EFFECTS OF CERTAIN ANIONIC, CATIONIC, AND NON-IONIC AGENTS ON GROWTH OF ESCHERICHIA COLI, SALMONELLA PARATYPHI B, AND STAPHYLOCOCCUS AUREUS

by

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I. INTRODUCTION

Surface-active agents are customarily defined as substances which alter the energy relationships at interfaces. In this category are included such agents as detergents, emulsifiers, wetting agents, and similar substances, some of which demonstrate bactericidal or bacteriostatic action. Because the term "surface-active agent" is such a general one, the present tendency is to classify these compounds as wetting agents, detergents, etc., according to the functions they best perform.

Since the effectiveness of synthetic surface-active agents as soap substitutes was discovered during World War I, an increasing amount of industrial research has been devoted to the development of these compounds. As a result, within the last 15 years a tremendous quantity of such products has appeared on the market. The rapidity with which they were developed, however, did not permit the thorough, basic research necessary to define clearly the relationship between these compounds and their biological activities.

Nevertheless, a vast amount of literature concerning them has been published. Many of these papers have been excellent, clarifying numerous aspects of the relationship between surface-active agents and bacteriological activity. Some others, however, have reported experiments demonstrating bacteriostatic and germicidal action of surface-active agents which could not be duplicated in other laboratories, or, for that matter, in the same laboratory. This was due, in some

cases, to inadequate definition of conditions under which the tests were made, and in others to the inapplicability of the test employed (for example, use of the Phenol-Coefficient Method with quaternary ammonium compounds).

The majority of the research on the influence of surface-active agents on bacteriological activity has been directed toward establishing the critical killing concentrations for these compounds, determining their optimum pH range, studying their effect on bacterial metabolism, or investigating the relationship between their chemical structure and their bacteriological activity. In several of the more recent studies, an attempt has been made to incorporate as many of these objectives as possible in one investigation. However, the procedure has been to observe the effects of surface-active agents on bacteriological activities only over periods of limited duration, varying from 5 to 90 minutes. In such a short time, killing concentrations or optimum pH ranges for the compounds could be established, but the effects on bacterial activity of prolonged exposure to weaker concentrations could not adequately be studied or interpreted. Moreover, in most investigations, arbitrary killing and non-killing concentrations of these synthetic agents have been selected for study. This has led to a restricted appraisal of the agents' effect on bacterial growth.

The chief purpose of this investigation is to present a systematic study of the effects of certain synthetic anionic, cationic, and non-ionic agents on the growth of

cultures of Escherichia coli, Salmonella paratyphi B, and and Staphylococcus aureus. The surface-active agents were tested in varying concentrations with a medium of varying pH. Measurements of bacterial growth, pH shift, and surface tension were made at definite intervals during 24-hour periods. Throughout, the intent has been to explain more fully the accepted generalizations concerning the influence these agents exert on bacterial activity, rather than to seek results having practical applications.

II. HISTORICAL SURVEY

Prior to 1914, the only surface-active agents known were soaps and sulfonated castor oil. Shortly thereafter, with the introduction in Germany of alkylated naphthalene sulfonates, an extensive development of these compounds took place in the United States. Production rose from zero pounds in 1928 to 125,000,000 pounds in 1945 (63). At present, there are over 500 different synthetic surface-active chemical products commercially available on the U.S. market (60).

Some of the earliest studies on the influence of surface-active agents on bacteria were made with sodium oleate. Lamar (42) demonstrated increased susceptibility of pneumococci to serum lysis after treatment of the organisms with sodium oleate. Avery (5) noted that its addition to culture media prevented the growth of pneumococcus and streptococcus, while increasing the growth of Bacillus influenzae. The work of Bayliss and Halvorson (9) substantiated the selective bactericidal action of the unsaturated soap.

A series of experiments on the effects of surface tension on bacterial growth were undertaken about the same time. Larson et al (43) noted that pellicle-formers ceased to grow at the surface when the tension of the medium was reduced below 45 dynes per centimeter by soaps. Poor growth of streptococci and pneumococci in media at 45 dynes per centimeter was also described. Frobisher (20) suggested a possible means of differentiating bacteria on the basis of their ability to grow at low surface tensions. Marshall (47) noted

that in media of different surface tension, bacteria appear to have different rates of growth and different gas metabolisms. However, the correlation of these variables revealed no systematic variation. He likewise pointed out that, "as regards relationship between surface tension and bacterial growth, the data can be considered as little more than suggestive. After all, the relationship between the surface tension at the air-medium interface is not necessarily a function of the surface energy relationships at the organism-medium interface in any way ..." Marshall's statement is considered valid to the present day.

The role of pH in the bacteriostatic and bactericidal properties of surface-active agents has merited a great deal of investigation. Eggerth (17), in 1926, reported that there were optimum pH ranges for bactericidal activity for a series of soaps. He found the lower members of the saturated series more active in an acid range, while the higher members were more bactericidal at increased pH levels. Dunn (15), in 1937, pointed out that, at an alkaline pH, alkyl dimethyl benzyl ammonium chloride was a more efficient bactericide than at neutral or acid pH. Gershenfeld (22) demonstrated greater bactericidal action for Aerosol OT with a decrease in pH. In a later paper (24), he showed that Triton K-12, a cationic agent, was most effective in an alkaline range, while the anionics Tergitol 4 and 4T were more efficient in an acid range. The work of Baker et al (6) substantiates these findings. Hoogerheide (31) contrasted the relative

bactericidal power of CTAB at pH 8 and pH 5, and concluded that at the alkaline pH level the quaternary ammonium salt could more easily withstand dilution and still maintain germicidal activity.

Since 1935, a number of papers have appeared in which the relationship of surface-active agents to biological activities is discussed. Katz (34) found that dilutions from 1-10T to 1-50T of the sodium salt of di-secondary butyl naphthalene sulfonic acid caused Mycobacterium smegmatis to assume involution forms. Bayliss (10) demonstrated the ability of sodium oleate and sodium linoleate to detoxify diphtheria toxin. In 1937, the same author (11) showed the lysing effect of sodium lauryl sulfate on bacteria. Gale and Taylor (21) likewise demonstrated lysis with subsequent release of amino acids. The destruction of pathogenic fungi by alkyl-dimethyl-benzyl ammonium chlorides (16), and the cysticidal power of cationic agents (19) extended the list of potential uses for these synthetic compounds. Anson (3), in his very important work, demonstrates the ability of synthetic detergents to denature such proteins as hemoglobin and egg albumin at their isoelectric points. Kramer (39) treated Streptococcus hemolyticus, Salmonella typhosa, and Bacillus subtilis with various wetting agents, and succeeded in rendering these bacteria filtrable through siliceous filters. Stock and Francis (59) were able to prove that influenza virus could be inactivated by certain of the higher fat acids. The inhibition of immunological reactions was pointed out by Holmes (30).

Many researchers have formulated theories (the majority of which are in agreement) concerning the mode of action of the synthetic surface-active agents on bacteria and biological systems. Baker et al (6) favor the theory that at the optimum pH for bactericidal action, there is an alteration of bacterial membrane or protoplasm, rendering the bacteria more susceptible to the surface-active agents. They also contend that at the optimum pH the formation of undissociated molecules of the synthetic agent is favored, and that these undissociated molecules have a greater ability to enter the bacterial cell and cause its destruction. Herein they concur with the findings of Osterhout (48) in his experiments on large plant cells. In a later paper, Baker et al (8) suggest that the disruption of the normal function of the bacterial cell is dependent on a twofold action: first, the cell membrane is disorganized as a result of the great surface activity of the synthetic agents, and second, certain proteins which are vital for metabolism and growth are denatured. Valko (61) suggests that two types of molecular forces are involved in the interaction between surface-active agents and proteins (bacterial cells) -- first, the intrinsic affinity and second, the electrostatic coulombic forces of both the protein molecule and the adsorbed ions, both of which carry free electric charges. The investigations of Hotchkiss (32) have led him to believe that there are three main stages in the bactericidal activity of surface-active agents. 1) Interaction of surface-active

agents and bacteria results from the attraction of opposite charges. 2) All the soluble nitrogen and phosphorus compounds are released from the cell, providing the hydrophobic group of the surface-active agent has the appropriate affinity for the bacterial surface. The cells are now dead, although they may still give evidence of a very low metabolism and appear unchanged morphologically. 3) Autolysis sets in, and a greatly increased release of nitrogen and phosphorous follows.

III. MATERIALS AND EQUIPMENT

A. Culture Medium

The basic medium was composed of the following ingredients*:

KH_2PO_4	- 0.1	percent
K_2HPO_4	- 0.1	percent
NaCl	- 0.1	percent
Na_2CO_3	- 0.1	percent
$(NH_4)_2SO_4$	- 0.1	percent
$MgSO_4$	- 0.005	percent
KCl	- 0.0025	percent
Peptones**	- 1.0	percent
Distilled H_2O	- 1000 ml.	

(Unadjusted pH of medium was 7.4.)

In order to effect complete solution of the Na_2CO_3, 5 cc. of 1 N HCl per liter of broth were added to the broth prior to autoclaving. The medium was autoclaved at 15 pounds pressure for 20 minutes.

This broth was used in all the experiments except those specifically noted otherwise.

Plain agar at approximately pH 7 ($\pm$.2) was used for all plating purposes. Its composition was as follows:

* Traces of Ca and Fe were also present.

** Only Difco Peptone, Lot No. 363113, was used in all media.

Peptone	- 5 grams
NaCl	- 5 grams
Beef Extract	- 3 grams
Agar	- 15 grams
Distilled H_2O	- 1000 ml.

B. Surface-Active Agents

The surface-active agents employed in the tests are listed in Table 1.

To facilitate understanding of these agents, a brief description of each, as supplied by the manufacturers, is herewith presented.

Duponol OS is an amber-colored, oily liquid, only partially soluble in H_2O. It is an oil-soluble emulsifying agent which combines the emulsifying properties of the alcohol sulfates with the homogenizing properties of the fatty alcohols.

Igepon AP Extra is a fine, light cream-colored powder which dissolves readily in hot water. It is a synthetic detergent used mainly for the processing of wool.

Tergitol 7 is a colorless, syrupy solution. It is used principally as a wetting agent in the processing of wool, leather, paper, etc. It is likewise a powerful penetrating agent.

Nacconol NRSF is a light, flaky compound. Although it is intended primarily to be used as a detergent, it also displays antiseptic, bacteriostatic, and moth-proofing properties in water solution or in oil emulsions.

Nekal BX High Concentration is a fine, white powder, very readily soluble in warm water with neutral reaction. It is characterized by strong wetting action and possesses dispersing and emulsifying properties.

Roccal is usually sold in a 10-percent water solution. It is recommended for sanitization of equipment associated with food-processing plants, as a bactericide, fungicide, etc. Phenol coefficients at 20 degrees C. against S. typhosa and S. aureus are 250 and 279 respectively.*

CTAB is a fine, white powder soluble in hot water. Its phenol coefficient against S. aureus is 200 to 250, and against S. typhosa is 125 to 175.

LPC is a colorless, 30-percent water solution of lauryl pyridinium chloride. Its phenol coefficient against S. aureus is 350 and against S. typhosa is 165. It is used as a germicide, fungicide, and wetting agent.

Emulphor ON is a wax-like, nonionogenic, water-soluble, organic substance with marked emulsifying, dispersing, and surface-active properties.

Triton X-100 is an amber, oily liquid. It is used as a wetting agent, emulsifier, detergent, and dispersant.

* The compound of Roccal used in most of the tests described in this paper, however, was a fine, white powder labelled as follows: 1 ounce of powder to 4 gallons of water gives a 1 to 3000 concentration of Roccal.

TABLE 1

SYNTHETIC SURFACE-ACTIVE AGENTS USED

Name of Compound	Chemical Designation	Percentage of Active Ingredient	Manufacturer
	Anionic		
Duponol OS (SH-2536)*	Mixture of two parts of oleyl alcohol and one part cyclic amine lauryl alcohol sulfate	95 to 100	E.I. DuPont de Nemours and Co., Inc. Wilmington, Del.
Igepon AP Extra (10339) (481-15916)	Sodium sulfonate of an oleic acid ester of an aliphatic compound $C_{17}H_{33}CO_2C_2H_4SO_3Na$	51	General Dyestuff Corp. Chicago, Ill.
Tergitol 7	$C_4H_9CHC_2H_4CHC_2H_4CH(C_2H_5)_2$ (with C_2H_5 and SO_4Na)	25	Carbide and Carbon Co. Detroit, Mich.
Nacconol NRSF (B.C. 86129)	Alkyl aryl sodium sulfonate	85	National Aniline Division Allied Chemical and Dye Corp. N.Y. 6, N.Y.
Nekal BX High Concentration	Sodium alkyl naphthalene sulfonate	80	General Dyestuff Corp. Chicago, Ill.
	Cationic		
Roccal	Alkyl dimethyl benzyl ammonium chloride (alkyl = C_8 to C_{18})	3 to 3.5	Winthrop Chemicals Co. N.Y. 13, N.Y.
CTAB	Cetyl trimethyl ammonium bromide	95 to 100	Rhodes Chemical Corp. Plainfield, N.J.
Liquid LPC	Lauryl pyridinium chloride	26	Hooker Electrochemical Co. Niagra Falls, N.Y.
	Non-Ionic		
Emulphor ON (S.O. 297)	Polyethylene ether of a long-chain fatty alcohol	100	General Dyestuff Corp. Chicago, Ill.
Triton X-100	Alkylated aryl poly-ether alcohol	100	Rohm and Haas Co. Philadelphia, Pa.

* All numbers in parentheses signify lot numbers and/or code numbers.

C. Bacterial Cultures

1. S. paratyphi B # 154 - furnished by The Michigan Department of Health Laboratory, Lansing, Michigan.

2. Hemolytic S. aureus #2 - furnished by The Michigan Department of Health Laboratory, Lansing, Michigan.

3. E. coli - isolated from a contaminated well-water sample submitted to the Michigan State College Department of Bacteriology for analysis.

All three organisms were rechecked for typical biochemical reactions and found to be pure, representative cultures.

D. Equipment

In addition to the regular laboratory equipment, a Number 70520 Simplified Cenco-du Nouy Tensionometer, a Beckman pH Meter (Model G), and a Lumitron Colorimeter, Model Number 400, manufactured by the Photovolt Corporation, N.Y.C., were employed.

