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MICROCALORIMETRIC MEASUREMENTS OF HEAT PRODUCTION IN RABBIT ALVEOLAR MACROPHAGES

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ABSTRACT

There is a need for short-term tests for characterization of toxic effects of environmental pollutants without employing expensive and time-consuming animal experiments. One approach is to monitor changes of the total metabolism of an exposed cell population in vitro. For this reason we have studied the heat production of rabit alveolar macrophages. A thermopile heat conduction microcalorimeter (LKB BioActivity Monitor) was used to measure the heat production rate from monolayers of macrophages (50 000 -180 000 cells) leading to a power value of 24 ± 3 pW/cell (37 °C, pH= 7.40).

INTRODUCTION

There are increasing needs for sensitive short-term tests for the toxicity of agents occurring in different production procedures and in our environments. One method for registering toxic effects is to monitor changes of the total metabolism of an exposed cell population. Modern microcalorimetric techniques can be used for a quantitative and continuous assessment of the metabolic activity for a cell sample through measurement of its heat production rate (the thermal power). Modern microcalorimetric instruments are easy to use and have a stability and sensitivity which make it possible to perform power measurements on the μW level in experiments which extend over many hours. For reviews of instrumentation and work in cellular fields see (refs. 1,2, and 3).

The inherent low specificity of calorimetric methods can be an advantage in studying the total toxic effects on cell populations, whereas by specific test methods unexpected toxic mechanisms might not be registered. Calorimetric methods are non-invasive and the cell sample is therefore intact after the measurement and can be assayed by various specific analytical methods.

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This work is part of a program where toxic effects from airborne particles on cells are investigated. Alveolar macrophages (AM) were chosen in this study due to the role they have in the defence of the respiratory system against exposure to toxic substances in our environments. Rabbit AM are used in this work but when further experience is attained it will be possible to use human AM.

In order to make correlations between toxic effects and the rate of heat production from the macrophages it is necessary first to characterize these cells calorimetrically when they are in a basal metabolic state AM participate in many important functions (ref 4) and the determination of their basal thermal power level is therefore also of general interest.

In the present preliminary report we describe results from a microcalorimetric study on rabbit AM which adhere in monolayer to a polystyrene surface. A more extensive report will be published elsewhere (ref. 5).

MATERIAL AND METHODS

AM were collected from rabbits by bronchopulmonary lavage (ref. 6) and the cell suspensions were transferred to sterile culture dishes where they were left to sediment and adhere on circular polystyrene plates. The plates, diameter 10 mm, had been exposed to concentrated sulphuric acid at 55 ^{o}C overnight according to Rubin (ref. 7). Non-adherent cells were rinsed out and the cells were incubated overnight in Minimum Essential Medium (MEM, Flow Laboratories, Scotland) supplied with 20% homologous serum Viability and morphology were examined by an immunofluorescence method (ref. 8) and the Giemsa staining procedure respectively. Before and after the calorimetric measurements concentrations of lactate dehydrogenase (LD) of the culture medium were determined.

A twin calorimeter of the thermopile heat conduction type (Bioactivity Monitor 2277, LKB Produkter, Sweden) (ref 9) was used. The polystyrene plates with cells and culture medium (pH 7.40) were placed into 5.0 ml stainless steel ampoules. After the calorimetric measurements, the number of cells on the plates was determined by use of an Image Analysing Unit (Mikro Measurement III) connected to an invert microscope (Olympus IMT/LWD) and a microcomputer (Apple II) with a printer (Epson MX 80 II). By an automatic step table mounted to the microscope the monolayers were scanned. From the viability index the number of viable cells of each plate was calculated and the thermal power per living cell could be estimated

For the experiments reported here cells from two rabbits were used. One batch of cells was measured simultaneously in 3 of the calorimetric

channels, whereas the other cell preparation was measured by use of all 4 channels of the instrument.

RESULTS

The cell cultures, 0.05-0.18 x 10^6 cells per plate, appeared as monolayers. No contaminating cells were observed. A calorimetric record from an experiment with cells adhering to a plate is shown in Fig. 1 Typically steady state conditions were obtained after a period of about 2.5 h. Steady state levels were stable up to at least 18 h. The distance between the baseline and the steady state level is directly proportional to evolved thermal power, P. Fig. 2 shows the calorimetric record from a control experiment where the ampoule was charged with water. The time for reaching a stable calorimetric signal was in this case about 1 h.

