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Title; FUNDAMENTAL FACTORS AFFECTING GERMICIDAL ACTIVITIES OF CERTAIN HYPOCHLORITE AND QUATERNARY AMMONIUM COMPOUNDS

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This study was undertaken to determine significance of a number of factors on germicidal activity of quaternary ammonium compounds (QAC). In order to provide a proper evaluation of their present comparative and potential value in dairy sanitation procedures, in general, the investigations included not only QAC, but also representative hypochlorite preparations.

The method for the determination of germicidal activity of QACs and hypochlorites was the Weber and Black technique. This test method was modified by the removal of bacterial clumps with filtration of the bacterial suspension through filter paper and by standardizing the number of cells in the test suspensions with a Beckman spectrophotometer.

Germicidal activity of alkyl dimethyl benzyl ammonium chloride, and para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride against E. coli, A. aerogenes, P. aeruginosa, Sarcina sp. and M. pyogenes var. aureus was determined. The QACs in concentrations of 50, 100 and 200 ppm were most active against A. aerogenes and least active against P. aeruginosa.

Concentrations of 50 ppm of sodium hypochlorite, lithium hypochlorite, alkyl dimethyl ethyl benzyl ammonium chloride and a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride destroyed all cells of E. coli in 15 seconds. Alkyl dimethyl benzyl ammonium chloride, para di-isobutyl phenoxy ethoxy benzyl ammonium chloride and methyl dodecyl benzyl trimethyl ammonium chloride followed in decreasing sequence in germicidal activity.

The germicidal activity of sodium hypochlorite against water suspensions of B. cereus spores and M. caseolyticus cells was not increased by the addition of 0.05 and 0.01 per cent surface active agents in the form of Nacconal NRCL, Stepanate, Alkanol DM, Santomerse # 1, or X-100. Concentration of 0.05 per cent Stepanate and X-100 appeared to decrease the activity of the sodium hypochlorite solution. The pH of a hypochlorite solution appeared to be the most important factor affecting its germicidal activity.

The germicidal activity of QAC varied markedly with a small change in pH level. The optimum pH value for greatest activity of any one individual QAC depended upon the bacterial species tested. This value appeared to vary for different QACs.

A low pH detergent sanitizer showed greater activity against P. aeruginosa than a high pH preparation. On the other hand, E. coli appeared more resistant under the same conditions at a low pH than at a high pH level.

QAC, in the form of a detergent sanitizer was more active than the QAC alone in the case of two different QACs in distilled water, Navy hard water and Navy hard water containing organic matter. Results indicated that the active agent accelerating QAC action was the tetrasodium pyrophosphate. Trisodium phosphate showed no pronounced accelerating effect on QAC germicidal activity.

In studies on effect of QAC action in presence of organic matter in the form of milk solids, it was found that acid production by lactic acid starter bacteria was inhibited by as little as five ppm QAC in milk. Inhibition of acid development was nearly complete with 25 to 30 ppm QAC in the milk when the organisms were incubated at temperatures near their maximum. At incubation temperatures normally used for culturing the organisms, 50 ppm QAC effected nearly complete inhibition. Milling time was delayed 15 minutes by presence of five ppm, and 45 to 60 minutes by 10 ppm QAC in the manufacture of Cheddar cheese.

A method was developed for determination of QACs in milk, based on extraction and precipitation of QAC in a tetrachloroethane-acetone-eosin indicator solution. Interfering factors in the solvent indicator were removed by successive washings with distilled water, and QAC then was titrated with a standard solution of anionic surface active agent. With suitable standards for comparison, the method determined quantities of QAC in the range of five to 100 ppm in milk.

FUNDAMENTAL FACTORS AFFECTING GERMICIDAL ACTIVITIES OF CERTAIN HYPOCHLORITE AND QUATERNARY AMMONIUM COMPOUNDS

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TABLE OF CONTENTS

FUNDAMENTAL FACTORS AFFECTING GERMICIDAL ACTIVITIES OF CERTAIN HYPOCHLORITE AND QUATERNARY AMMONIUM COMPOUNDS

INTRODUCTION

Milk provides an ideal culture medium for the growth of microorganisms. Therefore, milk and milk products must be handled in such a manner as to minimize their contamination by microorganisms. Another important objective of the proper handling is the prevention of disease transmission. One of the most important factors in proper processing of dairy products is the cleaning and sanitization of the various utensils and equipment with which they come in contact. This sanitization may be accomplished in various ways, but the most practical method is by chemical means. The chemicals commonly used for this purpose have been sodium and calcium hypochlorite and the chloramines. In recent years, quaternary ammonium compounds (QAC) have been advocated by some workers for this operation.

Many of the advantages and disadvantages of the hypochlorites for dairy sanitation have been fairly well established. Unfortunately, the QAC preparations were introduced to the dairy industry so rapidly that many factors affecting their germicidal activity have not been thoroughly studied. This study was undertaken to provide information on a number of factors affecting activity of QAC. In order to provide a proper evaluation of their present and potential value in food sanitation procedures in general, the investigations have included not only QAC, but also the representative hypochlorite preparations.

HISTORICAL

General information. Although chlorine is a very effective germicide and has given excellent service to the dairy industry, it does have certain disadvantages. As listed by Botwright (17, p.102), these disadvantages are: (a) its germicidal properties are destroyed by organic matter; (b) it imparts odors and tastes to foods; (c) it irritates hands; and (d) it corrodes equipment. Haller et al. (92, pp. 10-20) studied the effect of chlorine on 12 metals and alloys which were most likely to be used in dairy equipment. All metals studied showed corrosion by 100 and 200 ppm of available chlorine. The higher concentration had the greater effect. Another disadvantage of chlorine was implied by Dahlberg and Carpenter (38, p.550). They found that oxidized flavor of milk was most pronounced when chlorine sterilization was used. However, these findings have not been verified by other workers.

The first possibility that QAC preparations had germicidal properties was indicated in 1916 by Jacobs (111, pp.566, 567). He was able to show germicidal properties of hexamethylenetetramine. Jacobs et al. (112, p.574) (113, p.598) showed that the germicidal properties of the above compound were due to the hexamethylenetetramine nucleus, but that these properties could be enhanced by aliphatically bound halogen. No further important work was done on this type of compound until 1935, when Domagk (40, pp.830, 831) studied the germicidal properties of alkyl dimethyl benzyl ammonium chloride. He showed that this compound could destroy nine species of pathogenic bacteria when present in concentrations of 100 to 1000 ppm.

The concentration required for destruction depended upon the species of bacteria.

A number of reviews on the subject of QAC have been published in recent years (37, pp.133-139) (44, pp.25-27, 42-43) (61, pp.156-167) (62, pp.108) (104, pp.269-292) (188, pp.173-192) (220, pp.451-478). Davis (39, p.48) listed the following advantages for these compounds: (a) rapid and complete killing action against bacteria even in low concentrations; (b) practically non-poisonous; (c) non-corrosive; (d) odorless; (e) non-irritating to the skin; (f) great stability even in the presence of organic material; and (g) inhibit bacterial growth over long periods of time. However, Hirsch and Novak (100, p.378) showed that the toxicity index placed chlorine compounds as the most efficient germicides. This was followed by the phenolic compounds, and finally by alkyl dimethyl benzyl ammonium chloride. Subsequent investigations have raised some questions regarding claims made for destructive action of QAC against bacteria and on their stability in the presence of organic matter.

Mode of action of germicides. The fact that pH materially affected the efficiency of chlorine germicides led Marks et al.(159, p.303) to conclude that undissociated hypochlorous acid was the active form and that the hypochlorite ion was entirely inactive. The active form probably affects an enzyme system of bacteria. Knox et al. (136, pp.456, 457) showed that chlorine in bactericidal amounts or less inhibited various sulfhydryl enzymes and various other enzymes sensitive to oxidation. They used Escherichia coli as the

test organism. The inhibition of glucose oxidation was paralleled by the percentage of bacteria killed. The aldolase of the organism was shown to be one of the essential enzymes of glucose oxidation sufficiently sensitive to chlorine to explain its bactericidal effect. Green and Stumpf (88, p.1304) explained the difference in sensitivity to chlorine of vegetative cells and spores as being due to its effect on glucose oxidation. The spores were less sensitive because they required little glucose oxidation.

Various efforts have been made to determine the mode of action of anionic and cationic surface active agents on bacteria. Dyar and Ordal (53, pp.160,166) showed by electrokinetic studies that, in the presence of bacteria, cetyl pyridinium chloride yielded a pattern of decrease of charge, reversal of charge, and stabilization of charge. This would indicate that some type of chemical reaction took place. It also is known that anionic detergents will precipitate proteins only in the cationic form. Precipitation ceases above the isoelectric point of the protein. This study was accomplished by Putnam and Neurath (182, p.695). The reaction between protein and anionic detergents may be an adsorption rather than a chemical reaction. Pankhurst and Smith (176, p.569) showed that dodecyl sodium sulfate adsorbs around a gelatin molecule in a continuous unimolecular layer attached by its polar groups to the basic nitrogen atoms of the protein.

The QAC preparations probably destroy bacteria by inactivating the bacterial enzymes. Sevag and Ross (202, pp.679-681) determined that a 1:3500 (286 ppm) dilution of Zephiran completely inhibited the

oxygen consumption of p-phenylenediamine in yeast cells. Also aerobic and anaerobic oxidation of glucose by yeast cells was inhibited by Zephiran. The effect of Zephiran and Ceepryn on dehydrogenase, oxidase and catalase of E. coli was determined by Roberts and Rahn (196, pp.641, 642). Retardation of the enzyme systems occurred at a concentration of germicide that had little or no effect on the energy production. Concentrations that inhibited growth completely did not always inactivate or even retard the enzymes. However, at lethal concentrations, the enzymes were inactivated. Ordal and Borg (173, p.334) obtained an inhibition by cetyl pyridinium chloride on the lactate dehydrogenase of E. coli and Micrococcus pyogenes var. aureus. Miller et al. (164, p.706) showed that Zephiran exerted a pronounced inhibitory effect on both respiration and glycolysis of six different species of bacteria.

The possibility that surface active agents may act on bacteria in some method other than by inactivation of enzyme systems has been suggested by Gale and Taylor (75, p.549). They found that all internal lysine of bacterial cells was released by cetyl trimethyl ammonium bromide and aerosol OT (di-octyl sodium sulfosuccinate).

Hotchkiss (103, p.492) presented the theory that QAC preparations are adsorbed by the organism rather than uniting in chemical combination. Valko and DuBois (221, pp.20-24) reported that the killing action of surface active cations on bacteria could be reversed by detoxication with a high molecular anion. Similarly, Kivela et. al. (128, p.570) showed that the bacteriostatic effect of surface active cations on bacterial spores could be reversed by dilution and shaking

in distilled water or physiological saline solution.

Germicidal efficiency. Chlorine compounds have proven to be highly active germicides. Johns (116, pp.39-41) showed that 55 to 85 ppm of either calcium or sodium hypochlorite destroyed ropy and bitter milk organisms within 60 seconds. He tested five species that caused ropy milk and two species that produced bitter milk. It was reported by Long and Hammer (148, p.41) that Pseudomonas putrefacieus did not survive five seconds in sterile water containing one ppm available chlorine. Tonney et al. (218, p.1260) found that most pathogens failed to survive longer than 30 seconds in the presence of 0.1 ppm available chlorine. However, Mallman and Gelpi (153, pp.12-15) showed that E. coli developed a tolerance to chlorine in concentrations of 0.5 ppm. All of the above studies utilized suspensions of organisms containing less than 500 per ml. Wade et al. (223, p.192) determined the effect of calcium hypochlorite on tubercle bacilli in relation to milk bottle sterilization. They contaminated one-half pint bottles with 0.5 ml. of sputum containing four to six bacilli per microscopic field. A concentration of 100 ppm available chlorine required over three minutes exposure to destroy all bacilli.

The destruction by germicides of bacterial spores is much more difficult than that of vegetative cells. Tonney et al. (219, p.507) showed that 280 ppm available chlorine were required to destroy Bacillus vulgatus spores in 30 seconds. Bacillus subtilis spores required 160 ppm. However, Tilley and Chapin (214, pp.299, 300) were able to destroy anthrax spores in 15 minutes with 10 ppm of available

chlorine. The QAC preparations also destroy bacterial spores, but not so rapidly as do the hypochlorites. Dunn (51, p.48) demonstrated a bacteriostatic effect by these compounds on B. subtilis spores. DuBois and Dibblee (48, p.734) showed that 60 to 75 per cent of Bacillus metiens spores were destroyed by a 1:5000 (200 ppm) dilution of Tetrosan almost immediately. However, after six hours only 90 per cent of the original spores were destroyed. In other words, the 25 to 40 per cent of spores remaining after the initial kill were very difficult to destroy.

Mueller et al. (169, p.758) showed that six QAC preparations were effective in the destruction of organisms found in raw milk. Joslyn et al. (124, p.50) determined that Phemerol was an effective germicide against ten pathogenic species of bacteria. They found that Pseudomonas aeruginosa was the most resistant. The resistance to QAC of P. aeruginosa was verified by McCulloch (162, p.500). Gershenfeld and Milanick (77, p.322) reported that Triton K12 (cetyl dimethyl benzyl ammonium chloride) was more effective against Eberthella typhi than against M. pyogenes var. aureus. However, Johns (121, p.28) reported that QAC preparations were more effective against M. pyogenes var. aureus, Bacillus panis and Micrococcus candidus. Hypochlorites proved more effective against E. coli and P. aeruginosa. Cheese starter organisms were destroyed more readily by hypochlorites than by the QAC preparations. Dunn (50, p.428) found that alkyl dimethyl benzyl ammonium chloride was bacteriostatic to M. pyogenes var. aureus and B. subtilis in a dilution of 1:100,000 (10 ppm). E. coli required a dilution of 1:20,000 (50 ppm). A

meningococcus was destroyed by 0.025 per cent (250 ppm) Zephiran according to Miller (166, p.200). Freedlander (73, p.52) inhibited the growth of tubercle bacilli with 1:80,000 (12.5 ppm) dilution of Zephiran. Miller et al. (165, p.106) were able to prevent growth in glucose solution from dental plaque material with 1:3,000 (333 ppm) dilution of alkyl dimethyl benzyl ammonium chloride. Dilutions of 1:1000 (1000 ppm) and 1:3000 (333 ppm) of QAC were found by Whitehill (231, p.220) to be effective against M. pyogenes var. aureus.

The effectiveness of germicides against viruses is of interest to the dairy industry because of bacteriophage attacks of lactic acid starter cultures. Krueger (140, pp.628, 629) showed that a 1:10,000 (100 ppm) dilution of surface active agents failed to destroy influenza virus within one hour. The difficulty of destroying virus with QAC was substantiated by the fact that Maier (150, p.36) found no interference in the reproduction of M. pyogenes var. aureus phage by 1:50,000 (20 ppm) dilution of alkyl dimethyl benzyl ammonium chloride. In fact, Kalter et al. (125, p.239) were able to isolate coli phage from sewage by the use of Emulsol-607, Zephiran, and cetyl pyridinium chloride. On the other hand, Hunter and Whitehead (109, p.6465) found that 500 ppm chlorine completely inactivated within one minute the bacteriophage of the lactic streptococci. Prouty (180, p.219) studied the effect of six QAC preparations against Streptococcus cremoris phage. He found that a concentration of 200 ppm of these compounds destroyed this phage in two minutes. Parker and Elliker (177) showed that hypochlorites were more effective in destroying S. cremoris phage than the QAC preparations.

The possibility that the Gram reaction of an organism could be used as an index to predict the effect of QAC has been considered. Baker et al. (9, p.618), Dunn (51, p.50) and Johns (119, p.86) showed that these compounds had a greater germicidal effect against Gram positive than Gram negative organisms. Klein and Kardon (134, p.248) claimed Zephiran possessed several hundredfold greater activity against Gram positive than Gram negative organisms. However, Mallman and Zaikowski (158, p.208) found that alkyl dimethyl benzyl ammonium chloride killed both Gram positive and Gram negative organisms in 10 seconds at 120°F. Baker et al. (8, p.267) and Hoogerheide (101, p.283) found little difference in the effect on Gram positive and Gram negative organisms. Evidently the conclusions obtained by different workers are dependent upon the technique used in determining the germicidal effect of QAC.

Toxicity of quaternary ammonium compounds. An important factor to be considered about any germicide utilized in the dairy industry is its toxicity to man. There is always the possibility of an individual accidentally obtaining a large dose of the germicide. It is desirable, therefore, that a germicide be very active against bacteria, but not very toxic to man. Harshbarger (95, p.172) showed that white rats could have three per cent of their diet made up of Zephiran without causing any ill effects. Shelanski (203, pp.126-128) studied the effect of four different QAC preparations on white rats, guinea pigs and dogs. White rats survived 100 ml. of a 0.1 per cent solution for each kilogram of body weight. Guinea pigs survived 75

ml. Dogs received as drinking water 1:5000 (200 ppm) dilution of QAC. They showed no effect after six months treatment. Warren et al. (225, p.406) determined that one out of six rabbits died from oral injection of cetyl pyridinium chloride at the rate of 400 milligrams per kilogram body weight. No deaths occurred with lesser quantities. Death occurred when over 15 milligrams per kilogram body weight was injected intravenously. Intraperitoneal injection caused death above 3 milligrams.

It was shown by Miller et al.(163, p.175) and Rahn (187, p.3) that QAC formed a film over human skin, of which only the outside was germicidal. This film retained bacteria underneath it, but destroyed any new bacteria which came in contact with the surface. This condition permitted these compounds to be used as antiseptics for hands. Therefore, their irritation to skin is important. Barnes (12, p.530) showed that one per cent CTAB would not irritate skin. Heineman (99, pp.714, 715) was not able to obtain skin irritation with 10 per cent alkyl dimethyl benzyl ammonium chloride.

Practical application of germicides. The use of hypochlorites as mists in an effort to reduce the numbers of bacteria and bacteriophage in the air is important to the dairy industry. Particularly is this true in the reduction of the amount of bacteriophage present. Baker et al. (6, pp.579, 581) showed that mists containing one ppm of available chlorine were effective against E. coli. The same concentration was shown by Masterman (160, p.285) to be effective against the normal flora of bacteria found in the air. Challinor

(27, pp.30-40) obtained approximately the same results. Edward and Lidwell (54, p.199) found that one volume of hypochlorous acid gas to two million volumes of air was effective in destroying 99 per cent of the influenza virus particles in the air. The rapid inactivation of air-borne streptococci and staphylococci was obtained by Elford and Van Den Ende (60, p.11) with 0.1 to 0.3 ppm available chlorine in an atmosphere of 70 to 90 per cent relative humidity. The fact that hypochlorite mists are more effective at a high relative humidity than at a low was shown by Baker and Twort (7, pp.126, 127). Wolf et al. (235, pp.300-310) determined that three different lactic streptococcus bacteriophage races could be destroyed by 0.003 to 0.02 ppm available chlorine in the form of mists. However, Whitehead and Hunter (230, pp.70-76) believed that aerial disinfection was impractical for the dairy industry because of the large volume of air which must be treated to insure bacteriophage control.

The use of hypochlorites on the dairy farm has proven effective. Fay et al.(69, p.241) showed that 100 ppm available chlorine was as efficient in sanitizing milking machine tubing and teat cups as 0.3 per cent lye solution. Fouts (72, p.57) concluded that sodium hypochlorite was effective in sanitizing dairy equipment providing the concentration did not fall below 45 ppm. Byers and Ewalt (25, p.280) obtained very significant reductions in the bacterial content of the milk by using an udder and teat washing solution containing at least 50 ppm available chlorine.

Mastitis is an important problem of the dairy farmer. Bryan et. al. (21, p.83) found that one ppm available chlorine would destroy

alpha, beta, and gamma streptococci in less than one minute. Hughes and Edwards (108, p.449) applied one per cent CTAB in a lanette wax-oil base, twice daily to the teats of cows and the milker's hands for over two months at the time of milking. This treatment appeared to reduce the spread of infection by Streptococcus agalactiae. Spurgeon et al. (208, p.44) showed that both hypochlorites and QAC removed and destroyed sufficient S. agalactiae under laboratory conditions to suggest their use in milk procedure for disinfection of teat cups between cows. They recommended the use of a 500 ppm concentration. Another method of control of mastitis was suggested by Bryan et al. (22, p.419). They infused each infected udder with 75 ml. of 1:1000 (1000 ppm) solution of Phemerol (a QAC). They obtained 86 per cent reduction of infection.

QAC can also be used for sanitization of teat cups. Jensen and Bortree (114, p.337) determined no difference in germicidal efficiency between lye and a cationic germicide when used for this purpose. Krog (139, p.346) showed that alkyl dimethyl benzyl ammonium chloride was an efficient germicide for the sanitization of dairy equipment. Mueller et al. (170, p.128) used a solution of 200 ppm QAC to wash 30 to 40 udders before changing the solution with excellent results. No chapping nor cracked udders and teats developed on this procedure. Kesler et al. (127, p.180) and Reaves (190, p.767) were able to establish no difference between hypochlorites and QAC in germicidal efficiency when used as udder washes. Fabian and Nielsen (68, p.274) showed that a solution of 1:200 (500 ppm) Dowicide A (sodium ortho phenyl phenate) compared very favorably with a solution of 102 ppm

chlorine as a germicidal agent in sanitizing milking machines.

Effect of quaternary ammonium compounds on lactic acid starter organisms. DuBois and Dibblee (49, p.265) found that concentrations of BTC (alkyl dimethyl benzyl ammonium chloride) of 1:500 (2000 ppm) to 1:25,000 (40 ppm) did not influence the bacterial counts of pasteurized or raw milk stored at any temperature. However, concentrations of 1:500 (2000 ppm) and 1:5000 (200 ppm) did inhibit the growth of Streptococcus lactis but not E. coli. Mull and Fouts (171, p.103) determined that Roccal (alkyl dimethyl benzyl ammonium chloride) would have to be added to milk of low quality to the extent of 200 to 250 ppm to bring about a significant decrease in the bacterial count of the milk. Comparable results were obtained by McCulloch et al. (162, p.496). They found that Hyamine 1622 (para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride) had to be present in raw milk in concentration of 1:5000 (200 ppm) to cause a reduction in bacterial count after 24 hours storage. Johns and Pritchard (123, p.503) studied four QAC preparations as milk preservatives. They concluded that each compound exerted a slight but definite bacteriostatic action when added to milk in the highest concentration which might hope to escape detection organoleptically.

Johns (119, pp.79-81) (121, p.28) was able to show that, although QAC is usually more effective against Gram positive organisms, the lactic acid starter organisms were an exception. They appeared to have a resistance against this type of compound. However, Moore (167, p.63) showed that two different QAC preparations inhibited

acid production by a commercial starter culture when present at a concentration of 25 ppm. Concentrations of 50 to 75 ppm gave complete inhibition of acid production. Similar results were obtained by Barber et al. (11, p.56)

Germicide inactivators. In order to study properly the effect of a germicide on bacteria, it is necessary to employ an inactivator to stop germicidal action after the desired period of exposure. The inactivator must not adversely affect bacteria. Zoller (239, p.316) showed that milk absorbed chlorine rapidly at first, but the rate decreased until the milk became saturated. Saturation point was reached after addition of 500 ppm available chlorine. Wright (236, pp.526-530) found that various amino acids and proteins inactivated chlorine. However, the quantity of amino acid or protein determined the amount of inactivation. Caseinogenate and gelatin at 0.05 grams per 100 ml. decreased chlorine content to 30 per cent of the original. However, 0.2 grams per 100 ml. did not decrease the chlorine content nearly so much as the lower concentration. As the concentration increased, the chlorine content was again decreased. Sodium thiosulphate is commonly used to inactivate hypochlorites in studies on their germicidal activities (210, pp.98-100) (226, p.1409).

The problem of methods of inactivation of QAC still is under investigation. Quisno et al. (186, p.318) found that agar reduced the germicidal potency of these compounds. They suggested that the reduction was due to physical adsorption. Lawrence (141, p.58) showed that synthetic anionic detergents as well as soaps failed to

completely inactivate the surface active cationic detergents. Goetchius (82, p.132) suggested the use of Tamol-N as an inactivator for QAC. It required 1:750 (1333 ppm) dilution to inactivate 200 ppm QAC against M. pyogenes var. aureus and 1:4000 (250 ppm) dilution against Eberthella typhosa. The phospholipids lecithin, cephalin and sphingomyelin proved to be effective inactivators, according to Baker et al. (10, pp.630-635). Further proof of the value of lecithin was presented by Brewer (18, p.263) and Weber and Black (228, p.152). The latter authors also found that sodium naphuride would act as an efficient inactivator. However, Ridenour and Armbruster (195, p.121) found that neither lecithin nor naphuride prevented some bacteriostasis by QAC in the standard swab rinse test. As shown by Armbruster and Ridenour (1, p.120) and Weber and Black (227, p.142), the most efficient inactivator was lecithin in combination with Tween 80. The Tween 80 acted as a dispersing medium which enhanced the inactivation role of the lecithin. Recently, Collins et al. (32, p.308) suggested the use of congo red as an inactivator.

