ACTION OF SYNTHETIC DETERGENTS ON THE METABOLISM OF BACTERIA*

By ZELMA BAKER, Ph.D., R. W. HARRISON, Ph.D., and BENJAMIN F. MILLER, M.D.

(From the Walter G. Zoller Memorial Dental Clinic, the Department of Bacteriology and Parasitology, and the Department of Medicine, The University of Chicago, Chicago)

(Received for publication, October 14, 1940)

The bactericidal and bacteriostatic actions of soaps have been recognized for many years. Detailed studies by Eggerth (1) on the germicidal action of soaps have shown the importance of such factors as length of hydrocarbon chain, chemical configuration, effect of hydrogen ion concentration, effect of serum, etc. Avery (2) demonstrated the selective germicidal action of soap on microorganisms, and recommended for the isolation of influenza bacilli a medium containing sodium oleate. He noted that "this medium prevents the growth of certain Gram-positive organisms, principally pneumococcus and streptococcus, while the growth of *B. influenzae* is enhanced by the presence of this substance." The marked selectivity of soaps on bacteria has been studied further by Bayliss and Halvorson (3) and Bayliss (4). The work of Walker (5) indicates that although pneumococci and streptococci are unusually susceptible to certain soaps, staphylococci are very resistant. Because of the widespread occurrence of the staphylococcus on body surfaces, soaps have only a limited value as germicidal agents. Generally, they are employed when *detergent*, rather than *germicidal* action, is desired.

In addition to their selectivity, which is a disadvantage unless one wishes to attack or differentiate specific microorganisms, soaps have certain other undesirable characteristics which limit their usefulness. Ordinary soaps have too alkaline a reaction for application on sensitive tissues. Moreover, soap is precipitated by hard water.

Because of the tremendous demands of many industries for new and improved detergents, interface modifiers, wetting and emulsifying agents, and related compounds, and because of the competition for patents, at least a thousand individually different compounds with such properties have been synthesized in recent years.[1]

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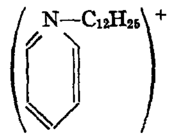
* A preliminary account of these experiments was given by Miller and Baker in *Science*, 1940, **91**, 624.

[1] Data concerning synthetic detergents and related compounds are scattered throughout the trade journals and patent literature. A discussion of the chemistry and applica-

These compounds are, in most cases, superior to soaps in wetting and cleansing abilities, and have the additional virtue of stability in acid and alkaline solutions. They may be classified in three main groups: (*a*) ***anionic compounds***, *e.g.* sodium lauryl sulfate, which ionizes with the hydrophobic group in the anion as follows: $(Na)^{+} + (C_{12}H_{25}OSO_3)^{-}$; (*b*) *cationic compounds*, *e.g.* lauryl pyridinium iodide, which contains the hydrophobic group in the cation:



$+ (I)^{-}$;

(*c*) ***non-ionized compounds***, such as the polyglycerol esters (7).

In recent years the biological effects of certain synthetic detergents (chiefly the alkyl sulfates) have been investigated, *e.g.* action on viruses (8–10), denaturation of proteins (11), extraction and modification of pigments (12, 13), secretion (14), enzyme action (15), inactivation of toxins (16), lytic action (17), effect on tissue cultures (18), effect on filterability of bacteria (19).

The bactericidal action of synthetic detergents was emphasized by Domagk (20) when he demonstrated in 1935 that the cationic, quaternary ammonium detergent, alkyl dimethyl benzyl ammonium chloride (Zephiran), possesses excellent germicidal properties. His initial report was followed by many laboratory and clinical studies demonstrating the effectiveness of this compound (21–31). Previously Harris and Bunker (32) had studied the action of methyl, triethanolamine and ephedrine ricinoleates on *Staphylococcus aureus*. Also, in 1935, Katz and Lipsitz (33*a*) reported that an anionic wetting agent derived from naphthalene sulfonic acid inhibited the growth of *Mycobacterium smegmatis*; later (33*b*), they studied the effects of one cationic and two other anionic compounds. According to Shelton *et al.* (34) and Blubaugh, Botts, and Gerwe (35), another cationic compound, cetyl pyridinium chloride, also possesses germicidal activity.

The germicidal action of several alkyl sulfates on pneumococci, *Streptococcus lactis*, *Eberthella coli*, and *Staphylococcus aureus* was investigated by Bayliss and Halvorson (3) and Bayliss (4). Cowles (36) and Birkeland and Steinhaus (37) observed that alkyl sulfates and triethanolamine lauryl sulfate (Drene) selectively inhibit the growth of Gram-positive organisms. Because of these results, Cowles (38) proposed the use of alkyl sulfates in selective media.

It is also of interest in connection with these studies on characteristic synthetic detergents to note the recent researches on the bactericidal effects of some long chain derivatives of sulfanilamide (39–43), and the striking trypanocidal action of long chain derivatives of diamidines and related compounds, reported by Yorke and collaborators (44–46).

It was shown by Miller, Baker, and Harrison (47) that a very low concentration of alkyl dimethyl benzyl ammonium chloride (Zephiran) inhibits the respiration and glycolysis of pure cultures of microorganisms associated with the early lesions of human

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tions, and of some theoretical aspects, may be found in "Wetting and detergency" (6). Dr. René Dubos has called our attention to a useful compendium entitled "Wetting agents, Bulletin No. 9," which may be obtained from the American Perfumer and Essential Oil Review, 9 East 38th Street, New York, N. Y.

dental caries. Miller, Muntz, and Bradel (48) demonstrated that the application of this compound *in vitro* or *in situ* suppresses the production of acid by dental plaques.

These experiments have made desirable a more detailed investigation of the action of synthetic detergents on bacterial metabolism. In this paper we present the results of experiments designed to correlate chemical structure and properties of the detergents with the effects on bacterial metabolism.

