

APPROVAL SHEET

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A Study of Quaternary Ammonium Compounds as
Disinfectants, With Special Reference to
Influence of Synthetic Detergents and Other
Factors.

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A STUDY OF QUATERNARY AMMONIUM COMPOUNDS AS
DISINFECTANTS, WITH SPECIAL REFERENCE TO
INFLUENCE OF SYNTHETIC DETERGENTS
AND OTHER FACTORS

By

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Thesis submitted to the Faculty of the Graduate School
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A STUDY OF QUATERNARY AMMONIUM COMPOUNDS AS
DISINFECTANTS, WITH SPECIAL REFERENCE TO
INFLUENCE OF SYNTHETIC DETERGENTS
AND OTHER FACTORS

One of the many problems which the Army has encountered in World War II is that concerned with laundering under the diverse conditions found in the far-flung theatres of war. The chief concern is that of producing laundered clothing free from pathogenic bacteria. In stationary laundry units and most mobile units removed from front lines, hot water and steam are used to achieve the desired results. However, on occasion the mobile laundry units are compelled to operate under an entirely different set of conditions. Frequently the units have to function close to the front lines and therefore must be completely unobserved by the enemy, either from air or otherwise, since supply and service installations are as important targets as are combat units.

The vapor which would rise from heated water and be visible from the air depends on the temperature of the atmosphere. There would be considerable variation from winter to summer in temperate climates, whereas in torrid regions supposedly vapors would not be evident unless the water was heated above the prevailing temperature. The chance of detection of activity of this nature eliminates sterilization by the use of hot water and steam.

Consequently the problem of discovering a substance as germicidal as steam which could be used safely in the mobile

laundry units was presented. At the request of the Committee on Medical Research of the Office of Scientific Research and Development, this investigation was undertaken.

The problem as outlined was to discover a safe germicidal substance that could be added to the final rinse water in laundering which would destroy pathogenic microorganisms in wearing apparel, as well as in field-hospital and dressing-station linens. Since local streams in the combat areas constitute the source of water supply for field-laundry units, the germicide must be lethal for both bacterial and protozoan life which might be present.

There are now several thousand mobile units in service overseas and the number is increasing. The conditions under which the mobile unit operated vary, but the main problems are as follows:

The water is obtained from local streams which may be heavily contaminated with diverse microbial life. Also the unit may be compelled at times to use a water source which contains considerable organic matter.

The temperature of the water for laundering may be as low as 20°C., but under certain conditions it may be safe to raise the temperature to 48° or 50°C. without indicating the position of the unit to the enemy.

The detergent used by mobile units is synthetic in nature, and under ordinary circumstances there would be a supply available with each mobile unit. In the event of a breakdown in the supply system, the situation may necessitate the use of a supply of soap procured locally.

It is the usual practice of laundries in recent time to include a "scouring" operation so that the pH of the rinse water and garments will be reduced to some point between pH 5.0 and 6.0, resulting in neutralization of residual alkalinity. This alkalinity may be due to the water itself or to residual soap.

The articles to be laundered include wearing apparel which may not only harbor the normal flora of the skin but also organisms in pus from wounds or sores, such as the pathogenic streptococci, staphylococci and pathogenic fungi. Clothing may be contaminated with such enteric organisms as Escherichia coli and possibly with Shigella dysenteriae, Vibrio parva, Eberthella typhosa and members of the Salmonella genus. The stream itself may well contribute many pathogenic bacteria and, in addition, dangerous protozoa such as Endamoeba histolytica and Schistosoma haematobium, the blood-fluke cercariae. Thus the microorganisms to be considered are highly diverse in nature and pathogenicity. Moreover, the public works in advanced positions may be called upon to launder hospital and field-dressing linens contaminated with blood and purulent materials.

The germicide should not bleach the olive-drab dyes in the wearing apparel and should have no deleterious effect on buttons, wearing apparel, or laundry equipment.

Chemicals which appeared to possess the necessary properties were obtained.

As complete information as possible was compiled on each chemical including the chemical formula, the phenol coefficient against the test organisms Eberthella typhosa and Staphylococcus aureus, degree of solubility, uses and toxicity.

The test organism, Staphylococcus aureus, was selected as the most suitable for this experimental work due to its resistance, wide distribution and universal use as a standard in disinfectant testing.

An understanding of the equipment and techniques used in the procedures of Laundry Battalions was obtained from the Technical Manual issued by the War Department. The experimental procedures were designed to duplicate actual laundry conditions.

Under mobile conditions the standard procedure is to suds the clothes in two 5-minute runs in the washer, using a synthetic detergent. This is followed by three 3 to 5-minute rinses, with the "sour" being added in the final rinse. The clothes are placed in the extractor, where 80-70 per cent of the water (by weight) is removed in 3 minutes time. The final step is to dry the clothes in a rotary tumbler with a hot-air blast at 140°-160°F. for 20 minutes. (Anonymous, 1942)

EXPERIMENTAL

Part I

Procedure

From the list of chemicals suggested, Roccal was selected as the substance with the most attributes for use under the conditions described. This substance is a mixture of high molecular alkyldimethylbenzyl ammonium chlorides, soluble in water and having no odor or toxicity.

Procedures were designed to determine the greatest dilution at which the chemical produces germicidal action under varied conditions. Briefly, to a 99-ml. water blank were added citrated plasma, soap, souring agent, a suspension of bacteria and disinfectant. This mixture was agitated to insure thorough mixing. After a 5-minute exposure, surviving organisms were detected by loop transfers to broth and by plating. The degree of inactivation of the chemical by organic matter was investigated, as well as varying amounts of inoculum, and a comparison of several souring agents was made.

Equal 99-ml. volumes of water contained in screw-cap bottles were obtained by use of an automatic pipette. Water for the dilution blanks was kept stored in 15-gallon amounts to obtain uniformity in diluting medium. To avoid variations in pH and volume, all water blanks were autoclaved at 15 pounds pressure for 30 minutes.

Since the disinfectant must be used in the final rinse to destroy the natural flora of streams when unheated stream water

is used, and since the souring agent or acidifier is also added to the final rinse, the disinfectant was tested at pH 5 to 5.5, depending on the type of souring agent employed. When acetic acid is used as the souring agent, the final pH should not be lower than 5.5, since lower values tend to leave clothes with a rancid odor. In the use of formic acid as a sourer, the final pH was adjusted to pH 5. Souring to pH 5 represents 95 to 100 per cent neutralization of the alkalinity, except in the case of acetic acid, where pH 5 represents a 25-per-cent excess of sour. (Special Report No.7) The Beckman pH meter was used to determine the pH values. The concentrated acid was diluted so that less than 1 ml. could be used to adjust to the desired pH.

The mobile laundry units adjacent to front lines are required to launder hospital and dressing-station linens which are contaminated with varying amounts of blood. Such organic matter may not be removed entirely by the sudsing and rinsing operations. For this reason the disinfectant was tested in the presence of different concentrations of citrated plasma. The amounts adopted exceed the organic matter apt to be present in the final rinse under field conditions. Moreover, the peptones and bacterial metabolites in the broth further increase the total concentration of organic matter in the test solution.

Since residual soap is likewise present in rinse water, the disinfectant was tested in the presence of a synthetic detergent, Nacconal NR. This detergent or one similar in nature

is commonly used by the Army in the laundry units. It consists of alkylaryl sodium sulfonates. The amount chosen was .005 per cent for the early experiments, which gives an alkalinity of approximately pH 8. This amount produces some sudsing so is probably in excess of that left in garments in the final rinse.

Staphylococcus aureus, strain 209, was used throughout these early experiments and was cultured according to F. D. A. technic. (Ruehle and Brewer, 1931) The test culture was transferred daily in Armour's peptone medium for not more than one month. At the end of each month, a fresh transfer was made from the stock culture. The stock culture was carried on agar slants of the same composition as the broth medium, plus 1.5 per cent Bacto-agar (Difco) adjusted to pH 7.2 to 7.4. The stock culture was transferred once a month and the test organism was taken from the month-old stock culture. Four or five consecutive daily transfers in broth were made before using it for testing purposes, in order to be reasonably certain of its maintaining a uniform resistance. The early experiments were carried out using 1 ml. of a 24-hour broth culture of Staphylococcus aureus. However, comparison was made of tests in which 1 and 2 ml. of inoculum were used, and since the bacteria in the larger inoculum were killed in almost the same concentration of disinfectant as the 1 ml. of inoculum, the 2-ml. inoculum was adopted for later studies. Each milliliter of the inoculum contained from 400 to 800 million organisms, as determined frequently by plate counts. It was estimated that this number of organisms far

exceeds those likely to be contributed by stream water and those present in garments previously sudsed and rinsed. The concentration of organisms was many times in excess of numbers present in rinse water when low-temperature (90° -100°F.) commercial and home-laundry practices were analyzed by the American Institute of Laundering. (Service Bulletin No.53, 1942)

In the usual laundry practice, the final rinse requires about 3 to 5 minutes, and the latter was chosen as the exposure time in these studies. Except in special tests at elevated temperatures, all tests were made at room temperature, which varied from 70° to 80°F.

After the 5-minute exposure, loop transfers of test solution were made to duplicate tubes of Armour's peptone broth. A standard loop, 4 mm. in diameter, was used throughout the experiments. To differentiate between killing and bacteriostasis, Standard Methods Agar plates were poured in duplicate of 1:0, 1:100, and 1:10,000 dilutions of the test solution. These were incubated at 37°C. for 48 hours.

Table 1 shows the manner in which the data was assembled.

TABLE I
TYPICAL DATA SHEET OF EXPERIMENTS

Disinfectant - Roccal

99 ml. water blank

0.1 ml. citrated plasma

.87 per cent formic acid (amounts varied) pH 4.5

1 ml. Roccal (dilutions varied) pH 4.525

1 ml. Staphylococcus aureus, 24 hr. broth pH 5.5

Dilution of disinfectant	1:5000	1:6000	1:7000	1:8000	1:9000	1:10,000	1:11,000	1:12,000	1:13,000	1:14,000	1:15,000
Plates *	===	===	===	===	===	===	+++	+++	+=		
Armour peptone broth **	=	=	=	=	=	=	+	+	broken		
Plates						===	===	===	===	===	===
Armour peptone broth						=	=	=	=	=	+
Plates							===	===	===	===	===
Armour peptone broth							+	+	=	+	+

* Three dilutions, 1:0, 1:100, 1:10,000 of test solution in duplicate
 (-) No growth
 (+) Growth

** Armour's peptone broth in duplicate (-) No growth
 (+) Growth

Roccal was furnished as a 10-per-cent concentration of the active ingredient, alkyldimethylbenzyl ammonium chloride. Primary dilutions were made in sterile water, and then 1-ml. portions were added to the test solution. The disinfectant then was always contained in a total volume of 1 ml.

