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The Effect of Arsenite on the Endogenous Respiration of some Microorganisms

Wpływ arseninu na endogenne oddychanie niektórych drobnoustrojów

Влияние арсенина на эндогенное дыхание некоторых микроорганизмов

Arsenite is known to inhibit oxidative decarboxylation of both pyruvate and α-ketoglutarate. It also may act as un uncoupler of oxidative phosphorylation (3, 6, 7).

An addition of this poison to a cell suspension results in accumulation of pyruvate the amount of which may serve as an index of the extent of glycolysis. This fact was taken advantage of to observe inducible synthesis of some catabolic enzymes in *Mycobacterium* sp. 279 (10). The inhibitory action of arsenite on *M. phlei* was first reported by Edson (1, 2).

The present paper provides evidence for a stimulatory effect of arsenite on the endogenous respiration of starved mycobacteria and yeast.

MATERIALS AND METHODS

The following microorganisms were used: *Mycobacterium* sp. 279, *Nocardia minima*, *Escherichia coli* and *Saccharomyces cerevisiae*. The first two organisms were the strains maintained in this laboratory on appropriate media by serial mass transfers. *E. coli* was from the Department of Bacteriology, Medical School in Lublin, *S. cerevisiae* was commercial bakers' yeast.

For experiments, *M.* sp. 279 was grown for 3 days at 37° on the surface of Sym's medium (5) containing: glucose (4%), glutamic acid (1%), citric acid (0.2%), KH_2PO_4 (0.05%), $MgSO_4 \cdot 7H_2O$ (0.05%), ferric ammonium citrate (0.005%), $ZnSO_4 \cdot 7H_2O$ (0.002%) plus dilute KOH to adjust *pH* to about 7. Glucose solution (10%) was autoclaved separately. *N. minima* was grown for two weeks at 30° on Sym's medium solidified with 1.5% agar (8). *E. coli* was grown for 1—2 days at 37° in an aerated 3% nutrient broth.

Starvation was carried out as described before (9, 10) by incubating washed cells for appropriate times in either phosphate buffer or Sym's medium with glucose omitted.

Oxygen uptake was followed manometrically at 37° by the conventional Warburg method (11). Each Warburg vessel contained in a final volume of 2.5 ml: 1 ml of 0.2 M phosphate buffer (*pH* 7.2), 1 ml of cell suspension, and 0.5 ml of 0.05 M sodium arsenite (or 0.5 ml of water in controls). The center well housed 0.2 ml of 25% KOH. Equilibration lasted for 15 minutes, and readings were taken every 10 minutes throughout a 1.5 h period.

Where indicated in the text, pyruvate was determined in cell-free supernatant fluids by the 2,4-dinitrophenylhydrazine-toluene extraction procedure (4). To this end, the incubation mixture was acidified with 1 ml of 25% trichloroacetic acid and the precipitated material removed by centrifugation.

RESULTS AND DISCUSSION

Table 1 shows the effect of arsenite on the endogenous respiration of *Mycobacterium* sp. 279 as a function of starvation time. As one can see, the oxygen uptake decreased with time of starvation reaching after 24 h approx. 1/4 of the original value. In a parallel determination with arsenite added the corresponding quotient proved to be markedly higher. When the arsenite-caused changes in respiration (expressed in ± percent) were plotted against starvation time, a graph was obtained with an inversion point indicating the transition from the state of inhibition to that of stimulation (Fig. 1-A). In other words, the cells starved for more than 7 hours responded to arsenite in a paradoxical way by consuming more oxygen than the controls. This was particularly true in the case of cultures which had been grown in the presence of a reduced (2%) amount of glucose. The production of pyruvate decreased with the starvation time as well, attaining negligible values at the inversion point (Fig. 1-B).

Table 1. Endogenous respiration of starved *Mycobacterium* sp. 279 in the presence and absence of arsenite. Figures represent the results of one typical experiment *

Starvation time (hours)	Oxygen uptake µl O_2/h per mg dry weight	
	arsenite omitted (control)	arsenite added
0	36.8	21.6
3	17.9	13.5
6	17.2	16.5
12	12.0	15.4
24	8.4	12.6

* Three-day old surface cultures were underlayered consecutively with equal volumes of sterile water and glucose-free Sym's medium supplemented with 50 mM phosphate buffer, *pH* 7.2, then incubated at 37°. At indicated times, cells of the particular cultures were washed with water by centrifugation and used for manometric measurements (25 mg of wet weight cells per flask) as described in Methods.

The stimulatory effect of arsenite was also obtained with the use of starved yeast (Table 2).

Table 2. Endogenous respiration of starved yeast in the presence and absence of arsenite. Figures represent the results of three independent experiments

Cells	Oxygen uptake µl O_2/h per mg dry weight					
	arsenite omitted			arsenite added		
	I	II	III	I	II	III
Fresh	13.5	12.2	10.2	11.5	8.7	8.9
Starved*						
— in 50 mM phosphate buffer, *pH* 7.4	3.9	3.7	3.5	5.6	4.9	4.7
— in glucose-free Sym's medium		2.6	2.7		6.2	7.7

* About 2 g of yeast were suspended in 200 ml of indicated solution and shaken at 37° for 8 h. Thereafter, the cells were collected, washed with water by centrifugation and used for manometric measurements (20 mg of wet weight cells per flask) as in Methods.

