

EFFECT OF THE CHEMICAL CONSTITUTION OF SOAPS UPON THEIR GERMICIDAL PROPERTIES¹

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Studies on the properties of soaps are of importance because of their common use as detergents, in the preparation of vaccines, in the treatment of disease, and because of their natural occurrence in the animal body where they may have some effect in determining the resistance of the animal organism to infection.

Reichenbach (1908) studied the action of soaps on *Escherichia coli* and found that potassium stearate, palmitate, and myristate were effective in killing this organism, while the oleate, erucate, and linoleate were inactive.

Walker (1924–1926) showed that sodium and potassium soaps of the same acid did not vary greatly in their germicidal action. The lower members of the series such as the butyrate, valerate, caprylate, and caproate, had little or no germicidal effect. He found the pneumococcus to be very susceptible to laurates, oleates, linoleates, and linolenates. Streptococci were killed much like the pneumococci but at higher concentrations of the soap. *Escherichia coli*, *Eberthella typhi*, *Shigella paradysenteriae*, *Salmonella paratyphi* were killed by moderate concentrations (N/20 to N/40) of the saturated soaps (laurates to stearates) but were very resistant to the unsaturated soaps.

Eggerth (1926–1931) studied the germicidal action of alpha substituted soaps. He found that the soaps of the alpha bromo fatty acids were usually more germicidal than the unsubstituted. The alpha hydroxy soaps exhibited a high germicidal activity

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toward certain microorganisms. The effect of the hydroxyl in the alpha position was to increase the selective germicidal action of saturated soaps and diminish that of the unsaturated. Certain of the alpha mercapto and disulfo soaps proved to be powerful germicides.

Recently there has been considerable interest manifested in the germicidal properties of sodium ricinoleate. Larson (1921) found that pneumococci and streptococci would not grow in the presence of even small amounts of sodium ricinoleate. Larson and Nelson (1925) reported that pneumococci instantly lost their pathogenicity on treatment with castor oil soap of a final dilution of 0.1 per cent. Scarlet fever streptococci lost their power to grow after 5 minutes in 0.5 per cent sodium ricinoleate.

Miller and Castles (1931) found that sodium ricinoleate inhibited the growth of gonococci on artificial media in dilutions of 1:20,000.

Violle (1933) studied the effect of a 1:1000 solution of castor oil soap on many kinds of bacteria. The common and pathogenic bacteria of the intestinal tract were unaffected. Streptococci were killed, but staphylococci were not.

Barnes and Clark (1934) from their experiments determined that 0.004 per cent sodium ricinoleate and 0.0004 per cent sodium oleate were approximately the minimal pneumococcal concentrations of the soaps against three types of pneumococci.

Kolmer, Rule, and Madden (1934) reported that whereas a 20 per cent solution of sodium ricinoleate was completely bactericidal for *Staphylococcus aureus* in an exposure of 5 minutes, yet a 10 per cent solution was not completely bactericidal in exposures as long as 1 hour when tested according to the Reddish method.

Hettche (1934) found that the unsaturated soaps such as the oleate, linoleate, and linolenate, were germicidal toward the staphylococcus but not toward the colon bacillus.

EXPERIMENTAL

The soaps and closely related compounds studied in this investigation, along with their structural formulas, melting points or iodine numbers, and sources or methods of preparation are given in table 1.

The technique used in the experimental work was planned with the idea in mind that these soaps might have some therapeutic value. Sodium ricinoleate has been studied quite extensively in this direction by Kolmer, Rule, and Madden (1934) and others, as has sodium chaulmoograte and similar soaps on the acidfast bacteria by Adams and his co-workers (1932).

Each of the soaps has been tested for its germicidal effectiveness in 1 per cent solution, using *Staphylococcus aureus* (F. D. A. strain) and *Escherichia coli-communis* (U. of M. strain). The least amounts necessary to kill *Diplococcus pneumoniae* (type I) and *Streptococcus lactis* have been determined in two different manners. In all this work, the soaps and solutions have been adjusted as closely as possible to a pH of 8.0, as determined by the glass electrode method, except where otherwise stated.²

The soap solutions were in general prepared by dissolving weighed quantities of the fatty acids in a slight excess of dilute sodium hydroxide to make a 2 per cent solution, which was then adjusted to the proper pH by the addition of dilute hydrochloric acid. The solutions were sterilized by heating in boiling water. Dilutions were made with sterile distilled water. The germicidal tests using *Diplococcus pneumoniae* (type I) and *Streptococcus lactis* were performed with the soap solution containing N/30 phosphate buffer of pH 8.0.