Bacteriological tests for germicidal efficiency. In 1913, Rideal and Walker (193, pp.577-580) developed a technique for the determination of the efficiency of germicides. The basis of the test was to add five ml. test solution to 0.5 ml. of a 24 hour broth culture of E. typhosa. Loopfuls then were transferred at 2.5 minute intervals to broth and a record of growth was made. This test, with some modifications became known as the phenol coefficient. The test was satisfactory until phenol coefficients of hypochlorites and QAC

preparations were compared to the actual performance of these compounds (14, p.132) (191, p.1046) (192, p.129) (213, p.125). It was found that the above compounds, especially hypochlorite, were much more active than the phenol coefficient indicated. This fact led to many variations, as well as the development of new test methods. A review on this subject was presented by Mallman (152, pp.101-131).

Pressman and Rhodes (179, pp.139-142) found that transferring 0.05 ml. with a pipette yielded more consistant results with the phenol coefficient test than when a loop was used. Goetchius (83, p.131) and Klarmann and Wright (130, p.117) suggested changes in the culture medium. Goetchius (83, p.131) was able to show that three different sources of beef extract yielded different average phenol coefficients. With Hyamine 1622 (para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride), he obtained erratic phenol coefficients within the three beef extract lots and a different average between the lots. Cade (26, pp.141,155), however, claimed that the phenol coefficient method could be used if plate counts were made. Quisno et al. (184, p.150) (185, p.322) recommended the use of lecithin and Tween 80 in the broth to inactivate any QAC which might be carried over by the loop. The fact that the surface tension of the broth affects the size of the drop used in transfer by a loop was shown by Tobie and Orr (216, p.768). Aerosol OT (di-octyl sodium sulfosuccinate) greatly, but not uniformly, reduced the size of the drop. This difficulty could be overcome by using a 0.02 ml. pipette.

A theory explaining the difficulty in using the phenol coefficient on QAC was proposed by McCulloch (161, p.480). He believed that the germicide caused the organisms to clump and adhere to the sides of the test tube. Thus, they were not picked up in the loop transfer. Conversely, DuBois (46, p.141) thought that QAC was adsorbed by the glass which reduced the actual concentration. Klimek and Umbreit (135, p.142) found no evidence to support the theory that the bacteria were adsorbed by the walls of the test tube.

Klarmann and Wright (131, p.128) (132, p.142) (133, p.157) introduced two variations to the phenol coefficient method which they claimed yielded more accurate results with QAC. In one method, they added 0.5 ml. disinfectant to 0.05 ml. culture. After the desired time of exposure, they added 20 ml. of broth and noted the presence or absence of growth after incubation. In the other modification, they used the regular phenol coefficient method, but had strips of sterile filter paper in the test tube. These strips were removed at desired intervals and transferred to broth to determine growth.

A method utilizing developing chick embryo was used by Green and Birkeland (86, p.56) (87, p.34). They inoculated the chorioallantoic membrane of 11 day old embryo with 0.02 ml. of a 1:10 dilution of 24 hour culture of M. pyogenes var. aureus. After 18 hours, and on each day for five days, they added 0.2 ml. of the test solution. On the sixth day, they stroked the membrane with moist cotton and streaked plates. The method showed that cetyl pyridinium chloride had a definite curative effect on the experimental infection. Kenner et al. (126, p.450) used injection of white mice with Salmonella

typhimurin after exposing the organism to cetyl pyridinium chloride as a method of testing the efficiency of the germicide.

Jensen and Jensen (115, p.490) suggested drying the test organism on a cover slip. The dried cover slip was placed in the germicide for two minutes, removed, washed, and placed in beef broth to determine surviving organisms. A similar method was used by Mallman et al. (156, pp.132, 133). They used glass cylinders, which were exposed to the germicide for varying periods of time after the bacterial film had dried. At the desired exposure time, the cylinders were dropped into 10 ml. broth, shaken thoroughly, and the broth plated on TGE medium. Johns (120, pp.1324, 1325) presented a glass slide technique. He used a bacterial suspension containing 200 x 10^6 organisms per ml. One ml. of this suspension was added to 60 ml. of 1:10 dilution of sterile skim milk. A sterile slide was dipped into this mixture, removed and dried. After drying, the slide was exposed to the test solution for the desired time, removed, rinsed with water, and plated.

Methods utilizing germicidal inactivators were proposed by Costigan (34, p.7) and Mueller et al. (170, p.129). The principle was based on exposing the test organism to a germicide for varying periods of time. A portion then was transferred to an inactivator solution and then plated in serial dilution. This principle also was used by Weber and Black (226, pp.1405-1417). Since this test method with slight modification was used in many of the experiments presented in this thesis, the following is a fairly complete description of the method. The desired test organism was grown for 24

hours on nutrient agar. Five ml. from a sterile water blank were added to the slope, the organism was loosened by a loop, and the contents poured back into the water blank. This suspension should contain 200 x 10^6 organisms per ml. After it was thoroughly shaken, five ml. were transferred into a 25 x 150 mm. sterile test tube with glass cap. The above suspension and the desired germicide solution were kept at 25° C. Five ml. of the germicide solution were added to the bacterial suspension. This mixture was swirled thoroughly and at 15, 30, 60, 120, and 300 seconds it was swirled again and one ml. transferred to 9 ml. of inactivator solution. This solution then was plated in TGE medium. After incubation, the plates were counted to determine the number of surviving organisms. The inactivator tubes contained lecithin at the rate of 2.222 grams per liter and Tween 80 at the rate of 15.8 ml. per liter when QAC was being tested. If chlorine compounds were being studied, the tubes contained sodium thiosulfate at the rate of 80 milligrams per liter. As a final control, the germicide was inactivated by addition of one ml. of lecithin solution containing 40 milligrams and the entire remaining contents of the medicinal tube itself were plated with TGE agar containing 75 per cent normal water. In the case of chlorine compounds, sodium thiosulfate was used for inactivation of germicide.

Effect of chemical configuration on germicidal efficiency. Walker (224, pp.561-563) showed that the germicidal properties of soaps differed markedly when made from different fatty acids. Soaps made from lauric, oleic, linoleic, and linolenic acids were the most

efficient. Bayliss (13, p.498) found that pneumococci were especially susceptible to certain unsaturated soaps; such as, sodium oleate, sodium linoleate, sodium linolenate, and sodium clupanodonate. Other unsaturated soaps as well as hydroxylated and saturated soaps were less effective. Eggerth (59, p.34) demonstrated that soaps in the a-mercapto series were most germicidal when they contained 12 and 14 carbon atoms. Eggerth (57, p.60) also showed that the a-brom fatty acids were usually more germicidal than the unsubstituted soaps. Their activity increased to a maximum as the carbon chain increased to 12 or 14 carbon atoms for Gram negative organisms and to 16 or 18 carbon atoms for Gram positive organisms. Eggerth (58, pp.310, 311) showed that the effect of the hydroxyl group in saturated soaps was to increase selective germicidal action. However, the effect of the hydroxyl group in an unsaturated soap was to decrease this selectivity.

Stanley et al. (212, p.1265) found that a ring structure was unnecessary in order that certain organic acids have a germicidal action. Browning et al. (20, p.1282) found no germicidal difference between the saturated and unsaturated acids. Armendt and Adams (3, p.1290) showed that dodecanoic acid had no germicidal effect toward Bacillus leprae. Tridecanoic acid had practically no effect and tetradecanoic had only slight effect. Against the same organism, Ford and Adams (71, p.1260) found that the alkyl acetic acids containing 16 and 18 carbon atoms were most effective. According to Greer and Adams (89, p.2542), pentadecanoic and heptadecanoic acids were active germicides, but not to such a degree as hexadecanoic

acids.

The germicidal activity of alcohols against Bacterium typhosum and M. pyogenes var. aureus was dependent upon the length of the carbon chain, according to Cowles (35, pp.129-133). The activity increased as the chain length increased from two to eight carbons.

A great deal of research work has been conducted on the effect of chemical configuration of QAC preparations on their germicidal activity. Jacobs et al. (112, pp.571-575) showed that germicidal activity of hexamethylenetetramine was enhanced by methyl, chloride, bromide, iodide, cyanide, and nitrous groups. The ortho position was more active than the meta or para position. Shelton et al. (204, p.754) found that substitution of benzyl, butyl, or ethyl groups for the methyl groups in alkyl trimethyl ammonium bromide had no effect on the germicidal properties. However, Shelton et al. (205, p.756) showed that when the lauryl group was present, the germicidal activity could be increased by the substitution of a carbethoxymethyl or B-acetoxymethyl group. Kolloff et al. (137, p.52) could not increase the germicidal activity of pyridinium alkyl halides and picolinium alkyl halides by the addition of a methyl group into the aromatic nucleus. Shelton et al. (206, p.758) showed that unsaturated cyclic amines reach their peak of germicidal activity with cetyl pyridinium salts. In the unsaturated series, this peak was reached with cetyl methyl piperidinium.

The number of carbon atoms in the alkyl portion of QAC preparations is evidently important for germicidal activity. Baker et al. (8, pp.258-264) claimed that maximum activity was obtained when the

alkyl group contained 12, 14, or 16 carbon atoms. Highest phenol coefficients were obtained by Epstein et al. (66, p.173) when the carbon chain contained 14 carbon atoms. Hoogerheide (101, pp.282, 283) found that the bactericidal potency of cetyl trimethyl ammonium bromide increased greatly as the carbon chain increased from eight to 16. However, Green (85, p.39) found no difference in germicidal efficiency of Emulsol 606, Emulsol 607, and Catol 2, when the carbon chains were varied between 12 and 18 atoms. Valko and DuBois (222, p.483) showed that aliphatic dimethyl ethyl ammonium chloride was most effective when the aliphatic groups contained 12 carbon atoms. However, aliphatic dimethyl ethyl ammonium bromide was most effective with 14 and 16 carbon atoms in the group. It was suggested by Rawlins et al. (189, p.14) that benzene rings could be counted as four atoms, and the long chain should contain 12 to 16 carbon atoms. This latter fact was verified by Lawrence et al. (142, p.357). The following scheme suggested by Epstein et al. (65, p.240) is probably as accurate as most conclusions. They found that C_{14} possessed maximum germicidal activity and the others possessed germicidal activity in the following decreasing order: C_{14}, C_{12}, C_{16}, C_{18}, C_{10}, and C_8.

The effect of the chemical configuration of organic compounds on surface tension possibly affects the germicidal activity of these compounds. Stanley and Adams (221, pp.1551-1556) studied the surface tension and germicidal properties of 120 aliphatic acids. They found, without exception, that all of the germicidally effective acids were marked surface tension depressants. Cowles (35, p.132)

showed that the germicidal activity increased as the carbon chain increased from two to eight and that the surface tension over this range decreased with increase in carbon chain length. Similarly, Dreger et al. (41, p.616) found that the surface tension of sodium alcohol sulfates decreased as the carbon chain increased from 10 to 16 atoms. Lawrence et al. (142, p.355) found that, as the alkyl group of QAC preparations was increased from C_6 to C_8, the surface tension decreased approximately 10 dynes per cm. The same phenomenon occurred from C_8 to C_{10}, but no further change occurred for C_{12}, C_{14}, C_{16}, and C_{18}. The surface tension of various dilutions of cetyl pyridinium chloride was determined by Huyck (110, p.56). He found that a 1:1000 (1000 ppm) dilution had a surface tension of 40.8 dynes per cm., and a 1:10,000 (100 ppm) dilution had a surface tension of 51 dynes per cm. According to his method of determination, water had a surface tension of 71.2 dynes per cm.

Effect of temperature on germicidal efficiency. An efficient germicide must be effective over a wide range in temperature. It is particularly important that the germicide has activity at room temperature and slightly lower, because, in many cases of sanitization, heat cannot be applied. Butterfield et al. (23, p.1860) found that increasing the temperature from 2° C. to 25° C. increased the germicidal activity of chlorine. The effect of 50 ppm chlorine on Mycobacterium tuberculosis at various temperatures was studied by Costigan (33, p.61), who found that the chlorine destroyed the bacteria in 2.5 minutes at 50° C., in one minute at 55° C., and in

0.5 minute at 60° C. Weber and Levine (229, p.724) showed that a drop of 10° C. within the range 20° C. to 50° C. caused a two-fold increase in period of exposure for chlorine to destroy B. metiens spores. Chloramine required a three to four-fold increase in period of exposure. Within the same temperature range, Rudolph and Levine (197, p.32) found that the killing time of hypochlorite solution was reduced 40 to 60 per cent for each 10° C. rise in temperature.

Dunn (50, p.428) showed that the germicidal efficiency of alkyl dimethyl benzyl ammonium chloride was not adversely affected by freezing or storage above 50° C. for 18 days. Temperatures above 70° F. were shown by Krog and Marshall (138, p.346) to not adversely affect the stability and germicidal efficiency of the above compound. Quisno and Foter (182, p.115) found that cetyl pyridinium chloride was active at 20° C. and 37° C. However, Hoogerheide (101, p.283) showed that cetyl trimethyl ammonium bromide increased in efficiency as temperature was increased from 20° C. to 37° C. Similarly, Ridenour and Armbruster (194, p.509) found that QAC became more efficient as sanitizing agents as the temperature increased from 20° C. to 50° C.

Johns, (119, p.88) (121, p.28) showed that increased temperature increased the germicidal efficiency of both hypochlorites and QAC preparations. However, the effect was much greater on the hypochlorites.

Effect of pH on germicidal efficiency. As was shown by Mudge and Lawler (168, p.379) and Levine et al. (146, p.64), alkali

solutions contained some germicidal activity because of the high pH obtained. Levine et al. (145, p.1339) found that sodium hydroxide, trisodium phosphate and sodium carbonate had a germicidal efficiency as a direct function of pH.

Activity of most germicides is affected by pH. Goshorn et al. (84, p.647) found that benzoic acid increased in germicidal efficiency as the pH decreased. Bittenbender et al. (15, p.743) (16, p.997) showed that chlorazene, gentian violet, hexylresorcinol, iodine, Listerine, Lysol, malachite green, mandelic acid, mercurochrome, Pepsodent antiseptic, phenylmercuric nitrate, phenol, and potassium permanganate became more effective antiseptics as the pH was decreased from 8.0 to 3.0.

It is fairly well agreed that chlorine is more active germicidally in an acid pH, but is much less stable (143, p.123). Costigan (34, p.6) found that hypochlorites were more active against E. typhosa and M. pyogenes var. aureus at pH 6.8 than at pH 8.4. Butterfield et al. (23, pp.1851-1858) showed that the germicidal activity of chlorine decreased as the pH increased from 6.5 to 10.7. At pH 8.5, 9.8, and 10.7, E. typhosa was more sensitive to chlorine than E. coli or Pseudomonas pyocyania. However, at pH 6.5, 7.0, and 7.8, the opposite was true. Rudolph and Levine (197, p.41) found that hypochlorite solutions above pH 8.0 were greatly affected by slight increases in pH. On the other hand, in solutions below pH 8.0, the effect was distinctly less marked. Mallman and Schalm (157, p.19) claimed that the germicidal activity of chlorine decreased as the pH decreased until pH 11.0 was reached. At this

point the alkali itself became germicidal. Charlton and Levine (28, p.168) found that chloramine T and calcium hypochlorite destroyed B. metiens spores more rapidly as the pH changed from 8.8 to 6.0. Additional work by Charlton and Levine (29, p.54) showed that 1000 ppm available chlorine at pH 11.3 required 64 minutes to destroy B. metiens spores. The same concentration at pH 7.3 required only 20 seconds. Johns (116, p.39) found that at 20° C., the destruction of E. coli was decreased by increased pH. However, at 50° C., the reverse was true. M. pyogenes var. aureus, on the other hand, was destroyed less rapidly as the pH increased at both temperatures.

Wolf and Cousin (234, p.755) determined that the optimum pH for the destruction of M. pyogenes var. aureus by hypochlorites was dependent upon the concentration of the germicide. Optimum kills for 25, 50, 100, and 200 ppm chlorine were obtained at pH 9.4, 9.8, 10.5, and 11.0 respectively. The fact that the influence of the pH of the chlorine solution was even greater than that of the concentration of the germicide was shown by Johns (117, pp.589, 590). Scales and Kemp (200, p.219) found by laboratory and plant tests that 50 ppm chlorine at pH 6.0 would produce as satisfactory germicidal results as 255 ppm at pH 10.0.

Wright (237, p.1666) concluded that the mode of action of chlorine was dependent upon the pH. In an acid solution the mode of action was believed to be chlorination; whereas, in an alkaline solution, it was believed to be oxidation.

Eggerth (55, p.158) showed that soaps containing short chain fatty acids were most active germicidally in acid solution. However,

the long chain fatty acid soaps were most active in alkaline solution. Several workers (8, p.269) (77, p.324) (78, p.91) (79, p.253) (201, p.518) found that the anionic wetting agents had greater germicidal activity in acid solutions. An exception to this rule was obtained by Sreenivasaya et al. (209, p.1708). They showed that tobacco mosaic virus was not disintegrated by sodium dodecyl sulfate below pH 7.0. The best disintegration was obtained at pH 8.0.

Baker et al. (8, p.269) and Gershenfeld and Milanick (77, p.324) claimed that the cationic germicides were most active in alkaline solutions. Yenson (238, p.813) showed that cationic detergents produced the greatest amount of protein precipitation at pH 9.2 to 11.0. Johns (121, p.28) demonstrated that the germicidal activity of QAC increased as the pH rose. According to Ridenour and Armbruster (194, p.510) the efficiency of these compounds against E. typhosa was greater as the pH increased with a marked increase at pH 9.0. Similarly, Gershenfeld and Ibsen (76, p.298) found the greatest activity against M. pyogenes var. aureus at pH 9.0. The activity progressively decreased to pH 5.0. Alkyl dimethyl benzyl ammonium chloride was proven by Dunn (51, p.50) to be more active against M. pyogenes var. aureus, B. subtilis, and E. coli at pH 8.9 than at pH 6.4 or pH 1.3. Quisno and Foter (183, p.115) showed that cetyl pyridinium chloride was an active germicide between pH 2.0 to 10.0. However, the relative activity over this range was not determined.

Probably the most comprehensive study on the effect of pH on QAC was made by Hucker et al. (107, pp.10-20). They found that the

optimum pH was dependent upon the compound studied. Those most active in the alkaline range were BTC, Quartol, and QB. Ceepryn, Emulsept, and Hyamine 1622 were most active in the acid range. CTAB, Tetrosan, Q Cl, and Hyamine 10 X were active in either alkaline or acid solution. All compounds were least active at neutrality. The pH's used in this study were 3.0, 5.5, 7.0, and 9.0. The buffers to obtain these values were glycocoll-sodium chloride for pH 3.0, sodium citrate for pH 5.5, sodium borate for pH 7.0, and glycocoll-sodium hydroxide for pH 9.0.

Effect of organic matter on germicidal efficiency. Hart and Stabler (96, p.48) showed that the addition of a small amount of milk to a calcium hypochlorite solution rapidly dissipated the available chlorine, due to the presence of organic matter. However, Neave and Hoy (172, p.49) were able to add 0.2 per cent whole milk to 200 ppm hypochlorite solution without materially reducing its germicidal efficiency. These findings indicate that small amounts of organic matter probably will not affect hypochlorites adversely, but that larger amounts are very detrimental. In fact, Johns (122, p.102) showed that the presence of 0.05 per cent skim milk potentiated the germicidal activity of these germicides.

Organic material also affects the germicidal efficiency of organic germicides. Soaps, as shown by Eggerth (56, p.683) have their germicidal effect reduced by animal serum. Hoogerheide (101, p.282) found that blood serum and milk would reduce the effectiveness of cetyl trimethyl ammonium bromide. Quisno and Foter (183, p.115)

also found that animal serum lowered the germicidal properties of cetyl pyridinium chloride. Similar results were obtained by Ridenour and Armbruster (194, p.510). Mueller et al. (170, p.129) demonstrated that 0.3 per cent cow dung or non-fat milk solids were required to first decrease the germicidal potency of 200 ppm QAC. MacPherson (149, p.200) claimed that normal organic material would not greatly affect the activity of these compounds.

A few comparisons of the effect of organic material on hypochlorites and QAC have been made. Klarmann and Wright (129, p.105) added 10 per cent horse serum and 10 per cent horse blood to chlorine and QAC solutions. They found that the chlorine retained only 1.5 per cent of its original activity when exposed to the horse serum and 0.5 per cent when exposed to the horse blood. One QAC retained 3.0 per cent and 0.6 per cent, respectively. The other QAC retained 15 per cent and 10 per cent, respectively. Dvorkovitz and Crocker (52, p.117) showed that 1500 ppm milk solids affected the germicidal activity of hypochlorites and QAC equally. Johns (122, p.102) demonstrated that both germicides retained their effectiveness in concentrations of skim milk up to two per cent. Above this concentration, hypochlorite activity decreased rapidly, whereas QAC activity decreased gradually. The concentration of the germicides was 200 ppm.

Effect of water ions on germicidal activity. Johns (121, p.28) found that hard water decreased the activity of both hypochlorites and QAC. Shere (207, p.68) showed that increasing water hardness to

400 ppm had a great and variable effect in reducing the germicidal power of QAC. As shown by Ridenour and Armbruster (194, p.509), 275 ppm water hardness decreased the efficiency of these compounds. They found that the calcium and magnesium ions caused the decrease in efficiency. Sodium and potassium ions had very little effect. In later work, Armbruster and Ridenour (2, p.105) demonstrated that 40 ppm calcium or magnesium reduced the effectiveness of 35 ppm alkyl dimethyl benzyl ammonium chloride from 99.9 per cent to 50 per cent kill. Increasing the calcium ion above 40 ppm did not show an additional effect. Also, 10 ppm ferric ion in 200 ppm of the germicide decreased its ability to destroy bacteria. Water softening compounds increased the germicidal activity of water containing 75 ppm calcium, 25 ppm magnesium, and 2 ppm ferric ions. Mallman and Harley (154, p.129) also found that 85 ppm and 450 ppm hard water allowed good germicidal activity with alkyl dimethyl benzyl ammonium chloride when softened with lime-soda.

Potentiation of germicidal activity. Tobey and Orr (217, p.743) showed that 0.1 per cent Aerosol OT (di-octyl sodium sulfosuccinate) increased the phenol coefficient of phenol from 1.0 to 1.8, USP cresol from 2.4 to 4.4, and phenylmercuric nitrate from 166 to 1300. Ordal et al. (175, p.124) found that the addition of wetting agents to buffered solutions of phenolic compounds increased the germicidal activity of such solutions. Ordal and Deromedi (174, p.298) also demonstrated that sodium lauryl sulfonate and the dioctyl ester of sodium sulfosuccinate enhanced the germicidal activity of solutions

containing 2,4-dichlorophenol or 2,4,6-trichlorophenol. Their evidence indicated that the enhancement of the germicidal activity was primarily due to a synergistic action between the wetting agent and the undissociated phenol. Gershenfeld and Perlstein (79, p.253) showed that Aerosol OT (di-octyl sodium sulfosuccinate) increased the germicidal activity of phenol, mercuric chloride, Merthiolate and hexylresorcinol. However, 39 other wetting agents were tested by Gershenfeld and Witlin (81, p.234) and they found no increased germicidal efficiency with 16 phenolic, 10 mercurial and two halogen compounds. In more recent work, Gershenfeld and Sagin (80, p.233) were able to show that Tergitol 4 (sodium tetradecyl sulfate) and Tergitol 08 (sodium octyl sulfate) had a definite synergistic effect against E. typhosa with sulfaguanidine.

The effect of alkyl aryl sulfonate on chlorine solutions was studied by Scales and Kemp (199, pp.206, 207). They found that it tended to increase activity in high pH chlorine solutions. Fabian et al. (67, pp.1172, 1173) added 0.5 per cent sodium carbonate, sodium hydroxide or trisodium phosphate to sodium hypochlorite. They concluded that the trisodium phosphate in hypochlorite solution yielded the most effective germicide. The sodium carbonate proved to be the least effective. Sodium hydroxide is itself germicidal (144, p.1365) (147, p.180) (215, p.102). Trisodium phosphate and sodium carbonate appear to be less germicidal than sodium hydroxide.