In the control tests in which organisms were exposed for 5 minutes to either acetic or formic acid at the pH of the experiments, i.e., pH 5.5 for acetic acid and pH 5.0 for formic, no consistent bactericidal action could be detected.

Influences of Blood Plasma, Detergents, Numbers
of Bacteria, and Types of Souring Agent
Upon the Effectiveness of Roccal.

Table 2 shows the results of a series of preliminary experiments carried out to determine (1) to what degree the disinfectant was inactivated when varied amounts of organic material in the form of citrated plasma were used, (2) the effects of the detergent on the germicidal potency, (3) the action of varied amounts of inoculum and (4) the comparative effects of two souring agents.

TABLE II

SUMMARY OF TESTS OF ROCCAL SHOWING EFFECTIVE DILUTIONS

VARIOUS INOCULUM, PLASMA, "SOUR" AND MACCONAL

Experiment numbers	Souring agent	Amount of culture inoculum ml.	Amount of citrated plasma per cent	Concentration of Macconal per cent	Final pH \pm .2	Time of exposure minutes	Range of dilutions tested	Effective dilution showing no growth in either broth tubes or on agar plates
1	Acetic acid	1.0	- - -	- - -	5.5	5	1:2000-1:17,000	1:3000
	Acetic acid	1.0	0.1	- - -	5.5	5	1:1000-1:9000	1:8000
	Acetic acid	1.0	0.1	.005	5.5	5	1:2000-1:12,000	1:4000
	Acetic acid	2.0	- - -	- - -	5.5	5	1:5000-1:14,000	1:6000
to	Formic acid	1.0	- - -	- - -	5.0	5	1:8000-1:17,000	1:11,000
	Formic acid	1.0	0.1	- - -	5.0	5	1:5000-1:15,000	1:10,000
	Formic acid	1.0	0.1	.005	5.0	5	1:5000-1:10,000	1:6000
	Formic acid	1.0	0.2	- - -	5.0	5	1:8000-1:15,000	1:10,000
140	Formic acid	1.0	0.2	.005	5.0	5	1:8000-1:15,000	1:9000
	Formic acid	2.0	- - -	- - -	5.0	5	1:8000-1:12,000	1:11,000
	Formic acid	2.0	0.1	- - -	5.0	5	1:8000-1:12,000	1:10,000
	Formic acid	2.0	0.1	.005	5.0	5	1:8000-1:12,000	1:9000
	Formic acid	2.0	0.2	.005	5.0	5	1:5000-1:10,000	1:6000

* Dilutions of 1:1000 intervals were made. When inconsistent results were obtained the dilutions were checked and rechecked.

In the first two experiments with acetic acid in table 2 the presence of 0.1 per cent citrated plasma did not change the bactericidal property of Roccal, 1:8000 being effective in both cases. On the addition of the detergent Nacconal, the bactericidal property was reduced to 1:4000. When the same souring agent (acetic acid) was used, but with 2 ml. of inoculum and no detergent, the effective dilution was 1:6000.

When formic acid was the souring agent, Roccal was bactericidal in a dilution of 1:11,000. When 0.1 per cent citrated plasma was added, Roccal was an effective bactericide in dilution of 1:10,000. Under the same conditions with the addition of Nacconal, the effective dilution dropped to 1:6000.

Without the detergent but with increased organic matter, the bactericidal dilution was again 1:10,000. Again when Nacconal was added, the effective dilution became 1:9000.

When organic matter and Nacconal were both omitted but the inoculum increased to 2 ml., the bactericidal property was the same as for 1 ml. of inoculum, i.e., 1:11,000. When organic matter was added to the above conditions (0.1 per cent citrated plasma), the effective dilution decreased to 1:10,000. With Nacconal added, the bactericidal dilution became 1:9000. Finally, with Nacconal added, and with increased inoculum and organic matter present, the bactericidal dilution was decreased to 1:6000.

The variation in the small amounts of plasma likely to be encountered in the disinfection of cloth materials, that of 0.1 to 0.2 per cent, appeared not to affect the bactericidal

property of Roccal appreciably. The presence of the detergent Nacconal reduced the bactericidal action considerably. Formic acid sour exerted a less deleterious effect on the killing action of Roccal than did acetic acid.

Both broth tubes and pour plates were used to detect survival. It was noted in certain cases that broth cultures showed growth while plates did not. This was due in part to a longer exposure to the disinfectant when agar plates were poured after the loop transfers were made. The random sampling error is to be taken into consideration in loop transfers.

In those instances when colonies were found on 1:100 dilution plates and not on 1:0 of the test solution, this, no doubt, represented bacteriostasis. Sufficient disinfectant was carried over in the 1:0 dilution to inhibit growth, while in 1:100 the disinfectant was so diluted that the bacteria were not inhibited.

As a result of the above study of the effectiveness of Roccal in varied concentrations of plasma and inoculum, it was decided to keep these substances constant at the maximum amounts used and to investigate the bactericidal action of Roccal in varied concentrations of detergent exposing the organisms a shorter period of time. Since the presence of soap or synthetic detergent so drastically decreased the action of the disinfectant, it was felt important to concentrate on this factor. In addition, it was ascertained that the rinsing process, according to Army laundry specifications should be limited to 3 minutes. This would allow

for only 3 minutes contact with the disinfectant in full strength. It will be pointed out later that during the extracting and drying processes there is a continued contact of garments with the disinfectant but in unknown and undetermined concentration since it is decreasing constantly. Furthermore, there will be occasions when the mobile units will be forced to operate without a synthetic detergent and will be obliged to use a local supply of soap. Inasmuch as the base of most soaps is a mixture of fatty acids of which stearic acid is typical, sodium stearate was compared with Roccal.

A series of experiments was designed to determine the effectiveness of Roccal at a higher pH, in case no sour were available. Table 3 shows the results of experiments carried out with the above factors in mind.

TABLE III

SUMMARY OF TESTS OF EFFECTIVE DILUTIONS OF ROCCAL
SHOWING VARIATIONS OF NAACONAL CONCENTRATION USING DIFFERENT "SOURS".

Experiment numbers	Souring agent	Amount of culture inoculum ml.	Concentration of citrated plasma percent	Concentration of Naacconal per cent	Final pH $\pm .2$	Time of exposure minutes	Range of dilutions tested	Effective dilution showing no growth in broth tubes on an agar plate
221-224	Acetic	2.0	0.2	.005	5.5	3	1:1000-1:4000	1:2000
194-198	Formic	2.0	0.2	.005	5.0	3	1:2000-1:6000	1:3000
183-188	Formic	2.0	0.2	.025	5.0	3	1:1000-1:6000	1:2000
214-220	Acetic	2.0	0.2	.025	5.5	3	1:1000-1:4000	1:2000
207-219	Acetic	2.0	0.2	-*	5.5	3	1:1000-1:6000	1:1000
225-234	-	2.0	0.2	.005	6.2-7.8**	3	1:1000-1:7000	1:2000

* Sodium stearate; .025 per cent, used instead of Naacconal

** Since no acid was added the pH varied according to the concentration of Roccal; e.g. in higher concentrations of Roccal the pH was more acid.

It may be observed in the preceding table that when acetic acid and formic acid were the only variables Roccal was less affected by formic acid, the bactericidal dilution being 1:3000, compared with 1:2000 when acetic acid was present. When the detergent was increased from .005 per cent to .025 per cent, Roccal was bactericidal in the 1:2000 dilution for either formic or acetic acid as souring agent.

In the comparison of Naccoral and sodium stearate under similar conditions, it was found (see table 3) that in the presence of sodium stearate (.025 per cent) the dilution of Roccal which was effective was 1:1000, whereas a dilution of only 1:2000 was effective with Naccoral. It should be emphasized that at pH 5.5 when sodium stearate is present in a concentration of .025 per cent, stearic acid precipitates, producing a suspension which is extremely turbid. This undoubtedly affects the germicidal capacity of the disinfectant.

The final experiment with Roccal was designed to determine the germicidal power in those instances when no sour is available. The bactericidal property of Roccal was found to be the same without a sour, i.e., 1:2000. The pH range was 6.2 to 7.8 depending on the concentration of Roccal, being more alkaline in the more dilute solutions.

A comparison of the results in table 2 with those of table 3 shows that the decrease from 5 to 3 minutes exposure necessitates using the disinfectant in double the amount for complete bactericidal action, all other conditions being constant, i.e., formic acid, 2 ml. inoculum, 0.2 per cent organic matter and .005 per cent detergent.

Influence of Temperature Upon Germicidal Power of Roccal

Further studies were made on Roccal under varied temperature conditions, as it is quite possible that in field operations the mobile laundry units will function with some latitude in this respect. Since transportation is an important factor, it would seem advisable to know the minimum amount of disinfectant which would effectively eliminate pathogenic microorganisms under different temperature conditions. The following table summarizes this data for Roccal.

TABLE IV

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF ROCCAL
SHOWING EFFECT OF TEMPERATURE VARIATIONS

Experiment numbers	Souring agent	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Nacconal per cent	Time of exposure minutes	Temperature C.°	Final pH ± .2	Range of dilutions	Effective dilution showing no growth in either broth tubes or on agar plates
304-309	Acetic acid	2.0	0.2	.005	3	10-12	5.5	1:5000	1:5000
316-319								1:1000	
221-224	Acetic	2.0	0.2	.005	3	23	pH 5.5	1:1000 1:4000	1:2000
320-326 332-336	Acetic	2.0	0.2	.005	3	37	pH 5.5	1:1000 1:7000	1:5000
299-303 310-315 326-331 336-341 342-347 351-354	Acetic	2.0	0.2	.005	3	48	5.5	1:1000 1:17,000	1:8000

In the investigation of the temperature relationships with respect to the disinfectant Roccal, the results were most striking. At a reduced temperature, i.e., 10° - 12° C., 1:500 dilution of Roccal was bactericidal while 1:1000 failed to kill. The bactericidal action was increased four fold by raising the temperature to that of room temperature, 1:2000 being effective. In contrast, at 37° C. the bactericidal dilution was 1:5000, whereas at 48° C. the dilution may be increased to 1:8000 and still exhibit total killing. Because of the dangers of possible skin irritations in sensitive individuals resulting from the disinfectant which may be adsorbed on garments in a laundry rinse, as well as the transportation problem, it would seem advisable to use the least amount of disinfectant which gives protection.