The organisms used in performing the tests were the Insecticide Board strain of *Staphylococcus aureus*, University of Minnesota strain of *Escherichia coli-communis*, University of Minnesota strain of *Diplococcus pneumoniae*, type I, pathogenic to mice and rabbits, and *Streptococcus lactis* which was isolated from milk and grew well at 37°C. The *Streptococcus lactis* had a tendency to lose its ability to ferment lactose without any change in its other fermentation reactions or in its resistance to phenol or soaps. Once it had lost its ability to ferment lactose, it did not regain this attribute under any of the conditions to which it was subjected. The test cultures were grown in nutrient broth, transferred every 24 hours, and incubated at 37°C. The nutrient broth was prepared in accordance with the recommendations

² The glass electrode instrument was kindly furnished by Dr. Allen Hemingway (1935) of the Department of Physiological Chemistry, University of Minnesota.

TABLE 1
Structural formulas of compounds

SUBSTANCE	FORMULA	MELTING POINT	IODINE NUMBER	SOURCE OR METHOD OF PREPARATION
Stearic acid	$\text{CH}_2(\text{CH}_2)_{16}\text{COOH}$	69-70°		Eastman Kodak Company
Palmitic acid	$\text{CH}_2(\text{CH}_2)_{14}\text{COOH}$	61-62°		Eastman Kodak Company
Myristic acid	$\text{CH}_2(\text{CH}_2)_{12}\text{COOH}$	52-53°		Eastman Kodak Company
Lauric acid	$\text{CH}_2(\text{CH}_2)_{10}\text{COOH}$	42-43°		Eastman Kodak Company
Oleic acid	$\text{HC}(\text{CH}_2)_7\text{COOH}$ 			Eastman Kodak Company
Linoleic acid	$\text{HC}(\text{CH}_2)_7\text{CH}_2$ $\text{CH}_2(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$		175	Reduction tetrabromide (M. P., 113-114°) from cottonseed oil
Linolenic acid	$\text{CH}_2\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$		268	Reduction hexabromide (M. P., 179-180°) from linseed oil
Clupanodonic acid	$\text{CH}_2(\text{CH}_2)_2\text{C}\equiv\text{C}(\text{CH}_2)_6\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$		312	Reduction octobromide (M. P., greater than 200) from cod liver oil
Alpha elaeostearic acid	$\text{CH}_2(\text{CH}_2)_4\text{CH}=\text{CHCH}=\text{CHCH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	47-48°		Crystallization of fatty acids of tung oil
Beta elaeostearic acid	$\text{CH}_2(\text{CH}_2)_4\text{CH}=\text{CHCH}=\text{CHCH}=\text{CHCH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	71-72°		Action of crystal of iodine on alpha elaeostearic acid
Undecylenic acid	$\text{CH}_2=\text{CH}(\text{CH}_2)_9\text{COOH}$			Vacuum distillation of castor oil
Ricinoleic acid	$\text{HC}(\text{CH}_2)_7\text{COOH}$ $\text{HCCH}_2\text{CHOH}(\text{CH}_2)_6\text{CH}_2$		84	Purification of castor oil fatty acid

