

## QUATERNARY AMMONIUM COMPOUNDS

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The quaternary ammonium compounds (called quats in this discussion for the sake of brevity) are characterized by their property of greatly depressing the surface tension of water. This property puts them in the group of chemicals variously described as wetting agents, detergents, emulsifiers, or dispersing agents. In that group of surface-active agents, we must distinguish between anionic detergents of acid nature, and cationic detergents, or substituted ammonia compounds. In addition, there are a few neutral surface-active agents. Only the cationic detergents or quats are discussed in this review.

### FUNDAMENTAL STUDIES

The first observations of germicidal properties in this group were made in 1916 by Jacobs and associates (1, 2, 3) who studied the relation between structure and bactericidal effects in the hexamethylene tetramine group. This was followed in 1928 by Hartmann & Kägi's work (4) with other quaternaries. However, the great possibilities of this class of compounds were not realized until Domagk (5) in 1935 reported on the long-chain quaternary ammonium salts. He found that germicidal properties were possessed by all quaternary ammonium salts in which at least one radical was a long-chain aliphatic group ( $C_8H_{17}$  to  $C_{18}H_{37}$ ), whether plain or substituted. Two or even three N-valencies may serve as part of a cyclic compound as in hexamethylene-tetramine, pyridine, isoquinoline, etc., and the anion may be halide, sulfate, acetate, or other similar group. Domagk's report opened up a wide field of exploration and has resulted in a rapidly growing literature, both in this country and abroad.

*Constitution and germicidal action.*—Domagk (5) found notable germicidal action only when at least one of the four radicals had a long carbon chain. He worked mostly with Zephriol which is alkyl dimethyl benzyl ammonium chloride. The alkyl group could be a chain containing from eight to eighteen carbon atoms. The same compound is sold in the United States as Zephiran, Roccal, or

BTC. Detailed data on many similar quaternaries are given by Valko & DuBois (6).

Compounds of the Hyamine group contain two radicals with benzene rings (7). When cyclic nitrogenous compounds are used, only one radical remains to be substituted. Shelton (17, 18, 19) found that with pyridine, the cetyl radical conferred strongly germicidal properties. This compound (cetyl pyridinium chloride) has the trade name Ceepryn. Some of the Emulsept products are also substituted pyridines. Shelton also studied the germicidal efficiency of other substituted cyclic amines, such as picolin, lutidine, quinoline, piperidine, pipercoline and morpholine.

Limitation of space prohibits any discussion of the relationship between chemical structure and germicidal efficiency. This relationship in homologous series has been recently correlated with thermodynamic activity (20). This theory has not been applied as yet to quats. [For details on homologous series, see (6 to 16, 41).]

In the following discussion, trade names will be used for the sake of simplicity and shortness. The most commonly used quats mentioned below are:

CTAB (Cetavlon) = cetyl trimethyl ammonium bromide

Zephiran = Roccal = BTC. = alkyl dimethyl benzyl ammonium chloride

Phemerol = Hyamine 1622 = Polymine D = p-tert. octylphenoxy ethoxy ethyl dimethyl benzyl ammonium chloride

Ceepryn = cetyl pyridinium chloride.

*Toxicity.*—Many experiments with a great diversity of quats have led to the unanimous conclusion that they are not toxic to animals in the concentrations in which they are commonly applied. A summary of these investigations was recently given by Valko (36).

*Bacteriostasis.*—When Dubos wrote [(21), p. 290]: "the difference between bacteriostatic and bactericidal effect is often of a quantitative rather than of a qualitative nature," he expressed a common conception [see also (22, 23) and others]. However, bacteriostasis is treated here separately from bactericidy because (a) with gentian violet bacteriostasis and bactericidy are quite different reactions (24), (b) Roberts & Rahn (25) observed complete enzyme inactivation at bactericidal concentrations but not at bacteriostatic doses, and (c) many very efficient bacteriostats do not kill bacteria even at very high concentrations.