Material and Methods

Preparation of Bacterial Suspensions.—For the measurement of metabolic activity in the Warburg apparatus, it is desirable to employ young cultures. Therefore, the period of incubation was limited to 14 to 16 hours. The inoculations were made at approximately 6 p.m.; the microorganisms were collected at 8 a.m. the following day, and used on the day of collection. All microorganisms were grown on veal infusion agar except *Micrococcus tetragenus*, for which brain-heart agar was employed, and lactobacillus,[2] which was cultured in 1 per cent glucose meat infusion broth, according to Jay (49).[3] For all organisms except lactobacillus, the inoculations were made from stock agar slants into Kolle flasks. The microorganisms were removed from the flasks by washing with a stream of sterile distilled water; they were then centrifuged, washed twice, and resuspended in sterile water. The number of bacteria per cc. was estimated by means of a Petroff-Hausser counter; for manometric experiments, the suspensions were diluted so that 1 cc. contained 15 to 25 billion cells.[4] 1 cc. of this diluted suspension caused an oxygen uptake or carbon dioxide evolution of 100 to 200 c.mm. per hour.

Manometric Technique.—The rate of respiration or glycolysis was determined in standard Warburg vessels. Each vessel contained 1.0 cc. of bacterial suspension, 1.7 cc. of appropriate buffer, 0.1 cc. of 10.8 per cent glucose solution (making the final concentration of glucose 0.02 M) and, in the side-arm, 0.2 cc. of water or detergent solution. For the measurement of oxygen consumption, M/15 phosphate buffer (pH 7.0) was used; respiratory carbon dioxide was absorbed by 0.15 cc. of 20 per cent KOH in the middle well. These experiments were conducted in air. Ringer-Krebs medium (50) containing bicarbonate was used for the measurement of aerobic acid production, in an atmosphere

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[2] The lactobacillus culture employed in these experiments was isolated from the oral cavity. This microorgânism was morphologically and biochemically identical with other strains of oral origin which have been frequently referred to as *L. acidophilus*. However, because of some doubt concerning the identity of such strains with intestinal *L. acidophilus*, in this paper the organism is referred to as lactobacillus, without species designation.

[3] Difficulty was sometimes encountered in obtaining actively glycolyzing suspensions of lactobacillus from agar cultures. Therefore, the organism was grown in 1 per cent glucose meat infusion broth, pH 5.0. After transfer for 3 successive days in this broth, a 4 hour culture of the microorganism was employed finally for inoculation of flasks containing the same medium.

[4] This corresponds to a dry weight of 0.8 to 5.0 mg. per cc., depending on the microorganism.

of 95 per cent O_2 + 5 per cent CO_2. By the techniques employed in these experiments, aerobic glycolysis can be determined only for microorganisms which have a respiration

TABLE I

Composition of the Detergents

	Trade name	Structure	Manufacturer
Cationic	Zephiran	Alkyl dimethyl benzyl ammonium chloride (alkyl = C_8 to C_{18})	Alba Pharmaceutical Co.
	Triton K-12	Cetyl dimethyl benzyl ammonium chloride	Röhm and Haas
	Triton K-60	Lauryl dimethyl benzyl ammonium chloride	" " "
	Retarder LA	Stearyl trimethyl ammonium bromide	E. I. du Pont de Nemours Co.
	Hydrocide	Alkyl hydroxy benzyl dimethyl ammonium phosphate	Röhm and Haas
	Damol	N-N-N′-N′-tetramethyl-N-N′-didodecyl-β-hydroxy-propylene-diammonium bromide	Alba Pharmaceutical Co.
	Emulsol-605	$C_{11}H_{23}$—COO—C_2H_4—NH—CO—CH_2—$N(CH_3)_3Cl$	Emulsol Corp.
	Cepryn chloride	Cetyl pyridinium chloride	Wm. S. Merrell Co.
	Emulsol-660 B	Lauryl pyridinium iodide	Emulsol Corp.
	Emulsol-606	Lauryl ester of glycine hydrochloride	" "
Anionic	Cetyl sulfate	Sodium cetyl sulfate	Proctor and Gamble Co.
	Duponol LS	Sodium oleyl sulfate	E. I. du Pont de Nemours Co.
	Triton W-30	Sodium salt of alkyl phenoxy ethyl sulfonate	Röhm and Haas
	Triton 720	Sodium salt of alkyl phenoxy dialkoxy sulfate	" " "
	Igepon T	R—CO—$N(CH_3)(CH_2)_2$—SO_3Na	General Dyestuffs
	Igepon AP	R—COO—$(CH_2)_2$—SO_3Na	" "
	Nopcocastor	75 per cent sulfonated castor oil	National Oil Products Co.
	Tergitol-7	Sodium alkyl sulfate (alkyl = 3,9 diethyl tridecanol-6)	Carbon and Carbide Corp.
	Drene	Triethanolamine lauryl sulfate	Proctor and Gamble Co.
	Sodium taurocholate	Sodium taurocholate	Pfanstiehl Co.

negligibly small in comparison with the aerobic glycolysis. In our experiments, only the lactobacillus and one strain of *M. tetragenus*, designated as *M. tetragenus* A, satisfied this requirement. All experiments were performed at 38°C. for 1 hour.

Source of Microorganisms.—Staphylococcus aureus, *Staphylococcus albus*, lactobacillus, and two strains of *Micrococcus tetragenus* were all oral strains, isolated from saliva cultures; *Escherichia coli* (No. 26) was obtained from the American Type Culture Collection; *Proteus vulgaris* (X 19) and *Salmonella paratyphi* (No. 339) were from the collection of Dr. Edwin O. Jordan (1933); *Sarcina lutea* was isolated in 1901 from Mississippi River water; *Pseudomonas pyocyanea* was isolated from a stitch abscess in 1934; *Aerobacter aerogenes* and *Shigella dysenteriae* were strains from the curator's stock of the Department of Bacteriology of The University of Chicago.

