A STUDY OF THE ACTION OF SYNTHETIC DETERGENTS ON BACTERIA

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Mary Georgia Blanchard
1942

A STUDY OF THE ACTION OF

SYNTHETIC DETERGENTS

ON BACTERIA

by

MARY GEORGIA BLANCHARD

*

* *

*

A THESIS

Submitted to the Graduate School of Michigan
State College of Agriculture and Applied
Science in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Bacteriology

1 9 4 2

THESIS

ACKNOWLEDGMENT

The writer wishes to thank Dr. W. L. Mallmann, under whose direction this work was carried out, for his guidance and advice.

TABLE OF CONTENTS

INTRODUCTION

The bacteriostatic and bactericidal activity of soaps have been known and studied for many years. This activity varies with the type of soap, with the pH of the solution in which it is used, and with the type of microörganism. The effect of these natural detergents has been thought to involve two factors--the toxic effect of the soap molecule and its surface tension reducing properties.

In recent years there has been developed another group of wetting agents, the synthetic detergents. Certain of these compounds have exhibited a startling bactericidal efficiency--far superior to that shown by the soaps. The literature of the last few years contains many references to the various types of activity displayed by the synthetic detergents of different types, under varying pH conditions, and in various concentrations. In view of the systematic variations in properties reported for these compounds, their bactericidal action seemed worthy of further investigation.

HISTORICAL SURVEY

The study of the activity of the soaps against bacteria was begun in 1911, when Lamar[1] noted that pneumococci treated with sodium oleate became more subject to autolysis and to complete serum-lysis with anti-pneumococcus serum. Nichols[2] in 1919, as a result of his work in the army during the World War, presented bacteriologic data on the epidemiology of respiratory diseases. He observed that sodium oleate had a selective action--killing streptococci readily in two minutes, but having no effect on the typhoid bacillus in ten minutes. Further, the antiseptic action of the soaps was lost if the reaction was changed from a pH of about 8.5 to 7 by the addition of acid.

At about this time Avery[3] noted that soaps of the unsaturated fatty acids were bactericidal for certain bacteria. He reported that the addition of sodium oleate to mediums prevented the growth of certain Gram-positive organisms, principally pneumococcus and streptococcus, while the growth of _Bacillus influenzae_ was enhanced by the presence of this substance.

Walker,[4] in 1924, found that soaps prepared from the pure fatty acids differed markedly in their germicidal properties, with the lower members of the series possessing no (or limited) germicidal properties against the organisms tested. He also observed their selective action against different organisms. _Staphylococcus aureus_, for example, was not killed by any of the soaps; pneumococci and streptococci were killed by the laurates, oleates, linoleates, and linolenates; while the typhoid bacillus was unaffected by these soaps.

Eggerth conducted a series of experiments which illustrated different phases of the germicidal activity of soaps. He reported in 1926[5] that soap, in a concentration that was not in itself germicidal, could considerably increase the titre of acriflavine. With increasing molecular weight of the soap,[6] the germicidal titre increased to a maximum, and then diminished. This was also true of the fatty acids, which were often more germicidal than the corresponding soap. The lower members of the saturated series of soaps were most germicidal in an acid reaction, whereas the higher members showed greater germicidal action when the pH was alkaline. Lipoids were found to be actively inhibitory to the action of soaps.[7] So far as the substituted soap compounds are concerned, he found[8] that the toxicity of the alpha-brom soaps for all species of bacteria increased rapidly with the length of the fatty acid chain.

Bayliss and Halvorson[9] confirmed the work of Nichols, Avery and Walker regarding the selective bactericidal action of the soaps. They reported that the pneumococcus was especially susceptible to the action of certain unsaturated soaps, such as sodium oleate, linoleate, and linolenate. _Streptococcus lactis_ was considerably more resistant to the action of soaps than the pneumococcus, while _Escherichia coli_ and _Staphylococcus aureus_ were even more resistant. These workers also noted that the ability to neutralize diphtheria toxin is a property common to all soaps.

The study of the synthetic wetting agents was initiated within the last ten years. In 1935 Domagk[10] pointed out the bactericidal action of these detergents with his demonstration that the quaternary ammonium compound, long-chain alkyl dimethyl benzyl ammonium chloride possessed excellent germicidal properties. Dunn[11] confirmed these observations the following year, reporting that both Gram-positive and Gram-negative organisms were readily destroyed by this compound in high dilution, which was most effective in alkaline solution.