IV. EXPERIMENTAL PROCEDURE

A. Preliminary Studies

1. Choice of Medium

It was thought desirable to use a single, simple medium which would permit good growth for all three organisms used in this study. At first, an attempt was made to employ a chemically defined medium. Sodium malate, casamino acids, dextrose, and peptone were used individually and in combinations with a basic salt solution. The simplest medium which permitted the three types of bacteria to grow luxuriantly in a 24-hour period was the one described in the section entitled "Materials and Equipment."

The choice of salts was based on a modification of those suggested by den Dooren de Jong (12) and Koser (38).

2. Selection of pH Ranges

On the basis of the data presented in the following table, the three pH ranges used in this study were established.

	pH Ranges								
	4.8	5.0	5.2*	5.4	7*	7.8	8.0*	8.2	8.6
E. coli	++++	++++	++++	++++	++++	+++	+++	+++	+
S. paratyphi B	++++	++++	++++	++++	++++	+++	+++	++	++
S. aureus	+	++	+++	++++	++++	+++	+++	++	++

* Ranges selected for this study.

B. Maintenance of Cultures

The stock bacterial cultures were kept on plain agar slants (with a parafilm covering over the lip of the test tube) at 5 degrees C. Stock subcultures were made every four weeks.

The cultures used in the recorded experiments were employed only after a minimum of four daily subtransfers into the proper broth after coming off the stock agar slant. After four weeks of use, test organisms were once again taken from the month-old stock cultures. Since most of the tests were made at three pH levels, the organisms were subtransferred and grown only in the broth medium adjusted to the proper pH, i.e., those organisms tested at pH 5.2 were daily subtransferred in broth at pH 5.2, etc. Subtransfers were made by inoculating 5 cc. of the broth medium with exactly 0.1 cc. of broth culture. It was found that, as a result of this procedure, it was possible to ascertain within two million the number of organisms per cc. of each of the cultures after 24 hours' growth at 37 degrees C. A further check was made with the Lumitron colorimeter.

C. Growth Curves and Related Phenomena

The growth-curve technic used in this experiment was theoretically divided into two stages. In the first stage, the stability of the organisms was established through testing their resistance to phenol* by the Phenol-Coefficient

* This procedure was followed prior to making any of the tests undertaken in this study. At the same time, the organism suspension was streaked on agar plates (for isolated colonies) to insure the fact that the organisms were mainly in the smooth phase.

Method. A chemical-range test was also made at the particular pH range under consideration. This was done by inoculating 0.1 cc. of a 24-hour organism suspension into a series of dilutions of the surface-active agent in 5 cc. of broth. Since many of the surface-active agent solutions were turbid, after 24 hours' incubation at 37 degrees C., subtransfers (2 loopfuls) of the inoculated solution were made into F.D.A. broth and incubated at 37 degrees C. for 24 hours. The results of this latter incubation constitute the data presented in the chemical-range tables. Information gained from these chemical-range tests was utilized in the second stage of the experiment, the actual study of organism growth after exposure to the various synthetic agents.

The organisms were grown in 250-cc. erlenmeyer flasks etched at a 175-cc. mark. The mark was made at the meniscus after adding, by volumetric pipettes, 175 cc. of the broth medium at 20 degrees C. The proper dilutions of the surface-active agents were made on the basis of 175 cc. of broth.

As a preliminary step, the pH of the sterile broth was adjusted to approximately the desired range by the addition of concentrated HCl, 1 N HCl, or 1 N NaOH, as the case might be. The particular surface-active agent was then added to this broth in the erlenmeyer flasks, and the flasks were heated for 20 minutes in an Arnold steamer. After complete solution was effected and the flasks cooled to approximately 20 degrees C., sufficient solution was removed to bring the

total volume back to the 175-cc. mark. This was necessary only in concentrations of surface-active agent greater than 1-100. The solution was again adjusted to the pH level at which the experiment was to be conducted. Fifteen cc. of the solution were used in this pH adjustment testing. The flasks were then incubated at 37 degrees C. for 15 hours to insure sterility.

Just prior to inoculating the surface-active agent solution with organisms, 1 cc. of a 24-hour suspension of the organisms was plated, after adequate dilutions were made. The count from these platings constitutes the "0-hour" bacterial count (after computing it on the basis of the ensuing proportion). Since 160 cc. of the surface-active agent solution remained at the time the test was to be initiated, 3.2 cc. of a 24-hour organism suspension were introduced into the solution (at 37 degrees C). This proportion of organisms to surface-active agent solution was the same as that used in the chemical-range test. Immediately after the addition of organisms, surface-tension and pH measurements were taken. This constituted "0-hour" data for both measurements.

The flask was then well shaken and placed in the incubator at 37 degrees C. Thereafter, the flask was shaken at 45-minute intervals for the first 10 hours. At the specified periods when platings were to be made, the flask was again shaken, 1 cc. of the solution withdrawn, and proper dilutions made.* Plates were poured with plain agar, allowed

* To compensate partially for possible bacteriostatic action, dilutions up to 1-1M were made.

to harden, and then incubated for 48 hours at 37 degrees C. Gram stains were made periodically to insure absence of contamination.

The method for determining the effects on bacterial growth of exposure to cationic agents for periods of short duration was essentially the same as that described above. The only difference was that the flasks were kept in a water bath at 37 degrees C. instead of in an incubator at 37 degrees C. In both types of tests, the surface-active agent solutions were at 37 degrees C. prior to inoculation of the organism suspensions.

A 12-cc. aliquot was likewise withdrawn and used for both the surface-tension and pH determinations. Surface tension was measured by the du Nouy Tensionometer with standard watch glasses of uniform size. The solutions for measurement of surface tension were kept at 37 degrees C. in a water bath, and readings were taken approximately 10 seconds after the sample was introduced into the watch glass. The pH was determined last of all. Prior to determinations for each time interval, the tensionometer was recalibrated and the Beckman pH meter was checked against a standard buffer solution.

In the foregoing series of experiments, an attempt was made to present the data in as uniform a manner as possible. The difficulties involved in such a presentation lie mainly with the inherent differences between the organisms themselves and between the compounds used, i.e., differences in physical state and in percentage of active ingredient.

Nevertheless, wherever possible, comparable concentrations of the surface-active agents were employed to indicate the relative resistance of the organisms. Such a procedure was most successful with certain of the less effective anionic agents and the non-ionic compounds. However, in order to indicate the gradual effect on bacterial growth of the more germicidal agents, it became necessary to use wide ranges in concentration of the synthetic agents when comparing the differences between the gram-positive and gram-negative organisms.

Wherever possible, an attempt was made to include in the tables the approximate dilution of synthetic agent which just killed completely the entire bacterial population. However, in those instances where this dilution has been omitted from the table, it is to be assumed that the next lower dilution from that first appearing in the table completely killed the organisms in 24 hours or less.* Since S. paratyphi B was used primarily for purposes of comparison with E. coli, no attempt was made to determine the effects of critical concentrations of the surface-active agents on the former organism.

It should be noted that gradations of dilution proceeded by increments of 100 within the hundred range, by

* The only exceptions to this statement occur where compounds are not germicidal for the bacteria in question, or where concentrations higher than those recorded are too viscous to permit complete solution of the organism suspension. Because of the extreme viscosity of the anionic compounds in concentrations stronger than 1-50, the 1-50 dilution was selected as the starting point for these agents. A 1-20 concentration of non-ionic compounds was selected for the same reason.

increments of 1000 within the thousand range, and by increments of 10,000 within the ten-thousand range.

An additional explanation is necessary for the interpretation of the results. Since the vast majority of published data concerning the relationship of synthetic surface-active agents to bacteria are based on the assumption of a 100-percent active ingredient of the synthetic compounds, it was deemed advisable to maintain this type of reporting. However, since it might be desirable to interpret the results on the basis of actual percentage of active ingredient, a listing of the densities of the liquid synthetic agents is presented below to aid in such an interpretation. All figures are based on the weights of 1 ml. of the agent at 20 degrees C.

Basic broth medium	0.996 gram
Duponol OS	0.843 gram
Tergitol 7	0.990 gram
LPC	0.985 gram
Triton X-100	0.960 gram

For example, in computing the exact concentration, as nearly as possible, of Tergitol 7 (25 percent active ingredient), the dilution stated in the various tables is multiplied by the factor 4.024. This factor was obtained by the following computation:

$$4\left(\frac{\text{weight of Tergitol per cc.}}{\text{weight of basic broth per cc.}} = \frac{1}{x}\right)$$

Thus the actual effective concentration of this compound is approximately four times greater than that stated in the tables. The factors for the other compounds are as follows:

Duponol OS	1.205
Igepon AP	1.96
Nekal BX	1.25
Nacconol NRSF	1.176
CTAB	1.02
LPC	3.888
Roccal (powder)	31.25
Emulphor ON	1.0
Triton X-100	1.037

V. RESULTS

A. Control Tests

In preparing these studies, it was necessary to determine growth rates, pH shifts, and surface-tension measurements in the absence of synthetic agents so that these data might serve as a reference group for comparison with data obtained in the ensuing experiments. The results are presented in Table 2.

B. Anionic Compounds

The first series of synthetic surface-active agents tested for their effects on bacterial growth were the anionic compounds Duponol OS, Igepon AP, Tergitol 7, Naccon-ol NRSF, and Nekal BX. The results of these tests appear in Tables 3 through 7.

When the results presented in the control table are compared with those obtained with the anionic agents, it becomes apparent that, in every instance, use of the latter produced a diminution of bacterial count during a 24-hour period. It is also clear that the shift in pH is likewise curtailed.

For the main part, the accepted generalization that anionic agents exert their maximum bactericidal effect in an acid range (22)(23)(24)(6) is substantiated by the data derived from these tests. The one exception is the action of Igepon AP on the gram-negative organisms. This compound appears more effective against *E. coli* and *S. paratyphi B* in an alkaline medium. Although the type of test

TABLE 2

DATA SHEET ON CONTROLS

pH 7

Phenolic Ranges									Hrs.	E. coli			S. paratyphi B			S. aureus		
Min.	E. coli			S. paratyphi B			S. aureus			pH	Surface* Tension	Bact.** Count	pH	Surface Tension	Bact. Count	pH	Surface Tension	Bact. Count
	1-60	1-70	1-80	1-80	1-90	1-100	1-60	1-70	1-80									
5	-	+	+	+	+	+	+	+	+									
10	-	-	+	-	+	+	-	+	+									
15	-	-	+	-	-	+	-	+	+									
									0	7.0	56.49	13M▲	7.0	56.58	9M	7.0	56.58	7M
									4	6.9	56.49	62M	6.9	56.63	36M	7.0	56.58	27M
									7	6.9	56.11	80M	7.0	56.63	78M	7.0	56.49	70M
									10	7.0	56.02	135M	7.2	56.49	220M	7.1	56.11	160M
									24	7.5	55.88	670M	7.7	55.88	500M	7.3	56.02	330M

pH 5.2

Min.	E. coli			S. paratyphi B			S. aureus		
	1-60	1-70	1-80	1-80	1-90	1-100	1-60	1-70	1-80
5	-	+	+	+	+	+	+	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Hrs.	E. coli			S. paratyphi B			S. aureus		
	pH	Surface Tension	Bact. Count	pH	Surface Tension	Bact. Count	pH	Surface Tension	Bact. Count
0	5.2	56.11	14M	5.2	56.11	9M	5.2	56.02	6M
4	5.4	55.88	40M	5.6	56.11	30M	5.3	56.02	19M
7	5.6	55.74	98M	6.2	56.02	90M	5.3	55.88	33M
10	5.7	55.74	190M	6.4	55.74	180M	5.4	55.88	39M
24	6.6	55.65	630M	6.9	55.65	385M	6.3	55.74	320M

pH 8

Min.	E. coli			S. paratyphi B			S. aureus		
	1-70	1-80	1-90	1-80	1-90	1-100	1-60	1-70	1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	+	+	-	-	-

Hrs.	E. coli			S. paratyphi B			S. aureus		
	pH	Surface Tension	Bact. Count	pH	Surface Tension	Bact. Count	pH	Surface Tension	Bact. Count
0	8.0	56.58	14M	8.0	56.58	9M	8.0	56.58	7M
4	7.7	56.58	72M	7.7	56.58	50M	7.8	56.58	27M
7	7.6	56.49	109M	7.8	56.11	100M	7.8	56.58	70M
10	7.7	56.49	152M	8.0	56.02	195M	7.9	56.49	118M
24	7.9	56.11	400M	8.2	55.88	320M	8.0	56.38	290M

* Surface tension is measured in dynes per centimeter.

** Bacterial count is measured in organisms per cc.

▲ M signifies millions.

TABLE 3

DATA SHEET ON DUPONOL OS

pH 7

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	-	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-100	1-200	1-300	1-400	1-500	1-600
E. coli	+	+						
S. paratyphi B	+	+						
S. aureus			-	-	-	-	+	+

	E. coli						S. paratyphi B						S. aureus					
	1-50			1-100			1-50			1-100			1-500			1-700		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	28.86	13M▲	7.0	28.55	13M	7.0	28.86	9M	7.0	28.59	9M	6.9	28.56	7M	7.1	28.56	7M
4	6.8	28.86	3.8M	6.9	28.05	15M	6.8	29.04	12M	6.8	28.59	6.3M	6.9	28.56	60T	7.1	28.56	180T
7	6.9	29.04	300T✱	6.8	28.86	2.5M	6.9	29.04	14M	6.9	28.59	7M	6.9	28.56	9T	7.0	28.56	17T
10	6.9	29.04	11M	6.9	28.86	8M	7.0	29.04	9M	7.0	28.15	22M	6.9	28.56	13T	7.0	28.56	85T
24	7.0	28.86	15M	7.0	28.61	43M	7.0	28.86	14M	7.2	28.15	17M	6.9	28.56	40T	7.0	29.04	4.2M

pH 5.2

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	+	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	-	+	-	+	+

Chemical Range

	1-10	1-20	1-1T	1-2T	1-3T	1-4T	1-5T	1-6T
E. coli	+	+						
S. paratyphi B	+	+						
S. aureus			-	-	-	-	+	+

	E. coli			S. paratyphi B			S. aureus					
	1-50			1-50			1-5T			1-6T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	28.61	15M	5.2	28.61	9M	5.2	30.74	7M	5.2	30.74	7M
4	5.4	#	57M	5.6	#	49M	5.2	#	1.9M	5.2	#	4.2M
7	5.6	#	75M	6.2	#	90M	5.2	#	1.2M	5.2	#	1.6M
10	5.7	#	49M	6.4	#	51M	5.2	#	200T	5.2	#	900T
24	6.0	28.36	46M	6.6	28.71	37M	5.2	30.13	39T	5.2	30.32	58T

pH 8

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	+	+	-	-	-

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli								
S. paratyphi B	All Tubes Positive							
S. aureus								

	E. coli			S. paratyphi B			S. aureus		
	1-50			1-50			1-50		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	28.86	13M	8.0	28.86	9M	8.0	28.86	7M
4	7.7	#	54M	7.8	#	17.5M	8.0	#	400T
7	7.7	#	23M	7.7	#	41M	8.0	#	500T
10	7.7	#	30M	7.8	#	34M	7.9	#	130T
24	7.7	28.86	30M	7.9	28.86	28M	7.9	29.04	300T

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✱ T signifies thousands.