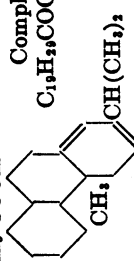
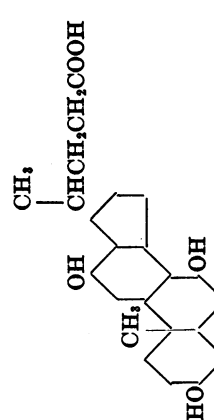
Ricinelaic acid	$\begin{array}{c} \text{HC}(\text{CH}_2)_7\text{COOH} \\ \\ \text{CH}_2(\text{CH}_2)_7\text{CHOHCH}_2\text{CH} \\ \\ \text{C}(\text{CH}_2)_7\text{COOH} \\ \\ \text{C}(\text{CH}_2\text{CHOH}(\text{CH}_2)_7\text{CH}_2 \end{array}$	52-53°	Action of HNO ₃ on ricinoleic acid
Ricinstearic acid	$\begin{array}{c} \text{CH}_2(\text{CH}_2)_7\text{CHOHCH}_2\text{CH} \\ \\ \text{C}(\text{CH}_2)_7\text{COOH} \\ \\ \text{C}(\text{CH}_2\text{CHOH}(\text{CH}_2)_7\text{CH}_2 \end{array}$	51-52°	Action of alcoholic KOH on ricinoleic dibromide
Abietic acid	<p>Complex salt C₁₉H₂₉COONa · 3C₁₀H₁₆O₂</p> 	166-167°	Purification of rosin
Chaulmoogric acid	$\begin{array}{c} \text{CH} = \text{CH} \\ \\ \text{CH}_2 - \text{CH}_2 \\ \\ \text{CH}(\text{CH}_2)_{12}\text{COOH} \end{array}$	69-70°	Eastman Kodak Company
Trihydroxystearic acid	$\begin{array}{c} \text{HCOOH}(\text{CH}_2)_7\text{COOH} \\ \\ \text{HCOHCH}_2\text{CHOH}(\text{CH}_2)_7\text{CH}_2 \\ \text{CH}_2(\text{CH}_2)_7\text{CHOHCHOHCH}_2\text{CHOHCHOH} \\ (\text{CH}_2)_7\text{COOH} \\ [\text{CH}_2\text{OH}(\text{CHOH})_7\text{COO}]_2\text{Ca} \end{array}$	140-141°	Alkaline oxidation of sodium ricinoleate
Satvic acid	$\begin{array}{c} \text{HCOHCH}_2\text{CHOH}(\text{CH}_2)_7\text{CH}_2 \\ \text{CH}_2(\text{CH}_2)_7\text{CHOHCHOHCH}_2\text{CHOHCHOH} \\ (\text{CH}_2)_7\text{COOH} \\ [\text{CH}_2\text{OH}(\text{CHOH})_7\text{COO}]_2\text{Ca} \end{array}$	172-173°	Alkaline oxidation of linoleic acid Eastman Kodak Company
Calcium gluconate	$[\text{CH}_2\text{OH}(\text{CHOH})_7\text{COO}]_2\text{Ca}$		Eastman Kodak Company
Cholic acid			Sodium cholate

TABLE I—Concluded

SUBSTANCE	FORMULA	MELTING POINT	IODINE NUMBER	SOURCE OR METHOD OF PREPARATION
Dehydrocholic acid Sodium taurocholate	—OH s in cholic acid replaced by —O CHCH ₂ CH ₂ COOH in cholic acid replaced by CHCH ₂ CH ₂ CONHCH ₂ CH ₂ SO ₂ Na CHCH ₂ CH ₂ COOH in cholic acid replaced by CHCH ₂ CH ₂ CONHCH ₂ COONa			Riedel de Haen Company Pfanstiehl Company Pfanstiehl Company
Salicylic acid	$\begin{array}{c} \text{COOH} \\ \\ \text{C}_6\text{H}_4 \\ \\ \text{OH} \end{array}$ $\begin{array}{c} \text{COOH} \\ \\ \text{C}_6\text{H}_3(\text{I})_2 \\ \\ \text{OH} \end{array}$	155–157°		Eastman Kodak Company
3,5 diiodosalicylic acid	$\begin{array}{c} \text{COOH} \\ \\ \text{C}_6\text{H}_3(\text{I})_2 \\ \\ \text{OH} \end{array}$	235–236°		Eastman Kodak Company
Sodium lauryl sulfate Sodium oleyl sulfate	$\text{CH}_2(\text{CH}_2)_{10}\text{CHOSO}_2\text{Na}$ $\text{CH}_2(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CHOSO}_2\text{Na}$			Extraction of Gardinol WA with C ₂ H ₅ OH Same with Gardinol CA Gardinols obtained from Dupont de Nemours Company

for the Reddish tests, and the nutrient agar used for the final plates was of the same composition, except that 1.5 per cent agar was added.

Plate counts were used in the case of *Staphylococcus aureus* and *Escherichia coli* because of the high resistance of these two organisms to soaps. Plate counts aid in revealing slight bactericidal action, and help to overcome the errors of random sampling, as shown by Cade and Halvorson (1934). Phenol controls were run with each of the tests in order to determine whether the organism had a constant resistance equal to that recommended by Reddish (1928).

