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Cellular thermogenesis in L-929 mouse fibroblasts. The influence of oxygen availability on growth, heat production and intermediary metabolism ☆

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Abstract

Cellular thermogenesis in L-929 mouse fibroblasts growing in glass ampoules was monitored over 72 h in three different systems: (1) closed ampoules with 2.5 ml of control medium; (2) closed ampoules with 2.85 ml of medium and a reduced oxygen content; and (3) ampoules kept open. Thermogenesis was determined as heat production (HP) and the pH, and oxygen, glucose and lactate contents in the medium as well as the cellular growth were assessed in order to evaluate whether small gaseous volumes are sufficient to facilitate adequate oxygen supply.

Oxygen deficiency resulted in lower HP, decreased growth and enhanced anaerobic rate of glycolysis. Low gas volume was associated with decreased HP after 40 h and a steady increasing rate of aerobic glycolysis similar to that shown during O_2 deficiency. In the open system a more pronounced decrease of HP was observed.

Keywords: Cell culture; Crabtree effect; L-cell; Microcalorimetry; Oxygen availability; Thermogenesis

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1. Introduction

Microcalorimetry is a valuable tool for direct, continuous and sensitive assessment of the net energy production in cultured cells.

The present study is intended to elucidate whether small gaseous volumes can facilitate adequate oxygen supply and if the occurence of the "Crabtree" effect [1,2] might reduce the oxygen requirement due to diminished availability. Accordingly, we investigated the influence of oxygen availability on growth, heat production (HP) and intermediary metabolism of mouse fibroblasts, line L-929, growing in a monolayer in 3-ml glass ampoules.

2. Methods

2.1. Cells and culture conditions

Mouse fibroblasts NCTC clone 929, clone of strain L, were purchased from Flow Lab. Ltd., Bonn, Germany. Cells were grown in BME with 10% newborn calf serum (Biochrom, Berlin, Germany; Boehringer, Mannheim, Germany), 20 mM Hepes and 15 mg achromycine per l (Lederle, Munich, Germany).

Cells were suspended by trypsinization and transferred into 3-ml glass ampoules (2.5×10^5 cells per ampoule) and inoculated in monolayer culture as described previously [3]. To facilitate the desired experimental conditions, the ampoules were filled with medium in one of the following ways: (1) with 2.50 ml according to the routine procedure [3,4]; control experiments ($n = 5$), or (2) with 2.85 ml, facilitating O_2 deficiency. This cell preparation was kept under an atmosphere of argon for 2–3 days and the pH of the medium was adjusted by gassing with CO_2 ($n = 5$). (3) Parallel ampoules kept open in a CO_2 incubator (Heraeus Instruments, Hanau, Germany) were analysed in the microcalorimeter for 24 h ($n = 5$).

Cell numbers were determined in duplicate cultures at 0, 20, 24, 42, 48, 68 and 72 h applying a Fuchs–Rosenthal hemocytometer. Cellular viability was assessed by the trypan-blue exclusion method.

2.2. Microcalorimetry

Calorimetric assays were performed with an LKB Bio Activity Monitor 2277 (Bromma, Sweden) and Thermal Activity Monitor (Thermometric AB, Järfälla, Sweden) fitted with 4 and 2 ampoule measuring cylinders, respectively [5]. Heat production was recorded in μW per culture at 37°C for 72 h. The error of the method in single determinations, based on duplicates, was ± 0.1 μW [4]. The stability of the baseline was 0.44 ± 0.3 μW. By ampoule exchange in the open system, the deviation of the power–time curve was 0.42 ± 0.38 μW as measured in the medium only, corresponding to 3.6% of the mean power signal derived from all measurements in the system with cells.

2.3. Determination of pH, and oxygen, glucose and lactate contents in the medium

All variables were determined from reference cultures at 0, 24, 48 and 72 h.

The pH was determined with a WTW pH-meter (WTW, Weilheim, Germany). Oxygen was measured polarographically with an Orion electrode (Colora, Lorch, Germany). Details of both procedures were described previously [4]. The medium was deproteinized with ice-cold 1.6 M perchloric acid and the supernatant neutralized with a saturated solution of K_3PO_4. Glucose and lactate were measured enzymatically [6,7].

