Comparative Studies of the Heat Production of Different Rat Hepatoma Cells in Culture


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ABSTRACT

Heat production of H35, HTC, and RLC rat hepatoma cells were measured under identical conditions by differential thermal analysis. Production of thermal energy was determined after sedimentation at various cell densities. Heat production was dependent on cell density and was compared with heat production measurements in suspension cultures by using an isoperibol calorimeter. Thermal energy production of the well-differentiated H35 cell line was approximately three times lower than that of the poorly differentiated HTC and RLC cell lines. A relationship with carbohydrate metabolism is discussed.

INTRODUCTION

Living cells produce thermal energy. Cellular thermal energy production is dependent on cell type. In suspension culture, determinations have been made of KB cells (3), L929 mouse fibroblasts (6), H35 rat hepatoma cells (8), and N2A mouse neuroblastoma cells (9). No literature is available about the comparative heat production of different cell lines derived from a common parental cell type and incubated under similar culture conditions. On the basis of their degree of differentiation, as judged by both biochemical and histological criteria, rat hepatoma cell lines have been divided into well differentiated and poorly differentiated cells (13, 15–17). In this paper, we describe the heat production of the well-differentiated rat hepatoma Reuber H35 cells and the poorly differentiated rat hepatoma cell lines HTC and RLC. Production of thermal energy has been determined with DTA at various cell densities after sedimentation and compared with thermal energy production of stirred suspension cultures in an isoperibol calorimeter. In sedimented cultures, heat production was found to depend on cell number up to a critical cell density. It is further shown that heat production of the poorly differentiated cell lines was approximately 3 times that of the well-differentiated H35 cells.

MATERIALS AND METHODS

Cells and Growth Conditions. The rat hepatoma cell lines Reuber H35 (11), RLC (10), and HTC (14) were grown as monolayer cultures in plastic Falcon flasks with a surface area of 75 sq cm, containing 15 ml of standard growth medium. Growth conditions have been described previously (13).

Determination of Heat Production of Cells. Preparation of the cell suspension used for the determination of heat production has been described previously (8, 9). The diathermic isoperibol reaction calorimeter has been described by Loesberg et al. (8), and the determination of heat production was started by addition of the cell suspension through the inlet of the incubation vessel into equilibrated medium.

For heat measurements with DTA, a calorimeter with 2 heat-flow meters was used (Technisch Fysische Dienst, Delft, The Netherlands). On one of these meters was placed the sample vessel, and on the other was placed a reference vessel containing water. The vessels were closed airtight with a Viton ring because otherwise the evaporation of water disturbed the measurements. The heat-flow meters were calibrated with small electrical heaters. A sensitivity of 3 watts/V was found. The noise level, defined as the S.D. of a long-term blank, was 0.5 x 10~4 V, or 1.5 microwatts. The base line could generally be reproduced within 1 x 10~4 V. In the reaction vessel, 0.5 ml of medium containing the cells at an appropriate dilution was used. Measurements were started after 15 min equilibrating. During 60 min of measurement, the heat production slowly decreased in a linear way. This enabled determination of initial heat production to be made by extrapolation.

RESULTS

Heat Production in H35, RLC, and HTC Cells. Cell densities ranging from 0.3 x 10^6 to 6 x 10^6 cells/0.5 ml were used to determine heat production in the DTA vessels. All the cell lines were assayed under similar conditions. Chart 1 shows that initial heat production was dependent on both cell density and cell type. Heat production increased with up to 1.5 x 10^6 cells ranging from 0.3 x 10^6 to 6 x 10^6 cells present in the experiment. At cell densities exceeding this value, the initial heat production remained at maximal level. The maximal level depended on the cell type and was 15, 40, and 50 microwatts for H35, RLC, and HTC cells, respectively. For H35 cells, it was difficult to establish the accurate relationship between heat production and cell density, because of its relatively low heat production in the low cell density range. The accuracy of values less than 10 microwatts (corresponding to a signal of about 3 x 10~6 V) could be influenced by the 0 level stability.

