INHIBITION OF THE RESPIRATION OF CULTURED MAMMALIAN CELLS BY OLIGOMYCIN[1]

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Studies on the effect of oligomycin on the respiration of intact cells have given two distinctly different results. On the one hand, the respiration of a variety of tissue slices (Van Rossum, 1964; Whittam et al., 1964; Wu, 1964; Tobin and Slater, in press) is inhibited only 15-30 percent by the antibiotic. On the other hand, ascites tumor cells (Dallner and Ernster, 1962; Minikami and Yoshikawa, 1963) and renal tubule cells (Arnaud and Rasmussen, 1964) show 70-85 percent inhibition of the endogenous respiration by comparable concentrations of oligomycin.

We present here what we believe to be the first report of complete inhibition of the respiration of intact cells by oligomycin. The concentrations required to inhibit completely are as much as 4 fold lower than generally employed. Moreover, the response of the cultured cells to oligomycin yields

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a titration curve distinctly different from that obtained with intact mitochondria.

Methods

The cell lines were HeLa S-3, L-929, Stoker's C13, Fischer's L-5178Y, and Tjio's Chinese hamster ovary (CHO) grown by standard suspension culture techniques (Petersen and Anderson, 1964). The nutrient media were BME, L_1, and Ham's F_{10} supplemented with calf serum and antibiotics. Calcium was omitted in all cases to facilitate monodisperse growth in suspension culture. Exponentially growing cells were removed from the growth medium by centrifugation (400 x g), washed twice by suspension in saline-phosphate solution (KCl, 0.06 M; NaCl, 0.15 M; KH_2PO_4, 0.01 M; pH 7.4), and finally resuspended in the same solution. Respiration was measured with a Clark oxygen electrode at 37°C. Protein was determined by the method of Gornall et al. (1949) using bovine serum albumin as a standard. Oligomycin was obtained from the Wisconsin Alumni Research Foundation as a mixture of 19 percent oligomycin A and 81 percent oligomycin B. Solutions of the inhibitor were prepared in absolute ethanol and stored frozen, or at 5°C. Both oligomycins A and B have the same quantitative effects on respiration and are stable under the conditions employed here (Lardy et al., 1965). The amount of ethanol added with the oligomycin had no effect on the respiratory activity.

It was noted that if cells were added to a reaction vessel already containing oligomycin, complete inhibition of respiration could not be obtained at any concentration of the antibiotic. It appears that the respiration of these cells

may become loosely coupled[4] during storage at high cell density in cold saline-phosphate. Complete inhibition of respiration by oligomycin cannot then be obtained unless the drug is added 1-2 minutes after dilution into warm medium.

Results

Figure 1 shows a titration of the endogenous respiratory activity of the Chinese hamster ovary cells by oligomycin.

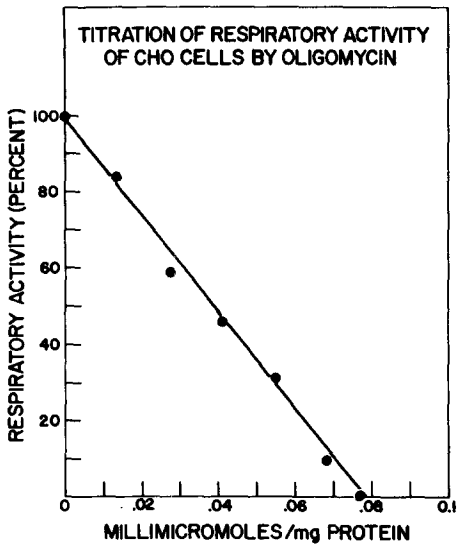

Fig. 1. Titration of the endogenous respiratory activity of Chinese hamster ovary cells by oligomycin. Five-microliter aliquots of a 23-μM solution of oligomycin in absolute ethanol were added to a vessel containing cell suspension (3.42 mg protein) in saline-phosphate. Additions were repeated until maximum inhibition of respiration (measured with a Clark electrode at 37°C) was obtained. The total volume was 2.0 ml.