2.4. Calculations

Glucose consumption and lactate production were assessed for three 24-h periods during the study by dividing concentration differences from glucose and lactate in the medium between the start and the end of a 24 h period with the mean cell number, according to Bradley et al. [8]. The total consumption and production is the sum of the fractional glucose uptake and lactate release obtained during the three 24-h periods.

The calorimetric-respirometric (CR) ratio was estimated by dividing the mean value of the cellular HP over 24 h by the decrease in oxygen concentration in the medium within the same time.

Statistical calculations were performed by the Wilcoxon test.

3. Results

As expected, oxygen concentrations were lower in O_2-deficient medium throughout the experiment than in the control (Fig. 1A). Similarily to the control cells, oxygen concentrations were initially reduced in open ampoules but were then maintained at the same level for the remaining days of the study. In open ampoules, the pH of the medium decreased less than in the control and during O_2 deficiency (Fig. 1B).

Oxygen-deficient cells revealed reduced growth (Fig. 2A) and exhibited lower cellular HP during the initial phase of the study (20–24 h) as compared with the control. In contrast, cellular HP was higher in open ampoules than in control cells

Table 1
The influence of oxygen availability on glucose consumption and lactate production during a 72-h culture period of L-929 mouse fibroblasts

	Glucose consumption/ (pM cell^{-1} h^{-1})	Lactate production/ (pM cell^{-1} h^{-1})
Control	1.01 ± 0.16	1.99 ± 0.87
O_2 deficient	1.52 ± 0.12 $p < 0.05$	2.45 ± 0.33
Open	1.35 ± 0.29 $p < 0.05$	1.79 ± 0.34

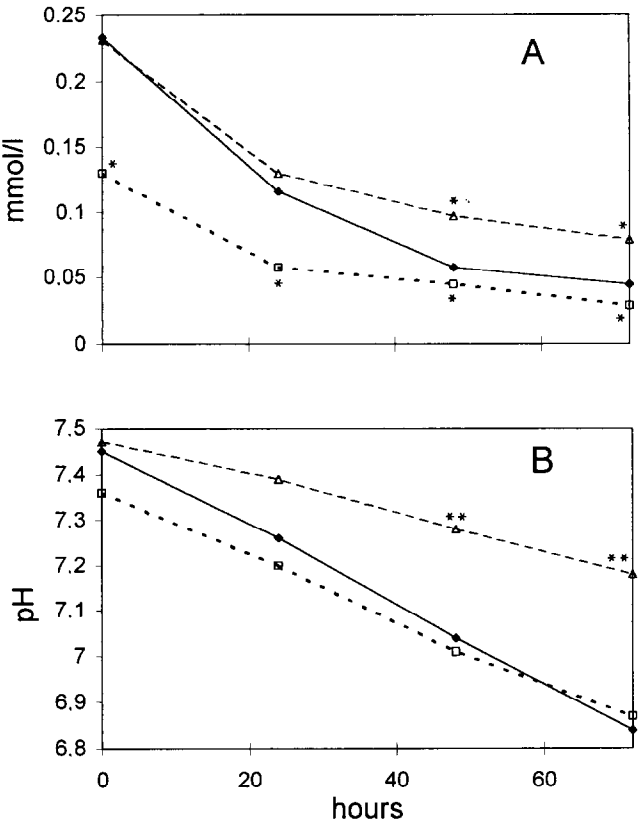

Fig. 1. A. The actual oxygen content in the medium of L-929 mouse fibroblasts cultured with different O_2 availability. B. Changes in the pH of the medium: control (◆), O_2 deficient (□), open ampoules (△). * = $p < 0.05$; **, $p < 0.01$ (significantly different from control).

at 48 and 72 h. In O_2-deficent cells and in cells cultured in open ampoules, glucose consumption was higher than in control cells (Table 1).