DISCUSSION

In this investigation, the germicidal action of 27 soaps and other sodium salts of carboxylic acids and sulfate esters has been tested on four different organisms. Carboxylic acids containing in their structural makeup aromatic rings, iodine, 1 to 4 double bonds, 1 to 5 hydroxyl and cyclopentenyl rings, as well as two sulfate esters were included among those tested. Most of the fatty acids studied in this series occur naturally in mixtures containing several individuals. A few, such as ricinelaidic, ricin-stearolic, trihydroxy stearic, sativic and beta elaeostearic, have been synthesized in order to determine how changes brought about in the molecule alter the effect of the soap on microorganisms. The salicylates have been included because of their wide use in acute rheumatic fever. The sulfate esters are new commercial products being sold under the trade names of "Dreft," "Gardinol," and "Brilliant Aviol." These esters are excellent surface tension depressants whose chief feature is their solubility in the presence of calcium, magnesium, hydrogen, or hydroxyl ions.

A comparison of the pneumococidal properties of the saturated soaps, laurate, myristate, palmitate, and stearate, shows that there is a maximum in this property with the soap containing fourteen carbon atoms. The low activity of the sodium stearate is probably due to its low solubility. The 1 per cent solution of this soap at 37°C. was a semi-rigid gel. The

TABLE 2
Concentrations of chemical sterilizing in 10 but not in 5 minutes

SUBSTANCE	DIPLOCOCCUS PNEUMONIAE	STREPTOCOCCUS LACTIS
	<i>per cent</i>	<i>per cent</i>
Germicide:		
Phenol, 20°.....	1 (1-100)	1.25 (1- 80)
Phenol, 37°.....	0.588 (1-170)	0.833 (1-120)
Saturated soaps:		
Sodium stearate.....	1.0	1.0
Sodium palmitate.....	0.1	1.0
Sodium myristate.....	0.01	0.1
Sodium laurate.....	0.04	0.1
Unsaturated soaps:		
Sodium oleate.....	0.004	0.1
Sodium linoleate.....	0.005	0.02
Sodium linolenate.....	0.005	0.02
Sodium clupanodone.....	0.005	0.02
Sodium alpha elaeostearate.....	0.03	>5.0
Sodium beta elaeostearate.....	0.4	>2.0
Sodium undecylenate.....	0.2	0.6
Unsaturated hydroxylated soaps:		
Sodium ricinoleate.....	0.025	0.06
Sodium ricinelaide.....	0.05	0.6
Sodium ricinostearate.....	0.07	2.0
Unsaturated alicyclic soaps:		
Sodium abietate.....	0.06	0.2
Sodium chaulmoograte.....	0.04	0.5
Hydroxylated salts:		
Sodium trihydroxy stearate.....	>2.0	>2.0
Sodium sativate.....	0.05	1.0
Calcium gluconate.....	>10.0	>10.0
Bile salts:		
Sodium cholate.....	7.0	>10.0
Sodium dehydrocholate.....	>4.0	>4.0
Sodium taurocholate.....	7.0	>10.0
Sodium glycocholate.....	10.0	>10.0
Aromatic carboxylic acid salts:		
Sodium salicylate.....	5.0	>10.0
Sodium diiodosalicylate.....	0.1	0.3
Sulfate ester salts:		
Sodium lauryl sulfate.....	0.01	0.02
Sodium oleyl sulfate.....	0.01	0.02

TABLE 3
Effect upon *Staphylococcus aureus* (F. D. A. strain)