In the addition of other compounds to QAC, it is important that the two substances are compatible. Powney (178, p.76) showed that sodium hexametaphosphate caused a precipitate with cetyl pyridinium

bromide. Tetrasodium pyrophosphate yielded a slight precipitate and trisodium phosphate gave no precipitate. Mallman et al. (156, p.133) claimed that wetting agents or polyphosphates had no effect on QAC.

Mallman (151, p.469) showed that sodium hexametaphosphate aided detergent mixtures in the removal of bacteria. Guiteras (90, p.636) demonstrated that when cation-active agents were used as germicides in detergent compositions, it was essential that the detergent was emulsifying but not saponifying. If the alkalinity of the detergent was sufficiently high to saponify fat, the resulting soap would inactivate the cation-active agent and render the solution completely ineffective germicidally. The use of detergent sanitizers containing QAC to sanitize dairy farm equipment was favorably considered by several workers (31, p.45) (104, p.35) (105, p.44)(181, p.137). Mallman et al. (155, p.177) indicated that these compounds yielded results comparable to conventional cleaning methods using standard cleaner and hypochlorite germicides. Elliker et al. (64, p.223) reported that satisfactory results were obtained on dairy farm sanitation when either the standard conventional cleaner and hypochlorite germicide or a detergent sanitizer was used.

Chemical determination of germicides. The iodometric method for the determination of available chlorine in water solution is given in "Standard Methods for the Examination of Water and Sewage" (210, pp.98-100). This test is dependent upon the release of iodine from potassium iodide by the chlorine. The quantity of iodine can then be titrated with sodium thiosulfate. Tests to determine free

chlorine in milk have been developed by Butterworth (24, p.36) and Rupp (198, pp.2-5) based on the above principle.

Various test methods have been recommended for the determination of QAC. Review articles were presented by DuBois (45, pp.125-141) (47, pp.122-125). Flanagan et al. (70, p.164) recommended the use of Tamol N containing a brilliant blue dye. A precipitate determined the quantity of QAC present in a water solution. However, the test is limited to certain compounds. Gain and Lawrence (74, p.526) used horse serum to produce a precipitate. DuBois (43, p.745) developed an argentimetric method using eosin and dichlorofluorescin indicators. He recommended the test as a supplementary control over the quality of commercial compounds. Iodine titration methods were suggested by DuBois (42, p.246) and Hager et al. (91, pp.886, 887) when the type of compound is known.

Most test methods are based on the findings of Hartley (97, 444-450) and Hartley and Runnicles (98, pp.424-438). They found that paraffin chain cations had a significant effect on the color of certain indicators. Bromphenol blue proved to be most suitable in determining the presence of these compounds. Brooks and Hucker (19, p.137), Cucci (36, p.130) and Krog and Marshall (138, p.346) utilized this principle in developing tests for the determination of QAC preparations. All three methods were recommended as rapid field tests, and therefore, are not so accurate as might be desired. Auerbach (4, p.493) (5, p.739) developed a laboratory test for QAC in dilute solution. His principle was to dissolve the cation in ethylene dichloride and determine with a photocolorimeter the color derived

by the addition of bromphenol blue. The quantity present was demined by comparison with known standards. The quantity had to be less than 20 ppm. Colichman (30, p.431) modified this method to include quantities between 0 and 500 ppm. Wilson (232, pp.315-326) (233, pp.481-483) developed a test for the presence of QAC in various foods including milk. His test for fruit juices was modified by Harris (93, pp.310, 311). The test was dependent upon the reaction of the quaternary nitrogen in weakly alkaline solution with bromphenol blue to form a product soluble in ethylene chloride. However, on the basis of data obtained by cooperating investigators, Wilson (233, p.483) suggested that further studies be carried out before recommending this procedure as an official method for milk. Harper et al. (94, pp.159, 160) developed a procedure using eosin-yellowish as an indicator and tetrachlorethane as a solvent. The test was based on the formation of a red precipitate between the dye and the QAC. The quantity of cation can be determined by titration to a colorless end point with an anionic surface active agent.

DuBois and Dibblee (49, p.265) claimed that the Hartley-Runnicles method could be used to detect QAC in milk at dilutions of 1:1000 (1000 ppm) to 1:20,000 (50 ppm). However, this claim has not been verified by other workers.

PART I

GERMICIDAL ACTIVITY OF VARIOUS HYPOCHLORITES AND QUATERNARY AMMONIUM COMPOUNDS ON DIFFERENT SPECIES OF BACTERIA

Any study on the activity of germicides must consider the fact that there may be variations in efficiency when different species of organisms are used. Also, differences may occur with the same species when a particular type of germicide varies in its chemical configuration. The available QAC preparations vary greatly in this property. The literature on these compounds is not in full agreement as to their effectiveness when different species of bacteria are tested. Some of this lack of agreement is due to the method of determining germicidal activity. Some, apparently, is due to the failure to study sufficient numbers of bacterial species. This study was undertaken to provide additional information on relative germicidal activity of different QACs on selected species of bacteria representative of a number important in the dairy industry.

There has been a need for a direct comparison of germicidal activity of QACs and the conventional hypochlorites used for dairy and food plant sanitation. With this in mind, a representative sodium hypochlorite (NaClO) was included in the germicidal studies. Lithium hypochlorite (LiClO) has shown some advantages in solubility over some commercial hypochlorite preparations. It has not been accepted for use as a sanitizer on dairy and food equipment. However, studies on its germicidal activity were carried out because of its possible future importance.

MATERIALS AND METHODS

Organisms. The organisms selected for this study were E. coli, Aerobacter aerogenes, P. aeruginosa, M. pyogenes var. aureus, and a Sarcina species. The cultures were grown on nutrient agar slopes, and were transferred daily throughout the experiment. Periodical microscopic examinations were made to make certain that the cultures remained pure.

Germicides. The QACs used in this experiment were commercial preparations of methyl dodecyl benzyl trimethyl ammonium chloride, (QAC 1), para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride (QAC 2), alkyl dimethyl benzyl ammonium chloride (QAC 3), and alkyl dimethyl ethyl benzyl ammonium chloride (QAC 4). A detergent sanitizer (DS) containing 10.7 per cent para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride also was used. The remainder of this preparation included about five per cent nonionic wetting agent and the remainder was tetrasodium pyrophosphate. The NaClO employed for comparison with QACs was a liquid preparation. Previous studies indicated its germicidal activity to be representative of a number of the more active commercial products. The trade name for this product was X-4, and it carried 4.62 per cent available chlorine. The LiClO was provided in the form of a powder by Klenzade Products, Inc., and carried 50 per cent available chlorine.

The desired concentration of germicide was obtained by the addition of the amount recommended by the manufacturer to distilled water. The concentration of this solution was established by

titration. For the QACs, the test method recommended by Harper et al. (94, pp.159, 160), and for the hypochlorites, the thiosulfate titration (210, pp.98-100) were used.

Inactivator solutions. The inactivator for the QACs contained 2.222 grams of lecithin and 15.8 ml. Tween 80 per liter of distilled water. The hypochlorites were inactivated by 80 milligrams sodium thiosulphate per liter of distilled water. Each inactivator contained 1.25 ml. M/4 phosphate buffer per liter, to give a pH of 7.2. This pH was adjusted with a Beckman pH meter. The inactivator was pipetted in nine ml. portions into a test tube and sterilized in an autoclav for 20 minutes at 15 pounds pressure.

Method of testing germicidal activity. The test method reported by Weber and Black (209, pp.1406-1415), with a few modifications was used to determine the efficiency of the germicides. The test organism was grown for 24 hours in nutrient broth at 32° C. It then was transferred to a nutrient agar slope, which was incubated for 24 hours at 32° C. About five ml. of sterile water from a water blank was added to the slope, the organisms were removed by the use of a sterile needle, and then were poured back into the water blank. The suspension was filtered by suction through filter paper to remove clumps. It then was adjusted with a Beckman spectrophotometer at 440 mu wave length so that it would contain 200 x 10^6 organisms per ml. Five ml. of this suspension was pipetted into a sterile glass-capped 25 x 150 mm. test tube. Care was taken to insure that the pipette did not touch the wall of the tube.

The germicide and concentration of bacterial cells to be tested both were made up to double the desired concentration. Five ml. of the germicide was rapidly pipetted into the medicinal tube containing five ml. of the bacterial suspension. Test materials were maintained at 25° C. throughout the experiment. By the use of an electric timer, one ml. of the mixture was transferred to nine ml. portions of inactivator at 15, 30, 60, 120, and 300 seconds.

After the germicide had been inactivated, the inactivator tube was plated in tryptone, glucose, beef extract (TGE) agar containing one gram lecithin and seven ml. Tween 80 per liter. The plating dilutions were 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. The plates were incubated for 48 hours at 32° C. and counted. Plate counts of the original bacterial suspensions also were made. The per cent of organisms killed was calculated from the number of original bacteria in the suspension and the number of surviving bacteria.

RESULTS

Concentrations of 50, 100, and 200 ppm of NaClO produced 100 per cent kill in every instance with E. coli, A. aerogenes, P. aeruginosa, M. pyogenes var. aureus, and Sarcina sp. exposed for the minimum period of 15 seconds.

The activity of various concentrations of the other 4 germicides on five different bacterial species are shown in tables 1 to 4. The tabulated figures are the average of three separate determinations. The activity of lithium hypochlorite on the same organisms at the same concentrations is shown in table 1. In all cases except with 50 ppm on P. aeruginosa, 100 per cent kill was obtained with an exposure of 15 seconds. P. aeruginosa required 60 seconds to effect 100 per cent kill. However, at 15 and 30 seconds, 99.999 per cent of the organisms were destroyed.

Table 2 shows the effect of alkyl dimethyl benzyl ammonium chloride on the same organisms at the same concentrations as that of the sodium hypochlorite. At a concentration of 50 ppm, the QAC required 30 seconds for A. aerogenes, 60 seconds for Sarcina sp.,and M. pyogenes var. aureus, 120 seconds for E. coli, and 300 seconds for P. aeruginosa to produce 100 per cent kill. With the exception of P. aeruginosa, 100 ppm and 200 ppm concentrations destroyed 100 per cent of all organisms in 15 seconds. This organism required 60 and 30 seconds, respectively, for 100 per cent kill. These results indicate that P. aeruginosa was very much more resistant to this compound than were the other four organisms. E. coli was next most

TABLE 1

Rate of destruction of five different bacterial species by a lithium hypochlorite germicide

Organism	Conc. of LiClO (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
E. coli	50	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		99.999	99.999	100.000	100.000	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000
E. coli	100	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		100.000	100.000	100.000	100.000	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000
E. coli	200	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		100.000	100.000	100.000	100.000	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000

TABLE 2

Rate of destruction of five different bacterial species by alkyl dimethyl benzyl ammonium chloride

Organism	Conc. of QAC (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
E. coli	50	98.362	99.881	99.998	100.000	100.000
A. aerogenes		99.999	100.000	100.000	100.000	100.000
P. aeruginosa		99.795	99.983	99.998	99.997	100.000
Sarcina sp.		99.993	99.999	100.000	100.000	100.000
M. pyogenes var. *aureus*		95.296	99.961	100.000	100.000	100.000
E. coli	100	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		99.998	99.999	100.000	100.000	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. *aureus*		100.000	100.000	100.000	100.000	100.000
E. coli	200	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		99.999	100.000	100.000	100.000	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. *aureus*		100.000	100.000	100.000	100.000	100.000

resistant, and A. aerogenes was least resistant. The Sarcina sp. and M. pyogenes var. aureus showed about equal resistance. These two species were more resistant than A. aerogenes, but less resistant than P. aeruginosa and E. coli.

The activity of para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride at three concentrations against five organisms is shown in table 3. A concentration of 50 ppm did not destroy 100 per cent of the P. aeruginosa cells in 300 seconds. It did destroy 100 per cent of the E. coli, Sarcina sp., and M. pyogenes var. aureus cells in 120 seconds. A. aerogenes required only 30 seconds for 100 per cent kill. The 100 ppm concentration did not destroy 100 per cent of the P. aeruginosa cells in 300 seconds. It did destroy 100 per cent of the cells of E. coli and M. pyogenes var. aureus in 30 seconds, and of A. aerogenes and Sarcina sp. in 15 seconds. A period of 300 seconds was required to destroy 100 per cent of the cells of P. aeruginosa with 200 ppm. However, the 4 other species tested were destroyed in 15 seconds by 200 ppm.

Table 4 shows the effect of three concentrations of a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride on five species of bacteria. A QAC of 50 ppm killed 100 per cent of the cells of E. coli, A.aerogenes, and M. pyogenes var. aureus in 15 seconds. P. aeruginosa required 120 seconds, and Sarcina sp. 30 seconds for 100 per cent destruction. The latter two organisms were completely destroyed in 30 seconds by 100 ppm of the QAC. A concentration of 200 ppm killed all species in 15 seconds. Again, P. aeruginosa showed the greatest resistance to

TABLE 3

Rate of destruction of five different bacterial species by para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride

Organism	Conc. of QAC (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
E. coli	50	99.760	99.995	99.999	100.000	100.000
A. aerogenes		99.999	100.000	100.000	100.000	100.000
P. aeruginosa		92.231	93.615	95.846	98.208	99.231
Sarcina sp.		99.983	99.998	99.999	100.000	100.000
M. pyogenes var. aureus		96.300	98.720	99.980	100.000	100.000
E. coli	100	99.998	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		94.692	98.131	99.267	99.739	99.654
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		99.340	100.000	100.000	100.000	100.000
E. coli	200	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		99.812	99.938	99.995	99.999	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000

TABLE 4

Rate of destruction of five different bacterial species by a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride

Organism	Conc. of QAC (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
E. coli	50	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		99.998	99.999	99.999	100.000	100.000
Sarcina sp.		99.996	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000
E. coli	100	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		99.998	100.000	100.000	100.000	100.000
Sarcina sp.		99.999	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000
E. coli	200	100.000	100.000	100.000	100.000	100.000
A. aerogenes		100.000	100.000	100.000	100.000	100.000
P. aeruginosa		100.000	100.000	100.000	100.000	100.000
Sarcina sp.		100.000	100.000	100.000	100.000	100.000
M. pyogenes var. aureus		100.000	100.000	100.000	100.000	100.000

the QAC germicide.

An attempt to show the variation in germicidal activity by different compounds is presented in table 5. QAC 1, QAC 2, DS, NaClO and LiClO values are the same as those found in tables 1 to 4 for E. coli. QAC 3 and QAC 4 values were obtained from other experimental problems presented in this thesis. The values given are the average of three separate trials.

The 50 ppm concentration figures show most strikingly the difference in activity of the various germicides. QAC 1 did not destroy 100 per cent of the E. coli cells in 300 seconds. QAC 2 and QAC 3, on the other hand, killed 100 per cent of the cells in 120 seconds. QAC 4, as well as both the hypochlorites and the detergent sanitizer completely destroyed the organism in 15 seconds. The detergent sanitizer contained QAC 2 as the germicidal agent. It appeared that some factor in the detergent sanitizer must have potentiated the activity of the QAC against the organism.

QAC 1 also was less active at a concentration of 100 ppm. It required 120 seconds to kill 100 per cent of the E. coli cells. QAC 2 required only 30 seconds to destroy 100 per cent of the cells, and all other compounds required only 15 seconds. QAC 1 was the only compound which did not destroy 100 per cent of the organisms in 15 seconds when a concentration of 200 ppm was used. It required 30 seconds.

A comparison of effect of NaClO on P. aeruginosa in this section and results in tables 19 and 29 demonstrate that NaClO was more germicidal under all conditions of exposure than the most active QAC.

TABLE 5

Rate of destruction of Escherichia coli by various quaternary ammonium compounds and hypochlorites

Germicide	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
QAC 1	50	91.860	99.541	99.983	99.994	99.998
QAC 2		99.760	99.995	99.999	100.000	100.000
QAC 3		98.362	99.881	99.998	100.000	100.000
QAC 4		100.000	100.000	100.000	100.000	100.000
DS		100.000	100.000	100.000	100.000	100.000
NaClO		100.000	100.000	100.000	100.000	100.000
LiClO		100.000	100.000	100.000	100.000	100.000
QAC 1	100	99.946	99.998	99.999	100.000	100.000
QAC 2		99.998	100.000	100.000	100.000	100.000
QAC 3		100.000	100.000	100.000	100.000	100.000
QAC 4		100.000	100.000	100.000	100.000	100.000
DS		100.000	100.000	100.000	100.000	100.000
NaClO		100.000	100.000	100.000	100.000	100.000
LiClO		100.000	100.000	100.000	100.000	100.000
QAC 1	200	99.997	100.000	100.000	100.000	100.000
QAC 2		100.000	100.000	100.000	100.000	100.000
QAC 3		100.000	100.000	100.000	100.000	100.000
QAC 4		100.000	100.000	100.000	100.000	100.000
DS		100.000	100.000	100.000	100.000	100.000
NaClO		100.000	100.000	100.000	100.000	100.000
LiClO		100.000	100.000	100.000	100.000	100.000

DISCUSSION

Although the results in this section do not show it, various changes in resistance of an organism can be observed over a long period of time, even though every attempt is made to keep all factors constant. This phenomenon was particularly noted with P. aeruginosa, which slowly became more resistant over a period of several months with daily transfer of the stock culture on nutrient agar. This shift in resistance did not affect the results of this study, but it is considered significant where comparisons are made in sections of an investigation carried out over extended periods.

The available literature is not in complete agreement as to the relationship of the Gram reaction and the efficiency of QACs. In general, it seems to favor the belief that these compounds are more effective against Gram positive than against Gram negative organisms. Unfortunately, many of the conclusions have been based on the use of E. coli and M. pyogenes var. aureus alone. In such comparisons, E. coli usually has shown greater resistance to the QACs.

A second factor which has led to erroneous conclusions in evaluation of germicidal activity of QACs is the method of testing employed. Most early experimental work on QACs used the phenol coefficient. It now is fairly well agreed that the phenol coefficient does not yield accurate results for the activity of QACs and hypochlorites. The consistent results obtained with the Weber and Black procedure in these studies indicate that it should provide a more reliable estimate of germicidal activity of QAC and hypochlorites in

laboratory trials than the phenol coefficient or other earlier methods.

The data presented here indicates that the germicidal efficiency of QACs is not necessarily related to the properties of the cell contributing to the Gram reaction. Of the five organisms studied, one Gram negative species was less resistant and two Gram negative species more resistant to QAC action than the two Gram positive organisms. There is no apparent explanation for the markedly greater resistance to QAC of P. aeruginosa than the other species studied. It is possible that the critical enzyme systems of P. aeruginosa are less affected than those of other species. Work of Knox et al. (125, p.456) suggests lactic acid oxidase of E. coli to be the critical enzyme system affected by QAC with this organism. Enzyme studies on P. aeruginosa may establish the difference in effect of QAC on this species and a more susceptible organism as, for example, E. coli.

The superiority of the NaClO (and LiClO) over all QAC preparations is contrary to reports of some investigators. However, the results have been highly consistent and have been repeated in a number of different trials. The Weber and Black method is believed to provide a truer picture of activity by germicidal solutions. At least some of the discrepancy in previous reports may be traced to faults in method of assessing germicidal activity. The high resistance of species such as P. aeruginosa, which closely resembles a number of food spoilage bacteria suggests that the QAC carries some liability when employed as the sole sanitizing agent in a dairy or food equipment sanitizing program. There always exists the

possibility that, unless cleaning procedures are thorough, certain Pseudomonas species may survive the sanitization step and, by an adaptation process, gradually accumulate in increasing numbers on QAC sanitized equipment. Such an accumulation of resistant strains or adaptation to increasingly greater concentrations of germicide should be less likely to develop in the case of NaClO. This may be related in part to the fundamental action of the two types of germicides on specific enzyme systems of bacterial cells.

Differences in germicidal activity between the various QACs are, without question, related to structure of the QAC molecule. The alkyl dimethyl benzyl type appears to produce a more germicidal compound than the para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride and methyl dodecyl benzyl trimethyl ammonium chloride. The alkyl dimethyl benzyl molecule with the addition of an ethyl group appears to show a marked increase in germicidal activity. The results suggest that other modifications in structure of the QAC molecule are possible, and that the limit of germicidal activity by the QAC as such have not necessarily been reached.

The effectiveness of the detergent sanitizer studied indicates that some factor present in the mixture enhanced its activity. There are three possibilities as to reasons for this greater activity. (1) The pH is higher than normally found in a QAC solution. (2) The mixture contains a wetting agent. (3) Presence of tetrasodium pyrophosphate may enhance activity. The role of these factors will be discussed more fully in Section IV.

SUMMARY AND CONCLUSIONS

Sodium hypochlorite in a concentration of 50 ppm destroyed all cells of E. coli, A. aerogenes, P. aeruginosa, Sarcina sp., and M. pyogenes var. aureus in 15 seconds when tested by the Weber and Black method. LiClO was almost as effective as the NaClO. The one exception was that LiClO required 60 seconds to kill all P. aeruginosa cells; whereas, NaClO destroyed them in 15 seconds.

Alkyl dimethyl benzyl ammonium chloride and para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride were most effective against A. aerogenes and least effective against P. aeruginosa.

A detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride destroyed all cells of E. coli, A. aerogenes and M. pyogenes var. aureus in 15 seconds when present in a concentration of 50 ppm. P. aeruginosa required 120 seconds and Sarcina sp. required 30 seconds.

Concentrations of 50 ppm sodium hypochlorite, lithium hypochlorite, alkyl dimethyl ethyl benzyl ammonium chloride, and a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride destroyed all cells of E. coli in 15 seconds. Alkyl dimethyl benzyl ammonium chloride, para di-isobutyl phenoxy ethoxy benzyl ammonium chloride and methyl dodecyl benzyl trimethyl ammonium chloride followed in decreasing sequence in germicidal efficiency.

There did not appear to be a close relationship between the Gram reaction and susceptibility of the species to QAC germicides.

The detergent sanitizer showed strikingly greater germicidal activity than the unfortified QAC from which it was prepared.

PART II

EFFECT OF WETTING AGENTS ON THE GERMICIDAL ACTIVITY OF HYPOCHLORITE.

Information in the literature indicates the possibility that the activity of various germicides can be enhanced by the presence of wetting agents. (81, p.234) (175, p.124) (199, p.206) (217, p.743). Most of the work has been done on phenolic type compounds. The reports available suggest that germicidal activity of hypochlorites might be increased by presence of a wetting agent, provided the wetting agent did not inactivate the chlorine compound. This would be particularly important under conditions which require the chlorine to destroy organisms that have dried on equipment in the form of a film. The wetting agent should allow the germicide better access to the organism by loosening the film from the surface of the equipment.

It is also possible that surface tension reduction would affect the action of chlorine on the organism itself. Therefore, the following experiment was attempted to determine whether or not wetting agents would affect the activity of hypochlorites against organisms in a water suspension.

MATERIALS AND METHODS

Organisms. The organisms selected for this study were Micrococcus caseolyticus and a spore suspension of Bacillus cereus. The M. caseolyticus was transferred daily throughout the entire experiment on nutrient agar slopes and was incubated at 32° C. The B. cereus cultures were grown for seven days in nutrient broth at 28° C. The culture was agitated on a mechanical shaker during the entire growth period. After seven days of growth, the culture was heat shocked at 80° C. for 10 minutes. The spores were removed by centrifugation, and were washed three times with sterile water to remove any organic material which might inactivate chlorine. They finally were suspended in sterile water. This suspension contained 170 x 10^4 spores per ml. and was used during the entire experiment.

Germicides and wetting agents. A commercial liquid sodium hypochlorite (X-4) was used during most of the experiment. A few trials were completed using 2 commercial calcium hypochlorites. One of these was LoBax Special, a 50 per cent available chlorine calcium hypochlorite. The second was LoBax 21, a 21 per cent available chlorine calcium hypochlorite with 6 per cent sodium, decyl benzyl sulfonate.

The following anionic wetting agents were used: Nacconal MX (alkyl aryl sulfonate), Nacconal NRCL (alkyl aryl sulfonate), Nytron (mixture of sulfonated ketones, amines, and alkyl sulfamates), Stepanate (alkyl aryl sulfonate), Alkanol DM (alkyl aryl sulfonate), Kleer Mor (alkyl aryl sulfonate), Kleer Mor Special (alkyl aryl

sulfonate), Duponal Paste (sodium sulfated mixed alcohol), and M189 (saturated hydrocarbon sodium sulfonate). The nonionic agents available were: Igepal CA Extra (alkyl aryl polyethylene glycol ether), Santomerse # 1 (alkyl aryl sodium sulfonate), Neutronyx 600 (aromatic poly glycol ether), Sterox CD (polyoxyethylene ether) and X-100 (alkylated aryl polyether alcohol). All germicide and wetting agent solutions and combinations of these were prepared with distilled water.