Description of the Detergents Employed.—Since the detergents[5] are manufactured for specific industrial uses where purity is secondary to effectiveness, it is difficult to obtain products which satisfy the usual requirements of chemical purity. The contaminants are, however, usually inert materials, frequently inorganic salts. It is our feeling that although many of the compounds we have employed are impure, they are satisfactory for this initial orientating study of the action of synthetic detergents on bacterial metabolism. It is to be hoped that this investigation will encourage manufacturers to prepare compounds of known purity for research purposes. Already Proctor and Gamble Company, E. I. du Pont de Nemours and Company, and The Emulsol Corporation have made a definite effort in this direction. We are indebted to these and other companies listed in Table I for their cooperation. This table gives the names of the detergents, their chemical structure, and the manufacturers.

Solutions containing 5.0 or 0.5 mg. per cc. of active constituent were prepared and neutralized to the desired pH.[6] 0.2 cc. of this solution was pipetted into the side-arm of the Warburg vessel and added to the contents of the vessel at zero time. This provides a concentration of detergent of 1:3000 or 1:30,000 (approximately M/1000 or M/10,000).

RESULTS

Cationic Detergents.—

The effects of ten cationic detergents on the respiration or glycolysis of six Gram-positive and six Gram-negative microorganisms are shown in Table II. The detergents were studied at two concentrations, 1:3000 and 1:30,000. The figures in the table represent results obtained in separate experiments. In many cases where the inhibition was practically complete at the lower concentration, the higher concentration was not studied.

Table II shows that at a concentration of 1:3000 all the cationic detergents exerted a marked inhibitory action on all the microorganisms studied. At the 1:30,000 dilution a number of the detergents, *e.g.* Zephiran, Triton

[5] For the sake of convenience, the term "detergent" is applied to all the compounds which we have studied. All of these are synthetic except sodium taurocholate, and all possess at least one polar and one non-polar, long chain portion, giving the characteristic hydrophilic-hydrophobic structure.

[6] All of the compounds except Hydrocide are soluble at these concentrations and neutral pH; in the stronger solution, Hydrocide forms an emulsion on the addition of alkali, and tends to crystallize out on standing. Therefore, it was freshly prepared for each experiment. Some of the higher alkyl sulfates also crystallize on standing but can be redissolved by warming.

TABLE II

Effect of Cationic Detergents on Bacterial Respiration and Glycolysis

Detergents	Per cent inhibition of bacterial metabolism*											
	Microorganisms											
	Gram-positive						Gram-negative					
	S. aureus	*S. albus*	*Sarcina lutea*	*M. tetragenus* B	*M. tetragenus* A†	Lactobacillus	*E. coli*	*Proteus vulgaris*	*Ps. pyocyanea*	*S. paratyphi*	*A. aerogenes*	*Sh. paradysenteriae*
	Concentration of detergent = 1:3000											
Zephiran	93 90	100	92	86	93	93	93	86	97 97			
Triton K-12	93 90	90	86	100	95	90	93	87	96 98			
Triton K-60	88 89	90	83	100	90	81	93	96	91 87	90 92	93	90
Retarder LA	91	94		86	93	89	93	83	95 95			
Hydrocide	100 86	91 90	83 87	85 49 91	74	90	61 92	62 100	83 96	98 100	97 97	94
Damol			90 91		90		92	91	89			92
Emulsol-605	91 86	92 97	91 93	94	94	82	92 100	91 94	96 93	100	97	90
Cepryn chloride			90		96	92	91	84*	95			100
Emulsol-660 B	95		90		93	95	89	100	96			90
Emulsol-606	87	90 87	92 89	94	91	75 77	93 98	89 49	27 86 53	92	99	85

K-12, Retarder LA, Damol, and the pyridinium compounds (Cepryn Chloride and Emulsol-660B) were, with very few exceptions, effective against all the microorganisms. The others were active at this concentration only against certain of the microorganisms. The results obtained with lactobacillus and *M. tetragenus* A indicate that glycolysis is as readily inhibited as respiration. It is apparent from the results assembled in Table II that *the cationic detergents inhibit the metabolism of Gram-positive and Gram-negative microorganisms to the same degree.*

TABLE II—*Concluded*

Detergents	Per cent inhibition of bacterial metabolism*											
	Microorganisms											
	Gram-positive						Gram-negative					
	S. aureus	*S. albus*	*Sarcina lutea*	*M. tetragenus* B	*M. tetragenus* A†	Lactobacillus	*E. coli*	*Proteus vulgaris*	*Ps. pyocyanea*	*S. paratyphi*	*A. aerogenes*	*Sh. paradysenteriae*
	Concentration of detergent = 1:30,000											
Zephiran	93 95	93 100	100 91	96 93	87 96	73 92	92 92	90 98	56 90 46	97 100	94 100	100
Triton K-12	91 90	92 86	12 89 81	86 92	96 91	88 92	94 93	85 87	78 71 33	91 91	91 89	92
Triton K-60	53 38 55	82 90	85 58	73 78 95	79 94	94 86	72 83	15 65 48	6 37	39 86 33	49 55	43 61
Retarder LA	65 95	100 86	96 100	93 93	93 67	87 79	95 76	87 85	54 15 12	100 100	100 88	98
Hydrocide	31 10 34	6		13	20	+19‡	8 10	15 17	0	25	+24	4
Damol	89 90	96	84 63 89	82	95	93	93 62	81 24	86	92	94	85
Emulsol-605	15 37	92 88	81 56	2	91	+26	54 94	15 1	10 11	44	86	79
Cepryn chloride	93 95	91	91 76	90	79	83	89 65	81 41	3 29	85	93	92
Emulsol-660 B	73 81	89	89 50	82	68	46 43	75 72	32	82	87	78	76
Emulsol-606	79 73	63 92	+4 +37	0	65	+5	54 100	42 7	+6 2	5	95	11

* Unless otherwise indicated, the values represent inhibition of respiration.
† Aerobic glycolysis.
‡ Plus represents stimulation.