Katz and Lipsitz,[12] in 1935, wrote that the highly resistant *Mycobacterium smegmatis* was inhibited in its growth in dilutions of the sodium salt of the di-secondary butyl naphthalene sulfonic acid, and in 1937[13] they reported that cyclic compounds were more effective in such inhibition of growth than the aliphatic compounds.

The visible action of a detergent, sodium lauryl sulfate, on microörganisms was described by Bayliss[14] in 1937. Cultures of Gram-negative organisms grown in liquid media were cleared by appropriate concentrations of this compound, and there was at the same time a marked increase in the viscosity of the medium. The majority of Gram-positive bacteria, with the exception of the pneumococci, remained unchanged. There was no correlation between clearing action and the lethal action of the sodium lauryl sulfate, for some of the organisms which were cleared were killed while others were not. The same was true of those which were not visibly affected.

In 1939 Maier[15] found that the new detergent, long-chain alkyl dimethyl benzyl ammonium chloride could be used very practicably in the preservation of vaccines and venom solutions, and that it was remarkably free from harmful effects when applied to the cornea of rabbits.

Another compound, cetyl pyridinium chloride, was studied by Blubaugh, Botts, and Gerwe.[16] They reported that both tincture and aqueous solutions of this detergent, in the absence or presence of organic matter, were highly bactericidal for virulent organisms.

Freedlander[17] observed that while certain commercial wetting agents, namely Zephiran (long-chain alkyl dimethyl benzyl ammonium chloride), Nacconol NR, and Aerosol OT 100 (mono-sodium sulfonate of dioctyl succinate), exhibited marked bacteriostatic effects on the growth of Mycobacterium tuberculosis, they were completely ineffective in killing the organisms.

Wetting agents may also be used effectively as aerosols, according to Robertson, Bigg, Miller, and Baker.[18] Using certain glycols, they could sterilize the air of a small chamber containing a suspension of Staph. albus. Ordal, Wilson, and Berg[19] found that the addition of wetting agents to buffered solutions of phenolic compounds did, in general, increase the germicidal activity of such solutions.

The selective action of different types of detergents was observed in 1938 by Cowles.[20] He found that, by and large, the Gram-negative organisms are not prevented from growing by the alkyl sulfates (anionic detergents), whereas the Gram-positives, for the most part, were inhibited.

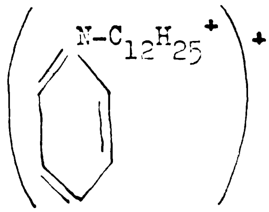
After studying the action of synthetic detergents on the metabolism of bacteria, Baker, Harrison, and Miller[21] concluded that all cationic detergents were very effective inhibitors of bacterial metabolism, and that few anionic detergents inhibit as effectively. Both Gram-positive and Gram-negative organisms were sensitive to the action of cationic detergents, which exhibited their maximum activity in the alkaline pH range, whereas the anionic detergents selectively inhibited the metabolism of the Gram-positive microörganisms, especially in the acid range, and had little or no effect on Gram-negatives. They then[22] checked the bactericidal action of these detergents and reported that the cationic detergents, as a group, exhibited marked bactericidal action on Gram-positive microörganisms and somewhat less pronounced action on Gram-negative organisms. The anionic detergents were germicidal only against Gram-positive organisms and were considerably less effective than the cationic compounds.

Gershenfeld and Perlstein[23] emphasized the importance of the hydrogen ion concentration in its effect on the action of detergents, showing that the anionic detergents were much more effective in acid than in neutral solution.

EXPERIMENTAL WORK

Problem

The synthetic detergents may be roughly divided into three groups: (a) anionic compounds, e.g. sodium lauryl sulfate, which ionizes with the hydrophobic group in the anion as follows: (Na^+) + $(C_{12}H_{25}OSO_3^-)$; (b) cationic compounds, e.g. lauryl pyridinium iodide, which contains the hydrophobic group in the cation:



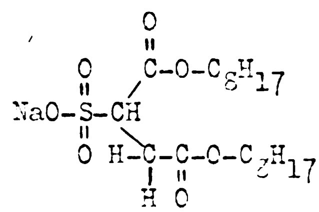
+ (I^-); (c) non-ionized compounds, such as the polyglycerol esters and other non-electrolyte compounds.[21]

It has been observed that these compounds differ from one group to another in their killing action toward bacteria. Thus, while the cationic detergents have been found to be effective against almost all types of bacteria, particularly in alkaline solution, the anionic compounds were far more effective against Gram-positives, especially in acid solution. Further study was indicated for a more complete demonstration of the bactericidal action, and of the effects of changing pH.

