PASTEUR EFFECT AND THE DPN-FLAVOPROTEIN REGION OF THE RESPIRATORY CHAIN[1]

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Nearly a century ago Pasteur discovered that fermentation is inhibited in the presence of oxygen. Since then a considerable amount of work concerned with an explanation for this phenomenon has been published. Although some of the proposed theories are quite attractive, it is the general consensus that a complete understanding of the "Pasteur effect" is still lacking.

The most widely accepted explanation is that respiration competes with glycolysis for some component of the phosphorylation system, inorganic phosphate or adenosine diphosphate (ADP) (1-6). The respiratory requirement for inorganic phosphate and ADP could be located at three phosphorylation sites in the respiratory chain, namely DPN-flavoprotein, cytochrome c, and cytochrome a. The cofactor requirements for these three sites of phosphorylation should have equal importance in their ability to compete with glycolysis for inorganic phosphate and ADP. However, we found that respiration restored by succinate in brain homogenates[2] or in intact ascites tumor cells (7) treated with amytal

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did not inhibit glycolysis or had little effect. The finding that amytal decreases the P/O ratio of succinate respiration (8), and the observation that high concentrations (3×10^{-3}M) of the inhibitor are needed to block the oxidation of reduced diphosphopyridine nucleotide (DPNH), made us interpret our findings with caution. The inability of succinate respiration to inhibit glycolysis could be due to the fact that such respiration is not tightly coupled to phosphorylation.

The recent report that rotenone blocks DPN-flavoprotein-linked electron transport in a more specific manner than does amytal, and without its side effects of P/O ratio, ATPase reactions and Pi-ATP exchange reaction (9), offered us the possibility of repeating the experiment under conditions which seemed free of that objection.

METHODS AND MATERIAL

The hyperdiploid Ehrlich Lettré ascites cell (ELD) was maintained in Ha/ICR Swiss males. The mice were sacrificed between the 9th and 11th day after inoculation, and the ascites fluid was removed and the tumor cells were isolated by low-speed centrifugation. The cells were washed twice with Ca^{++}-free Krebs-Ringer phosphate (0.1M, pH 7.4) and resuspended in this medium.

Rotenone was dissolved in 95% ethanol and diluted so that the desired concentration could be added in a maximum volume of 10λ to avoid secondary effects of ethanol. The procedures of incubation and methods of analyses were essentially the same as described in a previous paper (10).

RESULTS AND DISCUSSION

The lack of impairment of the phosphorylation reactions in the presence of rotenone was tested in whole ELD cells by measuring the ratio of 2-deoxyglucose (2-DOG) disappearance to oxygen consumed. Such a ratio can be expected to be lower in the intact cell than it would be in isolated mitochondria since energy-requiring reactions occurring in the cell, such as synthetic reactions, active transport through membranes, maintenance of cellular structures, etc., can be considered as endogenous high-energy phosphate acceptors able to compete with 2-DOG for respiratory ATP.

Two factors which tend to lower the value of the $\frac{\text{2-DOG}}{\text{oxygen}}$ ratio are the incubation time and the amount of phosphate present. The deleterious effect that the trapping of high energy phosphate by this sugar exerts on the cellular metabolism decreases the $\frac{\text{2-DOG}}{\text{oxygen}}$ ratio as the time of incubation increases. However, in short-term experiments a ratio as high as 1.2 was obtained. Although an increase in phosphate concentration produced an increase in 2-DOG uptake, it did not appear advisable to employ phosphate concentrations which were highly unphysiological.

For these reasons, the ratio $\frac{\text{2-DOG}}{\text{oxygen}}$ could not be used to measure the total efficiency of the oxidative phosphorylation reactions in the whole cell. However, this ratio appears to provide a reasonable comparison of the phosphorylative efficiency of whole cells incubated under different experimental conditions. In addition, the ratio of $\frac{\text{2-DOG}}{\text{oxygen}}$ in ELD cells fell to zero in the presence of 2.5×10^{-5}M dinitrophenol which suggests that changes in the P/O value of the cells can be expected to be reflected in the $\frac{\text{2-DOG}}{\text{oxygen}}$ ratio.