The cationic detergents exert their inhibitory effects very rapidly; usually the inhibition is complete at the time of the first manometric reading

(15 minutes after the addition of the compound). The curves in Fig. 1 illustrate the rapidity of action of 1:30,000 Zephiran on the respiration of *Staphylococcus aureus* and the aerobic glycolysis of lactobacillus. Furthermore, the effect is irreversible under the following experimental conditions: if microorganisms are exposed to 1:3000 Hydrocide, Emulsol-605, or Cepryn Chloride, or to 1:30,000 Zephiran or Damol for 5 to 10 minutes, then

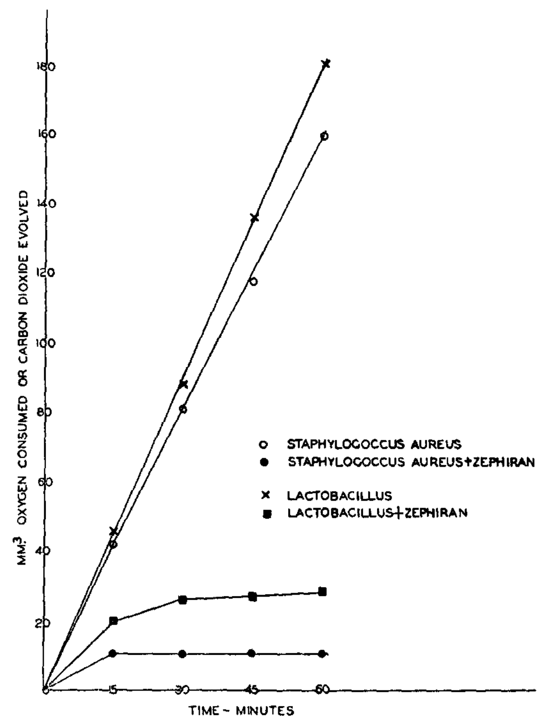


FIG. 1. Effect of Zephiran (1:30,000) on the metabolism of *Staphylococcus aureus* and lactobacillus.

centrifuged, washed, and resuspended in fresh glucose-buffer solution, they do not regain their metabolic activity.[7]

In one experiment, microorganisms were treated with Zephiran, washed, etc., and finally resuspended in a glucose solution containing an anionic detergent (which because of its opposite charge might neutralize or elute the cationic detergent); the inhibitory effect of the cationic detergent was not reversed.

[7] The irreversibility effect was studied only on the detergents listed. Presumably the other compounds would behave similarly.

Anionic Detergents.—

The results obtained with the anionic detergents are presented in Table III. It is apparent that this type of detergent exerts a much less general effect on bacterial respiration and glycolysis than the cationic derivatives. At a concentration of 1:3000, a number of the anionic compounds inhibited the metabolism of certain microorganisms, but at the 1:30,000 dilution they were considerably less active than the cationic compounds. In sharp contrast to the cationic compounds, *the anionic detergents inhibit only Gram-positive microorganisms.* Except for the inhibition of *Proteus vulgaris* by Tergitol-7, the maximum effect on any Gram-negative microorganism by an anionic detergent was an inhibition of 39 per cent of *S. paratyphi* by Triton W-30; in two other experiments with the same detergent and microorganism, the values obtained were −5 per cent and +8 per cent.

Tergitol-7 warrants special attention, since this compound consistently inhibited the metabolism of each of the six Gram-positive microorganisms almost completely at the 1:3000 dilution. Only one Gram-negative microorganism, *Proteus vulgaris*, was inhibited by Tergitol-7.

Stimulation of Bacterial Metabolism by Detergents

Several of the detergents stimulate bacterial metabolism. Thus, at 1:3000 concentration, Igepon AP in three experiments activated the respiration of *Sarcina lutea* 30, 56, and 83 per cent; of *Ps. pyocyanea* 23 and 35 per cent; and of *A. aerogenes*, 49 and 87 per cent. With *Sarcina lutea*, Drene activated as much as 114 per cent, and Nopcocastor, 134 per cent. Other instances of activation may be seen in Table III. It is apparent that *Sarcina lutea* exhibited this response most frequently.

Table IV shows the effect of concentration of the detergent on the degree of activation. *Sarcina lutea* was exposed to a wide range of concentrations of Drene or Igepon AP at a pH of 7. The results show clearly that the activation caused by triethanolamine lauryl sulfate (Drene) reaches a maximum at a concentration of 1:3000 and begins to fall at higher concentrations of the detergent; at still higher concentrations, 1:1500 to 1:375, the compound exerts an inhibitory effect. With Igepon AP, the activating effect had apparently not reached its maximum even at a concentration of 1:100.

As can be seen by a comparison of Tables II and III, stimulation is produced infrequently by cationic detergents. To compare further the cationic and anionic detergents, an experiment was performed on *Sarcina lutea* in which the concentration of two cationic detergents, Zephiran and Emul-

TABLE III

Effect of Anionic Detergents on Bacterial Respiration and Glycolysis

Detergents	Per cent inhibition of bacterial metabolism*											
	Microorganisms											
	Gram-positive						Gram-negative					
	S. aureus	*S. albus*	*Sarcina lutea*	*M. tetragenus* B	*M. tetragenus* A†	Lactobacillus	*E. coli*	*Proteus vulgaris*	*Ps. pyocyanea*	*S. paratyphi*	*A. aerogenes*	*Sh. paradysenteriae*
	Concentration of detergent = 1:3000											
Cetyl sulfate	31 16 13	25 98 12	84 75 91	10 5	71	86 89	14 +2	3 +2	+5	13 +3	0 8	7
Duponol LS	+7‡ 0	32 35	95 90 19	4 7	92 78	85 84	0 6 41	0 6	0 8	2	5 15	0
Triton W-30	10 9	82 88 100	80 82 23	82 29 +6	94 91	89 93	12 2	2 5	2 +2	5 39 +8	5 5	0
Triton 720	+12 +14	9 +8	4 9 +39	+2 +5	+9 +25	24 14	4 3	3 +18	+4 +5	5 7	2 +5	+4
Igepon T	+2 2	9 8	6 +6	+3 +18	41 55	16 37	3 +4	4 10	2 8	2 3	1 5	0
Igepon AP	+18 +14	23 11	+30 +56 +83	+10 +18	76 78 8	15 50	+14 +27	10 13	+23 +35	1 +9	+49 +87	+21
Nopcocastor	+20 +16	11 12	+70 +134	0 +20	48	3 +29	13 +1	3 4	3 4	9 +5	4 9	0
Tergitol 7	91 70	89 92	84 89	81 99	94	92 87	12	54 68 97	4 13	4 15	3 +3	+2
Drene	42 45	32 13	+26 +51 +114	0 1	74 77	77 72	0 3	1 3	+5	9 45 +4	1 +3	+2
Sodium taurocholate	4 2	24 3 +4	+31 +35	+5 +13	16	6 +33	14 +2	7 13	1 +10	+10	2 8	0

* Unless otherwise indicated, the values represent inhibition of respiration.

