Microcalorimetric Investigations of Animal Cells

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Calorimetric measurements on living animal cells normally involve determination of heat production rates under essentially isothermal conditions. Experiments are usually performed by use of twin heat conduction microcalorimeters. Combination of results from calorimetric measurements and analytical determinations lead to valuable thermochemical information. But in most studies conducted so far, the calorimeters are used solely as analytical tools, "process monitors". Experiments are easy to perform but several critical experimental parameters should be taken into account.

INTRODUCTION

Current microcalorimetric work on living systems includes investigations of microorganisms, small animals (in particular aquatic animals and insects, pieces of animal tissue, animal cells and materials from plants (2, 7, 16). A large part of the cells studied are of human origin. Most common are the investigations performed on the main fractions of blood cells: red cells, natural mixtures of lymphocytes, granulocytes and platelets (10, 16). Other mammalian cell systems which have been investigated include skin cells, fat cells, liver cells, heart cells, sperm cells, muscle fibre bundles, tumour cells and cultured tissue cells (8, 16).

As may be expected, many investigations of human cells have been connected with work in the fields of clinical and pharmacological analysis. Such studies have therefore often been concerned with comparison between properties of cells from patients and healthy subjects or with the effect of drugs on cells (10, 13).

THERMODYNAMIC AND ANALYTICAL MEASUREMENTS

It is often desirable that calorimetric measurements on cellular systems are accompanied by extensive and careful measurements of changes in medium composition (including consumption of oxygen and release of carbon dioxide) and of biomass. The combined results will lead to a thermochemical characterization of the system, i.e. an account of its material—energy balance. Results of such investigations are of basic interest in cell physiology and they will also lead to an improved understanding of the connections between the calorimetric signals and different metabolic properties of the cells. However, in most cases so far, microcalorimetric measurements of animal cells have been on a rather primitive level, from a thermochemical point of view. The instruments have mainly been used as general process monitors and little use has been made of the fact that thermodynamic properties have been determined. It is felt that a more pronounced thermodynamic approach in some investigations in this field would give a significant support also to the further development of the purely analytical use of the technique.

CALORIMETRIC TECHNIQUES

Microcalorimetry in the sense we now use this term, was introduced in the 1950s, but it was not until the early 1970s that microcalorimetric measurements started to be performed on animal cells, mainly on blood cells. Much
methodological work directed towards the special problems connected with measurements of animal cells have since been performed (16, 17) and during the 1980s significant instrument development of importance for this field took place (16).

In work on animal cells (like work on e.g. microorganisms), it is normally the heat production rate, the "thermal power", which is measured. Measurements take place under essentially isothermal conditions and in most cases thermopile heat conduction calorimeters, in twin configuration, are used (17). In most work conducted so far, static ampoules, volume about 1-5 ml, have been used as reaction vessels. Typically, 10^6 cells are required for a precise (1-5%) determination of a thermal power value. Measurements have also been performed with flow vessels, using flow-through, flow-mixing or stopped flow techniques. Recently small stirred vessels (1-3 ml) with possibilities for injections of reagents and flow-through (perfusion) of medium have been described (5, 17). With such vessels it is possible to keep cells in uniform suspension or the cells can be attached to e.g. a plastic film or to microcarriers kept in suspension. Small pieces of tissue (e.g. 50 mg) can be housed in a rotating cage in intimate contact with the medium, which may perfuse through the vessel (4). Very recently a "titration-perfusion vessel" of this kind has been equipped with miniature electrodes for the determination of pH and oxygen concentration, simultaneously with the measurement of the thermal power (1). The level of thermal power in current experiments on animal cells is typically in the range of 5-50 μW. The duration of the experiments varies but it is often of interest to follow the heat production during several hours. Such measurements will therefore require a very stable instrument baseline. Baseline stability values for some of the instruments in current use are about ±1 μW per 24 h.

**SOME PROBLEMS**

Although microcalorimetric measurements on animal cells often involve the determination of very small heat quantities or low heat production rates over long periods of time, it is not difficult to perform such experiments any more. But there is a number of problems which should be recognized. First, there are some general rules to be observed in all types of solution microcalorimetry (15, 17). It should also be noted that electrical calibrations of some microcalorimetric vessels might be problematic. Chemical test and calibration processes should therefore be used more frequently (3). There are also several experimental parameters which can be very critical in quantitative work with living cells. Some of them will be briefly discussed below, with particular reference to work with animal cells.

The preparation method and the purity of cell preparations can be very important for the thermal power value of a cell sample. The same applies to the time and the temperature at which the cells have been stored before the measurement (4, 6, 11).

Naturally, the medium composition is of great importance for the metabolic rate and thus for the rate of heat production. In a batch experiment, the pH of the medium normally will decrease with time, primarily due to the production of lactate. The metabolic rate of animal cells can change significantly following a very small change of pH. In particular red cells have been found to be very pH-sensitive: a change in pH by 0.01 unit at pH 7.4, results in a change of the thermal power by 1.2% (11).

In work with animal cells, using static calorimetric vessels, most of the cells normally will rest on the bottom of the vessel or there will at least be large gradients in the cell concentration. In cases where there will be some sort of "crowding effect" it will obviously not be studied under well defined conditions. When cells sediment in a non-stirred vessel, there will also be concentration gradients of nutrients and metabolites including the pH.

Sometimes it is difficult to sterilize calorimetric vessels and long term experiments with cells are therefore often conducted in the presence of antibiotics, usually gentamicin. Recent methodological work on human blood cells (12) and T-lymphoma cells (9), indicates that gentamicin, at low concentration, will not interfere with the metabolism of these cells. Further work on other cell types could be performed. Cultured tissue cells are frequently contaminated by mycoplasma which might significantly influence their rate of heat production.

Static microcalorimetric vessels are very convenient to use in many applications, including
work on living cells. However, particularly in work on animal cells, they should be used critically. Flow vessels of different types (17) can be useful in work with animal cells, but one must watch out for the possibility that cells may sediment in the flow line or in the flow vessel. Animal cells are often sticky, which in combination with the sedimentation effect, can make it particularly difficult to use flow vessels. It is concluded that stirred vessels, with or without perfusion of medium, make it possible to conduct the cell experiments under the accurately defined conditions needed for determination of reliable metabolic data.

Use of different calorimetric techniques can often lead to different results, because the physiological conditions may not be the same. Particularly striking examples are given in a report on human muscle fibre bundles, performed in a static vessel, in a non-stirred perfusion vessel and in a stirred perfusion vessel (4).

CONCLUSION

Recent developments of microcalorimetric instruments have led to improved methods for measurement of thermal power produced by different kinds of animal cells. Microcalorimetry forms an important method for the characterization of such cells in basic as well as in applied work.

REFERENCES
1) Bäckman P, Schön A and Wadsö I. To be published