THE MECHANISM OF BACTERIOSTATIC ACTION OF CHLOROCRESOL (CC) ON STAPHYLOCOCCUS AUREUS

S P Denyer, Valerie D Harding and W B Hugo, Department of Pharmacy, University of Nottingham, Nottingham NG7 2RD.

Many phenolic preservative agents are known to promote cytoplasm leakage as a result of an interaction with the bacterial cytoplasmic membrane (Hugo 1976). This structure is also the locus of considerable metabolic activity providing the link, via a transmembrane pH gradient, between substrate oxidation, phosphorylation and active transport. In this communication we report the action of CC on some of these metabolic processes and their relationship to the phenomenon of cytoplasm leakage.

Buffered, washed suspensions of **S. aureus** (4×10^9 cells/ml) were challenged with increasing concentrations of CC up to, and including, the minimum growth inhibitory concentration (MIC) of 0.035%w/v. Effects of the preservative on metabolic activity were judged from studies of respiration, active transport of radiolabelled glutamic acid and ATP synthesis using glucose as energising substrate (Harding **et al** 1984). Glucose uptake was determined enzymically after extraction of CC with chloroform. Respiration-driven proton expulsion was monitored by following the change in extracellular pH that occurred in a lightly buffered medium on addition of glucose. Changes in membrane permeability to potassium and phosphate ions, and the interaction of CC with a phosphatidylglycerol (PG) monolayer (a major phospholipid in **S. aureus**) were followed as before (Harding **et al** 1984). The results for two concentrations are presented in Table 1.

Table 1 Percentage difference between control response and response in the presence of CC (0.015%w/v and 0.035%w/v)

Effect	0.015%	0.035%	Effect	0.015%	0.035%
Growth	-20	-100	Proton expulsion	-50	-68
Respiration	+23	-79	PG monolayer expansion	+1	+45
ATP synthesis	-40	-91	Potassium leakage	+41	+190
Glutamic acid uptake	-62	-81	Phosphate leakage	+3	+394
Glucose uptake	0	-47			

+ = stimulation; - = inhibition; 0 = no difference from control

For concentrations of CC at, or below, 0.015%w/v an apparent uncoupling of respiration from ATP synthesis was observed. Furthermore, generation of a proton gradient and its utilisation in the active transport of glutamic acid was impaired. These effects were not caused by inhibition of substrate (glucose) uptake or by gross changes in membrane permeability which were only demonstrated at concentrations in excess of 0.015%w/v. At the MIC, expansion of the monolayer was evident and pool material was lost indicating substantial changes in membrane integrity.

In this study a distinct separation of the effects of CC on metabolism from its gross action on membrane permeability was observed. These findings suggest that CC-induced bacteriostasis may result from an inability of the cell to initiate energy-dependent repair mechanisms and to actively transport back leaked material. A reduced ability to generate and sustain a transmembrane pH gradient in the presence of CC may be central to this action.

Harding V D, Hugo W B and Denyer S P (1984) **J. Pharm. Pharmacol. 36,** 58p.
Hugo W B (1976) **Symp. Soc. Gen. Microbiol. 26,** 383