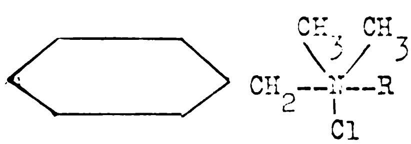
For this problem, four Gram-positive and five Gram-negative organisms were selected.

Gram-positive	Gram-negative
Bacillus subtilis	*Aerobacter aerogenes*
Alpha-hemolytic streptococcus	*Escherichia coli*
Beta-hemolytic streptococcus	*Eberthella typhosa*
Staphylococcus aureus	*Shigella dysenteriae*
	Pseudomonas aeruginosa

The wetting agents were selected according to their electrolytic structure--an anionic compound, a cationic compound, a non-electrolytic phospholipid, and a synthetic non-electrolyte. The anionic detergent was Aerosol OT 100,* a chemically pure mono-sodium sulfonate of dioctyl succinate:[24]

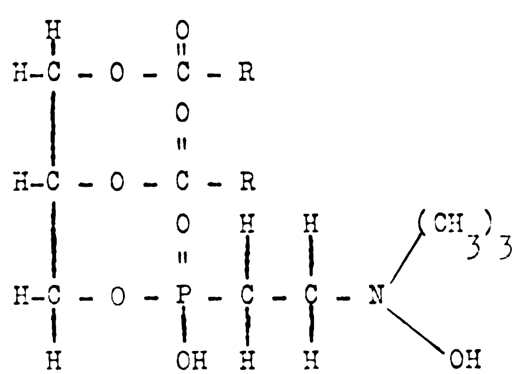


The cationic compound, Zephiran,** is a mixture of long-chain alkyl dimethyl benzyl ammonium chlorides.[25] The chemical structure is:



in which R represents alkyl radicals ranging from C_8H_{17} to $C_{18}H_{37}$.

The phospholipid was a crude egg-yolk lecithin, with a general lecithin formula:



in which R represents alkyl radicals of varying sizes.

The synthetic non-electrolytic detergent was Triton NE.*** It is an octyl methyl phenoxy polyethoxy ethanol:

---

* American Cyanamid and Chemical Corporation.

** Alba Pharmaceutical Company.

*** Röhm and Haas.

$$\begin{matrix} C_8H_{17} & & \\ & \diagdown & \\ & & C_6H_4 - O - (C_2H_4O)_n - C_2H_4 - OH \\ & \diagup & \\ CH_3 & & \end{matrix}$$

in which n represents a fairly large number.

These compounds were tested for their relative bactericidal efficiencies in neutral solution, and at a pH 5 and pH 9. The possible inhibiting action of the two non-electrolytes on the two electrolytic compounds was also determined.

Technique

The compound to be tested was prepared in the desired concentration in a liter of sterile distilled water. 100 ml. of the solution was then transferred to a sterile 250 ml. Erlenmeyer flask. The solution was adjusted to the desired pH by adding NaOH or HCl immediately prior to use, as determined by indicators on a spot plate. The pH was checked again immediately after the addition of the broth culture to be sure that the value had not changed. The temperature ranged from 23-26° C.

To 100 ml. of detergent solution was added 1 ml. of a filtered 24-hour broth culture. The broth used was a Tryptose medium containing 20 gm. Tryptose, 5 gm. NaCl, and 1000 ml. water. The mixture was shaken thoroughly, the pH checked, and after 1, 5, and 10 minute intervals a 1 ml. sample was transferred to a 99 ml. saline blank, so as to obtain a 1-100 dilution. Further dilutions of 1-1000, 1-10,000, and 1-100,000 were then made. 1 ml. of each of these dilutions was transferred to a sterile Petri dish, and 45° C. agar was poured into the plate. The agar used was a Tryptose agar, containing 20 gm. Tryptose, 2 gm. dextrose, 5 gm. NaCl, and 15 gm. agar to 1000 ml. distilled water.