Influence of U.S.G. "Sour" on the Bactericidal Action of Roccal.

Although acetic and formic acids are widely used as souring agents by commercial laundries to obtain desirable acidity, it seemed important to consider the action of the substance actually in use in the mobile laundry units in relation to the activity of the disinfectant under study. The souring agent used is a mixture of 10 per cent sodium acid fluoride and 90 per cent sodium silica fluoride, which hereafter will be designated as U.S.G. This designation is given to the mixture provided for Laundry Battalions.

U.S.G. is a white solid, rather insoluble in water. A 1-per-cent concentration provided a suitable dilution to

use, since less than 1 ml. could be employed to obtain the desired pH without materially changing the concentration of the disinfectant in the test solution. A slightly wider range of final pH determinations was obtained due to the fact that a 1-per-cent concentration is not entirely in solution, and a homogeneous portion therefore is difficult to deliver. A more dilute suspension was not feasible since it would require too much volume to bring the pH of the test solution to the desired point.

Reccal was tested using the sour U.S.G. under varying conditions of temperature, first with .005 per cent Macconal then checked against .025 per cent of this detergent.

The following table summarizes the results.

TABLE V

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF HOGGAL
 USING U. S. G. "SOUR" WITH BOTH NAACONAL CONCENTRATIONS AND TEMPERATURE VARIED

Experiment numbers	Souring agent	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Naacconal per cent	Time of exposure minutes	Temperature C. ± 1°	pH range	Range of dilution tested	Effective dilution showing no growth in either broth or on agar plates
391-395	U. S. G.	2.0	0.2	.005	3	23	5.5 ± .2	1:1000-1:5000	1:4000
396-401 404-409 414-416 424-427	U. S. G.	2.0	0.2	.005	3	37	5.5 ± .3	1:2000-1:10,000	1:4000
383-390 401-403	U. S. G.	2.0	0.2	.005	3	48	5.5 ± .3	1:1000-1:14,000	1:11,000
460-465	U. S. G.	2.0	0.2	.025	3	23	5.5 ± .3	1:1000-1:5000	1:4000
431-435	U. S. G.	2.0	0.2	.025	3	37	5.5 ± .3	1:1000-1:4000	1:2000
439-444 435-438 428-430	U. S. G.	2.0	0.2	.025	3	48	5.5 ± .2	1:1000-1:10,000	1:4000

From table 5 it may be noted that, when the sour U. S. G. was used with the low concentration of Nacconal (.005 per cent), there was little or no difference in the effective bactericidal dilution in comparing 23°C. with 37°C. since 1:4000 was obtained for both. On the other hand, at 48°C. in the same concentration of Nacconal the disinfectant Roccal can be diluted to 1:11,000 and still retain its bactericidal properties.

The effect of the temperature elevation in the presence of increased Nacconal appears inconsistent in table 5, in that at 23°C. with .025 per cent Nacconal, the effective dilution (1:4000) was the same as with .005 per cent detergent. Instead of an increased bactericidal action at 37°C. a decrease was obtained. The increase of Nacconal completely canceled the effect of elevation of temperature. However, at 48°C. the bactericidal action of the disinfectant was increased (1:4000), as would be expected.

To determine whether the U. S. G. sour is bactericidal in itself, control experiments were run under somewhat the same conditions as the test solutions with the exception that the disinfectant was omitted, the time was adjusted to approximate that time in which the bacteria and sour were in contact, and only 1 ml. of the test organism was present.

The following table summarizes typical counts obtained.

TABLE VI
 SUMMARY OF TESTS ON THE BACTERICIDAL PROPERTY
 OF THE SOURING AGENT, U. S. G. AT VARIED TEMPERATURES

Souring agent U. S. G. ml	Concentration of culture inoculum ml	Concentration of citrated plasma per cent	Concentration of Nacconal per cent	Time of exposure minutes	Temperature C.°	pH	Bacterial Count millians per ml. average of duplicate plates
1	1.0	0.2	.025	4	23	5.4	460
none	1.0	0.2	.025	4	23		515
1	1.0	0.2	.025	4	37	6.0	310
none	1.0	0.2	.025	4	37		405
1	1.0	0.2	.025	4	48	5.4	195
none	1.0	0.2	.025	4	48		455

It may be observed from the data in table 6 that the souring agent used did not materially reduce the bacterial population at either 23°C. or at 37°C. However, at 48°C. there did seem to be a considerable reduction due to the combined effects of heat and acid; but no doubt this could be ascribed more to heat disinfection than to the acid.

Phemerol Studies.

While the investigations with Roccal were being made, parallel studies were instituted using a bactericidal substance known as Phemerol. This disinfectant exhibited no undesirable qualities on preliminary experiments. Phemerol is p-tert-octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride. It occurs in colorless, odorless crystals which are extremely soluble in water, giving solutions which have a pH range of 5 to 6. According to the literature, Tincture Phemerol, 1:500, has been used to prepare the skin for surgical operations and in ante partum preparation of obstetrical patients. In 1:5000 dilutions it has been used for wet dressings; and for nose and throat spray 1:1000 is recommended. To disinfect surgical instruments 1:1000 dilution, with a 30-minute exposure, has been found to kill all vegetative cells, though it cannot be relied on to kill spores. On the basis of the above information a detailed study of Phemerol was made along the same general plan as was carried out with Roccal although not in such complete detail.

Phemerol is marketed in bottles in 24-per-cent concentration of the active ingredient. Appropriate dilutions were

made in sterile water, from which 1-ml. portions were delivered to the test solution. The results of the initial examination as to the bactericidal dilution are found in table 7.

TABLE VII

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF PHENOL
WITH NACCONAL CONCENTRATIONS VARIED

Experiment numbers	Souring agent	Amount of culture inoculum ml	Concentration of citrated plasma per cent	Concentration of Nacconal per cent	Time of exposure minutes	Temperature C.°	Final pH $\pm .2$	Range of dilutions tested	Effective dilution showing no growth in either broth tubes or on agar plates
169-182	Acetic acid	2.0	0.1	-	5	23	5.5	1:1000-1:9000	1:4000
159-168	Acetic acid	2.0	0.1	.025	5	23	5.5	1:1000-1:5000 1:6000-1:8000 1:10,000-1:12,000 1:14,000	1:3000

In table 7 it is demonstrated that Phemerol, in 5 minutes without the detergent Nacconal, was bactericidal in a dilution of 1:4000; but in those tests in which .025 per cent Nacconal was present, comparable results were obtained when the bactericide was present in 1:3000 concentration. Since this substance is recommended as a non-toxic and non-irritating germicide, and is found to be effective in low concentrations, it was felt that it merited further study.

The maximum inoculum of culture and organic matter and minimum Nacconal, adopted in the Roccal studies, was used, and the next determination was made with regard to varying the temperature. The time of exposure was kept at 3 minutes.

Table 8 summarizes the information obtained.

TABLE VIII

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF PHENEMOL
SHOWING THE EFFECT OF DIFFERENT TEMPERATURES

Experiment numbers	Souring agent	Amount of culture inoculum ml	Concentration of citrated plasma per cent	Concentration of Naocornal per cent	Time of exposure minutes	Temperature C.°	Final pH $\pm .2$	Range of dilutions tested	Effective dilution showing no growth in either broth tubes or on agar plates
245-252 241-244 253-255	: : Acetic : acid	2.0	0.2	.005	3	23	5.5	1:250;1:333;1:500; 1:625;1:700;1:300; 1:900;1:1000;1:2000; 1:3000;1:4000.	1:700
355-359	: : Acetic : acid	2.0	0.2	.005	3	37	5.5	1:1000-1:5000	1:3000
348-50 360-364	: : Acetic : acid	2.0	0.2	.005	3	48	5.5	1:1000-1:5000	1:4000

As would be expected, a similar increase in germicidal capacity with increased temperatures was obtained with Phemerol. At room temperature, i.e., approximately 23°C., the killing dilution was 1:700. At 37°C. the effective dilution was increased to 1:3000, while at 48°C. the germicidal dilution was 1:4000. The effective dilutions of Roccal are approximately twice those of Phemerol at equivalent temperatures and under exactly the same conditions except for the disinfectant used. That is, at 23°C., 1:2000, at 37°C., 1:5000 and at 48°C., 1:8000. (See table 4)

At this point it was decided to ascertain whether or not Phemerol as well as Roccal was inactivated by increased detergent and, if so, to what extent. In all of these studies the quantity of detergent present affected the germicidal power of the disinfectant more than any other variable with the possible exception of temperature.

Table 9 gives this information for Phemerol.

TABLE IX
 SUMMARY OF TESTS OF VARIOUS DILUTIONS OF PIEMEROL
 WITH TEMPERATURE VARIED AND INCREASED NACCONAL

Experiment numbers	Souring agent	Amount of culture inoculum ml	Concentration of citrated plasma per cent	Concentration of Nacconal per cent	Time of exposure minutes	Temperature C.°	Final pH + .2	Range of dilutions tested	Effective dilution showing no growth in either broth or on agar plates
365-368 375-376	: Acetic acid	2.0	0.2	.025	3	37	5.5	1:1,000-1:6,000	1:4000
369-374 377-382	: Acetic acid	2.0	0.2	.025	3	48	5.5	1:2000-1:10,000	1:7000

In a comparison of tables 8 and 9 it will be seen that at 37°C. the only variable was that of Nacconal, which is increased five fold in table 9. The bactericidal property of Phemerol increased slightly with the increased concentration of detergent, the effective dilution being 1:3000 when .005 per cent Nacconal was used, and 1:4000 when .025 per cent was present. At 48°C. the bactericidal property was enhanced to a considerable degree in spite of the greater amount of Nacconal; i.e., the effective strength was decreased from 1:4000 to 1:7000.

This is in striking contrast to the results obtained with Roccal, when an increase of detergent increased the concentration required for killing.

The above finding suggests that improvement in rinsing operation might be expected if a larger amount of detergent remained in the clothing. This is contrary to all experience and information so it became important to determine whether the same phenomenon would occur in the presence of the souring agent U.S.G. instead of acetic acid. Again the variables were temperature and Nacconal concentration. The results of this study are summarized in table 10.

