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

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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

size distributions in penicillin treated cultures partially inhibited by these agents and in control cultures treated with penicillin alone were followed for periods of up to 3 h.

The cells in cultures of *E. coli* treated with isopropyl or benzyl alcohols and of *Ps. aeruginosa* treated with gentamycin all increased in size with time but at rates slower than in control cultures; the coefficients of variation of the size distributions remained virtually constant. These results indicate that these agents act by uniformly slowing the growth rates of all cells in cultures as reported for the action of phenol, chloramphenicol and tetracycline against *E. coli*, (Rye & Wiseman, 1968).

In penicillin treated cultures partially inhibited by the other agents studied, cell size distributions widened with time and each slowly resolved into a bimodal distribution corresponding to two populations of cells one having a mean size similar to that of the cells at the commencement of the experiment and the other similar to that of cultures treated with penicillin alone for an equivalent time. All these membrane active agents thus partially inhibit cultures by completely arresting the growth of some of the cells whilst allowing the remainder to grow at an unhindered rate in a manner analogous to that previously reported for cetyltrimethylammonium bromide (Rye & Wiseman, 1968).

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Enhancement of lincomycin activity against *E. coli* by alcohols

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Lincomycin is active against most Gram-positive and some Gram-negative organisms with a minimum inhibitory concentration (MIC) of about 3 μg ml^{-1} but not against *Escherichia coli* or *Pseudomonas aeruginosa*, because it cannot penetrate the cell envelope. Richards & McBride (1973) have shown that benzyl, 2-phenylethyl and 3-phenylpropyl alcohols enhance the activity of benzalkonium chloride and chlorhexidine acetate against *P. aeruginosa*.

The effect of various aliphatic and aromatic alcohols on *E. coli* was assessed by measurement of the growth rates of nutrient broth cultures containing subinhibitory concentrations of the alcohols and determination of the MIC as described by Richards & McBride (1973). The results obtained ranked the alcohols in order of effect as phenylpropyl > phenylethyl > benzyl > n-amyl > n-butyl > n-propyl > ethyl > methyl. Concentrations of lincomycin greater than 100 μg ml^{-1} had a significant effect on the growth rate of log phase cultures of *E. coli*, but the MIC could not be determined because growth occurred in medium containing 600 μg ml^{-1} (highest concentration used) of lincomycin.

The effects of combinations of the various alcohols and lincomycin were evaluated by determination of the growth rate of *E. coli* in media containing single chemicals as well as combinations. The effect of the chemical was measured as the percentage decrease in the growth rate of the organism in the presence of the chemical compared with that of the control culture containing no chemical. When the effects of the alcohols and lincomycin estimated as single chemicals were added, the sum was less than the effect of the combination determined experimentally, indicating that the combination had a greater than additive or synergistic effect. The difference between the experimental effect and the summed individual effects varied with the alcohol used in the combination in the same ranking order as for the MIC.

In summary, the enhancing effect of the aromatic alcohols on the activity of antibacterials is also shown by the aliphatic alcohols. The enhancement may be due to the antibacterial being able to penetrate the bacterial cell more readily in the presence of the alcohol.

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