In the 7 calorimetric experiments where $50 \cdot 10^3$-$180 \cdot 10^3$ cells were used, recorded power levels were in the range of 2-4 µW. However, in control experiments with the culture medium without any cells present, significant power values were also found, mean value 0.7 µW. This power value (presumably due to processes in the serum) was subtracted from the values determined in the experiments with cells leading to a power value for the macrophages, under the stated experimental conditions, of $P = 24.0 \pm 3.0$ pW/cell ($\pm$ S.D.). No significant differences could be found between the concentrations of LD before and after the calorimetric experiments

DISCUSSION

There are several important advantages with the monolayer technique. The measurements can be made with a small number of cells and the washing procedure removing non-adherent cells and non-bound particles can be performed easily and rapidly. Furthermore, the monolayers can easily and repeatedly be examined microscopically. The most important drawback with the method is the uncertainty in the cell-counting (refs. 10 and 11).

The polystyrene plates showed some irregularities in the surfaces which were not due to any biological matter. These irregularities interfered with the cell-counting and will contribute to S.D. The long exposition of the polystyrene material to the concentrated sulphuric acid probably transforms the originally hard and smooth surface (refs 12 and 13). A new method of constructing the plates has now been developed, by which it is possible to reduce the exposition time to sulphuric acid and still obtain a sufficiently high negative charge density.

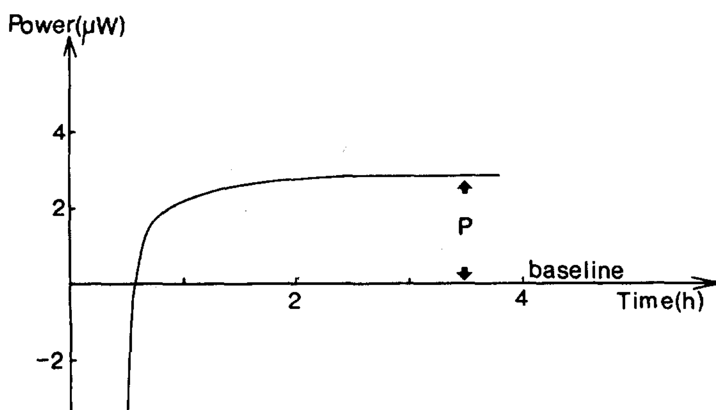

Fig. 1 A typical power-time curve obtained for rabbit AM in monolayer on a polystyrene plate in a standard culture medium with 20% homologous serum. The sample ampoule and the reference ampoule were inserted into the calorimeter at zero time.

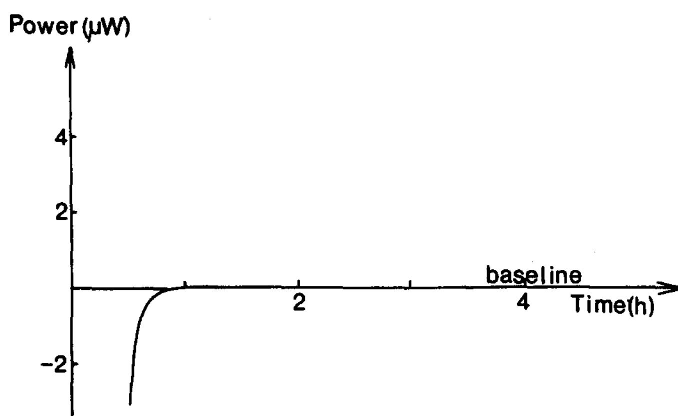

Fig. 2. Calorimetric curve from a control experiment with both ampoules charged with water. The ampoules were inserted into the calorimeter at zero time.

We conclude that the present calorimetric technique is well-suited for determination of power values for small quantities of macrophages adhered to a surface. The high power value, 24 pW/cell, reflects a high metabolic rate As a comparison, power values for human blood cells are much smaller (ref. 3), ca 9 fW/cell for erythrocytes (fW = 10^{-15} W), ca 60 fW/cell for trombocytes and 1-5 pW/cell for lymphocytes and granulocytes.

Earlier Loike et al. (ref. 14) reported from a calorimetric study on activated murine peritoneal macrophages which are known to have a different metabolic pattern from AM (ref. 4). These experiments were carried out with a Privalov scanning calorimeter where a large number of cells ($5 \cdot 10^6$-$60 \cdot 10^6$) were allowed to sediment in multilayer before the scan started. Maximum power values, observed at $\geq$ 30 °C,were significantly lower than those found in the present work.

The high power values for macrophages make it possible to study very small samples. It is likely that their high metabolic activity will make them suitable for studies of disturbances of the metabolism, e.g. by toxic materials, as it presumably will lead to large changes in the power values.

A special interest for application of the present method is to study the combined effects of different agents. Thus we believe it can be used to investigate intra- and intercellular reactions of cells in monolayers exposed to components of interest in, for instance, the working environment.

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