Thermochimica Acta, 172 (1990) 75–80
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

THE EFFECT OF TEMPERATURE ON METABOLISM IN 3T3 CELLS AND SV40-TRANSFORMED 3T3 CELLS AS MEASURED BY MICROCALORIMETRY *

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ABSTRACT

The rate of heat production was measured at different temperatures for nontransformed and SV40-transformed 3T3 cells. Both cell lines were very similar with respect to metabolic temperature dependence in the lower temperature range. The apparent activation energy for 3T3 cells and SV40-transformed 3T3 cells was 60 kJ/mol and 66 kJ/mol, respectively. A large difference was found at high temperatures. The transformed 3T3 cell line did not exhibit any "normal" metabolism above 41 °C, whereas the 3T3 cells showed a functioning metabolism also at 42 °C.

INTRODUCTON

The transformation of cells to a neoplastic state is accompanied by many physiological changes, e.g. new membrane properties, higher anaerobic to aerobic metabolic ratio, increased rate of nutrient transport etc. Especially changes in the membrane, such as the acquirement of new membrane structures and increased fluidity, have been proposed to be crucial for the physiological behaviour of tumour cells. In culture, altered membrane properties can be observed as e.g. decreased requirement of anchorage for growth (ref. 1) and changed affinity for molecules such as lectins (see e.g. ref. 2). The increased sensitivity of tumour cells to high temperatures has also been suggested to reside in the membrane but so far no accepted description of the mechanism behind this seems to exist (ref. 3). The concentration of cholesterol in the membrane correlates with fluidity and cell death caused by hyperthermia, but it has not yet been proved that the cholesterol level is increased after transformation (ref. 4). Hyperthermia is, however, already accepted as a method in treatment of cancer.

The aim of this work was to investigate the effect of temperature on the rate of metabolism in nontransformed cells compared to tumourigenic transformed cells by use of microcalorimetry. The parameter obtained is the rate of heat production, i.e. the thermal power, P, which reflects the overall metabolic rate. A well-known cell system for comparative studies was used, *viz* 3T3 cells from a Balb/c mouse embryo and a simian virus 40 (SV40) transformed

* Presented at the 7th International Symposium on Microcalorimetric Applications in Biology, Egham, U.K., 9–11 April 1990, and Dedicated to Ingemar Wadsö on the Occasion of his 60th Birthday.

0040-6031/90/$03.50

cell line derived from the same clone. The nontransformed 3T3 cells exhibit contact inhibition in contrast to the SV40-transformed cell line which is tumourigenic. Balb/c 3T3 cells and several cell lines derived from this origin by *in vitro* transformation with different agents have been used extensively by e.g. Pardee and co-workers (refs 5-7).

The study presented here was partly undertaken in order to see how the rate of metabolism increases with temperature in the lower temperature region for each cell line. The intention of the experiments was also to observe any detectable difference between the cell lines with respect to the critical temperature at which the metabolism collapses.

MATERIALS AND METHODS

Cell lines and culture conditions:

The nontumourigenic 3T3 cells were derived from a Balb/c mouse embryo by Aaronson and Todaro in 1968 (ref. 8). The tumourigenic cells (SV-3T3) had been obtained by *in vitro* transformation of the same clone (A-31) with SV40 by Scher and Nelson-Rees (ref. 9). Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4 mM glutamine, 0.05 g/l gentamicin, 20 mM hepes, 0.10 mM $NaHCO_3$ and 10 % foetal calf serum. Cell lines, medium and supplements were from Flow Laboratories, Rockville, USA.

For the calorimetric experiments microcarrier cultures of the cells were set up. The microcarriers (Cytodex 1, Pharmacia/LKB, Uppsala, Sweden) were hydrated and autoclaved in calcium and magnesium free phosphate buffered saline and finally washed with DMEM. A sub-confluent 75 cm^2 monolayer culture was trypsinized and transferred to a DMEM-suspension of microcarriers in a 100 ml spinner flask (Bellco Co., New Jersey, USA). The adhesion time was 5 h with stirring for 2 min every half hour. The concentration of microcarriers was 3 g/l.