\# No measurement was made.

TABLE 4

DATA SHEET ON IGEPON AP

pH 7

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-60	1-70	1-80	1-80	1-90	1-100	1-60	1-70	1-80
5	-	+	+	+	+	+	-	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-40	1-60	1-70	1-80	1-90
E. coli								
S. paratyphi B	All Tubes Positive							
S. aureus								

	E. coli						S. paratyphi B						S. aureus					
	1-50			1-100			1-50			1-100			1-50			1-100		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	29.83	12M▲	7.0	30.53	14M	7.0	29.83	9M	7.0	30.53	9M	7.0	29.83	6M	7.0	30.53	6M
4	6.9	29.83	33M	6.8	30.86	126M	6.9	29.83	30M	6.9	30.43	109M	6.9	29.83	30T*	7.0	30.86	84T
7	6.9	30.06	4M	6.9	30.39	40M	6.9	30.06	8.5M	7.1	30.39	57M	6.9	30.01	5T	7.0	30.43	8T
10	7.0	29.59	8.5M	6.9	30.67	80M	7.2	29.97	49M	7.2	30.48	145M	6.9	30.06	2T	7.0	30.62	30T
24	7.2	30.06	52.5M	7.2	30.67	31M	7.5	30.06	23M	7.5	30.57	57M	6.9	29.73	400T	6.9	30.39	800T

pH 5.2

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-60	1-70	1-80	1-80	1-90	1-100	1-60	1-70	1-80
5	-	+	+	+	+	+	-	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-40	1-60	1-70	1-80	1-90
E. coli								
S. paratyphi B	All Tubes Positive							
S. aureus								

	E. coli			S. paratyphi B			S. aureus					
	1-50			1-50			1-50			1-60		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	29.78	14M	5.2	29.78	9M	5.2	29.73	7M	5.3	29.83	7M
4	5.4	#	57M	5.6	#	38M	5.2	#	29T	5.3	#	800T
7	5.4	#	111M	6.1	#	114M	5.2	#	210	5.3	#	4T
10	5.5	#	127M	6.2	#	145M	5.2	#	7	5.3	#	4T
24	6.1	29.73	134M	6.4	29.69	90M	5.2	29.73	0	5.3	29.73	10⊕

pH 8

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-60	1-70	1-80	1-80	1-90	1-100	1-60	1-70	1-80
5	-	+	+	+	+	+	-	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	+	+	-	-	-

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli								
S. paratyphi B	All Tubes Positive							
S. aureus								

	E. coli			S. paratyphi B			S. aureus		
	1-50			1-50			1-50		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	29.87	13M	8.0	29.87	9M	8.0	29.99	6M
4	8.0	#	12M	8.1	#	7.5M	8.0	#	2.4M
7	7.9	#	17M	8.1	#	16M	7.9	#	400T
10	7.9	#	17.5M	8.0	#	18M	7.9	#	200T
24	7.9	29.83	31M	8.0	29.73	36M	7.9	29.62	340T

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

* T signifies thousands.

⊕ Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 10 organisms per cc. in this case.

\# No measurement was made.

TABLE 5

DATA SHEET ON TERGITOL 7

pH 7

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-70	1-80	1-90	1-80	1-90	1-100	1-60	1-70	1-80
5	-	+	+	-	+	+	-	+	+
10	-	+	+	-	-	+	-	+	+
15	-	-	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-50	1-10T	1-20T	1-30T	1-40T	1-50T
E. coli	+	+	+	+					
S. paratyphi B	+	+	+	+					
S. aureus					-	+	+	+	+

	E. coli						S. paratyphi B						S. aureus					
	1-50			1-100			1-50			1-100			1-20T			1-30T		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	27.69	15M▲	7.0	27.79	15M	7.0	27.69	8M	7.0	27.78	8M	7.0	35.85	6M	7.0	35.93	6M
4	6.9	27.59	35M	6.9	27.79	60M	6.9	27.55	27M	6.9	27.55	45M	7.0	35.64	1.4M	7.0	36.79	1.2M
7	6.9	27.64	50M	6.9	27.59	87M	7.0	27.55	25M	7.0	27.73	49M	7.0	35.64	22T✱	7.0	36.79	4M
10	7.0	27.50	30M	7.0	27.59	96M	7.3	27.59	5M	7.3	27.55	96M	7.0	35.69	500⊕	7.0	36.85	6M
24	7.1	27.46	20M	7.1	27.69	41M	7.5	27.59	4M	7.5	27.64	42M	7.0	35.69	28	6.9	36.58	72M

pH 5.2

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-60	1-70	1-80	1-70	1-80	1-90	1-60	1-70	1-80
5	-	+	+	-	+	+	+	+	+
10	-	-	+	-	-	+	-	+	+
15	-	-	+	-	-	-	-	+	+

Chemical Range

	1-10	1-20	1-30	1-50	1-10T	1-20T	1-30T	1-40T	1-50T
E. coli	+	+	+	+					
S. paratyphi B	+	+	+	+					
S. aureus					-	-	-	+	+

	E. coli						S. paratyphi B			S. aureus		
	1-50			1-60			1-50			1-40T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	27.59	13M	5.2	27.59	13M	5.2	27.45	9M	5.2	37.45	6M
4	5.3	#	70T	5.2	#	37T	5.4	#	5.2M	5.2	#	700T
7	5.3	#	91	5.2	#	2T	5.6	#	7M	5.2	#	200T
10	5.3	#	8	5.3	#	80T	5.9	#	29M	5.2	#	30T
24	5.3	27.54	0	5.4	27.64	2.7M	6.6	27.45	52M	5.2	38.02	150

pH 8

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-60	1-70	1-80	1-70	1-80	1-90	1-60	1-70	1-80
5	-	+	+	-	+	+	-	+	+
10	-	-	+	-	-	+	-	+	+
15	-	-	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-50	1-10T	1-20T	1-30T	1-40T	1-50T
E. coli	+	+	+	+					
S. paratyphi B	+	+	+	+					
S. aureus					-	+	+	+	+

	E. coli			S. paratyphi B			S. aureus					
	1-50			1-50			1-10T			1-20T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	27.72	13M	8.0	27.72	8M	8.0	34.64	6M	8.0	35.88	6M
4	7.8	#	61M	7.8	#	11M	7.9	#	2T	7.9	#	2M
7	7.8	#	11.8M	7.8	#	15.3M	7.9	#	200	7.8	#	1.9M
10	7.8	#	17M	7.8	#	18M	7.9	#	0	7.8	#	5M
24	7.8	27.72	40M	8.0	27.72	15M	7.9	34.58	0	7.9	36.02	14.7M

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✱ T signifies thousands.

⊕ Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 500 organisms per cc. in this case.

\# No measurement was made.

TABLE 6

DATA SHEET ON NACCONOL NRSF

pH 7

Phenolic Range

Min.	E. coli 1-60	E. coli 1-70	E. coli 1-80	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	-	+	+	+	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	+	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-50	1-10T	1-20T	1-30T	1-40T	1-50T
E. coli	+	+	+	+					
S. paratyphi B	+	+	+	+					
S. aureus					-	-	+	+	+

	E. coli 1-50			E. coli 1-100			S. paratyphi B 1-50			S. paratyphi B 1-100			S. aureus 1-30T			S. aureus 1-40T		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	31.89	13M▲	7.0	31.89	13M	7.0	31.89	8M	7.0	31.89	10M	7.0	36.05	7M	7.0	37.02	7M
4	6.9	31.89	74M	6.9	31.89	91M	6.9	31.89	15M	6.9	31.89	35M	7.0	35.49	1.3M	7.0	37.02	2M
7	6.9	31.89	37M	6.9	31.75	83M	7.0	31.89	35M	7.1	31.75	98M	7.0	36.24	1.6M	7.1	37.02	8.5M
10	7.0	31.75	8M	7.0	31.75	64M	7.3	31.75	5M	7.5	31.75	88M	7.2	36.51	7M	7.3	37.02	14M
24	7.1	31.75	20M	7.2	31.68	30M	7.6	31.68	13M	7.6	30.93	10M	7.2	36.51	14.5M	7.3	37.02	10M

pH 5.2

Phenolic Range

Min.	E. coli 1-60	E. coli 1-70	E. coli 1-80	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	-	+	+	+	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Chemical Range

	1-10	1-20	1-30	1-50	1-10T	1-20T	1-30T	1-40T	1-50T
E. coli	+	+	+	+					
S. paratyphi B	+	+	+	+					
S. aureus					-	-	+	+	+

	E. coli 1-50			S. paratyphi B 1-50			S. aureus 1-30T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	31.29	12M	5.2	31.31	8M	5.2	36.01	7M
4	5.4	#	7T*	5.4	#	2.6M	5.2	#	5.8T
7	5.4	#	30T	5.5	#	27.5M	5.2	#	3T
10	5.4	#	300T	5.7	#	99M	5.2	#	1T
24	5.7	30.97	29M	6.5	30.90	47M	5.2	35.98	4●

pH 8

Phenolic Range

Min.	E. coli 1-60	E. coli 1-70	E. coli 1-80	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	-	+	-	+	+	-	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-50	1-10T	1-20T	1-30T	1-40T	1-50T
E. coli	+	+	+	+					
S. paratyphi B	+	+	+	+					
S. aureus					-	+	+	+	+

	E. coli 1-50			S. paratyphi B 1-50			S. aureus 1-10T			S. aureus 1-20T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	31.75	12M	8.0	31.75	8M	8.0	34.47	7M	8.0	35.52	7M
4	7.8	#	76M	7.8	#	38M	8.0	#	119T	8.0	#	1.8M
7	7.7	#	89M	7.7	#	86.5M	7.9	#	2T	8.0	#	1.3M
10	7.8	#	72M	8.0	#	114M	7.9	#	40	7.9	#	1.1M
24	7.9	31.36	12M	8.2	31.36	12.5M	7.9	34.27	0	7.8	35.49	420T

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

* T signifies thousands.

● Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 4 organisms per cc. in this case.

\# No measurement was made.

TABLE 7

DATA SHEET ON NEKAL BX

pH 7

Phenolic Range

Min.	E. coli 1-60	E. coli 1-70	E. coli 1-80	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	-	+	+	-	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	+	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-50	1-5T	1-10T	1-20T	1-30T	1-40T
E. coli	-	-	+	+					
S. paratyphi B	-	-	+	+					
S. aureus					-	-	+	+	+

	E. coli 1-50			E. coli 1-100			S. paratyphi B 1-50			S. paratyphi B 1-100			S. aureus 1-20T			S. aureus 1-30T		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	31.72	14M▲	6.9	31.76	14M	7.0	31.72	9M	6.9	31.76	9M	7.0	42.16	7M	7.0	44.58	7M
4	6.8	31.72	8M	6.6	31.62	40M	6.8	31.72	10M	6.7	31.57	18M	6.8	42.23	10M	7.0	44.62	9M
7	6.8	31.66	2.5M	6.6	31.52	12M	6.8	31.72	12M	6.6	31.47	18.5M	6.8	42.31	10M	6.9	44.71	13M
10	6.9	31.66	2M	6.9	31.52	11M	7.0	31.62	8M	7.1	31.47	8M	7.0	42.31	28M	7.0	44.71	52M
24	7.0	31.66	1.5M	7.1	31.52	11M	7.2	31.62	15M	7.3	31.47	10M	7.2	42.16	18M	7.3	44.62	10M

pH 5.2

Phenolic Range

Min.	E. coli 1-60	E. coli 1-70	E. coli 1-80	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	+	+	+	+	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	-	-	-	+	-	+	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-8T	1-10T	1-20T	1-30T
E. coli	-	-	-	+	+				
S. paratyphi B	-	-	+	+	+				
S. aureus						-	-	+	+

	E. coli 1-50			E. coli 1-60			S. paratyphi B 1-50			S. paratyphi B 1-60			S. aureus 1-10T			S. aureus 1-20T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	31.66	14M	5.2	31.66	13M	5.2	31.66	8M	5.2	31.66	9M	5.2	41.16	8M	5.2	42.09	6M
4	5.3	#	300⊕	5.2	#	3.1M	5.2	#	6M	5.2	#	2M	5.3	#	70T	5.2	#	650T
7	5.3	#	26	5.3	#	3M	5.2	#	1T✠	5.3	#	4M	5.3	#	8T	5.2	#	500T
10	5.3	#	0	5.4	#	5M	5.2	#	10T	5.4	#	7M	5.3	#	11O	5.2	#	600T
24	5.3	31.57	0	5.6	31.72	7M	5.2	31.66	10	5.8	31.72	7M	5.3	40.66	0	5.2	42.16	100T

pH 8

Phenolic Range

Min.	E. coli 1-60	E. coli 1-70	E. coli 1-80	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	+	+	+	+	+	+
10	-	-	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Chemical Range

	1-10	1-20	1-30	1-50	1-1T	1-5T	1-10T	1-20T	1-30T
E. coli	-	-	+	+					
S. paratyphi B	-	-	+	+					
S. aureus					-	-	+	+	+

	E. coli 1-50			S. paratyphi B 1-50			S. aureus 1-10T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	32.06	13M	8.0	32.06	9M	8.0	41.23	7M
4	7.7	#	12M	7.9	#	2.7M	8.0	#	3.2M
7	7.6	#	19M	7.8	#	6.4M	7.9	#	8M
10	7.6	#	9M	7.8	#	9M	7.9	#	5M
24	7.7	31.20	4M	7.8	31.20	18M	7.9	40.66	20M

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✠ T signifies thousands.

