The effect of some antibacterial agents on proton flux across the membrane of *Clostridium welchii*

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So far there has been no thorough investigation of membrane function in an obligate anaerobe, though studies have been made of *Streptococcus faecalis* under anaerobic conditions (for a comprehensive review see Harold, 1972). It is of interest to note that membrane-active antibacterials have been extensively used as tools for research on membrane function.

Recent theories suggest that the cell membrane is essentially impermeable to most ions, including H^+ and OH^-, and therefore has low electrical conductivity. In anaerobic organisms the activity of a membrane adenosine triphosphatase (ATPase) could cause extrusion of protons from the cell, leading to formation of a gradient of pH and electrical potential across the membrane (interior alkaline and electrically negative). Mitchell postulates that most nutrients are accumulated in response to the gradients across the cell membrane, e.g. K^+ in response to the gradient of electrical potential. Any collapse of such gradients would have a serious effect on nutrient uptake and therefore on cell survival.

The following study examines the effect of some antibacterials on proton flux across the cell membrane, the method used was that of Harold & Baarda (1968). On addition of HCl, 0·12 ml, 10 mM, a drop in extracellular pH of 1·0 units occurs immediately. The addition of chlorhexidine, 10 μg ml^{-1}, or cetyltrimethylammonium bromide (CTAB), 10 μg ml^{-1}, failed to promote a flux of protons into the cell, indeed raising the CTAB concentration to 100 μg ml^{-1} produced a further flux of protons *out* of the cell, indicated by a drop in pH. This may be due to more severe membrane damage and subsequent leakage of acidic substances from the internal environment of the cell.

Tetrachlorosalicylanilide (TCS) 3×10^{-6} M and carbonyl cyanide-*m*-chlorophenyldrazone (CCCP) 5×10^{-6} M caused only small fluxes of protons into the cells. Prior addition of valinomycin, 0·5 μg ml^{-1}, did not alter the response, suggesting that *C. welchii* is not susceptible to this antibiotic which normally facilitates passage of K^+ ions across the membrane in exchange for H^+ ions. When the effect of phenols on proton flux was investigated it was found that phenol itself had no effect while ethylphenol, $5{\cdot}75 \times 10^{-2}$ M, produced a similar response to that of TCS. Ethylphenol is thought to have some uncoupling action in aerobes (Hugo & Bowen, 1973). 2,4-Dinitrophenol (DNP), 5×10^{-5} M, caused an instantaneous influx of protons seen as a rise in pH.

DNP is an established uncoupling agent for aerobic metabolism and the inhibition of active transport under anaerobic conditions by DNP has also been noted (Galeotti, Kovac & Hess, 1968). The effects of DNP upon *C. welchii* may represent indirect evidence that collapse of a proton gradient is involved in inhibition of active transport in this organism.

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The effect of chloroquine on the growth and viability of *Escherichia coli*

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It has been reported the exponential growth of cultures of *Escherichia coli* can be inhibited by the addition of chloroquine and that the inhibitory activity increases with increase in pH. (Wiseman 1972). The pattern of inhibition described was biphasic; the addition of chloroquine causing an immediate decrease in growth rate followed by a further decrease after about 60 min.

The present communication describes the changes in total and viable cell numbers that follow the addition of inhibitory concentrations of chloroquine to exponentially growing