Calorimetry:

Samples of microcarriers with adhered cells could be taken from the spinner flask after 2-3 days. The beads were allowed to sediment and the old medium was replaced by fresh supplemented medium without bicarbonate. Calorimetric vessels were each loaded with 2.7 ml of suspension and inserted into the calorimeter (Thermal Activity Monitor, Thermometric AB, Järfälla, Sweden) preset at the desired temperature (28 - 43 °C). One vessel was always used in parallel for measurements of the power at 37 °C, thus serving as a reference. During the measurement a stirring rate of 90 rpm was used in order to keep the culture as a homogeneous suspension. The number of cells in each vessel was determined at the end of the experiment by counting stained, released nuclei

according to van Wezel (ref. 10).

For a description of the calorimetric equipment see ref. (11). For details concerning the calorimetric procedure with fibroblasts attached to microcarriers, see ref. (12).

RESULTS AND DISCUSSION

The temperature dependence of the rate of heat production, P, can be expressed as an Arrhenius relation according to equation (1):

$$\log P = -E_a/R \cdot 1/T + \log A \qquad (1)$$

where E_a is the activation energy; R = 8.314 $J \cdot mol^{-1} \cdot K^{-1}$; A is the frequency factor; and T is the absolute temperature in kelvin. For cellular systems where differences between batches might exist, it is better to use a normalized rate, P/P^o, where P^o is the thermal power at 37 °C for a cell sample run in parallel. This way of expressing the temperature dependence of cellular thermal power was suggested by Wadsö and co-workers. The first work was published in 1976 for erythrocytes (ref 13). (In this context the unit for E_a ($kJ \cdot mol^{-1}$) may not have any molecular meaning).

Figure 1 shows a power-time curve from a calorimetric experiment with nontransformed 3T3 cells. Since the power is constant with time, growth can be considered to be negligible during the experiment. All curves for this cell line had the same shape at temperatures between 28 and 42 °C. However, the rate of metabolism (here expressed in terms of thermal power) increased with temperature up to 41 °C where it reached the maximum. This is shown in figure 2a, where the temperature dependence is expressed as an Arrhenius relation. From the linear part between 28 and 40 °C, an apparent activation energy of 60 $kJ \cdot mol^{-1}$ can be calculated.

Power-time curves from experiments with SV40 transformed cells showed increasing power with time, i.e. these cells were growing much faster than the 3T3 cells. The metabolic temperature dependence between 28 and 41 °C was, however, almost identical for the two cell lines. The activation energy for SV-3T3 cells calculated from the Arrhenius diagram shown in Fig.2b was 66 $kJ \cdot mol^{-1}$.

The difference between the two cell lines can be found in the high temperature region. At 42 °C the 3T3 cells have a normal, albeit somewhat lower rate of metabolism, whereas the SV-3T3 cells are dying as shown by the drastic decrease in power with time (Fig. 3a). Thus, the rate of metabolism for SV-3T3 cells does not exhibit a bend between 40 and 42 °C (cf. Fig.2a and

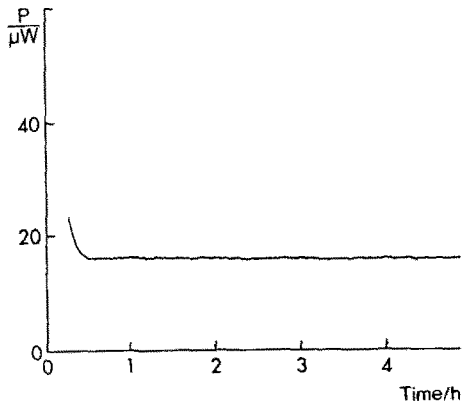

Fig. 1. Power-time curve at 37 °C for a 2.7 ml sample of nontransformed 3T3 cells attached to microcarriers. The number of cells was $5.0 \cdot 10^5$.