⊕ Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 300 organisms per cc. in this case.

\# No measurement was made.

used offers no means for explaining this phenomenon, it is interesting to note that Stock and Francis (59) found oleic, linoleic, and linolenic acids more effective in inactivating influenza virus at pH 7.6 than at pH 6. Baker, Harrison, and Miller (6) pointed out that Igepon AP (pH 7) stimulated bacterial metabolism even at a concentration of 1-100 (approximately the dilution of active ingredient of Igepon AP used in the experiment discussed in this thesis). This fact is substantiated by the results obtained with E. coli and S. paratyphi B at pH 5.2 for the first 7 hours of the growth period.

Surface tension alone appears to have little significant influence on the growth-curve data. This is in agreement with the results presented by Marshall (47) and by Mallmann and Darby (45). It has sometimes been assumed that the degree to which a surface-active agent can lower surface tension is a measure of its effectiveness as a germicide. The data from this study do not support this contention. No positive correlation appears to exist between germicidal activity and reduction of surface tension for the anionic agents acting at surface tensions between 27 and 32 dynes per centimeter. Although Nekal and Nacconol lowered the surface tension to approximately 31 dynes per centimeter in contrast to 29 dynes per centimeter for Igepon and Duponol, the first two compounds are far more germicidal than the latter two. In all the experiments, there was a negligible shift in surface tension over the 24-hour test.

Since the chemical-range test served as the initial basis for using the proper dilutions in the growth-curve studies, it is significant to note that there was no absolute correlation between the results of the two methods. With Igepon AP, in contrast to the chemical-range test result, S. aureus at pH 5.2 failed to survive a 24-hour exposure in a 1-50 concentration of the anionic agent. Both E. coli and S. paratyphi B at pH 5.2 displayed inconsistencies when reacting with Nekal BX at a 1-50 concentration. The same was true for E. coli at pH 5.2 with Tergitol 7 in a 1-50 concentration. The surprising fact is that all these discrepancies occurred at pH 5.2, the range where these anionic compounds were most effective against the organisms mentioned. Since the factor of exposure time was carefully checked, a possible explanation for these variations may be that in the chemical-range test, the organisms have a better chance of survival, insofar as the limiting area of the test tube may be sufficiently small (in comparison with an erlenmeyer flask) to afford protection for some of the organisms against the synthetic agents. Since this method is an "all or none" survival test, it is quite possible that one or two surviving bacteria may have been subtransferred successfully, or that one or two very small clumps of organisms withstood the bactericidal effect of the anionic agents. Further evidence of these variations will be presented in the discussion of the cationic agents.

Of the anionic agents tested, Tergitol 7 at pH 5.2 was the most bactericidal (on the basis of effective concen-

tration) for E. coli and S. aureus. This is in agreement with the findings of Gershenfeld (24) on a similar compound (Tergitol 4) and Baker et al (6)(7). Nekal BX at pH 5.2 was germicidal against all three organisms (at a 1-40 concentration it was germicidal against S. paratyphi B). Nacconol NRSF was germicidal only for S. aureus. Finally, Duponol OS and Igepon AP follow, in that order of effectiveness, as germicidal agents against S. aureus.

Perhaps the most important fact in these studies with anionic agents is that at pH 5.2, with the proper concentration of the synthetic compounds, Tergitol 7 and Nekal BX are germicidal for the gram-negative organisms. With the exception of the paper by Gershenfeld and Milanick (24), in which they indicate that Aerosol OT and Tergitol 4 at pH 4 and pH 5 can kill S. typhosa, the common belief has been that anionic agents are only bactericidal for gram-positive organisms. Bayliss (11) reported that sodium lauryl sulfate cleared suspensions of gram-negative organisms, but he failed to find a positive correlation between the lysing and lethal action.

The importance of pH is dramatically presented by Duponol OS. At pH 8, S. aureus survived a 24-hour exposure to a 1-50 concentration of this synthetic agent. At pH 7, a 1-400 concentration was effectively germicidal against the same organism, while at pH 5.2, a 1-4000 dilution completely killed S. aureus in 24 hours. The only positive correlation between the trend of pH and bacterial count over the various periods of the growth curve is that once the bacterial count

goes below the one-million mark and stays below that mark, the shift in pH is either negligible or non-existent. In all other instances, the change in pH falls short of the level reached in the controls.

C. Cationic Compounds

Domagk's (13) publication on the long-chain quaternary ammonium salts stimulated a tremendous interest in these compounds. Working mainly with zephirol (alkyl dimethyl benzyl ammonium chloride), he reported its germicidal activity, as well as germicidal activities for other quaternary ammonium salts which possessed a long-chain aliphatic group (C_8H_{17} to $C_{18}H_{37}$).

The methods for preparing the three types of cationic agents used in the investigation reported in this thesis are described in the papers by Kuhn et al (41) (for alkyl dimethyl benzyl ammonium chloride), and by Shelton et al (56) (57) (for CTAB and alkyl pyridinium chloride).

The concentrations of quaternary ammonium compounds used in these experiments either reduced the counts of all three types of bacteria or killed them completely. They also inhibited the normal shift in pH over the 24-hour period.

The results presented in Tables 8, 9, and 10 are in agreement with the general theory of maximum germicidal activity of cationic agents in an alkaline range. The work of Kuhn and Bielig (40) gave strong support to this generalization. They pointed out that proteins could only be precipitated when they were in the form of anions, i.e., on the basic side of the isoelectric point. They likewise proposed

TABLE 8

DATA SHEET ON ROCCAL

pH 7

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	-	+	+	-	+	+
10	-	+	+	-	-	+	-	+	+
15	-	+	+	-	-	+	-	-	+

Chemical Range

	1-1T	1-2T	1-3T	1-4T	1-10T	1-20T	1-30T	1-40T
E. coli	-	-	+	+				
S. paratyphi B	-	+	+	+				
S. aureus					-	+	+	+

	E. coli									S. paratyphi B				S. aureus					
	1-3T			1-4T			1-5T			1-3T			1-5T			1-40T			1-50T	
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	43.71	13M▲	7.0	46.02	15M	7.0	47.31	15M	7.0	43.66	8M	7.0	47.17	8M	7.0	53.99	6M	7.0	55.07	6M
4	7.0	44.13	2T✝	7.0	46.13	46T	7.0	47.92	10M	7.0	43.99	1M	6.9	47.17	17M	7.0	54.35	2M	7.0	56.04	2.2M
7	7.0	43.54	20⊕	7.0	45.97	10T	7.0	46.75	100T	7.0	43.99	14M	6.9	47.17	40M	7.0	53.76	480T	7.0	54.97	1M
10	7.0	43.48	0	7.0	45.84	2T	6.9	46.66	4.5M	6.9	43.66	29M	7.1	46.34	104M	7.0	53.57	450T	7.0	54.97	1M
24	7.0	43.48	0	6.9	45.84	5M	7.0	46.66	95M	7.2	43.66	39M	7.5	46.34	260M	6.9	53.99	21T	7.0	55.07	600T

pH 5.2

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	+	+	+
10	-	+	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Chemical Range

	1-1T	1-2T	1-3T	1-4T	1-5T	1-10T	1-20T	1-30T
E. coli	-	-	-	+	+			
S. paratyphi B	-	-	+	+	+			
S. aureus						-	+	+

	E. coli									S. paratyphi B			S. aureus					
	1-4T			1-5T			1-6T			1-5T			1-20T			1-30T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	45.97	15M	5.2	46.75	15M	5.2	47.92	15M	5.2	46.71	8M	5.2	52.26	6M	5.2	53.02	6M
4	5.3	#	32T	5.3	#	240T	5.3	#	3M	5.2	#	46T	5.2	#	800T	5.3	#	2M
7	5.3	#	5T	5.4	#	4T	5.3	#	220T	5.2	#	3T	5.2	#	500T	5.3	#	600T
10	5.3	#	260	5.4	#	800	5.3	#	2T	5.2	#	18T	5.2	#	200T	5.3	#	400T
24	5.3	45.08	0	5.4	45.97	112	5.4	46.75	800T	6.0	46.75	52M	5.2	51.80	5T	5.3	52.78	65T

pH 8

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	-	+	-	-	+

Chemical Range

	1-1T	1-2T	1-3T	1-4T	1-5T	1-10T	1-20T	1-30T
E. coli	-	-	-	-	+			
S. paratyphi B	-	+	+	+	+			
S. aureus						-	+	+

	E. coli						S. paratyphi B			S. aureus								
	1-5T			1-6T			1-5T			1-30T			1-40T			1-50T			1-70T	
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	46.98	13M	8.0	47.91	12M	8.0	47.48	9M	8.0	53.29	6.5M	8.0	54.51	6M	8.0	55.49	6M	8.0	57.83	6M
4	8.1	#	3T	8.0	#	91T	7.8	#	13M	8.0	#	15T	7.9	#	120T	7.8	#	700T	8.0	#	2M
7	8.1	#	3T	8.0	#	15T	7.8	#	64M	8.0	#	2T	7.9	#	30T	7.8	#	200T	8.0	#	1.3M
10	8.1	#	12	8.0	#	3T	8.0	#	85M	8.0	#	115	7.8	#	15T	7.8	#	100T	8.0	#	400T
24	8.1	46.13	0	8.0	47.49	10T	8.1	47.13	19M	8.0	51.80	0	7.8	53.29	7	7.8	53.97	100	8.0	56.94	75T

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✝ T signifies thousands.

⊕ Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 20 organisms per cc. in this case.

\# No measurement was made.

TABLE 9

DATA SHEET ON LPC

pH 7

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	-	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	-	+

Chemical Range

	1-5T	1-10T	1-20T	1-30T	1-40T	1-100T	1-200T	1-300T
E. coli	-	-	+	+	+			
S. paratyphi B	-	-	+	+	+			
S. aureus						-	+	+

	E. coli						S. paratyphi B						S. aureus								
	1-30T			1-40T			1-30T			1-40T			1-200T			1-300T			1-800T		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	44.88	15M▲	7.0	46.87	15M	7.0	44.33	8M	7.0	46.49	9M	7.0	53.04	6M	7.0	55.76	6M	7.0	57.36	6M
4	7.0	44.17	2M	6.9	45.56	7M	7.0	44.33	5M	6.9	46.27	16M	7.0	52.29	2.1M	7.0	53.56	6.9M	7.0	58.06	9M
7	7.0	43.81	400T✝	6.9	45.95	5M	6.9	43.38	47M	6.9	46.51	65M	7.0	53.56	2.1M	7.0	54.90	5.5M	7.0	58.39	48M
10	7.0	42.87	60T	6.9	46.37	3M	7.0	44.83	47M	7.2	46.96	110M	7.0	53.23	800T	7.1	54.50	5.6M	7.2	58.14	60M
24	7.0	43.81	24⊕	7.3	46.81	43M	7.5	45.34	75M	7.6	47.37	192M	7.0	52.29	96T	7.1	53.47	460T	7.4	56.75	90M

pH 5.2

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	+	+	+
10	-	+	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Chemical Range

	1-5T	1-10T	1-20T	1-30T	1-40T	1-100T	1-200T	1-300T
E. coli	-	-	+	+	+			
S. paratyphi B	-	+	+	+	+			
S. aureus						-	+	+

	E. coli			S. paratyphi B			S. aureus					
	1-30T			1-30T			1-100T			1-200T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	42.83	12M	5.2	42.27	9M	5.2	51.23	6M	5.2	53.56	6M
4	5.2	#	5.7M	5.2	#	9M	5.2	#	1T	5.2	#	7.8M
7	5.2	#	1.6M	5.7	#	9M	5.2	#	6	5.3	#	5.9M
10	5.2	#	500T	6.3	#	78M	5.2	#	0	5.3	#	3.2M
24	5.2	41.35	88	6.8	43.38	136M	5.2	50.95	0	5.4	51.59	700T

pH 8

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	-	+	-	-	+

Chemical Range

	1-5T	1-10T	1-20T	1-30T	1-40T	1-100T	1-200T	1-300T
E. coli	-	-	-	+	+			
S. paratyphi B	-	-	+	+	+			
S. aureus						-	+	+

	E. coli			S. paratyphi B			S. aureus					
	1-40T			1-40T			1-200T			1-300T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	45.95	14M	8.0	45.87	9M	8.0	53.16	6M	8.0	55.76	6M
4	8.0	#	51T	7.8	#	13M	8.0	#	200T	8.0	#	400T
7	8.0	#	15T	7.8	#	13M	8.0	#	6T	8.0	#	160T
10	8.0	#	700	7.7	#	47M	8.0	#	1T	8.0	#	82T
24	8.0	45.56	19	8.2	45.87	13M	8.0	53.56	0	8.0	56.75	20⊕

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✝ T signifies thousands.

⊕ Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 24 organisms per cc. in this case.

\# No measurement was made.

