STIMULATION OF GROWTH AND METABOLISM IN TETRAHYMENA PYRIFORMIS BY ANTIMYCIN A[1]

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The mechanism by which oxygenation decreases glyconeogenesis and represses glyconeogenic enzymes in Tetrahymena pyriformis has been further investigated by using electron transport inhibitors and uncouplers of oxidative phosphorylation. In general, these agents markedly inhibited the growth of the organism and produced non-specific effects as previously described (Shrago, et al., 1967). However, the addition of Antimycin A to growing cultures of Tetrahymena was surprisingly different in that it stimulated growth and metabolism of the organism. Recently the induction of carotenoid synthesis by Antimycin A in Mycobacterium marium has been reported (Batra, 1967).

Methods and Results. Maintenance of the cultures, processing of the organisms, preparation of extracts and enzymatic assays were performed as previously described (Shrago, et al., 1967). In the present experiments, 100 ml of 2% proteose peptone media containing 1% inoculum was grown for two days in 500 ml Erlenmeyer flasks either standing (partially aerated) or shaking (well aerated) in a New Brunswick Metabolyte shaker at 150 cycles/min. The temperature was maintained between 23-25°. Antimycin A (Sigma Chemical Co., St. Louis) dissolved in a volume of ethanol which did not affect

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growth was added to the flask at time of inoculation to give a final concentration of 10 μg/ml. Cultures with and without Antimycin A were harvested at the same time during the logrhythmic growth phase.

Table 1 compares the effect of Antimycin A on various metabolic parameters under conditions of poor and good aeration. There is marked stimulation by Antimycin A of growth, protein, and glycogen in the shaking cultures. By contrast, standing or poorly aerated cells do not show an Antimycin A effect. Diminished growth and increased glycogen synthesis in poorly aerated cultures of Tetrahymena have been well documented (Levy and Scherbaum, 1965; Shrago, et al., 1967). Rotenone, which acts in the electron transport chain at the reduced pyridine nucleotide level prior to Antimycin A, and which might be expected to produce a similar response, is shown for comparison. Not only did Rotenone produce a generalized inhibition of growth and metabolism, but its inhibitory effect was partially overcome by the simultaneous addition of Antimycin A.

Table 1. Effect of Antimycin A on growth, glycogen formation, and protein synthesis in aerated and non-aerated cultures of Tetrahymena pyriformis.

Conditions	Additions	OD_{660}	Dry wt total mg	Protein total mg	Glycoge: total mg	mg%
Shaking	None	100	122	74	2.3	1.9
Shaking	Antimycin A	131	177	106	16.2	9.1
Shaking	Rotenone	27	16	12	0.1	1.0
Shaking	Rotenone + Antimycin A	61	67	40	0.8	1.1
Standing	None	71	72	53	14.3	20.0
Standing	Antimycin A	74	80	53	17.7	22.0

Table 2 compares the effect of Antimycin A on glyconeogenic enzyme activity in the cytosol and the total extract which also includes the disrupted mitochondrial fraction. The repression of phosphoenolpyruvate carboxykinase (PEPCK) and malate dehydrogenase (MDH) in the cytosol of well aerated cultures is partially reversed by Antimycin A. Total extractable enzyme activities show less change indicating that the mitochondrial fraction is not affected.

The Antimycin A effect on glyconeogenesis is somewhat similar to diminished aeration, however, as was shown in Table 1, it cannot be reproduced by Rotenone and in addition stimulates growth of the organisms.

Table 2. Effect of aeration and Antimycin A on the activity of phosphoenolpyruvate carboxykinase and malate dehydrogenase in the cytosol and total extract from Tetrahymena pyriformis.

Conditions	Additions	Cytosol Enzyme		Total extractable enzyme	
		PEPCK	MDH	PEPCK	MDH
		(μmoles/min/mg P)			
Standing	None	1.00	1.40	1.50	2.50
Shaking	None	0.10	0.14	0.75	0.83
Shaking	Antimycin A	0.47	0.46	0.97	0.74

