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Microdiver Studies on the Respiration of Burkitt Lymphoma Cells (EB-3)[1]

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SUMMARY

The effects of several sugars on the respiration of Burkitt lymphoma cells (EB-3) were studied by the micro-Cartesian diver technique. Results demonstrated that respiration was inhibited by glucose, fructose, and mannose, whereas galactose and sucrose produced no effects. Inhibition was more pronounced at higher concentrations of glucose and fructose. Respiratory inhibition was maintained after 4 hr of determination for the glucose- and fructose-exposed cells, and respiration declined for cells in the absence of substrate. The effects obtained with the sugars are considered to be due to the hexokinase-type substrates and the general metabolic characteristics of the cells.

INTRODUCTION

Burkitt lymphoma has been of special interest to the oncologist since its original description by Burkitt (8) in Africa. Of more recent interest was the establishment of Burkitt lymphoma cells in tissue culture and the demonstration of an Epstein-Barr-type viral association (15). Although much has been reported concerning the possible viral role of this cancer, little quantitative information exists regarding the metabolic properties of the tissue culture cell lines. This study was designed to elucidate some of the general metabolic characteristics of these cells and, in particular, to determine the effects, if any, of several sugars on aerobic respiration. Such a study could lead to a better understanding of the characteristics of cells associated with a possible viral influence as compared to other neoplastic cells of unknown origin. Because of the extreme sensitivity of the system and the ability to use microquantities of cells, the micro-Cartesian diver (ampulla diver) of Zeuthen (28) was of special interest. Such an apparatus allows one to use 100 cells or less, as compared to millions for conventional gasometric techniques. This is of special importance with respect to crowding effects and alterations in available substrates when one is making determinations over extended periods of time.

MATERIALS AND METHODS

Burkitt lymphoma cells (EB-3), obtained from Flow Laboratories, Inc. (Rockville, Md.), were cultured in Bellco spinner flasks with RPMI[3] 1640 medium plus 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). Cells were maintained in a log phase of growth by subculturing every 7 to 10 days. All experimental cultures were approximately 5 days old. Cells to be used for experiments were removed, centrifuged, and resuspended in RPMI 1636 media with or without the desired substrate.

For microgasometry, ampulla microdivers were made from Pyrex glass with the use of 0.8-mm-thick glass as starting material, from which capillaries were pulled to a thickness of 0.08 mm. Microdivers were then prepared from the capillaries by a method similar to that of Zeuthen (28) and selected for suitable brake values for assurance of proper filling by capillary action (9). All filling procedures were conducted in a temperature-regulated chamber that was made of Plexiglas and contained an inner chamber that could be observed through the clear top when the chamber was placed on a dissecting scope stage. A microforge heating element was also included within the chamber. After the diver was filled, the diver buoyancy was adjusted so that the diver could be made to rise or fall in the flotation medium (0.1 N NaOH) by minor adjustments with a manometer. Details of some of the procedures have been described previously (9, 19, 28). Average cell numbers were estimated directly in the diver (9, 21), and viability was routinely checked by the 0.4% trypan blue exclusion technique.

The thermostat bath was regulated by a Yellow Springs Instrument Model 72 proportional controller (37 ± 0.005°), and the buret manometers and flotation vessels were made by Ole Dich (Hvidore, Denmark). The calibration value of the manometers was 1 μl/mm, and modifications were made as suggested by Hertz (18).

After divers were placed in flotation vessels and equilibrated, the diver equilibrium was established by changing the amount of fluid in the manometer and thus the pressure in the system. Therefore, oxygen uptake was measured by the decrease of pressure in the diver. The respiratory rate was calculated by the equation (19):

$$\Delta O_2 / \Delta t = [(X)(v)(g_D)(f)(B + h - e)(273)] / [(V)(10300)(273 + t)]$$

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[3] The abbreviation used is: RPMI, Roswell Park Memorial Institute.

where X is the movement of the manometer indicator (mm), v is the volume (μl)/mm manometer, g_D is the diver dry weight (mg), f is a glass factor (20), B is barometric pressure (mm H_2O), h is the initial equilibrium pressure, and e is the vapor tension of H_2O at t°. The divers used had an air space of 0.1 to 0.5 μl, and v/V was about 1×10^{-5}.

Determinations were made every 30 min for 5 hr, and control divers, filled with every component except cells, were tested simultaneously with the experimental divers. The total ΔV/hr in the control diver was subtracted from the total ΔV/hr in the experimental diver in order to obtain a corrected value. After each experiment, divers were removed from flotation vessels, cell viability was checked, the divers were dried in an oven, and the dry weight was determined with a Cahn electrobalance.

RESULTS

Data demonstrating the influence of sugars on respiration of EB-3 cells are presented in Tables 1 and 2. Table 1 represents respiration determinations over a 4-hr period, and Table 2 represents determinations during the 5th hr period. Chart 1 shows a typical experiment in which the actual buret-manometer readings are shown. The ΔV in control experiments without cells varied from 8.4 to 20.0 pl/hr, for an average drift of 11.0 ± 3.3 pl/diver/hr. Respiration of cells in balanced media without glucose ranged from 5.1 to 7.0 pl/cell/hr, or an average rate of 6.3 ± 0.6 pl/cell/hr. Respiration of EB-3 cells in the presence of 10^{-3} M sodium azide and glucose was significantly inhibited (55%) when compared to cells in glucose medium without inhibitor (Table 1).