TABLE 10

DATA SHEET ON CTAB

pH 7

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	+	+	-	-	+

Chemical Range

	1-5T	1-8T	1-10T	1-20T	1-100T	1-200T	1-300T	1-400T
E. coli	-	+	+	+	|			
S. paratyphi B	-	+	+	+	|			
S. aureus					|	+	+	+

	E. coli									S. paratyphi B						S. aureus					
	1-10T			1-20T			1-30T			1-10T			1-20T			1-700T			1-1M		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	37.29	13M▲	7.0	37.47	13M	7.0	37.61	15M	7.0	37.34	9M	7.0	37.52	9M	7.0	55.54	8M	7.0	57.69	8M
4	7.0	37.29	14⊕	7.0	37.47	4M	6.9	37.52	10M	7.0	37.34	120T	7.0	37.47	5.4M	7.1	56.29	400	7.0	57.69	1M
7	7.0	37.16	0	7.0	37.41	270T✱	6.9	37.47	14M	7.0	37.29	200T	7.0	37.47	82M	7.1	55.66	3	7.0	57.78	90T
10	7.0	37.06	0	7.0	37.38	40T	7.0	37.47	6.6M	7.0	37.29	6M	7.2	37.38	125M	7.1	54.31	6	7.0	56.87	10T
24	7.0	37.06	0	7.0	37.38	70T	7.1	37.47	1.5M	7.3	37.15	31M	7.6	37.38	82M	7.1	55.66	40	7.0	56.35	1T

pH 5.2

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	-	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Chemical Range

	1-5T	1-10T	1-20T	1-30T	1-100T	1-200T	1-300T	1-400T
E. coli	-	-	+	+	|			
S. paratyphi B	-	-	+	+	|			
S. aureus					|	+	+	+

	E. coli						S. paratyphi B			S. aureus					
	1-20T			1-30T			1-20T			1-700T			1-900T		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	37.38	15M	5.2	37.61	15M	5.2	37.38	9M	5.2	56.87	7M	5.2	57.65	7M
4	5.2	#	169	5.3	#	75T	5.2	#	11T	5.2	#	400T	5.2	#	1.3M
7	5.2	#	19	5.3	#	159	5.2	#	160T	5.2	#	36T	5.2	#	800T
10	5.2	#	0	5.3	#	14	5.2	#	4.8M	5.2	#	10T	5.2	#	720T
24	5.2	37.38	0	5.3	37.74	280	5.2	37.61	8.2M	5.2	55.31	36	5.2	57.65	100T

pH 8

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	-	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	-	+	-	-	+

Chemical Range

	1-8T	1-10T	1-20T	1-30T	1-100T	1-200T	1-300T	1-400T
E. coli	-	+	+	+	|			
S. paratyphi B	+	+	+	+	|			
S. aureus					|	+	+	+

	E. coli						S. paratyphi B			S. aureus					
	1-20T			1-30T			1-20T			1-800T			1-1M		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	37.52	13M	8.0	37.74	13M	8.0	37.61	9M	8.0	56.87	7M	8.0	57.13	7M
4	8.0	#	170T	7.9	#	5.1M	7.9	#	12M	7.9	#	320	7.9	#	500
7	8.0	#	7	7.9	#	3.6M	7.7	#	29M	7.9	#	41	7.9	#	65
10	8.0	#	0	7.9	#	170T	7.7	#	14M	7.9	#	20	7.9	#	48
24	8.0	37.52	0	7.8	37.61	200T	8.1	37.74	2.1M	7.9	54.31	13	7.9	55.66	68

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✱ T signifies thousands.

⊕ Where no M or T follows the enumeration, the figure is the actual bacterial count per cc., that is, 14 organisms per cc. in this case.

\# No measurement was made.

that this capacity to react with proteins and to split protein conjugates eventually causes the destruction of essential components of living bacteria, resulting in death of the cells. However, with the possible exception of the paper by Quisno and Foter (50), very few reports have been made on the comparable efficiency of the quaternary ammonium compounds in acid ranges. These authors demonstrated that Ceepryn was as efficient at pH 3 as at pH 8 against both gram-positive and gram-negative organisms. Although acid ranges as low as pH 3 were not explored in the study herein presented, the results in Table 10 indicate that CTAB was certainly as efficient against E. coli at pH 5.2 as it was at pH 8.

An important point that should enter into an evaluation of quaternary ammonium compounds is their ability to perform effectively over an extended period of time. For example, in comparing the counts of S. paratyphi B at pH 5.2 and at pH 8 in Tables 8 and 10, it will be noted that employing the same concentration of cationic (1-5T with Roccal and 1-20T with CTAB) at both pH levels, the end result (after a 24-hour period) was a higher count at pH 5.2. However, if one should evaluate these germicides after a 4-hour exposure period, the result would be a 99-percent reduction in count at pH 5.2 and an actual increase in count at pH 8. To a lesser degree, this was true of lauryl pyridinium chloride (Table 9) where there was no increase in count at pH 5.2 and a rise in count at pH 8. Naturally, this would serve as an unfair evaluation of relative germicidal activities.

First, the concentrations at which the comparisons were made were non-germicidal concentrations (so far as complete kill was concerned). Second, the possibility of bacteriostatic rather than bactericidal activity may be the explanation of the results. If the hypothesis is correct that bacteriostasis is characterized by a period of no visible development followed by multiplication at a rate which shows no inhibition by the chemical agent (29), then the reduction in bacterial numbers during the first 7 or 10 hours at pH 5.2 can be explained by bacteriostatic rather than by bactericidal action. The property of these compounds to maintain the bacterial population at a level which is 99 percent below the initial inoculum for periods of 7 to 10 hours, might be considered additional proof of stasis. However, under the conditions of this test, no positive statement concerning bacteriostasis can be made.

There is no absolute correlation of results between the chemical-range tests and the growth-curve tests.* This observation is similar to that noted in the anionic studies. Quisno et al (51) present a possible explanation for this discrepancy. They point out that when the test-tube method is used for testing synthetic cationic agents, it is quite possible for a significant proportion of quaternary ammonium molecules near the extreme upper margin of the meniscus to be attracted to the air-water and glass-water interfaces. These molecules are so arranged that the lethal lipophilic portion is oriented towards these interfaces and therefore

* This lack of agreement will be discussed in the section entitled "Bacteriostatic Tests."

is not available to act on bacteria that may float towards the region of the meniscus. As for other possible explanations of the difference in results between the two tests, the same reasons presented in the discussion of anionic agents would be applicable here.

In comparing the three cationic agents on the basis of percentage of active ingredient, it becomes apparent that Roccal was the most germicidal and CTAB the least germicidal against the gram-negative organisms. However, all three compounds are approximately equal in their bactericidal activity against S. aureus. Since the Roccal powder used in these tests only contained approximately 3.5 percent of active ingredient, it became necessary to employ a more concentrated Roccal solution, in order to check on the validity of the large conversion factor. For this purpose, a commercial 10-percent liquid solution of Roccal was used. Growth-curve tests showed the liquid solution to be between two-and-one-half and three times more germicidal than the powdered compound at the three pH levels. Consequently, in addition to substantiating the potency of Roccal as a germicide, it also proved that the filler material used in the powdered Roccal preparation did not materially hinder the performance of the active ingredient.

The role of concentration of synthetic agents in bacterial growth is best exemplified by the cationic agents. When only those concentrations which permitted S. aureus to survive for 24 hours were used, it became apparent that a

wide range of low concentrations of the quaternary ammonium compounds were capable of maintaining a very limited bacterial population. Roccal (pH 8), in dilutions of 1-40T, 1-50T, and 1-70T, reduced the S. aureus population from 6M organisms per cc. to 7 organisms, 100 organisms, and 75T organisms per cc., respectively, in 24 hours. CTAB (pH 8) reduced the numbers of bacteria from 7M per cc. to 13 organisms per cc. at a 1-800T concentration, while a 1-1M dilution gave a final count of 68 organisms per cc. LPC (pH 7) at concentrations of 1-200T and 1-300T decreased the counts from 6M per cc. to 96T per cc. and 460T per cc., respectively. On the other hand, comparable gradations of concentration used with E. coli usually resulted in large differences in bacterial numbers, e.g., LPC (pH 7) at concentrations of 1-30T and 1-40T resulted in 24-hour counts of 24 organisms per cc. and 43M organisms per cc., respectively. The anionic agents did not consistently parallel the cationic compounds in their effects on S. aureus. The only two anionic compounds which afford an opportunity for comparison are Duponol OS and Tergitol 7. Duponol OS (pH 5.2) at concentrations of 1-5T and 1-8T reduced the bacterial numbers to 39T and 58T organisms per cc., respectively. However, Tergitol 7 (pH 7) at concentrations of 1-20T and 1-30T decreased the counts to 28 organisms per cc. and 72M organisms per cc., respectively.

Since the cationic agents displayed such marked germicidal activity, it was thought advisable to ascertain their bactericidal or bacteriostatic effects on the basis of relatively short exposure periods. Those concentrations of

TABLE 10a

GROWTH OF E. COLI, S. PARATYPHI B, AND S. AUREUS AFTER SHORT PERIODS OF EXPOSURE TO ROCCAL, CTAB, AND LPC

All counts are in terms of bacteria per cc.

Compound	Concentration	pH	Organism	Time Interval in Minutes				
				0	15	30	60	120
Controls		5.2	E. coli	13M	13M	16M	18M	25M
		8.0		14M	14M	17M	18M	28M
		5.2	S. paratyphi B	8M	8M	10M	12M	16M
		8.0		7M	7M	8M	10M	25M
		5.2	S. aureus	7M	7M	8M	9M	10M
		8.0		6M	6M	6M	7M	15M
Roccal	1-6T	8.0	E. coli	14M	6M	5M	3M	1.5M
	1-5T	8.0	S. paratyphi B	9M	3M	3M	3M	3M
	1-40T	8.0	S. aureus	7M	5M	3.6M	3M	3M
CTAB	1-30T	5.2	E. coli	15M	1.7M	700T	600T	400T
	1-30T	8.0		15M	9M	6.8M	3.9M	3.8M
	1-20T	5.2	S. paratyphi B	9M	12T	3T	2T	800
	1-30T	8.0		9M	5.3M	2.4M	1.2M	2M
	1-800T	8.0	S. aureus	8M	1M	500T	100T	20T
LPC	1-40T	8.0	E. coli	13M	5M	5M	560T	200T
	1-40T	8.0	S. paratyphi B	8M	2.5M	2M	2M	2M
		8.0	S. aureus	7M	6M	6M	6M	2M

cationics which did not completely kill the organisms in 24 hours were selected for this study. The results of these short growth-curve tests are presented in Table 10a.

An unqualified correlation does not appear to exist between the results of the 2-hour exposure period in the short growth-curve tests (see Table 10a) and those of the comparable 4-hour exposure period in the 24-hour growth-curve tests (see Tables 8, 9, and 10). However, it is apparent from the results in Table 10a that the majority of the compounds tested caused a sharp decrease in bacterial numbers during the first 15 minutes, followed by a lag period lasting from 45 minutes to 105 minutes. This lag period might be indicative of bacteriostatic activity.

D. Non-Ionic Compounds

In the literature on non-ionic agents, there is general agreement that the germicidal activity of these compounds is either negligible or non-existent. Unfortunately, the writer has failed to find any published data to support this generalization, other than simple statements to this effect. Although a complete survey of the different types of non-ionic agents was not attempted in this study, the data on the two compounds used (see Tables 11 and 12) substantiate the accepted generalization.

By employing strong concentrations of the non-ionic agents, it was possible to show their activity in reducing bacterial numbers of all three types of organisms, when compared with the counts in Control Table 2. There was likewise

TABLE 11

DATA SHEET ON EMULPHOR ON

pH 7

Phenolic Range

Min.	E. coli 1-80	E. coli 1-90	E. coli 1-100	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-70	S. aureus 1-80	S. aureus 1-90
5	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli	+	+	+	+	+	+	+	+
S. paratyphi B	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+

	E. coli 1-20			E. coli 1-50			S. paratyphi B 1-20			S. paratyphi B 1-50			S. aureus 1-20			S. aureus 1-50		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	37.61	12M▲	7.0	37.99	13M	7.0	37.61	8M	7.0	37.94	8M	7.0	37.64	6M	7.0	37.94	6M
4	6.7	37.56	45M	6.7	38.03	75M	6.8	37.56	68M	6.8	37.94	70M	6.9	37.64	5.7M	6.9	38.03	6M
7	6.8	37.56	82M	6.8	38.08	105M	7.1	37.56	100M	7.1	38.03	124M	6.9	37.89	3.5M	6.9	38.03	4.1M
10	6.8	37.56	55M	6.8	38.08	68M	7.2	37.52	152M	7.2	38.03	180M	6.9	37.89	2.2M	6.9	38.03	2.8M
24	6.9	37.56	65M	7.0	38.08	130M	7.5	37.52	169M	7.5	38.03	214M	6.9	37.89	11.7M	7.0	38.08	23M

pH 5.2

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	+	+	-	+	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli	+	+	+	+	+	+	+	+
S. paratyphi B	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+

	E. coli 1-20			S. paratyphi B 1-20			S. aureus 1-20		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	37.75	15M	5.2	37.75	8M	5.2	37.75	6M
4	5.3	#	86M	5.5	#	38M	5.2	#	175T✚
7	5.4	#	106M	6.0	#	107M	5.2	#	2.5M
10	5.5	#	120M	6.4	#	203M	5.2	#	11M
24	5.8	37.89	214M	6.8	37.89	239M	6.1	37.75	90M

pH 8

Phenolic Range

Min.	E. coli 1-70	E. coli 1-80	E. coli 1-90	S. paratyphi B 1-80	S. paratyphi B 1-90	S. paratyphi B 1-100	S. aureus 1-60	S. aureus 1-70	S. aureus 1-80
5	+	+	+	+	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	+	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli	+	+	+	+	+	+	+	+
S. paratyphi B	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+

	E. coli 1-20			S. paratyphi B 1-20			S. aureus 1-20		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	37.56	15M	8.0	37.56	8M	8.0	37.56	6M
4	7.8	#	110M	8.0	#	51M	8.0	#	20T
7	7.7	#	117M	8.0	#	123M	7.9	#	2.1M
10	7.8	#	138M	8.1	#	239M	7.9	#	6M
24	8.0	37.61	142M	8.2	37.61	161M	8.1	37.56	52M

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✚ T signifies thousands.

\# No measurement was made.