SUBSTANCE	CONCENTRATION	PLATE COUNTS		
		5 minutes	10 minutes	15 minutes
	<i>per cent</i>			
Germicide:				
Phenol.....	1, 11 (1-90)	300	50	10
	1 (1-100)	600	200	50
Saturated soaps:				
Sodium stearate.....	1	10,000+	10,000+	10,000+
Sodium palmitate.....	1	10,000+	10,000+	10,000+
Sodium myristate.....	1	10,000+	10,000+	10,000+
Sodium laurate.....	1	10,000	8,000	6,000
Sodium laurate (pH 8.8).....	1	9,000	7,000	4,000
Unsaturated soaps:				
Sodium oleate.....	1	10,000+	10,000+	10,000+
Sodium linoleate.....	1	10,000+	10,000+	10,000+
Sodium linolenate.....	1	10,000+	10,000+	10,000+
Sodium clupanodionate.....	1	10,000+	10,000+	10,000+
Sodium alpha elaeostearate.....	1	10,000+	10,000+	10,000+
Sodium beta elaeostearate.....	1	10,000+	10,000+	10,000+
Sodium undecylenate.....	1	600	250	100
Unsaturated hydroxylated soaps:				
Sodium ricinoleate.....	1	10,000+	10,000+	10,000+
Sodium ricinelaideate.....	1	10,000+	10,000+	10,000+
Sodium ricinistearolate.....	1	10,000+	10,000+	10,000+
Unsaturated alicyclic soaps:				
Sodium abietate (pH 9.0).....	1	5	1	0
Sodium abietate (pH 8.7).....	1	150	70	30
Sodium chaulmoograte.....	1	10,000+	10,000+	10,000+
Hydroxylated salts:				
Sodium trihydroxy stearate.....	1	10,000+	10,000+	10,000+
Sodium sativeate.....	1	10,000+	10,000+	10,000+
Calcium gluconate.....	1	10,000+	10,000+	10,000+
Bile salts:				
Sodium cholate.....	1	10,000+	10,000+	10,000+
Sodium dehydrocholate.....	1	10,000+	10,000+	10,000+
Sodium taurocholate.....	1	10,000+	10,000+	10,000+
Sodium glycocholate.....	1	10,000+	10,000+	10,000+

TABLE 3—*Concluded*

SUBSTANCE	CONCENTRATION	PLATE COUNTS		
		5 minutes	10 minutes	15 minutes
	<i>per cent</i>			
Aromatic carboxylic acid salts:				
Sodium salicylate.....	1	10,000+	10,000+	10,000+
Sodium diiodosalicylate.....	1	5,000	1,500	500
Sulfate esters:				
Sodium lauryl sulfate.....	1	10,000+	10,000+	10,000+
Sodium oleyl sulfate.....	1	10,000+	10,000+	10,000+

palmitate soap shows an increased destructive action on the pneumococcus, most probably because of its increased solubility. The myristate exhibits the most germicidal activity against the pneumococcus of these saturated soaps, with the laurate soap second in this property.

The presence of unsaturated groupings in the molecule in some cases alters tremendously the effect of the soap upon pneumococci. The sodium oleate which comprises approximately 50 per cent of ordinary tallow soap is more than 100 times as effective as either sodium stearate or phenol in destroying the reproductive ability of the pneumococci. The addition of one, two, or three more double bonds as in the linoleate, linolenate, and clupanodionate soaps, respectively, causes no change in this property. However, the two soaps, sodium alpha and beta elaeostearates, although isomeric with sodium linolenate, are relatively ineffective as pneumococicides. The former of these two soaps, which occurs naturally in tung oil, is as soluble as the sodium linolenate and has much the same physical and chemical properties. Yet a mere change in position of the unsaturated linkages markedly altered the property of destroying this organism.

The three unsaturated soaps containing hydroxyl groups, as well as the two alicyclic unsaturated soaps, show pneumococidal properties considerably less than those of sodium oleate, etc. The ricinelaidate, a geometric isomer of sodium ricinoleate which has physical properties much like the stearate and palmitate, is

TABLE 4
Effects upon Escherichia coli communis (University of Minnesota strain)