Respiration in the Presence of Glucose. Oxygen uptake in the presence of 0.018 M glucose (Table 1) ranged from 2.3 to 4.3 pl/cell/hr (average, 3.1 ± 0.7 pl/cell/hr), and it ranged from 2.2 to 3.1 pl/cell/hr (average, 2.6 ± 0.4 pl/cell/hr) in the presence of 0.025 M glucose. In all determinations, glucose reduced respiration approximately 50%, as compared to cells without glucose (2.6 to 3.1 *versus* 6.3 pl/cell/hr).

Table 1

Respiration of EB-3 cells in RPMI 1636 media with or without sugars, with 0.1 N NaOH as an absorbant, and with flotation medium at 37°
Results represent average values from 4-hr determinations.

No. of cells in diver	Diver wt (mg)	Av. ΔP (mm)	Total ΔV_{O_2}/hr (pl)	Substrate	ΔV_{O_2}/cell/hr (pl)	ΔV_{O_2}/cell/hr
310	1.07	246.0	2130	0	6.5	
157	2.66	46.4	1097		7.0	
118	1.44	55.0	713		6.0	6.3 ± 0.6[a]
489	8.13	35.0	2500		5.1	
255	4.90	41.0	1740		6.8	
1042	4.90	80.0	2805		6.5	
250	2.73	46.2	1085	18 mM glucose	4.3	
500	4.50	44.2	1740		3.5	
405	2.43	50.0	1066		2.6	3.1 ± 0.7
1012	1.32	200.0	2345		2.3	
300	0.73	143.0	928		3.1	
76	0.65	26.0	1685	25 mM glucose	2.2	
1042	4.90	80.0	2805		2.8	
421	1.20	106.0	1128		2.7	2.6 ± 0.4
135	0.89	55.0	426		3.1	
580	0.50	280.0	1170		2.0	
330	1.32	100.0	1165	18 mM fructose	3.5	
150	1.62	45.2	635		4.2	3.8 ± 0.3
200	1.00	108.0	800		3.8	
225	1.05	110.0	838		3.7	
372	1.80	85.7	1345	25 mM fructose	3.5	3.5
100	1.45	35.5	511	18 mM galactose	6.4	
75	1.60	34.0	490		6.3	6.4 ± 0.5
35	2.00	7.5	330		6.0	
61	2.00	25.0	435		7.1	
207	0.92	85.2	691	18 mM mannose	3.3	3.3
210	2.13	73.0	1332	18 mM sucrose	6.3	6.2
175	1.09	110.5	1081		6.2	
150	0.95	23.5	235	1 mM azide + glucose	1.5	1.4
98	0.91	17.3	137		1.3	

[a] Mean ± S.D.

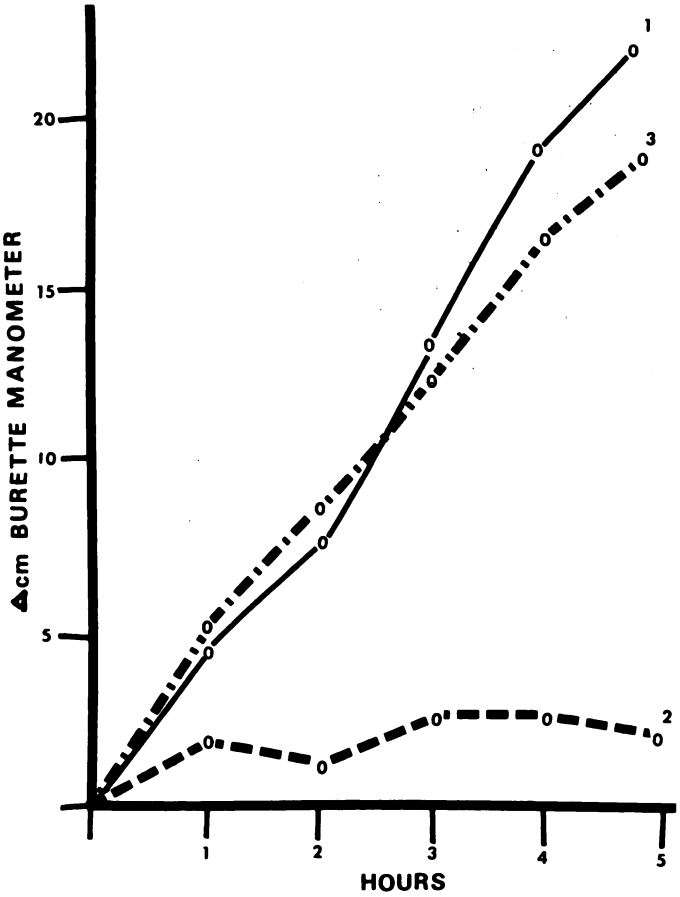

Chart 1. A typical experiment. *Curve 1*, diver with 250 EB-3 cells in RPMI 1636 medium + 18 mM glucose; diver weight, 2.73 mg. *Curve 2*, diver with control with RPMI 1636 medium + 18 mM glucose; diver weight, 4.20 mg. *Curve 3*, diver with 500 EB-3 cells in RPMI 1636 medium + 18 mM glucose; diver weight, 4.5 mg.