TABLE 12

DATA SHEET ON TRITON X-100

pH 7

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-80	1-90	1-100	1-80	1-90	1-100	1-60	1-70	1-80
5	+	+	+	-	+	+	-	+	+
10	-	+	+	-	-	+	-	+	+
15	-	+	+	-	-	+	-	-	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli	+	+	+	+	+	+	+	+
S. paratyphi B	+	+	+	+	+	+	+	+
S.aureus	+	+	+	+	+	+	+	+

	E. coli						S. paratyphi B						S. aureus					
	1-20			1-50			1-20			1-50			1-20			1-50		
Hrs.	pH	S.T.*	B.C.**	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	7.0	32.66	12M▲	7.0	32.75	14M	7.0	32.66	8M	7.0	32.75	8M	7.0	32.62	6M	7.0	32.71	6M
4	6.8	32.62	56M	6.9	32.62	72M	6.9	32.71	10M	6.9	32.66	18.5M	7.0	32.62	170T✠	7.0	32.71	280T
7	6.9	32.62	31M	6.9	32.57	53M	7.1	32.71	50M	7.1	32.66	57M	7.0	32.57	60T	7.0	32.71	60T
10	7.0	32.62	15M	7.0	32.57	67M	7.3	32.66	60M	7.3	32.66	61M	7.0	32.57	100T	6.9	32.71	600T
24	7.0	32.75	90M	7.0	32.66	112M	7.5	32.85	52M	7.5	32.66	100M	6.9	32.75	5M	6.8	32.66	10M

pH 5.2

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-70	1-80	1-90	1-80	1-90	1-100	1-70	1-80	1-90
5	-	+	+	-	+	+	+	+	+
10	-	+	+	-	+	+	-	+	+
15	-	-	+	-	-	+	-	+	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli	+	+	+	+	+	+	+	+
S. paratyphi B	+	+	+	+	+	+	+	+
S.aureus	+	+	+	+	+	+	+	+

	E. coli			S. paratyphi B			S. aureus		
	1-20			1-20			1-20		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	5.2	32.71	12M	5.2	32.71	8M	5.2	32.71	6M
4	5.3	#	38M	5.4	#	20M	5.2	#	2.1M
7	5.4	#	47M	5.8	#	51M	5.2	#	1.7M
10	5.8	#	54M	6.3	#	94M	5.2	#	3.1M
24	5.8	32.62	58M	6.7	32.62	120M	5.1	32.75	8M

pH 8

Phenolic Range

Min.	E. coli			S. paratyphi B			S. aureus		
	1-80	1-90	1-100	1-80	1-90	1-100	1-70	1-80	1-90
5	+	+	+	-	+	+	+	+	+
10	-	+	+	-	+	+	-	+	+
15	-	+	+	-	-	+	-	+	+

Chemical Range

	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80
E. coli	+	+	+	+	+	+	+	+
S. paratyphi B	+	+	+	+	+	+	+	+
S.aureus	+	+	+	+	+	+	+	+

	E. coli			S. paratyphi B			S. aureus		
	1-20			1-20			1-20		
Hrs.	pH	S.T.	B.C.	pH	S.T.	B.C.	pH	S.T.	B.C.
0	8.0	32.62	12M	8.0	32.62	8M	8.0	32.62	6M
4	7.8	#	55M	7.8	#	14M	8.0	#	4.8M
7	7.7	#	64M	7.8	#	44M	8.0	#	140T
10	7.8	#	42M	7.9	#	34M	8.0	#	3M
24	7.9	32.57	31M	8.0	32.57	25M	7.9	32.71	14M

* S.T. signifies surface tension in dynes per centimeter.

** B.C. signifies bacterial count per cc.

▲ M signifies millions.

✠ T signifies thousands.

\# No measurement was made.

a curtailment of the normal shift in pH, although to a much lesser extent than that occurring with either anionic or cationic agents.

The role of pH exerts some influence on the activities of these compounds. Emulphor ON was most active against S. aureus at pH 8 and against E. coli and S. paratyphi B at pH 7. On the other hand, Triton X-100 was most effective against S. aureus at pH 7, and against E. coli and S. paratyphi B at pH 8.

Although the data presented in Tables 11 and 12 are by no means conclusive, it is interesting to note the complete reversal of the inhibitory powers of the two agents against gram-positive and gram-negative organisms at pH 7 and pH 8. Functioning at their optimal pH ranges, both compounds are capable of reducing the S. aureus population by better than 97 percent for 4 hours or less. No attempt was made to establish whether this activity is bacteriostatic or bactericidal.

Information concerning commercial uses, methods of preparation, etc., of non-ionic compounds can be found in the papers by Goldsmith (26)(27).

E. Bacteriostatic Tests

Desired dilutions of surface-active agent were prepared in test tubes in 5-cc. quantities. To each tube, 0.1 cc. of a 24-hour organism suspension was added. Tubes were well shaken and then incubated for 24 hours at 37 degrees C. After 24 hours, subtransfers (2 loopfuls) were made into

F.D.A. broth, and both sets of test tubes were incubated for 24 hours. Each 24 hours thereafter, for 2 additional days, subtransfers from the original set of tubes were made into F.D.A. broth. Positive growth was recorded on the basis of visual turbidity. This method is a modification of that suggested by Kolmer (37).

Since the quaternary ammonium compounds, as a group, are considered more active bacteriostatic agents than the anionic compounds, bacteriostatic titres on the former agents were determined at the three pH ranges. The results presented in Tables 13 and 14 are in agreement with the accepted generalization concerning relative bacteriostatic powers. The acid range only was used for the anionic agents, since they are generally most active against bacteria at this pH level.

In the bacteriostatic tests, the number of organisms initially introduced into the test tubes was almost identical with the number initially inoculated in both the chemical-range tests and the growth-curve tests. Such control of the quantity of organisms originally introduced more clearly defines the conditions of these tests and makes possible a more exact duplication of results with all three methods of testing. The agreement between the results of the chemical-range tests and the 24-hour readings of the "subtransfer" tubes in the bacteriostatic tests* is very good in spite of the fact that the tests were made 1 to 6 months apart.

* It should be noted that the chemical-range tests and the 24-hour "subtransfer" determinations in the bacteriostatic series are identical tests.

TABLE 13

DATA FROM BACTERIOSTATIC TESTS AT pH 5.2

Duponol OS

Original

	Hrs.	1-10	1-20	1-1T	1-3T	1-4T	1-5T	1-6T	1-7T	1-8T	1-9T	1-10T	1-20T
E. coli	24		Turbid										
	48												
	72												
S. paratyphi B	24												
	48												
	72												
S. aureus	24			Flocculation									
	48												
	72												

Subtransfer

	Hrs.	1-10	1-20	1-1T	1-3T	1-4T	1-5T	1-6T	1-7T	1-8T	1-10T	1-20T
E. coli	24	+	+									
	48	+	+									
	72	+	+									
S. paratyphi B	24	+	+									
	48	+	+									
	72	+	+									
S. aureus	24			-	-	-	+	+	+	+	+	+
	48			-	-	-	+	+	+	+	+	+
	72			-	-	-	-	-	-	-	+	+

Igepon AP

Original

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80	1-90	1-100	1-200	1-300
E. coli	24		+	+	+	+	+	+	+	+	+	+	+
	48		+	+	+	+	+	+	+	+	+	+	+
	72		+	+	+	+	+	+	+	+	+	+	+
S. paratyphi B	24		+	+	+	+	+	+	+	+	+	+	+
	48		+	+	+	+	+	+	+	+	+	+	+
	72		+	+	+	+	+	+	+	+	+	+	+
S. aureus	24		++	++	++	+	+	+	+	+	+	+	+
	48		++	++	++	+	+	+	+	+	+	+	+
	72		++	++	++	+	+	+	+	+	+	+	+

Turbid

Subtransfer

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-70	1-80	1-90	1-100	1-200	1-300
E. coli	24												
	48				All Tubes Positive								
	72												
S. paratyphi B	24												
	48				All Tubes Positive								
	72												
S. aureus	24												
	48				All Tubes Positive								
	72												

Tergitol 7

Original

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-10T	1-20T	1-30T	1-40T	1-50T	1-60T
E. coli	24												
	48				Turbid								
	72												
S. paratyphi B	24												
	48				Turbid								
	72												
S. aureus	24											+	+
	48							Granular				+	+
	72											+	+

Subtransfer

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-10T	1-20T	1-30T	1-40T	1-50T	1-60T
E. coli	24	+	+	+	+	+	+						
	48	+	+	+	+	+	+						
	72	+	+	+	+	+	+						
S. paratyphi B	24	+	+	+	+	+	+						
	48	+	+	+	+	+	+						
	72	+	+	+	+	+	+						
S. aureus	24							-	-	-	+	+	+
	48							-	-	-	+	+	+
	72							-	-	-	+	+	+

Nacconol NRSF

Original

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-1T	1-5T	1-10T	1-20T	1-30T	1-40T
E. coli	24	+	+	+	+	+	+						
	48	+	+	+	+	+	+						
	72	+	+	+	+	+	+						
S. paratyphi B	24	+	+	+	+	+	+						
	48	+	+	+	+	+	+						
	72	+	+	+	+	+	+						
S. aureus	24							-	-	-	-	+	+
	48							-	-	-	-	+	+
	72							-	-	-	-	+	+

Subtransfer

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-1T	1-5T	1-10T	1-20T	1-30T	1-40T
E. coli	24	+	+	+	+	+	+						
	48	+	+	+	+	+	+						
	72	+	+	+	+	+	+						
S. paratyphi B	24	+	+	+	+	+	+						
	48	+	+	+	+	+	+						
	72	+	+	+	+	+	+						
S. aureus	24							-	-	-	-	+	+
	48							-	-	-	-	+	+
	72							-	-	-	-	-	+

Nekal BX

Original

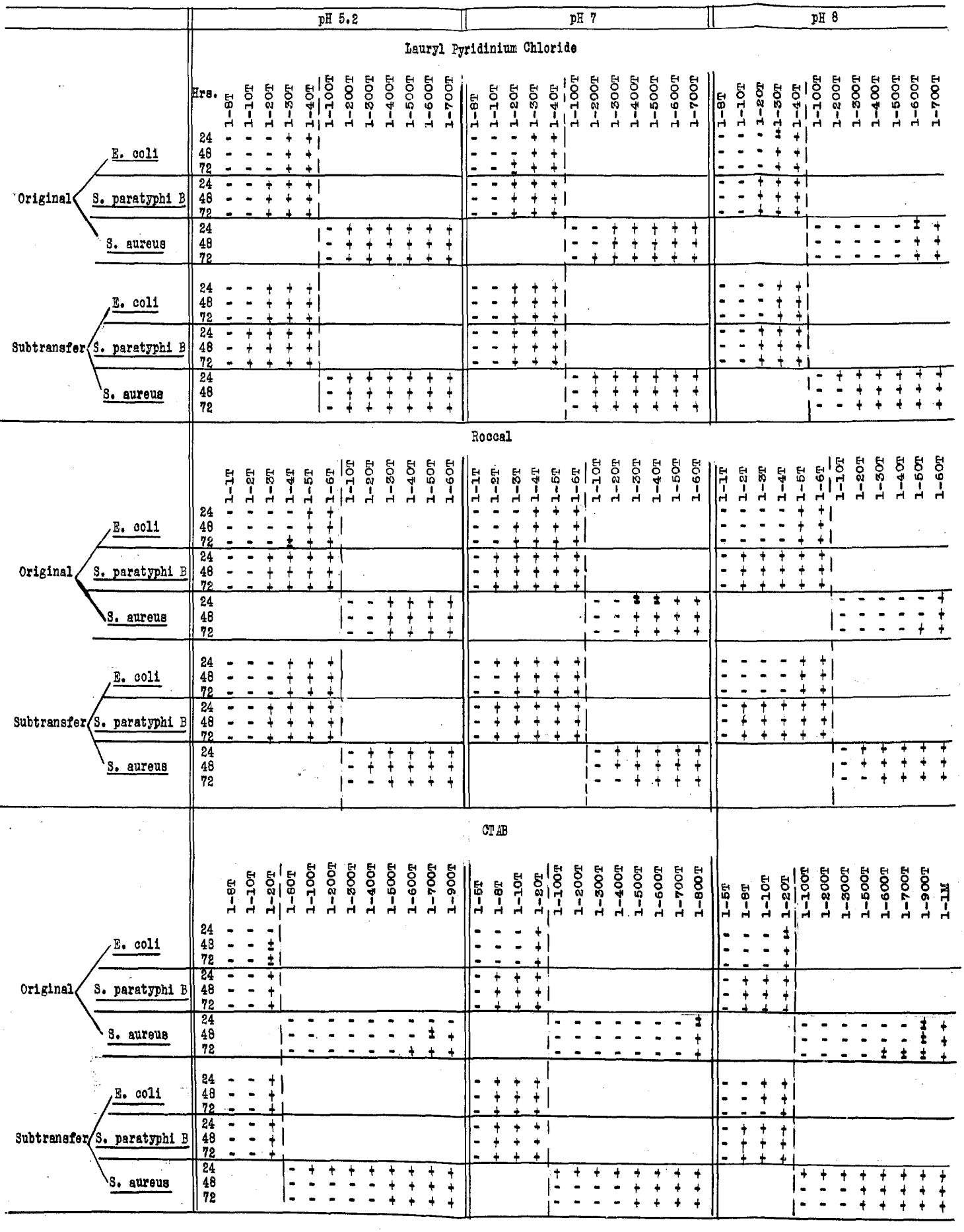
	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-1T	1-5T	1-8T	1-10T	1-20T	1-30T
E. coli	24	-	-	-	+	+	+						
	48	-	-	-	+	+	+						
	72	-	-	-	+	+	+						
S. paratyphi B	24	-	-	+	+	+	+						
	48	-	-	+	+	+	+						
	72	-	-	+	+	+	+						
S. aureus	24							-	-	-	-	-	+
	48							-	-	-	-	-	+
	72							-	-	-	-	-	+

Subtransfer

	Hrs.	1-10	1-20	1-30	1-40	1-50	1-60	1-1T	1-5T	1-8T	1-10T	1-20T	1-30T
E. coli	24	-	-	-	+	+	+						
	48	-	-	-	+	+	+						
	72	-	-	-	+	+	+						
S. paratyphi B	24	-	-	+	+	+	+						
	48	-	-	+	+	+	+						
	72	-	-	+	+	+	+						
S. aureus	24							-	-	-	+	+	+
	48							-	-	-	-	+	+
	72							-	-	-	-	-	+

TABLE 14

DATA FROM BACTERIOSTATIC TESTS



No bacteriostatic effects were observed in preliminary tests with the non-ionic agents.

Under the limitations imposed by the turbid and granular appearance in solution of some of the anionic agents, as well as by the nature of the test employed, it is impossible to make any absolute statement concerning the general extent of bacteriostatic activity of these anionic compounds. The results with Nekal BX, Nacconol NRSF, and Igepon AP indicate that no static effect was apparent against the gram-negative organisms. However, the results in Table 5 indicate that Tergitol 7 might possibly have exerted a bacteriostatic effect on E. coli. The strongest evidence of bacteriostasis was displayed by Nekal BX against S. aureus.

All the cationic agents (see Table 14) exhibit bacteriostasis against S. aureus, the most marked effect always occurring at pH 8. These compounds similarly affect E. coli, although no generalization concerning the pH range can be made. With the exception of LPC at pH 5.2, no cationic agent displayed bacteriostatic activity against S. paratyphi B.

Hoogerheide (31), in his experiments with CTAB in the presence of serum, observed that "A concentration of two to five times the critical bacteriostatic dilution was found to be bactericidal." This observation is substantiated (see Table 14) by results with S. aureus alone when exposed to all three cationic agents at pH 8 and with Nekal BX at pH 5.2 (the only pH range at which Nekal BX was subjected to bacteriostatic tests).