The plates were rotated, allowed to solidify, and incubated for 48 hours at 37° C. At the end of this time the dilution plate containing from 30 to 300 colonies was counted in its entirety. At the time of each test a control count was made by adding 1 ml. of the broth culture to 99 ml. of sterile water, diluting in blanks, and plating out.

Results

Several counts of surviving cells were made for each organism tested, and for each solution of varying concentration and pH value. These counts were averaged, and the result divided by the control count to obtain the percentage of survivers. This figure was then subtracted from 100 per cent for the percentage of organisms killed. It was felt that these percentage figures showed relative values better than the numerical counts themselves. Obviously these values cannot be considered except as showing the trends of the action taking place when there is not complete killing.

The cationic detergent, Zephiran, was the most effective bactericidal agent of the compounds tested. At a concentration of 1-10,000 it killed both Gram-negative and Gram-positive organisms at pH values of 5, 7, and 9. By using a higher dilution, (Table I) however, (1-100,000) it was found that Zephiran failed to kill more than 80 per cent of the _Staph. aureus_ present at a pH value of 5 in 10 minutes, though it was more effective against _Pseud. aeruginosa_ and _Esch. coli._ At a pH of 9 all these organisms were killed.

Aerosol OT (Table II), the anionic detergent, was not effective at such high dilutions as Zephiran, so that it was necessary to use a concentration of 1-1000 to demonstrate its selective action. At a pH of 7 this compound was completely effective against the strongly

TABLE I

PERCENTAGE OF ORGANISMS KILLED BY 0.001 PER CENT ZEPHIRAN SOLUTION UNDER VARYING CONDITIONS OF TIME OF EXPOSURE AND pH

Organism	pH5 Minutes			pH7 Minutes			pH9 Minutes		
	1	5	10	1	5	10	1	5	10
	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
Staph. aureus........	55.6	81.0	75.0	96.2	98.7	98.6	100	100	100
Esch. coli...........	98.4	100	100				100	100	100
Aero. aerogenes......				100	100	100			
Pseud. aeruginosa...	100	100	100	100	100	100	100	100	100

TABLE II

PERCENTAGE OF ORGANISMS KILLED BY 0.1 PER CENT AEROSOL OT SOLUTION UNDER VARYING CONDITIONS OF TIME OF EXPOSURE AND pH

Organism	pH5 Minutes			pH7 Minutes			pH9 Minutes		
	1	5	10	1	5	10	1	5	10
	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
B. subtilis.........				100	100	100			
α-streptococcus.....				36.2	19.2	45.2			
β-streptococcus.....	100	100	100	0	32.0	36.3			
Staph. aureus........	100	100	100	52.0	71.5	80.6	33.0	80.7	33.8
Aero. aerogenes......	94.5	96.8	98.3	0	0	35.0			
Esch. coli..........	79.1	97.8	98.4	47.7	55.3	66.2			
Eberth. typhosa......	92.6	99.1	99.6	0	0	0			
Shig. dysenteriae....	93.4	100	100	0	47.0	15.0			
Pseud. aeruginosa....	66.5	66.4	74.0	0	0	0	0	0	0

Gram-positive B. subtilis, partially effective against Staph. aureus, and hardly active at all against the streptococci. Aerosol's complete ineffectiveness against gram-negative organisms, with the exception of Esch. coli, was apparent at a pH of 7. At a pH of 5, however, a 1-1000 dilution of this compound was relatively effective against all organisms tested, though complete killing action in 1 minute was demonstrated only with Staph. aureus and the beta-hemolytic streptococcus. At a pH of 9 Aerosol was less effective than at a pH of 7.

It was shown by Baker, Miller, and Harrison[26] that phospholipids such as lecithin, cephalin, and sphingomyelin prevented the inhibition of bacterial metabolism which was caused by synthetic detergents. They also found that lecithin made germicidal concentrations of the detergents ineffective. In checking this inhibitory action, a 1.0 per cent crude egg yolk lecithin was used with a 0.01 per cent Zephiran solution. No bactericidal action was apparent against Staph. aureus or Aero. aerogenes. The lecithin was also ineffective as a killing agent by itself against these organisms at all pH values.