Bacteriostasis may be due either to reversible inactivation of enzymes, or to reversible disruption of semipermeable membranes, or to reversible interference with the mechanisms of synthesis or cell division.

The relation between enzyme inhibition and bacteriostasis has rarely been studied. Sevag (26) found that 18 p.p.m. Zephiran retarded growth of yeast cells 65 to 83 per cent whereas the oxygen uptake was either not affected at all or even increased as much as 50 per cent. With 5 p.p.m., a 34 per cent decrease of growth was accompanied by a 60 per cent increase of oxygen uptake. This agrees with data by Roberts & Rahn (25) who tested *Escherichia coli* under conditions where energy could be obtained only through oxidase or dehydrogenase systems. These enzymes were not affected by concentrations of Zephiran which inhibited growth completely. Bacteriostasis must be caused by some other reaction. Semipermeability of the membranes cannot very well be reversible, because during the period when the membrane is completely permeable, the cell contents would diffuse out and cause the death of the cell. That leaves as the most probable explanation of bacteriostasis the interference with synthesis, i.e., interference with the doubling of essential molecules such as enzymes or genes or other proteins, or with cell wall construction, or interference with the more mechanical aspects of cell division. The microscope does not reveal abnormal cell morphology.

Inhibition studies with gram-negative bacteria by Randles & Birkeland (27) suggest that inhibition is due to preferential adsorption of detergent cations at that site of the cell which normally adsorbs the necessary nitrogen compounds. No data are given to indicate whether this is a mechanism similar to that functioning in inhibitions elicited by sulfa compounds.

Recovery from injury or inhibition by quats is slower than with most disinfectants because the adsorption layer of quats on the cell surface is not readily removed, and therefore injury to the cell may continue for some time after the disinfectant has been removed from the medium by dilution, adsorption or even by antidotes. This interferes with the appraisal of germicidal efficiency. Transfer of the bacteria to new broth frequently does not permit reproduction, while transfer combined with actual removal of the adsorbed quat shows that the bacteria were not really dead (28, 29, 30). Chemical removal, by an anionic detergent, results in

more complete recovery than physical removal by addition of charcoal (31). The use of media containing soap (5), anionics (29, 32), or lecithin or other phospholipids (30, 32, 33) to remove adsorbed quats has been widely recommended [cf. p. 181].

*The lethal reaction.*—Various explanations have been advanced for the death of bacteria resulting from the action of quats, namely, disruption of the cell membrane, inactivation of enzymes, or inactivation of other types of indispensable proteins.

Enzyme inactivation which had been accepted for many years as the general explanation for all kinds of sterilization, has been assumed by several authors to be the cause of death by quats. Baker *et al.* (34, 35) found that, as a rule, complete sterilization is accomplished

the oxygen uptake [see also (26, 43)]. However, in a few cases, loss of oxygen consumption is not accompanied by death, and any such exception makes the explanation rather doubtful. Roberts & Rahn (25) observed practically complete and irreversible inhibition of certain oxidase and dehydrogenase systems by lethal concentrations, but were in doubt whether this was the cause or the result of death.

The denaturation of enzymes within the cell requires the penetration of the cell membrane by quats. Valko [(36), p. 475] believed that "there is no reason to assume that the surface-active ions will not penetrate into the bacteria. Indeed, Kuhn & Jerchel (37) proved that surface-active ions penetrate into the living cell." Dubos (21) is of the same general opinion whereas Hotchkiss [(23), p. 490] wrote: "In the case of the usual surface-active agents, the experience of cell physiology would tend to predict that they could not penetrate the cell as long as it is alive." However, Dyar (49) observed that the cytoplasm of vegetative cells suspended in Ceepryn stained black with Sudan Black B, indicating that the quat had penetrated into the cell and that its hydrocarbon portion was stained by the fat dye.