The estimated calorimetric-respirometric (CR) ratio [9] revealed higher values in O_2-deficient than in control cells (Table 2). Indeed, this ratio could not be

Table 2
The influence of oxygen availability on the calculated calorimetric–respirometric (CR) ratio and oxygen consumption per unit of lactate production in cultures of L-929 mouse fibroblasts

	CR-ratio/(kJ per mmol O_2)			Mol lactate produced per mol O_2 consumed
	0–24 h	24–48 h	48–72 h	0–72 h
Control	-3.6 ± 1.9	-8.0 ± 3.11	-30.9 ± 16.4	32.2 ± 4.2
O_2 deficient	-6.6 ± 3.9	-32.2 ± 12.2 ($p < 0.05$)	-32.4 ± 18.7	86.5 ± 50.9 ($p < 0.05$)

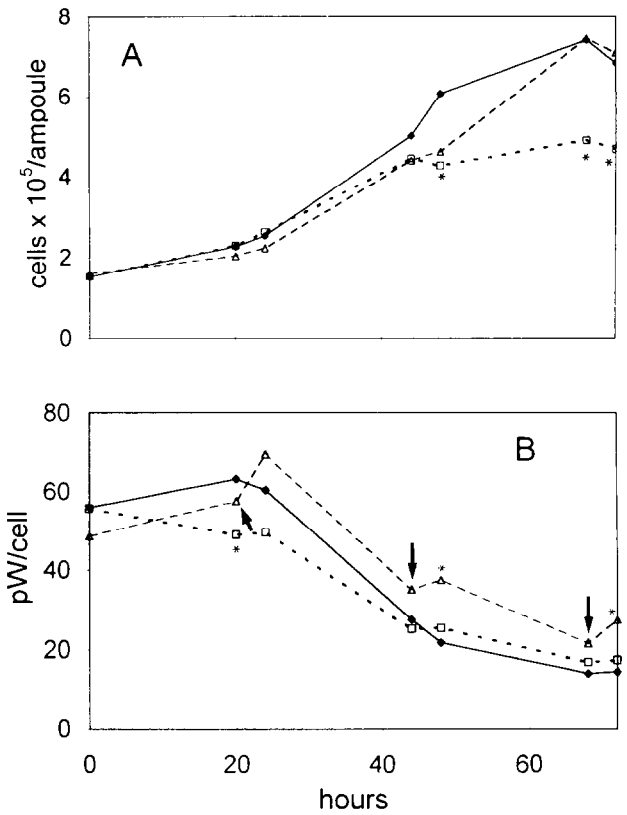

Fig. 2. A. The influence of oxygen availability on growth of L-929 mouse fibroblasts. B. Cellular heat production (HP). Arrows indicate the exchange of ampoules in the open system: control (◆), O_2 deficient (□), open ampoules (△). * = $p < 0.05$; **, $p < 0.01$ (significantly different from control).

calculated in open ampoules due to the continuous access of O_2 to the system. The highly increased ratio of moles of lactate produced per moles of O_2 consumed indicates anaerobic glycolysis in O_2-deficient cultures.

4. Discussion

The maintenance of cellular heat production in control cells during the first day of the study suggests that the oxygen availability was sufficient to facilitate intact energy metabolism of the cells despite an obvious reduction in oxygen concentration. Indeed, the thermogenesis reduced by O_2 deficiency was associated with diminished growth as compared to controls. The fact that the initial O_2 deficiency was accompanied by lower heat production and growth suggests an apparent additional need for O_2 in the closed cell culture system. This assumption is supported by the finding that heat production after 40 h was maintained at a low

level resembling control values. Furthermore it should be noted that in the late phase of the experiment higher heat production was observed with the open cell system.

The increased glucose consumption observed in O_2-deficient cells and in the open system might be explained, respectively, by a higher rate of anaerobic metabolism and possibly by futile cycles [9–13]. It is not clear, however, in what way the reduction in pH during the experiment contributes to the observed changes in thermogenesis as suggested by Wadsö and co-workers [14].

The calculated calorimetric-respirometric ratios in the control experiment considerably exceeded (10-fold) the theoretical value of the aerobic oxycaloric equivalent (435–496 kJ per mol O_2) [15]. L-cells are potential tumour cells; thus it is conceivable that they exhibit a pronounced Crabtree effect during the first 24 h of the culture [1,2].

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