EFFECT OF OLIGOMYCIN ON THE ARSENATE AND DNP STIMULATION OF MITOCHONDRIAL OXIDATIONS

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Crane and Lipmann (1953) have shown that mitochondrial oxidations could be stimulated by arsenate in the absence of inorganic phosphate. This has been suggested as due to the arsenolysis of an intermediate during the phosphorylation reactions. In a similar manner Loomis and Lipmann (1948) and Lardy and Wellman (1953) established that DNP (2,4 dinitrophenol) also stimulated mitochondrial oxidations. Recently Wadkins (1960) has reported on the arsenate stimulation of mitochondrial ATPase and suggested that the site of action of arsenate on the phosphorylation reactions may be "near or the same as that of dinitrophenol". Recent studies reinvestigating the mechanism of DNP and arsenate stimulation of mitochondrial oxidations and the influence of these reagents on the reactions of the phosphorylation sequence clearly showed that the mechanism of stimulation of oxidations by these two reagents occur at different loci. This is illustrated by studies in the presence of oligomycin. Oligomycin, as recognized by Lardy, Johnson, and McMurray (1958) and Lardy and McMurray (1959), is a potent inhibitor of the phosphorylation reaction sequence.

As shown in the oxygen electrode tracing presented in Figure I, the addition of ADP and succinate to a mitochondrial suspension diluted in a phosphate free medium causes only a slow rate of oxygen uptake. The subsequent addition of 0.5 mM sodium arsenate increases the rate of oxygen utilization to a rate comparable to that observed when phosphate and ADP are

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present. The subsequent addition of oligomycin causes a cessation of this arsenate stimulated oxygen uptake, after about a 10 second lag period. The high rate of respiration may be reinitiated by the addition of DNP, as shown in the figure. This clearly demonstrates that oligomycin inhibits arsenate stimulation but not DNP stimulation of mitochondrial oxidations. Similar results have also been obtained with betahydroxybutyrate as substrate and by altering the order of addition of inhibitor and reactants. This inhibitory effect of oligomycin on the phosphorylation reactions associated with the oxidation of pyridine nucleotide linked substrates is comparable in part to the effect of guanidine as reported by Hollunger (1955). Guanidine, however, does not appear to inhibit the oxidation of succinate.

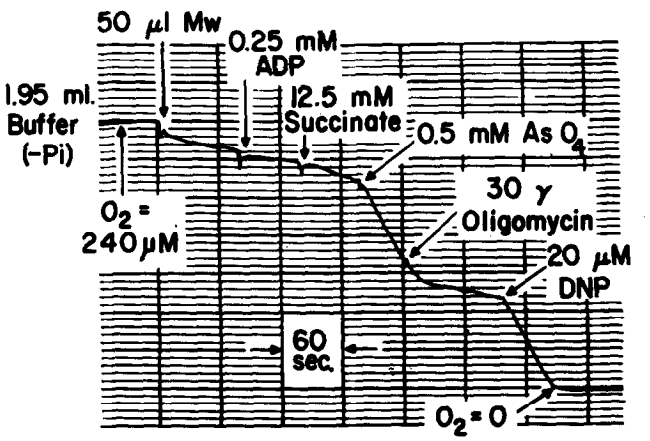

Figure 1. 50µl. of a rat liver mitochondrial suspension (2 mg. protein) were diluted to 2.0 ml. in a buffer composed of 80 mM KCl, 10mM triethanolamine hydrochloride, 5 mM $MgCl_2$ adjusted to pH 7.0. Subsequent additions of ADP, sodium arsenate (pH 7.0), oligomycin, and 2,4 dinitrophenol are as indicated. Temperature, 26°.

Also of interest in this type of experiment is the observation that pretreatment of the mitochondrial suspension with ADP prior to the addition of arsenate decreases the concentration of arsenate required to cause a comparable stimulation of respiration. This may result from the necessity to deplete the mitochondria of endogenous phosphate or to cause the release

of a phosphorylated intermediate. In the analogous situation, the addition of ADP to a system in which respiration has been activated by a sub-optimal concentration of sodium arsenate causes an immediate increase in the rate of oxygen uptake. In experiments similar to the one illustrated in Fig. 1 comparable results have been obtained when lower concentrations of oligomycin are employed except for a prolongation of the lag period before inhibition of the arsenate stimulated succinoxidase activity.

On the basis of the above experiments it appears that DNP and arsenate do not react at the same locus of the phosphorylation sequence. DNP acting between the electron transport chain and the site of oligomycin inhibition whereas arsenate reacts between this inhibition site and ATP. Since the reaction of arsenate is competitive with phosphate one may presume that these react at the same locus. Both arsenate and DNP do activate respiration by altering a rate limiting reaction, however, since the degree of stimulation of oxidative reactions by arsenate and DNP are additive.

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