† Aerobic glycolysis.

‡ Plus represents stimulation.

TABLE III—*Concluded*

Detergents	Per cent inhibition of bacterial metabolism*											
	Microorganisms											
	Gram-positive						Gram-negative					
	S. aureus	*S. albus*	*Sarcina lutea*	*M. tetragenus* B	*M. tetragenus* A†	Lactobacillus	*E. coli*	*Proteus vulgaris*	*Ps. pyocyanea*	*S. paratyphi*	*A. aerogenes*	*Sh. paradysenteriae*
	Concentration of detergent = 1:30,000											
Cetyl sulfate	4	52 +7	17	5	50	85	2	+4	+7	11	4	6
Duponol LS	0	+26	+9	6	66	39	+1	4			5	9
Triton W-30	+14	+7			56	41	1	11				
Triton 720	+2				+2	0	3	8				
Igepon T	+7			0	10	11	2	14				
Igepon AP	+2			+8	43	0	+13					
Nopcocastor	+4				1	8	+4	3				
Tergitol 7	13 47	54		9		84	7	18 81	13	3	+4	+3
Drene	0			2	16	30	0					
Sodium taurocholate	+2				+8	+8	1					

sol-605, was varied from 1:30,000 to 1:450,000. It was found that respiration was inhibited by the highest concentration, and that it was either inhibited or not affected by the weaker solutions. There was no evidence of stimulation of respiration despite the very wide range of concentration. A similar experiment with Zephiran upon *Staphylococcus aureus* gave essentially the same results.

Effect of Variation of pH

Some experiments were performed over a pH range of 5.3 to 8.0, employing M/15 phosphate buffers (Sörensen). The results obtained (Table V) demonstrate (*a*) inhibition by the detergents is a function of pH; (*b*) the *cationic* detergents show their maximum inhibitory action on the *alkaline* side; (*c*) the *anionic* detergents show their maximum inhibitory action on the *acid* side. Additional results with anionic detergents are shown in Table VI.

TABLE IV

*Effect of Drene and Igepon AP on the Respiration of Sarcina lutea**

Concentration of detergent	1:60,000	1:30,000	1:18,000	1:9000	1:6000	1:3000	1:2250	1:1500	1:750	1:375	1:100
Drene	+7†	+17	+25	+44	+66	+80	+60	−6	−67	−62	—
Igepon AP	0	+4	+12	+20	+8	+21	+28	+27	+46	+72	+107

* Each vessel contained 18 billion microorganisms.

† Minus values represent per cent inhibition; plus values represent per cent activation.

TABLE V

Effect of Variation of pH on Action of Detergents

Microorganism	Detergent	Concentration of detergent	pH				
			5.3	6.2	6.8	7.4	8.0
Staphylococcus aureus	Zephiran	1:60,000	−42*	−54	−67	−78	−85
" "	Emulsol-606	1:30,000	+18	+28	+10	−78	−73
" "	Drene	1:3000	−98	−43	—	−26	−15
" "	Sodium lauryl sulfate	1:3000	−90	−14	0	0	0
" "	Tergitol-7	1:30,000	−86	−62	−46	−29	−15
E. coli	Zephiran	1:60,000	−62	−79	—	−86	−96

* A minus sign indicates inhibition of respiration; a plus sign indicates activation.

TABLE VI

Action of Homologous Series of Detergents on Bacterial Respiration

Detergent	Microorganism	pH	Per cent inhibition by					
			Octyl C_8	Decyl C_{10}	Lauryl C_{12}	Myristyl C_{14}	Cetyl C_{16}	Stearyl C_{18}
Alkyl sulfate 1:3000	*Staphylococcus aureus*	5.3	5*	0	96	89	67	12
		8.0	13	12	2	65	62	9
"	" "	5.3	4	+6	84	86	81	25
		8.0	16	+6	39	65	60	13
"	*Sarcina lutea*	5.3	+4	1	90	86	89	49
		8.0	—	0	95	98	91	2
"	*E. coli*	5.3	1	+4	0	+2	2	0
		8.0	+1	+4	+3	6	+2	3
Alkyl sulfoacetate 1:3000	*Staphylococcus aureus*	5.3	2	3	88	86	37	9
		8.0	0	+5	+3	0	+3	+8
1:30,000	" "	5.3	—	0	28	0	—	—

* Unless otherwise indicated, the values represent inhibition of respiration; a plus sign indicates activation.

Studies on Homologous Series of Compounds

The influence on bacterial respiration of homologous series of alkyl sulfates and alkyl sulfoacetates (R—OOC—CH_2—SO_3Na) was investigated.[8] The alkyl groups in the two series varied from C_8 (octyl) to C_{18} (stearyl). Experiments were conducted on several microorganisms, using the compounds at a concentration of 1:3000, and at two different pH values, 5.3 and 8.0. The results are summarized in Table VI. It is evident that the chain length of the hydrophobic group modifies the degree of inhibition produced by these compounds. In both series, the lauryl, myristyl, and cetyl derivatives were most active. The sharp rise in activity from the decyl to the lauryl compounds is noteworthy.

Study of the action of the sulfoacetate series on *Staphylococcus aureus* at a lower concentration (1:30,000) and pH 5.3 demonstrated that only the lauryl compound (C_{12}) exerted an inhibitory effect under these conditions.

