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Cyanide-Resistant Respiration of a *Neurospora crassa* Membrane Mutant

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Choline starvation of the *Neurospora crassa chol-1* mutant leads to a decrease in respiration through the cytochrome chain and a concomitant induction of the alternate oxidase.

The *chol-1* mutant (no. 34486) of the mold *Neurospora crassa*, blocked in the methylation pathway synthesis of phosphatidyl choline (5), can be useful in elucidating the role of phosphatidyl choline in maintaining membrane-associated respiratory function. By controlling the choline supplementation, one can change the phosphatidyl choline content of this mutant in a wide range (1). In this study I have measured whole cell respiration as a function of variable choline supplementation.

A controlled number of spores was inoculated into 50 ml of Vogel's minimal medium (7) plus 1.5% sucrose (minimal medium). Choline was added as required. Growth at room temperature proceeded with shaking on a reciprocal shaker set at 140 strokes/min. Respiration was measured in micro Warburg vessels with 3 ml of Vogel's minimal medium plus 2% glucose, adjusted to pH 6.7 with phosphate buffer and equilibrated at 32 C. Results were expressed as microliters of oxygen consumed per hour per milligram (dry weight).

A potassium cyanide concentration of 3.3 mM was chosen with the purpose of distinguishing cyanide-resistant and -sensitive respiration (Fig. 1A and B). In the stationary phase (more than 24 h after inoculation) cyanide-sensitive respiration declines considerably faster for cholin-starved cultures, with parallel increase of the alternate respiration reaching 50% of the total respiration rate (Fig. 1B). Supplemented cultures maintain fairly high oxygen uptake rates (around 80 μl/mg per h) during the entire second day of growth, whereas their cyanide-resistant respiration never exceeds 10% of the total during the same time period (Fig. 1A).

When the choline concentration in the medium is varied, the alternate respiration rate fraction undergoes sharp change, from 1 to 0.5 μg of choline supplementation per ml (Fig. 2). This is also observed in antimycin A inhibition experiments (Fig. 2). It is interesting to note that the phosphatidyl choline content of *chol-1* membranes decreased significantly in the same range of choline supplementation (1).

It is known that alternate respiration can be induced in wild-type *Neurospora* (2) by various

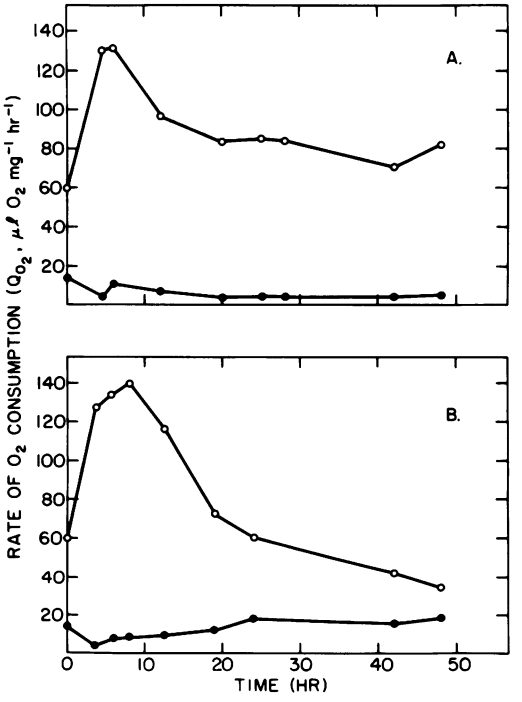

FIG. 1. *Respiration rate as a function of time after the inoculation of chol-1 spores into (A) supplemented medium (50 μg of choline per ml) and (B) minimal medium (without choline). Open circles represent total O_2 uptake rates, whereas filled ones denote corresponding O_2 uptake rates in the presence of 3.3 mM KCN. Zero time corresponds to approximately 1 h after spores were harvested with distilled water.*

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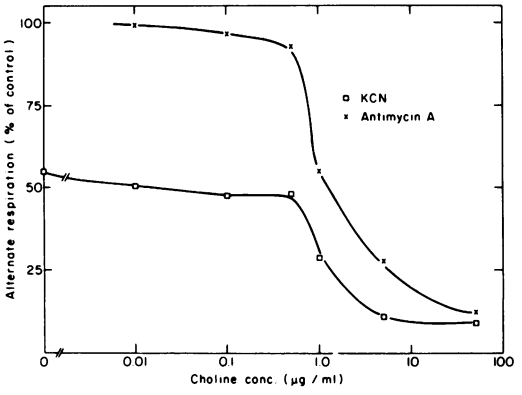

FIG. 2. *Effect of choline supplementation on the respiratory fraction of chol-1 cultures resistant to (i) 3.3 mM KCN (squares) and (ii) an antimycin A concentration of 0.1 μg/mg (dry weight) (crosses). All cultures were in the early stationary phase (day 2 of growth).*

drugs, such as cyanide and antimycin A, that interfere with the electron flow through the mitochondrial cytochrome chain, or drugs such as chloramphenicol, which hinders the normal process of mitochondrial synthesis. Choline starvation also interferes with the normal process of mitochondrial synthesis, resulting in a decrease in the ratio of mitochondrial phospholipid to protein (4). Some components of the cytochrome chain, in particular the cytochrome oxidase system, require phospholipids for their optimal function (6). Phospholipid depletion and phosphatidyl choline dilution can conceivably impair the electron flow through the cytochrome chain, leading to phenotypic behavior similar to that of mutations involving particular components of that chain (3).

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