

Staphylococcus aureus, *Escherichia coli*, and *Brevibacterium ammoniagenes*, and also demonstrated residual self-sanitizing activity against *Staphylococcus aureus* and *Escherichia coli*. Some of the miscellaneous applications for quaternary ammonium compounds are as preservatives. Lawrence (1950) evaluated a large number of chemicals as preservatives for ophthalmic solution and selected benzalkonium chloride at 1:5000 and 1:10,000 as superior. More recently, Stark (1985) used Polymeric quaternary ammonium compounds, namely Polyquat, for disinfecting and preserving solution for contact lenses, ointments, and other ocular medicaments. The polyquaterium-1 (Onamer M) has been approved by the FDA as a preservative in several brands of contact lens soaking solutions at 0.01 to 0.001% [Opti-Soft, Opti-Clean, Opti-Tears, Opti-Free, and Tears-Natural II]. This product has replaced benzalkonium chloride and Thiomerosal in these preparations. Bernarducci and Harrison (1988) have discovered that water-soluble cationic polymers (Busan 77, WSCP, Onyxspers 12S, Onamer M), when formulated in an aqueous medium with one or more nonionic surfactants, provide stable, isotropic liquid laundry detergent and sanitizer compositions having good detergency and bactericidal properties, and in addition are less irritating to the eye and practically non-irritating to the skin.

Like et al. (1975) examined a number of compounds, including a 50:50 mixture of alkylmethylbenzyl ammonium and alkylmethylbenzyl ammonium chlorides, as preservatives for cosmetic oil-in-water systems using amine oxides as emulsifiers. At 1000 or 2000 ppm, the quaternary ammonium compounds reduced the microbial count of the cosmetic system inoculated with bacteria and fungal spores from an initial count of several million per ml to less than 10 per ml over an 8-week incubation period, including a reinoculation after 4 weeks of incubation.

In another instance, as a fungistat for exterior latex paint films, Ramp et al. (1966) demonstrated that alkylmethyl ethylbenzylammonium cyclohexylsulfamate at about a 1% level in exterior latex paints would produce a paint film resistant to attack by fungi. In another instance, as a moth-proofing agent for wool fabric, Tolygesi et al. (1971) concluded that tricapyrylmethyl ammonium chloride was superior to all other quaternary ammonium compounds tested for this purpose. Both compounds applied to wool fabric at 0.15 to 0.40% add-on levels protected the fabric from the black carpet beetle and the webbing clothes-moth larvae.

MODE OF ACTION

The earliest accounts of the quaternary ammonium compounds related antimicrobial activity to chemical structure by utilizing homologous series of quaternary compounds and noting the effect on antimicrobial activity offered by variations in structure (Jacobs, 1916). This method of collecting information on the antimicrobial attributes of a quaternary compound is constantly em-

ployed to determine the most effective structure that may be used as an item of commerce. Beyond this purpose, this method contributes little to our understanding of the basic mechanism of quaternary ammonium compounds as antimicrobial agents. Although the mode of action has not yet been completely or definitively described in detail, there are well defined and accepted steps explaining the mode of action of cationic disinfectants or antiseptics as reported by Ikeda and Tazuke (1985) and Franklin and Snow (1989). Many techniques have been used in elucidating the mode of action of antimicrobial agents. Once the primary site of action is established the overall effect of the drug on the metabolism of microbial cells can often be explained. The most important site of adsorption is the cytoplasmic membrane. Spheroplasts or protoplasts lacking the outer cell wall layers will bind the cationic antiseptic and may be lysed or damaged. Adsorption by isolated cell membranes can be demonstrated.

The extent of killing of the bacteria is governed by five principal factors: (a) Concentration of antiseptic or disinfectant; (b) nature of bacterial cells and density; (c) time of contact; (d) temperature of medium; (e) the pH; and (f) the presence of foreign matter.

The adsorption of a given amount of the compound per cell leads to the killing of a definite fraction of the bacterial population in the chosen time interval. The lowest concentration of the antiseptic that causes death of the bacteria also brings about leakage of cytoplasmic constituents of low molecular weight. The most immediately observed effect is loss of K^+ ions. The increased permeability is a sign of changes in the membrane that are initially reversible but become irreversible on prolonged treatment. The necessary characteristic of an antiseptic is its bactericidal action, but there is often a low and rather narrow concentration range in which its effect is bacteriostatic. At this low concentration, certain biochemical functions associated with the bacteria membrane may be inhibited. In the presence of a higher concentration of antiseptic and after prolonged treatment, the compound usually penetrates the cell and brings about extensive ill defined disruption of normal cellular functions. The primary effect of these antiseptics on the cytoplasmic membrane is thus established beyond doubt, but secondary actions on the cytoplasmic processes are less defined and may vary from one compound to another.

A review of the most reliable evidence on the mode of action of cationic antiseptics suggests the following generalizations:

1. Adsorption of compound on the bacterial cell surface
2. Diffusion through the cell wall
3. Binding to the cytoplasmic membrane
4. Disruption of the cytoplasmic membrane
5. Release of K^+ ions and other cytoplasmic constituents

