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The Respiration and Aerobic Glycolysis of Mouse Embryo Cell Cultures Infected with the SE Polyoma Virus

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In mouse embryo cell cultures infected with SE polyoma virus the following changes were observed: decrease in endogenous respiration, increase in aerobic glycolysis, and increase of the Crabtree effect. Just before the cytopathic effect appeared the decrease of oxygen uptake reached its maximum, and the increase of glycolysis was arrested. Selected cells, surviving the cytopathic changes, showed a greater oxygen consumption and lactic acid production.

The polyoma virus described in 1953 by Stewart *et al.*[1] multiplies easily in cultures of mouse embryo cells. The metabolic effects of animal cells infected by oncogenic viruses have not yet been studied. The possible changes in respiration and glycolysis might be of particular value, since the transformation of the normal into the neoplastic cell is associated with changes in the metabolism of glucose described in the classical work of Warburg[2]. Therefore we have undertaken a study of the respiratory metabolism of cell cultures infected with SE polyoma virus.

EXPERIMENTAL

Tissue cultures. Embryos of the Porton strain mice were used for the experiments. The embryo cell suspension was prepared in 0.25 % trypsin solution (Difco), centrifuged, washed twice and resuspended with a sufficient volume of medium to obtain 10^5 cells per ml. The growth medium consisted of Earle's salt solution, 0.5 % lactalbumin hydrolysate (N. B. C. Cleveland, Ohio) and 10 % calf serum.

Virus and infection of cultures. The original SE polyoma virus was obtained from the G. Roussy Institute in Villejuif by courtesy of Dr. G. Barski, and a strain derived from the cell culture passage was used for the experiments. In 48-h cultures of mouse embryo cells the medium with 10 % calf serum was replaced by one containing 3 % serum. Immediately afterwards 1 ml of virus, infectious titre 10^5 TCID (tissue culture infectious dose) per ml, was added to each Roux flask, containing on the average 70×10^5 cells. TCID was calculated by Kärber's method[3], studying the cytopathic effect.

Biochemical studies. Cultures of uninfected cells (controls) and infected cells were prepared simultaneously, and were examined before infection and 1, 3, 5, 6, 7, and 9 days after infection. The cells growing as a monolayer on the walls of Roux flasks were detached by

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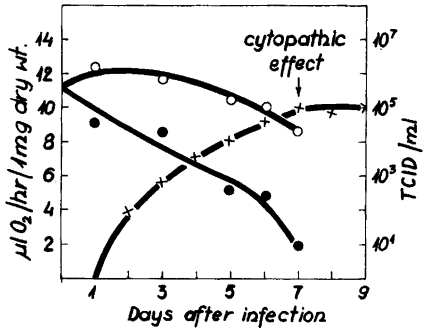

Fig. 1. Endogenous respiration (first 15 min) of mouse embryo cell culture normal o, and infected with SE polyoma virus •. The SE polyoma virus infectivity titre in mouse embryo cell culture medium ×.

scrubbing, washed twice, centrifuged, and suspended in a small volume of Krebs-Ringer buffer, pH 7.4, without glucose. The amount of cells in the suspensions was estimated by determining their dry weight. Endogenous respiration, and respiration in the presence of 10 mM glucose were measured by the method of Warburg[4] in Krebs-Ringer phosphate buffer, pH 7.4. Lactic acid was assayed by the Barker-Summerson method[5] after incubation of the cells with Krebs-Ringer solution containing 10 mM of added glucose.

RESULTS

The endogenous oxygen uptake by mouse embryo cell culture infected or uninfected with polyoma virus is shown in Fig. 1. The infected cells displayed diminished oxygen uptake when compared with the uninfected cells.