Method of determination. In order to determine the effect of the wetting agents on available chlorine, the 14 compounds were added to individual solutions containing 50 ppm of sodium hypochlorite to provide concentrations of wetting agents of 0.01 and 0.10 per cent in the final germicide solution. The solutions were titrated for available chlorine by the thiosulfate method (210, pp.98-100) immediately following the addition of the wetting agent and after 6, 24, 48, and 168 hours of exposure. The solutions were stored in the dark to prevent destruction of the chlorine by sunlight. Nacconal NRCL, Santomerse # 1, Stepanate, Alkanol DM and X-100 were chosen for further study on the basis of the data obtained in this experiment.

The method of determining the germicidal activity of the hypochlorite wetting agent mixture was similar to that described in PART I. Without adjusting the pH, effect of final concentrations of 0.05 and 0.01 per cent of wetting agent in 25 ppm of sodium hypochlorite was tested against M. caseolyticus. The activity was so great that no material difference could be noted between mixtures. Therefore,

the pH of the mixture was adjusted to 10.0 with sodium hydroxide, and the final concentration of sodium hypochlorite increased to 50 ppm in order to use the minimum concentration recommended for sanitizing procedures. The same quantity of wetting agent was added to the solution.

Solutions containing concentrations of 0.01 and 0.05 per cent wetting agent in 200 ppm sodium hypochlorite were tested against B. cereus spores. The only change in the test method was that the exposure times were 0.5, 2.0, 5.0, 10.0, and 20.0 minutes. The pH of the mixture was adjusted to approximately 9.0.

In order to obtain the desired pH of a germicidal solution, the mixture was adjusted just previous to use with hydrochloric acid or sodium hydroxide. The pH of the medicinal tube was determined after the test had been completed, and this latter figure was recorded as the pH of the reaction. A Beckman glass electrode pH meter was used.

The concentrations of hypochlorite were adjusted according to the directions of the manufacturer. The actual concentrations were verified with the thiosulfate test (210, pp.98-100).

RESULTS

The effect of 14 different wetting agents on the available chlorine concentration of sodium hypochlorite solution are shown in table 6. After an exposure time of 48 hours, Igepal CA Extra, Nytron and Sterox CD had caused material decreases in the available chlorine concentration, even though only 0.01 per cent of the wetting agent was present. All other agents tested did not show this decrease when present in such a low concentration. However, when 0.10 per cent wetting agent was added to the hypochlorites, only Stepanate and Duponal Paste did not cause a material decrease in available chlorine. The higher concentration of Igepal CA Extra, Neutronyx 600, Sterox CD, and X-100 affected the chlorine content almost immediately. The reduction in chlorine continued as the exposure time increased. Nytron, at this concentration, completely neutralized the available chlorine immediately. The reason for this is that the Nytron contains a small amount of thiosulfate.

Table 7 shows the activity of 25 ppm sodium hypochlorite plus 5 different wetting agents on M. caseolyticus at a pH of approximately 8.0. The values presented are the average of two separate determinations. The sodium hypochlorite, alone and in combination with Santomerse # 1 or X-100, destroyed all cells in 15 seconds. The original concentration of bacterial cells was 100 x 10^6 per ml. When Nacconal, Alkanol, and Stepanate were added to the hypochlorite, it required 30 seconds to destroy all bacterial cells. However, the number of cells remaining after 15 seconds exposure was so small that

TABLE 6

Effect of addition of various wetting agents on the available chlorine concentration of sodium hypochlorite solutions

Wetting agent		ppm of available chlorine after following hours of storage:				
Type	Conc. (%)	0 (ppm)	6 (ppm)	24 (ppm)	48 (ppm)	168 (ppm)
None	----	50	52	48	48	48
Nacconal MX	0.01	53	55	54	50	43
	0.10	48	41	10	0	0
Nacconal NRCL	0.01	52	53	50	56	42
	0.10	49	50	40	5	0
Igepal CA Extra	0.01	33	34	26	21	11
	0.10	28	18	0	0	0
Santomerse # 1	0.01	55	53	54	51	48
	0.10	53	50	38	17	0
Nytron	0.01	49	48	33	30	32
	0.10	0	0	0	0	0
Stepanate	0.01	50	48	44	48	48
	0.10	45	45	43	41	34
Alkanol DM	0.01	54	50	50	50	48
	0.10	51	40	27	28	25
Neutronyx 600	0.01	44	43	43	43	37
	0.10	30	24	19	18	0
Sterox CD	0.01	37	42	34	26	16
	0.10	16	11	0	0	0
Kleer Mor	0.01	49	51	53	49	50
	0.10	40	40	18	0	0
Kleer Mor Special	0.01	56	50	48	48	47
	0.10	45	46	24	9	0
Duponal Paste	0.01	49	51	48	48	45
	0.10	54	51	50	49	45
X-100	0.01	48	47	53	42	54
	0.10	19	16	12	4	0
MP 189	0.01	53	55	54	56	52
	0.10	40	20	17	16	2

TABLE 7

Rate of destruction of Micrococcus caseolyticus by combination of 25 ppm sodium hypochlorite with various added wetting agents

Wetting agent	Conc. of wetting agent (%)	pH	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
None	———	8.2	100.000	100.000	100.000	100.000	100.000
Nacconal	0.05	8.1	99.998	100.000	100.000	100.000	100.000
	0.01	8.8	99.999	100.000	100.000	100.000	100.000
Alkanol	0.05	8.0	99.999	100.000	100.000	100.000	100.000
	0.01	8.3	99.817	100.000	100.000	100.000	100.000
Stepanate	0.05	8.1	99.993	100.000	100.000	100.000	100.000
	0.01	8.0	99.994	100.000	100.000	100.000	100.000
Santomerse	0.05	8.0	100.000	100.000	100.000	100.000	100.000
	0.01	8.4	100.000	100.000	100.000	100.000	100.000
X-100	0.05	8.2	100.000	100.000	100.000	100.000	100.000
	0.01	8.2	100.000	100.000	100.000	100.000	100.000

no definite conclusions can be drawn.

The effect of adjusting a sodium hypochlorite to a pH of approximately 10.0 is shown in table 8. The values presented are the average of three separate determinations. Even though the concentration of the germicide was increased to 50 ppm, the destruction of M. caseolyticus was not so rapid as at pH 8.0 when 25 ppm was present. With the exception of X-100, there does not appear to be a material effect on the hypochlorite solution by the use of wetting agents. The X-100 mixtures appear to be slightly decreased in germicidal activity. This can be particularly noted in the mixture containing 0.05 per cent X-100.

Because hypochlorites act so rapidly against vegetative cells, B. cereus spores were used as a test organism. The results obtained when five different wetting agents were added to sodium hypochlorite are shown in table 9. A final concentration of 200 ppm available chlorine was used, and the spore content was 84×10^4 per ml. The listed values are the average of three separate trials.

The effect of pH on germicidal activity may be observed in the first two lines of the table. In both cases, no wetting agent was added to the sodium hypochlorite solutions, but the pH of the solutions was adjusted to approximately 10.0 and 9.0 respectively. After 20 minutes exposure, only 30.891 per cent of the spores were destroyed at pH 10.0; whereas, 99.662 per cent were destroyed at pH 9.0. Therefore, the pH of the various solutions containing wetting agents were adjusted to approximately 9.0. The results obtained in subsequent trials with these compounds must be compared with values of the

TABLE 8

Rate of destruction of Micrococcus caseolyticus by combination of 50 ppm sodium hypochlorite with various added wetting agents

Wetting agent	Conc. of wetting agent (%)	pH	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
None	----	10.1	99.962	99.999	100.000	100.000	100.000
Nacconal	0.05	10.0	99.997	100.000	100.000	100.000	100.000
	0.01	10.0	99.979	100.000	100.000	100.000	100.000
Alkanol	0.05	10.1	99.971	100.000	100.000	100.000	100.000
	0.01	9.9	99.964	100.000	100.000	100.000	100.000
Stepanate	0.05	10.0	99.705	100.000	100.000	100.000	100.000
	0.01	10.1	99.688	100.000	100.000	100.000	100.000
Santomerse	0.05	10.0	99.995	100.000	100.000	100.000	100.000
	0.01	10.0	99.859	100.000	100.000	100.000	100.000
X-100	0.05	10.0	98.987	99.961	100.000	100.000	100.000
	0.01	9.9	99.665	99.999	100.000	100.000	100.000

TABLE 9

Rate of destruction of Bacillus cereus spores by combination of 200 ppm sodium hypochlorite with various added wetting agents

Wetting agent	Conc. of wetting agent (%)	pH	Per cent spores killed at following exposure periods in minutes: 0.5 (%)	2.0 (%)	5.0 (%)	10.0 (%)	20.0 (%)
None	----	10.2	11.097	24.717	20.467	36.118	30.891
None	----	8.9	19.835	37.007	61.109	76.900	99.662
Nacconal	0.05	8.7	33.537	28.607	38.246	75.883	96.580
	0.01	9.0	6.481	18.473	26.626	70.578	98.576
Alkanol	0.05	8.7	20.395	28.627	38.844	81.590	99.744
	0.01	8.9	15.191	29.887	52.426	71.373	98.749
Stepanate	0.05	8.8	25.305	18.700	41.057	64.557	86.587
	0.01	9.0	12.136	32.054	44.425	62.750	97.450
Santomerse	0.05	8.6	19.071	28.978	27.059	59.257	98.134
	0.01	8.7	18.782	23.550	44.293	89.247	99.849
X-100	0.05	8.7	27.513	36.987	48.916	72.855	85.352
	0.01	9.0	13.890	19.546	42.663	54.720	97.608

control trial at this pH.

After 20 minutes exposure, all mixtures except those containing 0.05 per cent **Stepanate** and X-100 destroyed over 96 per cent of the spores. These two mixtures destroyed 86.587 and 85.352 per cent of the spores, respectively. These results indicate that none of the wetting agents increased the germicidal activity of the sodium hypochlorite under the test conditions. However, concentrations above 0.01 per cent of Stepanate and X-100 tend to decrease the effectiveness of the germicide. This decrease in activity caused by X-100 agrees with the information presented in table 9. It is possible that pH differences that existed between various samples may have been responsible for some differences between solutions in this experiment.

The effect of 200 ppm of a commercial calcium hypochlorite on B. cereus spores is shown in table 10. The germicide was available from the manufacturer with and without a wetting agent. A use dilution solution containing 200 ppm available chlorine contained 0.0056 per cent of sodium dodecyl benzyl sulphonate. The pH of the solution containing the calcium hypochlorite without the wetting agent was approximately 8.8. A comparable solution containing the wetting agent had a pH of approximately 10.3. A comparison of these two solutions is shown in lines 1 and 2 of table 10. After 20 minutes exposure, the low pH solution killed 100 per cent of the spores; whereas, the high pH solution destroyed only 34.510 per cent. However, when the solution from the commercial product containing the wetting agent was adjusted to approximately 8.8, its activity was

TABLE 10

Effect of pH and presence of wetting agent on rate of destruction of Bacillus cereus spores by 200 ppm preparations of commercial hypochlorite

Germicide preparation	Conc. of wetting agent (%)	pH of germ. sol.	Per cent spores killed at following exposure periods in minutes: 0.5 (%)	2.0 (%)	5.0 (%)	10.0 (%)	20.0 (%)
LoBax Special	----	8.8	5.882	42.353	73.058	99.069	100.000
LoBax 21	0.0056	10.3	5.490	12.549	25.490	27.259	34.510
LoBax 21 (pH adjusted)	0.0056	8.7	11.369	41.958	99.349	99.989	100.000

equal to that of the other product. It appears that, at equal pH, the activity of the commercial calcium hypochlorite was about the same with and without the wetting agent. However, as it is available on the market, the compound containing the wetting agent is much less effective. This difference in activity is due to lack of proper adjustment of the pH.

DISCUSSION

Two separate aspects of the action of wetting agents on chlorine solutions must be considered. The first is that these agents possibly may increase chlorine activity against vegetative cells and spores in a water suspension. Such increase would indicate that the wetting agent enhances the germicidal activity of chlorine. However, no material increase in germicidal activity was observed under the conditions of this experiment.

The second aspect which must be considered is the effect of wetting agents when the bacteria exist in a film on equipment. The formation of a dried or semi-dried film on equipment occurs frequently in dairy farm and plant operation. These films tend to protect the bacteria from germicides. Therefore, the possibility exists that wetting agents might loosen and break up this film, which would in turn allow access of the chlorine to the otherwise protected bacteria. This circumstance has been suggested by the data of Neave and Hoy (172, p.50)

Another factor that must be considered in the addition of wetting agents to hypochlorites is the effect of the surface active agent on the available chlorine itself. Since it is known that organic matter inactivates chlorine, and since wetting agents are organic compounds, there is a possibility that they may neutralize the germicide. This may explain decreased effectiveness of the hypochlorite and X-100 combination on M. caseolyticus. Some also may contain reduced sulfur radicals that would affect content of available chlorine. The results

of this study show that some of the wetting agents do have this property. However, some do not inactivate chlorine when they are present in low concentrations. These latter compounds (preferably Nacconal NRCL, Santomerse # 1, Stepanate, Alkanol DM and X-100) can be employed with chlorine without removing appreciable quantities of the germicide through reaction with organic material over a limited period.

The pH of a chlorine solution is one of the most important factors in determining its germicidal activity. Due to the increased corrosive activity and general instability of high acid chlorine solutions, the pH must not be too low. However, a high pH decreases greatly the germicidal activity of the compound. The results of this study suggest that a compatible buffer might be used to advantage with some commercial hypochlorites. The natural buffering constituents of some hard water supplies would tend to result in lower pH levels in use dilution than in the distilled water germicide solutions used in this investigation. The pH of the final dilution should be between 8.0 and 9.0 to give the greatest germicidal activity with a limited amount of corrosive ability. If the pH is raised from 9.0 to 10.0, the decrease in effectiveness of the germicide is tremendous. The pH of the germicidal solution appears to be the most important factor affecting activity of hypochlorites. Results obtained on the commercial calcium hypochlorites substantiated the other trials on effect of surface active agents on hypochlorite activity. They indicated further that the manufacturer actually decreased effectiveness of his product by attempting addition of the wetting agent.

SUMMARY AND CONCLUSIONS

The effect of 14 wetting agents at concentrations of 0.01 and 0.10 per cent on the available chlorine of a 50 ppm sodium hypochlorite solution was studied. At the low concentration, only three of the compounds materially lowered the available chlorine content in 48 hours. However, at 0.10 per cent, all except two of the wetting agents decreased the available chlorine content materially. These results indicated that certain wetting agents could be added to hypochlorite solutions without detrimental effects, providing the concentration of the agent was low.

The germicidal activity of sodium hypochlorite in combination with five separate wetting agents on M. caseolyticus was determined. At pH 8.0, the activity of all solutions with a concentration of available chlorine of 25 ppm was so rapid that no conclusions could be drawn. However, when the pH was adjusted to 10.0, and the chlorine content increased to 50 ppm, no material increase in germicidal activity appeared to be contributed by the presence of the wetting agents. A concentration of 0.05 per cent X-100 appeared to decrease the effectiveness of the germicide.

More than 96 per cent of the B. cereus spores were destroyed by all solutions of 200 ppm sodium hypochlorite in 20 minutes except those containing 0.05 per cent Stepanate and X-100. These agents allowed only 86.587 and 83.352 per cent destruction, respectively.

A commercial calcium hypochlorite, that had a wetting agent added by the manufacturer, was not as effective as the same compound

without the wetting agent. However, when the pH of the two solutions was adjusted to the same point, the germicidal activity of both solutions was equal.

Slight changes in pH of hypochlorite solutions have been shown to be far more important in determining their germicidal activity than presence of compatible surface active agents.

PART III

EFFECT OF pH ON THE GERMICIDAL ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS

The importance of pH of the germicidal solution with respect to hypochlorites has been emphasized in PART II of this study. However, the effect of pH on the QACs is not well understood. Most workers have assumed that the QACs are most active in an alkaline solution. Some data that are very incomplete (107, p.10-20) suggest that some exceptions to this rule may exist.

The difficulty in arriving at a definite conclusion hinges on the fact that most studies on this problem have not included enough pH levels. A possibility, not heretofore investigated, was that a slight variation in pH might cause a great variation in germicidal activity of a compound. Also, the type of QAC used might affect the results obtained at any one pH level. The effect of pH on germicidal activity of a QAC might vary with the species of bacteria tested. A consideration of these possibilities heretofore not adequately studied, led to the investigation in this section.

MATERIALS AND METHODS

Organisms. P. aeruginosa, E. coli, and M. caseolyticus were selected for this experiment. The cultures were carried on nutrient agar slopes at 32° C. and transferred daily.

Germicides. The following QACs were used: alkyl dimethyl ethyl benzyl ammonium chloride, methyl dodecyl benzyl trimethyl ammonium chloride, para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride, and alkyl dimethyl benzyl ammonium chloride. The germicide stock solutions were made to contain 100 ppm by following the directions of the manufacturer. The concentration was verified by titration (94, pp.159, 160).

Method of analysis. Boric acid, at the rate of 0.1 per cent was added to the stock solution of the germicide. Approximately 100 ml. portions were placed in beakers and the pH values were adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 with a Beckman glass electrode pH meter. The pH values of 3.0, 4.0, and 5.0 were obtained by the use of concentrated hydrochloric acid. The values 6.0, 7.0, 8.0, 9.0, and 10.0 were obtained by adding saturated sodium carbonate. These concentrated solutions were used to prevent excessive dilution of the germicide.

The bacterial culture to be studied was grown for 24 hours on a nutrient agar slope at 32° C. and washed into a water blank containing 0.1 per cent boric acid. After adjusting with a Beckman spectrophotometer to contain approximately 200 x 10^6 organisms per ml., portions

of the suspension were adjusted to the same pH values as the germicide solutions by the same method as that used for the germicide solution. The matching pH solutions then were tested bacteriologically by the Weber and Black (209, pp.1406-1416) technique.

After the germicide had been exposed to the bacterial suspension for 300 seconds and the necessary samples for plating had been removed, the pH of the remaining contents of the medicinal tube was determined. This value was taken as the actual pH of the test and is recorded in the results. The pH was constant for those solutions adjusted to 3.0, 8.0, 9.0, and 10.0. The solutions at pH 4.0, 5.0, and 6.0 rose approximately 0.5 pH unit and pH 7.0 increased about 0.3 pH unit.

RESULTS

The results obtained in the experiment are shown graphically in figures 1 to 4. Each curve represents one experimental trial. The trials on individual organisms and germicides were carried out on 2 separate occasions on different days. Slight changes in the resistance and initial concentrations of the organism would not permit superimposing duplicate curves on each other. However, the duplicates in all cases showed exactly the same response to changes in pH. One set of determinations is shown in figures 1 to 4, inclusive. Data from which these figures were prepared are shown in tables 11 to 14. The duplicate determinations are shown in tables 15 to 18, inclusive. Exposure periods selected for the figures were designed to bring out important differences related to change in pH of the solutions tested.

Figure 1 shows the effect of various pH values on the germicidal activity of alkyl dimethyl ethyl benzyl ammonium chloride against the three test organisms. Exposure periods selected for this figure were two minutes for P. aeruginosa and one minute for the other two organisms.

Against P. aeruginosa, the germicide was least active at a pH of approximately 9.0. Activity increased slightly above this value. Below pH 9.0, the activity increased rapidly until pH 7.5 showed no organisms surviving. All organisms were destroyed at pH values below 7.5.

When E. coli and M. caseolyticus were used as the test organisms, the number of surviving cells decreased as the pH value increased

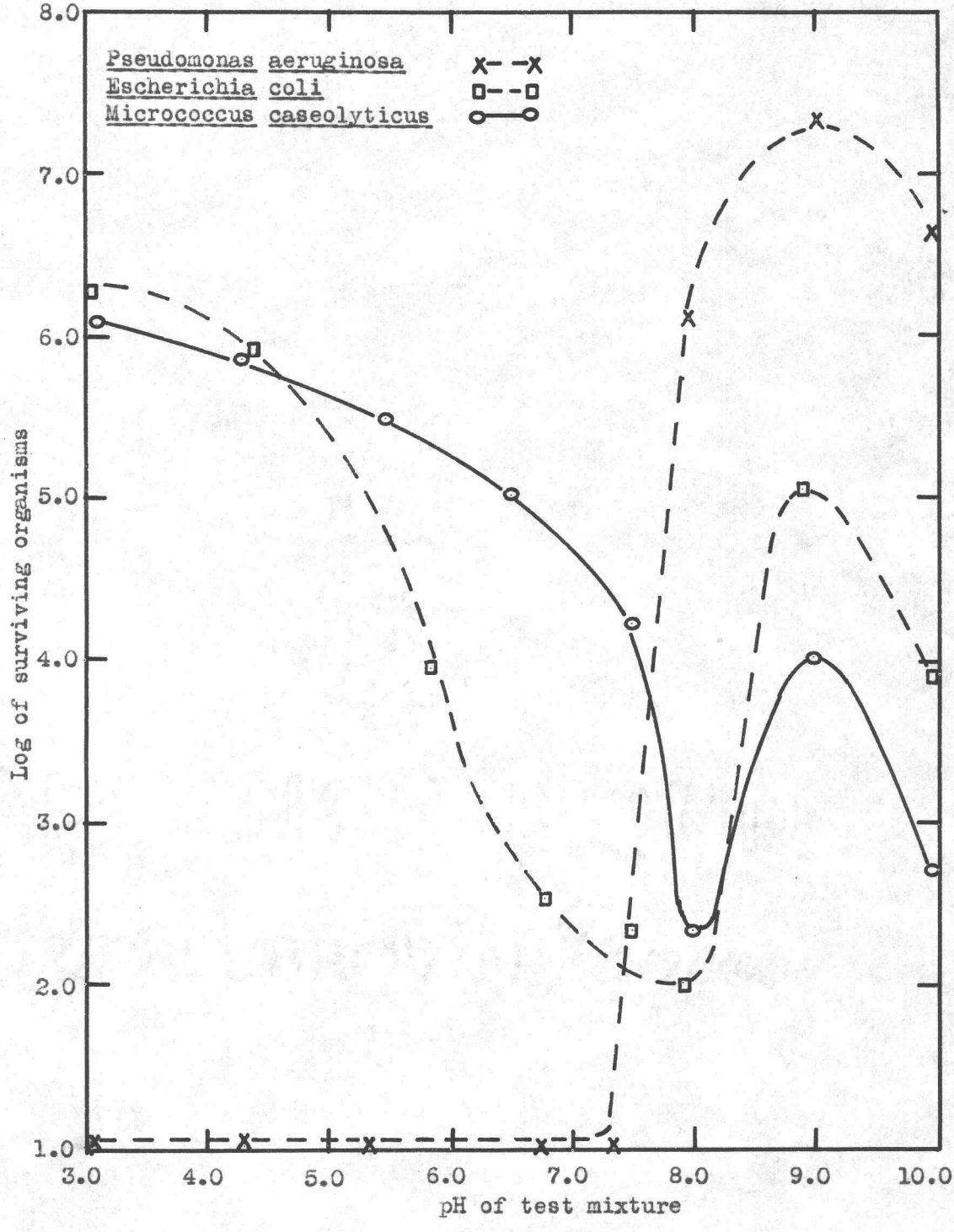

Figure 1. Effect of pH on the germicidal activity of 50 ppm alkyl dimethyl ethyl benzyl ammonium chloride over a two minute exposure period for Pseudomonas aeruginosa and a one minute exposure period for Escherichia coli and Micrococcus caseolyticus.

from 3.0 to 8.0. There was a sudden rise in rate of survival at pH 9.0, and another decrease at pH 10.0. The results indicate that this QAC was most active against these two bacterial species at pH 8.0.

The effect of pH on the germicidal activity of para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride against the same organisms is shown in figure 2. All curves represent the log of surviving organisms after five minutes exposure to the germicide. Against P. aeruginosa, the QAC was most active between pH 3.0 and pH 6.0. Above pH 6.0, the activity decreased rapidly to about pH 7.0. It then slowly decreased up to pH 10.0, the highest level tested.

The survival of E. coli decreased as the pH value increased from 3.0 to 8.0. A sudden increase in surviving cells was noted at pH 9.0, which was followed by another marked decrease at pH 10.0. Similar results were obtained on M. caseolyticus, except that the decrease in surviving cells did not occur until pH 7.0 was reached. These results indicate that pH 8.0 provides the most active level for the latter two organisms. A pH of 10.0 provided about the same activity on E. coli as pH 8.0.