6. Precipitation of cell contents and the death of the cells

It is well known that the bacterial cell surfaces are usually negatively charged, and then that adsorption of polycations on to the negatively charged cell surfaces (process 1) is expected to be enhanced with increasing molecular weight of the polymer due to the increasing charge density of the polycations. The binding of polymers to the cytoplasmic membranes and its disruption is expected to be facilitated by increasing the molecular weight of the polymer and by increasing the amount of the bound polymers to the bacteria cells. To examine this hypothesis Ikeda and Tazuke (1985) studied the effect of MW of the polymers on lysis of protoplasts, which are bacteria cells freed entirely of cell walls, so that the interactions processes 1 (adsorption) and 2 (diffusion) of the polymer with the protoplasts can be left out of consideration. The amount of cytoplasmic constituents released from protoplasts and from intact cells of *Bacillus subtilis* was measured at 260 nm after exposure to fractionated samples of polymers of various MW. Lysis of protoplasts was clearly enhanced with increase in MW of polymer[poly(n-butyl)dimethyl(vinylbenzyl)ammonium chloride]]. A bell-shaped curve relationship of the activity to the MW of fractionated polymer was observed in the case of intact cells of *B. subtilis*. Because separate experiments have shown that release of cytoplasmic constituents from intact cells correlates well with the death of the cells, these results support the concept that the mode of action of the polymeric biocides is disorganization of cytoplasmic membrane followed by rupture of membrane, leading to the death of the bacteria (Franklin and Snow, 1989).

Ikeda and Tazuke in 1985 reported an optimal MW region of 14,300 for the 6,6-ionene bromide with antibacterial activity with minimum bactericidal concentration of 6.6 ppm to 10 ppm against *Staphylococcus aureus*. The same workers reported that polymeric biquanides of MW 11,900 and poly[alkyl(C₄-C₁₂)dimethyl(vinylbenzyl)ammonium chloride] of similar MW exhibit better bactericidal action against gram-positive than gram-negative bacteria. The poly[dodecyl]dimethyl(vinylbenzyl)ammonium chloride) was the most active at 0.5 ppm against *Staphylococcus aureus*, which suggests that hydrophobicity plays an important role in bactericidal action.

A primary consideration in examining the mode of action is the characterization of quaternary compounds as surface-active agents, or surfactants. These are adequately defined by James (1965) as compounds with a structural balance between one or more water-attracting (hydrophilic) groups; depending on the nature of the charge or absence of ionization of the hydrophilic group, they may be classified as anionic, cationic, or nonionic. Quaternary ammonium compounds are cationic surface agents and possess such properties as a reduction of the surface tension at interfaces upon absorption; a ready attraction to an absorption on surfaces possessing a neg-

ative charge such as wool, glass, protein, and bacteria; the formation of ionic aggregates or micelles with attendant changes in electrical conductivity, surface tension, and solubility; a precipitation, complex formation, and denaturing effect on proteins; and an inhibiting or stimulating effect on enzyme activity (James, 1946).

With these demonstrable properties for cationic surfactants, it was natural for some investigators (Cowles, 1938) to attribute the antimicrobial activity of quaternary ammonium compounds entirely to the presence and amount of surface activity. However, this obvious explanation has been refuted (Gershenfeld and Milanick, 1941; Hotchkiss, 1946).

An excellent presentation on the mode of action of cationic surface-active agents on microbial cells was given by Hugo (1965). The concepts concerning the mode of action of surface-active agents on microorganisms may be divided into five broad categories.

1. *Effects on protein.* See Putnam (1948) for protein-denaturing agents, Khun and Dann (1940) for enzyme-disrupting agents. Quaternary ammonium compounds are surface-active agents and will denature protein or cause dissociation of an enzyme from its prosthetic group. This effect is usually caused at concentrations much higher than those which are lethal to the microbial cells, and it is unlikely that this effect is the primary cause of the antibacterial activity of surface-active disinfectants except at high concentration.

2. *Effects on metabolic reactions.* See Baker et al. (1941) on the aerobic and anaerobic respiration of glucose by a variety of bacteria and Ordal and Borg (1942) on the oxidation of lactate by *Escherichia coli* and *Staphylococcus aureus*. Attempts have been made to relate inhibition of metabolism with inhibition of growth. Such a correlation has been observed at high concentration, and almost any degree of agreement or disagreement may be demonstrated depending upon the enzyme system chosen and the test organism. It can be expected that those enzymes located in the cytoplasmic membrane will be the first to be affected. Penetration of the cell will follow, and cytoplasmic enzymes will then be inhibited. The enzyme inhibition is not the primary or main lesion caused by these compounds. A specific detergent-sensitive enzyme does not exist or has not been discovered.

3. *Effects on cell permeability.* See Armstrong (1957) on cytolytic damage and phosphorus loss and Scharff and Maupin (1960) on membrane damage and loss of potassium. The cytoplasmic membrane is probably the organelle most sensitive to surface-active agents within the cell of bacteria and yeasts, and the alteration in the semipermeable properties of this structure can lead to leakage of metabolites and coenzymes and disturbance in the delicate balance of metabolite concentrations within the cell. This lesion may be a major contribution to the death of a cell and cause an apparent loss in enzymic activity due to the loss or dilution of coenzymes or substrates. There is a well established relationship between cytolytic

action and surface tension. This lends support to the idea that cytolytic damage may in fact be the primary lesion caused by surface-active substances.

4. *Stimulatory effect of the glycolysis reaction.* This is suggested by the work of Strickland (1956) and Bihler et al. (1961). This reaction is of interest, but because the effect is elicited at concentrations well below those which are antibacterial, it cannot be considered of significance for the antibacterial action of surface-active agents.

5. *Effect on an enzymatically-maintained dynamic membrane.* This is offered as a speculative theory by Newton (1958). Results to support this interesting hypothesis are awaited. This concept of the dynamic cell membrane enzymatically maintained could well be the detergent-sensitive enzyme, which as it was stated before does not exist.

Hugo has selected the effect on the cytoplasmic membrane controlling the cell permeability as the mode of action for quaternary ammonium compound germicides. This, he feels, is most consistent with the data presented to date, and it is well accepted (Franklin and Snow, 1989; Ikeda and Tazuke, 1985).

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