The results of determination of the increase in the virus titre presented in Fig. 1, indicate that the first release of the virus into the medium was preceded by the reduction of oxygen uptake, observed after 24 h. The lowest oxygen uptake on the sixth day coincided in time with the presence in the medium of the largest amount of virus and with the appearance of necrotic changes in the cells. Endogenous respiration of the selected cells, after the detached necrotic cells had been discarded with the medium, was much higher on the ninth day after infection than just before the appearance of cytopathic changes in the whole material.

Oxygen uptake in the presence of glucose both by infected and uninfected mouse embryo cells, was lower than the endogenous oxygen consumption (Table 1). Inhibition of oxygen uptake by glucose, *i. e.* the Crabtree effect, was always greater in the cells infected with polyoma virus.

Infection augmented aerobic glycolysis (Fig. 2). The increase of lactic acid production was proportional to the increase in virus formation. In the uninfected culture aerobic glycolysis also increased with time, but this increase was slower and probably due to the long period of cultivation in an unchanged medium. The reduction in lactic acid formation on the seventh day after infection was correlated in time with the decrease in oxygen uptake, with the detachment of the cells from the glass, and with the appearance of cytopathic changes.

Table 1. The effect of 10 mM glucose on oxygen uptake by mouse embryo cell cultures uninfected and infected with polyoma virus. Mean values of 3 experiments are given.

Days	Cells	Mean values of oxygen uptake during 1st hour of experiment μl O_2/1 h/1 mg dry wt		Decrease in oxygen uptake %
		endogenous	in presence of glucose	
Before infection		11.3	8.3	19.8
After infection				
1	Uninfected	11.5	9.8	14.8
	Infected	6.2	4.1	33.9
2	Uninfected	8.9	6.6	25.8
	Infected	6.3	4.1	34.9
5	Uninfected	6.5	5.2	20.0
	Infected	5.7	1.7	54.0
6	Uninfected	10.4	6.2	40.3
	Infected	8.1	4.5	44.4
7	Uninfected	7.6	5.1	32.8
	Infected	3.6	2.0	44.5
9	Uninfected	15.2	12.2	19.7
	Infected	11.2	9.4	16.1

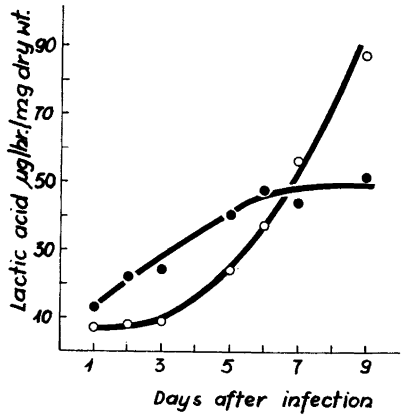

Fig. 2. Aerobic glycolysis in mouse embryo cell culture normal o, and infected with SE polyoma virus •.

DISCUSSION

The decrease in endogenous oxygen uptake and the increase in aerobic glycolysis already observed 24 h after infection, *i. e.* before the appearance of virus in the medium, might constitute evidence for alteration in the metabolism of the cells. When necrotic changes in the cells were first observed, the amount of virus reached its maximum and did not rise further, either because of the exhaustion of the cells necessary for the synthesis of virus, or as the result of development of defence mechanisms in the still undamaged cells. The surviving selected cells, investigated on the ninth day, were more resistant, or able to develop defence mechanisms, possibly leading to transformation into neoplastic cells. These cells may correspond to the fibroblast-type cells described by Negroni[6], more resistant to the virus and capable of surviving the period of cytopathic changes manifested mainly in sensitive embryo cells of the epithelial type.

The Crabtree effect observed in aerobically glycolysing neoplastic cells[7], as well as in those cultivated *in vitro*[8] was much higher in cells infected with oncogenic polyoma virus than in those uninfected. According to current views the Crabtree effect is due to a decrease of the phosphorus compounds which are essential for activation of the respiratory chain[9]. The increasing Crabtree effect in infected cells may be due to a shunt of the nucleotides and P_i which activate the respiration to the synthesis of viral nucleic acids. Further studies have been undertaken on these assumptions.

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