This inhibitory action is not characteristic of all non-electrolytic detergents, however. While Triton NE appears to prevent Aerosol from acting against Pseud. aeruginosa, Staph. aureus, and Aero. aerogenes even at an acid pH, it has no effect on the killing action of Zephiran with these organisms. Triton NE (Table III) also appears to have some effect as a bactericidal agent itself, but the concentration used was extremely high.

TABLE III

PERCENTAGE OF ORGANISMS KILLED BY 1.0 PER CENT TRITON NE SOLUTION UNDER VARYING CONDITIONS OF TIME OF EXPOSURE AND pH

Organism	pH5 Minutes			pH7 Minutes			pH9 Minutes		
	1	5	10	1	5	10	1	5	10
	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
Staph. _aureus_.......	15.3	7.0	13.2	0	25.0	27.8	13.9	5.6	37.5
Aero. _aerogenes_.....	58.4	78.5	83.2	57.1	77.6	76.8	54.5	54.5	61.5
Pseud. _aeruginosa_...	37.9	57.3	71.0	23.2	20.5	30.0	38.0	38.7	39.1

DISCUSSION

A number of factors should be included in a consideration of the data presented. These factors include: (1) the surface-tension reducing properties of the detergents; (2) the charge on the ion containing the hydrophobic group; (3) the pH of the solution; and (4) the general and specific characteristics of the microörganisms. The hydrophilic-hydrophobic balance of the molecule and the specific chemical structure of the molecule were not considered in this study.

Surface tension. The effect of surface tension as a factor in the growth of bacteria has been studied by several workers. Larson, Cantwell, and Hartzell[27] found that most of the common bacteria showed some growth in a medium whose surface tension was as low as 32 dynes. It was noted by Ayers, Rupp, and Johnson[28] that Esch. coli and Aero. aerogenes grew fairly well in a medium with a surface tension of 35 dynes. They observed that a particular surface tension could not be considered a critical tension which exhibited its effect solely through its influence on the permeability of the cell. If such were the case all depressants at a certain surface tension value should exhibit the same effect. They concluded that the nature of the surface tension depressant as well as the actual surface tension value in dynes must be considered. Gibbs, Batchelor, and Sickels[29] concurred with this view. Frobisher[30] also concluded that there seemed to be little relation between the ability of reducents, such as sodium oleate and sodium ricinoleate, to kill or inhibit the growth of Eberth. typhosa, and their ability to reduce surface tension. It was found in this experiment that lecithin and Triton NE, which lower the surface tension in

aqueous solution considerably have little or no effect on the organisms tested.

The difficulty with all such studies lies in the fact that it has been possible to measure only the air-solution interfacial tension, where in the case of a lowered surface tension there is an increased concentration of the substance in solution, i.e. positive adsorption occurs. In general, if there is positive adsorption at the air-solution interface, there will be at the solution-container interface. But it remains an open question whether there is a correlated lowering of the surface tension at the bacterial cell-solution interface, and thus a higher concentration of the solution on the bacterial surface. It has, however, been necessary to assume that a detergent which lowers surface tension at the solution-air interface will do the same for the solution-bacterial cell interface in order to explain, e.g. why the bactericidal action of a non-toxic solution such as hexylresorcinol is enhanced by small quantities of sodium oleate.[31] Fluids of very low surface tension may, under some circumstances, permeate or penetrate into minute crevices or interstices filled with air more readily than fluids of high surface tension. Assuming that there is a lowered surface tension at the solution-cell interface, the solution is probably adsorbed on the surface of the organisms, so that the solution exists in higher concentration at the most effective point. There would then be reason to believe that the surface tension reducent could induce changes in the permeability of the membrane which would facilitate the entrance of toxic molecules present in the solution. This possibility is borne out by the fact that when an excess of sodium oleate is added to hexylresorcinol, it inhibits the bactericidal action of the latter substance. This is believed to be the result of the adsorption of the soap on the surfaces of the cells, with the formation of a protective film or coating. These results may be compared with those in this experiment in which an excess of lecithin prevented a germicidal concentration of Zephiran from acting against the bacteria. Aside from its possible conjunctive action with toxic molecules, the surface tension value of itself seems to be of little importance in determining the germicidal action of the detergent. Aerosol OT solutions, for example, have a considerably lower surface tension value than Zephiran solutions. It is interesting to note, however, that in an acid solution, in which Zephiran is relatively ineffective, the surface tension value was noticeably higher than those obtained with neutral and alkaline solutions.

