THE ACTION OF 2-PHENYOXYETHANOL UPON POLYMER BIOSYNTHESIS IN ESCHERICHIA COLI NCTC 5933

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Sub-bactericidal concentrations of 2-phenoxyethanol (<0.6% w/v, LT 90% > 100 min) uncouple oxidative phosphorylation from respiration and specifically inhibit malate dehydrogenase in *Escherichia coli*. Higher, bactericidal, concentrations cause gross damage to the cell envelope (Gilbert, Beveridge and Crone 1977 a,b,c). Thus some inhibitory effect upon the biosynthesis of DNA, RNA, protein and other polymers was anticipated and examined further with *E. coli* cultures growing in the presence of sub-inhibitory levels of phenoxyethanol.

Inoculated aliquots of a mineral salts medium containing succinate (1% w/v) and yeast extract (0.2% w/v) were agitated at 35°. During early logarithmic growth (O.D. 0.2) graded concentrations of 2-phenoxyethanol (0.05 - 0.35% w/v) were added, followed after 5 min by ^{14}C labelled glucose (20 µCi) or thymidine, uracil or phenylalanine (each 10 µCi) as markers for general polymer, DNA, RNA and protein biosynthesis respectively. 2-Deoxyadenosine (10 mg/ml) was added to stimulate thymidine and uracil incorporation. Sufficient unlabelled monomers were also added to ensure steady incorporation of radiochemical into control cultures (no drug). Experiments were repeated, adding 2-phenoxyethanol after the radiochemical. The extent of polymer biosynthesis was assessed at intervals by removal of aliquots from the cultures, treatment with cold trichloracetic acid (40% w/v), to disrupt the cells and precipitate the polymers, collection of the precipitate by membrane filtration and measurement of radioactivity by a liquid scintillation counting technique.

The rates of biosynthesis of general polymers, DNA, and RNA in cells pretreated with drug were similarly sensitive to graded concentrations of phenoxyethanol and closely followed inhibition growth rate assessed photometrically. Protein biosynthesis however was relatively insensitive to drug levels which caused up to 40% inhibition of growth rate, significant inhibition occurring at the higher drug concentrations. The onset of inhibition of DNA biosynthesis in cells pretreated with radiochemical was almost immediate following drug addition, whilst with RNA and protein biosynthesis there was a slight delay before the onset of inhibition (2 min).

If the actions of phenoxyethanol upon polymer biosynthesis were solely indirect by interference with the supplies of ATP and metabolite precursors one might have expected a response analogous to that with step-down growth (sudden drop in nutritional status of the medium) where protein and RNA synthesis are rapidly inhibited followed, significantly later, by inhibition of DNA synthesis. Since this did not occur it is suggested that phenoxyethanol can exert an additional, more direct, inhibitory action upon DNA and RNA biosynthesis and possibly on protein biosynthesis.

Gilbert, P., Beveridge, E.G. and Crone, P.B. (1977a) Microbios *19*: 17-26
Gilbert, P., Beveridge, E.G. and Crone, P.B. (1977b) Ibid. *19*: 125-41
Gilbert, P., Beveridge, E.G. and Crone, P.B. (1977c) Ibid. *20*: 29-37