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THE USE OF MICROCALORIMETRY IN STUDIES OF MAMMALIAN CELLS

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Recent developments in instrumentation and working procedure in microcalorimetry have led to an increased interest in the direct measurements of heat production rates of living cells. Specific problems connected in particular with work on mammalian cells are briefly discussed and a system of microcalorimeters suitable for work on such cells is described. A few examples of its use in studies of *T*-lymphoma cells are briefly discussed.

Calorimetric work on living cells

A major part of the calorimetric work on cellular systems has been performed with microorganisms. [1–3] In most cases the studies have been made on pure cultures of bacteria or yeast but poorly defined mixtures such as those found in natural systems have also been investigated. The work on pure cultures has to a large extent been conducted on a thermodynamic level

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and the thermochemistry of microbial growth and maintenance is much better understood than that for mammalian cells. Most studies so far for these cells have dealt with the different major fractions of blood cells: erythrocytes, lymphocytes, granulocytes and thrombocytes. Other mammalian cells which recently have been studied by microcalorimetric methods include fat cells, macrophages, liver cells, skin cells, tumor cells and several kinds of cultured tissue cells from established cell lines. Aggregates of cells in the form of pieces of tissue such as muscle and fat tissue have also been investigated. To a large extent work on mammalian cell systems has been performed on human material as part of methodological and screening studies undertaken to explore the possible use of microcalorimetry in clinical analysis. Thus many studies have been made on cells from different categories of patients.

Some experimental parameters influencing the thermal power from mammalian cells

There are many experimental parameters which can greatly influence the thermal power produced by living cells. [4] Examples include preparation method, storage conditions and storage time, type of medium used and the cell concentration. For instance for some mammalian cells significantly different power values, measured at 37°, are obtained if the cells have been stored for a a few hours at 5° compared to storage at room temperature. As may be expected the measurement temperature and the *pH* of the medium will greatly affect the metabolic heat production rate. For instance, red blood cells suspended in a glucose medium at *pH* of about 7.4 will change their thermal power by 1.5% for a change of *pH* by 0.01 unit. [3] Mammalian cells require oxygen for their life functions and it is thus necessary that oxygen is present in the medium. This can be a difficult condition to meet if work is done on concentrated suspensions using static calorimetric ampoules (in contrast to stirred vessels or flow or perfusion vessels). Cells in suspension tend to sediment if a suitable stirring or flow arrangement is not provided for. In such cases the metabolic rate will usually decrease due to inhibited diffusion which will cause a local decrease in *pH* and possibly starvation effects (energy source, oxygen). In some cases a true "biological crowding effect" can occur. Many types of mammalian cells can easily be damaged by different kinds of mechanical treatment, for instance stirring. This can cause difficulties in keeping the cells uniformly suspended in a calorimetric vessel. In addition to the difficulties from a

purely calorimetric point of view to arrange for efficient stirring in a vessel which may be used for measurements on the microwatt level. Many types of mammalian cells tend to adhere to surfaces. This can create problems, in particular if one wishes to use a flow calorimetric method.

Factors like those given in the above examples call for carefully developed instrument designs and working procedures. It may then not be possible or practical to design the instrument in a way which would make it close to ideal from a calorimetric measurement point of view. This will frequently lead to situations where chemical calibration procedures are preferred before the more traditional electrical calibrations.

A microcalorimetric system

For a long time – about 25 years – our laboratory has been involved in the design and use of microcalorimeters, specially for use in biochemistry and cell biology. During recent years our attention has increasingly been directed towards the special problems connected with work on mammalian cells. Such considerations were taken into account when we designed our 4-channel microcalorimeter, which, like most current microreaction calorimeters, is based on the heat conduction principle. The basic unit consists of a thermostated water bath and an electric console, Fig. 1A. The temperature stability in the room temperature region is $\pm 1 \cdot 10^{-4}$ K over long periods of time (days) if the ambient temperature changes are kept within ± 1 K. The water bath can hold up to 4 cylindrically shaped twin calorimeters ("channels"), which can be fitted with different types of reaction vessels giving different functions to the calorimeters. Development work on such specialized calorimetric vessels continues at the present time.