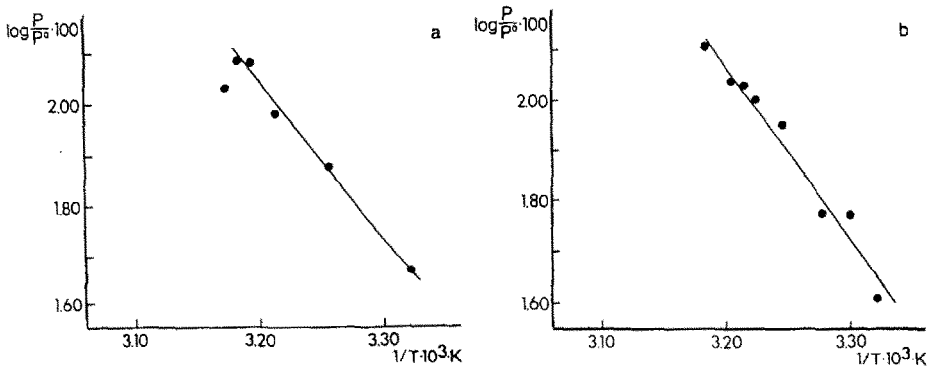

Fig. 2. Arrhenius diagrams for nontransformed 3T3 cells in the temperature range 28-42 °C (a), and for SV-3T3 cells in the temperature range 28-41 °C (b). Each point has been derived from a stable power-time curve (cf. Fig. 1).

2b). The thermal power increases straight up to 41 °C and any functioning metabolism above that does not exist. It should be mentioned that the degree of cell death at 43 °C has been determined for some different cell lines (ref 4). However, no attempt to determine the lowest temperature leading to cell death was made. It is interesting to note that the power-time curve obtained at 43 °C for the nontransformed cells indicates that other processes than just cell death are taking place (Fig.3b). At the present stage, we cannot make any interpretations of such a curve. It has been observed, however, that induction of thermotolerance occurs if cells are incubated at a temperature slightly above 42 °C (ref 14).

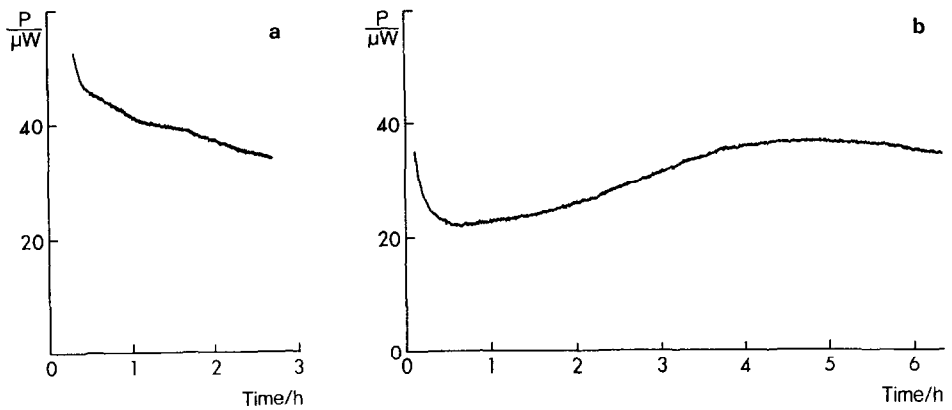

Fig.3. Calorimetric results obtained from experiments at high temperatures. The SV-3T3 cells exhibit cell death at 42 °C (a). Fig.3b shows the result obtained at 43 °C for 3T3 cells. Cf. the text.

The experiments reported here indicate the same metabolic temperature dependence for SV-3T3 cells as for nontransformed cells of the same origin. This is best described in terms of the activation energy which is within the range of 60-70 $kJ \cdot mol^{-1}$ for both cell lines. However, large differences in activation energy have earlier been obtained between cells of different origin. For erythrocytes, thrombocytes and lymphocytes the value for the activation energy was 83 $kJ \cdot mol^{-1}$(ref 13), 52 $kJ \cdot mol^{-1}$(ref 15) and 63 $kJ \cdot mol^{-1}$(ref 16), respectively. (In all of those studies static ampoules were used and it might be wrong to make direct comparisons with this work, where the cells are not sedimented.) Thus, the main difference between the two cell lines in this work can be found in the physiological properties of the tumour cell line at high temperatures, where it cannot maintain a normal metabolism

above 41 °C. If this is due to a higher concentration of cholesterol in the membrane of the SV-3T3 cells remains to be elucidated. It is also of interest to continue this work using 3T3 cells transformed by other means, since it has been reported that differences in parameters vary more among different transformed cells than between 3T3 cells and transformed cells (ref 7).

ACKNOWLEDGEMENT

This work was made possible by financial support from the Swedish Board for Technical Development.

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