Hotchkiss assumes that the quats first disrupt the cell membrane, and make it completely permeable, thereby markedly reducing enzyme activity and completely preventing multiplication, although morphologically, the cell appears unchanged. Hotchkiss supported his explanation with determination of the amounts of nitrogen and phosphorus compounds released from the treated

cells. Unfortunately, the experimental error of these measurements is very great.

Valko [(36), p. 478] believed in general protein denaturation as the lethal cause.

"Surface-active ions possess extraordinarily strong intrinsic affinity for proteins and combine readily with them. This combination causes disturbance of the intramolecular structure of proteins by upsetting the balance of the electrostatic forces as well as that of the non-Coulombic cohesion in the molecule. At the same time, the interaction of the proteins with the solvent molecules may undergo profound changes and the bonds between the components of the conjugated proteins may be disrupted."

The experiments on reactions of proteins with quats summarized by Valko [(36), p. 470] show, as a rule, that approximately equal weights must react to produce denaturation or precipitation of the proteins. In standard bacteriological technique, the culture, diluted 1:10, contains about 20 p.p.m. bacterial solids, and with the more efficient quats, about 20 to 100 p.p.m. are sufficient to kill in five to ten minutes. However, only a small fraction of the disinfectant in solution actually combines with the bacteria, so that death is probably not due to a general denaturation of all cell proteins. The necessity of general denaturation of all cell proteins appears improbable because of the logarithmic order of death (31) observed for 99 to 99.9 per cent of the cells. If death were due to a destruction of the surface, the order should be similar to that of multicellular organisms, as is the case with death by chlorine [(38), p. 93]. The survivor curves for quats and for chlorine bend in opposite directions. The initial logarithmic order with quats pertains to the single cells. The much slower death rate of the few remaining bacteria (40) is explainable by the clumping of the cells under the influence of quats (39).

In the opinion of Valko & Dubois (29), "the antibacterial behavior of surface-active cations is in agreement with that of toxic metallic ions and dye cations. They can be considered as a phenomenon of ionic exchange." The above refers largely to the explanation advanced by McCalla (45). The present authors hesitate to accept this analogy, because it has never been proved that quats act by their cations. Since most quats lose their efficiency almost completely in acid environment when they should be most completely dissociated, it may well be that their activity depends

primarily upon the undissociated molecules as is the case with many organic compounds (46, 47).

An interesting observation was made by Dyar & Ordal (44) when they studied the effect of surface-active compounds upon the electrokinetic mobility of different species of bacteria. As the Ceepryn concentration was increased, the negative electric charge of the bacterial cells remained constant at very low concentrations, but at about  $10^{-5}$  molar, the charge decreased to zero, then became positive and finally became stable at concentrations between  $10^{-4}$  and  $10^{-3}$  molar. While these experiments were made only to study the nature of the cell surface, it is interesting to note that with *Staphylococcus aureus* and *E. coli*, the bacteria died rapidly when the Ceepryn concentration was just strong enough to produce a noticeable decrease in mobility. This suggests a relation between surface charge and disinfecting power which has also been claimed by Bradbury & Jordan (48).

The "reversibility" of the "killing" action of these agents by anionic detergents or other means (29) is probably not due to the reversion of the lethal reaction by removal of the quat, but more likely to a last-minute prevention of the lethal reaction which would have taken place in routine transfer, but was interrupted by removal of the quat. Physical removal, by adsorption, is more effective in saving bacteria from death than chemical inactivation of the quat by anionics (31). All experiments, by all authors, have shown "recovery" of bacteria to be limited to a short time, about thirty minutes, after exposure; beyond that, cells are irreversibly killed.

#### FACTORS AFFECTING GERMICIDAL EFFICIENCY

As suggested above, surface-active agents are "temperamental." Their efficiency may be greatly decreased by conditions which hardly affect the so-called plasma-poison group of disinfectants. The quats are completely neutralized by anionic compounds, including the soaps, and by phospholipids, with which they form harmless compounds (5, 29, 32, 33, 57, 68, 73).