TABLE X

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF FEMEROL
WITH U. S. G. "SOUR", NACCONAL CONCENTRATIONS AND TEMPERATURE VARIED

Experiment numbers	Souring Agent	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Nacconal per cent	Time of exposure minutes	Temperature C.°	Final pH $\pm .2$	Range of dilutions tested	Effective dilution showing no growth in either broth or on agar plates
409-412	U. S. G.	2.0	0.2	.005	3	37	5.5	1:1000-1:5000	1:3000
416-423	U. S. G.	2.0	0.2	.005	3	48	5.5	1:3000-1:10,000	1:6000
455-459	U. S. G.	2.0	0.2	.025	3	23	5.5	1:500 1:1000-1:4000	1:1000
450-454	U. S. G.	2.0	0.2	.025	3	37	5.5	1:1000-1:5000	1:3000
445-449	U. S. G.	2.0	0.2	.025	3	48	5.5	1:1000-1:5000	1:4000

In summary of the data on Phemerol in table 10, it may be stated that at .005 per cent Nacconal the effective dilution at 37°C. was 1:3000, and at 48°C. was 1:6000. On increasing the concentration of Nacconal to .025 per cent, at 23°C. the killing dilution of Phemerol was 1:1000; at 37°C. the dilution may be increased to 1:3000; but at 48°C. only 1:4000 was found to be effective. The increased concentration of Nacconal inactivated the disinfectant more effectively at the highest temperature. If the results of table 10 are compared with those of table 9, it will be evident that all conditions were identical except for the souring agent. It would seem that acetic acid interfered less with the action of Phemerol than U. S. G. souring agent, inasmuch as the bactericidal dilution with acetic acid was 1:7000 and only 1:4000 with U. S. G.

The contrast between the effective dilutions of Roccal (table 5) and those of Phemerol (table 7) are interesting. At 37°C. with .005 per cent Nacconal Phemerol was effective at 1:3000 dilution, while Roccal was bactericidal at 1:4000. At 48°C. Phemerol may be diluted to 1:6000 while at the same temperature Roccal was effective when diluted to 1:11,000.

With concentration of the detergent increased to .025 at 23°C. Phemerol dropped markedly in effectiveness, in that 1:1000 was the critical dilution, whereas Roccal under these conditions remained as potent as in the lesser concentration of Nacconal, 1:4000. Roccal was four times as effective as Phemerol under these conditions.

At 37°C. and .025 per cent Nacconal, Phemerol appeared to

be a little more effective than Roccal; i.e., 1:3000 was the bactericidal dilution of Phemerol in contrast to 1:2000 for Roccal. This inconsistency may be due to errors in the technic or to the nature of Phemerol itself.

If the results at 48°C. are examined, it will be noted that the effective killing dilutions for the two substances are identical. Phemerol was inactivated by Macconal in a lesser degree than was Roccal.

Preliminary Study of Shirlan, Benchlophen and Sanitized Solution.

In answer to inquiries, the disinfecting potency of the substances Shirlan, Sanitized and Benchlophen were determined. The first two are water-soluble and all three are said to be non-toxic and non-irritating when left in a fabric.

The technic employed was the same as that previously outlined, with the exception that the culture inoculum was made last.

Table 11 gives the information obtained with Sanitized.

TABLE XI

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF SANITIZED

Experiment numbers	Souring agent	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Naccoonal per cent	Time of exposure minutes	Final pH	Range of dilution tested	Effective dilution showing no growth in either broth or on agar plates
189-193	Formic acid	2.0	0.2	.005	3	5.	1:2; 1:3; 1:4; 1:5; 1:6.	none

The material Sanitized was delivered in a small keg. The composition or concentration of the active ingredient was unknown. Preliminary experiments demonstrated that high concentrations would be necessary to produce germicidal action. As will be seen in table 11, the lowest dilution of Sanitized was 1:2. Fifty ml. of the stock solution were added to 49 ml. of sterile water to make the first dilution of the series. Subsequent dilutions were made by adding the stock solution to sterile water in the ratios indicated so that the total volume would be 99 ml. Since the substance was deficient in disinfecting power under the conditions of these experiments, it did not seem worth while to study the effects of other souring agents, varied amounts of detergent, or time and temperature relationships. In other investigations carried on in this laboratory results indicate that time is a factor of considerable importance in the tests with Sanitized, and this disinfectant could not be made effective in the short time available for the final rinse. Furthermore, it was noted that the test solution developed a flaky precipitate when the plasma was added to the diluted Sanitized solution, thus making it definitely objectionable.

Although all tubes showed growth in all dilutions used, inhibition or bacteriostasis was exhibited when contact was prolonged, as it is on agar plates. In the plates of 1:0 dilution of the test solution no bacterial growth occurred in dilutions from 1:2 through 1:4 of the test solution. However, colonies developed on the 1:100 and 1:10,000 agar plate

dilutions of the test solution. That there is slight bactericidal action is shown by the fact that in plates poured from the 1:2 dilution of the test solution, 44,000,000 bacteria survived in contrast to 116,000,000 in plates from 1:6 dilution.

The disinfectant next under consideration was Shirlan IN 2770. The active ingredient, sodium dimethyldithiocarbamate is prepared in 30-per-cent concentration. Appropriate amounts were added to sterile water to give the concentrations desired in a total volume of 99-ml. test solution, after which plasma, Nacconal, acid and S. aureus inoculum were added. The usual 3-minute exposure followed before surviving organisms were determined.

Table 12 contains the data for Shirlan.

TABLE XII

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF SHIRLAN IN 2770

Experiment numbers	Souring agent	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Naacconal per cent	Time of exposure minutes	Final pH	Range of dilutions tested	Effective dilution showing no growth in either broth or on agar plates
192-196 199-206	Acetic acid	2.0	0.2	.005	3	5.5	1:50; 1:75; 1:100; 1:150; 1:200; 1:250; 1:300-1:600	1:150

The disinfectant known as Shirlan IN 2770 has some value in that it may be diluted so that the active ingredient is 1:150 and still demonstrate bactericidal action. However, there are several disadvantages exhibited by this substance. In order to make the substance soluble in water, it is necessary to make a sodium salt of the compound. Therefore, when acid is added to bring the pH to the desired point, the disinfectant reacts with the acid and becomes insoluble, shifting the pH back to the alkaline range. Moreover, the disinfectant tends to precipitate the plasma, thus making the test solution exceedingly turbid. Perhaps the most undesirable feature of Shirlan, however, is that it has a disagreeable, fishy odor which, if left in wearing apparel, would be an added discomfort. Besides the complete germicidal effect obtained in dilutions of 1:150 on both plates and in tubes in three minutes, this disinfectant likewise exhibited bacteriostasis in the higher dilutions (1:600).

A series of experiments on Penchlophen, a mixture of monobenzylated orthochlorophenols and monobenzylated phenols, was carried out, since this substance is recommended as an external fungicide, germicide and antiseptic. Furthermore, this substance is supposedly non-toxic in dilutions as low as 1:50. Its value in laundering presumably is greatest if used in a 10- to 15-minute pre-soak of the garments. In a 100-per-cent solution this disinfectant has a heavy, colorless, oil-like appearance.

A preliminary study was first made in those dilutions which were not soluble in water in order to note the behavior in this diluent and to determine whether this substance was sufficiently

dispersed to have a germicidal effect. No sour was used throughout the Benchlophen study as it is unstable in the acid range. As mentioned previously, it could be used only in the pre-acid rinse in laundry procedures. The following table includes the information obtained.

TABLE XIII

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF BENCHLOPHEN
USING WATER AS THE PRIMARY DILUENT

Experiments numbers	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Naconal per cent	Time of exposure minutes	Temperature C. °	pH range	Range of dilutions tested	Effective dilution showing no growth in either broth tubes or on agar plates
255-267	2.0	0.2	.005	3	23	6.8-7.5	1:100-1:1400	1:800 in broth 1:1300 in plates

* Dilutions of 1:100 intervals were made.

Initial dilutions of the 100-per-cent concentration of Benchlophen were made with water. On shaking, an impermanent emulsion formed between disinfectant and diluent which, on standing, soon began to separate into the oil and water phases. An aliquot portion of the dilution was nonhomogeneous. When such aliquot part was delivered into the test solution, the final dilution was not necessarily in the amount designated.

A scum formed on the surface of the test solution which apparently contained plasma. In the making of loop transfers this scum was frequently inoculated into the peptone broth. The scum may have contained some organisms which had not come in contact with the disinfectant. After 3 minutes exposure the test solution assumed a milky appearance with a precipitate settling out.

Although Benchlophen was somewhat germicidal in this range of dilutions, the inconsistent results obtained and its insolubility make it entirely unsatisfactory for use as a laundry rinse as demonstrated under the conditions of this experiment. A further disadvantage of Benchlophen is that it is unstable in the presence of a souring agent. This disinfectant does have considerable bactericidal properties as shown by a dilution of 1:700 giving sterile tubes, and colonies of the test organism first appearing on 1:1300 dilution pour plates.

In the following study of Benchlophen primary dilutions were made with ethyl alcohol. One-ml. amounts of the various alcohol-Benchlophen dilutions were added to the test solution.

Using this diluent a homogeneous test solution was obtained which gave the appearance of an emulsion. The disinfectant did not exhibit a tendency to settle out as in the lower dilutions in which water was used for a primary diluent. The results are summarized in table 14.

TABLE XIV

SUMMARY OF TESTS OF VARIOUS DILUTIONS OF BENCHLOPHEN
USING ALCOHOL AS THE PRIMARY DILUENT

Experiment numbers	Amount of culture inoculum ml.	Concentration of citrated plasma per cent	Concentration of Nacconal per cent	Time of exposure minutes	Temperature C. °	pH range	Range of dilutions tested	Effective dilution showing no growth in either broth tubes or on agar plates
270-299	2.0	0.2	.005	3	23	6.6 9.0	1:1000-2500* 1:3000;1:3500 1:4000-15:000** 1:20,000-1:25,000 1:30,000	1:9000 in broth 1:10,000 on agar plates

* Dilutions of 1:500 intervals were made

** Dilutions of 1:1000 intervals were made

Although Benchlophen showed high bactericidal properties, i.e., no growth in tubes until 1:9000 dilution, and colony growth on plates poured from 1:11,000 test solution, the insolubility in water and instability in acid make it impractical as a laundry rinse germicide. It may be stated, however, that at 1:10,000 this substance is miscible in water but this dilution is barely sufficient for effective killing. Presumably, at a higher temperature the disinfectant would be slightly more soluble and the killing action more effective, but no such tests were made.

Part II

Tests with Bacteria Impregnated Into Cloth.

The purpose of this phase of the investigation was to discover, if possible, whether the information gained regarding germicidal dilution in the previous experiments could be applied to actual laundry practice with cloth.

Procedure.