SUBSTANCE	CONCENTRATION	PLATE COUNTS		
		5 minutes	10 minutes	15 minutes
	<i>per cent</i>			
Germicide:				
Phenol.....	.83 (1-120)	0	0	0
	.71 (1-140)	200	2	0
	.62 (1-160)	4,000	250	10
	.56 (1-180)	10,000+	10,000	1,500
Saturated soaps:				
Sodium stearate.....	1	10,000+	10,000+	10,000+
Sodium palmitate.....	1	10,000+	10,000+	10,000+
Sodium myristate.....	1	10,000+	10,000+	10,000+
Sodium laurate.....	1	10,000+	10,000	8,000
Sodium laurate (pH, 8.8).....	1	1,000	500	100
Unsaturated soaps:				
Sodium oleate.....	1	10,000+	10,000+	10,000+
Sodium linoleate.....	1	10,000+	10,000+	10,000+
Sodium linolenate.....	1	10,000+	10,000+	10,000+
Sodium clupanodonate.....	1	10,000+	10,000+	10,000+
Sodium alpha elaeostearate.....	1	10,000+	10,000+	10,000+
Sodium beta elaeostearate.....	1	650	400	300
Unsaturated hydroxylated soaps:				
Sodium ricinoleate.....	1	10,000+	10,000+	10,000+
Sodium ricinelaidate.....	1	10,000+	10,000+	10,000+
Sodium ricinostearolate.....	1	10,000+	10,000+	10,000+
Unsaturated alicyclic soaps:				
Sodium abietate (pH 9.0).....	1	10,000+	10,000	8,000
Sodium chaulmoograte.....	1	10,000-	10,000+	10,000+
Hydroxylated salts:				
Sodium trihydroxy stearate.....	1	10,000+	10,000+	10,000+
Sodium sativate.....	1	10,000+	10,000+	10,000+
Calcium gluconate.....	1	10,000+	10,000+	10,000+
Bile salts:				
Sodium cholate.....	1	10,000+	10,000+	10,000+
Sodium dehydrocholate.....	1	10,000+	10,000+	10,000+
Sodium taurocholate.....	1	10,000+	10,000+	10,000+
Sodium glycocholate.....	1	10,000+	10,000+	10,000+

TABLE 4—*Concluded*

SUBSTANCE	CONCENTRATION	PLATE COUNTS		
		5 minutes	10 minutes	15 minutes
	<i>per cent</i>			
Aromatic carboxylic acid salts:				
Sodium salicylate.....	1	10,000+	10,000	8,000
Sodium diiodosalicylate.....	1	1	0	0
Sulfate esters:				
Sodium lauryl sulfate.....	1	10,000+	10,000+	10,000+
Sodium oleyl sulfate.....	1	10,000+	10,000+	10,000+

somewhat less effective than the ricinoleate. The ricinstearolate, containing a triple bond instead of a double bond and with physical properties very similar to the ricinoleate, is even less active than the ricinelaideate. Sodium undecylenate containing eleven carbon atoms and a double bond at the end of the hydrocarbon chain requires a fairly high concentration in order to destroy this organism.

Sodium abietate, which is an alicyclic soap and the main constituent of rosin soap, has a destructive action on the pneumococcus in one-tenth the concentration necessary for phenol. The same is true of chaulmoograte, which has been widely heralded as a more or less specific chemotherapeutic agent in the treatment of leprosy. The hydroxylated salts, gluconate and trihydroxy stearate, seem to lack any ability to kill the pneumococci under the conditions employed. The sativate is peculiar in that the addition of a fourth hydroxyl group restores the power to destroy this organism in fairly low concentrations. The bile salts, in spite of the time honored publicity as to their ability to dissolve and kill this relatively delicate organism, are extremely inefficient in both respects as compared with other soaps. The sodium dehydrocholate, which Ziegler (1931) recommends for treating pneumonia, is in the same class as the naturally occurring bile salts in regard to its germicidal properties. Sodium salicylate requires a 5 per cent solution to kill the pneumococci in 10 minutes, while the addition of two iodine atoms to the molecule increases

its efficiency in this respect fifty times. The sulfate esters, which are now gaining wide popularity as hard water soaps, have an efficiency equal to the best of the saturated soaps in killing the organism which is the chief cause of lobar pneumonia.

The effect of the soaps upon the streptococci which cause souring of milk and which are among the most resistant to disinfection of the streptococci does not parallel the effect of the same soaps on the pneumococci. In no case, however, did it require a lower concentration to kill streptococci than to kill pneumococci, although in a few cases the values are very close. Of the saturated soaps, only the myristate and laurate exhibited killing action on *Streptococcus lactis*. The addition of one double bond as in sodium oleate gives the soap the ability to kill this organism in concentrations equal to that required by sodium laurate and myristate. The addition of a second double bond, as in linoleate, enhances this property considerably. Further addition of double bonds, however, confers no greater ability to kill streptococci. The hydroxyl group in the case of the ricinoleate enhances the bactericidal activity against the streptococcus, whereas there was a decrease against the pneumococcus. Sodium ricinelaide shows a decrease in potency to one-tenth the value of its geometric isomer, sodium ricinoleate. The replacement of the ethylenic linkage by an acetylenic linkage, as in the ricinostearolate, caused again a marked decrease in germicidal activity. The elaeostearates, which are isomeric with the linolenate, were unable to sterilize suspensions of streptococci in concentrations as high as 5 per cent. The two alicyclic unsaturated soaps, abietate and chaulmogrinate, were slightly less efficacious than sodium oleate as streptococicides. Neither the bile salts nor the hydroxylated salts, with the exception of the sativate, had any killing action in the concentrations tested. Sodium salicylate did not sterilize in a concentration of 10 per cent, but the addition of two atoms of iodine enabled it to do so in a fairly high dilution. The sulfate esters were almost as effective in killing streptococci as in killing pneumococci.