A comparison of the growth results in the "subtransfer" tubes with the 24-hour counts in the growth curves (Tables 3 to 10) indicates no steadfast correlation. In those instances where it was possible to observe growth in the "original" anionic tubes, the relationship is not clear-cut. The cationic agents are likewise variable.* In every instance with CTAB, the results from the "original" tubes more closely paralleled the results in the growth curves than did results from the "subtransfer" tubes. This was also the case for E. coli exposed to Roccal and LPC. On the other hand, for S. aureus reacting with Roccal and LPC, results from "subtransfer" tubes paralleled growth-curve results more closely than did results from "original" tubes.

Perhaps this apparent lack of positive correlation can partially be attributed to the lack of agreement among various authors as to what constitutes bacteriostasis. The term "bacteriostasis" was first introduced to indicate that the action of certain dyes on bacteria was more inhibitory than germicidal. Since that time, an attempt has been made to arrive at a more specific definition. This was necessary because, in studying the effect of chemicals on bacteria, no specific line of demarcation could be drawn between the inhibition of bacterial multiplication and germicidal activity (49).

Hoffmann and Rahn (29) stated that bacteriostasis is manifested by a long lag period, followed by a normal

* Since S. paratyphi B was used mainly for purposes of comparison with E. coli, the former organism does not enter into this particular discussion.

rate of multiplication. However, extended stasis leads to death. Roberts and Rahn (53) point out that retardation of bacterial growth may have little or no effect on enzyme activity. This observation is in agreement with the report of Sevag and Ross (55). Ely (18), attacking the matter from the opposite direction, showed that inhibition of respiration had no effect on bacterial numbers.

Present studies on quaternary ammonium compounds employ methods comparable to the Shippen modification of the F.D.A. Phenol-Coefficient Method. In this method, as well as in the method outlined by Kolmer, no visible growth in the original tube followed by visible growth in a sub-transfer tube indicates that the chemical agent exerted bacteriostatic rather than bactericidal action. The assumption is that by sufficiently diluting out the chemical agent, the bacteriostatic effect can be mitigated. Another method utilizes original tubes only. If these tubes fail to show growth in 1 or 2 days, but do show visible growth in 3, 4, or 5 days, the term bacteriostasis is applied. Actually, the definition by Hoffmann and Rahn appears to be most practical at present.

F. Surface Tension and pH Controls

The dilutions of synthetic agents selected for this study represented the concentrations which first permitted growth of *E. coli* and *S. aureus* respectively at the pH levels investigated in the growth-curve studies.

Erlenmeyer flasks containing the desired concentrations of surface-active agent were prepared in the same manner as that described under the section entitled "Growth Curves and Related Phenomena." However, only 1 hour elapsed between the time the surface-active agents were added to the broth in the flasks and the solution adjusted to the desired pH, and the time the "0-hour" readings were taken.

All flasks were incubated at 37 degrees C. Prior to taking surface-tension measurements* with the du Nouy Tensionometer, aliquot portions of the synthetic agent solution were transferred into test tubes immersed in a water bath at 37 degrees C. The measurements were made at 37 degrees C., since that was the temperature at which the organisms were grown in all the previous tests performed. Readings were taken approximately 10 seconds after the solution was placed in the standard watch glass. The remainder of the aliquot portion was employed for pH determinations in a Beckman pH meter.

The results of the surface-tension and pH control tests are presented in Tables 15, 16, and 17.

* The formula for surface-tension calibration was as follows:

$$\gamma = \frac{\frac{Mg}{r} \times R}{2L}$$

where γ is the surface tension in dynes per centimeter,
M is the total weight in grams added to the ring,
g is the acceleration of gravity,
r is the reading on the scale of the above weights,
R is the reading on the scale of the unknown solution,
and L is the mean circumference of the ring.

TABLE 15

MEASUREMENTS OF SURFACE TENSION* AND pH WITH CONTROL BROTH

Surface Tension in dynes per centimeter				pH			
Hrs.	pH 7	pH 5.2	pH 8	Hrs.	pH 7	pH 5.2	pH 8
0	56.04	54.45	56.27	0	7.05	5.25	8.05
4	56.04	54.45	56.27	4	7.1	5.3	8.1
7	56.04	54.45	56.27	7	7.1	5.3	8.1
10	56.04	54.45	56.27	10	7.1	5.3	8.1
24	56.04	54.45	56.27	24	7.1	5.3	8.1
30	56.04	54.45	56.27	30	7.1	5.3	8.1
48	56.04	54.45	56.27	48	7.1	5.3	8.1

* The surface-tension reading of singly distilled water (pH 6.5) at 37 degrees C. was 70.34 dynes per centimeter.

The purpose of the surface-tension and pH control tests was to observe any possible relationship between the stability of the solutions of synthetic agents and their effects on bacterial growth as noted in the growth-curve experiments.

The results in Tables 16 and 17 indicate that the time required for a solution of a surface-active agent to reach equilibrium is proportional to the concentration of the agent, i.e., the time required increases as the concentration decreases. This is in agreement with the findings concerning surface tension by Adam and Shute (1). The anionic agents (in concentrations greater than 1-700) and the non-ionic agents reached equilibrium immediately, whereas

TABLE 16

DATA SHEET ON SURFACE-TENSION AND pH CONTROLS FOR ANIONIC COMPOUNDS

SURFACE-TENSION CONTROLS

Surface tension was measured in dynes per centimeter at 37 degrees C.

Hrs.	Duponol OS						Igepon AP					Tergitol 7						Nacconol NRSF						Nekal BX					
	1-50 pH 7	1-700 pH 7	1-50 pH 5.2	1-8T pH 5.2	1-50 pH 8	1-60 pH 8	1-50 pH 7	1-100 pH 7	1-50 pH 5.2	1-60 pH 5.2	1-50 pH 8	1-50 pH 7	1-30T pH 7	1-50 pH 5.2	1-40T pH 5.2	1-50 pH 8	1-20T pH 8	1-50 pH 7	1-40T pH 7	1-50 pH 5.2	1-30T pH 5.2	1-50 pH 8	1-20T pH 8	1-50 pH 7	1-30T pH 7	1-50 pH 5.2	1-20T pH 5.2	1-50 pH 8	1-10T pH 8
0	28.95	28.56	28.56	30.82	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.85	27.36	37.30	27.23	35.48	31.75	38.04	31.75	35.91	31.89	35.54	31.52	44.62	31.52	42.09	31.62	40.66
4	28.95	28.56	28.56	30.82	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.85	27.36	36.75	27.23	35.26	31.75	38.04	31.75	35.41	31.89	35.23	31.52	45.54	31.52	42.64	31.62	40.21
7	28.95	28.56	28.56	30.13	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.85	27.36	36.57	27.23	35.17	31.75	39.33	31.75	35.41	31.89	35.18	31.52	45.66	31.52	42.98	31.62	40.21
10	28.95	28.56	28.56	30.13	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.71	27.36	35.39	27.23	35.03	31.75	39.01	31.75	35.23	31.89	35.04	31.52	45.77	31.52	43.17	31.62	41.12
24	28.95	28.56	28.56	30.13	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.71	27.36	37.30	27.23	35.03	31.75	38.73	31.75	35.23	31.89	35.04	31.52	46.0	31.52	42.92	31.62	41.12
30	28.95	28.56	28.56	30.13	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.71	27.36	35.62	27.23	35.03	31.75	38.56	31.75	35.18	31.89	35.04	31.52	46.0	31.52	42.68	31.62	41.12
48	28.95	28.56	28.56	30.13	29.04	29.62	29.31	29.72	29.04	29.44	29.72	27.32	35.71	27.36	37.30	27.23	35.03	31.75	38.41	31.75	35.18	31.89	35.04	31.52	45.04	31.52	42.23	31.62	40.66

pH CONTROLS

Hrs.	Duponol OS						Igepon AP					Tergitol 7						Nacconol NRSF						Nekal BX					
	1-50 pH 7	1-700 pH 7	1-50 pH 5.2	1-8T pH 5.2	1-50 pH 8	1-60 pH 8	1-50 pH 7	1-100 pH 7	1-50 pH 5.2	1-60 pH 5.2	1-50 pH 8	1-50 pH 7	1-30T pH 7	1-50 pH 5.2	1-40T pH 5.2	1-50 pH 8	1-20T pH 8	1-50 pH 7	1-40T pH 7	1-50 pH 5.2	1-30T pH 5.2	1-50 pH 8	1-20T pH 8	1-50 pH 7	1-30T pH 7	1-50 pH 5.2	1-20T pH 5.2	1-50 pH 8	1-10T pH 8
0	7.05	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.2	8.0	7.05	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.25	5.2	8.0	8.0
4	7.1	7.0	5.25	5.2	8.0	8.0	7.05	7.0	5.25	5.25	8.0	7.1	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.25	8.25	8.2	7.05	7.05	5.3	5.2	8.0	8.0
7	7.1	7.0	5.25	5.2	8.0	8.0	7.05	7.0	5.25	5.25	8.0	7.1	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.25	8.25	8.2	7.05	7.05	5.3	5.2	8.0	8.0
10	7.1	7.0	5.25	5.2	8.0	8.0	7.05	7.0	5.25	5.25	8.0	7.1	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.25	8.2	8.1	7.05	7.05	5.3	5.2	8.0	8.0
24	7.1	7.0	5.25	5.2	8.0	8.0	7.05	7.0	5.25	5.25	8.0	7.1	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.25	8.15	8.1	7.05	7.05	5.3	5.2	8.0	8.0
30	7.1	7.0	5.25	5.2	8.0	8.0	7.05	7.0	5.25	5.25	8.0	7.1	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.25	8.15	8.1	7.05	7.05	5.3	5.2	8.0	8.0
48	7.1	7.0	5.25	5.2	8.0	7.95	7.05	7.0	5.25	5.25	8.0	7.1	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.25	8.1	8.1	7.05	7.05	5.3	5.2	8.0	8.0

TABLE 17

DATA SHEET ON SURFACE-TENSION AND pH CONTROLS FOR CATIONIC AND NON-IONIC COMPOUNDS

SURFACE-TENSION CONTROLS

Hrs.	Roccal						LPC						CTAB						Emulphor ON				Triton X-100			
	1-4T pH 7	1-50T pH 7	1-5T pH 5.2	1-30T pH 5.2	1-6T pH 8	1-70T pH 8	1-30T pH 7	1-800T pH 7	1-30T pH 5.2	1-200T pH 5.2	1-40T pH 8	1-300T pH 8	1-20T pH 7	1-1M pH 7	1-30T pH 5.2	1-900T pH 5.2	1-30T pH 8	1-1M pH 8	1-20 pH 7	1-50 pH 7	1-20 pH 5.2	1-20 pH 8	1-20 pH 7	1-50 pH 7	1-20 pH 5.2	1-20 pH 8
0	45.84	54.90	46.89	53.66	47.76	57.19	44.17	56.56	44.01	52.64	47.33	55.58	37.97	57.69	37.84	53.01	37.97	56.35	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21
4	44.17	54.22	46.02	53.34	47.49	56.04	45.38	57.17	44.88	51.23	46.51	53.30	37.47	57.79	38.20	54.28	37.61	57.13	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21
7	43.92	54.81	46.25	53.62	47.81	56.50	44.74	56.58	43.74	52.90	46.65	54.08	37.42	56.87	36.69	53.91	37.52	56.35	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21
10	44.61	55.36	46.02	52.84	47.12	55.91	44.88	56.49	42.74	50.73	46.96	52.84	37.74	55.31	38.29	54.31	37.84	56.29	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21
24	44.61	53.07	45.84	52.02	46.44	56.96	42.87	54.81	42.38	52.38	47.34	52.38	37.38	54.31	37.52	55.20	37.52	55.31	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21
30	44.61	54.36	45.84	52.84	45.66	56.46	43.81	57.16	43.72	51.15	45.47	54.67	37.39	57.65	37.52	54.31	37.52	56.87	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21
48	44.61	54.36	45.84	52.84	45.66	55.69	42.66	58.39	44.49	53.39	45.32	55.83	37.38	56.29	37.52	52.44	37.52	55.66	38.87	38.87	38.87	38.87	32.21	32.21	32.21	32.21

pH CONTROLS

Hrs.	Roccal						LPC						CTAB						Emulphor ON				Triton X-100			
	1-4T pH 7	1-50T pH 7	1-5T pH 5.2	1-30T pH 5.2	1-6T pH 8	1-70T pH 8	1-30T pH 7	1-800T pH 7	1-30T pH 5.2	1-200T pH 5.2	1-40T pH 8	1-300T pH 8	1-20T pH 7	1-1M pH 7	1-30T pH 5.2	1-900T pH 5.2	1-30T pH 8	1-1M pH 8	1-20 pH 7	1-50 pH 7	1-20 pH 5.2	1-20 pH 8	1-20 pH 7	1-50 pH 7	1-20 pH 5.2	1-20 pH 8
0	7.0	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	5.2	8.0	8.0	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.0
4	7.1	7.1	5.3	5.3	8.2	8.1	7.1	7.1	5.15	5.2	8.05	8.1	7.0	7.1	5.2	5.2	8.1	8.0	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.1
7	7.1	7.1	5.3	5.2	8.2	8.1	7.1	7.1	5.15	5.2	8.05	8.1	7.0	7.1	5.2	5.2	8.1	8.0	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.1
10	7.1	7.1	5.3	5.2	8.2	8.1	7.1	7.1	5.15	5.2	8.05	8.0	7.0	7.1	5.2	5.2	8.0	8.0	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.1
24	7.1	7.1	5.3	5.2	8.2	8.1	7.1	7.1	5.15	5.2	8.05	8.0	7.0	7.1	5.2	5.2	8.0	8.0	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.1
30	7.1	7.1	5.3	5.2	8.2	8.1	7.1	7.1	5.15	5.2	8.05	8.0	7.0	7.1	5.2	5.2	8.0	8.0	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.1
48	7.1	7.1	5.3	5.2	8.1	8.0	7.0	7.0	5.15	5.2	7.95	7.95	7.0	7.0	5.2	5.2	7.9	7.9	7.0	7.0	5.2	8.0	7.0	7.0	5.2	8.1

those anionic compounds in concentrations of 1-10T or less reached equilibrium in from 10 hours to beyond the limit of the test period.