Table 2

Average respiration values during the 5th hr of determination

Substrate	ΔV_{O_2}/cell/hr (pl)	% change from 4th-hr value
0	5.0 ± 0.9	−21
0.018 M glucose	3.3 ± 0.5	+6
0.018 M fructose	4.0 ± 0.7	+5
0.018 M galactose	6.3 ± 0.7	−1

Respiration in the Presence of Fructose and Mannose. Oxygen uptake in the presence of 0.018 M fructose ranged from 3.5 to 4.2 pl/cell/hr (average, 3.8 ± 0.3 pl/cell/hr). Although inhibition was apparent when compared with cells without glucose (3.8 *versus* 6.3 pl/cell/hr), it was not as great as that produced by glucose. In addition, preliminary experiments with 0.018 M mannose and 0.025 M fructose demonstrated that mannose inhibited respiration (3.3 *versus* 6.3 pl/cell/hr), and that 0.025 M fructose inhibited respiration greater than did 0.018 M fructose (3.5 *versus* 3.8 pl/cell/hr).

Respiration in the Presence of Galactose and Sucrose. Respiration rates for galactose-exposed cells ranged from 6.0 to 7.1 pl/cell/hr, and no inhibition occurred when compared to cells without glucose (6.4 *versus* 6.3 pl/cell/hr). A similar effect was obtained with sucrose (Table 1).

Results in Table 2 demonstrate the influence of substrates on respiration during the 5th hr of determination. During this period, respiration of glucose- and fructose-exposed cells increased 6 and 5%, respectively. Cells in the absence of substrate demonstrated a 21% decline in respiration, while galactose-exposed cells remained relatively unchanged.

DISCUSSION

The reduced respiration rate produced by the addition of glucose and fructose demonstrates that a Crabtree effect (10) exists with the Burkitt lymphoma EB-3 cells. Such an effect has been reported in a variety of tumor tissues and tissue culture lines (4, 6, 7, 11, 12, 23).

Addition of excess glucose (0.025 M) inhibited respiration to a greater extent than did 0.018 M glucose. A similar phenomenon has been described by Medes and Weinhouse (23) and was considered to be related to changes in fatty-acid oxidation. They were able to conclude that, at high concentrations of glucose or fructose, fatty-acid oxidation decreased and was accompanied by respiratory inhibition. Furthermore, results here demonstrate that fructose exerts a similar effect on the respiration of Burkitt lymphoma cells. The inhibition produced by glucose and fructose suggests that a common metabolic mechanism may be involved for both sugars. Such a mechanism has been considered by some investigators to involve a similar hexokinase substrate (1–3, 7). Others think that a more appropriate explanation involves a competitive mechanism between mitochondrial and glycolytic mechanisms for ADP or P, (16, 17, 22, 26, 27). In addition, a preliminary experiment with mannose demonstrated a similar effect. Mannose is known to function as a hexokinase substrate under certain conditions (5, 7).

The lack of respiratory inhibition produced by galactose is not surprising in view of the report that galactose is not a hexokinase substrate (1–3). This does not mean, however, that galactose cannot be actively metabolized by the cells, since it has been reported that a variety of tissue culture lines utilize small amounts of galactose over extended periods of time (13, 24). A similar phenomenon may be true for sucrose, since it did not alter respiration.

The slight increase (5 to 6%) in respiration during the 5th hr of determination for glucose- and fructose-exposed cells suggests that the substrate levels of the sugars may be changing. In addition, cells without substrate declined considerably in respiration (21%), suggesting a lack of adequate metabolites. Furthermore, a variety of factors may be involved, such as pH, Krebs cycle intermediates, conditioning of the medium (11, 12, 21), or changes in the substrate levels of pyruvate (25). It is possible that respiration in the absence of added carbohydrate substrate may be maintained to some extent by the amino acids and other factors in the medium. Eaton and Scala (14) found that respiration of ascites cells could be maintained over a 10-hr period, provided that a combination of factors was present in the glucose-free medium. For this reason, complex media were used in all determinations, since preliminary experiments gave

considerable variations in respiration rates if balanced salt solution was used. The inhibition of respiration by azide suggests that a viable, functional cytochrome system exists with the cells.

The experiments presented here confirm earlier studies (11, 12) in that the Crabtree effect is maintained in the presence of hexokinase substrates, provided that adequate substrate levels are maintained with respect to cell density. It is possible that the changes in respiration obtained during the longer durations may be related to pH, changes in substrate concentration, or the metabolic characteristics of the cells, since cell density was kept minimal by the methods.

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