A glass tumbling jar of several liters capacity was used. This jar is fitted with a screw cap lined with tin foil and is hung in a metal stand by means of a metal band to which a rotating shaft is attached so that it can be tumbled manually or by motor.

Since the tumbling jar was not designed for steam sterilization, it was necessary to produce sterility by chemical means. This was accomplished after each test by washing the jar with soap and water, after which it was rinsed several times with tap water. Three hundred ml. of a very strong solution of calcium hypochlorite were used to sterilize the jar. The jar was rotated so that all inner surfaces would come in contact with the chlorine. This treatment was followed by three rinses, each with 300 ml. amounts of sterile water. These amounts gave sufficient volume for the size of the jar to insure thorough rinsing. Any residual chlorine was next neutralized by a rinse with 300 ml. of sterile 0.1 N sodium thiosulfate. Three subsequent rinsings with sterile water followed, after which a silver nitrate (0.1 N) test was used to determine any chlorine present. The silver nitrate test (Kolthoff and Sandell, 1943) detects chlorine in 3.5×10^{-5} parts per million. Chlorine is not bactericidal in this concentration. Two-tenths

to 0.4 parts of chlorine per million is considered bactericidal for water purification. (McCulloch, 1936). One ml. amounts of the final chlorine negative rinse were plated in duplicate to test for sterility.

The tumbling jar was contaminated with a heavy suspension of S. aureus, after which it was sterilized in the manner described above. No organisms were recovered on the agar plates poured from the rinse water; therefore it was assumed that this method of sterilization was satisfactory. This indeed proved to be the case as on repeated tests no organisms were detected subsequent to sterilization by the process outlined.

In the Army laundry procedures, approximately 2 pounds of clothes per gallon of water is considered the maximum for efficient cleansing. This ratio of cloth to water was found too large in the actual experiment due to the nature of the cloth used. The cloth was new, unused, rather heavy cotton toweling, which was somewhat resistant to wetting.

Strips of cloth 20 inches long and 6 inches wide were cut, folded and 2-inch squares were marked on the top fold. These were then wrapped in paper and autoclaved for 30 minutes at 15 pounds pressure. This procedure insured sterile cloth to be used in the tests. No organisms were ever recovered from cloth sterilized in this manner.

In order to determine how completely organisms could be recovered from such a cloth sample several 2-inch squares, after sterilization, were saturated with 2 ml. of a 24-hour broth culture of S. aureus. These control samples were then

cut from the cloth with sterile scissors and delivered into wide-mouth bottles containing 100 ml. of sterile water and glass beads. The bottles were shaken 100 times, after which appropriate dilutions were made in order to determine the numbers of organisms. Approximately 50 per cent recovery of the organisms was obtained.

Other squares, impregnated in the same manner with the same volume of inoculum, were cut into fine shreds, two or three threads wide. The number of organisms recovered on such a shredded sample was double that of the uncut samples, i.e., approximately 100 per cent instead of 50 per cent.

A 1-liter volume of water was used in the tumbling jar as a convenient multiple of the previous test solutions and a suitable amount for the size of the tumbling jar. To this were added sterile citrated plasma in appropriate per cent, sufficient Nacconal to obtain the desired concentration, a predetermined number of milliliters of U. S. G. sour to bring the pH down to pH 5.5, the sterile cloth on which the marked squares were impregnated with 2 ml. of 24-hour broth culture of S. aureus and finally an adequate amount of the disinfectant under investigation to produce the desired dilution.

The jar was then rotated manually forty revolutions a minute for almost 3 minutes. This speed gave maximum motion to the contents of the jar so that the disinfectant would come in intimate contact with the organisms impregnated in the cloth. At the end of the 3-minute period, a sample of cloth was removed with sterile forceps, and manipulated so that the square to be

recovered for testing did not become contaminated. The square was next cut out with sterile scissors and a bacterial count of surviving organisms determined as in the control sample described above.

Study of Effectiveness of Roccal Against

Staphylococcus aureus and Aerobacter

aerogenes in Cloth.

The first two experiments were carried out at 23°C. as this temperature is most likely to be maintained under field conditions. Previous experiments have shown markedly better results at slightly elevated temperature so in the next two experiments the temperature of the solution at the start of the test was raised to 37°C. During the operation of tumbling the temperature dropped to about 33°C.

The results are given in table 15.

TABLE XV

SUMMARY OF RESULTS USING ROCCAL IN TUMBLING JAR

AT TEMPERATURES; 23°C. and 37°C

Experiment numbers	Amount of culture inoculum on each 2 inch square ml.*	Concentration of citrated plasma per cent	Concentration of Naconal per cent	Time of exposure minutes	Temperature C.°	pH $\pm .2$	Dilution of disinfectant.	Bacterial survival in 2 inch square average of two countable plates
1	2.0	0.2	.025	3	23	5.5	1:4000	560 thousand
2	2.0	0.2	.025	3	23	5.5	1:4000	2 million
3	2.0	0.2	.025	3	37 to 33	5.5	1:4000	5 million
4	2.0	0.2	.025	3	37 to 33	5.5	1:4000	3 million

* Five two inch squares were impregnated with 2 ml. each of Staphylococcus aureus

As can be readily noted in table 15, 1:4000 dilution of Roccal is not bactericidal in 3-minute exposure to Staphylococcus aureus when the organisms are impregnated in cloth under the conditions of these experiments. That there is a great reduction of numbers is obvious, inasmuch as each 2-inch square of material was inoculated with approximately one billion organisms. Furthermore, the 37°C. temperature was not maintained during the period of exposure. The size of the tumbling jar and the operation of tumbling precluded the use of a water bath to control temperatures.

Nevertheless a further attempt was made to carry out the test at an elevated temperature. Table 16 shows these results and some further modifications in technic to take into consideration the fact that the disinfectant is in contact with the garment for a prolonged period of time even though the actual rinsing operation is terminated at the end of 3 minutes. The process subsequent to rinsing in the mobile laundry units is extracting by means of centrifugal force which removes 50-70 per cent by weight of the moisture in 3 minutes. The final step is to dry the clothes in a rotary tumbler with a blast of hot air from 140° to 180°F. Under conditions in which the hot-air drier could not be used, the clothes would be air dried. Thus the period of exposure is variable and the concentration of disinfectant is gradually decreased with the removal of water. Accordingly an attempt was made to simulate such conditions as nearly as possible.

The first determination was made on an impregnated square exposed 3 minutes to the disinfectant. Two other squares, similarly exposed, were dried on sterile filter paper in petri dishes overnight at 37°C. and surviving organisms determined by the usual method. Since the filter paper adsorbed a great deal of the moisture, bacterial counts were made on them also.

Table 16 shows the results.

TABLE XVI

SUMMARY OF RESULTS USING ROCCAL IN TUMBLING JAR

AT 48° to 40° C.

Experiment numbers	Souring Agent	Amount of culture inoculum on each 2 inch square ml.	Concentration of citrated plasma per cent	Concentration of Naocconal per cent	Time of exposure minutes	Temperature C.°	pH ± .2	Dilution of disinfectant	Bacterial survival on two inch squares		
									Average of 2 countable plates		
5	U.S.G.	2.0	0.2	.025	3	48 to 40	5.5	1:2000	Square 1 420,000		
6	U.S.G.	2.0	0.2	.025	3	48 to 40	5.5	1:200	Square 1 30,000	Square 2 dried overnight 2700 Filter paper none	Square 3 dried overnight 1200 Filter paper none

In the preceding table it is evident that 1:2000 Roccal did not kill the organisms although there was a definite decrease in surviving numbers compared to 1:4000 dilution. The surviving organisms possibly represented the more resistant cells and in contrast to previous experiments were found in the first dilution of the pour plates. On the other hand, the survival may merely signify that there were areas of less exposure in the cloth. The temperature, as may be noted, did not remain at 48°C.

Further work was carried out at 23°C. and in lower dilution of Roccal. Also Nacconal concentrations of .005 and .025 per cent were used. The 3-minute and 15-minute exposures were selected in an effort to determine a definite point when sterilization would be realized. The time of exposure for squares 3, 4, and 5 merely reflects the limitations imposed by time necessary for execution of the preceding steps in the experiment. Included in table 17 is a comparison of results obtained using an encapsulated strain of Aerobacter aerogenes which is rather resistant to sterilization by steam. The organism might be comparable in this respect to a pathogenic Gram negative organism such as Klebsiella pneumoniae.

TABLE XVII

SUMMARY OF RESULTS USING BOCCAL IN TUMBLING JAR
ORGANISMS AND BOCCAL VARIED

Experiment Numbers	Souring agent	Concentration of culture inoculum on each 2 in. square ml.	Concentration of citrated plasma per cent	Concentration of Naoccal per cent	Temperature C.*	pH	Dilution of disinfectant	Bacterial survival in two inch squares Average of two countable plates. Time of exposure given in minutes with subsequent treatment				
								Square 1	Square 2	Square 3	Square 4	Square 5
7	U.S.G.	2.0	0.2	.025	23	5.8	1:1000	3	20	25 dried over- night	26 dried over- night	28 dried
								none	500	none	none	none
8	U.S.G.	2.0	0.2	.025	23	5.8	1:1000	3	15	20 dried over- night	22 dried over- night	25 same as above
								none	none	none	none	none
9*	U.S.G.	2.0	0.2	.025	23	5.7	1:1000	3	15	22 dried over- night square cut in fine pieces	24 dried over- night	27 same as above
								1800	71,000	none	14,000	none

* Aerobacter aerogenes, 24 hour nutrient broth culture.

The results in table 17 clearly indicate that Roccal 1:1000 is bactericidal at 23°C. using both .005 and .025 per cent Nacconal, with the exception of one instance in experiment 7, square 2. The surviving organisms here may well have been clumped so firmly that the germicide did not penetrate, or they may have survived in the suds. Either condition could be encountered in actual laundry work. The fact that the rest of the plates were sterile shows efficient action of the bactericide. It should be emphasized too that the nature of the cloth was such as to resist wetting. In actual laundry rinse, garments will have passed through three sudings and two rinses before the souring operation; thus the resistance to wetting should not be encountered in the sour-disinfectant operation.

If the results in experiment 9, table 17, are examined, it will be evident that the encapsulated strain of Aerobacter aerogenes was more difficult to kill than the test organism S. aureus. It might be presumed, however, that the enteric pathogens would be less resistant than the encapsulated strain of A. aerogenes.