Figure 3 shows the effect of pH on the activity of methyl dodecyl trimethyl benzyl ammonium chloride. All curves were obtained from the five nimute exposure period. The compound was most active against P. aeruginosa between pH 3.0 and 6.5. The activity then decreased rapidly to pH 9.0. It increased again at pH 10.0. Against E. coli and M. caseolyticus, the activity was lowest at pH 3.0 to 4.0. It increased as the pH increased to about 7.5. A sudden decrease in activity then was noted to pH 9.0. An increase in effectiveness took

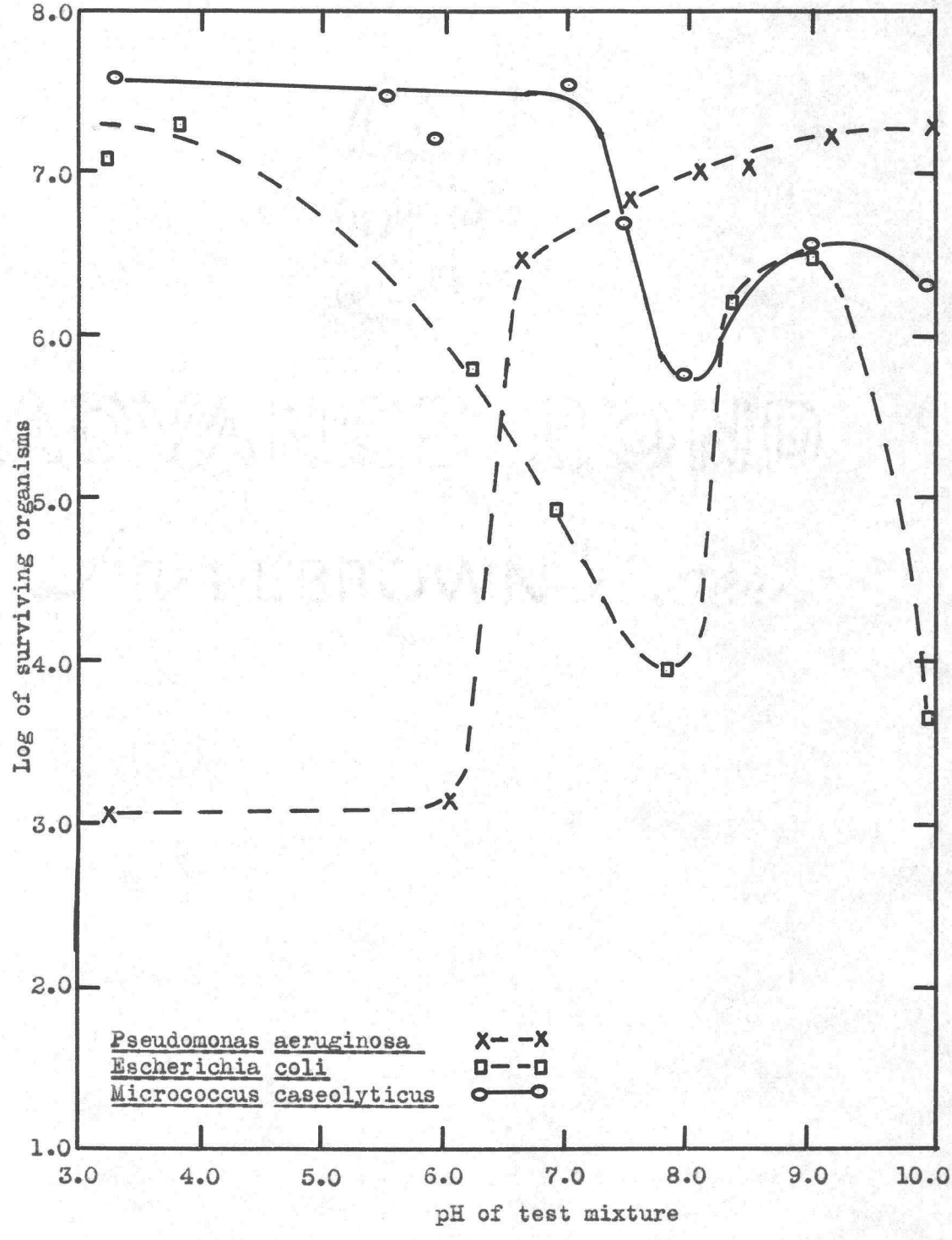

Figure 2. Effect of pH on the germicidal activity of 50 ppm para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride over a five minute exposure period for three test organisms.

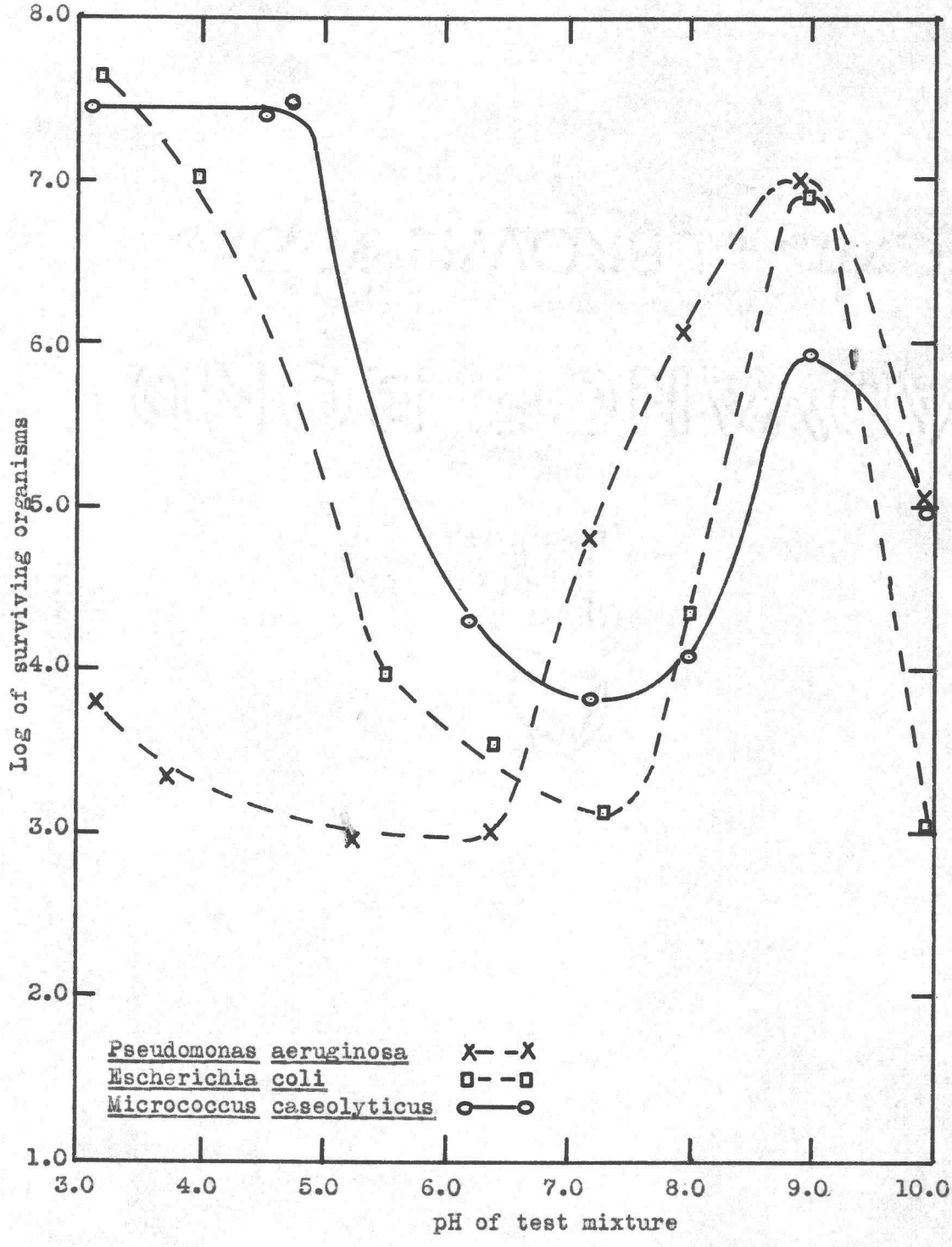

Figure 3. Effect of pH on the germicidal activity of 50 ppm methyl dodecyl benzyl trimethyl ammonium chloride over a five minute exposure period for three test organisms.

place at pH 10.0.

Alkyl dimethyl benzyl ammonium chloride responded differently than the other compounds as shown in figure 4. The curves represent an exposure of one minute against E. coli and five minutes against P. aeruginosa and M. caseolyticus. P. aeruginosa was destroyed at a moderate rate at pH 3.0 to 5.0. The rate of destruction increased rapidly to pH 6.5. It then decreased to pH 9.0, and again increased slightly at pH 10.0. The greatest number of surviving organisms was at pH 9.0.

The activity of the QAC against E. coli increased from pH 3.0 to 6.5. It then decreased up to pH 8.0, but not quite to the extreme of pH 3.0 and 4.0. Above pH 8.0, the activity increased to pH 10.0. The activity at pH 10.0 was not as great as at pH 6.5. Similar results were noted against M. caseolyticus, except that the decrease in activity stopped at pH 7.5 instead of pH 8.0. The activity at pH 9.0 and above was equal to that at pH 6.5.

Figure 5 shows the survival of the three species tested after five minutes exposure when no QAC was present in the test mixture. The various pH levels were obtained by the method used for testing QAC. No reduction in total bacterial count at any pH level was obtained for M. caseolyticus and E. coli. P. aeruginosa showed a slight reduction in surviving organisms at pH 3.0 and 10.0. No reduction for this organism was obtained at the other pH levels tested.

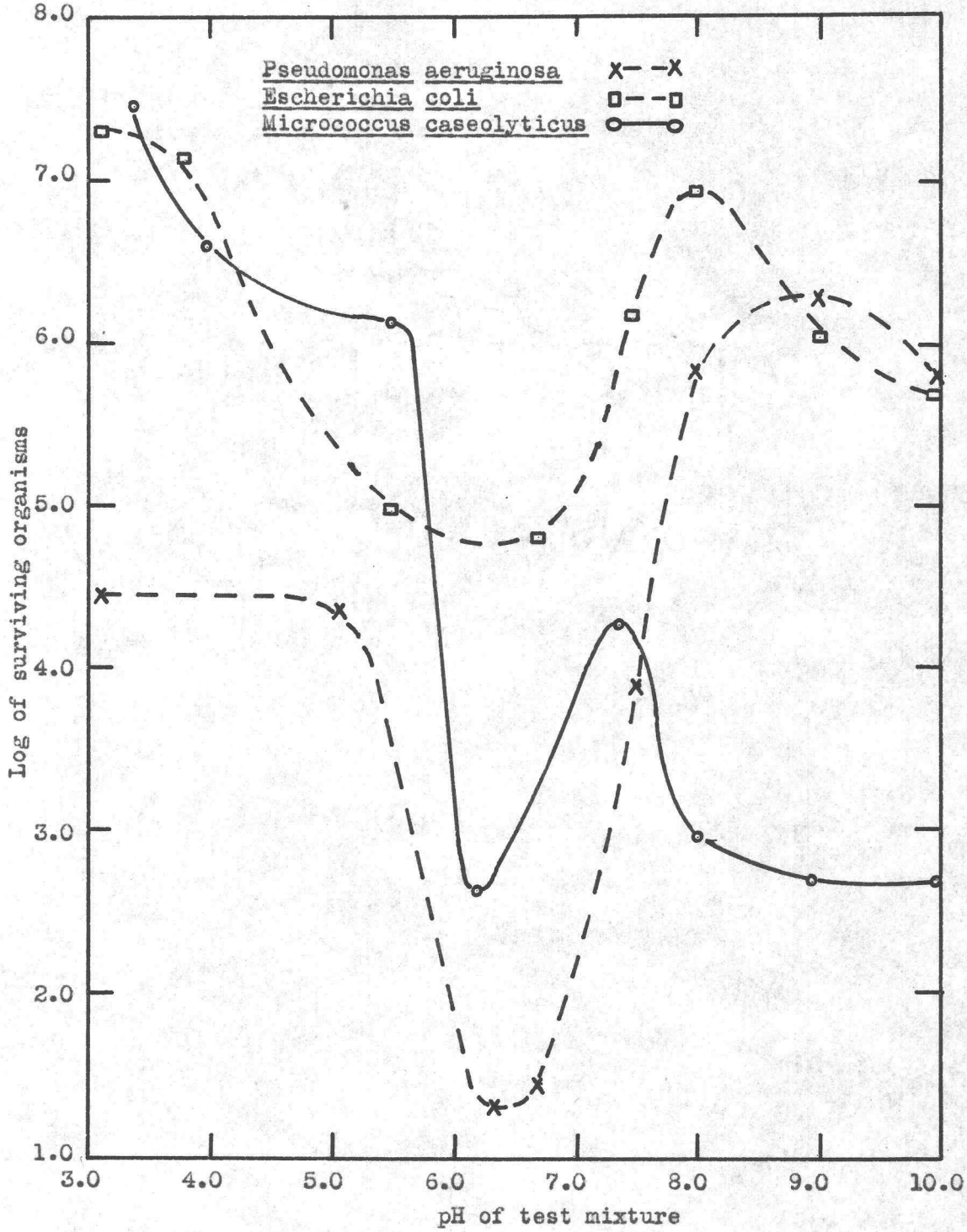

Figure 4. Effect of pH on the germicidal activity of 50 ppm alkyl dimethyl benzyl ammonium chloride over a five minute exposure period for Pseudomonas aeruginosa and a one minute exposure period for Escherichia coli and Micrococcus caseolyticus.

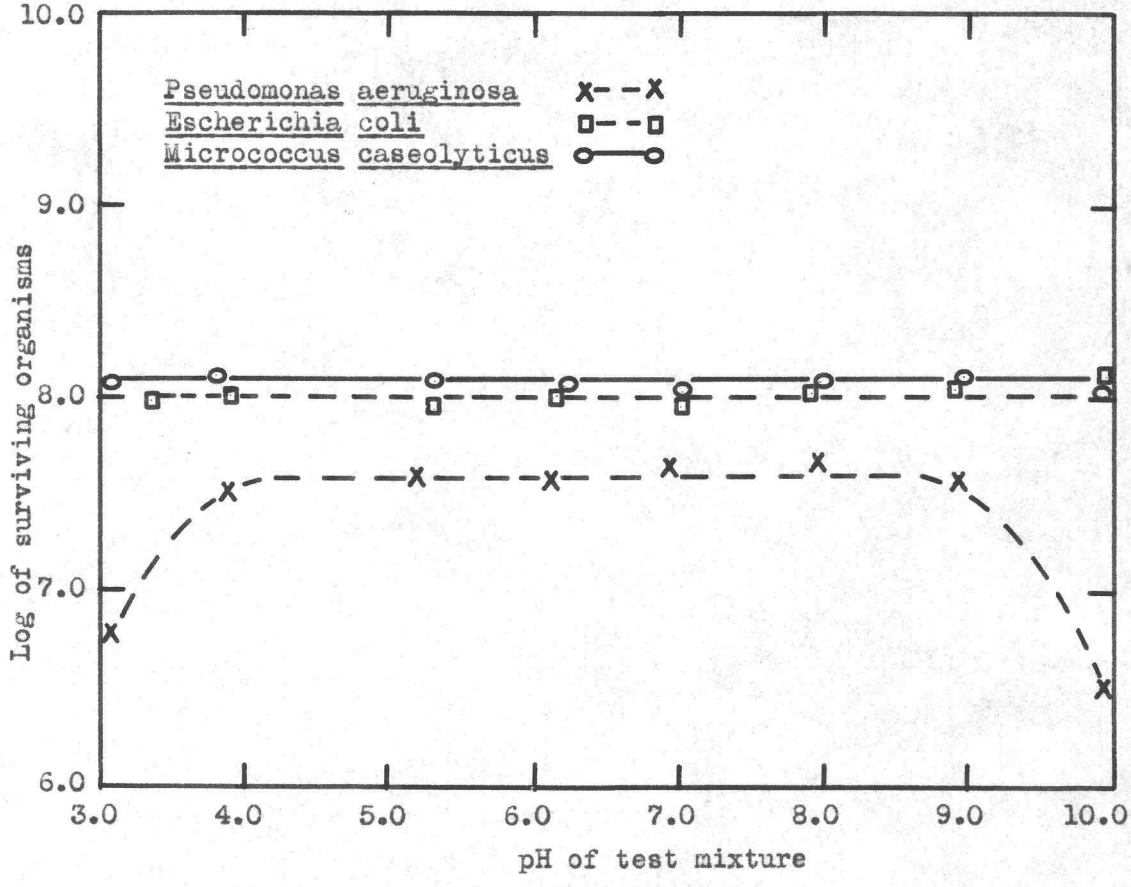

Figure 5. Effect on three test organisms of five minute exposure to 0.1 per cent boric acid buffer adjusted to various pH levels with hydrochloric acid or sodium carbonate.

TABLE 11

Survival of three bacterial species after exposure to alkyl dimethyl ethyl benzyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	9.96	2760.000	2280.000	1140.000	430.000	370.000
	9.03	3900.000	3340.000	2722.000	2220.000	1060.000
	7.96	3840.000	3120.000	1620.000	1320.000	850.000
	7.65	840.000	520.000	280.000	88.000	13.000
	7.37	0.015	0.000	0.000	0.000	0.000
	5.91	0.018	0.000	0.000	0.000	0.000
	5.32	0.008	0.000	0.000	0.000	0.000
	3.19	0.000	0.000	0.000	0.000	0.000
M. caseolyticus	10.02	0.440	0.200	0.054	0.008	0.000
	9.00	260.000	4.900	1.190	0.073	0.011
	8.01	0.195	0.043	0.022	0.014	0.007
	7.52	2.200	1.710	0.650	0.057	0.016
	6.89	174.000	16.000	1.410	0.019	0.005
	6.51	186.000	61.000	12.100	2.190	0.102
	4.30	900.000	255.000	72.000	4.900	0.108
	3.13	282.000	198.000	120.000	27.000	10.500
E. coli	9.92	10.500	3.400	0.840	0.003	0.000
	8.88	23.000	7.900	11.900	2.100	0.017
	7.88	6.800	0.205	0.110	0.003	0.000
	7.48	2.300	0.720	0.230	0.006	0.000
	6.72	38.000	8.400	0.360	0.280	0.020
	5.84	59.000	4.200	0.950	0.087	0.015
	4.32	2420.000	750.000	76.000	3.500	0.410
	2.98	1440.000	630.000	200.000	47.000	0.260

TABLE 12

Survival of three bacterial species after exposure to para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	10.16	6080.000	5550.000	4680.000	2760.000	1980.000
	9.14	4740.000	4640.000	3520.000	2020.000	1620.000
	8.52	4620.000	4560.000	4020.000	1320.000	1120.000
	8.10	4200.000	4300.000	2640.000	2400.000	1020.000
	7.48	3760.000	3300.000	2100.000	1920.000	720.000
	6.63	1440.000	1120.000	840.000	570.000	310.000
	6.03	83.000	21.000	9.000	1.000	0.150
	3.26	2.000	0.800	0.330	0.150	0.010
M. caseolyticus	9.97	5720.000	2460.000	1620.000	750.000	39.000
	8.96	9220.000	8120.000	3400.000	2200.000	410.000
	7.99	8640.000	6840.000	2580.000	610.000	37.000
	7.42	9020.000	8680.000	3960.000	1440.000	176.000
	6.80	9130.000	8030.000	5580.000	2820.000	2460.000
	5.78	9080.000	8000.000	4340.000	1810.000	1550.000
	4.03	9880.000	8210.000	5360.000	4440.000	2880.000
	3.22	9730.000	8460.000	6020.000	3950.000	1980.000
E. coli	10.11	960.000	720.000	430.000	157.000	0.300
	9.01	6600.000	3820.000	3480.000	2040.000	340.000
	8.48	6000.000	2760.000	1500.000	640.000	171.000
	7.81	3180.000	800.000	170.000	15.000	0.100
	6.91	3300.000	1500.000	900.000	290.000	10.000
	6.22	3480.000	3300.000	1740.000	420.000	65.000
	3.76	5320.000	4640.000	4060.000	3800.000	2400.000
	3.19	4820.000	4640.000	3100.000	2440.000	1430.000

TABLE 13

Survival of three bacterial species after exposure to methyl dodecyl benzyl trimethyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	9.99	184.000	120.000	51.000	39.000	12.000
	8.94	2360.000	1620.000	1380.000	1200.000	1020.000
	7.97	630.000	500.000	300.000	220.000	126.000
	7.24	580.000	88.000	42.000	2.100	0.580
	6.39	610.000	98.000	32.000	2.400	0.100
	5.31	600.000	120.000	21.000	1.900	0.080
	3.73	1260.000	520.000	26.000	8.000	0.220
	3.15	2400.000	1500.000	75.000	26.000	0.580
M. caseolyticus	9.98	2640.000	1860.000	1380.000	840.000	9.000
	9.00	3900.000	3700.000	2880.000	2160.000	87.000
	8.02	2220.000	1200.000	730.000	52.000	1.200
	7.22	502.000	450.000	131.000	6.300	0.690
	6.21	3960.000	2820.000	2200.000	1400.000	20.000
	4.72	7890.000	6580.000	5430.000	4680.000	3240.000
	4.60	7630.000	6320.000	4120.000	3860.000	2520.000
	3.14	8030.000	6850.000	5040.000	3060.000	2820.000
E. coli	9.91	2250.000	1240.000	940.000	91.000	0.120
	8.92	6480.000	5240.000	4560.000	2460.000	840.000
	8.03	2880.000	2640.000	1560.000	200.000	2.400
	7.28	2180.000	1220.000	890.000	33.000	0.133
	6.42	2280.000	1920.000	1240.000	65.000	0.380
	5.50	3840.000	3000.000	2400.000	1620.000	97.000
	3.92	5820.000	4200.000	3300.000	2460.000	1090.000
	3.23	4240.000	3720.000	3000.000	1200.000	450.000

TABLE 14

Survival of three bacterial species after exposure to alkyl dimethyl benzyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	10.01	780.000	680.000	520.000	400.000	59.000
	9.00	2100.000	1630.000	1350.000	1270.000	200.000
	8.01	1080.000	710.000	400.000	145.000	70.000
	7.54	720.000	430.000	140.000	22.000	0.900
	6.73	0.170	0.027	0.009	0.004	0.003
	6.32	9.000	0.280	0.031	0.003	0.002
	5.09	250.000	220.000	106.000	17.000	2.400
	3.09	390.000	250.000	110.000	7.000	2.800
M. caseolyticus	9.99	840.000	100.000	1.200	0.130	0.052
	8.95	1220.000	220.000	2.200	0.130	0.047
	7.99	1560.000	360.000	7.800	0.690	0.080
	7.36	2880.000	1260.000	180.000	7.400	1.900
	6.20	1580.000	880.000	40.000	1.600	0.410
	5.46	3660.000	2280.000	1800.000	830.000	140.000
	3.98	4740.000	3660.000	2640.000	1140.000	390.000
	3.43	5830.000	4380.000	4030.000	3300.000	2980.000
E. coli	9.89	960.000	320.000	60.000	0.190	0.007
	8.94	2140.000	1020.000	120.000	3.300	0.011
	8.02	1920.000	1140.000	960.000	180.000	0.053
	7.42	1040.000	380.000	150.000	15.000	0.190
	6.61	910.000	290.000	6.400	2.800	0.009
	5.49	1020.000	300.000	9.500	2.000	0.008
	3.81	6060.000	2220.000	1380.000	1080.000	660.000
	3.12	6320.000	4200.000	1900.000	1680.000	1120.000

TABLE 15

Survival of three bacterial species after exposure to alkyl dimethyl ethyl benzyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	9.87	2220.000	2050.000	690.000	340.000	230.000
	8.82	3340.000	2800.000	1620.000	1380.000	920.000
	7.96	2100.000	1020.000	790.000	390.000	230.000
	7.61	1020.000	500.000	290.000	100.000	39.000
	7.21	0.105	0.019	0.000	0.000	0.000
	6.83	0.029	0.012	0.000	0.000	0.000
	4.37	0.048	0.014	0.000	0.000	0.000
	3.11	0.051	0.016	0.000	0.000	0.000
M. caseolyticus	10.03	320.000	137.000	45.000	0.140	0.000
	9.00	2280.000	490.000	156.000	60.000	0.270
	8.05	0.083	0.007	0.002	0.000	0.000
	7.38	1200.000	320.000	46.000	33.000	2.500
	6.89	4620.000	1440.000	120.000	107.000	22.000
	5.80	4720.000	2220.000	320.000	38.000	0.470
	5.12	4680.000	2030.000	380.000	48.000	0.640
	3.14	5640.000	2220.000	1330.000	430.000	12.100
E. coli	10.00	14.000	3.600	0.910	0.011	0.000
	9.03	294.000	163.000	94.000	39.000	9.000
	8.08	35.600	5.600	0.460	0.290	0.008
	7.58	145.000	25.000	5.000	0.780	0.048
	6.98	382.000	312.000	13.800	3.500	0.080
	5.74	344.000	252.000	11.900	2.000	0.170
	4.16	3300.000	882.000	228.000	74.000	5.200
	3.35	5580.000	3600.000	266.000	64.000	7.900

