Effects of Dinitrophenol and Arsenate on the Respiration and Phosphorylation of Ehrlich Ascites Tumor Cells and the Mechanism of the Crabtree Effect

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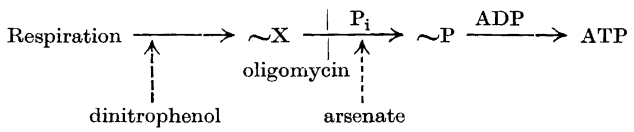
2,4-Dinitrophenol and arsenate both uncouple respiratory chain-linked phosphorylation in mitochondria. Yet recent evidence suggests that the two agents act at different levels of the energy transfer system. The evidence comes from studies with the inhibitor, oligomycin [1], whose effect on mitochondrial respiration is released by dinitrophenol but not by arsenate [2,3]. It has also been shown [4] that oligomycin inhibits the exchange of ^{18}O between P_i and H_2O in phosphorylating mitochondria. These and other findings have led to the conclusion [3,5-7] that oligomycin blocks the interaction of a high energy intermediate, ~X, with P_i, and that dinitrophenol and arsenate act above and below the level of this intermediate according to the following scheme:



Hexoses capable of undergoing hexokinase reaction depress the respiration of tumors. This phenomenon, known as the Crabtree effect [8], has often been attributed to a lack of, or lack of access to, P_i and/or ADP in maintaining a maximal activity of the mitochondrial respiratory chain (for reviews, see [9,10]). The Crabtree effect is abolished by dinitrophenol and, as has been demonstrated recently in this laboratory [11], also by vitamin K_3, which establishes an electron transfer shunt over the rate-limiting phosphorylation site of the respiratory chain. In the course of the latter studies it was found [12] that oligomycin induces a Crabtree-like effect in Ehrlich ascites tumor cells: it causes a respiratory inhibition which is non-additive to that induced by glucose and which can be overcome by dinitrophenol and by vitamin K_3. Prompted by these observations, and by current information about the mode of action of oligomycin, an investigation of the effect of arsenate on the Crabtree phenomenon was considered to be of interest.

When Ehrlich ascites tumor cells were incubated in an isotonic, efficiently buffered [13] salt solution in the presence of 0.1 mM $^{32}P_i$, they incorporated ^{32}P into organic phosphate at a linear rate (Table 1). Dinitrophenol (0.1 – 0.2 mM) and arsenate (2 – 10 mM) inhibited the incorporation to a considerable extent, indicating that both agents gained access to the mitochondrial respiratory chain. The endogenous respiration of the cells was virtually unaffected by either agent (Table 2). When glucose was added,

Table 1. Incorporation of $^{32}P_i$ into organic phosphate by Ehrlich ascites tumor cells. Each vessel contained ca 130 mg fresh weight Ehrlich (ELT) ascites tumor cells in 2 ml of a medium consisting of 112 mM NaCl, 4.48 mM KCl, 1.12 mM $MgSO_4$, 68 mM tris buffer, pH 7.5, and 0.1 mM $^{32}P_i$. Incubation at 37°C.

Addition	% organic ^{32}P after 5 min	10 min	20 min
None	6.3	11.6	22.1
2,4-Dinitrophenol, 0.1 mM	2.9	5.4	13.3
Arsenate, 2 mM	4.3	6.7	11.3
» 5 mM	2.4	4.4	6.6
» 10 mM	1.4	2.3	4.7

Table 2. Effect of dinitrophenol and arsenate on respiration and phosphorylation of Ehrlich ascites tumor cells. Conditions as in Table 1. Glucose and 2-deoxyglucose were added, when indicated, in a final concentration of 15 mM. Incubation for 20 min in Warburg vessels with air as the gas phase and 0.2 ml 2 M KOH as CO_2-adsorbent.

Addition	None		Glucose		2-Deoxyglucose	
	μat.O_2	% org.^{32}P	μat.O_2	% org.^{32}P	μat.O_2	% org.^{32}P
None	6.1	22.3	2.4	31.1		
2,4-Dinitrophenol,						
» 0.1 mM	6.9	7.7	8.3	26.2		
» 0.2 mM	6.0	2.1	7.9	19.4		
Arsenate, 2 mM	5.1	10.9	1.7	15.5		
» 10 mM	4.4	2.8	1.9	5.8		
None	4.9	23.7	2.8	33.7	1.7	27.8
Arsenate, 2 mM	5.8	13.5	2.7	15.1	1.8	12.8
» 10 mM	5.4	5.1	2.2	7.7	2.0	7.6

respiration was depressed, and this effect was counteracted by dinitrophenol. In contrast, arsenate did not release the Crabtree effect induced by either glucose or 2-deoxyglucose. The incorporation of $^{32}P_i$ into organic phosphate was about equally sensitive to arsenate in the presence and absence of hexoses. Dinitrophenol inhibited the phosphate uptake less in the presence of glucose than in its absence, in agreement with its known lack of effect on glycolytic phosphorylation.

The data reported above show that arsenate, although capable of uncoupling respiratory chain phosphorylation in Ehrlich ascites tumor cells, does not release the Crabtree effect. These results strongly suggest that the Crabtree effect is not due to a lack of, or lack of access to, P_i and/or ADP for respiratory chain-linked phosphorylation. The available information supports the view that the respiratory inhibition connected with the Crabtree effect resides at the level of a high energy intermediate situated between the dinitrophenol- and the arsenate-sensitive reactions of the energy-transfer system, *i.e.* at the level of $\sim$X in the above scheme.

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