Figure 1B shows schematically a "channel" for use with cylindrical insertion vessels, diameter 14 mm. The twin calorimetric unit, Fig. 1C, has two tube-shaped holders for the insertion vessels. Each of them is surrounded by semi-conducting thermocouple plates ("Peltier effect plates"). The upper and the lower parts of the vessel holders fit into bores in the main cylindrical blocks (the "heat sinks"). Several types of simple closed vessels have been used in our work on cells: 1, 3 and 5 ml steel ampoules or a glass vial. Such static vessels are convenient to use but as was discussed above they are often not adequate for work on living cells.

A twin channel essentially identical to that shown in Fig. 1 has been fitted with 24K gold tubes forming flow-through and flow-mixing vessels. Parts of the gold tubes are in contact with the heat sinks and the water bath where

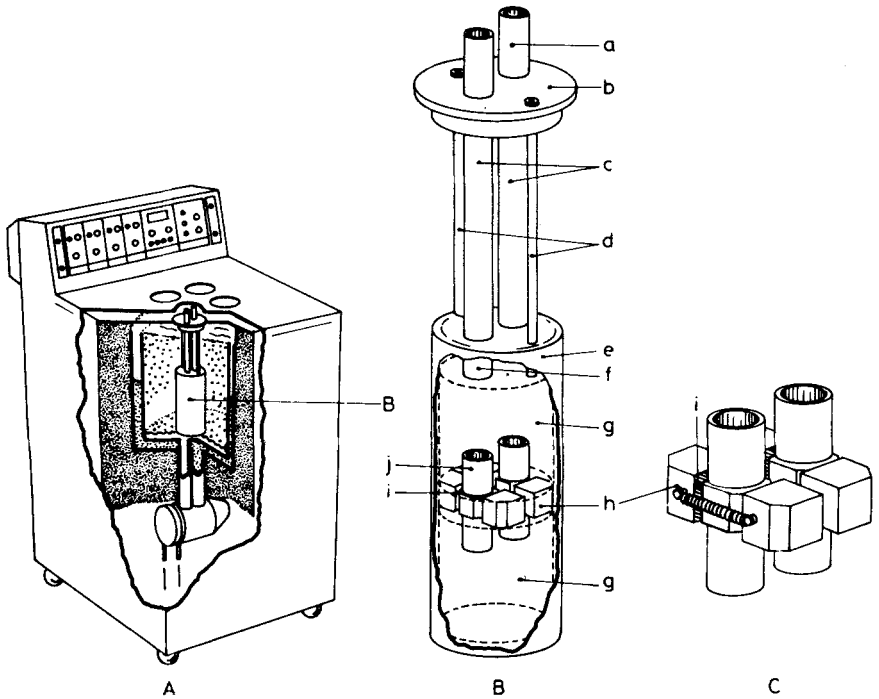

Fig. 1 A. The 4-channel microcalorimeter showing one measuring cylinder, B. immersed in the thermostated water-bath. C. is the twin calorimetric unit: a, connecting tubes; b, lid; c, 16 mm steel stubes; d, 6 mm steel tubes; e. steel vessel; f, connecting tubes; g, aluminium block; h, small aluminium block; Peltier effect plate; j, ampoule holder.

they serve as heat exchangers. Such flow vessels are of interest in work with many types of mammalian cells, but they cannot be used when the cells tend to adhere to the flow lines or to the flow vessels. Further flow calorimetry tends to require much larger quantities of cells than procedures where the cells are measured batchwise. Our experience with the static ampoules and the flow vessels led us to the design of the perfusion-titration vessel shown in Fig. 2A. Like the basic units of the calorimetric system this vessel forms a modular system which can be given different functions by using slightly different components. The version shown in Fig. 2 was mainly designed for use in work on mammalian cells [6, 7]. It consists of a sample compartment which is connected to a steel tube. Inside this tube there is a stirrer shaft rotated by a motor typically at a speed of 80 rpm. The brass bolts serve as thermal shortages to the water bath and to the upper heat sink, respectively. Experiments can be performed with or without liquid

