W. Hardy, Plant Physiol., 75 (1984) 304.
[33] I. Wadsö, Sci. Tools, 21 (1974) 18.
[34] C. Teixeira and I. Wadsö, Netsu Sokutei, 21 (1994) 29.
[35] B. Schaarschmidt and I. Lamprecht, Experimenta, 29 (1973) 505.
[36] A. Cooper and C.A. Converse, Biochemistry, 15 (1976) 2970.
[37] I. Tasaki and K. Iwasa, Biochem. Biophys. Res. Commun., 101 (1981) 172.
[38] I. Tasaki and T. Nakaye, Science, 227 (1985) 654.
[39] W.A. Hagins, P.D. Ross, R.L. Tate and S. Yoshikami, Proc. Natl. Acad. Sci. USA, 86 (1989) 1224.