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

DIANA C. DALTREY AND W. B. HUGO

Department of Pharmacy, The University, Nottingham NG7 2RD, U.K.

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*

K. R. MIDDLETON AND DAVID WISEMAN

Postgraduate School of Study in Pharmacy, Bradford University, Bradford BD7 1DP, U.K.

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

cultures of *E. coli* of pH 7·7. Conditions of cultivation were as previously described (Rye & Wiseman 1966). Overall growth was followed by absorbance measurements, total counts determined using a Coulter counter model B and viable counts by the pour plate method.

The absorbance of cultures treated with chloroquine showed the biphasic pattern of inhibition described by Wiseman and two concentrations, 0·4 and 1·0 $\times$ 10^{-3} M were selected for further study. The addition of 0·4 $\times$ 10^{-3} M chloroquine to cultures caused an immediate decrease in the rate of increase in absorbance equivalent to a reduction in the growth rate constant by about 50%, "growth" continuing at this new rate for a period of 50 min. After this primary phase of reduced growth the absorbance remained virtually constant for a period of at least 120 min, corresponding to a secondary inhibited growth rate of zero.

During the 50 min primary phase both viable and total cell counts increased slightly during the first 5 min but then remained constant for the remaining 45 min indicating that neither cell division nor death occurred to any extent. Although total cell counts remained almost constant during this early inhibition there was a substantial increase (45%) in the number of cells larger than the median size of the original cell population indicating an increase in cell size.

During the secondary phase of inhibition both total cell counts and cell size remained constant confirming the complete cessation of growth indicated by the absorbance measurements. Viable cell counts decreased during this phase at a slow exponential rate resulting in a 50% loss in viability after 120 min.

The addition of 1 $\times$ 10^{-3} M chloroquine to exponentially growing cultures caused an immediate and almost complete cessation of growth, the absorbance, total and viable cell counts and cell size remaining virtually constant for a period of 50 min. After this primary phase of neither cell growth nor death both absorbance and total cell counts declined slowly indicating a small amount of cell lysis whilst viable cell counts declined at a more rapid exponential rate resulting in 80% loss in viability after 120 min.

These results confirm the biphasic pattern of inhibition of *E. coli* by chloroquine reported by Wiseman and extend this biphasic principle to its bactericidal effect and also suggests that the process of cell division is particularly sensitive to this drug.

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The mechanics of inhibition of growth by some antibacterial agents

DAVID WISEMAN
Postgraduate School of Studies in Pharmacy, Bradford University, Bradford BD7 1DP, U.K.

The reduction in overall growth rate of a bacterial culture that follows the addition of partially inhibiting concentrations of antibacterial agents may reflect a uniform decrease in growth rate of all the cells in the culture, a non uniform effect on all the cells or the complete inhibition of some cells whilst the remainder grow at an unhindered rate. Rye & Wiseman (1968) devised a method of distinguishing between these mechanisms of inhibition based on studies of changes in the distribution of cell sizes in partially inhibited cultures of *Escherichia coli* in which cell division had been arrested by the addition of ampicillin. This communication reports further results obtained with *E. coli* and an extension of the method to a study of *Pseudomonas aeruginosa*.

Methods were as previously described (Rye & Wiseman, 1968) except that when using *Ps. aeruginosa* cell sizes were measured using a Coulter counter model T, the concentration of sodium chloride in electolyte solutions was increased to 2·0%, absorbance measurements were made at 450 nm and cell division was arrested with carbenicillin.

The antibacterial agents studied were isopropyl and benzyl alcohols, decyl-and dodecyltrimethylammonium bromides, benzalkonium chloride, chlorhexidine and polymixin B against *E. coli* and gentamycin and polymixin against *Ps. aeruginosa*. Changes in cell