The germicidal effect of each of these compounds was also tested by means of plate counts similar to those used in the tests

with *Staphylococcus aureus* and *Escherichia coli*, except that 1 per cent glucose was added to the agar for *Streptococcus lactis*, and a small amount of blood for *Diplococcus pneumoniae*. These additional tests were run in order to determine whether a zoning phenomenon, such as was observed by Eggerth (1929) in his studies, occurred with any of the soaps used in this investigation. Cade and Halvorson (1934) have also reported data which indicate such a phenomenon with certain soaps. However, in this investigation, no indication of such a phenomenon was found. The results of the tests using plates paralleled the results using growth in liquid medium as a criterion of germicidal effectiveness. These plate count experiments with the pneumococci and streptococci were run at pH values of 7.6 and 8.0, without any noticeable differences in the two hydrogen ion concentrations. The addition of sterile defibrinated blood greatly increased the amount of both the soaps and alkyl sulfate esters necessary to kill pneumococci and streptococci.

At a pH of 8.0, the effectiveness of most soaps as germicides against a resistant organism like the staphylococcus is very low. Sodium undecylenate was the most germicidal of the soaps tested. Sodium diiodosalicylate was also quite effective in killing this organism, although a 1 per cent solution did not sterilize in 15 minutes. The principal constituent of "salt water" soap, sodium laurate, also exhibited a small detrimental effect on the staphylococcus. Sodium abietate in a concentration of 1 per cent was almost completely precipitated at hydrogen ion concentrations of lower than pH 8.5. At a pH of 8.7 or greater, this soap in a concentration of 1 per cent rapidly destroyed *Staphylococcus aureus*. All the other soaps examined showed no germicidal action on this organism in a period of 15 minutes under the conditions employed in the experiment.

The three previous organisms used having belonged to a group of Gram-positive cocci, it was thought desirable to test the germicidal effectiveness of these soaps against one of the Gram-negative bacilli. Since Lutterloh and Stroud (1931) and Seely (1932) have recommended the use of sodium ricinoleate in abdominal toxemia and peritonitis, it was thought that the colon bacillus was the most

suitable of this group of microorganisms as a test organism. The effect in this case was much the same as with the staphylococci. The only compounds which killed this organism were the diiodosalicylate, undecylenate, laurate, and abietate. The action of the latter two was extremely slight.

SUMMARY AND CONCLUSIONS

Pneumococci are especially susceptible to the action of certain unsaturated soaps, such as sodium oleate, linoleate, linolenate, and clupanodone. The other unsaturated soaps, as well as the hydroxylated and saturated soaps, are less effective in killing this organism. The bile salts and sodium salicylate require high concentrations to kill pneumococci. The sulfate ester salts are pneumococidal in concentrations of 0.01 per cent.

Streptococcus lactis is more resistant to the action of soaps than the pneumococcus. The effectiveness of soaps in killing the latter microorganism does not parallel exactly its effectiveness against the former. The two saturated soaps, sodium myristate and laurate, the unsaturated soaps, sodium oleate, linoleate, linolenate, clupanodone, ricinoleate, and abietate, as well as the two sulfate esters, are very effective in killing this streptococcus.

Of these compounds, only sodium diiodosalicylate and undecylenate are effective germicides in a concentration of 1 per cent at a pH of 8.0, using *Escherichia coli* as the test organism.

Sodium diiodosalicylate and sodium undecylenate are also effective against *Staphylococcus aureus* at a pH of 8.0. Sodium abietate is effective above a pH of 8.5.

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