TABLE 16

Survival of three bacterial species after exposure to para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	9.87	3300.000	1920.000	1440.000	900.000	600.000
	8.91	4760.000	3340.000	2220.000	2160.000	1320.000
	8.06	3480.000	2280.000	1620.000	1040.000	860.000
	7.74	170.000	140.000	111.000	64.000	40.000
	7.24	21.000	8.000	7.000	4.000	2.500
	6.28	310.000	26.000	2.100	1.700	0.300
	3.75	7.000	3.600	1.400	0.800	0.300
	3.05	0.073	0.034	0.016	0.012	0.000
M. caseolyticus	10.02	4140.000	2580.000	1980.000	920.000	69.000
	9.00	4080.000	3720.000	1740.000	1040.000	360.000
	7.98	3220.000	2260.000	740.000	401.000	57.000
	7.45	4320.000	3200.000	2680.000	1460.000	510.000
	7.06	8540.000	6600.000	5100.000	4680.000	1780.000
	5.88	9030.000	8440.000	6240.000	4860.000	1620.000
	5.54	9120.000	8000.000	6060.000	5020.000	2020.000
	3.30	9940.000	8100.000	6840.000	5420.000	3080.000
E. coli	9.87	1140.000	750.000	310.000	18.000	0.009
	9.12	4320.000	3160.000	1560.000	400.000	83.000
	7.97	2040.000	1880.000	870.000	190.000	1.300
	7.77	2300.000	1140.000	980.000	111.000	1.300
	7.14	4640.000	3020.000	1960.000	1040.000	144.000
	6.46	4900.000	3100.000	2140.000	840.000	500.000
	5.32	4680.000	3940.000	2720.000	1060.000	540.000
	3.19	5400.000	4010.000	2800.000	1480.000	610.000

TABLE 17

Survival of three bacterial species after exposure to methyl dodecyl benzyl trimethyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	9.98	461.000	320.000	115.000	92.000	30.000
	9.01	2941.000	2220.000	1850.000	1320.000	960.000
	8.02	1020.000	930.000	520.000	202.000	124.000
	7.21	620.000	580.000	202.000	150.000	68.000
	6.54	630.000	610.000	350.000	240.000	86.000
	4.68	580.000	540.000	368.000	301.000	98.000
	3.87	730.000	660.000	399.000	346.000	120.000
	2.99	840.000	690.000	415.000	398.000	170.000
M. caseolyticus	10.02	2030.000	1550.000	1120.000	630.000	21.000
	9.02	3060.000	2840.000	2150.000	1590.000	112.000
	7.98	1590.000	830.000	640.000	122.000	8.000
	7.32	503.000	420.000	168.000	12.000	0.810
	6.33	2930.000	2650.000	2130.000	1680.000	132.000
	5.12	5460.000	4830.000	3890.000	3660.000	2840.000
	3.94	6330.000	5720.000	4060.000	3820.000	3240.000
	3.03	6880.000	6080.000	4610.000	4090.000	3260.000
E. coli	10.01	3060.000	2610.000	2220.000	1290.000	82.000
	8.98	4660.000	4480.000	3040.000	2680.000	960.000
	8.08	3120.000	2550.000	1660.000	870.000	15.000
	7.16	2090.000	1440.000	820.000	56.000	2.600
	6.46	2110.000	1880;000	1090.000	131.000	6.800
	5.50	3880.000	3270.000	2840.000	1610.000	430.000
	4.12	5950.000	5210.000	4320.000	2610.000	1110.000
	3.22	5690.000	5180.000	4620.000	4010.000	890.000

TABLE 18

Survival of three bacterial species after exposure to alkyl dimethyl benzyl ammonium chloride at eight different pH levels

Organism	pH	Number of organisms surviving after following exposure periods in seconds: 15 (10^4)	30 (10^4)	60 (10^4)	120 (10^4)	300 (10^4)
P. aeruginosa	10.03	930.000	730.000	610.000	410.000	48.000
	8.98	1850.000	1550.000	1110.000	980.000	176.000
	7.99	1110.000	830.000	480.000	206.000	69.000
	7.21	320.000	111.000	82.000	21.000	0.700
	6.40	10.000	0.500	0.002	0.000	0.000
	5.11	410.000	320.000	211.000	42.000	3.800
	4.50	560.000	430.000	250.000	31.000	5.300
	3.06	690.000	510.000	221.000	8.200	4.200
M. caseolyticus	9.99	1260.000	450.000	72.000	2.500	0.080
	9.01	2330.000	960.000	111.000	5.500	0.090
	7.96	2680.000	1130.000	321.000	11.000	0.200
	7.32	3720.000	1660.000	870.000	75.000	6.500
	6.51	1130.000	320.000	67.000	1.600	0.150
	5.02	4570.000	3060.000	2130.000	1220.000	468.000
	4.81	5080.000	4210.000	3000.000	2880.000	1560.000
	3.12	6930.000	5880.000	4220.000	3660.000	3110.000
E. coli	10.00	1060.000	410.000	86.000	0.890	0.009
	9.02	3220.000	1570.000	430.000	5.600	0.031
	8.01	3050.000	1690.000	980.000	115.000	0.082
	7.12	1140.000	390.000	95.000	1.210	0.010
	6.48	730.000	195.000	8.100	0.220	0.021
	5.46	850.000	201.000	9.600	0.190	0.032
	3.87	5320.000	3060.000	2120.000	1440.000	950.000
	3.01	6130.000	3240.000	2660.000	1630.000	1010.000

DISCUSSION

Other workers appear to have missed completely the marked drop and rise in germicidal activity of QACs as the pH varies over a wide range (such as pH 3.0 to 10.0). This undoubtedly is due to the fact that too few pH values have been studied in relation to QAC activity. Two factors must be considered in studying the effect of pH on the germicidal activity of QAC. First, a slight variation in pH may be critical because a change of one pH unit can cause a significant variation in the effectiveness of the germicide. The second factor is the type of buffer used to obtain the desired pH levels. More information is needed on the effect of various buffers on germicidal activity of QAC. There is a possibility that the germicidal activity of QAC may vary with the type of buffer used to adjust the pH. For this reason the variation in composition of buffers employed in this study was kept at a minimum.

This study has verified previous reports that indicate that QACs react differently in germicidal activity to change in pH of the germicide solution. The reason for these differences is not readily apparent from the available data. However, one possibility may be the degree of dissociation of the QAC molecule. There is a possibility that some QACs may show their greatest germicidal activity at the pH of greatest dissociation. This fact must be considered in conjunction with the difference in individual bacterial species.

Salton, (240, p.48) found that the germicidal activity of cetyltrimethylammonium bromide varied with pH but in a direction

dependent upon the species of bacteria studied. It also was shown that the inhibition of oxygen uptake at various pH levels had no relation to germicidal activity. There was no apparent explanation for the difference in susceptibility to germicide between bacterial species. Data presented here also show a difference in effect of an individual QAC on different bacterial species at different pH levels. There was no relationship between the optimum pH value for germicidal activity and the Gram reaction or bacterial morphology.

One possible explanation for the variation in the germicidal activity with varying pH against different organisms is the physiological difference between species. P. aeruginosa prefers alkaline conditions for growth and is not acid tolerant. E. coli, on the other hand, is more acid tolerant. This suggests a difference in critical enzyme systems. A low pH provides the most germicidal condition for QAC against P. aeruginosa and an alkaline pH against E. coli. M. caseolyticus is tolerant to both acid and alkaline conditions, but the effect of QAC on this organism at various pH levels is similar to its effect on E. coli. This suggests the possibility that the germicidal activity of QAC is greatest under pH conditions least favorable for the growth of the organism.

SUMMARY AND CONCLUSIONS

A study has been carried out on the effect of pH values between 3.0 and 10.0 on the germicidal activity of four different QACs. Three bacterial species were used as test organisms.

Alkyl dimethyl ethyl benzyl ammonium chloride was most active against P. aeruginosa at pH 3.0 to 7.5. The activity of the QAC decreased as the pH value increased from 7.5 to 9.0. A slight increase in activity occurred as the pH value increased from 9.0 to 10.0 . The germicidal activity of the QAC against E. coli and M. caseolyticus was least at pH 3.0 to 4.0. The activity against these two organisms increased at pH values above 4.0 to a maximum at pH 8.0. It then decreased from pH 8.0 to 9.0 and increased again from pH 9.0 to 10.0.

Para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride was most germicidal against P. aeruginosa at pH 3.0 to 6.0. The activity decreased as the pH rose to 9.0 and then increased slightly from pH 9.0 to 10.0. The germicidal activity of the QAC against E. coli and M. caseolyticus was poorest at pH 3.0 to 6.0. It increased as the pH increased above 6.0 to a maximum activity at pH 8.0. The germicidal activity then decreased at pH 8.0 to 9.0 and again increased at pH 9.0 to 10.0.

Methyl dodecyl benzyl trimethyl ammonium chloride activity against P. aeruginosa increased from pH 3.0 to 6.5. The germicidal activity of the QAC decreased rapidly as the pH rose from 6.5 to 9.0. A slight increase in activity was observed as the pH changed from 9.0 to 10.0. The poorest activity of the QAC against E. coli and M. caseolyticus

was observed at pH 3.0. Its activity against these two organisms increased as the pH increased from 3.0 to 7.5. A decrease in activity occurred from pH 7.5 to 9.0. A slight increase in activity was observed from pH 9.0 to 10.0.

Alkyl dimethyl benzyl ammonium chloride was moderately active against P. aeruginosa at pH 3.0 to 5.0. Its activity increased from pH 5.0 to a maximum at pH 6.5. The germicidal activity then decreased from pH 6.5 to 9.0. A slight increase in activity was observed at pH 9.0 to 10.0. The germicidal activity of the QAC against E. coli and M. caseolyticus was poorest at pH 3.0 to 4.0. Its activity against these two organisms increased from pH 4.0 to a maximum at pH 6.5. Against E. coli, the activity decreased rapidly as the pH level changed from 6.5 to 8.0. An increase in activity was observed from pH 8.0 to 10.0. Against M. caseolyticus, the activity decreased rapidly from pH 6.5 to a minimum at pH 7.5. An increase in activity occurred from pH 7.5 to 10.0.

The results of this study emphasize that any investigation on germicidal activity of QACs must control pH levels of the germicide solution within relatively narrow limits and must consider carefully the effect of pH in relation to the type of compound and bacterial species tested.

PART IV

FACTORS AFFECTING THE GERMICIDAL ACTIVITY OF DETERGENT SANITIZERS CONTAINING QUATERNARY AMMONIUM COMPOUNDS

Most detergent sanitizers on the market include a QAC as a germicide and a number of cleaner ingredients, such as nonionic wetting agent, trisodium phosphate, polyphosphates, sodium metasilicate, and sodium carbonate. The purpose of a detergent sanitizer is to provide both germicidal and cleaning properties when applied on dairy and food equipment and utensils.

During the study of various factors affecting the germicidal activity of QAC, it was noted that detergent sanitizers containing QAC were more active against bacteria than were the same germicides alone. This phenomenon was so consistent that it was apparent that some factor in the mixture was increasing the activity of the QAC against the organisms. Three factors might cause this increased germicidal activity. The increased pH of the solution could enhance the effectiveness of the germicide. However, results shown in PART III indicate that this probably is not the case. Another possibility might be presence of a wetting agent, and a third might be presence of phosphates in the detergent sanitizer. Preliminary work indicated that the most likely factor was the phosphates. The following study was conducted in three phases in an effort to determine the factors causing an increased germicidal activity in detergent sanitizers.

MATERIALS AND METHODS

Experiment 1. The effect of hard water and hard water plus organic matter on the germicidal activity of alkyl dimethyl ethyl benzyl ammonium chloride was determined in this experiment. This QAC was selected because it was the germicidal agent employed in one series of the detergent sanitizers under study. The effect of water hardness was studied because practical application of the detergent sanitizers requires their use in waters varying greatly in hardness.

The hard waters available for study were : Roseburg, which contained 2507 ppm hardness calculated as calcium carbonate; Starr ranch, which contained 168 ppm hardness calculated as calcium carbonate; and Navy specification no. 2000, which was prepared to contain 300 ppm hardness calculated as calcium carbonate. A stock solution of the Navy hard water, containing 4275 ppm hardness calculated as calcium carbonate was prepared by adding calcium chloride equivalent to 2.85 milligrams of calcium carbonate and magnesium chloride equivalent to 1.20 milligrams of magnesium carbonate to one liter of carbon dioxide-free distilled water. The stock solution was diluted with carbon dioxide-free distilled water so that the final test solution contained 300 ppm hardness calculated as calcium carbonate. The effect on the germicidal activity of the QAC of organic matter was determined by adding one per cent whole milk to a Navy hard water solution. A control test solution contained the QAC dissolved in distilled water.

P. aeruginosa and E. coli were chosen as the test organisms

because of the resistance to QAC of the former and of the importance to the dairy industry of the latter. The germicidal activity of the various solutions was determined with the Weber and Black (209, pp. 1406-1416) technique.

Experiment 2. Three types of detergent sanitizers were tested by the Weber and Black (209, pp.1406-1416) technique for their germicidal activity against P. aeruginosa and E. coli when dissolved in distilled, Navy hard, and Navy hard water plus one per cent whole milk. Each type of detergent sanitizer was prepared with three concentrations (50, 75, and 100 ppm, respectively) of alkyl dimethyl ethyl benzyl ammonium chloride. The pH of each of these solutions was determined with a Beckman glass electrode pH meter.

The low pH detergent sanitizer contained four per cent QAC, eight per cent nonionic wetting agent and 88 per cent hydroxy acetic acid and water. This mixture yielded a use dilution of 50 ppm QAC. The use dilutions of 75 and 100 ppm were obtained by increasing the QAC content to six and eight per cent, respectively, and by reducing the quantity of water proportionately.

The composition of the medium alkaline pH sanitizer was: four per cent QAC, four per cent nonionic wetting agent and 92 per cent tetrasodium pyrophosphate, sodium tripolyphosphate, sodium bicarbonate, and trisodium phosphate. This mixture yielded a use dilution of 50 ppm QAC. The mixtures that yielded use dilutions of 75 and 100 ppm QAC, increased the amount of QAC to six and eight per cent, respectively, and decreased the amount of detergent material

proportionately. The amount of nonionic wetting agent remained the same in all three mixtures.

The high pH detergent sanitizer contained four per cent QAC, four per cent nonionic wetting agent, 80 per cent tetrasodium pyrophosphate, sodium tripolyphosphate, sodium bicarbonate and trisodium phosphate and 12 per cent soda ash. This mixture yielded a use dilution of 50 ppm QAC. The use dilution of 75 and 100 ppm was obtained by a concentration of six and eight per cent QAC, respectively, with a corresponding decrease in detergent materials and soda ash. The quantity of nonionic wetting agent remained at four per cent in all mixtures.

Experiment 3. The effect of phosphates on the germicidal activity of QAC was determined with the Weber and Black (209, pp. 1406-1416) technique. P. aeruginosa was selected as the test organism because of its great resistance to QAC activity.

The first part of this experiment utilized para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride at 50, 100, and 200 ppm concentrations as the test QAC. This compound was chosen because it was the QAC employed in a very simple type detergent sanitizer. The composition of this detergent sanitizer was 10.7 per cent QAC, 81 per cent tetrasodium pyrophosphate, and 8.3 per cent nonionic wetting agent.

In the germicidal trials on effect of various detergent sanitizer ingredients on QAC action, the QAC was dissolved in distilled water, a borate buffer, or in solutions containing tetrasodium pyrophosphate

or trisodium phosphate. The pH values of the distilled water solutions were between 7.4 and 7.5. The borate buffer solutions contained 0.1 per cent boric acid adjusted to pH 9.5 with sodium carbonate. The germicidal solutions prepared with tetrasodium pyrophosphate or trisodium phosphate contained 1.6035 grams of these compounds per liter when the QAC concentration was 200 ppm. This weight was selected because it was the amount present in the detergent sanitizer and provided a concentration of approximately 1600 ppm in use dilution. The quantity was reduced proportionately when the concentration of QAC was reduced to 100 and 50 ppm. The pH of these solutions varied between 9.2 and 9.5. The pH of the trisodium phosphate solution was reduced with concentrated hydrochloric acid to these values. The complete detergent sanitizer also was used. The pH of its solutions varied between 9.2 and 9.5.

The effect of addition of tetrasodium pyrophosphate, trisodium phosphate and borate buffer also was determined on germicidal activity of alkyl dimethyl ethyl benzyl ammonium chloride solutions. Details of this experiment were similar to those for the above described trial on para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride.

RESULTS

Experiment 1. Table 19 shows that hard water affected the germicidal activity of 200 ppm alkyl dimethyl ethyl benzyl ammonium chloride against P. aeruginosa. The values are the average of three separate trials. The extent of decrease in germicidal activity varied with the increase in hardness of water. The Starr ranch water, with 168 ppm hardness reduced QAC activity as compared with the distilled water. The hardest water (Roseburg) decreased the effectiveness of the germicide markedly. Navy hard water fell between these extremes. The fact that the Navy hard water decidedly decreased germicidal activity demonstrated that a standard hard water of uniform composition was available for further experimental work.

The effect of Navy hard water and Navy hard water plus one per cent whole milk on the germicidal activity of alkyl dimethyl ethyl benzyl ammonium chloride against P. aeruginosa and E. coli is shown in table 20. The average of three separate trials are listed. A concentration of 50 ppm of the germicide destroyed 98.628 of the P. aeruginosa cells after 300 seconds exposure, when distilled water was used. When the Navy hard water was used, 89.752 per cent of the cells were killed. However, only 51.915 per cent of the cells were destroyed when one per cent whole milk was added to the Navy hard water. Similar results were obtained with a concentration of 100 ppm QAC. The percentage destruction was 99.997, 99.776, and 83.830, respectively.

E. coli was destroyed more rapidly than P. aeruginosa when

TABLE 19

Rate of destruction of Pseudomonas aeruginosa by 200 ppm alkyl dimethyl ethyl benzyl ammonium chloride in different types of water

Water	Per cent organisms killed at the following exposure periods in seconds:				
	15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	99.960	99.982	99.997	99.999	100.000
Starr ranch	99.316	99.791	99.933	99.988	99.998
Navy hard	94.250	97.607	99.058	99.712	99.972
Roseburg	60.000	71.798	75.263	87.121	88.831

TABLE 20

Rate of destruction of Pseudomonas aeruginosa and Escherichia coli by alkyl dimethyl ethyl benzyl ammonium chloride in distilled water, Navy hard water and Navy hard water plus one per cent whole milk

Water	Conc. of germ. (ppm)	Organism	Per cent organisms killed at the following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	P. aeruginosa	92.589	95.288	96.525	98.177	98.628
Navy hard			50.000	64.043	81.389	89.007	89.752
Navy hard plus milk			50.000	50.000	50.000	50.000	51.915
Distilled	100	P. aeruginosa	99.930	99.958	99.973	99.995	99.997
Navy hard			93.014	97.780	98.904	99.454	99.776
Navy hard plus milk			50.000	50.000	61.489	75.532	83.830
Distilled	50	E. coli	99.795	99.912	99.961	99.970	99.987
Navy hard			64.818	77.172	83.929	88.869	93.571
Navy hard plus milk			50.000	50.000	50.000	50.000	53.929
Distilled	100	E. coli	99.999	100.000	100.000	100.000	100.000
Navy hard			99.945	99.985	99.992	99.998	100.000
Navy hard plus milk			50.000	50.000	50.000	50.000	50.000

distilled or Navy hard water was used. However, the rate of destruction was slower when one per cent whole milk was added to the Navy hard water. Only 50 per cent of the organisms were destroyed in 300 seconds at 100 ppm when one per cent whole milk was present. P. aeruginosa had 83.830 per cent destruction at the same time and concentration. A similar trend can be noted when a concentration of 50 ppm QAC was used.

Experiment 2. The pH values of solutions of three different detergent sanitizers in three types of water are listed in table 21. The figures are the average of results obtained with two separate Beckman glass electrode pH meters. Navy hard water and Navy hard water plus one per cent whole milk did not materially affect the pH of the final solution. The pH of the low pH detergent sanitizer varied between 3.1 and 3.4, of the medium alkaline pH compound between 8.0 and 8.6, and of the high pH material between 9.5 and 9.8.

The effect of three different detergent sanitizers dissolved in various waters on E. coli is shown in tables 22 to 24. The values are the average of three separate trials. The activity of the low pH detergent sanitizer at three germicidal concentrations is presented in table 22. After 30 seconds exposure to any one germicide, no marked difference can be noted in rate of destruction between the distilled water, Navy hard water and Navy hard water plus one per cent whole milk germicides. Since the detergent sanitizer contained alkyl dimethyl ethyl benzyl ammonium chloride as the germicidal agent, these results can be compared with those of table 20. In the latter

TABLE 21

pH levels of nine different detergent sanitizer solutions prepared in distilled water, Navy hard water and Navy hard water plus one per cent whole milk

Compound	Conc. of germ. (ppm)	pH of solution in the following waters: distilled (pH)	Navy hard (pH)	Navy hard + milk (pH)
Low pH	50	3.3	3.4	3.4
Low pH	75	3.3	3.1	3.3
Low pH	100	3.2	3.3	3.2
Medium alkaline pH	50	8.4	8.5	8.0
Medium alkaline pH	75	8.6	8.6	8.4
Medium alkaline pH	100	8.1	8.2	8.0
High pH	50	9.6	9.7	9.5
High pH	75	9.7	9.7	9.6
High pH	100	9.7	9.7	9.8

TABLE 22

Rate of destruction of Escherichia coli by a low pH detergent sanitizer solution in different types of water

Water	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	93.143	98.145	98.414	98.926	99.995
Navy hard		81.607	98.290	98.712	99.555	99.949
Navy hard plus milk		73.559	92.816	99.181	99.947	100.000
Distilled	75	83.390	98.060	99.593	99.995	100.000
Navy hard		95.117	97.248	99.654	99.996	100.000
Navy hard plus milk		90.350	97.145	98.751	99.996	99.999
Distilled	100	97.226	98.748	99.477	99.990	100.000
Navy hard		92.699	98.248	99.007	99.985	100.000
Navy hard plus milk		93.431	99.059	99.976	99.998	100.000

case, there was a great difference in rate of kill between various types of water. The rate of destruction when distilled water was used was about the same in both cases. However, some factor in the detergent sanitizer increased the activity of the germicide in the presence of hard water and of milk. This increased activity is evident with all three concentrations of the germicide.

The results with the medium alkaline pH detergent sanitizer are shown in table 23 and with the high pH detergent sanitizer in table 24. In both trials, the detergent sanitizer increased the rate of destruction by the germicide. The increase was so pronounced that germicidal activity of Navy hard water or Navy hard water plus one per cent whole milk germicides approached that of distilled water solutions. The medium alkaline pH compound appeared to be slightly more active than either the low or high pH mixtures. This tendency probably was due to a more favorable pH for the activity of the germicide. However, the difference in effectiveness of all three mixtures was slight.