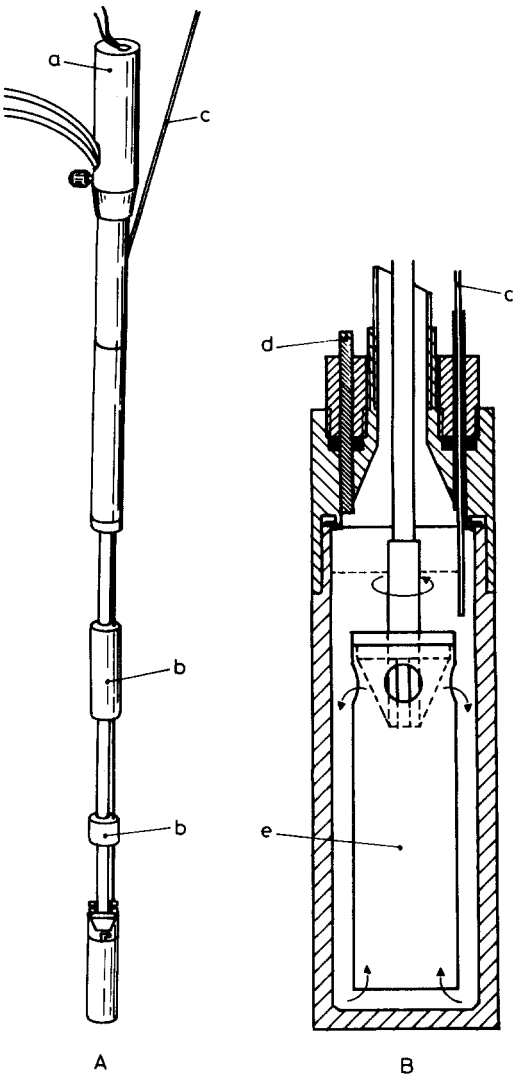

Fig. 2 A. Insertion vessel used for titration-perfusion experiments. B. Ampoule for the insertion vessel: a, stirring motor; b, brass bolts; c, injection tube; d, stopper (replacing the injection tube); e, turbine stirrer made from Kel F.

perfusing through the vessel, $\leqslant 25$ ml/h. During perfusion the liquid is introduced to the vessel through the space between the stirrer shaft and the steel tube and it leaves the sample cup through the stirrer shaft. Thus the steel tube and the stirrer shaft act as a counter-current heat exchanger. Sample

cups have a volume of 1 or 3 ml. In 3 ml vessels a "turbine stirrer" [7] is normally used, Fig. 2B. This has the advantage of gently circulating the cells up and down in the vessel, thereby keeping them in uniform suspension. Alternatively this stirrer can be used to keep microcarrier particles with attached cells in uniform suspension. In such experiments it is usually advantageous to perfuse medium through the vessel which can be done without losing any cells [7]. Perfusion can also be used with cells anchored to a film positioned around the inner wall of the sample cup. [6] For cells in free suspension there is usually a gas phase above the medium and no liquid perfusion takes place. Reagents can be added to the sample compartment during a calorimetric measurement. This is done by inserting a thin injection needle (i.d. 0.3 mm) into the titration tube.

With the simple closed ampoules baseline stability over a day is typically as good as $\pm$ 0.1 μW. The reproducibility of the baseline position between different experiments is about the same. With the stirred vessels, the baseline is somewhat less stable, typically $\pm$ 0.3 μW for several hours and the reproducibility of the baseline position is typically $\leqslant$ 0.5 μW.

Below a few examples are given from our recent work with *T*-lymphoma cells using the vessel shown in Fig. 2. In these experiments the commercial version of the 4-channel instrument was used (LKB Thermometric, Bromma, Sweden).

Figure 3a shows the power time curve from an experiment with $1.6 \cdot 10^6$ lymphoma cells in a medium where no growth takes place. It is seen that a good steady state value is obtained for the thermal power. 75% of the measured power could be accounted for by the measured values for rates of formation of lactate and bicarbonate from the glucose in the medium [8]. It was suggested that the residual power came from oxidation of intracellular material. Figure 3b shows a powertime curve for $1.9 \cdot 10^6$ lymphoma cells in a medium which allows growth. It is seen that the power in this case increases almost linearly (generation time was about 20 h). The thermochemistry of the growth process has not yet been clarified but is under investigation. Growth curves of the type shown in Fig. 3b have been used in some basic experiments concerned with the action of lectins [9] and in an analytical study with the long-range goal of finding a predictive test for antitumor drugs. [10] These experiments are reported briefly below.