The pH of the medium influences the inhibitory action as might be expected from the results given in the previous section. The compounds are most effective at an acid pH. This is particularly striking in the case of the sulfoacetates. The lauryl and myristyl derivatives inhibited 88 per cent and 86 per cent respectively at pH 5.3 and had no effect at pH 8.0.

The results in Table VI demonstrate again the capacity of the anionic detergents to differentiate sharply between the Gram-positive and Gram-negative microorganisms. None of the alkyl sulfates or the alkyl sulfoacetates inhibited the respiration of the Gram-negative organism, *E. coli*, even at the more favorable acid pH.

It has not been possible to obtain a series of cationic detergents with homologous variation of the alkyl group. However, three lauryl esters of amino acids[9] permitted observation on the effect of increased chain length in the hydrophilic portion of the molecule. These compounds, Emulsol-606, 608, and 609, are the lauryl esters of the hydrochlorides of glycine, alanine, and alpha-amino isobutyric acids, respectively. They were studied on three Gram-positive and three Gram-negative microorganisms at concentrations of 1:3000 and 1:30,000. At the higher concentration, inhibition was essentially complete with all three compounds. At the lower concentration, where inhibition is less marked,

[8] We are indebted to Proctor and Gamble Company for these compounds.

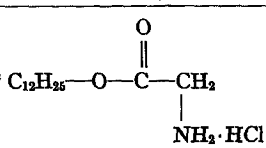
[9] We are indebted to Mr. A. K. Epstein and Mr. B. R. Harris, chemists of The Emulsol Corporation, for suggesting that compounds of the type 606, 608, and 609 would be characterized by relatively high bactericidal power and low local and systemic toxicity.

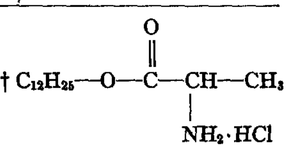
the results (Table VII) show that activity is enhanced in every case by an increase in length of the amino acid portion of the molecule.

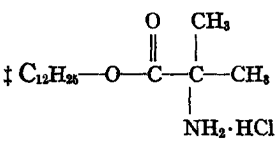
TABLE VII

Effect of Emulsol-606, 608, and 609 on Bacterial Respiration

	Microorganism	1:30,000		
		606*	608†	609‡
Gram-positive	*Staphylococcus aureus*	−22§	−38	−43
	Sarcina lutea	+22	+16	−3
	M. tetragenus B	+24	+12	−48
Gram-negative	*E. coli*	−19	−24	−69
	Proteus vulgaris	−46	−77	−92
	Ps. pyocyanea	−10	−19	−30
	A. aerogenes	−50	−77	−92

*


†


‡


§ A minus sign indicates inhibition of respiration; a plus sign indicates activation.

TABLE VIII

Effect of Zephiran on Oxidations by Staphylococcus aureus

Substrate (0.02 M)	Respiration of control	Zephiran, 1:60,000		Zephiran, 1:30,000	
	O_2 per hr.	O_2 per hr.	Inhibition	O_2 per hr.	Inhibition
	c.mm.	*c.mm.*	*per cent*	*c.mm.*	*per cent*
Glucose	144	47	67	0	100
Lactate	228	35	85	15	93
dl-Alanine	24	9	63	0	100
Succinate	59	12	80	4	93
Glutamate	75	11	85	5	93

Effect on Substrates Other than Glucose

The action of alkyl dimethyl benzyl ammonium chloride (Zephiran) on the oxidation of substrates other than glucose was studied to determine if the effect of the detergent is on respiration in general, or is confined to systems involved in the utilization of glucose. It was found that the oxidations by *Staphylococcus aureus* of glucose, lactate, *dl*-alanine, succinate, and glutamate were all inhibited to practically the same extent by 1:30,000 and 1:60,000 Zephiran. The results are given in Table VIII.

Action of Lecithin

Since lecithin contains a hydrophilic-hydrophobic structure and a quaternary ammonium nitrogen in the choline portion of the molecule, it was of interest to compare this naturally occurring substance with synthetic detergents of similar structure. Experiments were performed on a sample of lecithin, obtained from soy-bean. It was found that even at a concentration of 1:300 (0.3 per cent), this compound did not inhibit the metabolism of three Gram-positive and three Gram-negative microorganisms. In most cases there was stimulation of metabolism. With 1:300 lecithin at pH 7.0, the following results were obtained: *Staphylococcus aureus*, +15 per cent; *Staphylococcus albus*, +11 per cent; *Sarcina lutea*, +11 per cent; *Proteus vulgaris*, +5 per cent; *Ps. pyocyanea*, +20 per cent; *E. coli*, −6 per cent.

DISCUSSION

Our results show that the action of synthetic detergents on bacterial metabolism is influenced by a number of factors such as (*a*) charge on ion containing the hydrophobic group; (*b*) hydrophilic-hydrophobic balance of the molecule; (*c*) pH of the medium; (*d*) specific chemical structure; (*e*) general and specific characteristics of the microorganisms. The effects of protein and other organic materials, the influence of inorganic salts, age of microorganisms, etc., have not been considered in this study. Also, the bacteriostatic and bactericidal powers of the compounds have not been investigated.[10]

[10] Our experiments indicate that certain detergents are extremely potent in inhibiting the metabolism of a number of bacterial species, and that, under the experimental conditions employed, this inhibition is irreversible. Direct application of these results to a definition of the bactericidal or bacteriostatic power of these compounds is unwarranted. In a recent paper, Sykes (51) attempts to evaluate the relationship between the effect of germicides on bacterial metabolism (measured by inhibition of succinic dehydrogenase) and the effect on viability of the cells. He concludes that in general the viability of cells is affected at concentrations lower than those which inhibit bacterial metabolism. Likewise, Bronfenbrenner and collaborators (52) have concluded from their studies on certain standard germicides that inhibition of oxygen uptake

Ionic Configuration of Detergent Molecule

The work presented here clearly demonstrates that the cationic detergents inhibit Gram-positive and Gram-negative organisms to the same degree. Furthermore, in the group of compounds which we have studied, the cationic detergents are more effective inhibitors than the anionic, especially if one compares their activities at a physiological pH.