<u>The charge on the ion containing the hydrophobic group.</u> The factor of charge on the hydrophobic group of the detergent appears to be of considerable importance, as has been shown by Baker, Miller, and Harrison,[21] and by the results of this study. Zephiran ionizes with the positive charge on the ion which contains the long-chain hydrophobic group. Aerosol OT ionizes with the negative charge on the long-chain hydrophobic group. Lecithin does not ionize, except as a zwitterion, while Triton NE is a non-electrolyte. The simplest explanation for the bactericidal efficiency of Zephiran might be considered in terms of the mutual attraction between the negatively-charged bacterial cells and the positively charged hydrophobic group. For a clearer concept of this factor, however, it is necessary to consider first the pH of the solutions.

<u>The pH of the solution.</u> The effects of the pH on the action

of various compounds have been noted previously in connection with the action of soap.[7] Kligler,[32] Stearn and Stearn,[33] and others have noted that increase of pH favors the disinfecting power of basic dyes and decrease of pH, that of acid dyes. Stearn and Stearn[33] are inclined to believe that the hydrogen ion concentration directly affects the bacterial cell, altering the protoplasm so as to render the organisms more susceptible to the action of toxic substances.

Osterhout[34] has shown, in his work with large plant cells, that electrolytes pass through the non-aqueous protoplasmic surface chiefly in the molecular form, since its low dielectric constant would not permit much dissociation. It is true, he says, that some dissociation takes place and that ions can enter to some extent, but the concentration of the ions is very small. If this phenomenon can be applied to the bacterial cell, it is seen that the undissociated electrolyte should be a more effective bactericidal agent. Zephiran's greatest effectiveness is in an alkaline solution, which would favor the formation of the undissociated molecule, whereas Aerosol's greatest effectiveness would lie in the acid range, wherein its ionization would tend to be reversed to the molecular form. Aerosol OT and Zephiran are both strong electrolytes, however, and it is hard to imagine that their ionization could be prevented to any extent in the relatively weak acid and base solutions in which they were used. Furthermore, if an unionized molecule is more effective in penetrating the bacterial cell, why do not the non-electrolytes lecithin and Triton NE exhibit more action? There may also be some question as to whether Osterhout's observations on plant cells are directly applicable to all living cells.

Stearn and Stearn[35] report that the bacterial cell behaves like an ampholytic system, and that, while all bacteria are negatively charged, each organism has its own particular isoelectric point. They point out that for any organism, regardless of the value of its isoelectric point, cations should be retained to a greater extent at high pH values than at low ones, and the reverse should be true for anions.

It has been shown that the anion of Aerosol is more effective as a bactericidal agent in acid solution, while the cation of Zephiran is more effective in alkaline solutions--which would appear to bear out the hypothesis of Stearn and Stearn. One possible explanation for this behavior lies in the fact that a bacterial suspension may be considered to be a negatively-charged colloid. Cations, then, would tend to be attracted to the surface of a negatively charged particle. The addition of H^+ ions prior to the addition of this detergent might act to neutralize this negative charge on the bacterial cell (or even give the cell a positive charge), however, so that there no longer existed such a strong mutual attraction between the cell and the cation of the detergent. An anion, on the other hand, should be more efficient in an acid solution, for it should be much more readily attracted when the bacterial cell charges have been neutralized, or even better if the cell has become positively charged due to the adsorbed layer of positive hydrogen ions. It would be more difficult for an anionic detergent, then, to act through an electrical attraction mechanism than for a cationic detergent, and its only possible action would take place in an acid solution. This would explain in part the relative inefficiency of Aerosol OT as compared with Zephiran, and would also partially explain the almost complete lack of bactericidal action of lecithin and Triton NE.

The general and specific characteristics of the microörganisms. One of the most interesting results of this and of other studies on the bactericidal activity of the synthetic detergents has been the relative specificity of action of the anionic detergents for Gram-positive organisms, at least in neutral solution, as contrasted with the strong killing action of the cationic compounds for all organisms. The contrast in sensitivity to toxic substances between Gram-positive and Gram-negative organisms has been noted many times. Stearn and Stearn[33] wrote: "The popular idea that Gram-positive organisms are more sensitive to basic dyes than are Gram-negative is borne out only in the sense that the former find themselves at one end of a gradual series showing gradations in sensitivity rather than in a very distinct group showing sensitivity of a different order of magnitude from any member of the other group." In their study they found a gradual decrease in sensitivity to basic dyes from the strongly Gram-positive through the border-line organisms to the strongly Gram-negative, the order being: B. subtilis, Staph. aureus, Shig. dysenteriae, Esch. coli, Eberth. typhosa, and Aero. aerogenes.