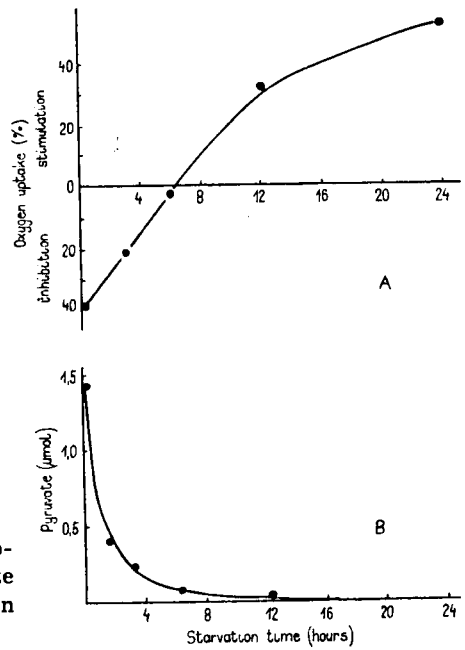

Fig. 1. Arsenite-induced changes in endogenous oxygen uptake (A) and pyruvate formation (B) as dependent on starvation time of *Mycobacterium* sp. 279

In the case of *N. minima*, arsenite diminished oxygen consumption regardless of whether the cells were starved or not (Table 3).

When *E. coli* was used, arsenite exerted neither inhibitory nor stimulatory effect (Table 3).

Table 3. Endogenous respiration of starved *Nocardia minima* and *Escherichia coli* in the presence and absence of arsenite

Cells	Oxygen uptake µl O_2/h per mg dry weight	
	arsenite omitted	arsenite added
N. minima		
— fresh	6.0	3.8
— starved* for 20 h		
— in buffer	3.1	2.2
— in glucose-free Sym's medium	5.4	3.0
E. coli		
— grown for 16 h		
— fresh	7.2	7.4
— starved* in buffer for 16 h	4.1	3.3
— grown for 24 h		
— fresh	8.7	9.0
— starved* in buffer for 6 h	5.6	6.1
— starved* in buffer for 24 h	2.2	2.3

* About 2 g of cells were suspended in 200 ml of 50 mM phosphate buffer, *pH* 7.2 or glucose-free Sym's medium and shaken at 30° (*N. minima*) or 37° (*E. coli*) for indicated times. Further procedure as in Table 2. For manometric measurements used were 25 and 20 mg wet weight cells (per flask) of *N. minima* and *E. coli*, respectively.

Interpretation of the results obtained in this study may in part be only speculative. In the case of *M.* sp. 279 which is a strongly aerobic organism arsenite must have caused inhibition of the Krebs cycle. The 7 h starvation exhausted endogenous carbohydrates to a degree allowing for only negligible production of pyruvate. The increase of oxygen uptake revealed by the sugar-depleted cells in the presence of arsenite may suggest a shift in the regulatory mechanism, enhancing Krebs cycle-independent oxidation reactions. It is of interest to note that *N. minima*, being a strain of the related genus, did not manifest such a peculiar response. The difference could be attributed to the relatively high endogenous respiration of the cells used. *N. minima* is a slowly metabolizing species and thus the 20 h starvation period might have been too short.

On the other hand, *S. cerevisiae* representing an eucaryotic organism with strong fermentative potentialities behaved like the mycobacteria in that it took up more oxygen with arsenite added. Unstarved cells, however, were almost insensitive to arsenite, apparently owing to small endogenous production of pyruvate. The latter assumption was confirmed by the results of pyruvate determinations performed as described earlier (10). In contrast, arsenite had no influence on *E. coli* in the conditions applied which may be interpreted to mean that the cells from the nutrient broth culture did not engage the Krebs cycle to a significant extent.

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STRESZCZENIE

Arsenin w stężeniu 10 mM dodany do zawiesiny komórek *Mycobacterium* sp. 279 powodował hamowanie endogennego oddychania bakterii świeżych i stymulację bakterii głodzonych. Efekt stymulacji stwierdzono również u głodzonych drożdży. W przypadku *N. minima* arsenin powodował tylko hamowanie, a u *E. coli* nie wywierał żadnego wpływu.

РЕЗЮМЕ

Арсенин добавляемый к суспенсии клеток *Mycobacterium* sp. 279 в концентрации 10 mM тормозил эндогенное дыхание свежих бактерий, а стимулировал голодных. Такой же эффект стимулирования получили у голодных дрожжей. В случае *Nocardia minima* арсенин только тормозил, а в случае *Escherichia coli* совершенно не влиял на эндогенное дыхание.