Pure & Appl. Chem., Vol. 65, No. 9, pp. 1973–1977, 1993.
Printed in Great Britain.

Illustrations of the value of calorimetry in biology

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Abstract

Calorimetry was used to measure heat generated by both living microorganisms and macroorganisms. This seemingly simple measurement can give an evaluation of the state of an organism and can differentiate between rest, sleep, under anaesthesia, activity and inactivity. The great advantage of calorimetry is that the measurement is not harmful and does not cause any damaging effect on the samples.

INTRODUCTION

Any organism has to perform chemical, physical and dynamic work for living and surviving. This includes not only anabolism, the synthesis of high molecular compounds such as nucleic acids and proteins, but also dynamic work such as cell membrane movement and muscle contraction. To perform this work, heat is eventually generated and can be measured. When organisms are active, the activity can easily be determined by various biochemical assays such as H^3-thymidine incorporation into DNA[1] chemical analysis such as quantitation of molecular compounds and microscopical observation by staining. In animals their movement and behaviour can be observed as is done in physiological experiments. On the other hand, it is rather difficult or almost impossible to determine how inactive organisms are. Inactivation of organisms can be achieved by placing them under pain and anaesthesia, which can be reversible under carefully controlled conditions. Heat dissipated was measured to determine the anaesthetic state of both a micro-organism using a cultured cell line and a macro-organism using mice.

MATERIALS AND METHODS

An Ehrlich ascites cell line, a gift from the Japanese Cancer Research Resources Bank, was used. The number of live cells was determined by MTT, 3-(4.5-dimethylthiozol-2-yl)-2.5-diphenyltetrazolium bromide, assay.[2] MTT is a tetrazolium compound and readily accepts hydrogen when dehydrogenase is in action. It has been established that the number of cells is directly proportional to the amount of reduced MTT. Mice used were male ICR, aged 8 to 20 weeks, and weighing 30-40g. The calorimeters used were the Thermoactive Cell Analyzer ESCO3000 (TCA) for cells and the BioDynamic Calorimeter BDC200 for mice. Calibration was made by giving electrically determined heat in uW for the TCA and Watts for the BDC. Sensitivity of the former was 1 uW and the latter was 20 mW. The BDC is equipped with a wheel for free exercise and a plentiful supply of food and water. Pain was induced in mice by

intraperitoneal(i.p.) injection of 0.25cm^3 0.1M acetic acid. Control mice were i.p. injected with physiological saline. Lidocaine (International Nonproprietry Names),a synthetic aminoacyl amide was used for cells at concentrations of either 1.5mM to measure the long term effect and 7.4mM for the short term effect. The cells were washed once with medium to remove lidocaine. Lidocaine treatment lasted for up to 2 weeks during which heat was measured. Isofluorane gas was introduced into the whole body calorimeter through the air duct at concentrations of 0.5%, 0.75% and 1.0% for four hours and 1.5% for three hours. A mouse was placed in the calorimeter at least three hours prior to the introduction of the gas to allow it time to settle down in its new environment. Heat dissipated was continuously measured for at least 48 hours.

RESULTS

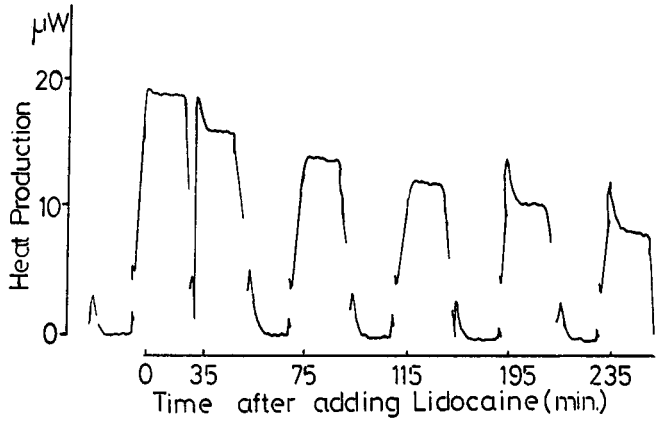
I Microcalorimetry

Cells under anaesthesia

1. Effect on heat production after Lidocaine treatment

A decrease in the quantity of heat produced could be detected 35 min after 7.5mM lidocaine treatment on Ehrlich ascites followed by a gradual decrease with time.(see fig. 1)