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[4]The terms tight and loose coupling refer to the respiratory response obtained when phosphate and ADP are added to a system capable of oxidative phosphorylation. In the first case, the respiratory rate increases 5-20 fold; in the second, there is no increase in respiratory rate. Uncoupled preparations are non-phosphorylating (cf. Lehninger and Gregg, 1963).

Response to the antibiotic is linear from 0-100 percent inhibition. In this experiment complete inhibition of respiration was obtained at 0.078 millimicromole oligomycin per mg protein. In 5 experiments with CHO cells the average value obtained was 0.070 $\pm$ 0.037.

In Table I the results obtained with 5 different mammalian cell cultures are compared. All the cell lines tested were highly sensitive to oligomycin; complete inhibition of respiratory activity occurred at concentrations of the antibiotic from 0.05-0.2 millimicromole per mg protein. There appears to be no correlation between respiratory rate (Q_{O_2}) and oligomycin sensitivity. In all cell lines the inhibition of respiration by oligomycin was completely relieved by 2,4-dinitrophenol at 1×10^{-5} M. Stimulation of respiration on addition of ADP to the intact cells has not been observed, presumably because of the impermeability of the cells to the nucleoside diphosphate. This point is being investigated.

TABLE I

Concentration of Oligomycin Required for Complete Inhibition of the Endogenous Respiratory Activity of Five Lines of Cultured Mammalian Cells

Cell Line	Q_{O_2} (microliter oxygen per mg protein per hour)	Oligomycin Concentration (millimicromole per mg protein)
Chinese Hamster Ovary	9.8	0.070
HeLa	13.0	0.065
L-5178Y	13.0	0.047
C13	5.6	0.074
L	7.8	0.180

Discussion

The antibiotic oligomycin is a true uncoupler of oxidative phosphorylation (Huijing and Slater, 1961). A corollary of this property is that oligomycin inhibits only that respiration which is tightly coupled to oxidative phosphorylation; loosely coupled or uncoupled respiration is unaffected by the antibiotic (Lardy et al., 1958; Huijing and Slater, 1961).

Thus, the simplest explanation of the available data on the action of oligomycin on intact cells is that all of the endogenous respiration of cultured mammalian cells is tightly coupled to phosphorylation, as is nearly all the endogenous respiration of ascites and renal tubule cells, while 70-80 percent of the endogenous respiration of the various kinds of tissue slices examined so far is uncoupled or loosely coupled. However, the possibility cannot be ruled out that the low oligomycin sensitivity of tissue slice respiration is due to the inability of the antibiotic to reach the bulk of the respiring cells. A case in point is provided by comparison of the isolated renal tubule cells (Arnaud and Rasmussen, 1964), which were strongly inhibited by oligomycin, with kidney cortex slices (Whittam et al., 1964; Wu, 1964), which were weakly inhibited, despite the fact that renal tubule cells make up the bulk of the kidney cortex (Ham, 1957).

The studies on oligomycin inhibition of the respiratory activity of intact mitochondria (Ernster et al., 1963; Lardy et al., 1964; Bruni et al., 1965) have yielded titration curves different from that presented here. In intact mitochondria the initial additions of oligomycin had no effect on respiration until a threshold level was reached, after

which respiratory activity was linearly inhibited by further additions of the antibiotic. In contrast, the respiratory response of the intact cells to oligomycin is completely linear from zero to complete inhibition. In no experiment was there evidence of a threshold level for oligomycin inhibition. This may indicate that these cells have lost either "excess" respiratory capacity or competitive sites to which oligomycin is preferentially bound (Potter and Reif, 1952; Thorn, 1956).

Further studies on the effects of oligomycin on dispersed cell preparations from rat liver and on ascites and cultured forms of the same cell line are in progress in this Laboratory.

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