*Adsorption.*—Surface-active compounds are readily adsorbed and filter paper, charcoal, bentonite (50) and agar (51, 83) remove part or all of the dissolved quats from solution. So do bacterial surfaces (50); those of dead cells probably being as efficient as those of living ones. Precipitated proteins also adsorb quats. Ad-

sorption has its good and bad features in the practical use of quats.

*Fat*.—Fat affects quats physically. On fatty surfaces, they are adsorbed in oriented layers, the hydrophobic, organic end of the molecule is directed towards the fat surface, while the  $\text{N—OH}$  group faces the aqueous phase. As the great reactivity of quats depends upon the  $\text{N—OH}$  group, such oriented adsorption films have two different sides of which the one, directed towards the fat, is non-toxic whereas the other, directed towards the aqueous phase, is strongly germicidal (50). This explains the peculiar effect observed in the disinfection of hands with quats (52) [cf. p. 185].

*Protein*.—Proteins combine with quats chemically, frequently producing a precipitate (36, 67). The reaction requires approximately equal weights of proteins and quats, so that a quat solution of 1:1,000 may be made completely harmless by a 1 per cent protein solution. Quats are inefficient in milk (53, 54). In presence of 10 per cent serum, the killing concentration of Ceepryn had to be increased four to eight times with streptococci or staphylococci, and twelve to twenty-five times with enterobacteria (55). Hence, the ease with which they react with proteins influences greatly the efficiency *in vitro* and especially *in vivo* [cf. p. 180]. Gelatin has been found to be an exception (70).

*pH*.—Acidity decreases the efficiency of many quats so decidedly that they are completely inefficient at pH 3, and only weakly bactericidal at pH 4 (42, 55, 56, 57). The only exception known so far is Ceepryn which is as efficient at pH 3 as at pH 8 (53, 55).

*Temperature*.—Temperature affects the action of this group as it affects all other disinfectants. An increase in temperature increases the efficiency, the temperature coefficient between 20° and 37°C. being less than 2 (55). Contradictory or variable results (62, 63) may be obtained at higher temperatures because it is sometimes overlooked that at the optimal temperature, bacteria are multiplying at the fastest possible rate which is a dangerous rate, and that a relatively slight disturbance may cause death. That would result in a seemingly abnormal temperature coefficient.

*Species*.—Generally speaking, the quats are not very selective in their efficiency (35, 58, 59, 60, 62, 63, 64). Commonly, somewhat larger doses are required to kill the gram-negative enterobacteria

than streptococci or staphylococci, but rarely more than double the amount (55). A striking exception is *Pseudomonas aeruginosa* for which ten to twenty times as much is needed as for staphylococci (55). *Mycobacterium tuberculosis* (65) and *M. phlei* (55) are even more resistant. Pathogenic fungi are somewhat more resistant than the gram-negative bacteria.

Bacterial spores are not easily killed. General statements which give no description of technique and no quantitative data (63, 66) are not convincing. The high concentrations required may produce bacteriostasis in the transfer tubes, unless special precautions are taken, and may simulate sterilization. According to DuBois & Diblee (40), a 1:10 dilution of Tetrosan is needed to kill all spores in a culture of *B. metiens* although a 1:5000 dilution kills 60 to 75 per cent almost immediately [see also (94, 95)].

*Potentiation.*—When two different disinfectants are applied simultaneously, it must be expected that their combined effect is greater than that of each disinfectant alone. The term potentiation, or synergistic effect, is justified only if the combined effect far exceeds the sum of the separate effects. Thus if *E. coli* is retarded by 1,000 p.p.m. sulfaguanidine, and completely inhibited when, in addition, 100 p.p.m. of Triton K 12 are added (61) this result can hardly be called potentiation. But when *E. typhi* is only slightly retarded by 1,000 p.p.m. sulfaguanidine, and not at all by 100 p.p.m. Triton K 12, but is completely inhibited by 100 p.p.m. Triton + 10 p.p.m. sulfaguanidine (61) that can be called potentiation (69). Since the effect of quats is inhibited by so many different factors (see above) it seems possible that potentiation may be accomplished by chemicals which remove some of the interfering or blocking compounds.

