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HEAT PRODUCTION AS A CELL CYCLE MONITORING PARAMETER

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Summary; A microcalorimetric method was applied to define the cell cycle by measuring heat production of mouse breast cancer cell line, FM3A. FM3A cells, were synchronised at the G_0 phase and produced 13.0 μWatts(W) per $1x10^6$ cells. Although the number of cells in fresh medium remained unchanged during the following 24 hour, a dramatic increase of heat production was observed and maximum heat (46.2 μW) was produced by the cells at 24 hours when the cell cycle was presumably at the G_2 phase. At 26 hours, although cell number increased, heat production decreased. Since the cells were not treated in any manner, this microcalorimetric method of measuring the cell cycle by monitoring heat production can be a very useful tool.

The analysis of the cell cycle has been tedious and time consuming[1]. Many unanswered questions remain pending, mainly due to unsatisfactory monitoring methods. The techniques involved in the cell cycle analysis are microscopic examinations, fluorocytometry[2], DNA labeling , fixing and staining of the cells etc. As only living cells but not dead cells release heat into their surroundings, measurement of such heat can be a useful tool for studying the cell cycle.[3] Although analytical application of calorimetry was extensively reviewed by Spink and Wadso[4], relatively little quantitative data

Abbreviations: W; Watts. HEPES;, N-2-Hydroxyethylpiperazine N'-2-ethylane sulfonic acid.

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was obtained from mammalian cells. With improvement of the micro calorimeter design (allowing detection of 1 μW of heat) and the development of a more accurate method for heat measurement, in which the experimental error within experiments was less than 1% , heat production during the cell cycle of a mouse breast cancer cell line FM3A cells was measured and an attempt was made to define the cell cycle by means of heat production.

Materials and Methods

A Mouse breast cancer cell line,(FM3A cells), kindly given by Dr.Hyodo of Tokai Univ. Sch. of Med., was used throughout this study. The cells were maintained in RPMI 1640 supplemented by 10% foetal calf serum and 20mM HEPES. The medium was changed every two to three days. The synchronisation of the cells into G_0 phase was achieved by culturing the cells for more than 96 hours but less than 120 hours. The cell cycle from G_0 phase was initiated by transferring the cells into fresh medium. Heat was measured by a twin-flow type microcalorimeter, Thermoactive Cell Analyzer ESCO 3000. The cells collected at G_0 phase and at appropriate time intervals of the cell cycle, were centrifuged, suspended in fresh medium, counted and adjusted to 2 x 10^6 per ml. When all the cells, which were suspended in a total volume of 0.5ml, were allowed to enter the heat detector part, the flow of the cell suspension was stopped[5] and heat was measured. [^{3}H]-thymidine incorporation was measured at times indicated in the figure. The number of cells were counted using a haemocytometer.

Results

Heat production during the cell cycle. 1 x 10^6 cells synchronised at the G_0 phase produced 13.0 μW . The heat produced steadily increased following 24 hours culture in fresh medium without increasing the cell number and a maximum heat of 46.2 μW was recorded at 24 hours. When the number of cells increased at 26 hours, heat production declined. So that the heat measured decreased, while the number of cells increased. A sudden increase of [^{3}H]-thymidine incorporation

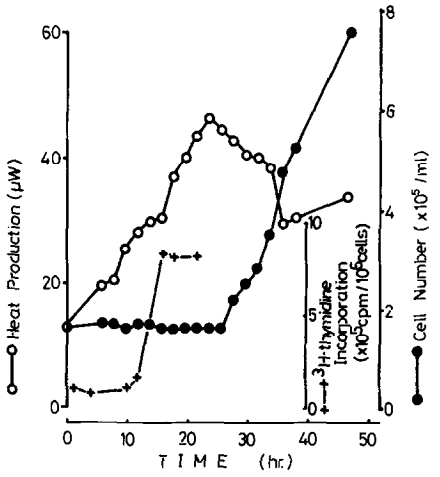

Fig.1 Heat production during the cell cycle.
Heat production indicated as ○——○, the number of cells indicated as ●——●, and [^{3}H]-thymidine incorporation indicated as +——+, were plotted against the incubation time after G_0 phase cells were transferred to fresh medium. The values at time 0 were those of cells at the G_0 phase.

into DNA was observed at 14hr. (see Fig.1)

Several experiments were carried out to determine the heat production of G_0 phase cells and the 24-hour-culture of G_0 cells in which the number of cells did not increase. The cells at G_0 phase produced $13.0 \pm 2.0 \mu W$ (n = 7) and the cells cultured for 24 hours produced $42.6 \pm 7.2 \mu W$ (n = 3).

Discussion

To the best our knowledge, this is the first time that the various phases of the cell cycle have been successfully detected by monitoring the cell cycle of a mammalian cell line.

Reliable results can now be otained as improvement in the design of the instrument allows the measurement of the whole volume of a cell suspension to be made at once by stopping the cell suspension within the heat detector part of the instrument. Hence, uneven flow of

cells within the flow line, which was the cause of non-quantitative microcalorimetric measurements, has been overcome.

By applying this method to measure heat production of mammalian cell lines such as FM3A, U937, HL60, it was possible to obtain results in which the heat production was directly proportional to the number of cells. It is therefore reasonable to assume that heat produced is a quantitative parameter for cell activity. Living cells produce de tectable heat, which can vary according to the incubation time. From the results of [^{3}H]-thymidine incorporation and the increased cell number, S phase and M phases started at 14 and 26 hours respectively Hence it may be that: first, the lowest heat production is at the G_0 phase, due to the lowest catabolic and anabolic activity of the G_0 phase: secondly, when the cells are catabolically and anabolically activated to enter the subsequent G_1,S and G_2 phases the heat pro duction would increase according to the energy requirements. Finally, when the cells at M phase are not synthesising any more protein, RNA and DNA[1], the heat production decreases.

The measurement of heat does not require any specific technique to treat cells except centrifugation and counting. Since the instrument used in this study was a flow type microcalorimeter, the time needed for one measurement, which included the preparation of cells, was less than 30min. The cells may be recovered from the instrument after measurement with more than 90% recovery without loss of via bility so that the cells at a known cell cycle phase can be utilised in further experiments.

Calorimetry can enable the quantitative measurement of cell func tions as a whole without manipulating cells, this will inevitably

add a new dimension to research in cell biology. Also the function of cell organelles such as mitochondria could be studied by this method.

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