DISCUSSION

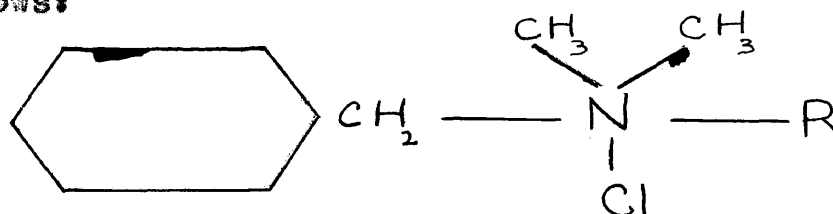
The need for a disinfectant suitable for use as a germicide in the final laundry rinse in Army Mobile Units presents a distinctly new problem. Evaluation of well-known bactericidal substances yields none that entirely meet the requirements of being non-toxic, soluble in water, effective in low concentrations in the presence of a detergent, stable in the acid range, and non-corrosive. Chlorine is used extensively in commercial laundries for bleaching, and incidentally reduces the bacterial population. Because of the bleaching effect of chlorine on dyes, and for other reasons, this method is not suitable for the problem considered here. Furthermore, in the usual laundry procedures, heat is an effective agent in producing sterile garments, but its use was not practical in the present problem.

The investigation thus resolved itself into a study of a few of the many substances recently introduced for which varied disinfecting properties have been described, and which appeared most nearly to meet the requirements.

Three of the substances, Shirlan, Benchlophen and Sanitized were eliminated after preliminary experiments due to characteristics of insolubility in water or lack of high germicidal power. The first two are sold as germicides, and the third one is a mild antiseptic that is impregnated into clothing commercially. Further study of Sanitized would have required tests of longer duration in the dried clothes. As a result, the experimental work was narrowed down to the study of two substances, Roccal and Phemerol. Both of these disinfectants are prototypes

of compounds known as quaternary ammonium salts, which are cationic in nature. The discovery of their germicidal nature is of relatively recent date.

The formula of such a quaternary salt may be illustrated as follows:



in which R may be a mixture of alkyl radicals found in fatty acids of coconut oil and may range from C_6H_{17} to $\text{C}_{18}\text{H}_{37}$. The above formula is that of Roccal.

Quaternary ammonium salts are soluble in water and give conducting solutions (Sidgwick, 1937). The salts ionize into the negatively charged chloride ion, while the four methyl or other groups remain attached to the nitrogen atom. The nitrogen in such a compound (as in ammonia) shares six of the eight valency electrons with three of the attached groups. When it unites with a fourth group, such as a methyl group, one electron is discharged leaving the nitrogen radical as a whole with a positive charge. Thus it is evident that in a quaternary compound four of the groups are attached by covalencies and the fifth is attached to the anion by electrovalency. Since such an ion carries a positive charge, it is cationic in nature. A compound of this structure also reduces the surface tension, when in dilute solutions, and is described as a surface-acting agent. This effect is due to the long-chain fatty-acid radical of the molecule.

In studies of other cations, e.g., Na, K, Li, Zn, and Cd, on viability of bacteria, Winslow and Haywood (1931) state that in low concentrations the action is that of stimulation, while in high concentrations the effect is that of inhibition. They postulate that the death of the bacteria is due to the coagulation of cell colloids by the cation.

The use of a cationic disinfectant of quaternary ammonium nature, Zephirol, was first mentioned by Domagk (1935). More recently a number of American investigators have studied these compounds in detail in an attempt to correlate the germicidal activity with the molecular structure. It was noted by Dunn (1937) that a mixture of alkyldimethylbenzyl ammonium chloride prevented spores of Bacillus subtilis from germinating. The germicidal action of the disinfectant was more rapid against B. subtilis in an alkaline medium as compared to an acid or neutral one. Also bacteriostatic action was pronounced toward B. subtilis and Staphylococcus aureus (Gram positive organisms), while it was of low order in respect to Escherichia coli, a Gram negative organism.

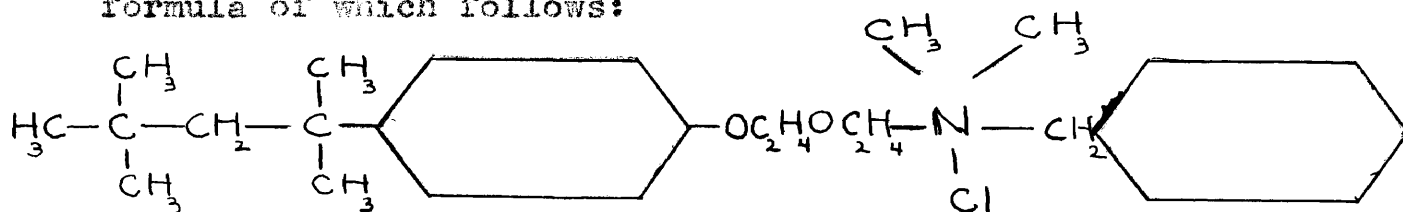
Baker, Harrison and Miller (1941a) used four Gram positive organisms and three Gram negative organisms and determined the effect of several cationic disinfectants, one of which was Phemerol. Their results indicate that cationic detergents show marked bactericidal effects on Gram positive organisms and somewhat less on Gram negative.

It has been reported by Valko and Du Bois (1944) that the bactericidal effect of a surface-active cation may be diminished

when another surface-active cation of less or no toxicity to the bacteria is present. They explain this phenomenon by assuming that the less bactericidal cations occupy certain spaces on the surface of or within the bacterial cell, thus protecting the organism from the more toxic cations.

Further studies have been reported by Joslyn, Yaw and Rawlins (1943) using Phemerol at three different temperatures against three Gram negative and four Gram positive organisms. Phemerol was more effective against Gram positive organisms than against Gram negative. Their results demonstrated that Pseudomonas aeruginosa was resistant to the bactericidal action of the cationic detergents. This exception also was pointed out by Brewer (1943).

An attempt to account for the bactericidal properties of the quaternary compounds has been made in a detailed study of the relationship of the chemical structure to germicidal activity by Rawlins, Sweet and Joslyn (1943). They studied varied lengths and composition of the cation by using different combinations of alkyl and aryl groups. The most effective bactericidal action was obtained with compounds which have a range of 12 to 16 atoms in the cation. The atoms of the cation for maximum germicidal activity should be composed of one long alkyl, one short aralkyl group and two lower alkyl groups. The maximum bactericidal activity was obtained with Phemerol, a p-tert-octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride, the formula of which follows:



Any increase or decrease in the number of atoms in the cation markedly decreases the bactericidal property.

Closed-ring substituents on the aromatic nucleus render the bactericidal property less potent. Likewise halogen substitution on the aryl nucleus tends to decrease the activity.

When the lower molecular weight substitutions are made on the pentavalent nitrogen, i.e., hydrogen, in place of the benzyl group, there is a drastic reduction in bactericidal power. It is reported also that, when the benzyl group which is attached to the pentavalent nitrogen is replaced by a nicotinic acid amide derivative, there is a similar reduction in bactericidal potency. (Epstein et al., 1943)

Another characteristic of the quaternary compounds which cannot be overlooked in the study of their mode of germicidal action on bacteria has to do with their action as a surface tension depressant. The compounds depend for this effect upon certain properties possessed by molecules which consist of a long chain of hydrocarbons attached to a terminal polar group (Langmuir, 1917). The polar group has an affinity for water and is frequently described as hydrophilic, whereas the long chain or hydrophobic has no such affinity, therefore the molecule, when in contact with water, is oriented with the polar group in the water and the carbon chain standing on end. The presence of the carbon group lowers the surface tension. When the carbon chain is 16 to 18 atoms, a valuable detergent is developed. The cationic quaternary ammonium compounds all lower the surface tension, as do the anionic agents which include soaps, and in

this study the detergent Nacconal NR falls in the latter class of compounds. It has been postulated that the decreased surface tension changes the permeability of the cell membrane rendering the cell more susceptible to bactericidal effects of disinfectants.

Inasmuch as the disinfecting power of the compounds under study has been greatly affected by Nacconal and sodium stearate, the nature of these substances merit some attention. Nacconal is a sodium salt of a sulfonic acid of the general formula of RSO_3Na where R (for maximum effectiveness) is composed of alkyl-aryl groups of 12 to 16 carbons. The anionic compounds dissociate into negatively charged RSO_3 ions and into positively charged sodium ions. The cleansing property of Nacconal and soap in general is attributed to the adsorption of dirt particles on the negatively charged colloidal particle. These compounds are also classed as wetting agents.

A number of studies on the effect of anionic substances on bacterial growth have been carried out. Due to the fact that the organisms of the acid-fast group are resistant to wetting agents, a study was made using Mycobacterium smegmatis (Katz, 1935). This organism was exposed to 1:10 dilution of an anionic substance. Morphological changes as well as lysis were exhibited. The appearance of spherical forms was attributed to reduced interfacial tension.

A further study, made by Lominski and Lendrum (1942), dealing with the surface-active agents on the swarming phenomenon exhibited by Proteus and Pseudomonas demonstrated that no

particular chemical grouping of the varied anionic compounds used accounted for the inhibition of swarming. The lytic property of sodium lauryl sulfate, one of the substances used, could be demonstrated at 1:500.

Baker et al. (1941a, 1941b) used the anionic compounds sodium lauryl sulfate (C_{12}), sodium myristil sulfate (C_{14}) and cetyl sulfate (C_{16}) as well as cationic compounds such as Lauryl Pyridinium Iodide, Phemerol and Zepheran. They published a series of papers in which they concluded that the action of these detergents on bacterial metabolism was influenced by a number of factors, such as the charge on the hydrophobic group, the hydrophobic - hydrophilic balance of the molecule, the pH of the medium, the specific chemical structure of the disinfectant and finally the general and specific characteristics of the organism.

They stated that the cationic compounds were more effective than the anionic, and that the influence of the cationic was more effective in alkaline range and the anionic in acid range. Both Gram positive and Gram negative organisms were sensitive to the cationic substances used.

That the process of dying may be a reversible process (Rahn, 1932) during certain stages of the act has been illustrated by a number of workers using the quaternary compounds. Valko & Du Bois (1944) report that after Staphylococcus aureus had been exposed for 5 minutes to a concentration of five times the killing concentration of alkyldimethylbenzyl ammonium chloride, the organisms were revived by a 5-minute treatment with one

equivalent of sodium dodecyl sulfate. Lominski and Lendrum (1942) found that dextrin, gum tragacanth, gum acacia and mucin neutralized the action of anionic compounds so that the swarming of *Proteus* was intensified.

In connection with the determination of the phenol coefficient of quaternary compounds, it has been reported by Brewer (1943) that phospholipids may inactivate to some extent certain of the synthetic detergents.