Mallmann, Botwright, and Churchill[36] reported that the slow oxidizing agents, potassium dichromate, and sodium azide, exerted a bacteriostatic effect on Gram-negative bacteria. By the use of different dilutions of these slow oxidizing agents the gradations in character from strongly Gram-positive to strongly Gram-negative organisms was again shown, the order being: Staph. aureus, B. cereus (comparable to B. subtilis), Pseud. aeruginosa, Esch. coli, Eberth. typhosa, and Aero. aerogenes.

In this study it is likewise apparent that there exist gradations in sensitivity to the anionic detergent, Aerosol OT, which are to be seen in the per cent killed figures in Table II. On the basis of these figures, the gradations from strongly Gram-positive to strongly Gram-negative would be: *B. subtilis*, *Staph. aureus*, *alpha-streptococcus*, *beta-streptococcus*, *Shig. dysenteriae*, *Esch. coli*, *Eberth. typhosa*, *Aero. aerogenes*, and *Pseud. aeruginosa*.

The results of these three studies cannot be directly correlated, but the importance of the general and specific characteristics of the microörganisms is evident. Further work along this line may throw more light on the question of the Gram-staining characteristics of bacteria.

The synthetic detergents appear to act in several ways to kill microörganisms. Because of their surface-tension lowering action they are probably adsorbed in a fairly high concentration on the bacterial cell, and may permeate the cell wall more readily as consequence. Either aiding or opposing this action may be the attraction or repulsion of the hydrophobic ion of the detergent by the negative charge on the bacterial cell, which is influenced by the presence or absence of hydrogen ions. And finally, the effect of certain detergents on different microorganisms may vary, depending on the general and specific characteristics of the organism, particularly whether it is Gram-positive or Gram-negative. In addition there must be considered the nature of the toxic substance itself, which was not a factor studied in this work.

CONCLUSIONS

1. The cationic detergent, Zephiran, in a 0.01 per cent solution killed both Gram-positive and Gram-negative organisms at pH values of 5, 7, and 9. In a 0.001 per cent solution it was considerably less efficient at a pH of 5 than at a pH of 9.
2. The anionic detergent, Aerosol OT, in a 0.1 per cent solution killed only strongly Gram-positive organisms at a pH of 7. At a pH of 5 it not only killed all Gram-positive organisms, but was also quite effective against Gram-negatives.
3. The non-electrolytic detergent, Triton NE, showed some killing action against both Gram-positive and Gram-negative organisms in a 1.0 per cent solution. A combination 1.0 per cent Triton-0.01 per cent Zephiran solution showed complete killing action. A combination 1.0 per cent Triton-0.1 per cent Aerosol solution showed no killing action.
4. The naturally occurring phospholipid, lecithin, showed no killing action against both Gram-positive and Gram-negative organisms. A combination 1.0 per cent lecithin-0.01 per cent Zephiran solution showed no killing action.

LITERATURE CITED

1. Lamar, R. V. Action on the pneumococcus and its experimental infections of combined sodium oleate and anti-pneumococcus serum. J. Exp. Med. 1911, 13:1.

2. Nichols, H. J. Bacteriologic data on the epidemiology of respiratory diseases in the Army. J. Lab. and Clin. Med. 1919-20, 5:502.

3. Avery, O. T. A selective medium for B. influenzae. J. Am. Med. Ass'n. 1918, 71:2050.

4. Walker, J. E. Germicidal properties of soaps. J. Infect. Dis. 1924, 35:557.

5. Eggerth, A. The bactericidal action of acridine dyes and the adjuvant effect of serum. J. Infect. Dis. 1926, 38:3.

6. Eggerth, A. The effect of pH on the germicidal action of soaps. J. Gen'l. Phys. 1926, 10:147.

7. Eggerth, A. The effect of serum upon the germicidal action of soaps. J. Exp. Med. 1927, 46:671.

8. Eggerth, A. The germicidal and hemolytic action of alpha-brom soaps. J. Exp. Med. 1929, 49:53.

9. Bayliss, M. and Halvorson, H. O. Germicidal and detoxifying properties of soaps. J. Bact. 1935, 29:9.

10. Domagk, G. Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. Deutsch Med. Woch. 1935, 61:829.

11. Dunn, C. G. A mixture of high molecular alkyl dimethyl benzyl ammonium chlorides as an antiseptic. Proc. Soc. Exp. Biol. and Med. 1936-7, 35:427.