It is unlikely that the superiority of one group over the other is due to physical factors such as "wetting" power, effectiveness in detergency, or effect on surface tension. In many cases, the anionic compounds are at least equally effective, and even frequently superior in these properties. Moreover, the germicidal action of surface active compounds cannot be attributed entirely to their effect on surface tension. Frequently compounds which have the same capacity to reduce surface tension, *but which differ in chemical configuration*, vary in their ability to inhibit bacterial growth (56–60).

In our earlier experiments, we were tempted to attribute the marked activity of cationic detergents to the quaternary ammonium structure, because of the well known biological activity of compounds containing this group. However, subsequent experiments with Emulsol-606, 608, and 609 made this appear unlikely. These compounds are all lauryl esters of amino acid hydrochlorides ($C_{12}H_{25}$—OOC—R—$NH_2 \cdot HCl$) and differ from the typical, fully alkylated quaternary ammonium compounds (see Sidgwick (61), page 27). Yet the results obtained with these amino acid esters show that they possess activity which is comparable to that of the quaternary ammonium cationic detergents. It should be profitable to study further the cationic group of detergents, employing compounds of widely different chemical structures.

It is tempting for the sake of simplicity to consider the effectiveness of the cationic compounds in terms of the positive charge on the ion which contains the long chain, hydrophobic group. It is a well established fact

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may be taken as a reasonably quantitative measure of germicidal action. The experiments of Ely (53) gave similar results. Christophers and Fulton (54) studied the effects of some new trypanocidal agents on the oxygen uptake of trypanosomes, and observed that the inhibition of metabolism fairly closely parallels the therapeutic effect.

From these investigations it appears likely that compounds which inhibit the metabolic processes of bacteria would also act as germicides. However, exceptions should be expected since, as Needham (55) has pointed out, there are instances of dissociation between metabolism and cell division, in which metabolic processes are more sensitive than cell division.

In a future paper we shall present data correlating effects on bacterial metabolism with germicidal action and animal toxicity.

that most bacteria are negatively charged and one might assume that the long chain positive ion is more effective because its opposite electrical charge facilitates adsorption (see Adam (62), Chapter VIII, also McCalla (63)).

Selective inhibition of Gram-positive organisms by anionic detergents is discussed on page 268.

Hydrophilic-Hydrophobic Balance

Solubility and balance are very important properties of wetting agents and detergents. If the compound has too many hydrophilic groups, it loses its effectiveness as a detergent or wetting agent; similarly, if the balance is overweighted on the side of the hydrophobic groups, the compound may become too insoluble to be effective. In detergents derived from straight chain alkyl compounds, the maximum efficiency is obtained when the chain contains from 12 to 16 carbon atoms (6). With higher homologues, the decrease in solubility usually limits effectiveness. Similar effects of chain length are demonstrable in biological activity. In our experiments with homologous series of alkyl sulfates and sulfoacetates, there is a dramatic increase in activity from the decyl (C_{10}) to the lauryl (C_{12}) compound. The activity is maintained in most cases at a high level by the myristyl (C_{14}) and cetyl (C_{16}) homologues, and decreases markedly with the stearyl (C_{18}) derivative. Our experiments were designed to determine the point of maximum effectiveness in both series. It is likely that at a higher concentration, the decyl and stearyl compounds would also inhibit.

Cowles (36), who studied the bacteriostatic effects of alkyl sulfates on Gram-positive organisms, also observed that the lauryl, myristyl, and cetyl compounds were the most active. Furthermore, he correlated germicidal power in an homologous series of alcohols with increasing surface tension and with "wetting ability." We agree with Cowles that such correlations are reasonable in an homologous series, but believe that other factors must be considered in a comparison of compounds of different chemical structures.

Effect of pH

Our results show that the cationic detergents are more active in the alkaline range, and the anionic, in the acid range. Previously, Dunn (23*b*) had reported that alkyl dimethyl benzyl ammonium chloride (Zephiran) exerts a greater germicidal action at an alkaline pH. The studies of Eggerth (1) clearly demonstrated the effect of pH changes on the germicidal action of soaps. It is interesting that many investigators (65–68) have found that increase of pH favors the disinfecting power of basic dyes and decrease of pH, that of acid dyes.

The pH effect may be due to one, or several, factors. Our data show that the pH activation is in the direction which could favor formation of undissociated molecules, which may be more effective than ions because of greater ability to enter the cell. The work of Osterhout (69) indicates that only undissociated molecules penetrate the cell membrane. Possibly the pH effect is due to greater adsorption. Thus, Phelps and Peters (70) have shown that the adsorption of fatty acids (and presumably detergents) on charcoal depends on the concentration of undissociated acid. It is interesting in this connection to note that Michaelis and Dernby (71) have concluded from their studies that the action of quinine alkaloids (including optochin) on bacteria depends on the free base, and not on the salt.

It is possible that hydrogen ion concentration directly affects the bacteria, altering either membrane or protoplasm in such a manner as to render the microorganisms more susceptible to the action of the detergent (72). It is conceivable that hydrogen ion concentration may influence the formation of micelles. It is known that detergents are very apt to change from molecular to micellar solution (73). In such a case the effective, antibacterial concentration of the detergent might be changed markedly.

There are many other possibilities which might be considered, *e.g.*, the effect of pH on formation of molecular films, or on penetration of films, etc. Without more experimental evidence, further speculation seems unwise. However, it is apparent from our results that special attention must be given to control of hydrogen ion concentration in any studies on the antibacterial effects of detergents. Obviously, phenol coefficients obtained at a stipulated pH may not indicate the maximal germicidal power of many detergents.