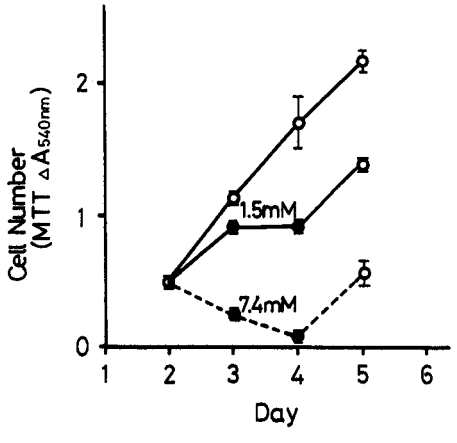
Fig.1



2. Recovery from Lidocaine treatment

Cells were cultured for 48 hours with 1.5mM and 7.4mM lidocaine and were observed after removal of lidocaine. The cells were then cultured for a further 24 hours. The increase in number and the rate of increase was similar to that of cells under normal culture conditions. (see Fig. 2)

Fig.2



II Whole body calorimetry

1. Mice under normal condition

The pattern of heat dissipation by two mice measured for 96 consecutive hours is shown in Fig. 3a. Mouse B exhibited a regular pattern throughout the measurement, while mouse A shows some disturbance in the pattern on day 2 and day 4. Thereafter all measurements were performed between the first and the second day. Because a good correlation was found between the degree of heat generated and the rate of revolution of the wheel, it was possible to conclude that a higher level of heat dissipation was the result of more exercise.
Fig.3b shows the integrated heat dissipation into kilo Joules(kJ) of 8 hour periods. Night(N) was defined between 18:00 hour of the first day to 02:00 of the following day, Morning(M) was between 02:00 to 10:00 and Day(D) was between 10:00 to 18:00. Each line represents one mouse and ten mice were measured. Averages and standard deviations of heat generated during 24 hour periods were 66.7±8.8kJ(100%), of which 29.0±5.1kJ(43.6%) was generated during the night, 20.6±2.3kJ(31.2%) was generated during the morning and 16.8±2.7kJ(25.3%) during the day.

Fig.3a

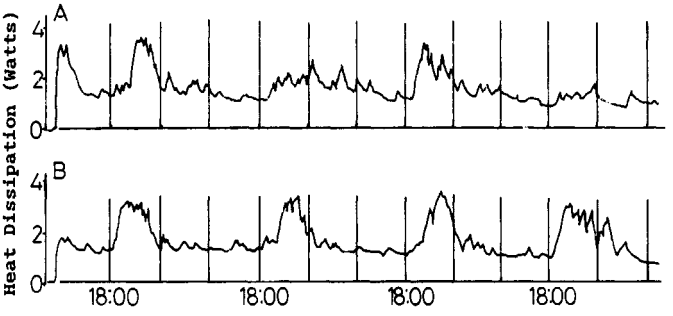
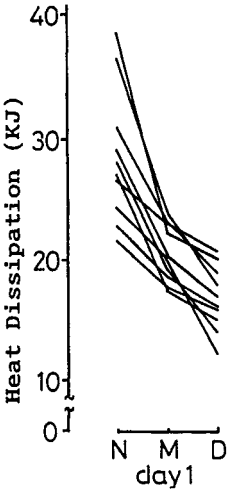


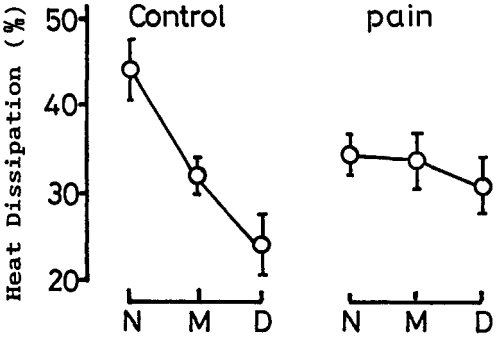
Fig.3b



2. Pain research

Heat dissipation was measured in nine mice with pain as well as in seven control mice. 24-hour integrated heat dissipation was 55.2±7.6 kJ and 61.0±7.3 kJ, respectively. In Fig.4, the percentage of heat dissipated during 8 hour periods(Night, Morning and Day) is shown. The pattern of 8-hour circadian rhythm in control mice was identical to that of normal mice as observed in Fig. 3b. However, the rhythm was completely disrupted in the presence of pain.

