INTERACTION OF 2-BROMO-2-NITROPROPAN-1,3-DIOL WITH SOME RESPIRATION AND DEHYDROGENASE ENZYME SYSTEMS IN ESCHERICHIA COLI

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Compounds possessing the 'gem-bromo-nitro' grouping, Br-C-NO_2, have intense antimicrobial activity. One of these, 2-bromo-2-nitropropan 1,3diol (Bronopol), has found successful application in the preservation of pharmaceutical products (Croshaw et al 1964). Concentrations of bronopol which cause growth inhibition (13µg/ml) and significant levels of killing (D,15min; 500µg/ml) for E.coli,do not affect envelope integrity. Early work has suggested that compounds such as Bronopol might interact oxidatively with -SH containing proteins, particularly those involved in catabolism and associated with the cytoplasmic membrane (Stretton & Manson 1973). We have further investigated the action of Bronopol upon cytochrome function in intact cell suspensions and oxygen consumption associated with the catabolism of various carbohydrate substrates. Additionally we have conducted an in vitro study of it's effects upon five isolated dehydrogenase (DH) enzymes of the TCA and associated pathways.

Oxygen uptakes were determined using a washed cell suspension of E.coli ATCC8739 (OD,E_{470},4.3) prepared from a stationary phase culture grown in a chemically-defined medium with glycerol as a sole carbon source. Suspensions were stirred at 35^{o} in a closed metabolic chamber (Rank Bros.Ltd. Cambridge) and oxygen tension was monitored following the addition of various carbohydrate substrates at concentrations determined to be in 5x excess. Suspensions were incubated, where necessary for 2min, with various concentrations of Bronopol prior to substrate addition. Sensitivity of oxidation of these substrates varied markedly. Glucose, acetate and pyruvate oxidation were equally sensitive (50% rate inhibition; 15µg/ml), whereas α-ketoglutarate and succinate oxidation were inhibited to 50% by concentrations of 52 and 102µg/ml respectively. Cytochrome difference spectra were unaffected by Bronopol at concentrations upto 1000µg/ml.

The effects of Bronopol upon various dehydrogenase enzyme systems were determined with substrate and coenzyme in excess (1,below) and with each in turn controlling the rate of reaction (2,below). This enables not only the relative sensitivity to be determined but also the nature of the inhibition to be assessed:-

Enzyme	ID50%(1)	Nature of Inhibition		Ki (2)	
		Substrate	Coenzyme	Substrate	Coenzyme
Lactate DH	1.8µg/ml	noncompet	compet	1.65µg/ml	0.5µg/ml
Isocitrate DH	16.5µg/ml	compet	noncompet	1.0µg/ml	3.0µg/ml
Succinate DH	17.5µg/ml	noncompet	*	0.8µg/ml	*
Glucose-6-PO4 DH	2450µg/ml	uncompet	uncompet	*	*
Malate DH	3420µg/ml	*	*	*	*

Compet; competitive. ID50%, Inhibitory dose 50%; *, not done.

The pattern of sensitivity observed in vivo was complex but might be explained in terms of -SH interaction and accessibility of various enzymes to drug. In vitro studies however showed competitive, noncompetitive and uncompetitive inhibition of the different enzymes. Since all of these contain -SH groups at their active centres, then if thiol-oxidation was the primary mechanism of killing, uncompetitive inhibition might also have been expected throughout. In view of the strong structural similarity between lactate DH and malate DH, it was surprising that these differred in sensitivity by some 100-fold.

We suggest that thiol-oxidation might be responsible for uncompetitive inhibition observed at high bronopol concentrations, but that some other mechanism accounts for the growth inhibitory response.

Croshaw B. et al (1964) J. Pharm. Pharmacol. 16:127T
Stretton R.J.,Manson T.W. (1973) J.Appl.Bact. 36:61