During the period of measurement, production decreased in a linear fashion. At 60 min after the start of measurement, heat production decreased to 60 to 75% of that of the initial value. Although the decrease varied in the various experiments, it was independent of initial cell density. Measurements of the rate at which cells settle out of solution were performed in cuvets and were derived from a plot of the decrease in absorbance versus time. In the experiment with the DTA vessels, settling out of cells was accomplished within 15 min. During the duration of mea-
Glucose consumption by H35 cells was approximately 4-fold lower than glucose consumption by HTC cells. In contrast, $^{14}$CO$_2$ production was identical in both cell lines.

### DISCUSSION

This paper demonstrates that the rat hepatoma cells Reuber H35, RLC, and HTC differed in their heat production under similar culture conditions. Thermal energy production of H35 cells was approximately 3 times lower than that of the HTC and RLC cell lines. The heat production at cell concentrations where heat production per cell is not dependent on cell concentration ranged from 15 to 50 picowatts/cell. This is in the same range as determined for other epithelial cell lines. HeLa cells (7) and KB cells (3) produced 20 to 40 and 25 picowatts/cell, respectively.

Differences between hepatoma cells derived from a common parental cell type and cultured under identical conditions have been described previously (15–17). Reuber H35 is considered to be a well-differentiated cell line in contrast to RLC and HTC. The ultrastructure of the cytoplasm and the mitochondrial volume per unit volume of cytoplasm of H35 are more hepatocyte-like than that of RLC and HTC. In H35, a number of normal hepatic functions appear to persist, as well as certain features of the regulatory circuits controlling these processes. Perhaps a poorly differentiated cellular state coincides with increased heat production. This is also indicated by a study of heat production of normal and transformed embryonic hamster cells (4, 5). Thus, heat production of benzo(a)pyrene-transformed cells was more than that of normal embryonic hamster cells.

The question can be raised whether heat production values of hepatoma cells are reasonable, when taking into account cellular metabolism. Comparative data of the glycolytic capacity and activities of enzymes belonging to the carbohydrate metabolism of these cells have been reported before (13). The well-differentiated H35 differs from the poorly differentiated HTC and RLC cell lines in its glucose utilization and lactic acid accumulation. The main difference is in the rate of glucose consumption. Our data showed that H35 cells utilize 28.5 nmol glucose per hr per 10$^6$ cells (Refs. 12 and 13; Table 1) and produce 65.2 nmol CO$_2$ per hr per 10$^6$ cells. In contrast, HTC cells utilize 121.0 nmol glucose per hr per 10$^6$ cells and produce 66.6 nmol CO$_2$ per hr per 10$^6$ cells. If we assume (a) that glucose is converted into either lactate or CO$_2$ and (b) and $\Delta H = -28$ kcal/mol for the conversion of glucose into lactate at most (1), and probably not more than about $-300$ kcal/mol for glucose respiration, then the theoretical heat production of H35 and HTC by glucose consumption can be calculated. The heat production for H35 and HTC cells is 4 and 7 picowatts/cell, respectively. Apparently, glucose metabolism could account for only a part (18 to 27%) of the heat production values.
shown that these cells remove lactic acid as a component of serum from the medium (13).

This paper has described a sharply defined plateauing of heat production at cell densities of $2 \times 10^6$ cells. This is in contrast to observations of Hansen et al. (4) using cells in suspension. Suspended cells, at increasing cell densities, demonstrate a gradually decreased heat production. In our suspension cultures, it was impossible to measure heat production at these high cell densities since extensive formation of cell aggregation then occurred. Our calculations suggested that the leveling out might be due to cell crowding. Cerretti et al. (3) studied the possible connection between heat production and cell settling. The rate constant of the process of cells settling out of solution was similar to the rate constant of the process of decay of heat production. Apparently, when crowding occurs, cells have poorer contact with medium. In those cases, pH might be changed locally, $O_2$ availability can become limited, or, in general, a high cellular activity in the use of substance exceeds the diffusion rate of this substance, thereby starving underlying cells.

In conclusion, the experiments performed here illustrate that the thermal behavior of various hepatoma cells can be differentiated, can be studied in suspension cultures or in settled cells at low densities, and might reflect their different metabolic behavior.

REFERENCES

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