With the meager information at hand regarding the mode of action of these new disinfectants, an attempt will be made to correlate the results of the foregoing experiments with the facts known regarding structure and behavior of the quaternary ammonium compounds studied, Roccal and Phemerol.

The test solution was composed of sterile tap water, a small amount of organic matter in the form of citrated blood, an anionic substance alkylaryl sodium sulfonate (Nacconal), a small amount of acid, a measured amount of cationic substance (the quaternary salt disinfectant) the composition of which is known, and lastly an inoculum of *Staphylococcus aureus* accompanied by the culture medium, Armour's peptone and bacterial metabolites. The exact nature of the three final substances is unknown.

In such a mixture of substances there will be many forces operating, some of chemical nature, others physical and still others unknown. A few of the physical and chemical reactions may be predicted, but others may only be surmised.

The mode of action which the quaternary compounds exert on

the metabolism of the bacterial cell, resulting in death, may be due to several factors. This action may be associated with electrovalency of the cell in part and in part with the action of lowering the surface tension. The quaternary compounds as well as the alkylaryl sulfonates are distinguished by the capacity of lowering the surface tension of water from 72 dynes per cm. to values ranging from 27 to 50 dynes. This may change the permeability of the cell wall, or may bring about other unknown alterations in composition, metabolic activity, etc.

That the bactericidal action of quaternary compounds is not due entirely to the surface tension relationship has been demonstrated by the examination of two compounds which produce surface tensions of equal magnitude in identical concentrations (0.1 per cent). Phenexol, for instance, is bactericidal if diluted 1:25,000, while a similar compound o-methyl-p-cetyl derivative is ineffective at 1:100 (Rawlins, Sweet and Joslyn, 1943).

Another property of the cationic detergents which must be taken into consideration and which may determine their germicidal effectiveness is that of architectural structure. When long-chain radicals are substituted for the octyl group, either in an ortho or para position on the phenyl radical, a sharp drop in germicidal activity is noted. It is in the realm of possibility that such configurations might well prevent the ion from coming in contact with the bacterial cell thru some mechanism related to spacial arrangement, bulk, or steric effects. If the lethal action is one of mere surface

phenomenon, an explanation of reduced bactericidal action may lie in such intermolecular interference. Furthermore, if such substituted cations have come in contact with the bacterial cell, the structural configuration may prevent the cation from penetrating the cell for the same reason of structure and size. If the death of the cell is due to chemical reaction between the cation and protoplasm, this reaction is therefore prevented.

In postulating the mechanisms which might govern the action of the organic cation on a bacterial cell, one is immediately confronted with the limitations of knowledge concerning the nature of single cells. A bacterial cell may be looked upon as a complex colloidal mixture of lipoids, proteins, and polysaccharides. The behavior of each species depends on its unique chemical and physical properties, as well as its environment. This bit of living protoplasm, for instance, when suspended in a neutral or slightly acid medium of low salt concentration, carries a negative charge. This charge can be reversed if the pH is lowered beyond the isoelectric point of the cell. The negatively charged bacterium will adsorb hydrogen ions (McCalla 1941) as well as metal and dye cations (McCalla, 1940). It may be assumed, therefore, that the organic cation of a quaternary salt is adsorbed by similar electrochemical forces. But this adsorption in itself may not be lethal to the cell, so the organic cation then either enters into a chemical reaction with the cell constituents, which if prolonged sufficiently, eventually brings about the death of the cell, or it

interferes with the metabolism of the cell. The latter is less probable because of the relatively rapid death of the cell.

The small increase of organic matter in the form of human plasma in the preceding experiments changed the bactericidal value of Roccal to a slight degree. This is due, no doubt, to the fact that there was little or no attraction between the cations and the substances found in citrated blood. This statement would not hold if larger amounts of organic matter were used (Klarman, 1944).

The increase of inoculum from 1.0 to 2.0 ml. of S. aureus increased the numbers of living cells from a minimum of 400 million to approximately 1 billion, which is not considered a large increase when so many cells are present. As one might expect, it reduced the germicidal action only in a small degree (table 2). The decrease was more pronounced with acetic acid than with formic acid.

Also the metabolic products of bacterial growth may interfere with the reactions between the cation and the bacteria. No doubt some of the constituents in the nutrient medium, if in sufficient amounts, as well as those in the serum may exercise a neutralizing action on the cation. It has been observed that phospholipids neutralize quaternary compounds whereas mucin and gum tragacanth display a neutralizing action on anionic compounds. Besides the possibility of this reaction due to a chemical neutralization, there may also be a physical phenomenon involved which has to do with adsorption

of the organic cation on the colloids present in citrated plasma.

It has been reported by other workers that these cationic substances are more germicidal in neutral or alkaline pH. The results of the foregoing experiments reveal that the quaternary ammonium cation is no more germicidal in a neutral or slightly alkaline medium (table 3) when an anionic substance is present. It would seem then that the deleterious influence of the anion on the cation has greater significance in these experiments, or that the anion has a neutralizing effect regardless of the pH. The neutralizing effect of the anion masks the effectiveness that the increase of pH might produce.

It is rather difficult to evaluate the effect of the acid in the test solution. Even if the ions of the acid react with those of the cation and anion, new acids or salts are formed which immediately ionize, so that the cation is left free for reaction with the bacterial cell. Since Roccal was more bactericidal at pH 5 in the presence of formic acid than it was at pH 5.5 with acetic acid, the possibility of toxic action due to the formic acid itself may be a factor of some importance. Or the difference in ionization of the two acids may play some role. The effect may be due to the charge on the bacterial cell, which changes with the pH of the suspending medium.

The most important effect shown by the detergent sodium alkylaryl sulfonate is that of the chemical reaction which presumably takes place between this anion and the cation of the disinfectant. This may be shown in the simplified

illustration:



When an organic cation of large molecular weight of approximately 320 reacts with an organic anion of similar size, approximately 248, a colloid is formed. This was demonstrated by adding .005 per cent Nacconal dropwise to a 0.1 per cent solution of Roccal. When saturated, a turbid solution resulted from which colloidal particles, on standing, settled out. Thus the effectiveness of the germicide depends on limiting the concentration of Nacconal. Indeed this fact is evident on examination of the tables in which the per cent of Nacconal is varied from .005 to .025 per cent (tables 3, 5, 7, 10). The germicidal efficiency of the disinfectant may depend on the outcome of competitive forces between the negatively charged bacterial cell and the anion, both contending for the positively charged cation. If the bacterial cell is to be made the successful rival, then the concentration of anionic substance should be reduced as much as possible.

When Nacconal is replaced with sodium stearate, a greater neutralization reaction of the cationic ion occurs. The sulfonates are to be preferred over soaps since they are stable in the range of pH of the laundry rinse.

Many biologists (Rahn et al., 1932) and more recently Katzin and Sandholzer (1943) propose that the order of death in bacteria approximates monomolecular reaction rates. The "rate of reaction" or "reaction velocity" is mathematically identical with the death rate in bacteria which are exposed to a

germicidal chemical. To reach an endpoint when all bacteria are dead necessitates a definite concentration of the disinfectant over a specified period of time. To accomplish this complete destruction in less time, either the temperature has to be increased, to increase the rate of reaction, or the time may be reduced by increasing the reacting substance. A comparison of tables 2 and 3 shows that by reducing the time from 5 minutes to 3, a greater concentration was necessary to produce killing.

Roccal and Phemerol both exhibit temperature coefficients in that the concentration necessary for killing was decreased 50 per cent by a 14° raise in temperature, i.e., from 23° to 37°C . (tables 4, 5, 8, 9, 10). At 48°C ., heat disinfection as well as bactericidal effects may be expected and from a practical standpoint a high temperature in laundering is to be recommended whenever possible.

A comparison of the bactericidal dilutions of Roccal and Phemerol would seem to show that Roccal is a more effective germicide (tables 3, 4, 5 and 8, 9, 10). A more exact comparison of the activity of the molecules could be demonstrated if the dilutions were made on a molar rather than a per cent basis. Ten per cent Roccal is approximately .32 molar whereas 10 per cent Phemerol is about .25 molar. Even with this correction, results of the experiments indicate that the molecules of Roccal are more efficient in producing that effect on the bacterial cell which ultimately terminates in death.

Phemerol also appears to be less reactive toward the anionic substance Nacconal, especially at higher temperatures. This

property of being less reactive might be attributed again to the architectural structure or steric effects which may make union between the organic anions and cations difficult or impossible.

That Roccal is more effective bactericidally for Staphylococcus aureus than it is for Aerobacter aerogenes and other Gram negative organisms is no doubt due to the nature of these bacteria, possibly in part to the same chemical characteristics which makes them Gram negative. If death of the bacteria is due to penetration of the cation into the cell with a subsequent chemical reaction, the insensitivity would indicate a lesser amount of reacting substances. The possibility also suggests itself that the nature of the negative charge is such that there is less electro-chemical attraction between the Gram negative organisms and cations. In encapsulated organisms the polysaccharide of the capsule may well interfere with bactericidal activity of the disinfectant.

The case of Pseudomonas aeruginosa may represent a specialized type of metabolism. The fact that this organism produces pyocyanin, a water-soluble basic substance, indicates unique metabolic activity. The study of the chemical nature of these substances in relation to quaternary cations might yield some pertinent information as to the basis for the resistance of this species to the quaternary compounds. Moreover, the change of oxidation-reduction potential produced by these organisms should not be neglected in the study of their resistance.

The organisms to be encountered in a laundry rinse will include all types of which the Gram positive *Staphylococcus* represents the most resistant of the coccus group. There will be Gram positive rods of pathogenic nature as well as the many Gram negative enteric organisms which will show varied resistance to these new types of disinfectants.

No study with disinfectants would be complete without mentioning errors involved. Just as there is no perfect disinfectant that can be used under all conditions, neither has a perfect method been devised for testing disinfectants. The method used in the preceding study is subject to the usual errors of measurements, random sampling, and variation in inoculum resulting from differences in lots of Armour's peptone, to mention a few. The technic used in the tests revealed some unusual conditions. It was felt that survival of the organisms in the foam or suds was the usual cause of the inconsistent results. Since both the cationic and anionic detergents used produce some foaming, this factor cannot be overlooked in actual laundry practice. The most effective remedy in such practice would be elimination of the neutralizing substance Naeconal in so far as possible.

It may be said in conclusion, that, of the products tested, the most suitable disinfectant for the Army Mobile Laundry Units under the conditions outlined is found among the quaternary compounds. Furthermore, Roccal best fits the requirements. This disinfectant, in so far as this problem carried the investigation, fits the specifications prescribed for the Bureau of Ships by the U. S. Navy as of May 1943.