#### APPRAISAL OF BACTERICIDAL EFFICIENCY

The customary evaluation of disinfectants by their phenol coefficients (87, 93, 94, 95) has proved unsatisfactory with quats (30, 39, 71, 72, 74 to 77). Different broths give different results (30, 71). Death time determinations are likely to give inconsistent results (55, 72). Cultures seemingly dead after five or ten minutes exposure may grow in the fifteen-minute transfer. Special precautions must be taken to prevent bacteriostasis by the disinfectant carried over with the transferred bacteria (85).

The bactericidal properties of quats are due to their chemical

reactivity and also to their great adsorbability. These same properties account also for their frequent failure. They are completely inactivated by soap (5), and by lecithin and other phospholipids (32, 33, 73); they are completely adsorbed by charcoal or bentonite (31, 50), also to a lesser degree by agar (51, 79, 83). They combine readily with proteins (5, 36, 91) and therefore are less efficient in presence of serum (55, 60) or milk (53, 54). Even some phosphates (103) appear to exert an inhibitory effect.

Another unusual feature makes the appraisal difficult. The logarithmic survivor curve is straight only for the killing of the first 99 to 99.9 per cent (77, 78). After that, the death rate decreases very noticeably, and the last survivors often display a surprising resistance (40). This has been explained, at least partially, by the agglutination of bacteria by quats (39). The bacteria inside the clumps remain alive longer. The clumps settle on the walls of the vessel and cause uneven sampling. To overcome this, Klarmann and Wright recommended using only 0.05 ml. of culture treating it with 0.5 ml. germicide, and after five, ten and fifteen minutes, adding 20 ml. of broth to the tubes, thus including survivors from clumps.

Another method developed by the same authors consists of using standard pieces of filter paper to which 0.5 ml. of culture and 5 ml. of disinfectant is added. After five, ten and fifteen minutes, one piece of filter paper is removed to 20 ml. of broth, and shortly afterwards transferred again to new broth to eliminate bacteriostasis. Both methods are far more severe than the phenol coefficient method (31).

In the standard phenol coefficient test, bacteria may retain for quite a while the film of adsorbed quats when transferred to a new medium, so that they cannot multiply and may be considered dead, unless this film is destroyed by soap, lecithin, or adsorbent charcoal. The addition of such compounds to the test medium has been recommended (28, 33, 79, 85). As no antidotes are used in assaying phenols, mercurials or sulfa drugs, it must be admitted that the use of antidotes only in the case of quats is quite arbitrary. A similar use of antidotes with phenols would show them to be less efficient, and it would indicate mercurials to be utterly useless. Mallmann (80) states "By selecting the proper laboratory technic, we can show that the cationics are either poor or unusually good disinfectants" Nevertheless, even the manufactures of quats

have recommended the use of antidotes to prove the efficiency of their products.

All these reactions greatly affect the usefulness of quats in the various applications, and for any specific case, the method for testing the germicidal efficiency should consider the kind or degree of efficiency that is needed. Absolute sterility, including the killing of spores, would be expected with surgical instruments, but in the cleansing of machinery and equipment for the manufacture of frozen foods, elimination of 99 per cent of the bacteria may be satisfactory. In still other cases, it may be immaterial whether the bacteria are killed or not, as long as they are prevented from multiplying. It is quite important in these latter cases to remember that a few cells or clumps may prove to be much more resistant than the large majority.