Non-calorimetric work with the lectines *PHA-L*$_4$ and *PHA-E*$_4$ has shown that the L_4 lectin is a much more active inhibitor of DNA synthesis than the E_4 compound. The picture became quite different following a study where the thermal power was recorded for cell suspensions incubated with different concentrations of the two isolectins. [9] The calorimetric results

showed that the inhibitory effect of the L_4-lectin did not change significantly with concentration. The E_4-lectin had virtually no effect at low concentrations but at higher concentrations the effect increased dramatically and became much higher than observed for L_4 at corresponding concentrations. These surprising results were observed thanks to the non-specific but sensitive calorimetric technique and led to a more detailed analysis and subsequent clarification of the actions of the two lectins tested.

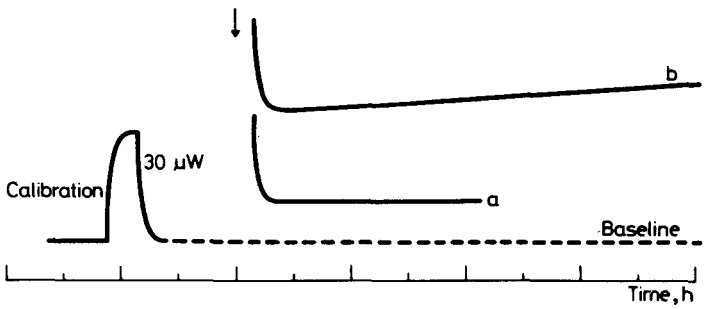

Fig. 3 Power-time curves for experiments with lymphoma cells under non-growing conditions (a) and under growing conditions (b). The amount of cells was $1.6 \cdot 10^6$ and $1.9 \cdot 10^6$ cells respectively.

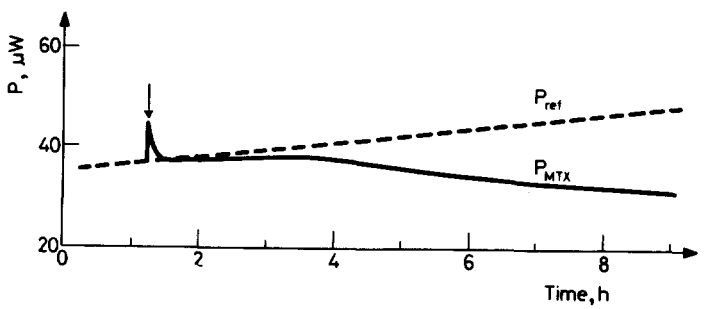

Fig. 4 Power-time curve for a sample of *T*-lymphoma cells where MTX was injected, P_{MTX}. The dashed line, P_{ref}, represents the power-time curve for a reference sample where no MTX was added.

The dashed curve in Fig. 4 shows the power time curve for a reference sample of lymphoma cells under growing conditions. The other curve shows a corresponding curve from an experiment where the antitumour drug methotrexate was added to a final concentration of 0.18 $^1/_2$ *M* at the time indicated by the arrow. [10] The two experiments were run in parallel

using two channels and the cells were from the same batch. It is seen that the two curves deviated markedly from each other soon after the drug injection. Comparison between results of the calorimetric experiments and of viability measurements suggest that the type of deviation observed between the two curves already a few hours after the addition of the drug can be useful in predictive in vitro testing of anti-tumour drugs.

Conclusion

Recent developments of microcalorimetric instruments have led to improved methods for measurement of thermal power produced by different kinds of mammalian cells. Microcalorimetry forms an important, although as yet not very videly used, method for the characterization of such cells is basic as well as in applied work.

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Zusammenfassung – In letzter Zeit führte die Entwicklung von Geräten und Arbeitsvorschriften in der Mikrokalorimetrie zu erhöhtem Interesse an direkten Messungen der Wärmeproduktionsgeschwindigkeit von lebenden Zellen. Spezifische Probleme im Zusammenhang mit der Arbeit an Säugetierzellen werden kurz dargelegt und ein zur Arbeit an solchen Zellen geeignetes Mikrokalorimetriesystem beschrieben. Einige Anwendungsbeispiele werden and Hand der Untersuchungen von *T*-Lymphozyten kurz beschrieben.

РЕЗЮМЕ — Недавние достижения аппаратурных и экспериментальных разработок в микрокалориметрии, привело к увеличению интереса в области измерений скоростей теплообразования в живых клетках. Кратко обсуждены специфические проблемы, касающиеся работы с клетками млекопитающих, и описана система микрокалориметров, приемлемых для работы с такими клетками. Кратко обсуждено несколько примеров использования таких микрокалориметров при изучении *T*-лимфоцитных клеток.