Roccal (Table 17) serves as a good example of the relationship between concentration of synthetic agent and rapidity of reaching equilibrium. At a concentration of 1-4T, equilibrium was reached in 10 hours; at 1-5T, equilibrium was attained in 24 hours; whereas at 1-6T, equilibrium was reached in 30 hours. It is interesting that at a concentration of 1-50T equilibrium was also achieved in 30 hours. However, at a 1-70T dilution no equilibrium was attained in 48 hours. An explanation for this last-mentioned phenomenon might possibly be found in the paper by Lundgren (44). He reports that in highly dilute solutions, long-chain hydrocarbon salts behave as single ions, whereas in more concentrated solutions these salts form micelles with colloidal dimensions. He arrived at these conclusions by taking measurements of conductivity and osmotic coefficients. Gonick and McBain (25) point out critical concentrations for micelle formation with non-ionic agents. Adam and Shute (1) likewise refer to critical concentrations. They indicate that above a critical concentration of 0.001 N for a 16-C and 0.01 N for a 12-C hydrocarbon chain, ionic micelles begin to form. They also add that equilibrium of surface tension is reached almost immediately at these concentrations. In other words, it is quite possible that between the Roccal concentrations of 1-50T and 1-70T, the alkyl dimethyl benzyl ammonium chloride molecules may be dissociated into simple

ions, thereby destroying the stability of the solution.

CTAB, at concentrations of 1-20T and 1-30T, attained equilibrium in 24 hours, whereas the lower concentrations of CTAB and all the concentrations of LPC tested did not succeed in reaching equilibrium during the 48-hour test period. Under the conditions of these control tests it is not possible to offer an explanation for the behavior of LPC. However, if Adam and Shute are correct in their contention concerning the critical concentration for a 12-C hydrocarbon chain, then it is quite apparent that the concentrations of LPC used in these tests were far below the 0.01 N range, and consequently the solution was in an unstable condition. The writer has not seen any literature on the relationship between the pyridine structure in a synthetic surface-active agent and the surface-tension stability.

On the basis of the surface-tension and pH control tests, the pH of the medium seemed to have little effect on the rapidity with which surface-tension equilibrium was attained. With the exception of Nacconol NRSF at pH 8, all the surface-active agents presented uniform stability in the pH control tests. There was no shift in pH to compare with the shifts taking place in surface tension. Moreover, CTAB solutions (1-30T) at both pH 5.2 and pH 8 achieved surface-tension equilibrium within 24 hours. This indicated that, within the pH ranges tested, the pH exerted little influence on the attainment of surface-tension equilibrium. Admittedly, however, the pH ranges tested were too narrow to justify making a broad generalization.

As for the relationship between surface-tension stability and bacterial growth, the only generalization that can be made, on the basis of the results in Tables 16 and 17, is that a stable solution of a synthetic surface-active agent is not necessary for germicidal activity against S. aureus. This statement in no way contradicts the findings of some authors (62)(33) that the undissociated molecules of the surface-active agent (concentrated solutions) are more germicidal than the ionized molecules (dilute solutions). However, it would be desirable to ascertain the possible potency of the dissociated hydrophobic portion of the molecule. Quisno and Foter (50) reported that Ceepryn (cetyl pyridinium chloride) in a 1-2T concentration was as effective a germicide at pH 3 as it was at pH 8. Since Ceepryn would most likely be dissociated at pH 3, it is quite possible that, with the proper concentration of the synthetic agent, the dissociated cation might also be germicidal.

VI. GENERAL DISCUSSION

A thorough review of the literature pertaining to the bactericidal or bacteriostatic effectiveness of synthetic surface-active agents indicates that the majority of researchers used a modification of the Phenol-Coefficient Method (54) in their studies. This technic was used mainly as a means of ascertaining the potential quick-killing power of the various compounds under test, and to conform with previous presentation of data in the literature. In 1943, Anderson and Mallmann (2) pointed out that the F.D.A. Method (Phenol-Coefficient Method) offers a fair means of comparing phenolic preparations but is not applicable when other types of germicidal preparations are used. Reddish (52) reaffirmed this limitation, and strongly warned against use of the Phenol-Coefficient Method in testing quaternary ammonium compounds. Hotchkiss (32), Klarmann and Wright (35), and DuBois and Dibblee (14) likewise point out discrepancies arising from use of this method.

It was not surprising to see a series of papers recently published dealing with suggestions for modifying the overworked F.D.A. technic. The purpose of all these papers was to arrive at a more correct evaluation of the germicidal effectiveness of the quaternary compounds under practical conditions. Reddish suggested the Use-Dilution Method (46) as the most promising at present. Quisno et al (51) and Armbruster and Ridenour (4) have proposed methods for avoiding misinterpretations due to bacteriostasis.

However, in all these methods, the short period of exposure limits a clear understanding of the gradual effects that are exerted by the synthetic agents on bacteria. With the exception of the Use-Dilution technic, all the modifications of the F.D.A. Method merely describe the all-or-none result, i.e., complete kill or survival. Even those papers dealing with the relationship of surface-active agents to metabolism of bacteria (18)(6) have likewise employed methods involving an exposure period of only 1 or 2 hours.

Another limiting factor in the evaluation of these compounds is the arbitrary choice of concentrations at which to observe the effects on growth or metabolism of bacteria. In many cases, such choices represent the extreme ranges of activity of the synthetic compound, i.e., complete kill or complete ineffectiveness, leaving unreported the potential activities of a whole series of dilutions between these extremes. One of the main purposes of the study undertaken here was to fill in such gaps and at the same time to define as many of the test conditions as possible, i.e., time, pH, surface tension, concentration of synthetic agent, and initial numbers of organisms.

The one point on which most of the investigators in this field agree is the importance of pH. It is now known that the mere reporting of a killing concentration of a particular synthetic compound is not sufficient. Whereas it is generally accepted that anionic agents are most effective against bacteria in an acid range and the quaternary ammonium compounds in an alkaline range, the latter compounds

do not always follow such a simple rule. These compounds are definitely most germicidal against S. aureus in the alkaline range; however, certain of the more concentrated solutions of Roccal and CTAB exert nearly as effective a bactericidal and bacteriostatic action on the gram-negative organisms at pH 5.2 as they do at pH 8. Lauryl pyridinium chloride follows the general rule of being more active in an alkaline range for all three organisms. As for the non-ionic compounds, the optimal pH levels varied with the gram nature of the organism, and with the compounds themselves (see section entitled "Non-Ionic Compounds").

The important papers by Gershenfeld et al (22) (23) (24) have definitely tied maximum bactericidal efficiency of synthetic surface-active agents to the optimum pH at which they are employed. An historical review of the relationship of pH to germicidal activity is presented in the last-mentioned paper by Gershenfeld. As to the explanation for the effect of pH on these germicidal agents, the literature offers many theories. Stearn and Stearn (58) suggest that, at optimum pH levels for germicidal action, the bacterial membrane or protoplasm is so altered as to make the bacteria more susceptible to the action of the chemical. Hartley (28) demonstrated the formation of micelles as a result of hydrogen-ion concentration. Baker, Harrison, and Miller (6) suggested that pH activation (for maximum germicidal effect) was in the direction which favored formation of undissociated molecules possessing greater ability to penetrate into cells. Gershenfeld and Milanick (24) present the hypothesis that the pH

effect may be responsible for alterations of the solubility of the reducing agents in solution. Unfortunately, very little is known of the relationship between the change in pH of the living bacterial protoplasm and the pH changes taking place in the medium.

The importance of the influence of dissociated and undissociated molecules of surface-active agents upon bacterial growth and inhibition has recently occupied the attention of a large number of workers in the field of synthetic surface-active compounds. It is now fairly well established that the undissociated molecule not only has greater penetrating ability (48), but that it also accounts for the germicidal nature of concentrated solutions. This theory is further strengthened by the fact that dissociated molecules in dilute solutions exhibit less effective bactericidal and bacteriostatic action. Physical-chemical measurements on solutions containing varying concentrations of synthetic surface-active agents have led to the acceptance of a critical concentration for micellar formation. It has likewise been established that above this critical concentration lie the most germicidal levels of the synthetic compounds. However, the report by Quisno and Foter (50) and the results of Roccal and CTAB against the gram-negative organisms, presented in Tables 8 and 10, indicate the necessity for a more comprehensive knowledge of the bactericidal or bacteriostatic role of the dissociated molecules in acid ranges.

Many attempts have been made to correlate chemical structure of synthetic compounds to bactericidal efficiency. The most successful results have been attained within an homologous series of a particular synthetic agent. The important factor in such studies has been the length of the hydrophobic carbon chain. Hoogerheide (31) and Shelton et al (56), working with quaternary ammonium salts of the general type $R-(CH_3)_2-N-Br$ found that as the straight-chain alkyl group (R) was increased from C_6 to C_{18}, germicidal activity likewise increased, with the maximum potency at C_{16}. In a series of alkyl pyridinium chlorides, the same germicidal relationship existed with the increase in the straight-chain alkyl group from C_8 to C_{18} (57)(36). Similar results were obtained from studies with dialkyl methylbenzyl ammonium chlorides (41). The work with anionic agents likewise substantiated the correlation between carbon-chain length and bactericidal efficiency (10)(59). However, the attempts to correlate the structures of different types of surface-active agents to bactericidal powers were not entirely successful. Hotchkiss (32) summarizes these efforts as follows:

"No unique active chemical groups have been recognized. Indeed, two active substances may bear dissimilar groups, and yet other substances bearing these groups may be relatively inactive. Nevertheless, within a homologous series, there is a tendency for an optimally bactericidal substance to exist..."

However, the literature is in agreement on the bactericidal nature of certain synthetic compounds. Baker et al (7) and Gershenfeld (24) report that Tergitol 7 and Tergitol 4, respectively, were the most active germicides of a series of anions tested. Domagk (13) and Baker et al (7) pointed out the bactericidal efficiency of the alkyl-dimethyl-benzyl ammonium chloride structure. The results in the growth-curve tables substantiate the findings mentioned above. Baker et al (7) suggest that the effectiveness of Tergitol 7 can be explained on the basis that it is derived from a branched-chain, secondary alcohol, whereas most of the other anionic compounds are derived from straight-chain, primary alcohols. They also suggest that the effectiveness of the alkyl dimethyl benzyl ammonium chloride compounds is based on their possession of a benzyl group attached to the quaternary nitrogen. It should be noted that in the concentrations employed in this study, Nekal BX was the only anionic agent which was bactericidal for all three types of organisms. A possible explanation as to why the bactericidal activity of this particular compound has been given very little attention in the literature is that only recently has it been manufactured in a very high concentration. However, on the basis of percentage of active ingredient, Tergitol 7 was far more efficient against E. coli and S. aureus than was Nekal BX.

The attempts to correlate curtailment of bacterial metabolism with germicidal activity have likewise failed to produce exact relationships. Contrary to the conclusions

drawn by Baker et al (6) concerning the positive relationship between respiratory and glycolytic inhibition and germicidal activity, a number of papers (55)(53)(18) have indicated the lack of correlation between these phenomena. Data were reported by Ely (18) showing as much as 50 percent inhibition of respiration, with no diminution of bacterial numbers. Only at complete cessation of respiration are all bacteria killed.

Although this writer has not found any steadfast relationship between the shift in pH and bacterial growth at similar time intervals, as evidenced in Tables 3 to 10, there still exist sufficient examples of deviations from the normal pH trend to merit further consideration.

It is conceivable that, in a medium which is fairly well defined, a control curve of pH readings, similar to acidity curves in microbiological assays, can serve as a partial indication of bacterial metabolism. With the medium used in this study, it became apparent that the normal tendencies of the three bacterial types were to approach neutrality when initially grown at pH 5.2, to remain fairly stable and then rise gradually toward alkalinity when grown in the medium at pH 7, and to drop toward neutrality only to rise again in the alkaline direction when subjected to the medium at pH 8. These shifts in pH might gain in importance if tests had been conducted to determine the extent of bacterial breakdown of the peptone under normal conditions and after exposure to synthetic surface-active agents. It

is quite likely that in the near future such concurrent experiments will be made in order to explore more thoroughly the differences between bacteriostatic and bactericidal action, as well as the significance of such related phenomena as long lag periods and toxic effects.

VII. SUMMARY

A systematic study was made of the effects of certain anionic agents (Duponol OS, Igepon AP, Tergitol 7, Nacconol NRSF, Nekal BX), cationic agents (Roccal, CTAB, LPC), and non-ionic agents (Emulphor ON, Triton X-100) on the growth of E. coli, S. paratyphi B, and S. aureus.

An attempt was made to establish the relationships of pH, surface tension, bacteriostasis, and concentration of synthetic agent, to bacterial growth.

Growth-curve studies over periods of 0, 4, 7, 10, and 24 hours showed the following:

1. Concentrations of 1-50 of Nekal BX and Tergitol 7 completely killed E. coli in 24 hours. Nekal BX reduced the count of S. paratyphi B by more than 99 percent in the same period. This germicidal activity of anionic agents against fairly resistant gram-negative organisms merits additional investigation. There is very little literature dealing with this relationship, and that which does exist is inconclusive.

2. The quaternary ammonium compounds displayed germicidal activities against all three types of organisms in accordance with the accepted generalizations appearing in the literature. These generalizations deal with optimum bactericidal activity at alkaline ranges, greater killing power against gram-positive organisms, and germicidal efficiency at low concentrations. However, it was noted that, in certain instances, the germicidal activities of

Roccal and CTAB against the gram-negative organisms were as marked at pH 5.2 as at pH 8.

3. The concentrations of all surface-active agents used in these studies arrested growth of the three bacterial cultures when compared with normal growth counts.

4. Determinations of pH made concurrently with growth-curve platings showed arrested deviations from the normal trend in pH shift. These determinations might serve as an adjunct to the indication of interference with the normal metabolic activities of the organism.

Test-tube bacteriostatic tests with the anionic agents indicated no absolute proof of stasis against the gram-negative organisms, although bacteriostasis was noted against S. aureus. Turbid and particulate nature of some of these solutions prevented exact conclusions. The three cationic agents exerted static effects against E. coli and S. aureus, whereas only LPC at pH 5.2 exerted bacteriostasis against S. paratyphi B.

Surface-tension controls, with the majority of the compounds, substantiated the general theory that rapidity of attaining equilibrium is a function of concentration. Solutions of LPC did not attain equilibrium in any of the concentrations tested within 48 hours.

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