In order to get a clear picture of the efficacy of a disinfectant, the phenol coefficient, as an all-or-nothing method, must be substituted by a procedure which gives information concerning the percentage of survivors. Such a method has been introduced by Mallmann (81) in his "use-dilution method". The test bacteria are dried on glass rods which are dropped into the disinfectant solution. After various exposure times, the rods are removed, shaken in broth, and the survivors counted by agar plating. This technique has been found satisfactory for sanitation purposes.

The agar cup method has been shown to give results which do not agree with the phenol coefficient method (51, 83).

*In vivo* tests have been made to prove the efficiency of quats for the sterilization of skin and mucous membranes, and to justify their application as skin disinfectants, mouth washes, nose drops, vaginal suppositories, etc. For this purpose, Green *et al.* (82, 83, 84) used the chorioallantoic membrane of the chick embryo; Whitehill (89) used the rabbit eye; Kenner *et al.* (88) injected cultures plus quats into mice; Nungester & Kempf (90) and also Spaulding & Bondi (92) applied the "infection prevention method;" Herrell (96) used tissue cultures.

#### APPLICATIONS OF THE QUATERNARY AMMONIUM COMPOUNDS

*General sanitation.*—Krog & Marshall (98), using a swab test technique, established the bactericidal action of Zephiran against bacteria commonly found on eating and drinking utensils. A one-

minute exposure to a 1:5,000 dilution was apparently sufficient to reduce bacteria surviving on tumblers to less than 100 per rim. Subsequent studies (80, 99 to 102) have confirmed these results for this and other quats. The reaction of quats with soap or with phosphates used in cleaning compounds (103) does not seem to lower their efficiency when properly used in 2- or 3-compartment washing procedures. The quats have an advantage in being relatively odorless and tasteless in recommended concentrations, and have been accepted as a sanitizing rinse after washing by more than one hundred and sixty cities in eighteen states throughout the country (102).

Recently there has been considerable interest in the possibility of developing a dishwashing compound which would be germicidal as well as having good detergent properties, and which would thus combine two operations into one. Quats have been suggested for this purpose, but they are incompatible with the best detergents which are anionic. Progress has recently been reported by Guiteras & Shapiro (104) who used a nonionic alkylated aryl poly-ether alcohol as the wetting agent and cetyl-dimethyl-ethyl-ammonium bromide as the germicidal agent.

Quats have been suggested for use on the farm in sterilizing milking machine cups, etc., (105) and have been found effective in reducing the bacterial count from washed and supposedly clean milk cans when one ounce of 10 per cent solution was used in one gallon of water (106, 151). Scales & Kemp (105) and Frayer (107) considered Zephiran or Roccal useful for sterilizing cows' udders prior to milking. Recently Hughes & Edwards (108) in England reported lesions developing on cows teats apparently as the result of repeated application of another quat, CTAB.

Krog & Marshall (109) made a study of the use of Roccal in a milk pasteurizing plant and reported that the use of a 1:5,000 to 1:8,000 dilution of the compound following the usual cleaning treatment resulted in 68 to 99 per cent reduction in bacterial counts obtained from swabbings made at specified stations throughout the plant. Several workers (78, 109, 113) have reported that the quats they studied are not harmful to those metals commonly used in the fabrication of dairy and food processing equipment. Krog (110) has suggested the use of 1:5,000 aqueous Roccal on wiping cloths for the sterilization of "novelty equipment" in the ice cream industry. Directions for the application of the quats in

the dairy plant have been given by Vignolo (111) and Levowitz (112). An important point to be emphasized is that these compounds become less efficient in the presence of milk and hence can be used only to sterilize equipment which has been washed free of milk (53). Health authorities have hesitated to permit quats as general disinfectants in milk plants because they might be used to cover up high bacterial counts in milk. This does not seem justified, however, because it has been shown (54) that Roccal does not influence the bacterial count of raw or pasteurized milk at concentrations as high as 1:500, and hence could not be used by unscrupulous dealers to cover up unsanitary practices. Quats have been accepted for dairy purposes in about thirty cities in the United States (102).