Importance of Specific Chemical Groups in the Detergent Molecule

It has been shown above that cationic or anionic structure, as well as hydrocarbon chain length, are important factors in determining the relationship between chemical structure and the effects on bacterial metabolism. In addition, it is apparent that particular chemical configurations modify activity. For example, Tergitol-7 was the most active of all the anionic compounds which we have had occasion to study. This compound is an alkyl sulfate derived from a secondary alcohol, 3,9-diethyl tridecanol-6.[11]

[11] Wilkes and Wickert (74) have compared the "wetting" abilities of several Tergitol compounds (all secondary alcohol sulfates) with those of other commercially available wetting agents. They found that secondary alcohol sulfates were better wetting agents

Since other alkyl sulfates derived from primary alcohols containing approximately the same number of carbon atoms are not nearly so effective as Tergitol-7, it would appear that the incorporation of this specific group has created a more active compound.

Another compound worthy of special consideration is Damol. This is the only diquaternary compound which we have had available. It is among the most active detergents studied, possessing activity equivalent on a weight basis to Zephiran or Retarder LA. On a molar basis, however, Damol is approximately twice as active as Zephiran, possibly because it contains two quaternary ammonium groups. It is conceivable, therefore, that the incorporation of more than one quaternary ammonium group into a detergent might be expected to enhance its activity.

The Emulsol compounds, 606, 608, and 609 are interesting since their hydrophobic portion (long chain alcohol) is linked to a naturally occurring substance, an amino acid. These lauryl esters of amino acids are all very effective in reducing bacterial metabolism and their effectiveness is a function of the number of carbon atoms in the amino acid. To what extent further substitution can increase activity must remain for future studies. It is noteworthy that preliminary studies (75) on one of these compounds, the lauryl ester of glycine hydrochloride, have shown a comparatively low toxicity. *These compounds are cationic detergents which do not possess the quaternary ammonium structure.* We believe that the results obtained in these experiments demonstrate for the first time that detergents of this type inhibit bacterial metabolism.

Lecithin, which is a naturally occurring hydrophilic-hydrophobic compound, shows no inhibitory activity even at very high concentrations. Yet, from a consideration of its structural formula as ordinarily represented, one might have predicted that lecithin would possess an activity com-

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than primary alcohol sulfates, and concluded that the position of the hydrophilic group of the molecule with respect to the hydrophobic portion can be correlated with distinct differences in wetting efficiency. Furthermore, their results indicate that the improved wetting efficiency of the secondary alcohol derivatives could not be attributed to greater reduction of interfacial tension.

It is of interest in this connection that Cowles (64*b*), using as an analogy the wetting of wool by various germicides, has recently discussed the possibility that the disinfection process by substances of this kind may be due to a "wetting" of the bacterial cell, and has concluded from his studies that "changes in the time of disinfection with changes in the concentration of disinfectant may be due to correlated changes in wetting power."

parable to that of cationic detergents containing the quaternary ammonium group.

Significance of Biological Characteristics of the Microorganisms

As has been pointed out in the introduction, it has been recognized for some years that soaps and related compounds have an unusual affinity for certain microorganisms. Moreover, Cowles and others (36, 37) have shown clearly that alkyl sulfates differentiate sharply between Gram-positive and Gram-negative organisms. Our experiments demonstrate further that this differentiating capacity is not confined to alkyl sulfates, but appears to be characteristic of anionic detergents. In addition they reveal the very important practical point that cationic detergents attack Gram-positive and Gram-negative organisms equally well.

It is not clear why anionic detergents affect only Gram-positive microorganisms. Wetting or detergent properties *per se* do not appear to be the only determinant, because *cationic* wetting and detergent compounds do not exhibit selective action. Furthermore, marked selectivity is shown by non-detergent compounds such as dyes (76).

The many explanations which have been offered for the Gram-staining ability of microorganisms and for the differences in behavior of Gram-positive and Gram-negative microorganisms have been reviewed fairly recently by Sander (77). It is to be hoped that the elucidation of the mechanism of action of synthetic detergents on bacteria will simultaneously provide insight into the differences between Gram-positive and Gram-negative microorganisms.

It is of interest to note individual differences among the bacteria in the larger classification of Gram-positive and Gram-negative microorganisms. For example, *Sarcina lutea* appears to be stimulated by certain detergents more frequently and more intensely than any of the other organisms studied. Some organisms were stimulated occasionally, others never.

Stimulation Effects

It is not uncommon to observe stimulation of metabolic processes by drugs or chemicals at low concentrations, and depression or inhibition of the same processes at higher concentrations. This phenomenon is encountered both in the animal body and in isolated tissue preparations or cells. Therefore, its manifestation in the case of the detergents would warrant little attention except for the fact that the stimulating effect has been much more marked in our series of anionic detergents than in the cationic group.

SUMMARY

A study of the effects of synthetic detergents and wetting agents on respiration and glycolysis of Gram-positive and Gram-negative microorganisms has led to the following conclusions.

1. All the *cationic detergents* studied are very effective inhibitors of bacterial metabolism at 1:3000 concentration, and several are equally active at 1:30,000. Few of the *anionic detergents* inhibit as effectively as the cationic compounds.

2. Gram-positive and Gram-negative microorganisms are equally sensitive to the action of the cationic detergents. On the other hand, all the anionic detergents included in our studies selectively inhibit the metabolism of Gram-positive microorganisms.

3. The inhibitory action of both types of detergents is influenced markedly by hydrogen ion concentration. Cationic detergents exhibit their maximum activity in the alkaline pH range, and the anionic, in the acid range.

4. Studies of homologous series of straight chain alkyl sulfates and sulfoacetates (C_8 to C_{18}) demonstrate that maximum inhibition is exerted by the 12, 14, and 16 carbon compounds (lauryl, myristyl, and cetyl).

5. It has been observed that three lauryl esters of amino acids are powerful inhibitors of bacterial metabolism. To our knowledge, the effects on bacterial metabolism of such cationic detergents (without the quaternary ammonium structure) have not been studied previously. Our results demonstrate that other cationic detergents can exhibit an inhibitory activity comparable to quaternary ammonium compounds.

6. Certain detergents *stimulate* bacterial metabolism at concentrations lower than the inhibiting values. This effect has been found more frequently among the anionic detergents.

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