The effect of Antimycin A on the incorporation of various radioactive precursors into protein, glycogen and lipid in growing cultures of Tetrahymena is shown in Table 3. Ten μc of the radioactive compound was added at the time of inoculation of the culture. After 48 hours, the cells were harvested and extensively washed with water. The packed cells were homogenized in 5.0 ml of 0.5 N perchloric acid, and the precipitate rehomogenized with another 5.0 ml of perchloric acid. Glycogen was separated by treating the combined supernatant fraction with an equal volume of 95% ETOH. The precipitated glycogen was suspended in 0.01 N NaOH, reprecipitated with 95% ETOH, washed twice with 60% ETOH, and finally resuspended in 5.0 ml. of H_2O. One ml was added to 20 ml Bray's solution (Bray, 1960), and counted in a Packard TriCarb Spectrometer. A protein fraction was obtained by treating the perchloric acid residue successfully with hot 95% ETOH, ETOH-ether (3:1), hot 0.6 N perchloric acid, and 95% ETOH (Hogg and Kornberg, 1963). The residue was suspended in 0.1 N NaOH and a small aliquot counted as above. In experiments with $2^{14}C$ acetate, 100 mg. of Na acetate was also added and the glycogen and lipid were isolated by the method of Gutman, et al. (1963), and counted as above. There is a marked stimulation of Antimycin A of radioactive incorporation into all the cell constituents studied.

Table 3. Effect of Antimycin A on the incorporation of radioactive precursors into protein, glycogen and lipid fractions of Tetrahymena pyriformis.

Radioactive precursor	Additions	Radioactive incorporation Protein	Glycogen (cpm x 10^3)	Lipid
^{14}C Protein Hydrolysate	None	472	13	
^{14}C Protein Hydrolysate	Antimycin A	1057	78	
3-^{14}C Aspartate	None	43	5	
3-^{14}C Aspartate	Antimycin A	92	64	
2-^{14}C Acetate	None		1	85
2-^{14}C Acetate	Antimycin A		10	350

An initial working hypothesis was that Antimycin A might preferentially inhibit a non energy linked electron transport pathway and thereby allow for more efficient oxidative phosphorylation. The fact that Antimycin A is ineffective in poorly aerated cultures is consistent with this proposed mechanism. It has been reported that mitochondria from Tetrahymena in good respiratory control are resistant to Antimycin A (Kobayashi, 1965). It, therefore, seemed desireable to carefully study the electron transport system of the organism in order to better understand the Antimycin A effect.

Although previous workers have detected cytochromes b and c in the mitochondria of Tetrahymena, one of the most puzzling aspects of its metabolism is the apparent lack of a cytochrome oxidase (Kobayashi, 1965). In a series of experiments aimed at determining the site of action of Antimycin A, the cytochrome components of Tetrahymena mitochondria were examined with a double-beam spectrophotometer (Aminco, American Instrument Co.). The spectra shown in Figure 1 indicates the presence of cytochromes b (peak at 563 mμ) and c (shoulder in 555 region), but in addition shows a peak at 617 mμ. Studies now in progress suggest that the latter compound may be a new type of cytochrome a which is the cytochrome oxidase of Tetrahymena pyriformis. Antimycin A was found to inhibit O_2 uptake in Tetrahymena only at very high levels (5×10^{-4}M). The site of action of Antimycin A in the electron transport system is not certain at present, but it does not appear to be between cytochromes b and c, and so differs from the mammalian system. Studies are now underway to determine the mechanism of

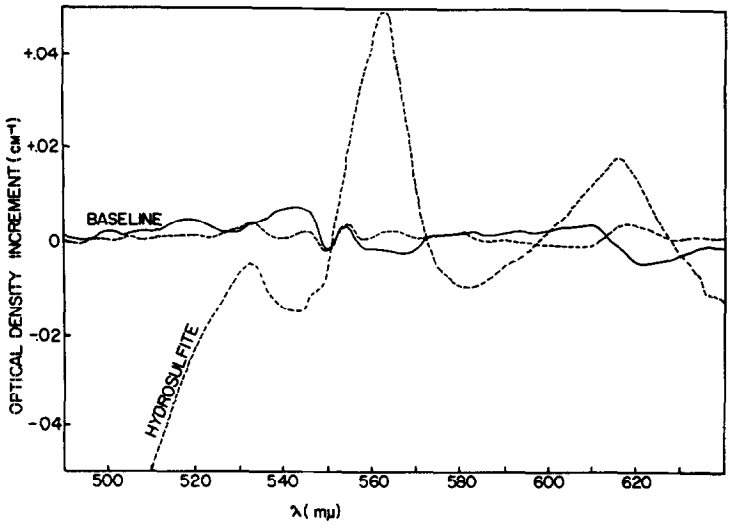

Figure 1. Difference spectra for respiratory components of Tetrahymena pyriformis mitochondria. Mitochondria equivalent to 75 mg protein. Baseline _____; oxidized minus oxidized ------; reduced (hydrosulfite) minus oxidized.

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action of Antimycin A and related compounds on electron transport and oxidative phosphorylation in the unusual electron transport system found in Tetrahymena pyriformis mitochondria. It should then be possible to better understand how this compound stimulates the growth and metabolism of the organism.

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