The success quats have enjoyed in the sanitation of dairy plant equipment has led to the suggestion that they be used in other food industries. Lehn & Vignolo (113) suggested that a quat in a dilution of one ounce of 10 per cent solution to four gallons of water could be used in breweries to sterilize lauter tubs, brew kettles, hop strainers, fermenting tanks, and surface coolers by spray application, and to sterilize plate coolers, beer and wort lines, fittings, and pumps by circulating the solution through the various pieces of equipment. Similar application has been suggested for the sterilization of lug boxes and various pieces of equipment in the food processing industry (114). In these nontechnical papers, no data as to the efficiency of the quats under the proposed conditions are given, but it seems likely that they should be as effective here as in the dairy plant, provided their use is restricted to previously cleaned equipment. Penniston & Hedrick (115) considered a mixture of long-chain pyridinium chlorides useful in preventing contamination of the shells of eggs intended for dehydration. The usual count of three to six million microorganisms found on eggs after washing in water could be reduced to a few thousand or less by a three to five minute rinse.

*Water supply disinfection.*—Fair *et al.* (116) studied the action of ten quats against *Entamoeba histolytica* cysts and found that with some quats the cysts could be killed by 30 p.p.m. in ten minutes or by 10 p.p.m. in two hours. The required concentration increased with increasing density of cysts to be killed, but suspended organic matter and pH seemed to have only slight effects. At

least four of the compounds were effective cysticides: Fixanol, Nopco QCL, Ceepryn, and Sapamine K<sub>w</sub>. Zephiran and Triton K60 may also be effective, but the data on these compounds are not complete. The authors suggest their possible usefulness in water disinfection, sanitation of eating utensils, disinfection of shellfish, cleansing and disinfection of contaminated vegetables and fruits intended for raw consumption, cleansing and disinfection of water mains, cleansing of water filters, and control of *Psychoda* in trickling filters.

Recently (117) Polymine-D, when used in solid form combined with mild alkalies, has proved effective for sterilizing two jute-packed water mains that had proved refractive to chlorine sterilization. For the larger main (3,000 ft.) one 7-day application of the germicidal solution was effective.

*Disinfection in surgery.*—Domagk (5) mentioned the sterilizing effect of Zephirol on skin, and suggested its use in surgery to disinfect hands before putting on rubber gloves. Since that time, a number of workers have reported various quats to be effective for sterilizing surgeon's hands in gloveless surgery, or for securing a sterile operative field (118 to 127, 149). However, Miller *et al.* (52) emphasize certain limitations of using quats for skin disinfection. Quats, specifically Zephiran and Phemerol, deposit an invisible, nonperceptible film on the hands, and this film retains bacteria underneath it and is very resistant to mechanical trauma. The inner surface of the film has hardly any bactericidal power, whereas the outer surface exerts a strong germicidal action. The formation of such a film has been explained by Rahn (50) as due to an oriented adsorption of the molecules of the detergent on an organophilic surface with the harmless organic ends directed towards the skin and the germicidal—NOH groups toward the outside. The outside of such a film remains sterile for some time, but beneath the film the bacteria on the skin remain alive.

Whenever swabbing techniques (122, 123, 124) or contact plate methods (121, 127) have been used to determine the efficiency of skin disinfection, only the outside of this film was touched and the results are of course good. Much better are the skin-sectioning techniques (118, 119, 126), but these too are not entirely reliable for no antidote was used, and this is essential when testing quaternary ammonium compounds (28). In surgery, quats could

not be expected to exert a bactericidal action in incisions where they would come in contact with various types of organic matter, phospholipids, etc., which would act as antidotes. It is possibly significant however that quats on the surgeon's hands and on the operative field have resulted in no infection, or in less infection than with iodine tincture (52, 119, 121). Kramer & Sedwitz (125) reported that 1:200 Ceepryn tincture had been used routinely in operative cases at the Youngstown, Ohio Hospital for three years (about 18,000 cases) with no observed irritation and no single instance of infection. Sterilization of the surgeon's hands in gloveless surgery depends upon the stability of the film of adsorbed germicide and upon the possibility of its neutralization by contact with tissue fluids and blood. Sterilization of the incision itself must be considered doubtful.