The effect of the same three detergent sanitizers against P. aeruginosa are presented in tables 25 to 27. These values also are the average of three separate determinations. Table 25 shows that the low pH detergent sanitizer was extremely active against this organism. Normally, P. aeruginosa is more resistant to QAC than is E. coli. With this detergent sanitizer preparation, however, it is very much less resistant. A concentration of 50 ppm germicide destroyed 99.999 and 100 per cent of the cells in 30 seconds when distilled and Navy hard water, respectively, were used. However, 300 seconds were

TABLE 23

Rate of destruction of Escherichia coli by a medium alkaline detergent sanitizer solution in different types of water

Water	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	98.781	99.949	99.982	99.999	100.000
Navy hard		99.548	99.765	99.915	99.996	100.000
Navy hard plus milk		60.000	88.743	99.233	99.789	99.964
Distilled	75	99.638	99.938	99.957	100.000	100.000
Navy hard		99.725	99.853	99.960	99.999	100.000
Navy hard plus milk		89.535	98.917	99.633	99.906	99.971
Distilled	100	99.771	99.924	99.969	100.000	100.000
Navy hard		99.827	99.845	99.952	99.998	100.000
Navy hard plus milk		99.535	99.755	99.873	99.940	99.995

TABLE 24

Rate of destruction of Escherichia coli by a high pH detergent sanitizer solution in different types of water

Water	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	99.917	99.969	99.991	99.996	100.000
Navy hard		99.737	99.808	99.839	99.977	99.999
Navy hard plus milk		79.103	89.295	99.568	99.868	99.869
Distilled	75	99.482	99.723	99.973	99.997	100.000
Navy hard		99.884	99.852	99.962	99.995	100.000
Navy hard plus milk		88.317	99.401	99.832	99.898	99.989
Distilled	100	99.759	99.887	99.976	100.000	100.000
Navy hard		99.851	99.875	99.959	99.998	100.000
Navy hard plus milk		99.261	99.722	99.889	99.958	99.987

TABLE 25

Rate of destruction of Pseudomonas aeruginosa by a low pH detergent sanitizer solution in different types of water

Water	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	99.997	99.999	100.000	100.000	100.000
Navy hard		99.998	100.000	100.000	100.000	100.000
Navy hard plus milk		99.880	99.992	99.996	99.998	100.000
Distilled	75	99.999	100.000	100.000	100.000	100.000
Navy hard		99.999	100.000	100.000	100.000	100.000
Navy hard plus milk		99.883	99.994	99.997	99.999	100.000
Distilled	100	100.000	100.000	100.000	100.000	100.000
Navy hard		99.999	100.000	100.000	100.000	100.000
Navy hard plus milk		99.993	100.000	100.000	100.000	100.000

required to produce 100 per cent kill when one per cent whole milk was added to the hard water. Table 22 shows that the rate of destruction of E. coli by the low pH detergent sanitizer was approximately equal for all three types of water. This indicates that the low pH detergent sanitizer was less effective in overcoming the protective action of milk from P. aeruginosa than from E. coli. The overall increased activity of the detergent sanitizer probably was due to the pH of the solution. PART III has shown that this organism was very susceptible to QACs in an acid range.

Tables 26 and 27 show that the medium alkaline pH and high pH detergent sanitizers increased the activity of alkyl dimethyl ethyl benzyl ammonium chloride against P. aeruginosa as compared with the results presented in table 20. However, the effectiveness of these detergent sanitizers was not as great as the low pH preparation. With both detergent sanitizers at all three germicidal concentrations, the effectiveness was not as great when one per cent whole milk was present in Navy hard water.

Experiment 3. Tables 28 and 29 show the effect of phosphates on the germicidal activity of two QACs against P. aeruginosa . The values presented are the average of three separate trials. The results obtained with para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride are shown in table 28. The non-buffered, buffered, and trisodium phosphate solutions showed about equal germicidal activity. The solutions containing tetrasodium pyrophosphate and the detergent sanitizer were much more active against P. aeruginosa than

TABLE 26

Rate of destruction of Pseudomonas aeruginosa by a medium alkaline detergent sanitizer solution in different types of water

Water	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	99.741	99.879	99.987	99.995	99.999
Navy hard		99.575	99.742	99.901	99.919	99.985
Navy hard plus milk		63.127	82.051	88.534	95.777	99.814
Distilled	75	99.917	99.979	99.987	99.998	100.000
Navy hard		99.687	99.861	99.967	99.989	99.999
Navy hard plus milk		67.071	86.369	97.649	99.405	99.935
Distilled	100	99.917	99.979	99.987	99.998	100.000
Navy hard		99.638	99.880	99.978	99.997	100.000
Navy hard plus milk		98.226	99.347	99.732	99.849	99.989

TABLE 27

Rate of destruction of Pseudomonas aeruginosa by a high pH detergent sanitizer solution in different types of water

Water	Conc. of germ. (ppm)	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	99.921	99.969	99.980	99.998	100.000
Navy hard		99.828	99.912	99.973	99.988	99.996
Navy hard plus milk		72.307	81.797	93.902	98.315	99.753
Distilled	75	99.791	99.970	99.987	99.993	100.000
Navy hard		99.791	99.845	99.940	99.977	99.997
Navy hard plus milk		84.205	93.246	97.963	99.626	99.964
Distilled	100	99.938	99.935	99.992	99.996	100.000
Navy hard		99.767	99.856	99.972	99.993	100.000
Navy hard plus milk		92.067	98.082	99.532	99.441	99.400

TABLE 28

Effect of pH and phosphate addition on the germicidal activity of para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride against Pseudomonas aeruginosa

Compound	Conc. of germ. (ppm)	pH	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	7.5	63.564	83.163	90.326	94.532	97.401
Borate buffer		9.5	67.942	79.815	87.028	93.840	97.818
Pyrophosphate		9.3	99.992	99.999	100.000	100.000	100.000
Trisodium phosphate		9.2	61.567	86.863	87.059	91.177	95.098
Detergent sanitizer		9.2	99.999	100.000	100.000	100.000	100.000
Distilled	100	7.4	96.927	98.275	98.800	99.364	99.727
Borate buffer		9.5	98.646	99.470	99.746	99.935	99.988
Pyrophosphate		9.4	99.999	100.000	100.000	100.000	100.000
Trisodium phosphate		9.3	94.902	96.556	98.360	99.190	99.772
Detergent sanitizer		9.4	100.000	100.000	100.000	100.000	100.000
Distilled	200	7.4	99.662	99.949	99.987	99.999	100.000
Borate buffer		9.5	99.999	100.000	100.000	100.000	100.000
Pyrophosphate		9.5	100.000	100.000	100.000	100.000	100.000
Trisodium phosphate		9.4	99.487	99.601	99.992	99.999	100.000
Detergent sanitizer		9.5	100.000	100.000	100.000	100.000	100.000

the non-buffered, buffered and trisodium phosphate solutions. The detergent sanitizer solution appeared to be slightly more active than the tetrasodium pyrophosphate plus QAC. However, the difference between the two solutions was slight.

Table 29 shows the results obtained with alkyl dimethyl ethyl benzyl ammonium chloride. There appeared to be no difference between the non-buffered, buffered and trisodium phosphate trials. However, the tetrasodium pyrophosphate increased the effectiveness of the germicide tremendously. A concentration of 50 ppm QAC destroyed between 97.5 and 98.2 per cent of the P. aeruginosa cells in the case of the non-buffered, buffered and trisodium phosphate solutions after 300 seconds exposure. Destruction was 100 per cent complete after 60 seconds exposure when tetrasodium pyrophosphate was present. The same difference was noted when 100 ppm QAC was used. A concentration of 200 ppm QAC was too active to allow differences between the experimental conditions to be observed.

TABLE 29

Effect of pH and phosphate addition on the germicidal activity of alkyl dimethyl ethyl benzyl ammonium chloride against Pseudomonas aeruginosa

Compound	Conc. of germ. (ppm)	pH	Per cent organisms killed at following exposure periods in seconds: 15 (%)	30 (%)	60 (%)	120 (%)	300 (%)
Distilled	50	7.4	90.822	93.715	96.007	96.853	97.508
Borate buffer		9.3	91.784	94.049	95.141	96.725	97.607
Pyrophosphate		9.2	99.997	99.999	100.000	100.000	100.000
Trisodium phosphate		9.1	90.117	92.680	95.740	96.824	98.215
Distilled	100	7.4	99.523	99.852	99.932	99.982	99.999
Borate buffer		9.4	99.980	99.989	99.997	99.999	100.000
Pyrophosphate		9.3	99.997	100.000	100.000	100.000	100.000
Trisodium phosphate		9.2	99.214	99.860	99.920	99.980	99.998
Distilled	200	7.2	100.000	100.000	100.000	100.000	100.000
Borate buffer		9.3	99.999	100.000	100.000	100.000	100.000
Pyrophosphate		9.5	99.999	100.000	100.000	100.000	100.000
Trisodium phosphate		9.3	100.000	100.000	100.000	100.000	100.000

DISCUSSION

Results of this study indicate that effect of calcium and magnesium hard water salts against QAC activity may be nullified if the QAC is used in combination with certain detergent sanitizer ingredients. Whether or not this same effect would operate with iron salts in water has not been established. Likewise, further studies would have to be carried out to establish whether or not natural hard waters would respond to this effect of detergent sanitizer ingredients on QAC action. Another effect of considerable importance is the effect of detergent sanitizer salts on QAC activity in distilled water germicide solutions. This suggests either some factor associated with a protective mechanism of the organism (possibly ions on the cell surface) or a potentiation of germicidal activity by the QAC. Results on addition of various detergent sanitizer ingredients to QAC solutions in distilled water demonstrated that the compounds chiefly responsible for acceleration of germicidal activity was the polyphosphate compounds, tetrasodium pyrophosphate. This suggests the possibility that a sequestering effect on ions, such as Ca and Mg, may be responsible , at least in part, for the greater activity of the QAC in presence of the polyphosphate.

Another observation of considerable significance is the fact that activity of a slower acting QAC from a germicidal standpoint may be accelerated in germicidal action to about the same degree as is possible with a more active QAC. For example, para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride has consistently

shown a slower rate of destruction of test organisms than alkyl dimethyl ethyl benzyl ammonium chloride in distilled water solutions of these two germicides. Yet, when both were prepared in a distilled water solution containing added polyphosphate, both were accelerated in germicidal activity, but their highest activity under such conditions was about equivalent.

There has been considerable controversy regarding the degree to which organic matter inactivates QAC action. Results of this study indicate a pronounced inactivating effect on germicidal action of QACs under all experimental conditions employed. They also suggest that studies on germicidal action of detergent sanitizers should include this factor in order to better simulate natural use conditions for these preparations. In these trials with two representative species, presence of organic matter in the form of one per cent milk exerted a protective effect on each. At both high and low pH levels, the protective factor appeared to be more pronounced with P. aeruginosa. This may be due to the greater inherent resistance of P. aeruginosa to QACs in general.

The studies again brought out the striking relationship between pH, species of organism and QAC activity. P. aeruginosa, a less acid tolerant species, showed greater susceptibility to QAC at low pH levels and E. coli, an acid tolerant species, showed slightly greater resistance to QAC at low pH levels. The reason for this relationship again is not clear, but it is significant that it occurred again in presence of the accelerating detergent sanitizer ingredients. Contrary to some reports in the literature, adjustment of QAC solutions

to a low pH level may be more effective in coping with resistant Pseudomonas species on food handling equipment than adjustment to high pH levels.

SUMMARY AND CONCLUSIONS

Use of synthetically prepared hard water (Navy specification no. 2000) enabled a study on effect of the hard water salts, calcium and magnesium, on germicidal activity of QACs alone and in the form of detergent sanitizers. Addition of one per cent whole milk provided organic matter simulating that encountered under practical use conditions for these preparations.

The QAC in the form of a detergent sanitizer was more active than the QAC alone in the case of two different QACs in distilled water, hard water, and hard water containing organic matter. Results indicated that the active agent accelerating QAC action was the tetrasodium pyrophosphate. Trisodium phosphate showed no pronounced accelerating effect on QAC germicidal activity.

A low pH detergent sanitizer showed greater activity against P. aeruginosa than a high pH preparation. On the other hand, E. coli appeared more resistant under the same conditions at a low pH than at higher pH levels.

The normally high resistance of P. aeruginosa to QACs appeared to be accentuated by addition of organic matter in the form of whole milk to the detergent sanitizer solutions. While E. coli showed greater resistance to QAC in presence of organic matter, this increase was not as pronounced as with P. aeruginosa under the same exposure conditions.

PART V

EFFECT OF QUATERNARY AMMONIUM COMPOUNDS ON ACTIVITY OF LACTIC ACID STARTER BACTERIA IN MILK AND CHEESE

The increased application of QACs for dairy sanitation purposes has stimulated interest in the effect of various concentrations of these compounds on the growth of lactic acid bacteria in milk. The problem is important from the standpoint of attempts to prevent souring of milk or to reduce bacterial counts prior to delivery to the dairy plant. Another important factor is the effect of QAC on lactic acid bacteria in starters, cheese or other cultured milk products for which such milk might be employed. This study deals with the effect of various concentrations of QAC on representative lactic acid starter bacteria.

MATERIALS AND METHODS

Organisms. The organisms selected for this study included a single strain lactic culture, Streptococcus cremoris (R-6), a mixed strain commercial lactic culture widely used in the manufacture of cottage and Cheddar cheese, and two Swiss cheese starter organisms, Lactobacillus lactis (39a) and Streptococcus thermophilus (C-3).

Germicides. The QAC preparations employed were as follows: para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride (QAC 1), Methyl dodecyl benzyl trimethyl ammonium chloride (QAC 2), alkyl dimethyl benzyl ammonium chloride (QAC 3), and a detergent sanitizer (DS) containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride. For purpose of comparison, a commercial sodium hypochlorite (NaOCl) also was included in the trials. Concentration of QAC stock solution was established by the titration method of Harper et al. (94, pp.159-160) and concentration of the hypochlorite solution by titration with sodium thiosulphate (210, pp. 98-100).

QAC or hypochlorite from the stock solution was added to 50 ml. quantities of sterile reconstituted skim milk to provide added concentrations in the milk of 0, 5, 10, 15, 20, 25, 30, 40, 50, 75, and 100 ppm of the compound under study. A concentration of 200 ppm available chlorine as hypochlorite was also used.

It was recognized that the hypochlorite in the above concentrations in milk dissipates rapidly due to reaction with organic matter, and thus the above concentrations do not necessarily represent final

content of available chlorine in the milk. The QACs are more stable in milk than the hypochlorites.

Milks containing the various added concentrations of germicide were inoculated with one per cent of the respective cultures and incubated as follows: Commercial lactic culture, 37.8° C.; S. cremoris, 37.8° C.; S. thermophilus, 48° C.; and L. lactis, 44° C. After six hours incubation, the titratable acidities were determined with N/10 NaOH to phenolphthalein end point. Incubation at temperatures near the maximum for the organisms involved has been employed as an activity test in other studies to more closely simulate manufacturing conditions to which the lactic acid organisms are subjected (63, p.811) (102, p.247).

The experiment also was repeated using sterile whole milk instead of skim milk to determine whether or not the presence of butterfat might affect the bacteriostatic action of the various germicides. Trials also were carried out using an incubation period of 16 hours at the following temperatures for the respective organisms: Commercial lactic culture, 21.1° C. ; S. cremoris, 21.1° C.; S. thermophilus, 37° C.; and L. lactis, 37° C.

Triplicate experimental lots of Cheddar cheese were made with milk containing 0, 5, and 10 ppm alkyl dimethyl benzyl ammonium chloride. The commercial lactic culture was used for these trials. One lot of cheese was made with 20 ppm in the cheese milk. Effect of the QAC was followed by acidity measurement at various stages of manufacture.

RESULTS

Results of the trials are shown in tables 30 to 32. Each value in the table represents the average of three experiments run on separate days. The values obtained in the three different experiments were practically identical throughout. It will be noted that titratable acidities of inoculated milks were slightly lower than the uninoculated control. This may be explained by the neutralizing effect of the cation of the respective germicide compounds added to the milk.

Table 30 shows the effect of the various germicides on growth and acid production of the starter organisms at temperatures near their maximum. Slight inhibition of acid production by 5 ppm of the respective QACs or the detergent sanitizer was evident in the case of each culture. The degree of inhibition increased progressively with an increase in the concentration of the compound. Inhibition was almost complete with 25 to 30 ppm of QAC in the milk. In general, the degree of inhibition of the organisms used was similar. The results suggest that S. thermophilus showed slightly greater resistance to bacteriostatic effect of the QACs than the other organisms. All titratable acidities, including the control, were somewhat low in the case of L. lactis because the culture was not fully activated following removal from the stock culture. However, the results are representative of the comparative inhibition of different concentrations and compounds against this type of organism.

The effect of the QACs and detergent sanitizer on acid production

TABLE 30

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a six hour incubation period in sterile skimmilk at temperatures near their maximum

Culture	Incub. temp. °C.	Germicide	Titratable acidity developed in sterile skimmilk containing the following ppm concentrations of germicide: 0 (%)	5 (%)	10 (%)	15 (%)	20 (%)	25 (%)	30 (%)	40 (%)	50 (%)	75 (%)	100 (%)	200 (%)
Commercial lactic culture	37.8	QAC 1	0.40	0.29	0.24	0.22	0.19	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 2	0.40	0.32	0.24	0.22	0.20	0.19	0.18	0.18	0.17	0.16	0.16	
		QAC 3	0.39	0.32	0.25	0.21	0.19	0.18	0.18	0.18	0.17	0.17	0.16	
		DS	0.40	0.31	0.24	0.22	0.19	0.18	0.18	0.17	0.17	0.16	0.16	
		NaOCl	0.38	0.37	0.37	0.37	0.36	0.36	0.35	0.31	0.30	0.24	0.21	0.16
S. cremoris R-6	37.8	QAC 1	0.34	0.32	0.30	0.26	0.23	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 2	0.35	0.33	0.28	0.25	0.22	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 3	0.36	0.33	0.30	0.27	0.25	0.21	0.20	0.17	0.17	0.16	0.16	
		DS	0.34	0.33	0.29	0.25	0.21	0.19	0.17	0.17	0.17	0.16	0.16	
		NaOCl	0.34	0.34	0.33	0.33	0.33	0.32	0.32	0.30	0.28	0.26	0.19	0.17
S. thermophilus C-3	48.0	QAC 1	0.34	0.30	0.29	0.27	0.25	0.24	0.22	0.19	0.17	0.15	0.14	
		QAC 2	0.31	0.29	0.27	0.27	0.24	0.24	0.21	0.19	0.16	0.15	0.14	
		QAC 3	0.32	0.28	0.27	0.26	0.24	0.23	0.23	0.19	0.17	0.15	0.14	
		DS	0.32	0.30	0.27	0.25	0.23	0.23	0.22	0.18	0.16	0.15	0.14	
		NaOCl	0.31	0.31	0.31	0.30	0.30	0.29	0.28	0.27	0.25	0.24	0.23	0.15
L. lactis 39a	44.0	QAC 1	0.30	0.29	0.26	0.23	0.20	0.20	0.20	0.18	0.18	0.18	0.16	
		QAC 2	0.31	0.28	0.28	0.26	0.23	0.21	0.19	0.19	0.18	0.17	0.16	
		QAC 3	0.32	0.30	0.27	0.24	0.21	0.21	0.19	0.19	0.18	0.17	0.17	
		DS	0.30	0.29	0.26	0.23	0.20	0.19	0.19	0.18	0.18	0.17	0.16	
		NaOCl	0.29	0.30	0.30	0.30	0.29	0.29	0.28	0.28	0.26	0.25	0.23	0.18

Titratable acidity of uninoculated control was 0.18 per cent.

in general was about the same. The sodium hypochlorite, however, showed no pronounced inhibition of acid production until its added available chlorine concentration exceeded 50 ppm. Complete inhibition apparently occurred at a concentration between 100 and 200 ppm.

As shown in table 31, the presence of the milk fat did not greatly affect the bacteriostatic action of the QACs. When the experiment, as shown in table 30, was repeated with whole milk instead of skim milk, identical results were obtained.

Other studies have shown that the inhibitory effect of QACs is not materially reduced by pasteurization of the milk after their addition. However, more severe heat treatment, such as sterilization, may reduce the inhibitory effect of these compounds.

Table 32 shows the inhibition by the various compounds at incubation temperatures normally employed for the different cultures. The relative effect of all types of germicides was less pronounced at the lower incubation temperatures than at near-maximum temperatures. With one exception, complete inhibition by the QACs present was not effected until the concentration reached 50 to 75 ppm. As much as 200 ppm sodium hypochlorite did not completely inhibit growth at the normal incubation temperature of the respective cultures. The S. cremoris and commercial lactic culture appeared to be more susceptible to bacteriostatic effect of the QACs than S. thermophilus or L. lactis. The methyl dodecyl benzyl trimethyl ammonium chloride appeared less bacteriostatic against the lactic acid bacteria studied on a ppm basis than the other QAC preparations used.

The effect of a QAC (alkyl dimethyl benzyl ammonium chloride) on

TABLE 31

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a six hour incubation period in sterile whole milk at temperatures near their maximum

Culture	Incub. temp. °C.	Germicide	Titratable acidity developed in sterile whole milk containing the following ppm concentrations of germicide: 0 (%)	5 (%)	10 (%)	15 (%)	20 (%)	25 (%)	30 (%)	40 (%)	50 (%)	75 (%)	100 (%)	200 (%)
Commercial lactic culture	37.8	QAC 1	0.30	0.28	0.26	0.23	0.22	0.21	0.21	0.20	0.18	0.17	0.16	
		QAC 2	0.30	0.27	0.25	0.24	0.23	0.22	0.21	0.19	0.19	0.18	0.17	
		QAC 3	0.31	0.29	0.26	0.24	0.23	0.22	0.21	0.19	0.19	0.18	0.16	
		DS	0.31	0.28	0.25	0.23	0.22	0.21	0.21	0.20	0.18	0.17	0.16	
		NaOCl	0.31	0.31	0.30	0.31	0.31	0.30	0.29	0.28	0.27	0.24	0.22	0.16
S. cremoris R-6	37.8	QAC 1	0.26	0.24	0.23	0.22	0.21	0.20	0.19	0.18	0.18	0.17	0.15	
		QAC 2	0.25	0.23	0.23	0.21	0.20	0.20	0.19	0.19	0.18	0.17	0.15	
		QAC 3	0.25	0.23	0.22	0.21	0.20	0.20	0.19	0.19	0.18	0.17	0.15	
		DS	0.26	0.24	0.23	0.22	0.20	0.20	0.19	0.18	0.17	0.16	0.15	
		NaOCl	0.24	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.22	0.20	0.20	0.15
S. thermophilus C-3	48.0	QAC 1	0.34	0.33	0.29	0.28	0.27	0.23	0.22	0.20	0.19	0.17	0.17	
		QAC 2	0.34	0.34	0.33	0.28	0.26	0.26	0.22	0.20	0.19	0.18	0.16	
		QAC 3	0.35	0.33	0.30	0.27	0.26	0.24	0.22	0.20	0.18	0.18	0.17	
		DS	0.34	0.33	0.27	0.26	0.25	0.23	0.22	0.20	0.19	0.17	0.16	
		NaOCl	0.36	0.35	0.36	0.35	0.36	0.35	0.33	0.34	0.33	0.31	0.26	0.18
L. lactis 39a	44.0	QAC 1	0.30	0.30	0.27	0.25	0.24	0.22	0.21	0.20	0.20	0.18	0.18	
		QAC 2	0.30	0.29	0.28	0.27	0.26	0.23	0.22	0.22	0.20	0.19	0.17	
		QAC 3	0.30	0.28	0.27	0.27	0.26	0.23	0.22	0.21	0.21	0.19	0.18	
		DS	0.30	0.28	0.27	0.26	0.23	0.22	0.22	0.21	0.20	0.19	0.17	
		NaOCl	0.28	0.29	0.29	0.29	0.29	0.29	0.29	0.27	0.28	0.26	0.24	0.17

Titratable acidity of uninoculated control was 0.18 per cent.