SUMMARY AND CONCLUSIONS

A technic was designed to determine the bactericidal dilution of a disinfectant which could be used in the final rinse in mobile laundry units. An attempt was made to anticipate actual field conditions as to numbers of organisms, amount of residual soap, organic matter and temperature variations. A comparison of different "souring" agents was made. Detailed studies of two quaternary ammonium compounds were carried out. The detailed studies indicated that with Roccal slight increases of organic matter in the form of citrated blood changed the bactericidal property very little. A small increase of inoculum likewise had very little neutralizing effects on this germicide. Formic acid interfered less than acetic acid with the germicidal property of Roccal. On the other hand, the detergent Nacconal (.005 per cent) neutralized markedly the bactericidal activity of Roccal. With the preceding information established, the higher concentrations of plasma and the larger inoculum of organisms were adopted for further tests.

A comparison of the effects of Nacconal and sodium stearate demonstrated that the latter reduced the potency of Roccal slightly more than did Nacconal. It might be expected, therefore, that any soap would have this effect. Although Roccal is said to be more bactericidal in an alkaline pH, this effect was not detected when Nacconal was present. Nacconal presumably destroyed or masked such a tendency.

With all conditions constant except time, it was found that a decrease of exposure time from 5 to 3 minutes required the utilization of a two-fold increase of Roccal concentration to obtain similar bactericidal action.

In the lower concentration of Nacconal (acetic acid sour) Roccal exhibited a remarkable increase in efficiency with increase in temperature. When U.S.G. sour (10 per cent sodium acid fluoride and 90 per cent sodium silica fluoride) was used under the same conditions, Roccal displays a greater germicidal effect at 23°C., less effective at 37°C. and increased again at 48°C. From results obtained using Nacconal at a higher concentration (.025 per cent), it was evident that the increase of Nacconal nullified the bactericidal effects to be gained by an increase of temperature.

When organisms were exposed to U.S.G. sour with all substances present except the disinfectant a 50 per cent reduction of organisms was obtained at the 48°C., indicating some bactericidal action due to heat sterilization fortified by acid. This is to be expected since heat disinfection rate is increased with lower pH.

To summarize the results of Phemerol, it may be said that this substance in a 5-minute exposure without Nacconal displays bactericidal efficiency at 1:4000, which is reduced to 1:3000 when Nacconal (.025 per cent) is added.

When the time was reduced to 3 minutes with only .005 per cent Nacconal, the bactericidal dilution was reduced drastically. There was marked increase of bactericidal action at both 37°C and 48°C. under these conditions. At the same elevated temperatures

when Nacconal was increased (.025 per cent), the bactericidal action still was increased, which is the opposite effect from that noted with Roccal. Thus Phemerol was less sensitive to the neutralizing action of Nacconal than was Roccal.

When the sour was changed from acetic to U.S.G., Phemerol tended to be less bactericidal (at similar concentrations of Nacconal and at the same temperatures) than when acetic acid was the sour.

In an attempt to compare Roccal and Phemerol as bactericides under identical conditions Roccal showed a distinct advantage at both 37° and 48°C. (.005 per cent Nacconal). However, when Nacconal was increased to .025 per cent, at 23°C. Roccal was four times as effective as Phemerol. In contrast, at 37°C. Phemerol was effective at 1:3000 and Roccal at only 1:2000. At 48°C. both Phemerol and Roccal were bactericidal at 1:4000. Thus it may be stated that at lower temperature and low concentration of Nacconal (.005 per cent) Roccal is more effective than Phemerol. If the temperature and Nacconal concentration is increased (.025 per cent), this characteristic is lost and the two substances approach equal values in action.

The disinfectants Shirlan and Benchlophen and the anti-septic solution known as Sanitized were eliminated after preliminary examination for reasons of insolubility or lack of germicidal activity.

In the second part an attempt was made to evaluate under more practical conditions the results obtained regarding bactericidal dilutions of Roccal. The effectiveness of the

disinfectant was tested against S. aureus impregnated into cloth swatches. The cloth was immersed in the solution in a jar designed so that the permeation of the disinfectant could be facilitated by a tumbling action. Since the size of the apparatus and the mechanical manipulation prevented the determinations being made at elevated temperatures, information was obtained only at 23°C. A dilution of 1:1000 was effective in killing S. aureus in 3 minutes when either .005 or .025 per cent Naconal was present. This dilution is considerably more concentrated than was found effective in the previous culture tests. It is important to emphasize that the nature of the cloth used was such that it resisted wetting. Presumably garments which had previously been sudsed and rinsed would absorb the water and disinfectant more readily.

In contrast, Roccal at 1:1000 did not completely kill the encapsulated strain of Aerobacter aerogenes in either a 3 or 15-minute exposure when tested in the cloth swatch.

If further studies were made using a large variety of organisms, a more complete evaluation of these disinfectants, Roccal and Themerol, could be obtained. Pertinent information regarding different pathogens, including such spore formers as Bacillus anthracis and the Clostridia, and the effects on the higher forms of Actinomyces and molds might well be developed. More information on the action on the Gram negative organisms, as well as on the protozoa and other forms of life in open streams, is necessary before the use of such a disinfectant in the laundry rinse may be relied upon to give safety to the wearer.

Since the disinfectant is to be used in the final rinse in laundry, some information as to the toxicity to the skin should be available before such a treatment is put into practice on a large scale. The results from some fourteen tests carried out to obtain information of this nature, hint that no such irritation would be expected, since after forty-eight hours, examination revealed no irritating effects nor was any physical discomfort reported during this time. Due to the small number of tests made the results merely suggest that 1:1000 Roccal would not be likely to be toxic when used in this manner.

In an attempt to postulate the mechanism by which the quaternary compounds produce death in the bacterial cell, the unique characteristics of these organic cations were pointed out. It was emphasized that their ionization with positive charge and surface tension depression tendencies with adsorption phenomena should be taken into consideration. Further, it was pointed out that the architectural structure and steric effects may control the efficacy with which the disinfectant is adsorbed by the cell. If death is due to penetration of the cell, this too may be prevented by such structural configuration.

It has been noted that a number of anionic substances show neutralizing effects on the bactericidal action of the cationic ions. Nacconal exhibits this type of reaction.

On the other hand, the characteristics of different species of bacteria and their behavior in a suspending medium

govern to some extent the effects of the germicide. These effects can be due to the charge on the bacteria at different pH values, the permeability of the cell, the specific chemical composition of the protoplasm, the type of metabolism, the presence of a capsule as compared with a naked cell, and the production of neutralizing metabolic substances.

LITERATURE CITED

- Anonymous, 1942. *Laundries, Laundry Battalions and Laundry Companies Technical Manual*. War Department.
- Baker, Zelma, Harrison, R. W. and Miller, Benjamin F. 1941b. Action of synthetic detergents on the metabolism of bacteria. *J. Exp. Med.* 53: 249-253.
- Baker, Zelma, Harrison, R. W. and Miller, Benjamin F. 1941a. The bactericidal action of synthetic detergents. *J. Exp. Med.* 74: 611-620.
- Brewer, C. M. 1943. Variations in phenol coefficient determinations of certain disinfectants. *Am. J. Public Health.* 33: 261-264.
- Domagk, G. 1935. Eine neue Klasse von Desinfektionsmitteln. *Deutsche. Med. Wochschr.* 61: 829-832.
- Dunn, Cecil G. 1937. Antiseptic and germicidal properties of a mixture of high molecular alkyldimethylbenzyl ammonium chlorides. *Am. J. Hyg.*, 26: 46-52.
- Epstein, Albert K., Harris, Benjamin R. and Katzman, Morris. 1943. Relationship of bactericidal potency to length of fatty acid radical of certain quaternary ammonium derivatives. *Proc. Soc. Exptl. Biol. Med.*, 53: 238-241.
- Joslyn, D. A., Yaw, Katherine and Rawlins, A. W. 1943. Germicidal efficacy of Phemerol. *J. Am. Pharm. Assoc.* 32: 49-51.
- Katz, J. and Lipsitz, Aaron. 1935. Studies on the effect of synthetic surface active materials on bacterial growth. *J. Bact.*, 30: 419-422.
- Katzin, L. I. and Sandholzer, L. A., 1943. Bacterial death rates and decimal reduction time. *J. Bact.*, 45: 19
- Klarman, E. G. and Wright, Eleanore S. 1944. Effect of organic matter on germicidal performance. *Soap and Sanitary Chemicals.* Feb. p. 103.
- Kolthoff and Sandell. 1943. *Textbook of quantitative analyses.* 2nd Ed., The Macmillan Company, New York.
- Langmuir, I. 1917. Fundamental properties of solids and liquids. *J. Am. Chem. Soc.*, 39: 1848.
- _____ Overturning and anchoring of monolayers. *Science.* 1938, 87: 493-500.

- Lominsky, I. and Lendrum, A. C., 1942. The effect of surface-active agents on B proteus. J. Path. and Bact., 54: 421-33.
- McCalla, T. M. 1940. Cation adsorption by bacteria. J. Bact., 40: 23-32.
- _____ The adsorption of H ion by bacteria as measured by the glass electrode. J. Bact., 41: 775-784.
- McCulloch, Ernest C. 1936. Disinfection and sterilization. Lea and Febiger, Philadelphia.
- Rahn, Otto. 1932. Physiology of bacteria. W. Blakiston's Son & Co., Inc., Philadelphia.
- Rawlins, A. L., Sweet, L. A. and Joslyn, D. A. 1943. Relationship of chemical structure to germicidal activity of a series of quaternary ammonium salts. J. Am. Pharm. Assoc., 32: 11-16
- Ruehle, G. L. A. and Brewer, C. W., 1931. U. S. Food and Drug Administration methods of testing antiseptics and disinfectants. U. S. Dept. Agr. Cir. 198.
- Service Bulletin #53, 1942. Sanitary aspects of commercial laundering. Department of Research and Textiles, American Institute of Laundering, Joliet, Illinois.
- Sidgwick's Organic chemistry of nitrogen. 1937. Taylor and Baker. New Edition. At the Clarendon Press, Oxford.
- Special Report #7, 1942. Control of the souring operation. Department of Research and Textiles, American Institute of Laundering, Joliet, Illinois.
- Valko, E. I. and Du Bois, A. S. 1944. The antibacterial action of surface active cations. J. Bact., 47: 15-25
- Winslow, C. E. A. and Haywood, Eloise T. 1931. The specific potency of certain cations with reference to their effect on bacterial viability. J. Bact., 22: 49-58