It has also been suggested that solutions of quats may be used to sterilize bowls, baths, and surgical instruments (118, 123, 124, 128). More information is needed with respect to their use on surgical instruments. In Germany, Brekenfeld (129, 130) and Kayser (131) recommended boiling surgical instruments in army field hospitals in 0.75 to 2.0 per cent Zephriol for fifteen minutes, while Zeissler & Günther (132, 133) were against using Zephriol on the ground that it would not kill spores from soil.

*Human medicine.*—Quats have been used in attempts to combat various infections. They also have been found to be fungicidal (150). Wright & Wilkinson (120) considered 1:1,000 Zephiran effective in preventing infection in injuries and specifically mention burns. On the other hand, Barnes (123) found of CTAB on burns. Forman (128) reported 1 per cent CTAB appeared effective in curing boils and dermatitis due to strapping and postulated the theory that it reduced the staphylococcal population of the skin so that natural bactericidal powers of the skin were sufficient to cope with the infection. Although one case appeared to show sensitivity, Forman found satisfactory progress in most cases in treating severe nummular eczema, chronic dermatitis, and impetigo of the face. Fischer (134) also used a quat in the prevention of impetigo in the newborn. Neter (135) showed that Zephiran 1:2,000 would inactivate tetanus toxin almost completely, which suggested its use in the prevention or treatment of infection by toxin-producing bacteria. But as Neter himself

pointed out, the addition of human serum to the Zephiran solution interfered with the toxin-destroying properties. Neter (136) has also shown that 1:10,000 Zephiran inhibits clotting of plasma by staphylococci, and 1:100,000 inhibits fibrinolysis by hemolytic streptococci. Sarber (137) reported some success with 1:200 cetylpyridinium chloride tincture in preventing experimental *Streptococcus* infections in mice. The same compound was used successfully in bronchial lavage (152).

Although quats have been reported to be highly virucidal *in vitro*, against various strains of bacteriophages and viruses (138, 139, 153), Klein & Stevens (138) could not prevent experimental influenza virus infections in mice when quats were administered by intranasal spray. Other workers (140) have reported that Zephiran 1:10,000 will not inactivate type B influenza virus in one hour at room temperature. In fact quats have been used to preserve virus and bacteriophage suspensions (141, 142, 143).

Zephiran has been found effective in inhibiting the metabolic activities of microorganisms associated with dental caries (144) and there is some evidence that this compound (145) and cetylpyridinium chloride (146) are effective mouth antiseptics.

*Veterinary medicine.*—On the basis of *in vitro* tests against mastitis organisms Scales & Kemp (105) suggested that a mixture of Zephiran, a dispersant (Triton 720) and 33 per cent milk might be effective as a treatment for chronic mastitis if injected into the udder at body temperature directly after milking. Bryan *et al.* (147) have subsequently reported success in treating chronic mastitis by infusing each quarter with 75 ml. of 1:1,000 aqueous Phemerol.

Bryan & Young (148) have also reported success in curing ringworm of calves by twice weekly applications of 1:1,000 aqueous Phemerol to the scabs.

It is evident that caution must be exercised in the application of quats for Hughes & Edwards (108) found lesions developing on cows' teats apparently as the result of continued application of CTAB. While 1 per cent CTAB cream appeared effective in preventing the spread of *Streptococcus agalactiae* mastitis when applied twice daily to milkers' hands and cows' teats, after three months lesions of the teats developed accompanied by a rapid increase in *S. agalactiae* infection of teats and milk. 1 per cent

CTAB cream failed to control this infection, and it was suggested that the increase in the number of cows with teat lesions was due to the use of this compound.

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