TABLE 32

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a 16 hour incubation period in sterile skimmilk at normal temperatures

Culture	Incub. temp. °C.	Germicide	Titratable acidity developed in sterile skimmilk containing the following ppm concentrations of germicide: 0 (%)	5 (%)	10 (%)	15 (%)	20 (%)	25 (%)	30 (%)	40 (%)	50 (%)	75 (%)	100 (%)	200 (%)
Commercial lactic culture	21.1	QAC 1	0.79	0.77	0.70	0.63	0.48	0.37	0.32	0.23	0.21	0.18	0.16	
		QAC 2	0.82	0.78	0.74	0.68	0.61	0.43	0.34	0.24	0.21	0.18	0.16	
		QAC 3	0.79	0.72	0.67	0.57	0.48	0.39	0.32	0.23	0.20	0.17	0.17	
		DS	0.79	0.75	0.72	0.63	0.46	0.34	0.30	0.23	0.19	0.16	0.15	
		NaOCl	0.79	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78	0.71	0.72	0.63
S. cremoris R-6	21.1	QAC 1	0.71	0.68	0.65	0.59	0.53	0.30	0.26	0.21	0.19	0.17	0.16	
		QAC 2	0.72	0.69	0.67	0.64	0.59	0.48	0.35	0.27	0.22	0.17	0.16	
		QAC 3	0.71	0.68	0.63	0.58	0.48	0.32	0.26	0.20	0.18	0.17	0.16	
		DS	0.69	0.67	0.63	0.58	0.43	0.30	0.25	0.21	0.18	0.16	0.15	
		NaOCl	0.71	0.71	0.72	0.72	0.71	0.70	0.71	0.69	0.70	0.68	0.67	0.59
S. thermophilus C-3	37.0	QAC 1	0.74	0.65	0.61	0.59	0.59	0.52	0.49	0.44	0.28	0.18	0.14	
		QAC 2	0.72	0.71	0.68	0.65	0.65	0.62	0.54	0.42	0.27	0.20	0.14	
		QAC 3	0.69	0.62	0.61	0.56	0.56	0.52	0.48	0.39	0.27	0.16	0.15	
		DS	0.73	0.71	0.64	0.60	0.53	0.48	0.42	0.36	0.25	0.16	0.14	
		NaOCl	0.72	0.72	0.71	0.71	0.71	0.70	0.68	0.67	0.65	0.59	0.55	0.40
L. lactis 39a		QAC 1	0.78	0.77	0.69	0.67	0.60	0.52	0.49	0.42	0.28	0.15	0.14	
		QAC 2	0.77	0.76	0.72	0.68	0.63	0.59	0.54	0.46	0.31	0.24	0.14	
		QAC 3	0.75	0.67	0.64	0.61	0.58	0.54	0.50	0.44	0.32	0.16	0.14	
		DS	0.77	0.74	0.70	0.63	0.57	0.52	0.48	0.46	0.31	0.15	0.12	
		NaOCl	0.75	0.75	0.75	0.75	0.76	0.75	0.75	0.74	0.72	0.65	0.63	0.46

Titratable acidity of uninoculated control was 0.18 per cent.

acid production during manufacture of Cheddar cheese is shown in table 33. Since only two experimental vats were available for a single trial, it was necessary to include a 0 ppm control vat for each concentration of QAC studied. Thus, on one day, one vat contained 0 and the second 5 ppm, and on the next day one contained 0 and the second 10 ppm QAC. The entire experiment including 0, 5, and 10 ppm was run three times on successive days. The results were highly consistent through all three experiments and table 33 represents a typical trial. A concentration of 5 ppm QAC in the cheese milk delayed milling time about 15 minutes over the control with no QAC. A delay in milling time of 45 to 60 minutes occurred with 10 ppm QAC in the cheese milk. In an additional trial with 20 ppm alkyl dimethyl benzyl ammonium chloride added to the cheese milk, the acidity of the whey reached only 0.20 per cent during a 7.5-hour manufacturing period.

TABLE 33

Effect of added quaternary ammonium compound in cheese milk on acid development during manufacture of Cheddar cheese

Operation	Time	Change in per cent titratable acidity with the following concentrations of quaternary ammonium compound:			
		Control 0 ppm	QAC 5 ppm	Control 0 ppm	QAC 10 ppm
	hours:minutes	(%)	(%)	(%)	(%)
Received milk	0:00	0.17	0.17	0.17	0.17
Added starter	0:15	0.18	0.18	0.17	0.17
Added rennet	1:15	0.19	0.19	0.18	0.18
Cut curd	1:45	0.12	0.12	0.13	0.13
Began cooking	2:00	0.12	0.12	0.13	0.13
Steam off	2:30	0.14	0.14	0.14	0.14
Drained whey	3:30	0.16	0.16	0.17	0.16
Packed curd	3:45	0.21	0.21	0.23	0.18
Cheddared	4:00	0.27	0.26	0.30	0.23
	4:15	0.34	0.31	0.40	0.27
	4:30	0.39	0.37	0.45	0.30
	4:45	0.44	0.42	0.52*	0.33
	5:00	0.52*	0.48		0.36
	5:15		0.52*		0.40
	5:30				0.48
	5:45				0.54*

* Milling time

DISCUSSION

The minimum concentration of 5 ppm QAC added to milk in these studies caused very slight inhibition of four different lactic acid starter cultures. If excessive quantities of QAC should enter the milk supply, through intentional adulteration or accident, definite inhibition of lactic acid starter bacteria may occur. The effect of higher concentrations of these compounds (5 to 10 ppm) would vary with the manufacturing conditions employed for a dairy product. For example, the effect might be more pronounced in manufacture of Cheddar cheese than in cottage cheese or buttermilk. Presumably, the near-maximum growth temperature employed in Cheddar cheese manufacture accentuates the bacteriostatic effect of the germicide on lactic starter organisms. The delay of 45 to 60 minutes in milling time caused by 10 ppm QAC is considered significant. Such an extension in time would be detrimental to economical operation of a cheese plant. A concentration of 5 ppm of these compounds in the cheese milk might not cause a noticeable delay in acid development in such a product. Another problem suggested, but not investigated in these studies, is the possible effect of QACs in the cheese milk on rate of ripening and final flavor of the cheese.

Results of these studies emphasize that a delay in souring of raw milk may be accomplished by as little as 20 ppm of QAC. Unless a milk grader is familiar with the bitter flavor contributed to milk by QACs, he may fail to detect such a quantity in the milk. However, concentrations as low as 5 ppm of these compounds in milk can be

detected by a modified eosin indicator titration method developed in the course of these studies.

SUMMARY AND CONCLUSIONS

A study was carried out on the effect of three QACs, a quaternary containing detergent sanitizer, and a sodium hypochlorite on acid development by four types of lactic acid starters in sterile skim milk and whole milk.

All of the cultures (S. cremoris, a mixed strain commercial lactic culture, L. lactis and S. thermophilus) were slightly inhibited by 5 ppm of each of the QACs added to the milk.

The inhibition of acid development was nearly complete in all cultures with 25 to 30 ppm of QAC in the milk, when the organisms were incubated at temperatures near their maximum. At incubation temperatures normally used for culturing the organisms, 50 ppm of these compounds in the milk effected nearly complete inhibition.

Milling time was delayed 15 minutes by presence of 5ppm, and 45 to 60 minutes by 10 ppm QAC in the manufacture of experimental Cheddar cheese.

Results obtained suggest the necessity of employing farm and plant sanitizing procedures that will avoid contamination of milk with inhibitory concentrations of QACs.

PART VI

A RAPID METHOD OF TESTING FOR QUATERNARY AMMONIUM COMPOUNDS IN MILK

The application of QACs for various dairy sanitative operations has resulted in a need for a simple, sensitive method of determining concentration of these compounds in milk. Presence of certain types of QACs in milk may be detected organileptically in concentrations as low as seven ppm; minimum concentration of other compounds detectable in milk by taste may be as high as 56 ppm (123, p.503). However, detection of these compounds by organoleptic test in general is neither sufficiently nor practical for dairy plants or inspectors.

As has been shown in PART V of this thesis, small amounts of QAC have a detrimental effect on lactic acid starter bacteria. Therefore, it is important that some simple method be available to determine the presence of these compounds in milk. Various test methods have been recommended to determine concentration of QAC in water solution and in certain foods. However, no known test procedure has proven accurate in determining low concentrations of these compounds in milk. The test method presented here is a modification of a procedure recommended by Harper et al. (94, pp.159, 160) for the determination of QACs in water solution.

MATERIALS AND METHODS

Indicator solution. (a) dissolve eosin yellowish dye (dye concentration of about 90 per cent, Biological Commission Color Index 768) in acetone (analytical reagent) at the rate of 0.5 mg. of dye to 1. ml. of acetone. (b) Add acetone-eosin solution to tetrachloroethane.(technical grade) at the rate of 1 ml. of acetone-eosin to 9 ml. of tetrachloroethane. (c) Remove the reddish color from the solution by adding citric acid crystals (monohydrate, analytical reagent) at the rate of 10 mg. of crystals to each ml. of dye solution. (d) Shake for 1 minute, or until the red color disappears. (e) Filter through filter paper.

Buffer. Prepare a solution of citric acid (monohydrate, analytical reagent) at the rate of 25 gm. to 100 ml. distilled water and adjust to pH 3.5 with 50 per cent NaOH (analytical reagent). Approximately 12 ml. of NaOH to 100 ml. of citric acid solution usually are required.

Anionic solution. Prepare a 0.01 per cent solution of active anionic compound from 10 per cent Fisher Laboratory Aerosol (10 per cent di-octyl sodium sulfosuccinate). This represents approximately a 1/1000 dilution of the 10 per cent Aerosol. Other anionic surface active agents may prove suitable for this solution.

Preparation of standards. Prepare standards for comparison by adding known concentrations of the desired QACs to unhomogenized milk samples to provide final concentrations covering the range suspected

in the test samples. This method of standardization is necessary because different QAC preparations yield different titration values, and interpretation of the titration end point varies with different individuals.

Procedure. 1. Place 1 ml. of the milk to be analyzed in a test tube. Add 5 ml. of distilled water, 1 ml. of indicator solution, and 0.2 ml. of buffer solution. Plug test tube with a cork stopper and shake vigorously for 10 seconds.

2. Centrifuge to separate solvent fraction from milk solids and water. This may be accomplished by centrifuging for 5 minutes at 3200 RPM in a 10-inch centrifuge. A Babcock centrifuge can be used, but may require as long as 25 minutes for the same operation. Three distinct layers should be apparent following the centrifuging. The top layer should be a liquid; the middle layer should consist chiefly of precipitated protein; and the lower layer should consist of the solvent containing most of the QAC in the sample. The procedure up to this point may be employed as a qualitative test for detection of QACs in milk. Presence of these compounds is indicated by development of a red color in the solvent layer. A pink to red color usually indicates at least 5 ppm QAC. The subsequent steps in the procedure outlined below are necessary for an approximate determination of the amount present.

3. Remove the top layer. Pour out the solvent layer into a second tube. The precipitated protein should adhere to the first tube when the solvent layer is transferred. Flush remaining solvent

fraction from this tube with 5 ml. of distilled water. Transfer this water to the solvent in the second tube and agitate for about 5 seconds to thoroughly mix contents. The rate of separation of solvent from the water layer can be acclerated by centrifuging for about 20 seconds. Remove the top layer. Wash the solvent layer in the tube twice more. Each of these washings should consist of addition of 5 ml. of water to the solvent in the tube, agitation of contents, centrifuging for 20 seconds, and removal of the water layer.

4. Add 1 ml. of distilled water and 0.1 ml. of buffer to the solvent layer after the last washing. Titrate this mixture with standard anionic solution. Add slowly and shake until the red color of the lower layer is removed. The quantity of QAC is indicated by the ml. of anionic solution required to remove the red color. A microburette facilitates this titration. In some samples, pigments such as carotene may slightly mask the red color formed by QAC and eosin. A few experimental trials, however, should enable accurate interpretation of the end point.

Test samples. In trials to evaluate the accuracy of the new method for determination of QAC in milk, each compound to be tested was made into a distilled water solution containing 500 ppm according to the concentration specified by the manufacturer. The concentration of this solution was checked by the test method of Harper et al. (94, pp.159, 160). The necessary quantity of the respective stock solution then was added to various milk samples so that the final QAC content would be 0, 5, 10, 25, 50, and 100 ppm, respectively. The recommended

test procedure outlined above then was used to determine the concentration of this compound in each of the milk samples. Samples were prepared in such a manner that their QAC content was not known to the operator conducting the titrations.

RESULTS

The quantity of anionic solution required to titrate 0 to 100 ppm of different types of QACs in milk by the new method is shown in table 34. Results indicate the method to be reasonable consistent for the types of QACs tested. Expected differences in titration values occurred between different types of these compounds. Results similar to those shown have been obtained on 11 different types of QACs and 3 detergent sanitizers added to milk. Some difficulty has been experienced in determining concentrations greater than 100 ppm. The most consistent results have been obtained when concentrations of 50 to 100 ppm QAC were present in milk.

The actual percentage recovery of the original QAC added to the milk samples was not determined in these studies. A number of trials indicate that more than half of the original compound added to the milk was recovered in the final, washed solvent fraction titrated in step 4 of the procedure. Results in table 34 and in a large number of other trials indicated the percentage recovery in this fraction to be fairly consistent. If desired, the recovery of QAC can be increased by subjecting wash water and milk solids fractions removed in step 3 of the procedure to further extractions by the indicator solution and washing until all QAC has been recovered. The recovered compound can be titrated as in step 4. However, under normal conditions, where suitable standards for comparison are included, the additional extractions are unnecessary.

There was a difference in the sharpness of the end point when

TABLE 34

Quantity of anionic solution required to titrate various concentrations of added quaternary ammonium compound in milk

QAC	ml. of anionic solution to titrate the following concentrations of QAC in milk: 0 ppm (ml)	5 ppm (ml)	10 ppm (ml)	25 ppm (ml)	50 ppm (ml)	100 ppm (ml)
Alkyl dimethyl benzyl ammonium chloride	0.00*	0.05	0.14	0.35	0.65	1.20
	0.00	0.05	0.12	0.37	0.67	1.21
	0.00	0.06	0.14	0.33	0.60	1.18
	0.00*	0.05	0.13	0.32	0.65	1.14
	0.00	0.04	0.13	0.33	0.62	1.19
Para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride	0.00*	0.05	0.14	0.29	0.61	1.19
	0.00	0.04	0.15	0.29	0.64	1.21
	0.00	0.05	0.13	0.36	0.61	1.17
	0.00*	0.03	0.12	0.32	0.62	1.20
	0.00	0.03	0.13	0.29	0.57	1.14
Methyl dodecyl benzyl trimethyl ammonium chloride	0.00*	0.03	0.04	0.21	0.30	0.70
	0.00	0.04	0.08	0.22	0.35	0.64
	0.00	0.03	0.07	0.22	0.34	0.68
	0.00*	0.03	0.07	0.23	0.34	0.66
	0.00	0.03	0.09	0.19	0.34	0.65

*The first three values for each QAC represents triplicate determinations on one set of unknown; the last two values represent duplicate determinations on another set of unknown samples prepared the following day.

different types of QACs were tested. However, little difficulty was experienced in establishing the true end point. The intensity of red color in the solvent layer decreased progressively as the concentration of QAC decreased from 100 to 5 ppm. Certain compounds (for example, alkyl dimethyl benzyl ammonium chloride) produced a red color in the solvent layer at concentrations below 5 ppm and levels 2 to 3 ppm of this compound in milk could be determined with a microburette.

Thus far, no false positive tests with this method have been encountered during several hundred determinations on various types of raw and pasteurized mixed herd milks, as well as on individual cow or herd milks. Results obtained on sour milk containing QAC were similar to those obtained on fresh milk. However, difficulty was encountered in determining concentrations of this compound added to homogenized milk.

A modification of this method for QAC determination in milk also may be applied to QAC determination of water solutions of detergent sanitizers. Detergent compounds in the preparation must be removed by successive washings by the same procedure used in the above method for determining this compound in milk.

DISCUSSION

The simplicity and consistent results obtained with the eosin-indicator titration method for QACs in milk should enable its application for detection of gross QAC contamination of milk. The first two steps of the procedure may be employed as a presumptive or qualitative test. Samples can be eliminated if no red color is present in the solvent layer at this stage. If the solvent fraction exhibits a red color, the subsequent washing steps and titration should provide an accurate indication of total quantity of QAC present. The type of QAC in the test sample must be known to prepare the appropriate standards for an accurate determination.

The most accurate range for QAC determinations for either milk or detergent sanitizers appears to be about 5 to 100 ppm. If concentrations higher than 100 ppm are expected, the milk may be diluted to bring the QAC content of the sample in this range. Further studies are needed to establish accuracy of this method for determining concentration of QAC in milk at levels lower than 5 ppm. The reason for differences in sensitivity with different types of QACs has not been investigated. The results obtained with this method indicate, however, that it should be sensitive enough to detect levels of QAC that might be inhibitory to lactic acid bacteria in milk.

Further investigation also is necessary to fully explain the reactions between QACs and eosin indicator. Apparently the successive washings remove certain compounds that interfere with the reversal of this reaction by the anion in the titration solution. The principle

of removal of interfering factors also is important for QAC determination on detergent sanitizer and buffered QAC solutions. Unless this washing is sufficiently thorough and complete, the final accurate titration of QAC present in the solvent fraction is difficult or impossible.

Preliminary observations and a consideration of the general procedure involved suggest that this method or modifications of it may be employed for detection of QACs in certain foods other than milk.

SUMMARY AND CONCLUSIONS

A method has been developed for determination of QACs in milk. It is based on extraction and precipitation of QAC in a tetrachloroethane-acetone-eosin indicator solution. Interfering factors in the solvent indicator are removed by successive washings with distilled water and QAC then is titrated with a standard solution of anionic surface active agent.

The first two steps of the procedure may serve as a presumptive or qualitative test for detection of QAC in milk. The subsequent steps are necessary for a determination of quantity of QAC present. The method has proven suitable for determination of concentration in milk of QAC preparations commonly employed in dairy sanitation procedures. With suitable standards for comparison, the method will determine quantities of QAC in the range of 5 to 100 ppm in milk.

CONCLUSIONS

Sodium hypochlorite in a concentration of 50 ppm destroyed all cells of E. coli, A. aerogenes, P. aeruginosa, Sarcina sp. and M. pyogenes var. aureus in 15 seconds when tested by the Weber and Black method. Lithium hypochlorite demonstrated germicidal activity almost as great as sodium hypochlorite with the one exception that it required 60 seconds to kill all P. aeruginosa cells; whereas, sodium hypochlorite destroyed them in 15 seconds. Experimental conditions permitted no further differentiation in germicidal activity of the two compounds.

Alkyl dimethyl benzyl ammonium chloride and para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride were most effective against A. aerogenes and least effective against P. aeruginosa.

Concentrations of 50 ppm of alkyl dimethyl ethyl benzyl ammonium chloride and a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride destroyed all cells of E. coli in 15 seconds. Alkyl dimethyl benzyl ammonium chloride, para di-isobutyl phenoxy ethoxy benzyl ammonium chloride and methyl dodecyl benzyl trimethyl ammonium chloride followed these two QACs in decreasing sequence in germicidal activity. There appeared to be no consistent relationship between the Gram reaction and susceptibility of various bacterial species to QAC germicides.

One (Nytron) out of nine anionic surface active agents (Nacconal MX, Nacconal NRCL, Nytron, Stepanate, Alkanol DM, Kleer Mor, Kleer Mor Special, Duponal Paste and M 189) added in concentrations of 0.01 per cent to 50 ppm sodium hypochlorite solution markedly lowered the

available chlorine content after an exposure period of 48 hours. Two (Igepal CA Extra and Sterox CD) nonionic surface active agents out of five (Igepal CA Extra, Santomerse # 1, Neutronyx 600, Sterox CD and X-100) decreased the available chlorine content of 50 ppm sodium hypochlorite when added in concentration of 0.01 per cent. Only Stepanate and Duponal Paste did not cause a material decrease in available chlorine when the surface active agents were added in a concentration of 0.10 per cent.

A concentration of 25 ppm sodium hypochlorite at pH 8.0 alone and in combination with five different surface active agents (Santomerse # 1, X-100, Nacconal NRCL, Alkanol DM and Stepanate) destroyed all cells of M. caseolyticus in the minimum test period of 15 seconds employed. Consequently, in order to obtain an end point to evaluate germicidal activity of the hypochlorite alone and in combination with surface active agents, the pH was adjusted to 10.0 and the chlorine content increased to 50 ppm. Under these conditions, no material increase in germicidal activity against M. caseolyticus appeared to be contributed by the presence of the surface active agent, and a concentration of 0.05 per cent X-100 appeared to decrease the effectiveness of the germicide.

More than 96 per cent of a standard suspension of B. cereus spores were destroyed in 20 minutes by all solutions of 200 ppm sodium hypochlorite alone and in combination with three surface active agents (Santomerse # 1, Nacconal NRCL and Alkanol DM), respectively. Two surface active agents (Stepanate and X-100) when added at the rate of 0.05 per cent allowed only 86.587 and 83.352 per cent destruction,

respectively, under the same test conditions. Combinations of 200 ppm sodium hypochlorite with 0.01 per cent of each of the five surface active agents, respectively, destroyed more than 96 per cent of the B. cereus spores in 20 minutes.

Slight changes in pH of hypochlorite solutions have been shown to be far more important in determining their germicidal activity than the presence of compatible surface active agents.

The effect of pH within the range of 3.0 to 10.0 on the germicidal activity of alkyl dimethyl ethyl benzyl ammonium chloride, para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride, methyl dodecyl benzyl trimethyl ammonium chloride and alkyl dimethyl benzyl ammonium chloride against P. aeruginosa, E. coli and M. caseolyticus was determined.

Alkyl dimethyl ethyl benzyl ammonium chloride was most active against P. aeruginosa at pH 3.0 to 7.5. The activity of the QAC decreased as the pH value increased from 7.5 to 9.0. A slight increase in activity occurred as the pH value increased from 9.0 to 10.0. The germicidal activity of the QAC against E. coli and M. caseolyticus was least at pH 3.0 to 4.0. The activity against these two organisms increased at pH values above 4.0 to a maximum at pH 8.0. It then decreased from pH 8.0 to 9.0 and increased again from pH 9.0 to 10.0.

Para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride was most germicidal against P. aeruginosa at pH 3.0 to 6.0. The activity decreased as the pH rose to 9.0 and then increased slightly from pH 9.0 to 10.0. The germicidal activity of the QAC against E. coli and M. caseolyticus was poorest at pH 3.0 to 6.0. It increased

as the pH increased above 6.0 to a maximum activity at pH 8.0. The germicidal activity then decreased at pH 8.0 to 9.0 and again increased at pH 9.0 to 10.0.

Methyl dodecyl benzyl trimethyl ammonium chloride activity against P. aeruginosa increased from pH 3.0 to 6.5. The germicidal activity of the QAC decreased rapidly as the pH rose from 6.5 to 9.0. A slight increase in activity was observed as the pH changed from 9.0 to 10.0. The poorest activity of the QAC against E. coli and M. caseolyticus was observed at pH 3.0. Its activity against these two organisms increased as the pH increased from 3.0 to 7.5. A decrease in activity occurred from pH 7.5 to 9.0. A slight increase in activity was observed from pH 9.0 to 10.0.

Alkyl dimethyl benzyl ammonium chloride was moderately active against P. aeruginosa at pH 3.0 to 5.0. Its activity increased from pH 5.0 to a maximum at pH 6.5. The germicidal activity then decreased from pH 6.5 to 9.0. A slight increase in activity was observed at pH 9.0 to 10.0. The germicidal activity of the QAC against E. coli and M. caseolyticus was poorest at pH 3.0 to 4.0. Its activity against these two organisms increased from pH 4.0 to a maximum at pH 6.5. Against E. coli, the activity decreased rapidly as the pH level changed from 6.5 to 8.0. An increase in activity was observed from pH 8.0 to 10.0. Against M. caseolyticus, the activity decreased rapidly from pH 6.5 to a minimum at pH 7.5. An increase in activity occurred from pH 7.5 to 10.0.

The QAC in the form of a detergent sanitizer was more active than the QAC alone in the case of two different QACs in distilled water,

hard water, and hard water containing organic matter. Results indicated that the active agent accelerating QAC action was the tetrasodium pyrophosphate. Trisodium phosphate showed no pronounced accelerating effect on QAC germicidal activity.

A low pH (3.3) detergent sanitizer showed greater activity against P. aeruginosa than a high pH (9.7) preparation. On the other hand, E. coli appeared more resistant under the same conditions at a low pH than at higher pH levels.

The normally high resistance of P. aeruginosa to QACs appeared to be accentuated by addition of organic matter in the form of whole milk to the detergent sanitizer solutions. While E. coli showed greater resistance to QAC in presence rather than absence of organic matter, this increase was not as pronounced as with P. aeruginosa under the same exposure conditions.

S. cremoris, a mixed strain commercial lactic culture, L. lactis and S. thermophilus were slightly inhibited by 5 ppm of each of the QACs added to the milk. The inhibition of acid development was nearly complete in all cultures with 25 to 30 ppm of QAC in the milk when the organisms were incubated at temperatures near their maximum. At incubation temperatures normally used for culturing the organisms, 50 ppm of these compounds in the milk effected nearly complete inhibition. Milling time was delayed 15 minutes by presence of 5 ppm and 45 to 60 minutes by 10 ppm QAC in the manufacture of experimental Cheddar cheese.

A method has been developed for determination of QACs in milk. It is based on extraction and precipitation of QAC in a tetrachloro-

ethane-acetone-eosin indicator solution. Interfering factors in the solvent indicator are removed by successive washings with distilled water and QAC then is titrated with a standard solution of anionic surface active agent. The first two steps of the procedure may serve as a presumptive or qualitative test for detection of QAC in milk. The subsequent steps are necessary for a determination of quantity of QAC present.

The method has proven suitable for determination of concentration in milk of QAC preparations commonly employed in dairy sanitation procedures. With suitable standards for comparison, the method will determine quantities of QAC in the range of 5 to 100 ppm in milk.

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