12. Katz, J. and Lipsitz, A. Studies on the effect of surface active materials on bacterial growth. J. Bact. 1935, 30:419.

13. Katz, J. and Lipsitz, A. Studies on the effect of synthetic surface-active materials on bacterial growth, II. J. Bact. 1937, 33:473.

14. Bayliss, M. Visible action of sodium lauryl sulfate on microorganisms. J. Lab. and Clin. Med. 1937, 22:700.

15. Maier, E. Preservation of biological fluids with alkyl dimethyl benzyl ammonium chloride. J. Bact. 1939, 38:33.

16. Blubaugh, L. V., Botts, C. W., and Gerwe, E. Study of germicidal properties of cetyl pyridinium chloride. J. Bact. 1940, 39:51.

17. Freedlander, B. L. Bacteriostatic action of various wetting agents upon growth of tubercle bacilli in vitro. Proc. Soc. Exp. Biol. and Med. 1940, 44:51.

18. Robertson, O. H., Bigg, E., Miller, B. F., and Baker, Z. Sterilization of air by certain glycols employed as aerosols. Science, 1941, 93:213.

19. Ordal, E. J., Wilson, J. L., and Borg, A. F. Action of wetting agents on microörganisms. J. Bact. 1941, 42:119.

20. Cowles, P. B. Alkyl-sulfates: thdr selective bacteriostatic action. Yale J. of Biol. and Med. 1938, 11:33.

21. Baker, Z., Harrison, B. W., and Miller, B. F. Action of synthetic detergents on metabolism of bacteria. J. Exp. Med. 1941, 73: 249.

22. Baker, Z., Harrison, R. W., and Miller, B. F. The bactericidal action of synthetic detergents. J. Exp. Med. 1941, 74:611.

23. Gershenfeld, L. and Perlstein, D. The effect of Aerosol OT and hydrogen ion concentration on the bactericidal efficiency of antiseptics. Am. J. Pharm. 1941, 113:237.

24. McBain, J. W., and Sharp, E. R. A comparative study of surfaces of solutions with the film balance and surface tension equipment. J. Am. Chem. Soc. 1941, 63:1425.

25. Alba Pharmaceutical Company, "Zephiran," page 5.

26. Baker, Z., Harrison, R. W., and Miller, B. F. Inhibition by phospholipids of the action of synthetic detergents on bacteria. J. Exp. Med. 1941, 74:621.

27. Larson, W. P., Cantwell, W. F., and Hartzell, T. B. The influence of the surface tension of the culture medium on the growth of bacteria. J. Infect. Dis. 1919, 25:41.

28. Ayers, S. H., Rupp, P., and Johnson, W. T. The influence of surface tension depressants on the growth of streptococci. J. Infect. Dis. 1923, 33:202.

29. Gibbs, W. M., Batchelor, H. W., and Sickels, T. N. Surface tension and bacterial growth. J. Bact. 1926, 11:393.

30. Frobisher, M. Relations of surface tension to bacterial phenomena. J. Infect. Dis. 1926, 38:66.

31. Frobisher, M. Interfacial tension and bacterial growth. J. Bact. 1927, 13:163.

32. Kligler, I. J. A study of the antiseptic properties of certain organic compounds. J. Exp. Med. 1918, 27:463.

33. Stearn, E. W., and Stearn, A. E. The role of hydrogen ion concentration in the action of dyes and bacteria. Am. J. Pub. Health, 1924, 14:409.

34. Osterhout, W. J. V. The adsorption of electrolytes in large plant cells. Bot. Rev. 1936, 2:283.

35. Stearn, E. W. and Stearn, A. E. University of Missouri Studies, 1928, 3, No. 2.

36. Mallmann, W. L., Botwright, W. E., and Churchill, E. S. The selective bacteriostatic effect of slow oxidizing agents. J. Infect. Dis. 1941, 69:215.