An experiment on the effect of rotenone on the $\frac{\text{2-DOG}}{\text{oxygen}}$ ratio in whole ELD cells is reported in Table I. The results suggested that the phosphorylative ability of the cell was not impaired by rotenone when succinate was present.

TABLE I

STIMULATION OF 2-DEOXYGLUCOSE UTILIZATION BY SUCCINATE IN ROTENONE-TREATED CELLS

ELD cells equivalent to 28 mg. dry tissue weight were incubated for 30 minutes at 38° in Ca^{++}-free Krebs-Ringer buffer containing 80 μmoles of TRIS buffer and 10 μmoles of phosphate buffer, pH 7.4. 2-DOG was tipped in from the side arm 10 minutes after temperature equilibration.

Additions	Rotenone (1.8×10^{-7}M)	Oxygen uptake μatoms		2-DOG utilized	2-DOG uti / oxygen up
		No 2-DOG	Plus 2-DOG (0.005 M)	μmoles	
None	-	12.4	5.8	2.2	0.38
	+	1.4	1.4	0.3	not signifi
Succinate (0.05M)	-	19.2	10.6	4.0	0.38
	+	6.4	8.6	3.4	0.39

The effect of rotenone and succinate on the glycolytic activity of ascites tumor cells was then investigated. It was found (Table II) that rotenone inhibited respiration and increased glycolysis, as measured by glucose disappearance and by lactate accumulation. The addition of succinate completely restored respiration, but this succinate respiration

TABLE II

EFFECT OF SUCCINATE RESPIRATION ON THE GLYCOLYSIS OF ROTENONE-TREATED ASCITES TUMOR CELLS

One ml of a 15% suspension of Ehrlich Lettre ascites tumor cells (19 mg. dry tissue weight) in Ca^{++}-free Krebs Ringer phosphate buffer, pH 7.4, was introduced into Warburg flasks containing 125 μmoles of Tris buffer, pH 7.4, and, when added, rotenone in the designated concentration and potassium succinate, 30 μmoles. The total volume was made up to 3.0 ml with Ca^{++}-free Krebs Ringer phosphate buffer. Glucose (60 μmoles) was tipped in from the side arm after 10 minutes temperature equilibration and incubation was carried out for 40 minutes.

Additions		Oxygen consumption	Glucose utilized	Lactate accumulated
Rotenone	Succinate			
(molar)		(μmoles)	(μmoles)	(μmoles)
0	-	4.3	9.9	11.2
	+	5.6	8.6	11.0
7×10^{-8}	-	2.0	15.2	21.6
	+	4.6	16.0	20.1
3.5×10^{-7}	-	0.8	16.2	22.6
	+	4.6	16.2	21.1
7×10^{-7}	-	0.7	17.3	25.0
	+	4.9	16.7	22.3

was incapable of inhibiting the rate of glycolysis. These experiments strongly suggested that the transfer of reducing equivalents from cytochrome b to oxygen, although linked with two phosphorylations, does not exert any control on glycolysis. It appears therefore that the inhibition

of glycolysis by oxygen is regulated at the DPN-flavoprotein step. The phosphorylation that takes place at this site, and/or the ratio of the reduced/oxidized carriers of this region of the respiratory chain, appears to be responsible for the aerobic inhibition of glycolysis.

The finding that succinate in the absence of rotenone induces an increase in oxygen consumption and a decrease in glucose uptake could be considered to favor the idea that an accompanying increase in phosphorylation is responsible for the inhibition of glucose disappearance. However, previous studies have demonstrated that succinate is able to compete with pyruvate for the electron transfer chain, lowering the P/O ratio and actually inhibiting pyruvate oxidation (10). Therefore, the increase in the rate of respiration induced by succinate does not imply that there is also an increase in the total amount of phosphate esterified, since it can be compensated for by the decrease in P/O ratio. A more detailed investigation of this problem is now underway.

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