Fig.4

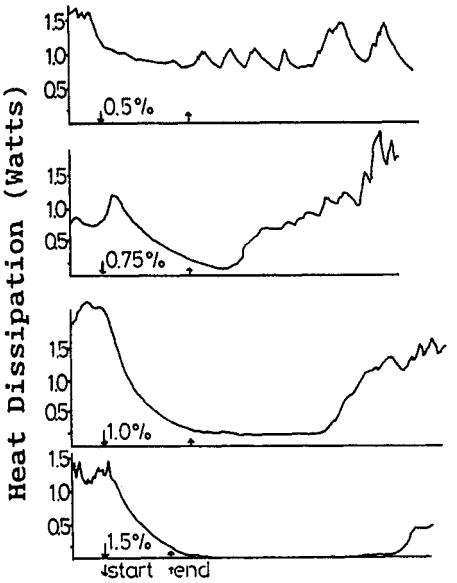


3. Mice under anaesthesia

A strikingly rapid decrease in heat production was seen when more than 0.75% isofluorane gas was passed through the chamber, while such a decrease was not in evidence when 0.5% gas was given(see fig. 5).

A constant amount of heat was still generated for a few hours even after 1.0% isoflurane was stopped. Interestingly, when 1.5% gas was used, although the mouse was alive, no heat was detected for a few hours. The mouse eventually recovered since the heat rose to the usual level. The finding that no heat was detected was confirmed in 4 out of 6 mice.

Fig.5



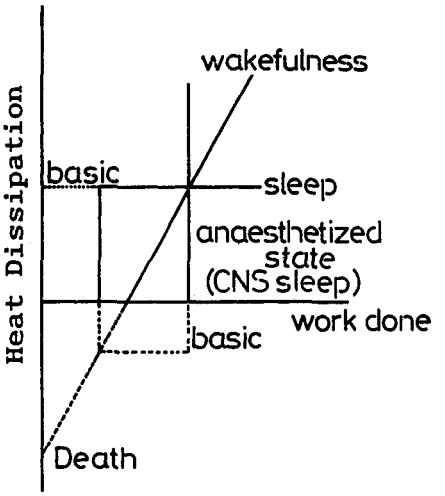
DISCUSSION

Calorimetry, which basically is the measure of heat generated by living organisms, was applied to cells as well as mice under anaesthesia. Important findings in cells were: a change in heat production can be detected as early as 35 min after cells were mixed with lidocaine. While under microscopical examination, the change could not be recognised at this stage. One should bear in mind that this detection was made without damaging or manipulating cells, which is more than often required if biochemical, chemical and microscopical methods were used. Furthermore the number of cells under anaesthesia remained constant and could resume normal growth. Practical application of anaesthesia in cells would be to store cells at room temperature for a limited time period. This may become very useful, especially in the clinical field of medicine, because it is sometimes difficult to collect cell samples from individuals or patients at a specific time.

Calorimetry in mice described here shows that first, the circadian rhythm of healthy mice is clearly evaluated not only by the pattern of heat dissipated but also by mathematical values in kilo Joules of 8-hour integration. Although circadian rhythm has been monitored by watching animal movements using infrared television monitoring systems in the field of wild life research, it seems that exact evaluation is difficult to obtain. Especially when an animal is in a resting state such as sleeping and anaesthesia, no other method so far has enabled any measurements of the state of somnolence. Values of heat dissipation, as seen in Fig.3a, 3b and Fig,5, can lead to a distinction between physiological sleep observed during the day time(Fig.3a and b) and anaesthesia(Fig.5). The deeper the degree of anaesthesia or depth of sleep, the lower the heat dissipated and the closer it reaches zero value. Calorimetry can thus give values for understanding the state of organisms; not only the normal state including consciousness and sleep but also the inactive state such as anaesthesia.

These findings can be summarized in Fig.6, in which a linear relationship between heat dissipation and work done is shown. Basic heat dissipation can easily be measured during physiological sleep. Zero or near zero heat production, although the animal is still alive and sound, can be achieved by anaesthesia.

Fig.6



Whole body calorimetry has also been used in the field of pain research. Another form of inactive state in terms of circadian rhythm can be observed in the presence of pain. The normal or active state is partially restored by the administration of analgesic agents for example aspirin.
All measurements described here were done without being injurious to whole organisms.

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