of inhibition of growth of a test organism when the antimicrobial chemical is added to the culture medium. In such cases the composition and pH of the medium may influence the result. The medium may contain substances that antagonize the action of the test compound, e.g. high concentrations of thymidine or para-aminobenzoic acid will interfere with sulfonamide activity.

The antimicrobial activities of several groups of chemical are influenced by the ease with which they cross the cell membrane and interfere with the metabolism of the cell. This, in turn, is influenced by the lipid solubility of the substance, because the membrane contains a high proportion of lipid and tends to permit the passage of lipid-soluble substances. Many antimicrobial chemicals are weak acids or weak bases, which are more lipid soluble in the unionized form. The pH of the environment therefore affects their degree of ionization, hence their lipid solubility and so, ultimately, their antimicrobial effect. Benzoic acid, for example, is a preservative used in several oral mixtures which has a much greater activity in liquids buffered to an acid pH value than those which are neutral or alkaline. Conversely, the aminoglycoside antibiotics, e.g. streptomycin, neomycin and gentamicin, which are weak bases, are more active at slightly alkaline pH values, although this is more a consequence of the transport systems by which the molecules enter the bacterial cell working better at alkaline pH than of enhanced lipid solubility. The presence of organic matter, e.g. blood, pus or serum, is likely to have a marked protective effect on the test organism and so antimicrobial chemicals may appear less active in the presence of such material. The activity of several antibiotics, notably tetracyclines and aminoglycosides, is reduced by the presence of high concentrations of di- or trivalent cations in the medium.

Exposure and incubation conditions

The temperature, duration and redox conditions of exposure to the antimicrobial chemical (or incubation of survivors after exposure) may all have a significant effect on its measured activity. Increasing the temperature of exposure of the test organism to the chemical increases the antimicrobial activity by a factor which is quantified by the temperature coefficient (Q_{10} value: the number of times increase in activity for a 10 °C rise in temperature). Phenols and alcohols, for example, may respectively exhibit Q_{10} values of 3–5 and > 10, and so a variation of 5 °C in the temperature of exposure (which is permitted by pharmacopoeial preservative efficacy tests) may lead to a markedly different rate of kill of the organism in question.

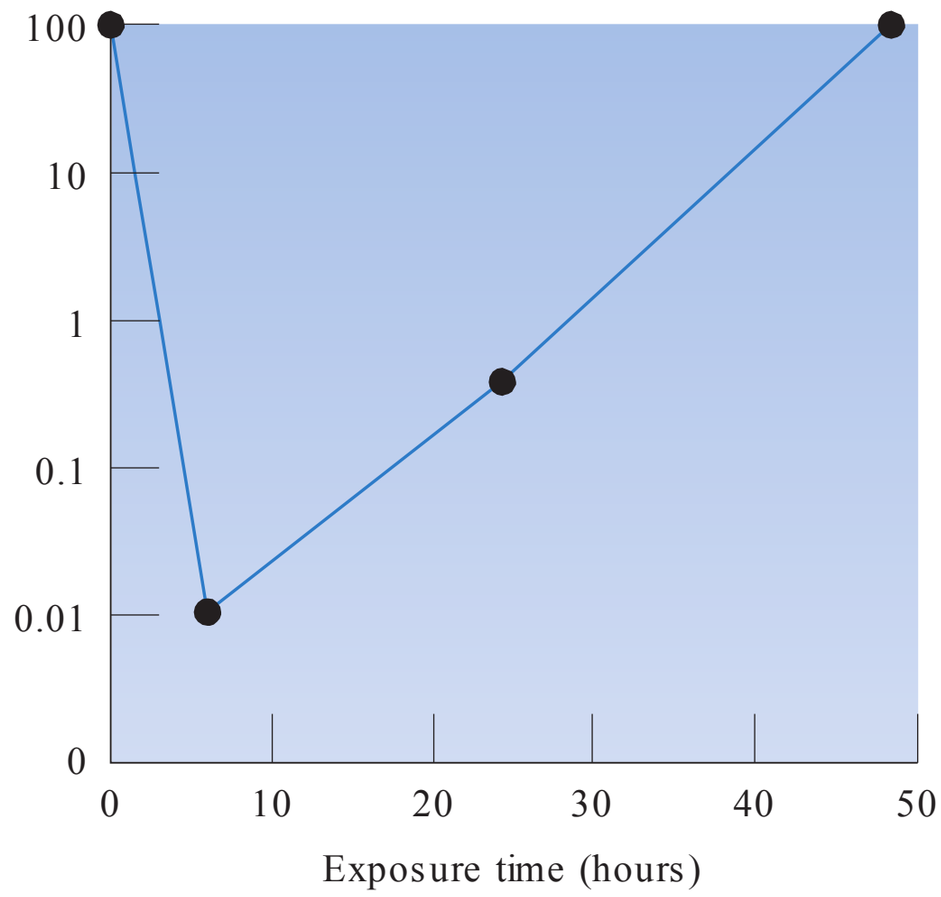

Fig. 14.1 • The survival and recovery of Pseudomonas aeruginosa exposed to benzethonium chloride during a preservative efficacy test.

The period of time for which the test organism is exposed to the antimicrobial chemical may influence the recorded result because it is possible for the organism to adapt and become resistant to the presence of the chemical. In preservative efficacy tests, the exposure period is normally 28 days, which is sufficient time for any cells that are not killed during the first 24–48 hours to recover and start to reproduce, so that the final bacterial concentration may be much higher than that at the start. This is illustrated in Figure 14.1, which shows the effect of the quaternary ammonium preservative benzethonium chloride on *Pseudomonas aeruginosa*. The concentration of bacteria was reduced to approximately 0.01% of the initial value during the first 6 hours, but the bacteria that survived this early period recovered to the original level within 2 days. There is the potential for a similar phenomenon to arise in other situations, e.g. in minimum inhibitory concentration (MIC) determinations of bacteriostatic agents (those that do not kill but merely inhibit the growth of the test organism), although it is not common in MICs because the exposure (incubation) time is much shorter than that in preservative testing.

The effect of some antibiotics may be influenced by the redox conditions during their period of contact with the test organism. Aminoglycosides, for